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Plenary Session

Global Status and Future Outlook of Table Grapes

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Summary
Approximately 76 million tons of grapes are produced in a total area of 6,725,000 hectares around the world. A significant portion of this production consists of grapes grown for table consumption, and the share of table grapes in total production is constantly increasing. Especially in recent years, wine consumption has declined due to changing consumption habits among young consumers, negatively affecting wine grape production. However, in table grape production, the high quality and yield of newly developed varieties have increased interest in this area. Many local table grapes are in low demand in international markets, encouraging growers to turn to alternative, high-quality grape varieties. In addition, the negative effects of climate change, increasing awareness of human and environmental health, and the need for new varieties to meet rapidly changing consumer demands on a global scale are contributing factors. Grapevine breeding programs to develop new, high-quality table grape varieties are ongoing in various institutions worldwide. Varieties developed by private breeding companies, in particular, sell at higher prices, but only growers who meet specific conditions and pay breeder's fees can cultivate them. This study evaluates the leading table grape breeding efforts worldwide, their main challenges, and opportunities.

Keywords: *Vitis* spp, biotic and abiotic stress, breeding, major problems, opportunities

Introduction

Viticulture and table grape cultivation have been a significant source of income for producers in many countries since ancient times (Atak, 2024a). Currently, grape production worldwide is approximately 6,725,000 hectares, yielding 76 million tons. A significant portion of this production is table grapes (FAOSTAT, 2026; Graph 1, 2 and 3). Furthermore, the share of table grapes in total production has been steadily increasing over the past few years. The countries with the highest grape production are, in order: China, Italy, France, Spain, the USA, Türkiye, India, Chile, and South Africa. Except for France, a significant portion of the grapes produced in all of these countries are grown for table consumption.

Grapes are grown in almost every region of the world except the equatorial and polar regions, although production quantities vary from year to year (Graph 2). Examining worldwide grape production area and quantity between 1994 and 2024 shows an increase in production quantity despite a decrease in production area. This situation is explained by the increase in yield per unit area, particularly in recent years, which has occurred in parallel with the increase in table grape production. Furthermore, the decrease in the use of traditional systems and their replacement with more modern systems, as well as the replacement of traditional varieties with new, more efficient, improved varieties, are among the main reasons for this increase (Dry et al., 2022).

Challenges and Opportunities to Growing Table Grapes

In table grape production, due to increasing competition in world markets, it is important to develop and bring to market high-quality new varieties that meet consumer demands. Conditions in the table grape market are rapidly changing due to climate change, and demand for new varieties is increasing (Sharma et al., 2013; Khan et al., 2020). In recent years, several breeding studies, conducted for different purposes, have been funded and carried out by consortia/companies comprising various government institutions and private-sector

representatives. The primary goals of these breeding studies are to develop varieties that are seedless, firm-fleshed, large-grained, with a unique aroma, suitable for cold storage, and with high yields (Shelton et al., 2017; Atak, 2023). However, in recent years, amid the negative effects of global climate change, more breeding studies have been conducted to develop varieties that are more tolerant/resistant to biotic and abiotic stressors (Louime et al., 2010). Furthermore, growers expect these new varieties to have lower production costs. With increasing public health awareness, consumers have begun to demand new varieties that are healthier and use fewer pesticides/fertilizers (Chávez-Dulanto et al., 2021). In addition, with the increase in the prices of basic production factors such as chemicals, fertilizers, and labor, more work has begun to develop new, more grower-friendly varieties (Töpfer and Trapp, 2022). There is a growing demand for new varieties that are more drought-resistant and suitable for organic cultivation, and table grape breeding programs are ongoing in various countries and institutions (Carratore et al., 2026).

Although these growers who cultivate newly developed grape varieties share 3-5% of their revenue with the organizations that hold the rights at the end of each season, they generate more income than local and standard varieties due to their higher yields and higher prices. In particular, higher income can be obtained from varieties that ripen at both early and late times (Novello and De Palma, 2008).

Table Grape Breeding Programs

When the grape production levels of the continents are examined over recent years, it is seen that production in Europe has decreased, while production in Asia and Africa has increased (Graph 4). Although climate change is among the main reasons, increasing labour costs is also effective. In addition, while a large part of European production consists of low-yielding vineyards for wine, most vineyard areas in Asia and Africa are high-yielding table and raisin varieties. Table grape cultivation is of great importance for China, Turkiye, the USA, Italy, Spain, Egypt, Iran, Chile, South Africa, India, and some other countries (Table 1). There are some seeded and seedless varieties of table grapes. The seedless table grape market has exploded over the past 50 years, and consumers are increasingly accepting of seedless grape varieties (Ibanez et al., 2015).

Controlled grapevine breeding studies began almost two hundred years ago. The modern era of grape breeding is closely linked to the arrival of North American fungal diseases and insects in Europe (Paul, 1996). Different breeding programs have been initiated to develop new grape varieties with different characteristics, especially in accordance with changing consumer demands (Reisch et al., 2012; Khan et al., 2020). In these breeding programs, especially seedless, large-berry, distinctive-flavoured varieties that are resistant/tolerant to various stress conditions are being developed. Due to young consumers' interest in seedless varieties, the selection of seedless cultivars in new breeding programs has significantly decreased (Puglisi et al., 2022). Although *V. vinifera* grapes constitute over 95% of the world market, they are generally highly susceptible to many diseases. It is expected that as interest in organic and sustainable viticulture increases, demand for disease resistant varieties will increase (Reisch et al., 2012).

Table grape breeding studies first began in California, USA, through a collaboration between the University of California-Davis (UC Davis) and the US Department of Agriculture (USDA) near Fresno, CA. Many table, dry, and wine grape varieties and rootstocks were developed and registered. From the 1980s onwards, private grapevine breeding companies began operating in the southern San Joaquin Valley of California. These companies focused particularly on table grape breeding, developing many new varieties preferred by consumers. As a result of the high demand for these new varieties, similar organizations in different countries (especially Spain, Italy, and Israel) began breeding programs. Most of these companies, especially through global

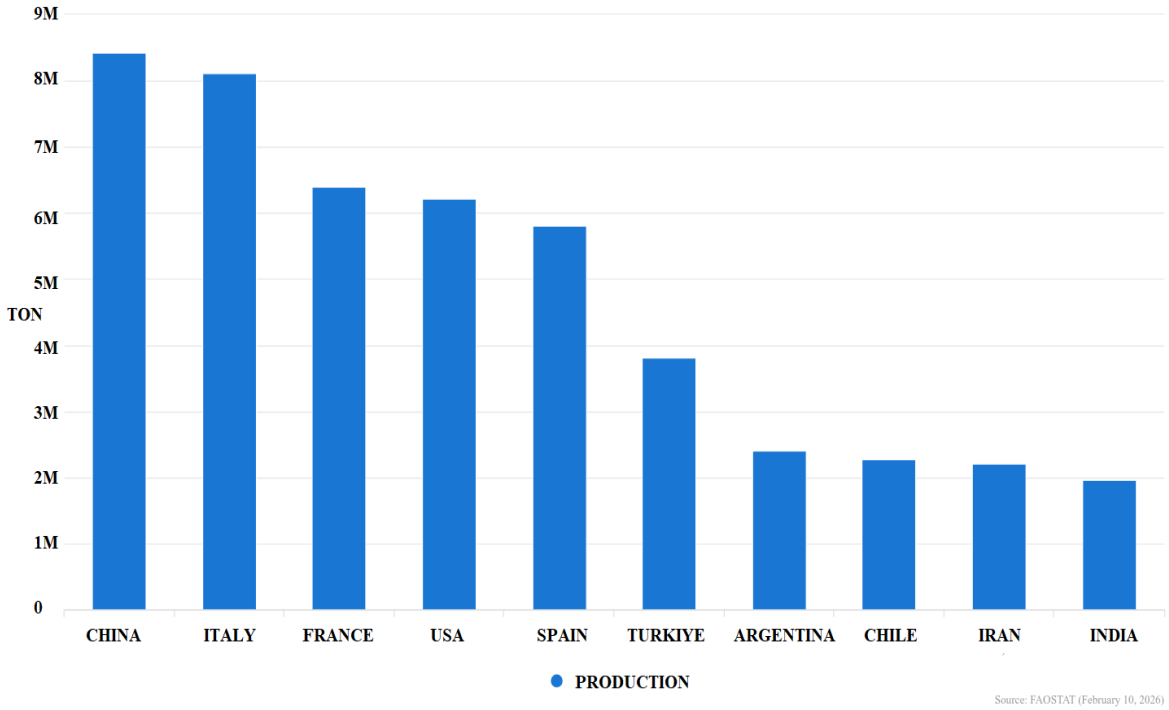
marketing techniques, have made effective use of the existing table grape market and now largely dominate it. (Lund, 2015; Atak, 2024a). In recent years, breeding studies have been continuing in many countries of the world for diferent purposes.
Today, among companies specializing in table grape breeding, Bloomfresh International, Sunworld International, Grapa Global, and Itum are the most prominent, with their production areas and volumes increasing year over year. While Bloomfresh International and Sunworld International also breed and develop for other species, Grapa Global and Itum focus solely on developing table grape varieties. In addition to these companies, various table grape breeding programs are ongoing, particularly in countries with significant table grape production, such as Italy, Chile, China, and Turkiye. In recent years, large growers and some companies have tended to collaborate, funding different research institutions to develop their own breeding programs. It is expected that in the near future, a significant portion of table grapes grown worldwide will be varieties developed through such breeding programs (Atak, 2024b).

Major Problems and Solutions in Table Grape Industry

Despite the high volume of table grape production worldwide, the sector has been negatively affected by climate change in recent years, resulting in several adverse consequences for vineyards. Climate change, which has become a global threat with increasingly significant effects, is causing numerous problems and negative impacts on the viticulture sector, as it does in all sectors. The decrease in vineyard areas and production quantities in 2020 and 2021 is reported to be significantly related to the increasing negative impacts of climate change (Fraga, 2009; Sgroi and Sciancalapere, 2022). Climate change poses significant challenges for both table and wine grapes. One of the most effective solutions to this situation is to develop new grape varieties and rootstocks that can adapt to climate change (Marin et al., 2021).
Rising temperatures due to climate change cause abiotic stress in plants, negatively affecting plant growth and fruit quality. The table grape sector is among the sectors most affected by the negative impacts of climate change, and unfortunately, these negative effects are increasing every year (Ollat et al., 2019; Grillakis et al., 2022).
With global warming, water resources are becoming limited, making it difficult to find sufficient water for vines. As a solution, it is primarily necessary to develop more drought-resistant varieties and rootstocks. In addition, practices that protect the vine from heat stress have become increasingly important (Venios et al., 2020).
Different breeding programs aimed at addressing climate change have the potential to largely solve the problems. Many practices, such as producing in the right climates, using protective covering systems if necessary, utilizing precision/smart viticulture technologies, selecting appropriate rootstocks, and correctly performing cultural practices, can significantly help table grape growers (Delrot et al., 2020; Magon et al., 2023; Sun et al., 2023).
Growing local or standard grape varieties in open fields is becoming increasingly difficult, and their profitability is decreasing year by year (Palotti et al., 2023). Grape growers are cultivating more and more newly developed grape varieties with superior characteristics each year. However, cultivating these new varieties with high quality requires higher initial planting costs, especially due to the need for netting or plastic coverings. Growers are forced to spend more to take precautions against increasing risks, which somewhat reduces the profitability of these high-yielding new varieties (Alekberova et al., 2023). In addition, insufficient government support significantly limits small growers' access to these varieties.

Table 1. Countries with the largest vineyard area and grape production quantity in 2024 (FAOSTAT, 2026).

COUNTRIES	VINEYARD AREA (HA)	PRODUCTION (TON)
Spain	903.980	5.387.000
China	770.850	16.821.000
France	741.330	4.747.000
Italy	715.790	7.637.000
USA	373.529	4.902.000
Turkiye	372.285	3.468.000
Argentina	199.946	1.918.000
India	179.629	3.904.000
Portugal	173.210	917.000
Chile	166.457	2.453.000
Romania	161.930	704.160
Australia	135.264	1.613.000
Iran	119.275	1.566.000
Uzbekistan	115.421	1.832.000
South Africa	103.955	1.870.000
Germany	101.560	1.059.000



Graph 1. The top ten grape-producing countries and their production quantities (Average from 1994 to 2024.

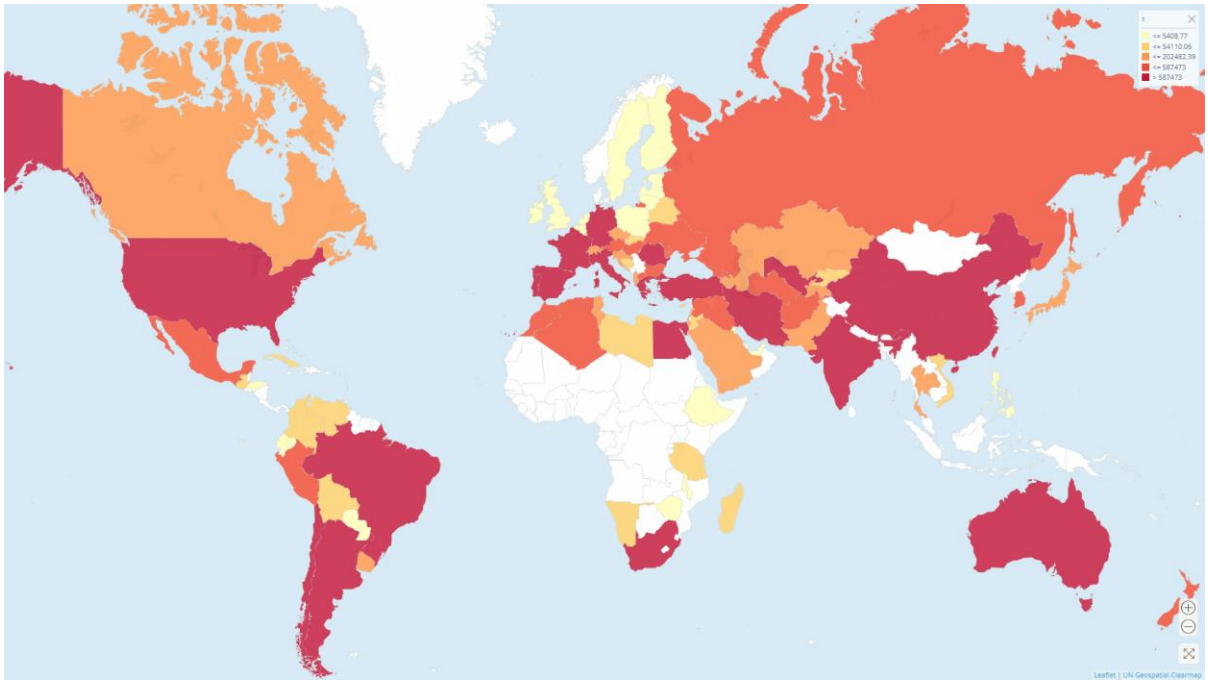
While table grape breeding programs in America and Europe are predominantly private-sector led, in countries like Turkiye, China, and Korea, breeding efforts are mostly conducted by state-owned organizations. Especially given the budget, personnel, and participation of client organizations in breeding programs, varieties developed by private-sector organizations are much easier to commercialize and meet expectations (Burris et al., 2025).

Conclusion

Although viticulture has a long history, most local grape cultivars cannot fully meet rapidly changing consumer demands. Globally, a significant segment of the population earns its income from table grape cultivation. However, in recent years, the effects of climate change, shifting consumer demands, and increasingly competitive market conditions have made it increasingly difficult for these producers to achieve their expected income. While new varieties developed through breeding programs serve as a kind of savior, the fact that not every producer has access to or the right to cultivate them is a significant problem. Grower unions and cooperative models, in particular, could offer solutions to overcome these difficulties.

Furthermore, with climate change, table grape production is increasingly shifting into areas previously suitable for wine grape production. Nowadays, demand for new table grape varieties that are more environmentally friendly, require fewer pesticides, and are beneficial to human health is increasing. Research increasingly shifting in this direction, increased government support, and the development of new varieties that meet the expectations of all parties are likely scenarios for the future of the table grape industry.

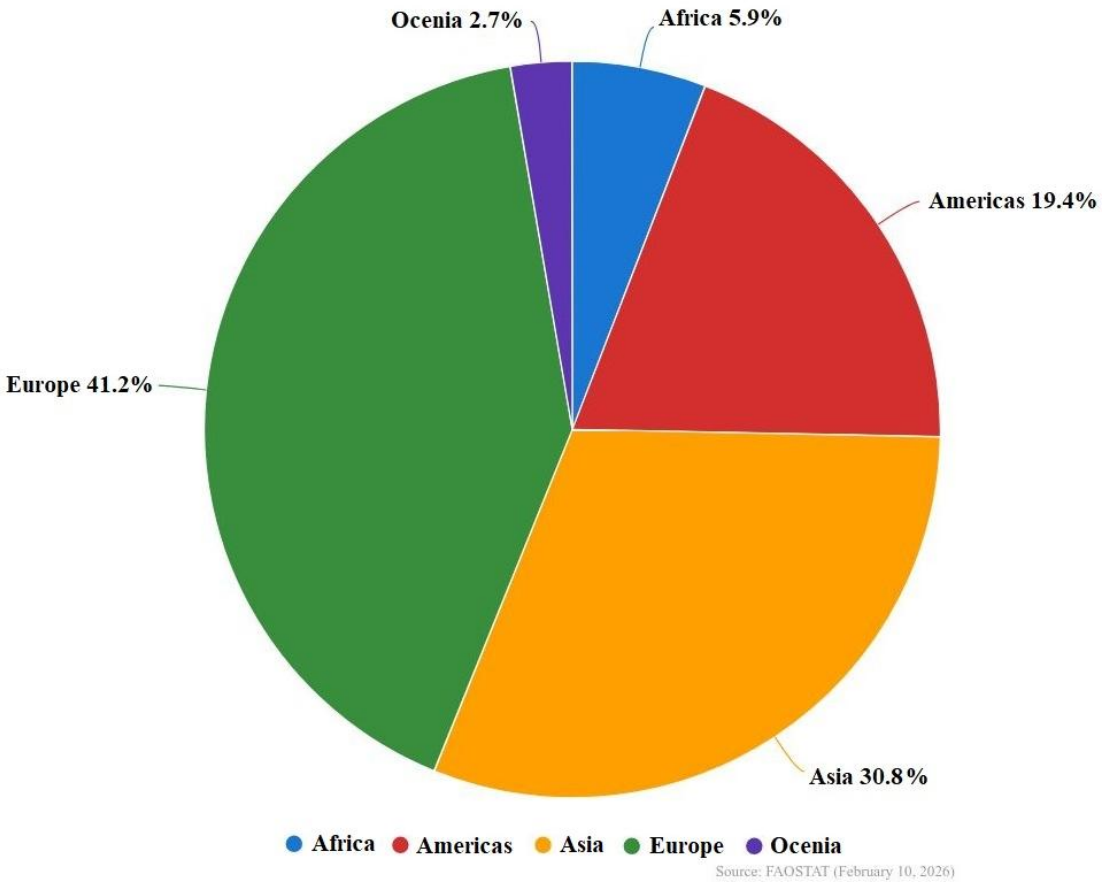
Multi-partner, high-budget breeding programs that also involve client organizations are proving to be much more successful globally. State-owned enterprises face significant difficulties in obtaining commercially viable varieties through breeding programs for various reasons. Thanks to advancements in biotechnology, new varieties that require far fewer pesticides and are richer in health-related components are becoming increasingly available on the market. Furthermore, as consumer awareness rises, it is predicted that the number of new varieties suitable for organic production will continue to increase.



Graph 2. Production quantities of Grapes by country (Average 1994 - 2024) (Faostat, 2026).



Graph 3. Total grape production area and quantities in the world between 1994 and 2024.



Graph 4. Production share of grapes by continents (average 1994-2024).

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Session 1

Plant and Food Production

ORIGINAL SCIENTIFIC PAPER

The effect of apical defoliation after veraison of Svrdlovina (*Vitis vinifera* L) grapevines on yield components and grape phenolic composition

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Summary
Modulating the vine source-sink relationship is a very practical strategy to mitigate effects of climate changes that can decouple tehnological from phenolic maturity. This study investigated the effects of the apical defoliation performed after veraison on 'Svrdlovina' vines in Northern Dalmatia, Croatia (2023), on yield, berry primary and phenolic composition. Apical defoliation reduced the content of total phenols and anthocyanins but did not affect the sugar content, as well as the content of total flavonols, flavan-3-ols, hydroxycinnamic and hydroxybenzoic acids. Apical defoliation is a timing-dependent practice, and under the climatic conditions of this study it did not result in delayed fruit ripening or enhanced phenolic content.

Keywords: apical defoliation, grapevine, phenolic content, yield

Introduction

The decision of when to harvest is an important subject for grape and wine producers and it is based on the sugar and total acidity levels, as well as phenolic and aromatic ripeness of the grapes. Today, many wine-growers are facing challenges in determing the optimal harvest date as a consequence of climate changes. Among the most significant consequences of climate change in viticulture are higher temperatures during grape ripening, earlier harvest dates, and increased sugar accumulation, resulting in elevated alcohol levels in wine. At the same time, a reduction in total acidity has been widely reported leading to a loss of varietal typicity and terroir expression (van Leeuwen and Darriet, 2016). It is becoming increasingly difficult to achieve phenolic maturity at the same time as sugars and total acidity reach desirable levels, a decoupling between technological and phenolic ripeness often occurs (Sadras and Moran, 2012). Managinig the vine source–sink relationship and balancing assimilate supply from leaves vs. demand from berries, shoots, roots, and reserves is a very practical way to buffer climate-change stressors, especially heat waves, drought, and accelerated sugar ripening that can decouple technological from grape phenolic maturity.

Numerous studies have focused on short-term adaptation strategies to mitigate the effects of climate change on viticulture, such as late winter pruning, minimal pruning and late apical defoliation (Netzer et al., 2022; O'Brian et. al., 2021; Zhen et al., 2016). While basal leaf removal is conducted around the fruit zone to reduce the leaf photosynthetic area and to increase sunlight exposure to the cluster, apical defoliation involves the removal of leaves located above the clusters, leaving a leafless window of approximately 50 cm by removing 30-35% of the leaves from upper two-thirds of each shoot. Several leaves are retained at the apex of each shoot, as well as few leaves immediately above the grape zone, reducing the photosynthetic activity of the vine, wihout modifying berry microclimate or cluster exposure (Zhang et al., 2017). When it is performed after veraison, its primary goal is to regulate vine vigor, limit carbohydrate allocation to shoot apices, and redirect assimilates toward berry ripening and secondary metabolite accumulation. Apical defoliation is an effective method to reduce wine

alcohol concentration with minimal influence on wine composition, expecially wine aromatic properties (Zhang et al., 2017).
While various other research have shown the potential of apical leaf removal to achieve slower grape sugar accumulation with little or no affect on the content of anthocyanins, phenols and tanins in grapes (Lu et al., 2022; Palliotti et al., 2013; Villango et al., 2025), other research were somewhere inconclusive (Gatti et al., 2019; O'Brian et. al., 2021; Vercesi et al., 2024), highlighting the need for investigation of this technique across different varieties and growing conditions. The aim of our study was to investigate the effect of apical defoliation performed after veraison on yield, berry primary and phenolic composition of 'Svrdlovina' *(Vitis vinifera* L.*)* grape variety grown in Northern Dalmatia, Croatia.

Materials and Methods

A one-year study (2023) was conducted on 'Svrdlovina' (*Vitis vinifera* L.) grape variety grown in Zadar wine-growing region, Northern Dalmatia, Croatia. The site has a northeastern exposure facing the Velebit mountain and is situated at an altitude of 140 m, with a slope of 10%. The soils of the Zadar wine-growing region are marly and calcareous, rich in minerals (Vrsaljko, 2008). The vines were cordon trained and shoots vertically positioned, with a trunk height of 0.9 to 1.0 m. Vine spacing within the row was 0.9 m, while row spacing was 2.5 m. The experiment was arranged in a randomized block design with three replications and consisted of two treatments: apical defoliation and control. Each replication comprised 10 consecutive vines, resulting in 30 vines per treatment. The apical defoliation treatment was conducted after the onset of veraison, at the phenological stage E-L 36, based on the modified Eichhorn and Lorenz system (Coombe, 1995), where approximately 33% of the leaf area above the cluster zone was removed, whereas no defoliation was applied in the control treatment. Harvest was carried out on 20 September 2023, and both treatments were harvested simultaneously. At harvest, yield per vine was measured. Average cluster weight was obtained by measuring 10 clusters per replication for each treatment. Sugar content were determined using a refractometer, total acidity was determined by titration, and must pH was measured using a pH meter, according to OIV methods (2001). From each replication, a sample of 100 berries was collected for the analysis of grape phenolic compounds using high-performance liquid chromatography (HPLC). A total of 150 g of frozen berries was weighed, and 5 mL of an aqueous ascorbic acid solution (200 g/L) was added. The berries were homogenized in the presence of dry ice, and 5 g of the obtained homogenate was used for further analysis. Immediately prior to analysis, the prepared samples were heated in a water bath at 50 °C for 20 min, after which 5 mL of extraction solvent containing 40% acetonitrile, 2% formic acid, and 58% water was added. The extraction mixture was placed on a preheated magnetic stirrer (50 °C) and stirred for 1 h. The resulting extracts were centrifuged at 12,000 rpm for 10 min, and the supernatant was filtered through a 0.22 µm membrane filter. Individual phenolic compounds were determined using reversed-phase high-performance liquid chromatography with an Agilent 1100 Series liquid chromatograph (Agilent, USA). Separation was performed on a Phenomenex Luna Phenyl-hexyl column (250 × 4.6 mm; Phenomenex, USA) using gradient elution with 0.5% (v/v) aqueous phosphoric acid as solvent A and a mixture of acetonitrile:water:phosphoric acid (50:49.5:0.5, v/v/v) as solvent B, at a flow rate of 0.9 mL/min. The injection volume was 20 µL, and the column temperature was maintained at 50 °C. Hydroxybenzoic acids were detected at 280 nm, hydroxycinnamic acids at 320 nm, flavonols at 360 nm, and anthocyanins at 518 nm. Flavan-3-ols were determined using a fluorescence detector at λex = 225 nm and λem = 320 nm. Peak identification was based on comparison of retention times and UV spectra with those of authentic standards, while quantification was performed using the external standard method. All data were analyzed by the t-test at a 95% confidence level. Statistical analysis was performed using the software XLSTAT (Addinsoft, 2020, New York, USA).

Results and Discussion

Apical defoliation performed after veraison did not affect yield per vine, cluster weight, or berry weight (Table 1) of the 'Svrdlovina' grapevines. Similar results have been reported in other studies (O'Brian et. al., 2021; Vercesi et al., 2024; Zhang et al., 2017), indicating that post-veraison canopy manipulation generally has limited effects on yield components.

Despite the fact that the active leaf area has a great influence on the sugar accumulation during the period between veraison and havest, it appears that the source limitation induced by apical leaf removal did not significantelly affect the accumulation of sugars in our study (Table 1), neather did it affect the total acidity, or must pH (Table 1), which is in accordance with O'Brian et. al. (2021). This may be attributed to non-limiting light conditions (O'Brian et. al. (2021) or the high capacity of grapevines for photosynthetic compensation (Poni et al., 2013). This is contrary to the resulty of some studies who reported the decrease in sugar accumulation following apical defoliation performed at veraison (Lu et al., 2022;Vercesi et al., 2024).

Table 1. Yield and basic composition of 'Svrdlovina' grape juice from apical defoliation and control treatments (2023)

	Apical defoliation	Control	Sign.
Yield per vine (kg)	2.46 ± 1.16	2.48 ± 0.76	n.s.
Cluster weight (g)	317.5 ± 100.7	294.5 ± 83.4	n.s.
Berry weight (g)	92.0 ± 14.9	86.7 ± 9.3	n.s.
Cluster number per vine	8.8 ± 3.9	8.9 ± 3.7	n.s.
Soluble solids (°Oe)	80 ± 0.6	76 ± 1.0	n.s.
Titratable acidity (g L^{-1})	5.6 ± 0.5	5.2 ± 0.6	n.s.
pH	3.41 ± 0.05	3.41± 0.03	n.s.

Data were analysed using a t-test. Differences were considered significant at $P < 0.05$; mean ± SD (n=30) for yield component and mean ± SD (n=3) for basic grape composition

A total of 34 individual phenolic compounds were extracted and quantified in the grapes of the variety 'Svrdlovina', including 10 anthocyanins, 8 flavonols, 5 hydroxycinnamic acids, 2 hydroxybenzoic acids, 8 flavan-3-ols, and 1 stilben- resveratrol-3-*O*-glucoside (Table 2). The most abundant anthocyanin was malvidin-3-O-glucoside, quercetine-3-O-glucoside was most abundant flavonol, while catechin was the predominant flavan-3-ol.

Apical defoliation performed after veraison resulted in a reduction in total phenolic and anthocyanin content, however, it did not significantly affect the content of total flavonols, flavan-3-ols, hydroxycinnamic acids, hydroxybenzoic acids, or resveratrol-3-*O*-glucoside in the grapes. Similar trends have been reported in other studies, where post-veraison apical defoliation altered vine canopy architecture sufficiently to indirectly alter berry UV exposure and temperature, leading to changes in anthocyanin accumulation, despite the absence of direct leaf removal in the fruiting zone (O'Brian et. al., 2021; Vercesi et al., 2024). Also, under the source limitation conditions, such as removing leaves apical to the grapes, berries can prioritize carbon allocation to sugar accumulation, maintaining sugar levels at the expense of secondary metabolites (Bobeica et al., 2015).

Several studies have reported that apical defoliation can reduce grape anthocyanin and total phenolic contents, sometimes also negatively affecting wine color parameters, particularly when applied around veraison (Buesa et al., 2019; Zhang et al., 2017; O'Brien et al., 2021). In contrast, other studies have shown that apical defoliation, especially when applied after veraison, can enhance the accumulation of specific phenolic compounds, including flavonols and total polyphenols in grape skins (Lu et al., 2022; Vallango et al., 2025).

Table 2. Phenolic composition of 'Svdlovina' grapes from apical defoliation after veraison and control treatments (2023)

	Apical defoliation	Control	Sign.
Cyanidin-3-*O*-(coumaroyl) glucoside	0.289 ± 0.06	1.363 ± 0.04	***
Delphinidin-3-*O*-glucoside	2.152 ± 0.85	3.757 ± 0.14	*
Malvidin-3-*O*-glucoside	91.342 ± 13.99	133.511 ± 8.04	*
Malvidin-3-*O*-(acetyl) glucoside	0.325 ± 0.14	2.091 ± 0.16	***
Malvidin-3-*O*-(caffeoyl) glucoside	1.568 ± 0.14	3.647 ± 0.26	***
Malvidin-3-*O*-(coumaroyl) glucoside	45.096 ± 4.16	75.867 ± 12.44	*
Peonidin-3-*O*-glucoside	4.944 ± 0.71	4.784 ± 0.50	n.s.
Peonidin-3-*O*-(acetyl) glucoside	2.978 ± 0.74	7.060 ± 0.61	**
Peonidin-3-*O*-(coumaroyl) glucoside	0.660 ± 0.10	2.763 ± 0.35	**
Petunidin-3-*O*-glucoside	2.884 ± 0.53	8.601 ±0.71	***
Total anthocyanins	**152.238 ± 15.24**	**243.44 ± 17.63**	**
Izorhamnetine-3-*O*-glucoside	1.679 ± 0.18	1.668 ± 0.31	n.s.
Kaempferol-3-*O*-glucuronide	1.224 ± 0.17	1.401 ± 0.24	n.s.
Kaempferol-3-*O*-glucoside	6.014 ± 0.39	6.463 ± 0.63	n.s.
Quercetin-3-*O*-galactoside	9.579 ± 1.43	10.544 ± 2.32	n.s.
Quercetin -3-*O*-glucuronide	2.252 ± 0.67	5.026 ± 0.81	**
Quercetin -3-*O*-glucoside	25.924 ± 2.03	33.667 ± 3.31	n.s.
Myricetin -3-*O*-galactoside	6.640 ± 0.66	14.081 ± 2.03	**
Myricetin-3-*O*-glucoside	11.065 ± 0.80	2.798 ± 1.13	***
Total flavonols	**64.377 ± 1.30**	**75.646 ± 8.65**	**n.s.**
Fertaric acid	0.417 ± 0.12	0.498 ± 0.05	n.s.
Ferulic acid	1.112 ± 0.38	1.531 ± 0.17	n.s.
Caffeic acid	1.391 ± 0.25	1.307 ± 0.07	n.s.
Caftaric acid	66.754 ± 3.31	66.549 ± 3.39	n.s.
Coutaric acid	8.702 ± 2.53	11.108 ± 1.75	n.s.
Total hydroxycinnamic acids	**78.375 ± 5.55**	**80.992 ± 5.36**	**n.s.**
Protocatechuic acid	3.981 ± 0.07	2.947 ± 0.87	n.s.
Vanillic acid	8.503 ± 1.26	7.885 ± 0.10	n.s.
Total hydroxybenzoic acids	**12.483 ± 1.21**	**10.831 ± 0.91**	**n.s.**
Epigallocatechin	12.826 ± 2.40	14.489 ± 1.34	n.s.
Epicatechin	18.609 ± 3.39	19.231 ± 2.06	n.s.
Gallocatechin	10.416 ± 0.88	15.872 ± 4.08	n.s.
Catechin	28.744 ± 4.47	23.741 ± 5.69	n.s.
Procyanidin B1	22.110 ± 1.66	21.590 ± 0.33	n.s.
Procyanidin B2	10.244 ± 2.46	12.713 ± 2.36	n.s.
Procyanidin B3	8.326 ± 1.02	7.328 ± 0.38	n.s.
Procyanidin B4	5.631 ± 0.97	6.092 ± 0.88	n.s.
Total flavan-3-ols	**116.906 ± 11.37**	**121.057 ± 3.75**	**n.s.**
Resveratrol-3-O-glucoside	**6.131 ± 0.93**	**5.751 ± 0.41**	**n.s.**
Total phenolic concentration	**430.51 ± 24.98**	**537.72 ± 23.48**	****

Data were analysed using a t-test; ns, *, **, and *** denote not significant, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; the results are expressed in mg/kg of fresh berry

Conclusion

There are various research in the last years indicating the potential of apical leaf removal to mitigate the adverse effects of climate change and to achieve slower grape sugar accumulation without affecting the concentration of phenolic compounds in grapes. Nevertheles, the results of this study do not support the use of this method for delaying grape ripening or improving grape phenolic content. Significant research still needs to be done to determine the optimal ratio of leaf to fruit ratio, the timing and a location of leaf removal relative to grapes, and the long-term impact on the vine physiology to determine the optimal apical defoliation tehnique suitable for commercial vineyards.

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ORIGINAL SCIENTIFIC PAPER

Kernel composition of maize hybrids under different environmental conditions

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Summary
Research on the influence of climate, soil (location), and the genetic effect of hybrids on maize grain yield and kernel composition was conducted at two sites, L1 Karanac and L2 Nuštar, during 2024. Maize hybrids from FAO maturity groups 390 to 500 were sown at both locations. Although the sites are 60 km apart, similar climatic conditions were recorded in terms of precipitation and average daily air temperatures. Based on the $LSD_{0.05}$ test for average starch kernel content, it can be concluded that there were no statistically significant differences among the tested hybrids at L1, as well as among hybrids H2, H3, H4, and H5 at L2. A statistically significant difference in crude protein content was observed between hybrid H4 and the other hybrids at L1, while at L2 a significant difference was found for H2 compared to the other hybrids. Regarding crude fat content, a statistical difference was determined for H3 compared to the other hybrids at L1, and for H2 compared to the remaining hybrids at L2.

Keywords: kernel composition, maize, grain yield, plant density

Introduction

Global shifts in climatic conditions, which increasingly limit the cultivation potential of certain agricultural crops, position maize (*Zea mays* L.) with its extensive production range as a crop that may soon become the most widely cultivated worldwide. Maize is characterized by versatile utilization of all plant components (stalk, ear, grain, cob, etc.). According to Erenstein et al. (2022), more than 50% of global maize grain production is used as livestock feed and in the food industry, with a growing share directed toward energy production (Stępień and Rejmer, 2023). The same authors report that maize grain consumption provides approximately 5% of the global population with their required intake of proteins and fats. The proportion of individual grain components results from a complex interaction among soil nutrient availability, site-specific conditions, and the genetic characteristics of hybrids, including their adaptation to environmental factors. As noted by Sibuea et al. (2023), the nutritional maize kernel content particularly protein content directly influences its suitability for human and animal nutrition. Chenlong et al. (2024) reported an average protein content of 9.79% in maize kernel consistent with typical values for standard maize, which range from 8.43% to 11.25%. Protein content is not solely determined by cultivar genetics; it is also influenced by climatic conditions, soil properties, fertilization practices, and crop protection measures. In contrast to most edible vegetable oils derived from oil-rich seeds, maize kernel contains only 3–4% fat (Ortíz-Islas et al., 2019). Serna-Saldivar (2016) state that maize kernel oil content ranges from 2% to 6%, with approximately 85% located in the germ scutellum. Fat plays an important role in animal nutrition, as maize grain is a major component of livestock diets. According to Klopfenstein et al. (2013), fat represents a concentrated, highly digestible energy source and provides essential fatty acids necessary for growth and development. Li et al. (2025), stated that the choice of the

right variety is crucial for achieving the desired kernel nutritional quality and that further study of the impact of external environmental stressors on the nutritional quality is needed. The objective of this study was to determine the potential relationship between production conditions in 2024 at two locations (L1 – Karanac and L2 – Nuštar), situated 60 km apart, and the content of selected kernel components in five maize hybrids (H1–H5) belonging to FAO maturity groups 390 to 500 (H1 – 390, H5 – 500).

Material and Methods

Research locations and basic sowing information

The study was conducted at two sites: Karanac (L1) and Nuštar (L2). Sowing of five maize hybrids was carried out on 12 April 2024 at L1 and on 13 April 2024 at L2, on fields where barley had been the preceding crop. Sowing was performed at a theoretical within-row spacing of 18.75 cm, corresponding to an expected plant density of 75,733 plants ha^{-1}. Fertilization included a total of 200 kg N, 120 kg P_2O_5, and 200 kg ha^{-1} K_2O. Soil at both locations was prepared using conventional tillage practices, and weed control was recorded using the active substances tembotrione-methyl at 45 g ha^{-1} and isoxaflutole at 112 g ha^{-1}. Grain yield (kg ha^{-1}) of the tested hybrids under standard sowing conditions was determined by hand-harvesting ears along a 20 m row segment in four replications. Harvested ears were weighed using an electronic scale (Kern electronic balance: d = 10 g). Ten representative ears were used to determine grain-to-ear mass ratio (shelling percentage) and grain moisture content. Grain yield (kg ha^{-1}) was calculated after adjusting moisture content to 14%.

Climatic Conditions During the Research Period

Climatic conditions during the 2024 growing season at both research locations did not deviate substantially from long-term averages. Table 1 presents the mean monthly air temperatures and total monthly precipitation (mm) for the 2024 vegetation period, along with long-term averages for both locations. Climatic conditions during the 2024 growing season at both research locations did not deviate substantially from long-term averages.

Table 1. Climatic conditions in 2024 recorded at the Grabovac climatological station (45°42'06.6", 18°44'10.3") and the Vinkovci climatological station (45°17'00.7", 18°46'23.8")

Month	Mean air temperature (°C)				Precipitation (mm)			
	Location (L_1)		Location (L_2)		Location (L_1)		Location (L_2)	
	2024.	2020 - 2024.	2024.	2016 - 2024.	2024.	1920 - 2024.	2024.	2016 - 2024.
I.	1.9	0.9	2.7	1.6	48.1	46.3	42.5	45.4
II.	9.1	3.1	9.8	5.4	14.6	45.7	16.2	44.4
III.	10.8	7.3	11.8	8.3	22.6	42.7	19.9	37.6
IV.	14.2	12.5	15.1	12.9	27.1	45.8	12.4	49.3
V.	18.3	17.4	19.0	17.6	131.3	76.4	142.1	71.4
VI.	23.0	21.4	23.9	22.6	80.4	81.5	88.0	76.4
VII.	25.3	23.1	26.8	24.2	41.0	68.3	46.1	70.8
VIII.	25.6	22.6	27.2	23.9	12.0	59.3	0.8	42.9
IX.	18.1	17.2	18.8	18.4	119.2	64.5	116.7	60.1
X.	12.6	12.1	13.5	13.0	39.7	55.1	42.7	47.9
XI.	4.6	6.8	5.0	7.5	52.5	55.0	71.4	66.2
XII.	2.4	2.1	2.9	3.5	63.7	51.9	89.8	55.9
Total IV.-IX.	20.7	19.0	21.8	19.9	411	395.8	406.1	370.9
Total I.-XII.	13.8	12.2	14.7	13.2	652.2	692.5	688.6	668.3

Source: *CMHS* (2025.); *IV.-IX. - maize vegetation*

From Table 1 and Figure 1, it is evident that during the first ten months of the 2024 growing season, both locations experienced periods of increased mean monthly air temperatures compared with long-term averages. An increase in mean monthly temperature in April was

recorded at both sites, ranging from +1.7 °C at L1 to +2.2 °C at L2. The largest deviations in mean monthly temperatures in 2024 occurred in August, amounting to +3.0 °C at L1 and +3.3 °C at L2. During August, only 22% of the long-term average precipitation was recorded at L1, and merely 1.8% at L2. In the first three months of 2024, both locations received only about 60% of the precipitation expected based on long-term climatic records. In May, the previously noted soil moisture deficit was compensated, with 131.3 mm of precipitation recorded at L1 and 142.1 mm at L2, which was highly favourable for the establishment of the experimental sowings. Although the experimental sites were more than 60 km apart (in a straight line), similar total precipitation amounts were recorded during the vegetation period: 411 mm at L1 and 406 mm at L2. The average mean monthly temperature during the vegetation period was higher at L2 by +1.1 °C.

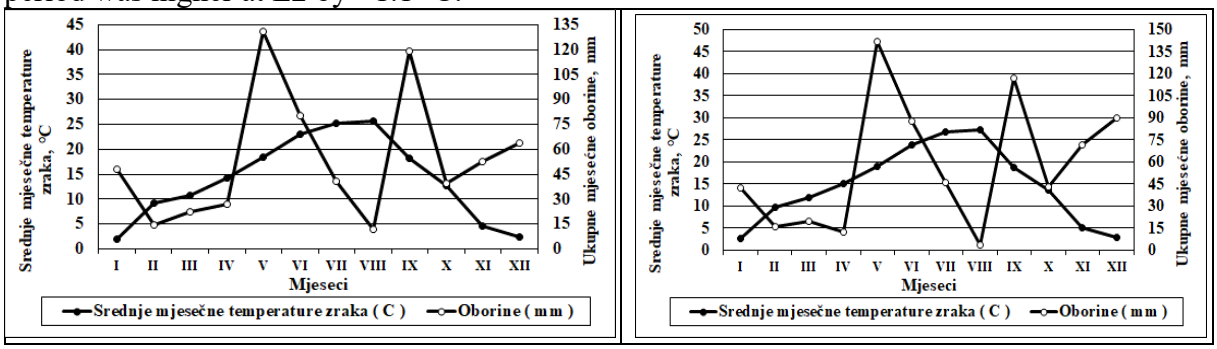

Figure 1. Walter climate diagram for 2024 – site L1 (left) and site L2 (right)

Results and Discussion

From Table 2, it can be concluded that at harvest, the combined trial at L2 showed an increase in recorded plant density of +3.35% (69,520 ± 2,625.2) compared with L1 (67,191 ± 2,232.9). At both experimental sites, the average plant density (plants ha^{-1}) was considerably lower than the theoretical value (L1: –8,549; L2: –6,213). According to Banaj et al. (2017), between 8,000 and 13,000 plants ha^{-1} are typically missing from the planned theoretical density by harvest.

Table 2. Mean values of plant density (plants ha^{-1}), grain yield (kg ha^{-1}), and other yield components at the investigated locations in 2024

Hybrid/ Location	Plant density (ha^{-1})		Grain yield* (kg ha^{-1})		Grains per cob (g)		Harvest moisture (%)		Hectoliter mass (kg/hl)	
	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
H_1/L_1	67417	3225.184	9417	361.953	139.8	6.789	17.3	0.918	73.6	0.826
H_2/L_1	65909	3174.090	8380	224.326	127.2	4.561	15.6	0.634	71.9	1.771
H_3/L_1	64112	3629.825	8290	176.318	129.6	7.496	15.8	0.337	70.2	1.262
H_4/L_1	68930	1493.712	10479	402.655	152.1	7.943	17.2	0.512	73.4	2.295
H_5/L_1	69588	1884.828	10532	854.530	151.4	12.765	17.6	0.572	73.0	1.744
H_1/L_2	67656	2526.479	9032	157.652	133.6	5.889	17.9	0.512	73.4	1.308
H_2/L_2	68647	1353.197	9638	127.530	140.4	4.286	18.5	0.443	74.7	1.037
H_3/L_2	67573	2740.984	9439	378.563	140.0	11.160	18.5	0.665	71.9	2.216
H_4/L_2	73932	2619.681	11971	564.828	161.9	4.662	17.9	0.386	72.7	1.485
H_5/L_2	69793	1301.625	9240	224.123	132.4	1.212	18.6	0.640	93.2	2.187

**- 14 % moisture*

Sowing hybrid H1 (Table 2, Table 3) at location L1 resulted in an average plant density of 67,417 plants ha^{-1} and a grain yield of 9,417 kg of dry grain ha^{-1}. The average protein content was 8.1%, with a starch content of 61.3% and fat content of 2.9%. The same hybrid at L2, under a similar plant density, produced a grain yield lower by approximately 400 kg ha^{-1}. Regarding kernel composition, a decrease in crude protein and crude fat content was observed, while starch content increased by +0.7%. Hybrid H2 at L1 had a plant density of 65,909 ± 3,174.090 at

harvest and a grain yield of 8,380 kg ha^{-1}. At L2, the same hybrid, grown at a slightly higher density (68,647 ± 1,353.197), recorded a somewhat higher yield of 9,638 kg ha^{-1}. A higher average crude protein content (+1.4%) was recorded at L2, whereas crude fat and starch contents were slightly higher in H2 grain at L1.

Table 3. Statistical values of absolute grain weight (g) and kernel composition at the investigated locations in 2024

Hybrid/ Location	Crude protein (%)		Crude fat (%)		Starch (%)		Absolute mass (g)	
	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
H_1/L_1	8.1	0.171	2.9	0.275	61.3	1.808	302.5	1.291
H_2/L_1	7.3	0.129	3.0	0.208	62.7	3.071	302.0	1.826
H_3/L_1	8.1	0.350	2.5	0.141	62.0	2.624	344.0	2.944
H_4/L_1	8.7	0.171	2.7	0.263	61.5	3.005	293.0	2.449
H_5/L_1	8.1	0.238	2.9	0.183	60.9	1.745	284.0	3.367
H_1/L_2	8.0	0.171	2.8	0.337	62.0	2.259	331.0	3.742
H_2/L_2	8.7	0.222	2.6	0.403	60.8	1.970	302.0	4.690
H_3/L_2	7.7	0.523	3.1	0.386	57.8	2.859	251.0	2.160
H_4/L_2	7.8	0.311	2.8	0.374	58.4	1.896	258.0	3.742
H_5/L_2	8.0	0.451	3.3	0.311	58.2	1.893	250.0	3.916

For hybrid H3 (FAO 430), the lowest plant density at L1 was recorded, amounting to 64,112 ± 3,629.825 plants ha^{-1}, accompanied by the lowest grain yield in the trial (8,290 kg ha^{-1}). At L2, the same hybrid recorded a slightly higher plant density of 67,573 plants ha^{-1} and a grain yield of 9,439 kg ha^{-1}, which is 13.8% higher than at L1. At L1, grain of hybrid H3 contained 0.4% more crude protein and 4.2% more starch, whereas at L2 a slightly higher average crude fat content (+0.6%) was observed. The highest average plant density in the trial at L2 was recorded for hybrid H4, amounting to 73,932 ± 2,619.681 plants ha^{-1}, with a grain yield of 11,971 kg ha^{-1}. At L1, the same hybrid had a plant density lower by 5,000 plants ha^{-1} and a grain yield of 10,479 kg ha^{-1}. Hybrid H4 at L1 had a slightly higher average crude protein and starch content, while a somewhat higher crude fat content was recorded at L2. Under comparable average plant densities at both locations, hybrid H5 recorded a grain yield of 10,532 kg ha^{-1} at L1. At L2, the average yield was lower by 1,290 kg ha^{-1}. Higher average crude protein content (8.1%) and starch content (60.9%) were recorded at L1, whereas the highest average crude fat content (3.3%) was observed at L2. The $LSD_{0.05}$ test results for the examined kernel traits are presented in Table 4.

Table 4. $LSD_{0.05}$ test for the main traits examined – L1 and L2 ($\alpha = 0.05$)

Feature	H1($\bar{x}$)	H2($\bar{x}$)	H3($\bar{x}$)	H4($\bar{x}$)	H5($\bar{x}$)	$LSD_{0,05}$
C. protein L_1	8.125^{B}	7.250^{C}	8.075^{B}	8.675^{A}	8.050^{B}	0.339
C. protein L_2	7.975^{B}	8.675^{A}	7.700^{B}	7.750^{B}	7.950^{B}	0.544
C. fat L_1	2.925^{A}	2.950^{A}	2.500^{B}	2.675^{AB}	2.900^{A}	0.331
C. fat L_2	2.800^{AB}	2.625^{B}	3.125^{AB}	2.800^{AB}	3.250^{A}	0.548
Starch L_1	61.275^{A}	62.725^{A}	64.950^{A}	61.525^{A}	60.850^{A}	3.792
Starch L_2	61.950^{A}	60.825^{AB}	57.825^{B}	58.400^{B}	58.175^{B}	3.325

Based on the $LSD_{0.05}$ test for average kernel starch content, it can be concluded that no statistically significant differences were observed among hybrids at L1, nor among hybrids H2, H3, H4, and H5 at L2. A statistically significant difference in crude protein was detected between hybrid H4 and the remaining hybrids at L1. At the same location, no significant

differences in protein content were found among hybrids H1, H3, and H5. At L2, a statistically significant difference was identified for hybrid H2 in comparison with the other hybrids.

Conclusion

Based on the findings obtained in this study, the following conclusions can be drawn:

According to the meteorological data for 2024, and considering the mean monthly air temperatures and total monthly precipitation, production conditions at both locations were essentially equivalent and did not exert significantly different effects on yield components or grain composition of the tested maize hybrids.

The average overall grain yield at L1 was 9,419.6 ± 1,058.6 kg ha^{-1}, which is 4.51% lower than at L2, where an average yield of 9,864 ± 1,125.9 kg ha^{-1} was recorded.

The average crude protein content of the tested hybrids at both locations (L1 and L2) was 8.0%. Average crude fat content amounted to 2.8% at L1 and 2.9% at L2.

Given that this study represents a single-year research of the effects of location (soil and meteorological production conditions) and hybrid on a number of interrelated factors including yield and kernel composition, the results cannot be considered statistically conclusive from a scientific standpoint. Continued research is therefore recommended.

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ORIGINAL SCIENTIFIC PAPER

Enhancing early vegetative performance of white clover (*Trifolium repens* L.) through magnetic seed priming

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Summary
The aim of the study was to examine the effect of seed pretreatment of white clover (*Trifolium repens* L. cv. Apolo) with magnetic fields of different strengths and exposure times on the growth and morphological traits of the above-ground part of the plants. The results showed that MF of different strengths significantly affected all tested properties. The most favorable effects were recorded at strengths of 50 to 150 mT, while higher strengths led to stabilization or a slight decrease in the effect. The exposure time affected all properties except the number of leaves. The optimal exposure duration was about 6 h. The results of the study suggest that pretreatment of seeds with a magnetic field can positively affect the growth of white clover, with the necessary precise optimization of the strength and duration of the treatment.

Keywords: white clover, seed, magnetic field, biomass

Introduction

White clover (*Trifolium repens* L.) is one of the most important perennial forage legumes of the temperate zone, which is primarily used in pasture mixtures. It is characterized by high nutritional value, the ability to symbiotically fix atmospheric nitrogen, but also by slow growth, especially in the first three months of growth (Brock and Hay, 2001). Leaf development, biomass accumulation and stolon formation in the early phase of development are key to the sward establishment, competitiveness in mixtures and later productivity. The low germination and uneven emergence of white clover compared to most field crops are associated with a high proportion of hard seeds (Kimura and Islam, 2012). According to the literature, the average germination of white clover is 63-66% (Chu et al., 2022), and it varies significantly between cultivars and is dependent on temperature (Bukvić et al., 2008; Baxter et al., 2019).

In modern agriculture, there is an increasing need to develop sustainable technologies that can improve early growth and productivity of field crops while reducing the use of chemicals. Physical seed pretreatments represent one of the promising methods in this context, and the magnetic field is attracting increasing attention from researchers. Magnetic seed pretreatment is based on short-term exposure of dry seeds to a static or alternating magnetic field of a certain strength and duration. Previous research suggests the possibility of stimulating physiological and biochemical processes, including changes in membrane permeability, increased enzyme activity, enhanced metabolism, and faster mobilization of reserve substances (Sarraf et al., 2020; Hafeez et al., 2023). As a result, germination can be accelerated and initial vegetative growth can be improved. However, the effect of the magnetic field depends on the intensity and time of exposure (Nyakane et al., 2019), and the response is often species-specific, which requires further research in agronomically important crops. Despite the great agronomic importance of white clover, data on the application of magnetic seed pretreatment to improve early growth of white clover are still limited.

Therefore, the aim of this study was to examine the effect of seed pretreatment with magnetic fields of varying strength and exposure time on early vegetative development of white clover under controlled conditions, assessing plant mass, leaf number and stolon number after five weeks of cultivation.

Material and Methods

The research was conducted under controlled conditions in a phytotron, with a programmed day/night regime. Light intensity varied from 0% (night) to 30–100% of LED lighting capacity (day), the temperature was 24°C during the day and 18°C during the night, with a relative humidity of 70–80%. Sowing was carried out in polystyrene containers with 24 cells (volume 180 cm^3 per cell). Eight seeds of white clover, cultivar Apolo, were sown in each seed cell. The commercial substrate Klasmann Potgrond H was used for cultivation. The experiment was set up in four replicates, with each replicate containing 96 seeds.

In addition to the control, seed pretreatment with a magnetic field (MP) was applied at five levels of intensity (50, 100, 150, 250 and 350 mT) and six exposure times (0.5; 1; 3; 6; 12 and 24 h), forming a total of 30 treatments. The magnetic field was generated using neodymium permanent magnets. Seeds were placed in the central zone between the magnets, and magnetic field intensity was measured using a teslameter (VICTOR 862B). Measurements indicated small variations, suggesting relatively uniform conditions within the treatment zone. After five weeks of growth, above-ground biomass was harvested. The number of leaves and stolons, as well as the mass of fresh and dry matter, were determined.

To examine the effect of magnetic field strength (mT), exposure time (h) and their interaction, a two-factor analysis of variance (ANOVA) was applied, and statistical data processing was performed in the SAS Enterprise Guide 7.1 software package. Differences between mean values were tested with the Tukey HSD test ($p \leq 0.05$).

Results and Discussion

The application of pretreatment of seeds with magnetic fields (MF) of different strengths significantly ($p < 0.05$) affected the growth and morphological traits of the plants examined (Table 1). The time of exposure of seeds to MF also significantly ($p < 0.05$) affected all traits examined, except for the number of leaves (Table 2). A statistically significant interaction between magnetic field strength and exposure time (MF × time) was observed ($p < 0.05$).

Averaged across exposure time, all MF treatments had a significantly higher number of leaves compared to the control, with the highest values recorded for the 50 and 100 mT treatments. The number of stolons was also the lowest in the control, and the highest at MF strengths of 50, 100 and 250 mT. With regard to the exposure time, averaged across all MF strengths, the number of stolons was the highest at 6 h and the lowest at 24 h.

All applied MF intensity treatments resulted in higher fresh mass compared to the control, with no significant differences between treatments, with stimulation being achieved already at 50 mT. Dry matter mass was also the lowest in the control, while the highest values were recorded at 100 and 150 mT. Higher intensities (250 and 350 mT) showed stabilization or a slight decrease. Since dry matter mass better reflects organic matter accumulation, the results indicate an optimal intensity range around 100 mT.

Table 1. Effect of magnetic field strength (MF) on the number of leaves and stolons and on fresh and dry above-ground biomass (g).

MF (mT)	Number		Above-ground biomass (g)	
	of leaves	of stolons	Fresh mass	Dry mass
0	512 ± 4.97^{c}	39 ± 0.70^{b}	49.24 ± 0.55^{b}	6.88 ± 0.06^{c}
50	606 ± 8.66^{a}	48 ± 2.94^{a}	63.06 ± 1.70^{a}	7.58 ± 0.17^{bc}
100	625 ± 8.81^{a}	48 ± 1.48^{a}	66.85 ± 1.83^{a}	8.64 ± 0.20^{a}
150	539 ± 6.39^{bc}	41 ± 2.29^{ab}	66.85 ± 1.83^{a}	8.52 ± 0.20^{a}
250	553 ± 7.86^{b}	50 ± 2.05^{a}	65.66 ± 1.73^{a}	8.29 ± 0.19^{ab}
350	536 ± 12.96^{bc}	45 ± 2.88^{ab}	62.31 ± 2.55^{a}	8.10 ± 0.25^{ab}

Bars represent means of four replicates ± standard error, differences were tested by Tukey HSD ($p \leq 0.05$) and indicated by letters, while magnetic field values are means averaged across all exposure times.

Table 2. Effect of exposure time (h) on the number of leaves and stolons and on fresh and dry above-ground biomass (g).

Exposure time	Number		Above-ground biomass (g)	
(h)	of leaves	of stolons	Fresh mass	Dry mass
0.5	578 ± 13.51^{a}	43.33 ± 2.02^{bc}	59.08 ± 1.49^{b}	7.54 ± 0.17^{c}
1	572 ± 12.98^{a}	45.58 ± 2.52^{abc}	60.95 ± 1.74^{ab}	7.91 ± 0.19^{bc}
3	540 ± 9.85^{a}	40.29 ± 2.15^{bc}	62.83 ± 2.51^{ab}	7.55 ± 0.23^{c}
6	582 ± 9.78^{a}	54.00 ± 2.02^{a}	68.17 ± 2.43^{a}	8.79 ± 0.25^{a}
12	560 ± 9.55^{a}	47.83 ± 2.17^{ab}	61.56 ± 1.77^{ab}	8.42 ± 0.20^{ab}
24	540 ± 12.63^{a}	38.67 ± 1.72^{c}	58.30 ± 1.93^{b}	7.80 ± 0.21^{bc}

Bars represent means of four replicates ± standard error, differences were tested by Tukey HSD ($p \leq 0.05$) and indicated by letters, while exposure time values are means averaged across all magnetic field strengths.

The number of leaves in white clover (*Trifolium repens* L.) is a highly plastic morphological trait that primarily depends on the genotype and climatic conditions. For the same genotype, it changes during the season, being smaller during winter and larger in summer (Hamilton et al., 1989). It also varies between different developmental stages (Kang et al., 1995). An increase in the number of leaves by applying MF seed pretreatment was determined by Vashisth and Joshi (2017) in maize, Vashisth et al. (2021) in sunflower, while Krawiec et al. (2018) recorded an increase in the length and area of leaves and the vegetation index in horticultural species.

The number and length of stolons enable the formation of new rooted nodes and thus affect the sward density and the stability of the crop under mowing and grazing conditions. These traits are largely influenced by the genotype and environmental factors (Frame and Laidlaw, 1998).

In this study, the control had the lowest values, while all other treatments increased the number of stolons. According to previous studies, exposure of seeds or plants to a magnetic field can affect physiological processes (germination, enzyme activity, photosynthesis intensity and cell division), which can indirectly result in changes in vegetative growth and an increase in the number of stolons formed in certain treatments. Germination of untreated seeds of the cultivar Apolo was 79%. However, germination was not assessed under magnetic field treatments, and early plant development parameters observed in this study can be considered as indirect indicators of seed response.

Based on the results obtained, a temporal pattern can be observed that indicates the existence of an optimal treatment duration (about 6 h). Shorter exposures are probably not sufficient to achieve a full physiological response, while longer exposures can activate adaptive mechanisms that limit stimulation, which is consistent with a nonlinear response model (Calabrese and Baldwin, 2002; Agathokleous et al., 2019).

Considering this interaction, the optimal combination was approximately 100 mT and 6 h. This combination resulted in the highest accumulation of dry matter and high values of

morphological parameters. The absence of a linear increase at higher strengths and longer exposures suggests that MF acts as a growth regulator within a narrow physiological range. Possible mechanisms include changes in redox balance, ion signaling and hormonal regulation (Galland and Pazur, 2005; Radhakrishnan and Kumari, 2012; Maffei, 2014), but additional experimental research is needed to confirm these mechanisms.

Conclusion

The results confirm that MF can positively influence the growth of white clover, but only within optimally defined parameters of strength and duration. Precise treatment optimization is therefore crucial for potential agronomic application.

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ORIGINAL SCIENTIFIC PAPER

Responses of sunflower to herbicide stress and post-treatment biostimulant application

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Summary
An outdoor pot experiment was conducted to evaluate the physiological effects of imazamox-based herbicides and the potential of a biostimulant (BS01) to mitigate herbicide-induced stress in sunflower. Treatments included imazamox and imazamox2, with BS01 applied 4, 7, and 10 days after herbicide application. Photosynthetic parameters, transpiration, water use efficiency, biomass, and leaf pigment content were measured. Herbicide treatments negatively affected biomass accumulation, chlorophyll content, and photosynthetic parameters. However, BS01 application, particularly 4 days after herbicide treatment, significantly alleviated these effects, resulting in increased plant height, biomass, and improved photosynthetic performance. In several cases, chlorophyll content exceeded control values, while water use efficiency and photosynthesis parameters approached those of untreated plants. Later biostimulant applications were less effective. Results indicate that optimal timing of biostimulant application plays a key role in mitigating herbicide stress. Overall, post-treatment application of BS01 represents a promising strategy to improve sunflower tolerance to herbicide-induced stress conditions.

Keywords: *Helianthus annuus*, photosynthetic parameters, biomass production, herbicide stress

Introduction

Herbicides are used to control weeds, but they may also affect sunflower growth and reduce its competitive ability, which can indirectly impact crop yield. Imazamox is a widely used herbicide in sunflower production, and it can cause temporary yellowing and growth retardation in sunflowers, particularly under stressful environmental conditions (Pfenning et al., 2008). It negatively affects photosynthetic processes, including light-dependent redox reactions and leaf gas exchange (Sangiorgio et al., 2020). Biostimulants can help maintain photosynthetic efficiency and protect against oxidative damage, which is crucial for plant recovery and growth. They can enhance the plant's defense mechanisms, improve water balance, stabilize cellular structures, and regulate hormonal and enzymatic pathways, contributing to better stress tolerance (Balabanova et al., 2016, Yuldasheva and Karabayeva, 2023). Several researchers have found that biostimulant treatments applied after the use of herbicides had a positive effect on sunflower growth, head diameter, yield, and oil content (Neshev et al., 2022). The combination of herbicides and biostimulants offers the potential for effective weed control while improving yield and quality (Jankauskienė et al., 2024). Our goal was to analyze whether it is possible to mitigate the effects of herbicide-induced stress by using a biostimulant.

Material and Methods

Outdoor pot experiment was established with six replications using 15 litres pots. We used chernozem soil for filling the pots. The sowing was 02 May 2023, with 3 seeds/pot. After

germination one plant was left in each pot. Genotype: Pioneer P64LP180. Treatments: 8 June: Pulsar (imazamox 40 g L^{-1}) 1.2 L ha^{-1}, Pulsar Plus (imazamox2 25 g L^{-1}) 2 L ha^{-1}.
The physiological effects of sunflower herbicides were tested on a tolerant genotype, and we also investigated whether the timing of biostimulant application influenced plant stress responses. The biostimulant (BS01) was applied 4, 7, and 10 days after herbicide treatment with dose 0.2 L ha^{-1}.
Biostimulant 01 (BS01): MTU (1-(2-methoxyethyl)-3-(1,2,3-thiadiazol-5-yl) urea) (2 g L^{-1}) + pyroglutamic acid (320 g L^{-1})
Irrigation: In May, applied on three occasions to promote emergence and early growth, at a dose of 300 mL per pot. Termination of experiments: July 31, 2023
Photosynthetic parameters and transpiration were determined on intact leaves using a LI-6800 (LI-COR, USA) portable photosynthesis system equipped with two high-precision infrared gas analyzers for measuring CO_2 and H_2O mole fractions in the air. The system automatically calculated net assimilation rate, transpiration, stomatal conductance, intercellular CO_2 concentration, and additional physiological parameters. Light conditions within the chamber were controlled and set to 1500 µmol photons m^{-2} s^{-1} PAR, consisting of 90% red (625 nm) and 10% blue (475 nm) light. The CO_2 concentration in the chamber was maintained at 400 µmol mol^{-1} using an injector and carbon dioxide cartridges. Measurements were performed on light-adapted leaves, with six replicates per leaf on two plants per plot. Water use efficiency was calculated from the measured data on the leaves, applying the formula proposed by Tanner and Sinclair (1983):

$$WUE = \frac{Ass*44}{Emm*18}$$

WUE: water use efficiency (kg m^{-3})
Emm: transpirated H_2O (mmol m^2 s^{-1})
Ass: assimilated CO_2 (µmol m^2 s^{-1})

At the end of the experiment, chlorophyll content of the leaves as well as the dry weights of shoots and roots were determined. Data processing and statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Chicago, IL, USA). Differences among treatments were evaluated using a univariate general linear model (GLM). Prior to analysis, the assumptions of analysis of variance were verified for the dependent variables, and homogeneity of variances was assessed using Levene's test. Pairwise comparisons of treatment means were conducted using the LSD post hoc test. Statistical significance was set at $p = 0.05$.

Results and Discussion

No significant differences in plant height were observed among treatments on 16 June; however, by 5 July, BS01 application 4 days after imazamox treatment resulted in significantly greater plant height compared to the control and other treatments. NDVI values clearly reflected the negative effect of imazamox, which was partially alleviated by BS01. The strongest effect was observed with application 4 days after herbicide treatment, while later applications (7 and 10 days) showed a gradual decline in efficacy, although values remained higher than in the imazamox-only treatment.
Similar trends were observed for dry root mass, dry shoot mass, and total plant dry biomass (Table 1). The highest biomass values were recorded when BS01 was applied 4 days after imazamox treatment. Compared to the control, root mass increased by 9.3% and shoot mass by 28.0%. When BS01 was applied 7 days after imazamox, root mass increased by 3.1% and shoot mass by 9.8% relative to the control.
Photosynthetic parameters of BS01-treated plants differed significantly from both the control and the imazamox-only treatments (Figure 1). Differences were most pronounced above 300 µmol m^{-2} s^{-1} PPFD in terms of photosynthetic rate, transpiration, and electron transport rate,

and treatment effects became increasingly distinct as the growing season progressed. Imazamox treatment significantly reduced stomatal conductance and water use efficiency at all light intensities (Figure 2). Although BS01 application did not fully compensate for the negative effects of imazamox, the measured values approached those of the control.

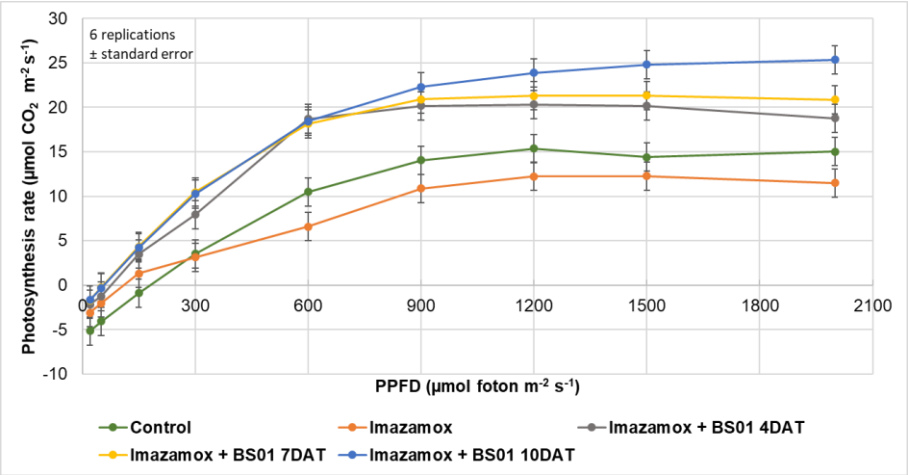

Figure 1. Effect of biostimulant treatments on the photosynthetic rate of sunflower after imazamox herbicide application (Debrecen, 2023)

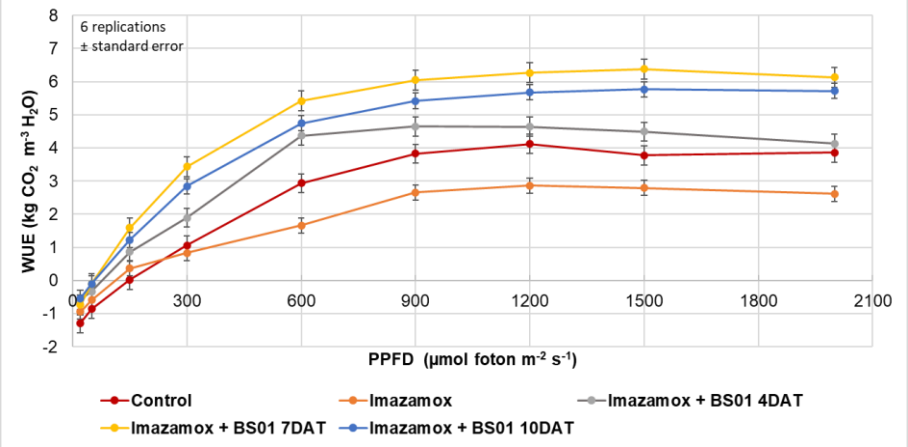

Figure 2. Effect of biostimulant treatments on the water use efficiency of sunflower after imazamox herbicide application (Debrecen, 2023)

At the end of the experiment, chlorophyll and carotenoid contents were determined. Imazamox reduced chlorophyll a, chlorophyll b, and carotenoid concentrations in the leaves. When BS01 was applied 4 days after imazamox, chlorophyll a and b contents increased by 25.2% and 21.1%, respectively, compared to the control, and by 42.5% and 76.0%, respectively, compared to the imazamox-only treatment. Carotenoid content was also higher following BS01 application, exceeding the control by 4.2% and the imazamox-only treatment by 13.7%. Later BS01 applications did not produce a consistent effect (Figure 3).
Imazamox2 treatments caused significant differences in plant height, application of this herbicide resulted in taller sunflower plants compared to the control and the imazamox2 + BS01 treatment. The lowest plant height at all measurement dates was recorded under the imazamox2 + BS01 treatment. Beside of this, highest dry biomass in this experiment was also measured in plants that received Biostimulant 01 following herbicide application.

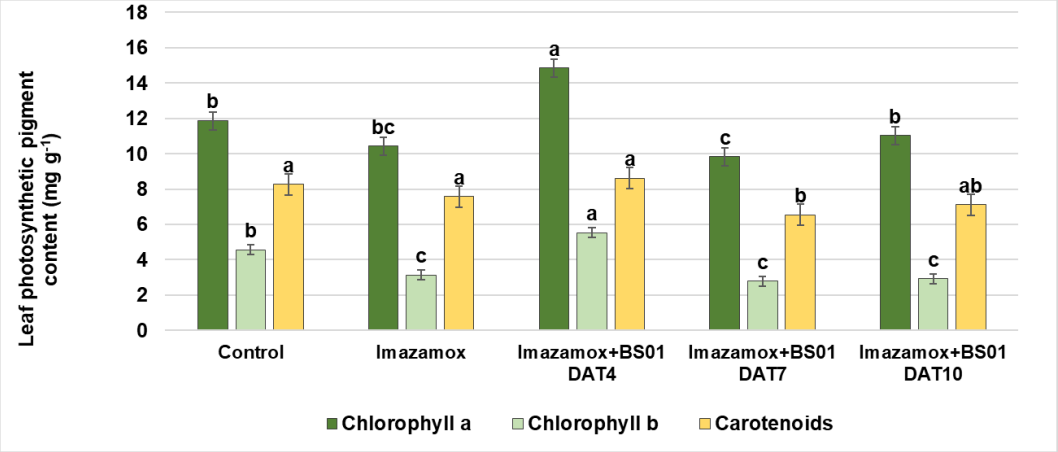

Figure 3. Effect of biostimulant treatments on the photosynthetic pigent content of sunflower leaves after imazamox herbicide application (Debrecen, 2023)

Table 1. Dry root mass, shoot mass, and total plant biomass of sunflower as affected by the treatments (Debrecen, 2023)

	Control	Imazamox	Imazamox +BS1 DAT4	Imazamox +BS1 DAT7	Imazamox +BS1 DAT10	Imazamox2	Imazamox2 +BS1 DAT4
Dry root weight (g)	21.1±7.1a	16.4±5.9b	23.0±5.0a	21.7±7.2ab	18.4±6.1ab	19.2±5.2ab	24.6±6.9a
Dry shoot weight (g)	35.4±6.7b	29.5±5.9bc	45.3±10.6a	38.9±7.9a	33.3±4.7b	28.8±1.7c	40.9±9.5a
Dry plant weight (g)	56.5±12.7ab	45.9±11.1b	68.3±13.6a	60.6±13.7ab	51.7±9.5b	48.0±5.1b	65.5±11.6a

Note: 6 replications, ± standard deviation; letters show significant differences between treatments in the lines at *p*=5% level.

Root biomass increased by 16.9% compared to the control and by 28.4% compared to the imazamox2-only treatment, while shoot biomass increased by 15.6% and 42.3%, respectively. In the experiment the imazamox2 herbicide reduced chlorophyll a and b as well as carotenoid content in the leaves (by 14.5%, 41.9%, and 3.9%, respectively). Application of the biostimulant 01 4 days after imazamox2 treatment increased chlorophyll a content by 2.1% compared to the control and by 19.4% compared to the imazamox2-only treatment. Chlorophyll b content remained lower than in the control both in the imazamox2 treatment (−41.9%) and in the imazamox2 + BS01 treatment (−31.5%). Carotenoid content was higher in leaves treated with BS01 4 days after imazamox2 application, increasing by 3.1% compared to the control and by 7.3% compared to the imazamox2 treatment.

The lowest photosynthetic rate in the experiment was recorded in the control plants at all light intensities. Both the imazamox2 and imazamox2 + BS01 treatments resulted in considerably higher photosynthetic activity (Figure 4). The differences between these two treatments became more pronounced at medium and high light intensities, where CO_2 assimilation in biostimulant-treated plants was 5.6–16.4% higher.

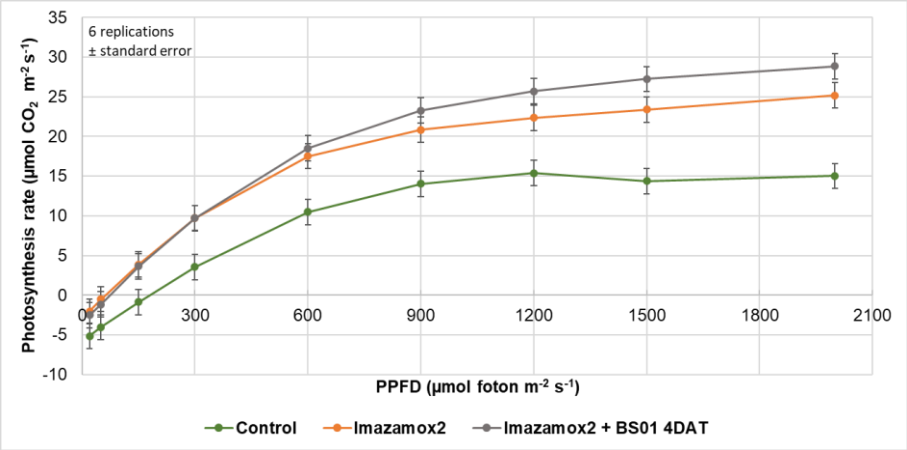

Figure 4. Effect of biostimulant treatments on the photosynthetic rate of sunflower after imazamox2 herbicide application.

Conclusion

The application of biostimulants post-herbicide treatment can significantly mitigate the negative effects of herbicides on sunflowers. By enhancing photosynthetic activity, reducing oxidative stress, and improving overall growth and yield, biostimulants offer a promising strategy for managing herbicide stress in sunflower cultivation. Further research is needed to optimize dosages, application timing, and understand the molecular mechanisms involved.

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PRELIMINARY COMMUNICATION

Preliminary evaluation of amino acid composition in selected soybean genotypes

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Summary
Soybean branched-chain amino acids (BCAAs) valine, leucine, and isoleucine play a key role in nutrition. This study assessed the effects of genotype, year, and their interaction on BCAA accumulation over two growing seasons. Analysis of variance revealed that all tested sources of variation significantly ($p < 0.01$) affected BCAA content. Genotype G53 exhibited the highest relative content of valine and leucine, while G44 had the highest isoleucine content. In contrast, genotype G14 had the lowest values for all amino acids. Year influenced accumulation, with higher values in 2025. Further testing is needed to confirm genotype stability for breeding.

Keywords: soybean, nutritional value, amino acid content, plant breeding

Introduction

Soybean (*Glycine max* (L.) Merr.) is among the most important legumes worldwide, valued for its high protein content in the seeds. It is particularly notable for its nutritional quality due to its rich amino acid profile. Soybean contains all essential amino acids (AAs), making it a valuable source of protein for both human and animal nutrition (Kudełka et al., 2021; Sudarić and Vratarić, 2008). Branched-chain amino acids (BCAAs), leucine (Leu), isoleucine (Ile) and valine (Val) represent a specific class of amino acids. They play a pivotal role in skeletal muscle and total-body protein synthesis, regulation of energy homeostasis, nutrient metabolism, gut health, and immune response to various diseases in humans and animals. In addition, BCAAs are involved in cellular signaling pathways related to glucose and lipid metabolism (Mann et al., 2021; Nie et al., 2018).

The composition and content of amino acids in soybean seeds are influenced by genetic background and environmental conditions. Therefore, identification of superior genotypes in terms of nutritional value plays a key role in soybean breeding programs aimed at improving protein and amino acid composition in soybean seeds (Singer et al., 2023; Josipović et al. 2012). In this context, the aim of this study was to conduct a preliminary evaluation of the branched-chain amino acid composition in selected soybean genotypes to identify candidates for further research and enhancement of seed nutritional quality.

Material and Methods

The field experiment was set up in the 2024 and 2025 growing seasons at the Agricultural Institute Osijek (AIO, 45°32’ N, 18°44’ E, Osijek, Croatia) in a randomized complete block design with three replicates. The individual plots sown by hand consisted of two 500 cm long rows, with an inter-row distance of 50 cm.

The plant material comprises eight genotypes, including two of each: (a) archived AIO varieties presently not in commercial production (AV; G2, G10); (b) the commercial AIO varieties (CV; G14, G24); (c) elite AIO lines (EL; G37, G40); (d) introductions (IV; G44, G53).
Both years of the field experiment (2024, 2025) were characterized by more or less unfavorable weather conditions for soybean cultivation (Figure 1). According to the data of the Croatian Meteorological and Hydrological Service (CMHS), the spring of 2024 was extremely warm with below long-term average (LTA) rainfall in April and above LTA rainfall in May. The summer of 2024 was extremely warm as well with below LTA rainfall. On the other hand, both temperatures and rainfall were above LTA in September of 2024. The spring of 2025 was warm with above LTA temperatures in April and below LTA temperatures in May. The precipitation in spring of 2025 was near LTA. The summer of 2025 was classified as very warm with well-below LTA precipitation, especially in June, when temperature averages were classified as extremely warm. September of 2025 was very warm and dry.

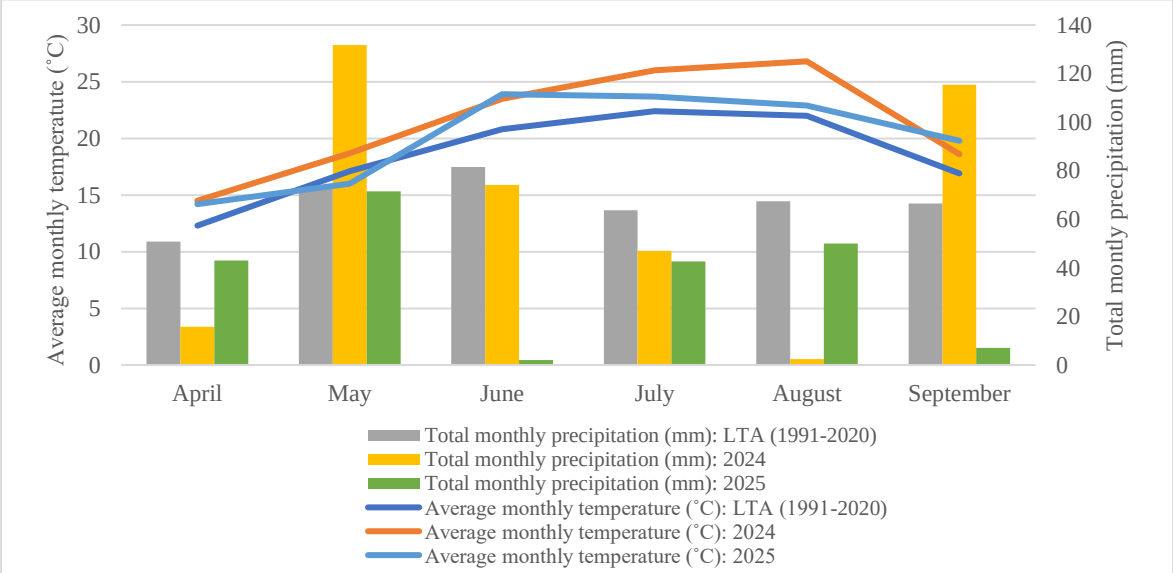

Figure 1. The long-term average (LTA) monthly temperatures and average monthly temperatures (˚C), total monthly precipitation LTA, and total monthly precipitation (mm) in 2024 and 2025 for the soybean growing season (April – September) in Osijek, Croatia (Croatian Meteorological and Hydrological Service)

During the field trial, all currently accepted agricultural management practices were applied. The experiment plots were harvested at full maturity (R8; Fehr and Caviness, 1977). After all impurities were removed from the grain lot, the relative content of amino acids was determined according to the method of Jajić et al. (2013) with minor modifications on a UPLC system (Shimadzu, Nexera series 40, XR model on a C18 column (Shim-pack GIST 5 um, 4.0x150 mm)) and on a DAD detector (A diode array detector). Quantification of amino acids was obtained using a five-point external calibration.

Statistical analyses included two-way ANOVA with Tukey post-hoc comparison for genotype and genotype and year interaction ($p < 0.01$) conducted in JASP 0.18.1.0.

Results and Discussion

Analysis of variance revealed that genotype, year, and their interaction were highly significant ($p < 0.01$) sources of variation for the relative content of branched-chain amino acids (BCAAs) in the eight evaluated soybean genotypes.

Table 1. ANOVA (mean squares and significance) for analysed traits (relative valine, leucine and isoleucine grain content) in eight soybean genotypes tested in two environments (2024–2025)

Source of variation	df	VALINE	LEUCINE	ISOLEUCINE
Genotype (G)	7	0.218**	0.401**	0.486**
Year (Y)	1	0.088**	1.181**	0.491**
G x Y	7	0.112**	0.147**	0.382**

** - Highly significant ($p < 0.01$)

The analyzed BCAAs included valine, leucine, and isoleucine. Previous studies have shown that soybean protein content and amino acid accumulation are strongly affected by genotype and environmental factors (Shi et al., 2023; Jumrani and Bhatia, 2019; Carrera et al., 2011; Kumar et al., 2006). For valine accumulation, genotype G53 exhibited the highest relative two-year average content, although genotypes G37, G44, G40, and G2 did not differ significantly (data not shown). A similar pattern was observed for two-year average leucine, where G53 showed the highest relative two-year average content, with no significant differences detected among genotypes G37, G44, G2, G10, G24, and G40. For two-year average isoleucine accumulation, genotype G44 exhibited the highest relative content, with genotypes G53, G37, G24, and G2 not differing significantly (data not shown).
These results indicate that genotypes G53 and G44 potentially represent promising candidates for improving BCAA content in soybean, depending on the target AA. Genotype G14 stood out across all analyzed traits, exhibiting the lowest relative contents for each of the evaluated amino acids. If these results are confirmed in further experiments, this genotype would be excluded as a parental component in breeding programs aimed at increasing BCAAs content.

Weather conditions during the two growing seasons differed notably (Figure 1). Shi et al. (2023) reported a significant correlation between the accumulation of soybean AAs with growing environmental factors such as temperature,humidity, biotic stresses, water availability, and soil quality. In this study, 2024 was characterized by extreme drought during seed filling period (July and August), while 2025 showed relatively more favorable moisture conditions in that period. The temperatures were above average in both years during seed filling. These differences influenced the accumulation of BCAAs, as environmental conditions during seed filling are known to play a pivotal role in determining seed composition. For example, Carrera et al. (2011) reported that amino acid composition in soybean is strongly influenced by temperature, solar radiation, and water availability during seed filling. The same study highlighted that most amino acids respond significantly to these climatic variables, whereas valine may show a weaker response, and that individual amino acids may exhibit distinct and potentially compensatory responses to environmental conditions. The relative content of BCAAs for eight soybean genotypes across the two study years is presented in Figure 2 (a, b, c) with Tukey post hoc test for genotype and year interaction.

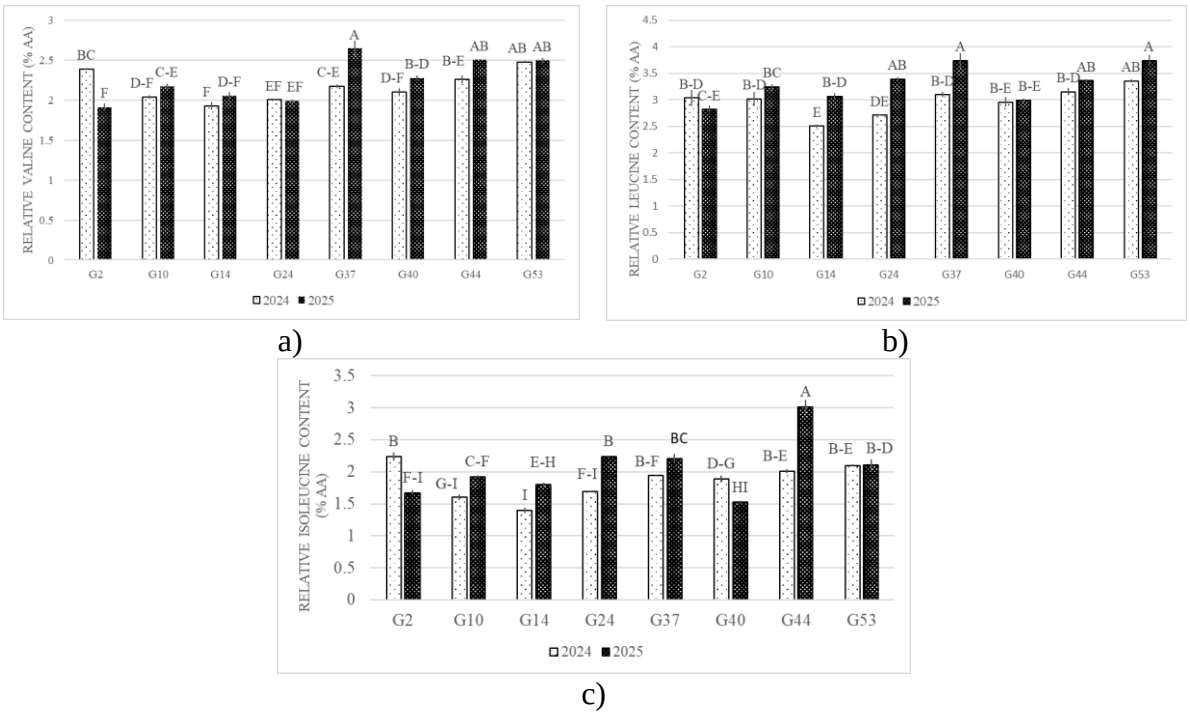

Figure 2. Relative valine (a), leucine (b), isoleucine (c) content (% AA) ± the standard error for eight soybean genotypes (G) tested in two environments (2024, 2025). Values with the same letter in the superscript are not significantly different based on the results of Tukey post hoc test for genotype and year interaction (ANOVA, Tukey test, $p < 0.01$).

In this study, mean relative valine content was significantly ($p < 0.01$) higher in 2025 than in 2024. Significant differences ($p < 0.01$) in relative valine contents between years were observed only for genotypes G2 and G37. In genotype G2, a decrease in valine content was reported in 2025 compared to 2024, whereas genotype G37 showed an increase in 2025 to 2024. Similarly, in the present study, year had a significant effect on leucine accumulation ($p < 0.01$), with higher values observed in 2025 compared to 2024, particularly in genotypes G14, G24, and G37, indicating that the environmental conditions during the seed filling period were more favourable for amino acid accumulation in 2025. Mean relative isoleucine concentration was significantly higher ($p < 0.01$) in 2025, as well. Statistically significant differences in relative contents between years were observed for all genotypes except G37 and G53. In genotypes G2 and G40, isoleucine accumulation was significantly lower in 2025 compared to 2024, whereas in the remaining genotypes G10, G14, G24, and G44, the opposite trend was observed. Given that Jumrani and Bhatia (2019) reported amino acid content in soybean declined with increasing growth temperatures, especially when combined with water stress, decreasing relative valine content in G2 should be further investigated. Furthermore, the lack of environmental impact on BCAA accumulation in some genotypes could indicate a probable positive genotype- dependent response to environmental variation, but this too necessitates further investigation.

Conclusion

These preliminary results demonstrate that BCAA accumulation in the analyzed soybean genotypes is influenced by year and genotype interaction, with each amino acid responding differently to environmental conditions. Although previously reported trends in valine, leucine, and isoleucine accumulation were observed over the two growing seasons, testing the material across multiple environments is necessary to draw reliable conclusions. Nevertheless, these

preliminary results can be used to further guide research aimed at providing a basis for identifying genotypes with high BCAA content and stability across years.

Acknowledgement
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PRELIMINARY COMMUNICATION

Biocontrol potential of essential oils against *Colletotrichum acutatum*

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Summary
Apple bitter rot, caused by *Colletotrichum*, leads to significant economic losses. Its control relies mainly on the chemical fungicides, whose use may promote pathogen resistance and pose risks to human health and the environment. Therefore, essential oils are being researched as potential biopesticides. This study evaluated the antifungal activity of cinnamon, orange, and tea tree essential oils against *Colletotrichum acutatum* using a fumigation bioassay. After seven days, cinnamon essential oil showed complete inhibition, orange oil reduced mycelial growth by 97%, while tea tree oil showed no inhibitory effect. These results indicate the biocontrol potential of the mentioned essential oils against *C. acutatum*.

Keywords: apple, *Colletotrichum spp.*, essential oils, effect

Introduction

The causal agents of the apple bitter rot are phytopathogenic fungi from the genus *Colletotrichum* (Shi et al., 1996). They can develop on apple leaves, fruits, trees and branches, where they cause necrosis and premature defoliation. The most important symptoms are necrotic lesions on fruits, which fall off trees or remain in the mummified form at the end of the vegetation, representing the reservoirs of infection in the orchards (Trkulja et al., 2024). This disease reduces yields, affecting fruits' nutritional value, quality and shelf life. Additionally, the latent infections in fruits can remain undetected until postharvest, making control particularly challenging. Another important characteristic of these pathogens is their biotrophic, hemibiotrophic, and necrotrophic stages, which make them highly infectious, destructive, and easy to spread (Münch et al., 2008). The wide host range and adaptability of these fungi further complicate control measures, as environmental and climatic factors can influence disease severity. Especially *C. acutatum* Simmonds and *C. gloeosporioides* (Penz.) Penz & Sacc may cause economically important postharvest losses during storage and transport (Freeman et al., 1998).

Regarding all the above, the current management strategies mainly rely on synthetic fungicide applications during the ripening stage. Currently, several chemical fungicides based on active substances fludioxonil, fluopyram, tebuconazole and cyprodinil are registered in Serbia for the control of *Colletotrichum spp.* in apple orchards (Petrović & Sekulić, 2023). However, reliance on chemical fungicides is increasingly questioned due to health and environmental concerns (Adkar-Purushothama et al., 2026). Improper or excessive use of these fungicides can often lead to the development of resistance in these phytopathogenic fungi, making their control difficult and increasing production costs (Goswami et al., 2018).

Considering these risks, a few environmentally friendly alternatives to chemical pesticides have already been identified and registered, while others are still being studied. Currently, only two biofungicides based on *Bacillus subtilis* are registered in Serbia for the control of *Colletotrichum spp.* in apple. Nevertheless, the limited spectrum of these biofungicides and their species-specific activity highlights the need for novel, broadly effective alternatives.

These limitations in recent years have increased interest in essential oils as effective biofungicidal agents. Their complex chemical composition, along with specific modes of

action, distinguishes them from other biopesticides and underlines their broad-spectrum activity (Gupta et al., 2023). Moreover, their low toxicity to mammals and biodegradability make essential oils particularly suitable for application in plant protection within organic agricultural production (Morkeliūnė et al., 2021).

The chemical composition of essential oils determines their antifungal activity. The most abundant component plays a key role, but also the minor constituents and their interactions contribute to the antifungal effect (Scariot et al., 2020).

The main components in cinnamon essential oil are cinnamaldehydes. In tea tree essential oil, the most abundant component is carvomenthenol, and orange essential oil has a large amount of limonene (Danh et al., 2021; Octaveus et al., 2024).

In light of this potential, the focus of the present study was the evaluation of the inhibitory effects of cinnamon, orange, and tea tree essential oils on *C. acutatum* under *in vitro* conditions.

Material and Methods

The experiment was carried out in laboratory conditions, using a fumigation bioassay. Three commercially available essential oils were tested: cinnamon (*Cinnamomum verum* J. Presl.), orange (*Citrus aurantium* L.) and tea tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel) essential oil. The laboratory experiment evaluating the antifungal activity was set up in four replications for each essential oil, along with the control treatment (biological replicates). *C. acutatum* was a part of the permanent collection of microbiological cultures at the Laboratory for Biological Research and Pesticides, Faculty of Agriculture, University of Novi Sad in Serbia. Before the experiment, *C. acutatum* was inoculated on potato dextrose agar (PDA) and kept at 23 °C. When the mycelium completely covered the Petri dish, 15 ml of PDA medium was poured on the base of each Petri dish for each replication. Mycelial agar plugs of 3 mm in diameter were cut from the peripheral region of the growing colony using a sterile cork borer. Then, three pieces of *C. acutatum* discs were placed on each Petri dish (representing technical replicates). A volume of 10 µl of each essential oil was applied to the inside of the lid of the Petri dish, which was then covered by the base part. Afterwards, they were sealed with parafilm and incubated in the growth chamber where ambient conditions were set up at 23 ºC and >90% humidity. After 7 days, the results were assessed.

Results and Discussion

When the mycelium in the control treatment had completely covered the Petri dish (after 7 days) (the growth of the three discs made contact), the diameters of the mycelial discs in all Petri dishes were measured. The mean value was calculated and used as a single replicate for statistical analysis (Table 1).

Table 1. Mycelium diameter and mycelial growth inhibition

Mycelium diameter (mm)					
Repetition	I	II	III	IV	**Efficacy**
Control	30.0	30.0	30.0	30.0	
Cinnamon EO	0	0	0	0	100 ± 0.0
Orange EO	1.1	0.8	0.9	1.0	96.8 ± 0.4
Tea tree EO	29.8	30.0	29.5	29.7	0.8 ± 0.7

The efficacy of each treatment (Table 1) was calculated as the percentage of mycelial growth inhibition using the formula:

Inhibition (%) = K-T/K x 100

where K is the mean mycelial diameter in the control and T is the mean diameter in the treated Petri dish.

The examined essential oils exhibited different antifungal activities against *C. acutatum*, which is explained by variations in their chemical composition and possible synergistic and antagonistic interactions among their chemical compounds.
Cinnamon essential oil showed the highest inhibitory effect on *C. acutatum* (100%), which is in agreement with the literature confirming its strong antifungal effects (Danh et al., 2021; Duduk et al., 2015; He et al., 2018), due to the presence of phenylpropenes (cinnamaldehydes), which are proven to have fungicidal properties (Marei & Abdelgaleil, 2018). Similarly, other essential oils rich in phenolic compounds, such as thyme, have also shown complete inhibition of *Colletotrichum spp*., confirming that oils with strong bioactive components often exhibit the highest antifungal activity (Morkeliūnė et al., 2021).
The orange essential oil reduced mycelial growth by 97% compared to the control, which is consistent with reports showing that it may be attributed to a large amount of antioxidant limonene, the monoterpene with antimicrobial characteristics (Octaveus et al., 2024; Danh et al., 2021). Comparable variability has been reported in previous studies where some essential oils showed moderate inhibitory effects, while others demonstrated higher efficacy, depending on their composition (Lozada et al., 2019; Danh et al., 2021). Although orange essential oil showed negligible inhibition in some studies (Danh et al., 2021), the high inhibition observed in this study suggests that differences in origin, chemical composition, concentration and pathogen sensitivity may significantly influence antifungal efficacy.
In contrast, tea tree essential oil did not affect *C. acutatum*, showing that not all essential oils exhibit strong antifungal effects (Jing et al., 2011). The absence of inhibition may be related to the predominance of carvomenthenol, a monoterpene with antibacterial and antiparasitic, but weaker antifungal activity. Similar variability was reported in studies where only selected essential oils, such as thyme or lemongrass, completely inhibited fungal germination and growth, while others showed limited or no activity (Lozada et al., 2019; Morkeliūnė et al., 2021).
Overall, these findings confirm the biocontrol potential of essential oils against *C. acutatum*, which strongly depends on their chemical composition, while the variability highlights the importance of selecting or combining the appropriate essential oils.

Conclusion

This study revealed the potential of cinnamon and orange essential oils as biocontrol agents against *C. acutatum*. Their activity is strongly connected with the presence of different chemical compounds with antifungal properties, particularly phenolic constituents, while tea tree oil, based mainly on monoterpenes such as limonene, showed no inhibitory effects under the tested conditions. These findings pinpoint the importance of chemical composition in determining the effects of essential oils as potential biocontrol agents, so further research should focus on in vivo experiments, detailed chemical characterization using GC-MS, and optimization of applied concentrations.

Acknowledgment

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ORIGINAL SCIENTIFIC PAPER

Effects of mowing date and cutting height on biomass production and grain yield of dual-purpose winter oat

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Summary
The effects of cutting height (6 and 9 cm) and mowing dates (four successive dates) was investigated on the dual-purpose use potential of two winter oat varieties (GK Arany and Mv Hópehely). Biomass yield was maximised at 6 cm with the second mowing date, whereas grain yield peaked at 9 cm with the earliest mowing date. Mv Hópehely consistently outperformed GK Arany under optimised biomass and yield. Our results demonstrate that cutting height and timing must be synchronised with variety choice to balance biomass and grain production in dual-purpose use.

Keywords: dual-purpose use, winter oats, cutting height, biomass, grain moisture content

Introduction

Cutting height and cutting time are fundamental agronomic factors in dual-purpose oat (*Avena sativa L.*) production. They interact with each other and with variety to determine biomass yield, grain yield and grain moisture dynamics. This assumption is strongly supported by available research. In an early study, Hasan and Shah (2000) demonstrated that cutting management, especially its timing, decisively influences oat biomass and grain yield, with delayed cutting leading to a drastic reduction in grain yield. Similar conclusions were reached by Indian researchers: in their master's theses, Pukhraj (2024) and Kumar (2022) investigated the dual-purpose use potential of different oat varieties and found that varietal differences manifest themselves depending on cutting management, and that both biomass and grain yield can be optimised by selecting the appropriate variety - cutting height - mowing date combination. The effect of cutting date was approached from a different perspective by Addaheri et al. (2021), who showed that early cutting favours grain yield and also affects lodging susceptibility. The role of cutting height in dual-purpose systems was highligted by the Brazilian study of Neres et al. (2012), according to which a higher cutting height (20 cm) is more advantageous for subsequent forage use because it results in a more favourable botanical composition and lower moisture content.
This study aimed to compare the biomass and grain yield of two winter oat varieties (GK Arany and Mv Hópehely) under two cutting heights (6 and 9 cm) across four successive mowing dates, and to identify the interactions between agronomic factors (cutting height and harvest date) and variety in order to optimise management practices for different utilisation purposes (forage versus grain production).

Material and Methods

The experiment was conducted in Hungary, at the Experimental Garden of the University of Debrecen, under small-plot conditions. Plot size was 3m x 3m (9 m^2) with two independent replications per treatment. The preceding crop was potato and maize. No NPK fertilizers were applied, because oats are prone to lodging and the soil of the Experimental Garden is rich in

potassium (366-638 mg kg^{-1} K_2O [AL] in the top 60 cm layer of soil) and phosphorus (1020-1538 mg kg^{-1} P_2O_5 [AL] in the top 60 cm layer of soil) (AL: ammonium lactate-soluble). The soil type is chernozem with good humus content (2.63-2.89% in the top 60 cm layer of soil) (Bytyqi et al., 2026).

We used two Hungarian-bred winter oat varieties in the experiment: 'GK Arany' and 'Mv Hópehely'. Sowing was carried out on 17 October 2024 at a seeding rate of 600 seeds per square metre, at a depth of 5 cm.

We examined four mowing dates with one-week offset in 2025. At each date, two cutting heights (6 cm and 9 cm) were applied. The phenological stages at the time of cutting were recorded as follows:

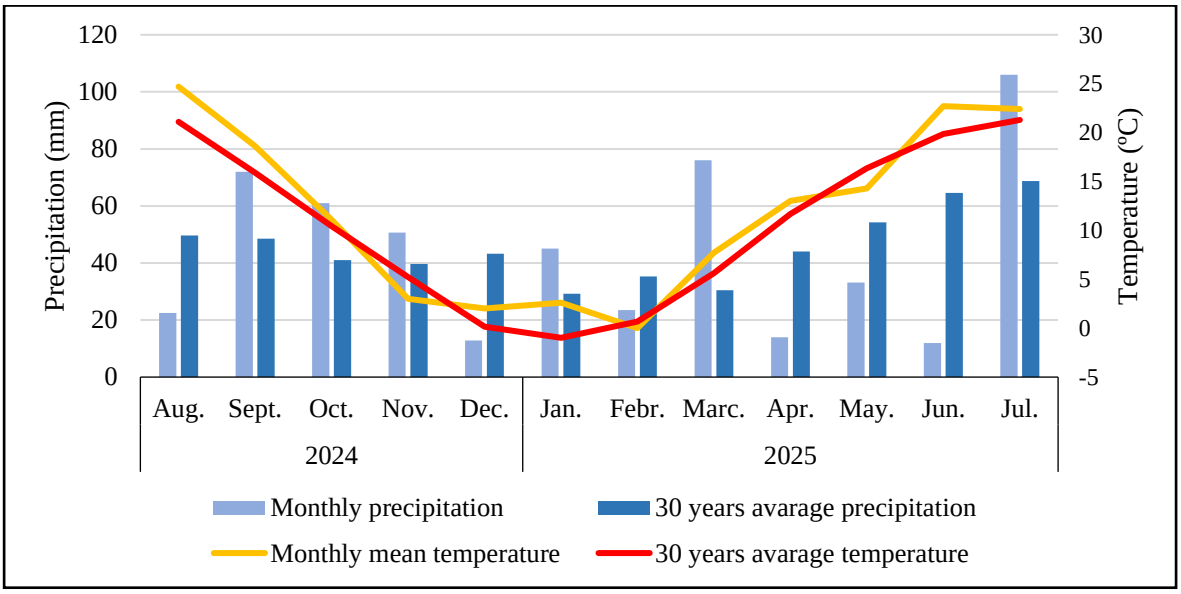
- 1st mowing date: 7 May (BBCH 37)
- 2nd mowing date: 14 May (BBCH 39-45)
- 3rd mowing date: 20 May (BBCH 49-51)
- 4th mowing date: 27 May (BBCH 58-59)

Our analysis encompassed biomass yield (corrected to 70% dry matter), grain moisture and grain yield. After mowing, we weighed the collected forage and determined its moisture content using a drying cabinet. Then we calculated the forage yield per hectare by converting the measured weight and adjusting it to a moisture content of 70%.

We followed a similar procedure to determine grain yield. After harvest, we weighed the grain from the harvested plots and then determined the moisture content. Based on these values, we calculated the yield per hectare, adjusted for a grain moisture content of 14%.

We applied chemical protection against cereal leaf beetle (*Oulema melanopus*) using the active substance lambda-cyhalothrin. Treatments were carried out on 25 April 2025 (BBCH 32-33).

As for the weather conditions, autumn was favorable for crop development. However, the above-average rainfall in September and October complicated optimal sowing time. Mild winter months prevented significant frost damage of the crop, and despite the modest precipitation, the plants developed well thanks to the slow but steady rise in temperature and the reserves stored in the soil. However, from June, the weather turned hot and dry, and this significantly stunted the plants' growth, particularly in the case of plants in plots mowed at a later date (Graph 1).



Graph 1. Weather conditions of the growing season of the winter oat (Debrecen, 2025)

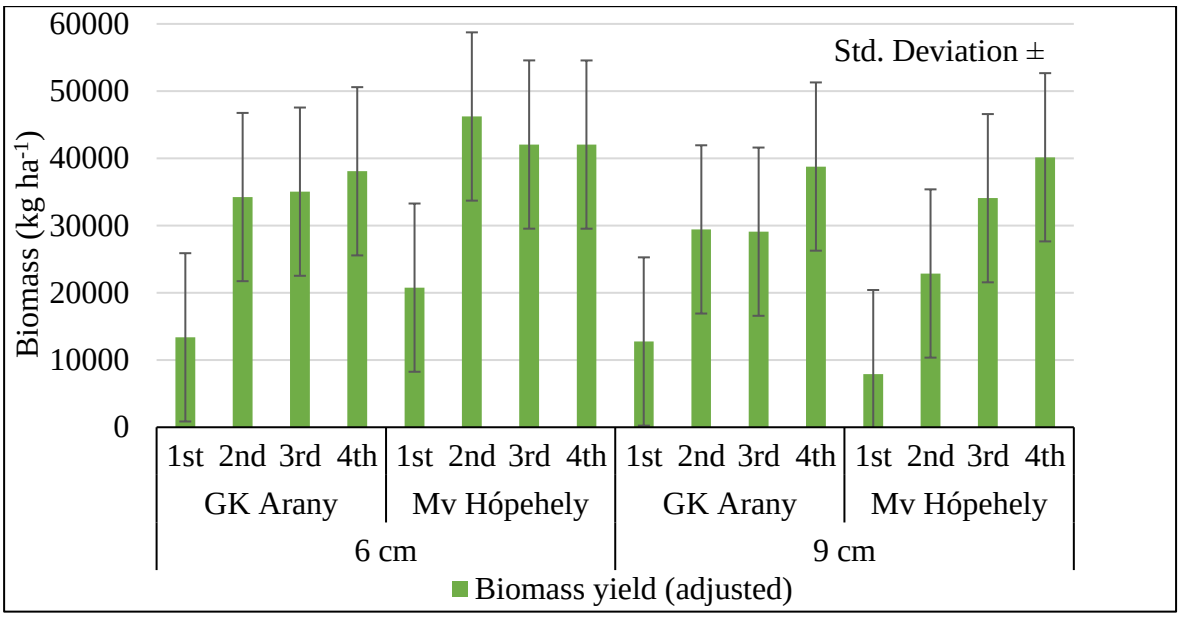
Statistical analysis was performed using IBM SPSS 22, and graphical representations were created with MS Excel 2016. Descriptive statistics were calculated for all measured parameters,

including mean values, standard deviation, minimum and maximum values. Group means were calculated using the Compare Means procedure in SPSS, evaluating the effects of variety, cutting height, and mowing date separately, considering one factor at a time. Due to the limited number of replications (n=2), no analysis of variance (ANOVA) was performed.

Results and Discussion

Biomass production (kg ha^{-1})

In all treatment combinations, the lowest biomass yields (7901–20767 kg ha^{-1}) were measured at the first mowing date. A marked increase occurred by the second mowing date, after which yields remained at a high and stable level during the third and fourth mowing dates. The longer regeneration period associated with later mowing dates likely promoted greater vegetative growth. In most cases, a cutting height of 6 cm resulted in higher biomass yields than 9 cm, particularly at the earlier mowing dates. At 6 cm cutting height, Mv Hópehely consistently outperformed GK Arany at all dates (the difference reached 12000 kg ha^{-1} at the second mowing date), while at 9 cm cutting height, GK Arany proved superior in the first (12757 kg ha^{-1} vs. 7901 kg ha^{-1}) and the second (29430 kg ha^{-1} vs. 22872 kg ha^{-1}) mowing dates, whereas Mv Hópehely outperformed it at the later dates. The datas can be seen in Graph 2. The results indicate the possibility of variety × mowing date and cutting height × mowing date interactions. For example, Mv Hópehely appeared more sensitive to the higher cutting height at the earlier mowing dates.



Graph 2. Biomass yield at harvest for the oat varieties studied corrected to 70% moisture content (Debrecen 2025)

(Note: The number under the colums show the mowing dates as it can be seen in the material and methods chapter).

Grain moisture (%)

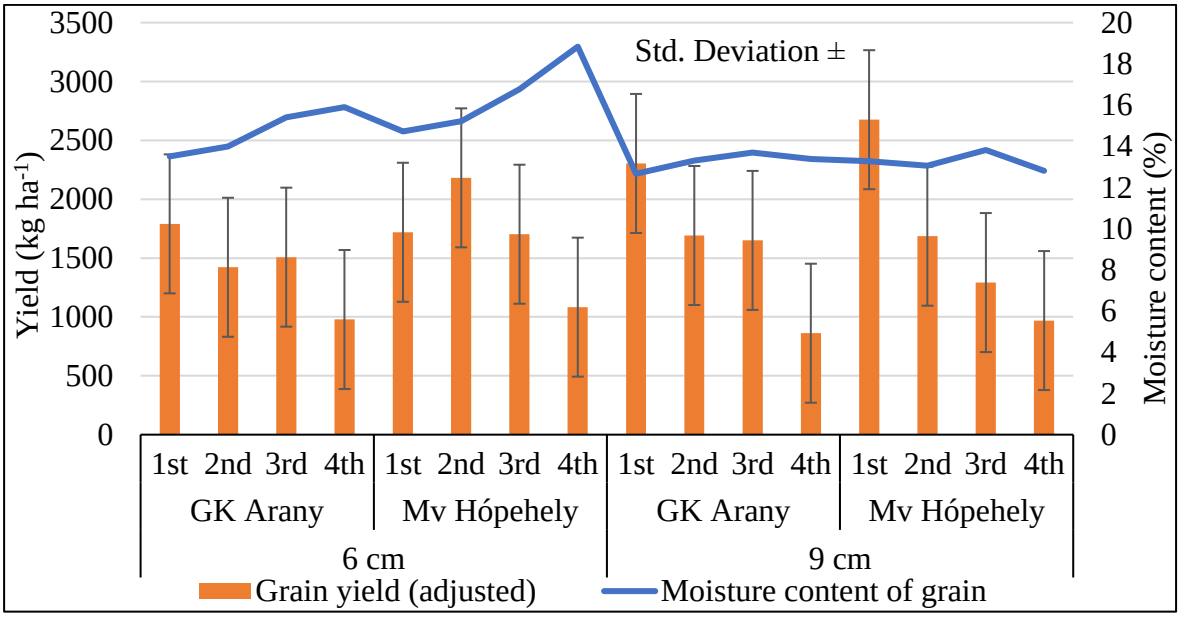
The moisture content of the grain is of particular importance from a practical standpoint, as it fundamentally affects the storability and quality of the harvested crop. For this reason, the seeds are typically dried to a moisture content below a certain level (usually 14%), which - depending on the initial moisture content - represents a significant expense for farmers.

The moisture content was fundamentally influenced by the cutting height. At a height of 6 cm, the moisture content clearly increased as time progressed (due to less mature grains resulting from later mowing), while at 9 cm, the moisture content remained stable or decreased slightly. The 9 cm cut resulted in lower moisture content at all mowing dates compared to the 6 cm cut,

suggesting that the higher cut may accelerate ripening. At the 6 cm cutting height, Mv Hópehely consistently showed higher grain moisture than GK Arany across all mowing dates. In contrast, at 9 cm cutting height, the difference between the two varieties were minimal, with values remaining close throughout the experimental period. The effect was most pronounced at the fourth date, where the moisture content was 13.4% vs. 15.9% for GK Arany, and 12.8% vs. 18.8% for Mv Hópehely at 9 and 6 cm cutting heights, respectively (Graph 3).

Grain yield (kg ha^{-1})

As shown in Graph 3, the trend in grain yield was the opposite of that of biomass: the highest values were recorded after the harvest of the parcels that were mowed first, while the lowest were observed at the harvest of the parcels that were mowed the latest. The longer regeneration and grain-filling period following early mowing ensured higher grain yield, while late mowing led to a decrease in yield due to the shortening of the generative phase. At the first mowing date, GK Arany slightly outperformed Mv Hópehely at the 6 cm cutting height (1791 kg ha^{-1} vs. 1719 kg ha^{-1}), whereas at the 9 cm cutting height Mv Hópehely produced higher yield (2676 kg ha^{-1} vs 2304 kg ha^{-1}) At the second mowing date, Mv Hópehely at 6 cm cutting height substantially exceeded GK Arany (2182 kg ha^{-1} vs. 1422 kg ha^{-1}), while the two varieties showed nearly identical yields at the 9 cm cutting height. At the third mowing date, Mv Hópehely maintained a yield advantage at 6 cm, whereas GK Arany outperformed it at 9 cm. By the fourth mowing date, yields had declined sharply in all treatments, although Mv Hópehely retained a slight advantage over GK Arany at both cutting heights. A cutting height of 9 cm resulted in higher grain yield for both varieties at the first mowing date compared to 6 cm; however, this advantage disappeared at later mowings, and by the fourth mowing date, the 9 cm treatment actually resulted in lower yield. GK Arany's performance was more consistent across the different treatments, while Mv Hópehely exhibited a higher response rate: in some combinations (6 cm + second mowing date; 9 cm + first mowing date) it produced outstanding yields, while in other cases yields were more moderate.



Graph 3. Grain yield and grain moisture content at harvest for the oat varieties studied (Debrecen 2025)

(Note: The number under the colums show the mowing dates as it can be seen in the material and methods chapter).

Conclusion

To achieve the highest grain yield, we recommend combining early (first) mowing with a cutting height of 9 cm, which ensures an outstanding yield at an acceptable moisture content (around 13%). Alternatively, a cutting height of 6 cm at the second cutting—especially for the Mv Hópehely variety—can also result in high grain yields, but the increased harvest moisture content and the associated drying costs must be taken into account. The use of late (third and fourth) cutting dates is not advantageous either for maximizing grain yield or for achieving low moisture content. In terms of variety selection, GK Arany is more stable, while Mv Hópehely can be grown with greater potential but at increased risk. Due to the low number of replicates in the experiment, the findings should be interpreted as indicative trends; confirmation through further studies is warranted.

Acknowledgment

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ORIGINAL SCIENTIFIC PAPER

Mitigating clover sickness: Spent mushroom substrate and symbiotic recovery

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Summary
Clover sickness reduces the vitality and persistence of red clover in repeated cropping systems. This study investigated the effects of spent mushroom substrate (SMS) and cattle manure (CM) on root symbiosis of red clover (*Trifolium pratense* L.) at a field site affected by clover sickness. SMS increased root nodulation (+55.6 %) compared to the control and CM. Mycorrhizal colonization was higher under SMS than under CM (+67.5 %), but did not differ significantly from the control. In addition, higher cover ratios of red clover were observed under SMS. These results suggest that SMS may support red clover performance under clover sickness conditions. However, causal relationships require further investigation.

Keywords: clover sickness, red clover, spent mushroom substrate, nodulation, mycorrhiza

Introduction
Red clover (*Trifolium pratense* L.), a member of the Fabaceae, plays a key role in grassland systems due to its ability to fix atmospheric nitrogen (N_2) through symbiosis with rhizobia. Biological N fixation is particularly important in organic farming systems, where external N inputs are limited (Cesarano et al., 2017). However, repeated cultivation of red clover can lead to reduced plant vitality and persistence, a phenomenon commonly referred to as clover sickness (Huang et al., 2013; Katznelson, 1972). This condition is associated with poorly developed clover stands and represents a major challenge for sustainable grassland management. The underlying mechanisms are complex and may involve interactions between soil properties, management practices, and plant–microbe interactions. Symbiotic interactions, such as root nodulation and mycorrhizal colonization, are central to the performance of legumes and may be impaired under clover sickness conditions. Organic amendments may influence these plant–soil interactions and thereby support the recovery of red clover. Spent mushroom substrate (SMS), a by-product of mushroom production based on *Pleurotus ostreatus*, contains high amounts of organic matter and nutrients and has been suggested as a soil amendment in agricultural systems (Medina et al., 2012). However, its potential to restore symbiotic interactions and improve red clover performance under clover sickness conditions remains largely unexplored. This study investigates the effects of cattle manure (CM) and SMS on root nodulation and mycorrhizal colonization of red clover at a field site affected by clover sickness. It was hypothesized that SMS enhances symbiotic interactions by promoting overall biological activity in the soil and thereby improves plant vitality.

Material and Methods
Study site
The study site is located in Friedland, Northern Germany (53°38′N, 13°22′E) on a loamy sand. Clover–grass mixtures have been cultivated under organic management since 2020. An area showing symptoms of clover sickness was divided into three plots representing different treatments: control (C), cattle manure (CM), and spent mushroom substrate (SMS). In May

2023, five red clover (*Trifolium pratense* L.) plants per treatment were sampled randomly within each plot (BBCH 39) at a soil depth of 0–10 cm. Samples were stored at 4 °C until further analysis.

Root nodulation
Roots were carefully washed and nodules were counted visually. Nodulation was recorded up to a maximum of 200 nodules per root sample to avoid bias from excessively high counts.

Mycorrhizal colonization
Mycorrhizal colonization of fine roots was determined following the modified staining method of Brundrett et al. (1984). Fine root segments (1 cm length) were cleared in 10 % KOH (24 h), acidified in 1 % HCl (15 min), and stained with Chlorazol Black E in lactoglycerol (24 h). Samples were stored in lactoglycerol after destaining. Colonization was assessed microscopically using the gridline intersect method described by McGonigle et al. (1990). A total of 200 intersections per sample were evaluated to estimate mycorrhizal colonization as percentage of root length.

Vegetation cover
The vegetation cover of red clover (*Trifolium pratense*), white clover (*Trifolium repens*), grasses, and other plant species was recorded for each plot in May 2023 and expressed as percentage cover.

Statistical analyses
Statistical analyses were conducted in R (RStudio). Data were analyzed using one-way ANOVA after testing for normality with the Shapiro–Wilk test. Significant differences between treatments were determined using Tukey's HSD post hoc test.

Results and Discussion

Root nodulation

Table 1. Root nodulation of red clover (*Trifolium pratense* L.) under control (C), cattle manure (CM), and spent mushroom substrate (SMS) treatments

Treatment	Mean nodules per root
C	124.4 b
CM	150.0 ab
SMS	193.6 a

Values represent mean nodules per root (n = 5 plants per treatment). Mean values marked with different lowercase letters are significantly different at $p \leq 0.05$. Results should be interpreted with caution due to subsampling within plots.

The number of root nodules of red clover differed between treatments (Table 1). Mean nodulation ranged from 124.4 in the control (C) to 193.6 per plant under SMS application. Compared to the control, nodulation was higher after SMS application, whereas application of CM showed no significant effect.

SMS is a by-product of mushroom cultivation and is characterized by a high content of organic matter and a wide C:N ratio due to its lignocellulosic composition (Sygula et al., 2019; Herrmann and Bauhus, 2018). This addition of organic material with a high C:N ratio may lead to temporary N immobilization during microbial decomposition. This may increase the N demand of the plant–soil system and potentially stimulate biological N_2 fixation, resulting in enhanced root nodulation (Nicolardot et al., 2001). Similar patterns have been reported by

Medina et al. (2012), who observed increased abundance of free-living N_2-fixing microorganisms following SMS application, along with reduced N mineralization.

Mycorrhizal colonization

Mycorrhizal colonization was higher under SMS than under CM, but did not differ significantly from the control (Fig. 1).

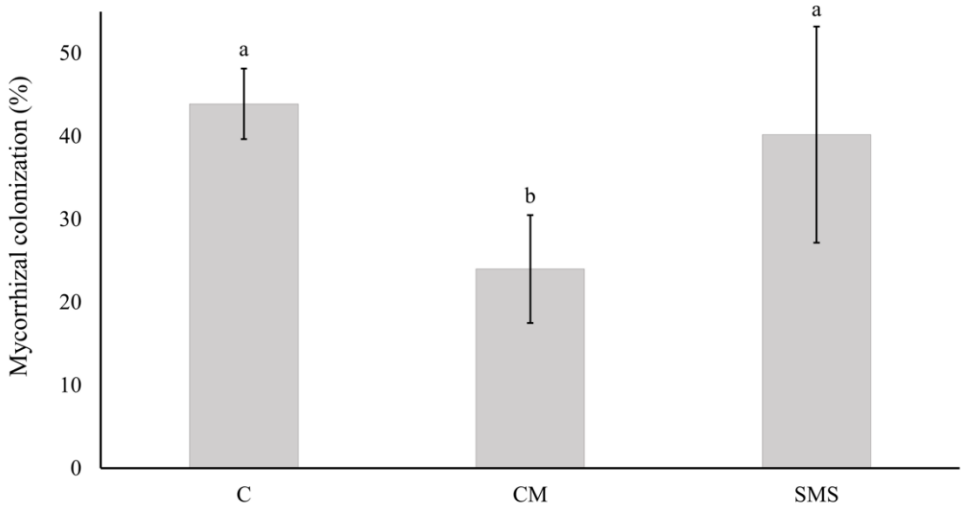

Figure 1. Mycorrhizal colonization of red clover (*Trifolium pratense* L.) roots under control (C), cattle manure (CM), and spent mushroom substrate (SMS) treatments

Values represent mean ± standard error (n = 5 plants per treatment). Mean values marked with different lowercase letters are significantly different at $p \leq 0.05$. Results should be interpreted with caution due to subsampling within plots.

Mycorrhizal associations are often promoted under conditions of limited nutrient availability, particularly phosphorus (P) (Abbott et al., 1984). SMS application may have altered soil nutrient dynamics and reduced nutrient availability, which could have favored the formation of mycorrhizal symbiosis. The concurrent increase in nodulation and mycorrhizal colonization suggests a coordinated response of symbiotic interactions, although causal relationships cannot be confirmed based on the present data.

Vegetation cover

Table 2. Vegetation cover (%) of red clover (*Trifolium pratense* L.) and associated plant groups under control (C), cattle manure (CM), and spent mushroom substrate (SMS) treatments

Plant species	C (%)	CM (%)	SMS (%)
red clover	15	30	35
white clover	15	10	5
grasses	65	45	55
weeds	5	15	5

Values represent plot-level observations.

The vegetation survey showed differences in plant composition between treatments (Table 2). The highest cover ratio of red clover was observed under SMS application, whereas the control plots showed lower clover abundance and higher grass dominance. The increased abundance of red clover under SMS may be related to enhanced symbiotic interactions, which can improve nutrient acquisition and competitiveness. However, as vegetation data were collected at plot level without replication, these observations should be interpreted as indicative rather than conclusive.

Conclusion

Application of spent mushroom substrate (SMS) increased root nodulation of red clover under clover sickness conditions. Mycorrhizal colonization was higher under SMS than under CM, but did not differ significantly from the control. This was accompanied by a higher cover ratio of red clover in the vegetation under SMS treatment. These findings indicate that SMS may contribute to mitigating clover sickness by enhancing root nodulation and supporting beneficial root–soil interactions. However, the results are limited to the conditions of this study and should be interpreted with caution due to the experimental design. In addition, SMS can vary widely in its chemical composition and biological properties, which may influence its agronomic effects. Further studies across multiple sites and growing seasons are needed to confirm the long-term agronomic benefits and broader applicability of SMS in red clover systems.

Acknowledgement

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REVIEW ARTICLE

Requirements of CPVO technical protocol for DUS testing in maize (*Zea mays* L.)

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Summary
Distinctness, Uniformity and Stability (DUS) testing represents a fundamental requirement for granting plant breeders' rights within the European Union. The aim of this paper is to present the requirements and methodological principles of the *Community Plant Variety Office* (CPVO) Technical Protocol used for DUS testing in maize (*Zea mays* L.), along with a critical evaluation of its application and future development perspectives. The protocol defines standardized procedures for field trial design, observation of morphological characteristics and assessment of varietal uniformity and stability. Candidate varieties are evaluated based on plant, tassel, ear and grain traits using predefined scales of expression. However, the application of morphological descriptors may be limited by environmental influences, particularly in the case of quantitative traits, which can complicate the reliable differentiation of closely related genotypes. In this context, molecular approaches, especially the use of SNP markers, are increasingly considered as complementary tools to conventional methods, as they provide higher precision and objectivity in variety identification. Although the current regulatory framework still prioritizes morphological characteristics, future developments in DUS testing are expected to involve the integration of phenotypic and genomic approaches.

Keywords: maize, DUS testing, CPVO, plant variety protection, morphological traits

Introduction

The objective of this paper is to summarize the key requirements of the CPVO protocol and highlight the most important morphological descriptors used in maize DUS testing. Maize breeding (*Zea mays* L.) plays a crucial role in ensuring food security and sustainable agricultural production. The development of new maize varieties with improved yield potential, resistance to biotic and abiotic stresses and improved grain quality is essential for modern agriculture (Meng et al., 2024; Deribe, 2025; Tang et al, 2025; Mushayi et al., 2025; Ocwa et al., 2024). In order to protect the intellectual property rights of plant breeders, international and regional systems for plant variety protection have been established. In the European Union, plant variety protection is administered through the Community Plant Variety Office (CPVO) (2010). According to Council Regulation (1994), a new plant variety must fulfil three essential criteria in order to obtain Community Plant Variety Rights: distinctness, uniformity and stability (DUS). These criteria ensure that newly developed varieties can be clearly distinguished from existing varieties, that they exhibit sufficient uniformity in their characteristics and that their traits remain stable after repeated propagation cycles. Maize (*Zea mays* L.) is one of the most important cereal crops in the world and represents a major component of global food and feed production. Continuous breeding efforts have resulted in a large number of maize hybrids with diverse morphological and agronomic characteristics (Brkić, 2023). As a consequence, standardized testing systems are required to accurately identify and distinguish candidate varieties. The CPVO Technical Protocol TP/002/3 (2010) defines the methodology for conducting DUS tests in maize. This protocol provides detailed

instructions for field trial design, observation of morphological characteristics and assessment of varietal distinctness, uniformity and stability.

Methodological Framework for DUS Testing in Maize

The methodological framework presented in this paper is based on the Community Plant Variety Office Technical Protocol TP/002/3 (2010). DUS testing is conducted through officially organized field trials carried out by authorized testing institutions, where candidate varieties are grown alongside reference varieties representing the closest comparable genotypes. Field trials are typically performed over at least two independent growing cycles to minimize environmental effects and ensure reliable assessment of varietal characteristics. The number of plants observed in each trial is defined by the protocol and depends on the type of variety being tested. Observations are performed on a representative sample of plants to evaluate the expression of morphological characteristics, which are grouped according to plant organs and developmental stages. Assessments are conducted either visually (VG – visual assessment of a group of plants) or through measurements (MG – measurement of a group of plants), while in some cases individual plant observations (VS or MS) may also be required. Each characteristic is described using predefined states of expression coded on a numerical scale from 1 to 9, enabling objective comparison between candidate and reference varieties. The Key DUS characteristics for maize (CPVO TP/002/3) are summarized in Table 1.

Table 1. Key DUS characteristics for maize (CPVO TP/002/3)

Characteristic		Type	States of expression	Growth stage
Plant	Plant length (height)	QN*	very short – very long	75–85
	Ratio: ear insertion height / plant height	QN	very small – very large	75–85
Tassel	Time of anthesis	QN	very early – very late	61–69
	Anthocyanin coloration at base of glume	QN	absent – very strong	65–69
	Number of primary lateral branches	QN	few – very many	65–75
Ear	Time of silk emergence	QN	very early – very late	65–75
	Ear length	QN	very short – very long	75–93
	Number of rows of grain	QN	few – many	75–93
Grain	Type of grain	QL	flint – dent – intermediate – sweet – pop – waxy	92–93
	Colour of grain (top or dorsal side)	PQ	white – yellow – orange – red – purple	92–93

*QN – quantitative characteristic; QL – qualitative characteristic; PQ – pseudo-qualitative characteristic. Growth stages are presented according to the BBCH scale (decimal code for growth stages). Observations are conducted in accordance with the Community Plant Variety Office Technical Protocol TP/002/3 (2010).

In addition to morphological descriptors, supplementary methods such as electrophoretic analysis of enzymes may be applied in certain cases to support variety identification; however, morphological characteristics remain the primary basis for DUS testing in maize.

Current Challenges and Future Perspectives in DUS Testing

Although the Community Plant Variety Office technical protocol defines a comprehensive set of morphological characteristics for the differentiation of maize varieties, its practical

implementation is associated with several limitations. Despite their standardized nature, morphological descriptors can be influenced by environmental conditions, which may complicate the consistent interpretation of results across different locations and growing seasons. In particular, quantitative traits such as plant height or ear insertion height are highly responsive to agroecological factors, potentially reducing their discriminatory power under certain conditions. In contrast, reproductive traits, including tassel morphology and ear characteristics, generally exhibit higher genetic stability and are therefore considered more reliable indicators of varietal distinctness. However, in modern breeding programs, especially those involving closely related hybrids, phenotypic differences are often subtle, making it increasingly challenging to clearly establish distinctness within the framework of DUS criteria. In this context, molecular approaches are gaining importance as complementary tools to traditional morphological assessment. Molecular markers offer higher resolution and are less affected by environmental variation, thereby enhancing the precision and objectivity of variety identification (Jamali et al., 2019; Tian et al., 2021; Yu et al., 2022). In maize, several studies have already demonstrated the practical applicability of SNP-based approaches for variety identification. For example, a large-scale SNP fingerprinting study based on 200 core markers showed high discriminatory power among more than 20,000 maize genotypes, with strong correlation between reduced SNP sets and high-density genotyping platforms, confirming their suitability for variety authentication and protection of plant breeders' rights (Tian et al., 2021). Šimić et al. (2009) investigated the usefulness of SSR and SNP molecular markers for genetic mapping in a maize population adapted to Southeast European conditions. Total of 294 F2:3 lines derived from a biparental mapping population were genotyped using 121 polymorphic SNP and SSR markers. The main finding was that combining SSR and SNP data produced a high-quality genetic map consistent with existing maps, confirming that both marker systems are suitable for further QTL analysis. Nevertheless, despite their potential, their broader implementation in official DUS testing remains constrained by regulatory limitations and the need for international harmonization. According to the International Union for the Protection of New Varieties of Plants, molecular data should primarily support, rather than replace, morphological descriptors, which continue to serve as the fundamental basis for DUS evaluation (UPOV, 2017). Future developments in DUS testing are therefore expected to focus on the integration of morphological and molecular approaches, aiming to improve the robustness, reproducibility and discriminatory capacity of varietal assessment while maintaining compliance with existing regulatory frameworks.

Closing remarks

DUS testing in maize remains fundamentally based on standardized morphological descriptors as defined by the Community Plant Variety Office and aligned with UPOV principles. While this approach ensures regulatory consistency and international harmonization, it faces increasing challenges due to environmental variability and the limited discriminatory power of certain traits in modern breeding materials. Future progress in DUS testing will depend on achieving a balance between maintaining standardized phenotypic evaluation and incorporating genomic approaches. Strengthening international cooperation and revisiting existing guidelines, particularly within the framework of the International Union for the Protection of New Varieties of Plants, will be essential for enabling the transition toward more robust and efficient next-generation DUS systems.

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ORIGINAL SCIENTIFIC PAPER

Spent mushroom substrate: nutrient source or limitation in oilseed rape?

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Summary
Spent mushroom substrate (SMS) is a potential organic fertilizer, but its agronomic value is inconsistent. A field experiment with winter oilseed rape (*Brassica napus* L.) was conducted in a randomized complete block design. SMS was applied before sowing and compared with a control treatment without SMS under equal mineral nitrogen fertilization. Shoot nutrient concentrations (N, P), seed yield, and oil content were evaluated. No significant effects were observed. The results indicate limited short-term nutrient availability, likely related to the high C:N ratio and predominant lignocellulosic composition of the substrate. SMS was not an effective short-term nutrient source under the given conditions and may require prior composting to improve its agronomic value.

Keywords: spent mushroom substrate, organic amendments, C:N ratio, nitrogen immobilization, nutrient availability

Introduction

Several studies have reported that spent mushroom substrate (SMS) can improve soil properties, such as increasing soil organic matter content and microbial activity (Martín et al., 2023; Baptista et al., 2023). These improvements may also enhance plant growth and yield. However, the effects of SMS are not consistent and depend on factors such as substrate composition, mushroom species, and processing. In particular, plant growth promotion was often associated with processed or composted materials, while the effects of fresh SMS remain unclear. SMS derived from lignocellulosic substrates may reveal low short-term nutrient availability due to slow decomposition and potential nitrogen immobilization.
Winter oilseed rape (*Brassica napus* L.) has a high nutrient demand and is considered a suitable crop for evaluating the effects of organic fertilizers. However, the short-term effects of fresh SMS on nutrient availability and crop performance in oilseed rape have not been sufficiently investigated.
The aim of this study was to evaluate the effects of SMS on shoot nutrient concentrations (N and P), seed yield, and oil content of winter oilseed rape. The study further assesses whether SMS acts as a nutrient source or limits nutrient availability under field conditions.

Material and Methods

The field experiment was conducted in Gülzow-Prüzen, Germany (53°47′N, 12°03′E) in 2024. The soil type was classified as a Stagnic Luvisol with a sandy to loamy sand texture. Weather conditions during the growing season were characterized by below-average precipitation (495 mm vs. 552 mm long-term mean) and elevated temperatures (10.6 °C vs. 9.1 °C long-term mean) compared to the regional average for Gülzow-Prüzen. The experiment was established as a randomized complete block design with four replicates. The experimental setup included several organic fertilization treatments. For the present study, only the control treatment (without organic fertilization) and the treatment with spent mushroom substrate (SMS) were considered.

The SMS originated from *Pleurotus ostreatus* cultivation and consisted mainly of beech wood chips. It was applied prior to sowing at a rate of 2.6 t ha^{-1} and incorporated into the topsoil. Concentrations of carbon (C), nitrogen (N), and phosphorus (P) of SMS were 401.1, 7.7, and 2.3 g kg^{-1} dry matter, respectively, resulting in a C:N ratio of approximately 52.
Winter oilseed rape (*Brassica napus* L.) was grown as model crop. Sowing was carried out on 25 of August 2024. All treatments received identical mineral nitrogen fertilization of 130 kg N ha^{-1} in spring.
To assess plant nutrient status, shoot samples were collected at a late growth stage (BBCH 60 - 65). Five plants per plot were sampled and analyzed individually. The plant material was dried, homogenized, and analyzed for nitrogen (N) and phosphorus (P) concentrations. Harvest was carried out on 30 July 2025. Seed yield and seed oil content were determined on a plot basis. Seed oil content was determined using standard laboratory analysis at the State Institute for Agriculture and Fisheries (LFA, Germany).
Differences between treatments were tested for significance at $p \leq 0.05$. Results are presented as mean ± standard deviation. Statistical analyses were performed using R (version 4.3.1) in RStudio.

Results and Discussion
The applied spent mushroom substrate (SMS) was characterized by a high carbon content and a wide C:N ratio (≈ 52), indicating a stable organic material with limited short-term nutrient availability. High C:N ratios are typically associated with slow decomposition and may promote microbial nitrogen immobilization, as microbial decomposition of carbon-rich organic materials requires additional nitrogen (Geisseler et al., 2010).
No significant effects of SMS application on the nutrient status of oilseed rape shoots were observed (Figure 1). Concentrations of nitrogen (N) and phosphorus (P) were similar in both treatments, indicating that SMS did not enhance plant nutrient uptake during the growing season. This may be related to the lignocellulosic composition of the substrate, which decomposes slowly and delays nutrient release. Stewart et al. (1998) reported that inorganic N release from spent mushroom compost was initially limited by nitrogen immobilization and followed by only slow mineralization of organic N, resulting in delayed nutrient availability.
In addition, nitrogen in SMS is predominantly present in organic forms and is released gradually during mineralization processes, resulting in a delayed nitrogen supply to plants (Lou et al., 2017). In contrast to fresh SMS, composted materials usually show a lower C:N ratio and a higher degree of decomposition, which improves mineral nitrogen availability and short-term nutrient release (Lou et al., 2017; Yagüe et al., 2025). This may explain why positive agronomic effects are more frequently reported for processed SMS than for fresh materials such as the substrate used in the present study.

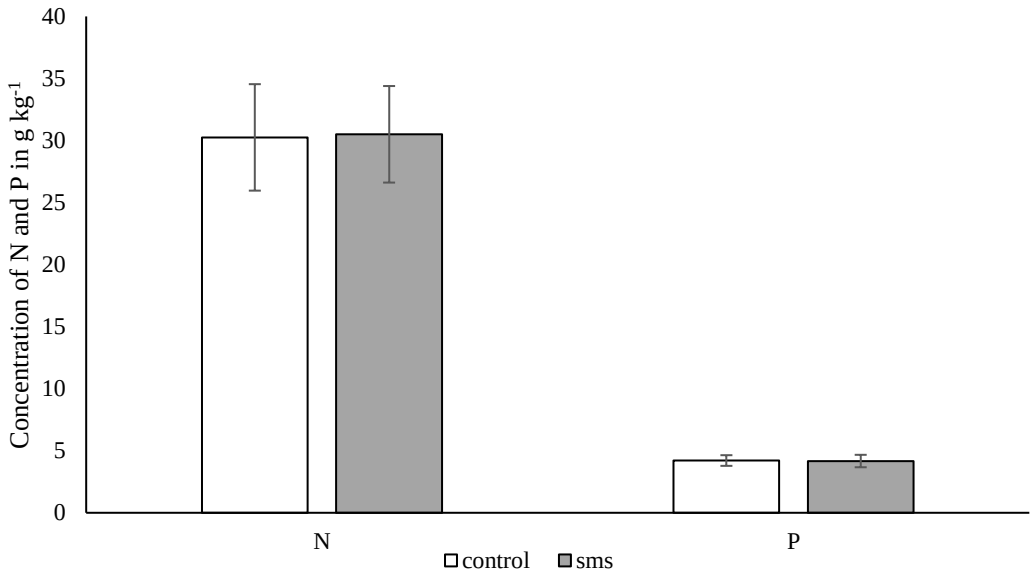

Figure 1. Mean nitrogen (N) and phosphorus (P) concentrations (g kg^{-1}) in shoots of winter oilseed rape under control and SMS treatments. Error bars represent standard deviation.

Seed yield was not significantly affected by SMS application (Fig. 2). Mean seed yield was 1.00 t ha^{-1} in the control and 1.09 t ha^{-1} in the SMS treatment. Although yield was slightly higher under SMS application, variability was also increased and differences were not statistically significant. Seed oil content was likewise not affected. Similar results have been reported in previous studies, where the agronomic effectiveness of SMS depended strongly on its degree of decomposition, with more pronounced positive effects observed after composting or further processing (Martín et al., 2023; Baptista et al., 2023).

The absence of seed yield effects is consistent with the observed nutrient concentrations and indicates that SMS did not improve short-term nutrient supply under the given conditions. The experimental site is considered a marginal site with sandy soil conditions, while the growing season was characterized by below-average precipitation and elevated temperatures. In addition, the relatively high mineral nitrogen fertilization (130 kg N ha^{-1}) may have reduced the short-term agronomic relevance of SMS as an additional nutrient source.

Under the conditions of this study, fresh SMS showed limited short-term agronomic effects in winter oilseed rape. Its stable lignocellulosic composition and wide C:N ratio likely restricted nutrient mineralization and delayed nutrient release during the growing season. This supports the assumption that further processing, such as composting, may be necessary to improve mineral nitrogen availability and enhance agronomic performance (Ma et al., 2023; Yagüe et al., 2025).

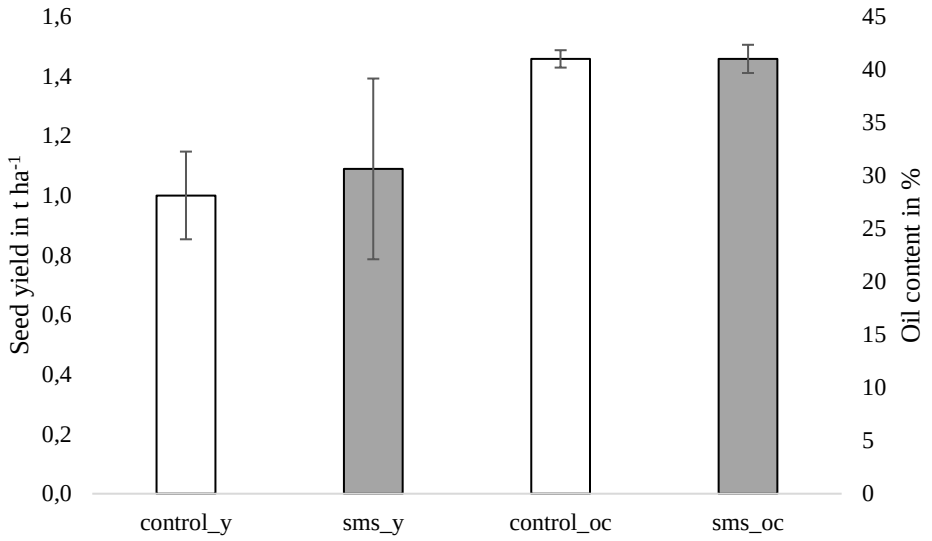

Figure 2. Mean seed yield (t ha^{-1}) and oil content (%) of winter oilseed rape under control and SMS treatments. Error bars represent standard deviation.

Conclusion

Fresh spent mushroom substrate (SMS) was not an effective short-term nutrient source for winter oilseed rape under the conditions of this study. No significant improvements in plant nutrient status, seed yield, or oil content were observed. The wide C:N ratio and stable lignocellulosic composition of the substrate likely limited nutrient mineralization and delayed nutrient release during the growing season. These findings indicate that pre-treatment, particularly composting, may be necessary to improve mineral nitrogen availability and enhance the agronomic value of SMS.

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ORIGINAL SCIENTIFIC PAPER

Ecotype x foliar nutrition interactions in oats: implications for climate resilience

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Summary
The aim of this study was to evaluate the yield performance and stability of winter and spring oat genotypes. Between 2021 and 2025, we conducted a small-plot field trial to investigate the effects of foliar application of silicon and sulfur on the productivity and adaptability of oats. Winter oats outperformed spring varieties, with up to 31% higher yield under severe drought. The combined Si+S treatment significantly improved yield stability in winter cultivars. Silicon alone increased yield under mild drought conditions but was ineffective in dry years. In spring oats, Si and S had no significant effect on stability. Winter oat cultivation may offer a more reliable option under climatic risks, while Si+S treatments can help reduce yield fluctuation.

Keywords: *Avena sativa* L., foliar fertilization, silicon, sulfur, yield stability

Introduction

Oat (*Avena sativa* L.) is an important cereal crop used both as concentrate and bulk feed, as well as for human consumption (Marshall et al, 2013). Its high biological value protein (avenin), favorable fatty acid composition, and significant dietary fiber (β-glucan) and antioxidant contents make it an essential component of a healthy diet (Allwood et al., 2021; Stewart and McDougall, 2014). In recent decades, both the cultivation area and total production of oat have gradually declined worldwide; however, demand has increased due to the recognized nutritional benefits of whole-grain cereals (Stewart et al., 2014; Achleitner et al., 2008). As in other cereals, oat yield, quality, and physiology are influenced by several production factors, including nutrient supply, genotype, and environmental conditions (Wilson et al., 2021; Kutasy et al., 2025). Under increasingly extreme climatic conditions, the selection of genotypes adapted to specific ecological conditions can further improve adaptability, as the yield of winter ecotypes may exceed that of spring varieties by 25–30%; however, considerable differences in productivity may also occur among cultivars within the same ecotype (Kutasy et al., 2023; Bytyqi et al., 2026).
Our research focused on assessing and evaluating the effects of foliar applications of silicon and sulfur on yield performance and the adaptibility of oats. A further objective of our study was to identify those winter and spring oat varieties that demonstrate superior adaptation to changing environmental conditions and exhibit above-average yield stability, resilience, tolerance to abiotic stress, and high yield potential.

Material and Methods

A small-plot field experiment was established at the Experimental Garden of the University of Debrecen, Hungary (47°33′02” N; 21°35′56” E), using a randomized complete block design (RCBD). The four treatments were arranged in four consecutive blocks, with three replications per treatment within each block. The plot size was 3 × 4 m, corresponding to an area of 12 m^2.

The experimental site is characterized by homogeneous chernozem soil with high humus content (2.7-3.66%), an approximately 80 cm thick humus layer, and a clay loam texture (Arany plasticity index, K_A=45), providing a favorable water regime. The nutrient status of the upper 0-60 cm soil layer is very good, with high levels of available phosphorus (P_2O_5 (AL): 1020-1538 mg kg^{-1}) and potassium (K_2O (AL): 366-638 mg kg^{-1}) (Kutasy et al., 2025).
We used 550 seeds m^{-2} seeding rate, and a sowing depth of 5 cm. Fertilizers (NPK) were applied uniformly in the experiment as follows: $N_5P_{40}K_{120}$ kg ha^{-1} in October 2020, followed by 50 kg ha^{-1} nitrogen in March 2021. In 2022 and 2023 March also 50 kg ha^{-1} nitrogen fertilizer was applied. In 2023, substantial lodging occurred, likely due to the efficient nutrient uptake of oat, the beneficial effect of the legume preceding crop, and adequate water availability. Therefore, no additional NPK fertilization was applied in 2024 or 2025.Table 1 shows the forecrop and the fertilization in the examined period, while in Table 2 the varieties can be seen.

Table 1. The crop rotation and nutrient supply for the experiment

Year	Forecrop		Fertilization
	Winter oat	Spring oat	
2020/2021	potato	winter wheat	autumn: $N_5P_{40}K_{120}$ spring: N_{50}
2021/2022	winter wheat	winter wheat	spring: N_{50}
2022/2023	soybean	bean	
2023/2024	bean	soybean	-
2024/2025	soybean	proso millet	

Table 2. Winter and spring oat varieties for the experiment

Winter oat	Year	Spring oat	Year
GK Arany	2021-2025	Mv Kengyel	2021-2025
Mv Hópehely		Mv Ménes	
Mv Imperiál		Mv Pehely	
Mv Kincsem		Mv Szellő	
Mv Istráng		GK Kormorán	
Mv Hóka	2023-2025	GK Pillangó	
Mv Fergeteg	2025	Lota	
		Panna	2022-2025

The treatments included a control, silicon (0.5 L ha^{-1}), sulfur (0.5 L ha^{-1}), and combined silicon + sulfur (0.5+0.5 L ha^{-1}) foliar fertilization. Foliar applications were carried out at three phenological stages (BBCH13, BBCH37, BBCH73). The applied foliar fertilizers were Jello Fluid: liquid foliar fertilizer with high content sulfur (lignosul-fonate formulation, 1000g L^{-1} SO_3, 30g L^{-1} N, 30g L^{-1} MgO, 27g L^{-1} B, 0.003g L^{-1} Mo) and Optysil (200 g SiO_2 L^{-1})
The study covered five growing seasons (2021–2025), which showed considerable differences in their climatic conditions. To evaluate water availability during the growing season, the

modified Pálfai Drought Index (PaDI) was calculated for each experimental year (Pálfai and Herceg, 2012), and based on this, the years were classified into the following drought categories: mild drought (2023), moderate drought (2024), medium drought (2021, 2025), and severe drought (2022) (Table 3).

Table 3. The calculated modified Pálfai Drought Index during five growing seasons in the experimental site in Debrecen (2021-2025).

Year	**$P_{36\ month}$** (mm)	**Monthly average P for previous 36 month** (mm)	**Sum of P Apr-Aug** (mm)	**MIN(P_{jun}, P_{jul}, P_{aug})** (mm)	**$PaDI_0$** (°C 100 mm^{-1})	**PaDI** (°C 100 mm^{-1})	**Drought category**
2021	1401.2	38.9	180.6	6.0	7.10	**9.7**	medium drought (8-10)
2022	1519.7	42.2	160.9	10.5	8.75	**11.6**	serious drought (10-15)
2023	1567.3	43.5	**359.8**	35.9	3.76	**4.3**	mild drought (4-6)
2024	1761.7	48.9	282.2	17.0	5.19	**6.5**	moderate drought (6-8)
2025	1823.0	50.6	191.9	17.9	8.21	**9.4**	medium drought (8-10)

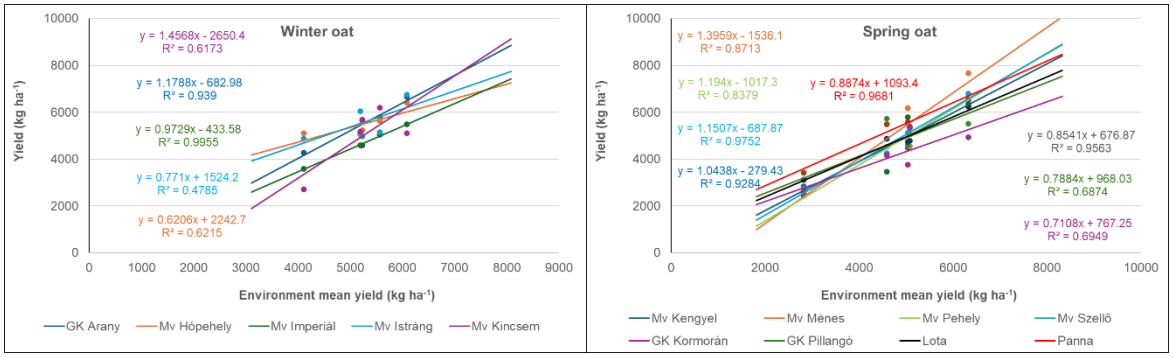
Note: P=precipitation; $P_{36\ month}$= precipitation sum for period October-September for previous three years; $MIN(P_{jun}, P_{jul}, P_{aug})$ = the lowest value from annual precipitation sum of three summer months (June, July, August)

Statistical analyses were performed using IBM SPSS Statistics 22.0 software. One-way analysis of variance (ANOVA) was conducted within the General Linear Model (GLM) framework. Differences between treatments were evaluated using an F-test, and mean comparisons were performed using the least significant difference (LSD) post hoc test at a significance level of p = 0.05. Stability analysis was applied to assess the effects of genotypes and treatments on yield stability.

Results and Discussion

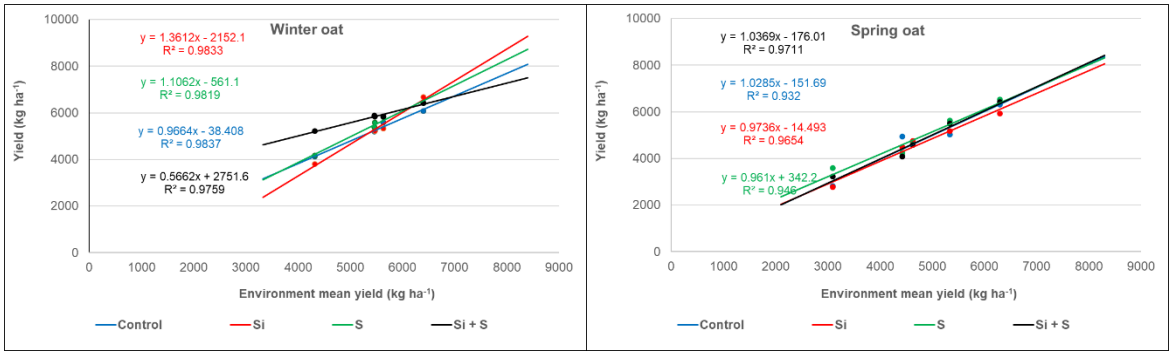
Significant differences in yield stability were observed among both winter and spring oat genotypes (Graph 1). Among winter cultivars, Mv Hópehely exhibited outstanding yield stability. Under serious drought (PaDI value: 11.6 °C 100 mm^{-1}) conditions (2022), its yield exceeded the cultivar mean by 24%; however, under improved conditions, its yield level was considered average. Similarly high stability was observed for Mv Istráng. In contrast, Mv Kincsem showed lower adaptability to drought but performed well under mild drought conditions. The GK Arany cultivar also demonstrated high yield potential and superior adaptability under dry conditions. Differences among spring oat cultivars were less pronounced. Although at a lower yield level, GK Kormorán showed the highest stability, while the Panna

cultivar combined relatively high yield with good stability. The high yield potential of Mv Ménes was expressed only under better water supply, as its drought tolerance proved to be limited.



Graph 1. Yield stability of winter and spring oat genotypes without silicon and sulfur fertilization (Debrecen, 2021-2025)

The effects of Si and S treatments on yield stability of spring oats were generally not significant; however, under drought conditions, sulfur treatments showed a yield-increasing effect, whereas under better water supply, the combined Si+S treatment proved more effective. Based on our previous studies, we found that sulfur and silicon+sulfur treatments also positively influenced the macro- and micronutrient content of the grain (Bytyqi et al., 2026). The response of winter oats to Si and S treatments was more pronounced in terms of yield performance. The combined Si and S treatment significantly improved yield stability. Silicon alone had no effect under drought conditions, but under more favorable environmental conditions, its positive effect on yield was clearly demonstrated. An advantage of sulfur treatment is that its effect is expressed even in dry years, whereas under favorable water supply, it was less effective than the combined treatment (Graph 2).



Graph 2. Effect of silicon and sulfur treatments on the yield stability of winter and spring oat genotypes in the average of genotypes (Debrecen, 2021-2025)

Conclusion

According to our results – except for 2023 at better water supply – the yield of winter oat genotypes exceeded that of spring varieties, with a yield advantage reaching 31% in the severely drought-affected year. The combined Si+S treatment significantly improved yield stability in winter oat cultivars. Silicon (Si) application increased yield under mild or moderate drought conditions; however, it was not effective when applied alone in dry years. In the case of spring oats, Si and S treatments did not significantly affect yield stability. Under chernozem soil conditions in the Hajdúság region, the winter cultivars Mv Hópehely, Mv Istráng, and GK Arany, as well as the spring cultivars Mv Ménes, Mv Szellő, Lota, and Panna, exhibited higher

yield levels and yield stability. Based on these results, winter oat cultivation represents a more reliable alternative under climatic risk conditions, and Si+S treatments contribute to reducing yield variability.

Acknowledgement
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ORIGINAL SCIENTIFIC PAPER

Preliminary investigation on the cultivation of *Physalis peruviana* in decoupled aquaponics

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Summary
Thirty plants were assessed using decoupled aquaponics by measuring Soil-Plant Analysis Development (SPAD), Normalized Difference Vegetation Index (NDVI), and yield parameters, light intensity, air humidity, and temperature.
SPAD and NDVI increased rapidly, peaking by the end of July, followed by gradual decline. Pearson's correlation analysis using environmental data from one and four weeks before harvest showed weak negative correlations between SPAD and increasing temperature. NDVI exhibited weak positive correlations with humidity and light intensity.
Temperature and light intensity in the week preceding harvest showed moderate positive correlations with yield parameters, while four-week humidity data showed weak to moderate positive relationships.

Keywords: aquaponics, physalis, cultivation, yield

Introduction

Physalis peruviana is an annual herbaceous plant belonging to the *Solanaceae* family (Damu et al., 2007). Cultivation of plants in the *Physalis* genus occurs primarily in the tropical and temperate climate zones of the American continent, and a smaller portion in Southeast Asia (Feng et al., 2018). These plants possesses potent pharmacological effects (Liang et al., 2022). Widely used in Asia to treat skin inflammation, bronchitis, pneumonia, hepatitis, and rheumatism (Liang et al., 2024). Wild populations have declined significantly, efforts are needed to protect them and ensure their sustainable cultivation (Luo et al., 2015).
In agricultural production, we must remain mindful of changes in climatic factors even as we adopt innovative technologies. Aquaculture is one of the fastest-growing sectors of the food industry (Rastegari et al., 2023). Wastewater these systems could be a potential environmental problem (Piedrahita, 2003), Aquaponics is a complex system that simultaneously enables the reuse of wastewater from intensive aquaculture systems (Schneider et al., 2005) and effective crop production where field production is limited. The production and distribution of food to cities cause significant environmental pollution. Aquaponics can provide a solution to these challenges by supplying locally grown vegetables and aquatic organisms, while also promoting social and economic development (Love et al., 2015; Santos, 2016).
In our experiment, we investigated the potential for growing *Physalis peruviana* in aquaponics system.

Material and Methods

The study was conducted in the aquaponics system of the Fish Biology Laboratory University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, in a double-walled, semi-shaded greenhouse. The plant growing medium was baked clay media bed

(10 m^2). The system was decoupled, so the water does not return to the fish tanks. The units were supplied with water and nutrients from a fish-rearing system with a usable water volume of 12 m^3, in which there waswe a mixed-age carp stock at a general density of 20-25 kg m^{-3}. Water was replenished every two weeks.

We measured the environmental factors affecting the plants on a daily basis: light intensity (PeakTech5065 DIGITAL 155 LUX-METER, PeakTech Prüf- und Messtechnik GmbH, Ahrensburg, Germany, spectral range: 0.1-100000Lux), air humidity and temperature (PCE-THB 40 Thermo Hygrometer Barometer, PCE-THB 40 Humidity/Baro/Temp. Data Recorder (PCE Holding GmbH, Meschede, Germany). The average temperature during the experimental ranged from 23.5 to 36.3°C, but daily temperature fluctuations were high (16°C morning and 51.5°C afternoon in July). The humidity fluctuated between 70% and 20% from morning to afternoon. Light intensity averaged over 20000 lux in July and August.

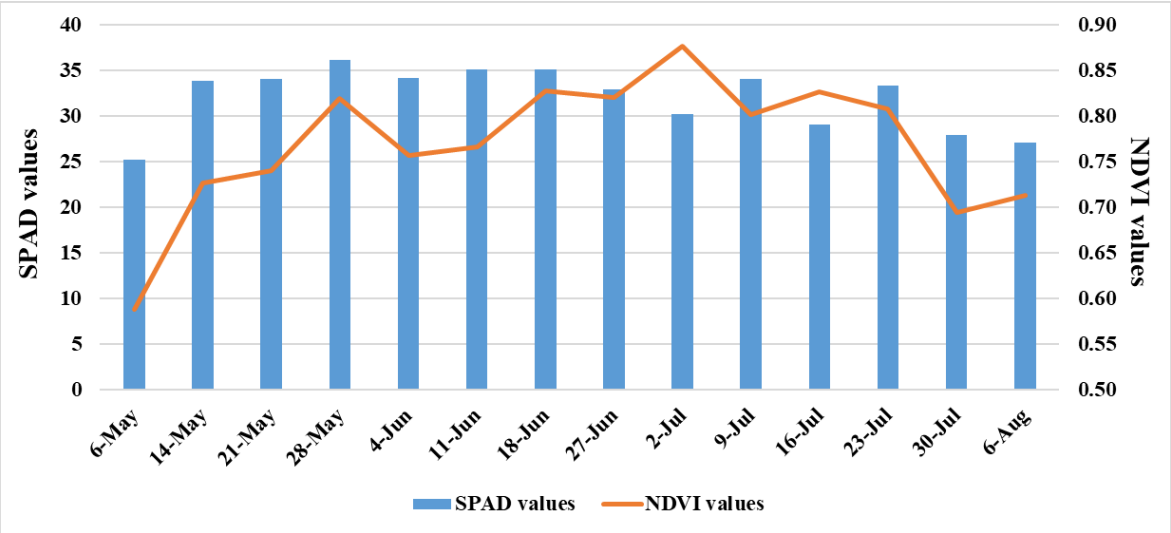
Seeds were sown on March 5 and planting took place on April 23, with 30 seedlings. After a two-week accumulation period, weekly SPAD (SPAD-502 Plus Konica Minolta, Konica Minolta Sensing Europe B.V., Warrington, United Kingdom) and NDVI (Trimble HCS-100 GreenSeeker, Westminster, CO 80021 USA) measurements began. The crop was harvested five times between July 9 and August 6. At that time, we measured the total berry weight (Waage OHAUS Scout Pro SPU6000, 6000g, d:1g portable, OHAUS Europe GmbH, Nänikon, Switzerland), length, and diameter (Digital caliper, DC-1 Artikel-Nr. 650080, Ovibell GmbH & Co. KG, Mülheim an der Ruhr, Germany) in a sample of 10, and the total yield (Waage OHAUS Scout Pro SPU6000, 6000g, d:1g portable, OHAUS Europe GmbH, Nänikon, Switzerland).

We used Microsoft Excel (2019 MSO 64-bit) and IBM SPSS Statistics 22.0 (64-bit version) to analyze the data. We used Pearson's correlation analysis to examine the relationships between the measured plant parameters and the environment. We used an ANOVA test to analyze variations in yield data.

Results and Discussion

We sought to monitor the growth of the plant population by conducting weekly, non-destructive chlorophyll measurements. SPAD values ranged on average between 25.24 and 36.14; in most measurements, the plants' values were above 30.

NDVI values ranged on average between 0.59 and 0.87. The low values began to rise early from the second week and quickly reached the maximum. Favorable SPAD and NDVI values were typical in the stand until the end of July, after which they began to decline slowly (Graph 1).



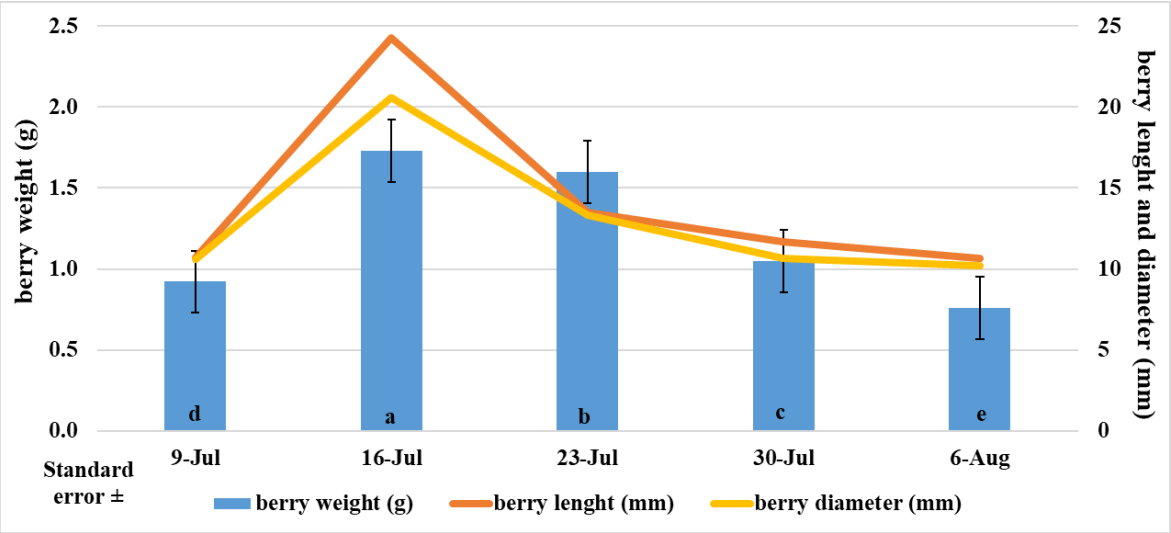
Graph 1. Changes in SPAD and NDVI values during the experiment

Using Pearson's correlation analysis and the average environmental parameters one and four weeks before harvest, it was determined that, over a four-week interval, rising temperatures had a weak negative correlation with plant SPAD values (r= -0.314, P=1%). NDVI values show a positive weak correlation with air humidity (r 1 week before harvest = 0.363, r 4 weeks before harvest = 0.428, P=1%) and light intensity in lux (r 1 week before harvest = 0.423, r 4 weeks before harvest = 0.286, P=1%). The long-term harmful effects of high temperatures are also confirmed by the research of Aparecido et al. (2019); this condition is characteristic of 80% of Brazil's growing regions.

The crop was harvested five times between July 9 and August 6. The total yield was 3,936.8 kg ha^{-1}. The yield per plant ranged from 0.27 to 1.60 kg. The individual berry weight ranged from 0.76 to 1.73 grams, with berry lengths ranging from 10.64 to 24.28 mm and berry diameters ranging from 10.18 to 20.58 mm. The weight and size of the berries peaked during the second harvest and then began to decline, but remained significantly larger than the values of the first harvest until the last (Graph 2).

In Fischer's (2000) study, the average weight of the berries was 4-10 grams, and their diameter was approximately 12.5-25.0 mm; the yield from the aquaponics falls short of these measurements. According to Lima et al., 2012, the yield per plant is 2-3 kg, which is close to our results. The growing season can last up to 8 months, while flowers and fruits develop simultaneously. We observed this phenomenon as well.

According to Muniz et al. (2014), in Colombia, where physalis is grown on 800-1,000 hectares, the yield is 15-28 tons per hectare. If we extrapolate our 4-week yield results over 8 months, this amounts to 31.49 tons per hectare, which suggests promising future prospects. This is supported by the finding of Saha et al. (2016) that aquaponics is capable of achieving high yields.



Graph 2. Changes in yield parameters of the 10-sample sets during the experiment

In our Pearson correlation analysis, we found that both the temperature and light intensity during the week preceding harvest showed a moderate positive correlation with the specific yield parameters we measured. For weight measured in samples of 10 berries, r= 0.735, P= 1%. For length, r= 0.554, and for diameter, r= 0.557; we measured a correlation with the average temperature at a 1% significance level. For the lux values, the berry weight showed r= 0.763, the length r= 0.784, and the diameter r= 0.774 (P= 1%). Only the air humidity data showed a positive correlation over the four-week period. We detected correlation values of r= 0.707 for berry weight, r= 0.469 for length, and r= 0.532 for diameter (P= 1%).

We found only a weak negative correlation between berry diameter and temperature (r= -0.305) at the 5% significance level, suggesting that, in the long term, a drastic rise in temperature will have a negative impact on yield parameters.

Several researchers have attempted to determine the ideal temperature range for growing physalis. According to the findings of Salazar et al. (2008), and Fischer et al. (2014), the ideal temperature for cultivation ranges between 13 and 18°C, and fertility remains stable up to 30°C. Above 30°C, both the number of flowers and the yield decrease significantly. These temperature values were also confirmed by Muniz et al. (2014). Furthermore, they explained in their study that heat itself does not inhibit fruit set. This is also demonstrated by the example of Hawaii, where plants produced fruit even at daily temperatures of around 27-30°C. Low temperatures (below 10°C at night), however, likely inhibit plant growth. Late frosts can cause significant damage, especially if nighttime temperatures drop below -2°C. According to their hypothesis, temperature and light play an important role in determining the size, color, nutritional composition, flavor, and stage of ripeness of fruits.

Fischer (2000) and Aparecido et al. (2019) described in their work that, to prevent fruit spoilage, relative humidity must be between 70-80%, with an evenly distributed annual precipitation of 1,000-2,000 mm. In addition, physalis is highly sensitive to drought and strong winds, so its cultivation must be protected with windbreaks (Muniz et al., 2014). The advantages of aquaponic greenhouse cultivation can reduce or completely eliminate these problems.

Conclusion

Overall, the aquaponics system provided suitable conditions for *Physalis peruviana* cultivation, particularly where field production is limited. Optimizing environmental parameters may further improve yield stability. It would be worthwhile to expand the experiment to include an analysis of nutritional values in addition to yield parameters.

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PRELIMINARY COMMUNICATION

Sensory analysis and young consumer perceptions of PIWI wines

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Summary
The aim of this study was to assess the acceptability of wines produced from PIWI varieties (fungus-resistant grape cultivars) and to determine whether younger consumers are able to differentiate wines from different grape varieties, particularly when the wines are made from grapes originating from the same region using identical vinification techniques. The study was conducted on a sample of 15 undergraduate students in the Enogastronomy program, with three repetitions, and comprised a sensory evaluation using the 100-point scale and a structured questionnaire. The correspondence of the evaluated wines with the typical profile of Graševina was assessed. Wines from PIWI varieties achieved the highest overall scores (86 points). Souvignier gris was rated as the most similar to typical Graševina (8/10), supporting the hypothesis that younger consumers may have difficulty distinguishing between varieties. Participants exhibited no bias against novel grape cultivars, and the majority expressed willingness to purchase wines from PIWI varieties.

Keywords: Young consumers, wine, PIWI, sensory analysis

Introduction

The term PIWI is an acronym of the German word *"Pilzwiderstandsfähige (Rebsorten)"*, which means "fungus-resistant grape varieties," and refers to grape cultivars developed through breeding to achieve resistance to fungal diseases (PIWI International, n.d.). For the viticulture of the future, it is essential to manage grapevine diseases with reduced use of chemical agents (Zini et al., 2019). Consumer perceptions of products are continuously evolving and are influenced by numerous factors, ranging from generational characteristics to the incorporation of scientific knowledge (such as awareness of environmental degradation) and prevailing market trends (Borrello et al., 2021).

The aim of this study was to assess the acceptability of wines produced from PIWI varieties. It was hypothesized that younger consumers might have difficulty distinguishing wines from individual grape varieties, especially when the grapes originate from the same growing region and the wines are produced using identical vinification technologies, particularly if the wines are of similar quality. In comparative tastings, wines from PIWI varieties have been shown to be equivalent or even superior to wines from *Vitis vinifera L.* varieties (Pedanault & Provost, 2016). For example, Santana et al. (2025) demonstrated that varying maceration durations in Caladris blanc and Felicia provided a basis for the development of enological practices that can contribute to wines with a distinctive sensory identity, whose secondary aromas resemble those of *Vitis vinifera L.* wines.

The motivations driving consumers toward organic wines vary from health concerns to ecological awareness, interest in terroir, and the general perception that organic wines have higher value than conventional wines (Bonn et al., 2015; Borrello et al., 2024). According to Peglarini et al. (2013), 61% of respondents would be willing to pay more for wines produced using organic methods. Consumers also believe that the quality of sustainably produced wines will be equal to or higher than that of wines produced using conventional methods (Forbes et al., 2009).

Material and Methods

The study was conducted on three groups (repetitions) of five students each from the Enogastronomy undergraduate program, first and second-year students, at the Faculty of Tourism and Rural Development in Požega. The study consisted of two parts.

In the first part, as part of the Food and Beverage Sensory Analysis course, students evaluated four wines (treatments) from the 2024 vintage. The tasting included two Graševina wines fermented with different commercial yeast strains and two PIWI variety wines, namely Souvignier gris (SG) and Caladris blanc (CB). Graševina fermented with Anchor Vin 13 yeast was labeled GR-Vin13, while Graševina fermented with Fermol Cryarome yeast was labeled GR-Cry. Grapes for all wines were harvested on the same day, and the entire vinification process was carried out identically for all four musts. PIWI musts were fermented using Fermol Cryarome yeast. Wines were evaluated using the official 100-point scoring method in accordance with the current Wine Regulations. Subsequently, the panelists were asked to rate, on a scale from 0 to 10, how closely each wine corresponded to the typical profile of Graševina from the 2024 vintage.

In the second part, the same panelists completed a questionnaire to collect general information on wine consumption and perception. Finally, the panelists' attitudes toward PIWI wines were assessed. Questionnaire results are expressed as percentages (%), while results from the sensory evaluation are presented as medians. Although the study was conducted in three repetitions, no statistical analysis of the data was performed.

Results and Discussion

Table 1 presents the results of the sensory analysis of Graševina wines fermented with two different yeast strains and wines from two resistant PIWI varieties (Souvignier gris and Caladris blanc). The treatment GR-Vin13 received the lowest score and was the only one classified within the traditional category of "kvalitetno vino". The remaining three treatments fell into the traditional category of "vrhunsko vino" with the highest scores awarded to both PIWI variety wines, achieving an average of 86 points. Similar results were reported by Pedanault and Provost (2016).

Table 1. Sensory analysis – 100 points: Graševina - Anchor Vin13, Graševina - Cryarome, Souvignier Gris, Caladris Blanc, vintage 2024.

Sample		GR-Vin13	GR-Cry	SG	CB
Appearance	Clarity	5	5	5	5
	Color	10	10	10	10
Flavor (aroma)	Cleanliness	5	5	4	5
	Intensity	6	7	7	6
	Quality	12	12	12	14
Taste	Cleanliness	4	5	5	5
	Intensity	7	7	7	6
	Durability	6	7	7	6
	Quality	16	16	19	19
Harmony		9	10	10	10
Overall rating		80	84	86	86

The correspondence of each treatment with the typical profile of Graševina from the 2024 vintage is presented in Table 2. Respondents rated each wine on a scale from 0 to 10 points. The Souvignier gris wine was perceived as most similar to typical Graševina, receiving an average score of 8 points, whereas the two Graševina wines were rated lower, with GR-Cry scoring 6 points and GR-Vin13 scoring 7 points.

Table 2. Match with the typical profile of Graševina, on a rating scale from 0 to 10, Graševina - Anchor Vin13, Graševina - Cryarome, Souvignier Gris, Caladris Blanc, vintage 2024.

Sample	GR-Vin13	GR-Cry	SG	CB
	7	6	8	4

Table 3 presents the general habits and attitudes of respondents regarding wine consumption. It is evident that all respondents consume wine, with 86.68% preferring white wines.

Table 3. General information and perceptions of wine expressed in percentages (%)

1.	How often do you consume wine?					
	Never	Rarely	Occasionally	Often		
	0	26,67	46,67	26,67		
2.	Which type of wine do you consume most often?					
	White	Rosé	Red	Sparkling		
	86,67	0	13,33	0		
3.	How important is wine in your everyday life?					
	Not important	Slightly	Moderately	Very important		
	6,67	33,33	46,67	13,33		
4.	What are the most important criteria when buying wine? (multiple answers possible)					
	Price	Quality	Origin	Variety	Recommendation	Organic production
	33,33	100,00	6,77	26,27	33,33	0
5.	How familiar are you with different wine varieties?					
	Not at all	Slightly	Partially	Very well		
	0	20,00	60,00	20,00		
6.	Do you consider wine to be part of cultural and gastronomic tradition?					
	Yes	Not	Not sure			
	100,00	0	0			
7.	How important is the method of wine production (e.g., organic, sustainable) to you?					
	Not at all	Partly	Important	Very important		
	6,67	53,33	33,33	6,67		
8.	Would you be willing to pay more for higher-quality wine?					
	Yes	No	It depends			
	73,33	0	26,67			
9.	How often do you choose wine based on recommendations (friends, sommelier, internet)?					
	Never	Rarely	Sometimes	Often		
	0	6,67	53,33	40,00		
10.	How important is the impact of wine on health when choosing?					
	Not at all	Slightly	Important	Very important		
	13,33	40,00	33,33	13,33		

The most important criteria when selecting a wine were quality, followed by price and recommendation, and only then the grape variety. None of the respondents considered organic cultivation as a relevant criterion for wine selection, despite the option for multiple responses

being provided. Although all respondents (100%) indicated that wine price is important, only three-quarters (73.33%) were willing to pay a higher price for a higher-quality wine.
Table 4 presents questions and responses related to the knowledge and perception of PIWI wines. Prior to the study, 73.33% of respondents were familiar with PIWI wines, while 40.00% encountered wines from resistant varieties for the first time during the tastings. One-third of respondents (33.33%) believed that PIWI wines could compete with traditional varietal wines, whereas 60.00% were uncertain. None of the respondents expressed prejudice against lesser-known grape varieties. Regarding purchase intent, 66.67% of respondents indicated they would probably buy wines from resistant varieties, and 26.67% stated they would very likely purchase them, similar to the findings of Peglarini et al. (2013), where 61% of respondents were willing to pay more for PIWI wines if their quality was equal to that of *Vitis vinifera* wines. Only 26.67% of respondents considered the ecological benefits of PIWI wines as a reason to buy them, which contrasts with the results reported by Bonn et al. (2015) and Peglarini et al. (2013).

Table 4. Percentage (%) distribution of attitudes toward wines from PIWI cultivars

1.	Had you heard of wines from PIWI varieties prior to the tasting conducted as part of this study?				
		Yes		No	
		73,33		26,67	
2.	Where did you first hear about PIWI wines?				
	Media	Friends	Tasting	Internet	Other
	0	13,33	40,00	6,67	13,33
3.	How would you rate the taste of PIWI wine?				
	Very poor	Poor	Average	Good	Excellent
	0	6,67	33,33	33,33	20,00
4.	Do you think PIWI wines can compete with traditional wines?				
		Yes	No	Not sure	
		33,33	6,67	60,00	
5.	How important is the fact that PIWI varieties are more disease-resistant (fewer pesticides)?				
	Not ta all	Slightly	Important	Very inportant	
	0	46,67	26,67	26,67	
6.	Would you prefer to buy PIWI wine because of its environmental sustainability?				
		Yes	No	Depends on price	
		26,67	40,00	33,33	
7.	Do you have prejudices against new or lesser-known wine varieties?				
		Yes	No	Not sure	
			80,00	20,00	
8.	How likely are you to buy PIWI wine in the future?				
	Very unlikely	Unlikely	Probably	Very probably	
	0	6,67	66,67	26,27	

Regarding purchase intent, 66.67% of respondents indicated they would probably buy wines from resistant varieties, and 26.67% stated they would very likely purchase them, similar to the findings of Peglarini et al. (2013), where 61% of respondents were willing to pay more for PIWI wines if their quality was equal to that of *Vitis vinifera* wines. Only 26.67% of respondents considered the ecological benefits of PIWI wines as a reason to buy them, which contrasts with the results reported by Bonn et al. (2015) and Peglarini et al. (2013).

Conclusion

The sensory analysis showed that wines from PIWI varieties (Souvignier gris and Caladris blanc) received the highest scores (86 points) and, together with one Graševina sample, would be classified in the "vrhunsko vino" category, whereas Graševina Vin13 received the lowest score (80 points) and falls into the traditional "kvalitetno vino" category. Interestingly, Souvignier gris was perceived as the most typical Graševina (8/10), even more so than the actual Graševina samples. Although some respondents considered that PIWI wines could compete with traditional wines, the majority were uncertain. Overall, respondents did not express prejudice against new grape varieties, and there is a high interest in purchasing PIWI wines in the future; however, the ecological aspect plays a lesser role in the purchase decision.

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ORIGINAL SCIENTIFIC PAPER

Pomological performance of the European plum cultivar ‘Nada’

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Summary
This study evaluated the pomological performance of the European plum cultivar ‘Nada’ in comparison with ‘Čačanska Lepotica’ and ‘Stanley’ during 2020–2022 under Serbian growing conditions. The results showed that ‘Nada’ had similar vigor and yield to ‘Čačanska Lepotica’, while ‘Stanley’ achieved higher productivity. However, ‘Nada’ was distinguished by larger fruit size and favorable physical characteristics. In addition, it exhibited lower acidity and a higher ripening index, indicating a favorable balance between sugars and acids. These results indicate that ‘Nada’ represents a promising cultivar for modern plum production, with potential for both fresh consumption and processing.

Key words: plum, new cultivar, late-season, fruit quality, yield

Introduction

European plum (*Prunus domestica* L.) is one of the most important fruit species in Serbia, characterized by high production, wide adaptability and diverse utilization, including fresh consumption and processing. In addition to its economic importance, plum fruit represents a valuable source of sugars, organic acids, and bioactive compounds (Milošević et al., 2025; Tomić et al., 2019).

In recent decades, the need for new cultivars has increased due to changing climatic conditions, market demands and the spread of diseases, particularly Plum pox virus (PPV), which significantly limits production (Hartmann and Neumüller, 2006; Neumüller et al., 2021). Therefore, modern plum breeding programs are focused on developing cultivars with improved productivity, fruit quality and adaptability.

One of the leading breeding centers in Southeast Europe is the Fruit Research Institute, Čačak, where numerous cultivars with significant commercial value have been developed and widely adopted in production (Milošević et al., 2021). Among recently developed cultivars, ‘Nada’ has shown promising characteristics, including large fruit size and high fruit quality, as well as Sharka tolerance and good adaptation to local growing conditions.

Therefore, the aim of this study was to evaluate the growth, yield and fruit quality characteristics of the cultivar ‘Nada’ in comparison with the standard cultivars ‘Čačanska Lepotica’ and ‘Stanley’.

Material and methods

The study was conducted during the period 2020–2022 in an experimental orchard located at Preljinsko brdo, near Čačak (43°53′ N, 20°20′ E, 250 m a.s.l.), Serbia. The orchard was established in 2012 with trees grafted on Myrobalan (*Prunus cerasifera* Ehrh.) seedlings, planted at a spacing of 5 × 2 m. Trees were trained as a modified central leader and managed under standard cultural practices without irrigation.

The experiment included three plum cultivars: ‘Nada’, ‘Čačanska Lepotica’ and ‘Stanley’. The trial was set up as a randomized block design with three replicates, each consisting of five trees.

Tree vigor was assessed using trunk cross-sectional area (TCSA, cm^2), calculated from trunk diameter measured at the end of the growing season. Yield per tree (kg) was determined after harvest from each tree in all examined years, using an ACS System Electronic Scale (Zhejiang, China), while yield efficiency (kg cm^{-2}) was calculated as the ratio between averaged Y per tree and TCSA.

For fruit analysis, 25 fruits per cultivar were sampled at commercial maturity. Fruit weight (g) and stone weight (g) were measured using an Ohaus Adventurer technical scale (Parsippany, NJ, USA). Flesh percentage (%) was calculated by subtracting the stone weight from the total plum fruit weight. Fruit dimensions (length, width and thickness; mm) were determined using a digital caliper Kronen (Kronen GmbH, Kehl am Rhein, Federal Republic of Germany), and geometric mean diameter and sphericity were calculated according to Mohsenin (1980).

Chemical properties included soluble solids content (SSC, %) determined using a refractometer (Carl Zeiss, Germany) at 20°C, total acids (TA, %) determined by titration and expressed as malic acid, and ripening index calculated as SSC/TA ratio. Total sugars (TS) and reducing sugars (RS) were determined using the Luff–Schoorl method.

Data were subjected to analysis of variance (ANOVA), and mean comparisons were performed using the LSD test at $P \leq 0.05$. All statistical analyses were performed using standard software package Microsoft Office Excel 2019.

Results and discussion

Significant differences in vigor and yield parameters were observed among the examined cultivars (Table 1). The observed differences were statistically significant for most of the examined parameters, indicating clear cultivar variability. ‘Stanley’ exhibited the highest tree vigor and yield, while ‘Nada’ and ‘Čačanska Lepotica’ showed similar values. These results confirm that genotype plays a dominant role in determining tree growth and productivity in plum, although environmental conditions and orchard management may also contribute to variability (Reig et al., 2018; Milošević et al., 2013; Iglesias and Echeverría, 2009). These results further highlight the stability of the cultivar ‘Nada’, which maintained consistent performance across years despite potential environmental variability.

Table 1. Vigour and yield parameters of tested plum cultivars during examined period (2020–2022)

Cultivar	TCSA (cm^2)	Yield per tree (kg)	Yield efficiency (kg cm^{-2})
‘Čačanska Lepotica’	63.39±1.20 b	24.23±0.78 b	0.38±0.01 b
‘Nada’	63.09±1.30 b	23.86±1.37 b	0.38±0.00 b
‘Stanley’	64.89±1.19 a	27.21±0.43 a	0.42±0.00 a

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

Fruit characteristics varied significantly among cultivars (Table 2). The cultivar ‘Nada’ was distinguished by larger fruit size compared to ‘Čačanska Lepotica’, while ‘Stanley’ showed intermediate values. Differences in stone weight and flesh percentage followed a similar pattern. These findings are consistent with previous studies indicating that fruit size and related traits are primarily genotype-dependent and strongly influence market value and consumer acceptance (Crisosto et al., 2004; Walkowiak-Tomczak et al., 2008).

Table 2. Fruit and stone weight and flesh percentage of tested plum cultivars during examined period (2020–2022)

Cultivar	Fruit weight (g)	Stone weight (g)	Flesh percentage (%)
'Čačanska Lepotica'	40.88±0.32 c	1.26±0.01 c	95.90±0.04 a
'Nada'	48.54±0.20 a	1.91±0.01 b	96.04±0.03 a
'Stanley'	44.38±0.29 b	2.05±0.01 a	95.38±0.01 b

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

Regarding fruit physical properties (Table 3), 'Nada' exhibited favorable dimensions and shape characteristics, confirming its suitability for both fresh consumption and processing. Fruit morphology, including sphericity and size, represents an important quality attribute mainly determined by genetic background (Jannatizadeh et al., 2008).

Table 3. Fruit physical properties of tested plum cultivars during examined period (2020–2022)

Cultivar	Fruit length (mm)	Fruit width (mm)	Fruit thickness (mm)	Geometric mean diameter	Sphericity
'Čačanska Lepotica'	48.35±0.32 b	39.34±0.16 a	37.21±0.41 b	41.35±0.18 b	0.85±0.00 a
'Nada'	49.44±0.50 b	40.25±0.28 a	41.42±0.04 a	43.51±0.20 a	0.88±0.01 a
'Stanley'	52.38±0.26 a	40.54±0.19 a	40.66±0.34 a	44.19±0.19 a	0.84±0.00 a

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

Chemical properties also showed significant differences among cultivars (Table 4). Such differences are particularly important from a consumer perspective, as they directly influence flavor perception and market acceptance. 'Stanley' had the highest soluble solids content, while 'Nada' was characterized by lower acidity and consequently a higher ripening index. This indicates a more favorable balance between sugars and acids, which is one of the key determinants of fruit taste and overall quality (Colarič et al., 2005; Usenik et al., 2008; Drkenda et al., 2022).

Table 4. Content of soluble solids and total acids and ripening index of tested plum cultivars during examined period (2020–2022)

Cultivar	Soluble solids content (%)	Total acids (%)	Ripening index
'Čačanska Lepotica'	16.14±0.24 b	0.89±0.00 a	18.18±0.23 c
'Nada'	17.44±0.36 b	0.58±0.01 c	29.90±0.31 a
'Stanley'	20.53±0.19 a	0.78±0.01 b	26.46±0.51 b

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

The content of sugars followed similar trends (Table 5), with 'Nada' showing intermediate values between the examined cultivars. However, the relatively low acidity in 'Nada' contributes to improved sensory perception, emphasizing the importance of the sugar–acid ratio rather than individual components alone. Recent studies confirmed that sugars and organic acids are the main determinants of plum flavor and their balance directly defines fruit quality (Crisosto et al., 2004; Xiao et al., 2024; Ba et al., 2025).

Table 5. Content of sugars of tested plum cultivars during examined period (2020–2022)

Cultivar	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars
'Čačanska Lepotica'	9.11±0.06 c	7.06±0.10 b	1.94±0.04 c
'Nada'	11.52±0.18 b	8.88±0.11 a	2.50±0.10 b
'Stanley'	13.98±0.04 a	9.07±0.02 a	4.66±0.04 a

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

Overall, the obtained results indicate that 'Nada' combines favorable fruit size and quality traits with acceptable productivity, making it a competitive cultivar under the given growing conditions. This combination of traits positions 'Nada' as a cultivar with balanced agronomic and quality performance.

Conclusions

The results of this study indicate that the cultivar 'Nada' exhibits favorable pomological characteristics under the given growing conditions. Although its productivity was slightly lower compared to 'Stanley', it showed comparable vigor and yield to 'Čačanska Lepotica'. A key advantage of 'Nada' is its large fruit size and favorable balance between sugars and acids, which contribute to good fruit quality and consumer acceptance. In addition, its physical fruit characteristics further support its suitability for both fresh consumption and processing.

Overall, 'Nada' can be considered a promising cultivar for modern plum production, representing a valuable addition to existing cultivar assortments. In addition, its favorable fruit quality traits make it suitable for diversification of plum production under changing environmental conditions. Therefore, 'Nada' can contribute to improving both production efficiency and fruit quality in contemporary plum orchards.

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ORIGINAL SCIENTIFIC PAPER

The influence of different temperature on oat seed germination

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Summary
The aim of this study was to assess the influence of 15 °C and 25 °C on germination and early seedling development of ten oat (*Avena sativa* L.) cultivars (Lovčen, BC Marta, Kučev, Pierce, Kamil, Iniap 82, Kupa, Dolphin, Posejdon, Charlotte) under laboratory conditions. Parameters included germination energy, total germination (means 58.46% vs. 67.4%), shoot/root lengths, and fresh/dry masses in a factorial design. The 25 °C regime yielded higher values across parameters with reduced variability. Cultivars Lovčen, Dolphin, and Kučev excelled at 25 °C (e.g., longest shoots ~15 cm, highest biomass ~5.5 g fresh mass), while Kamil performed best at 15 °C (e.g., 93.33% total germination). These findings highlight genotypic temperature adaptation for breeding resilient cultivars.

Keywords: oat (*Avena sativa* L.), temperature, germination

Introduction

Oat (*Avena sativa* L.), a hexaploid member of the Poaceae family, ranks among the top cereal crops globally, primarily used for animal feed (~70%) and human food products (~30%) (FAO, 2024; Barcchiya et al., 2017). Originating around 2000 BC as a weed in wheat/barley fields before independent domestication, cultivated *A. sativa* now predominates among diverse Avena species spanning ploidy levels (Webster, 1996; Köse et al., 2021). Seed germination, a critical phase determining crop establishment, is highly sensitive to temperature alongside moisture and seed quality (Bewley et al., 2006). Optimal germination for oats typically occurs at 20–25°C, yielding highest rates and uniformity, while suboptimal temperatures (<15°C) delay emergence and amplify genotypic differences valuable for breeding (Willenborg et al., 2005; Sonego, 2000). Climate projections indicate increasing frequency of temperature extremes during sowing periods, potentially disrupting early growth phases (IPCC, 2022). Despite extensive research on oat germination optima, comparative cultivar responses to contrasting temperatures (15°C vs. 25°C) relevant to early sowing scenarios remain limited, particularly regarding vegetative growth parameters beyond basic germination rates. This study addresses this gap by evaluating ten diverse oat cultivars for germination energy, total germination, shoot/root elongation, and seedling biomass under controlled 15°C and 25°C regimes, providing insights for temperature-adaptive cultivar selection amid climate variability.

Material and Methods

The research aimed to investigate the effects of varying temperature regimes on seed germination and early seedling development of ten oat (*Avena sativa* L.) cultivars. Selected cultivars - Lovčen, BC Marta, Kučev, Pierce, Kamil, Iniap 82, Kupa, Dolphin, Posejdon, and Charlotte, represent diverse genetic and geographical origins, including those developed in Croatia, Europe, and South America, enabling broader interpretation of genotypic resilience to germination stress, particularly thermal extremes. Post harvest, seeds of each cultivar were

carefully packaged in breathable paper bags labeled with essential data for subsequent analysis: harvest year, lot number, and cultivar name. Paper bags were chosen for their permeability, preserving microclimatic conditions around the seeds. Samples were stored in an MB FRIGO cooling chamber compliant with international standards for biological material storage, featuring modular panels of non-toxic materials suitable for food and laboratory use, with precise temperature and humidity control. Storage conditions maintained a constant 5°C and 10–12% relative humidity (RH), minimizing physiological aging and oxidative changes to ensure seed viability for laboratory assays.

The laboratory experiment assessed seed responses to two germination temperatures (15°C and 25°C), simulating early and late sowing conditions or climate-induced stress. A factorial design incorporated two temperature levels as the main factor and ten cultivars as the subfactor, with three independent replicates per combination for statistical robustness. Samples were prepared and analyzed per the Croatian Regulation on Seed Sampling and Quality Testing Methods (NN 69/20) and ISTA guidelines. Key parameters included germination energy, total germination, root length, shoot length, fresh and dry seedling mass, providing comprehensive evaluation of seed vitality and early vigor under thermal variation. Standard pleated filter paper (type 21/N, 80 g/m², 580 × 580 mm) was pre-moistened with 66 mL tap water to ensure uniform moisture without excess risking anaerobiosis. Fifty seeds per cultivar were placed in four even rows on each paper using sterile forceps, maintaining spacing, then rolled, placed in transparent PVC bags labeled with cultivar, replicate, and lot number. Rolls were vertically positioned in a Memmert ICH260C climate chamber set to 15°C or 25°C, with the closed end downward to prevent seed displacement and uneven germination. Readings occurred on day 5 (germination energy) and day 10 (total germination), counting all normal seedlings.

Post germination, 20 seedlings per cultivar-treatment-replicate were randomly selected, carefully removed from paper, and straightened without mechanical damage. Measurements used a precision plastic ruler with millimeter graduations: root length from seed base to primary root tip, shoot length from seed base to coleoptile tip.

Seedlings from morphological measurements were grouped by replicate, weighed for fresh mass using an analytical balance (0.001 g precision) to assess biomass accumulation differences by temperature. They were then dried in labeled paper bags at 60°C for 5 days in a seed drying oven, reweighed for dry mass, revealing tissue water content and metabolic activity during germination, aiding cultivar-temperature comparisons.

Descriptive statistics were performed using SAS Enterprise Guide 7.1..

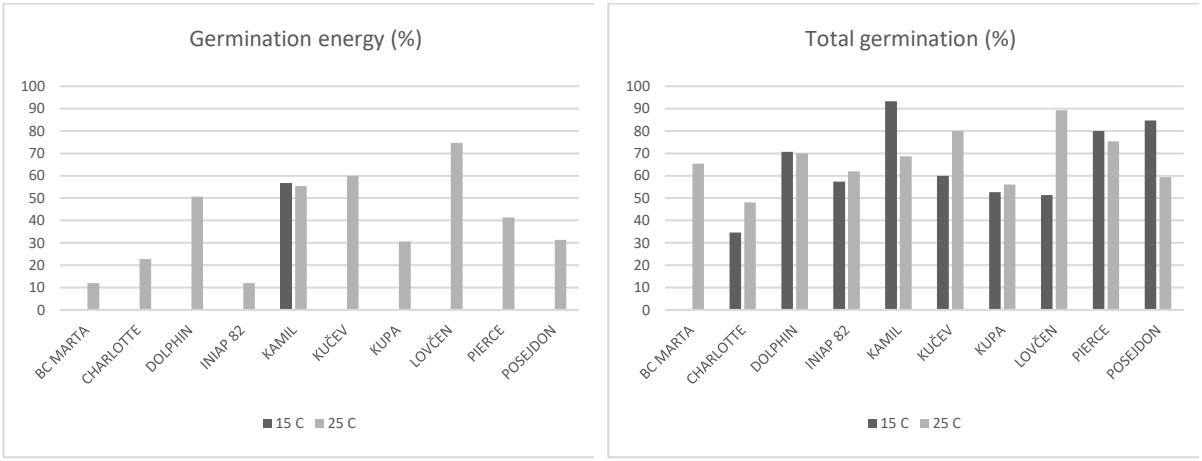
Results and Discussion

Laboratory experiments demonstrated a pronounced temperature dependence of oat germination and early seedling development. All evaluated parameters: germination energy, total germination, shoot length, root length, fresh mass, and dry mass - were higher at 25 °C than at 15 °C across most cultivars, indicating a generally favorable effect of warmer conditions on seed vigor and establishment.

Germination energy, an indicator of initial seedling development rate, showed marked temperature sensitivity. The cultivar Kamil maintained relatively high germination energy at 15 °C, suggesting enhanced metabolic activation under cooler conditions. In contrast, cultivars Lovčen, Kučev, and Dolphin exhibited stronger responses at 25 °C, consistent with their potential suitability for environments with elevated soil temperatures.

Total germination, reflecting the proportion of seeds producing normal seedlings, averaged 58,46% at 15 °C and 67,4% at 25 °C. Kamil achieved the highest value at 15 °C (56.6%), while Posejdon and Pierce also exceeded the mean under cooler conditions. At 25 °C, Lovčen (51.33%) and Kučev (60.0%) performed best. These patterns align with Catelan et al. (2021),

who reported optimal germination speed and total germination for white oat cultivars at 20–25 °C, with reduced vigor at 15 °C and increased abnormalities at 30 °C.



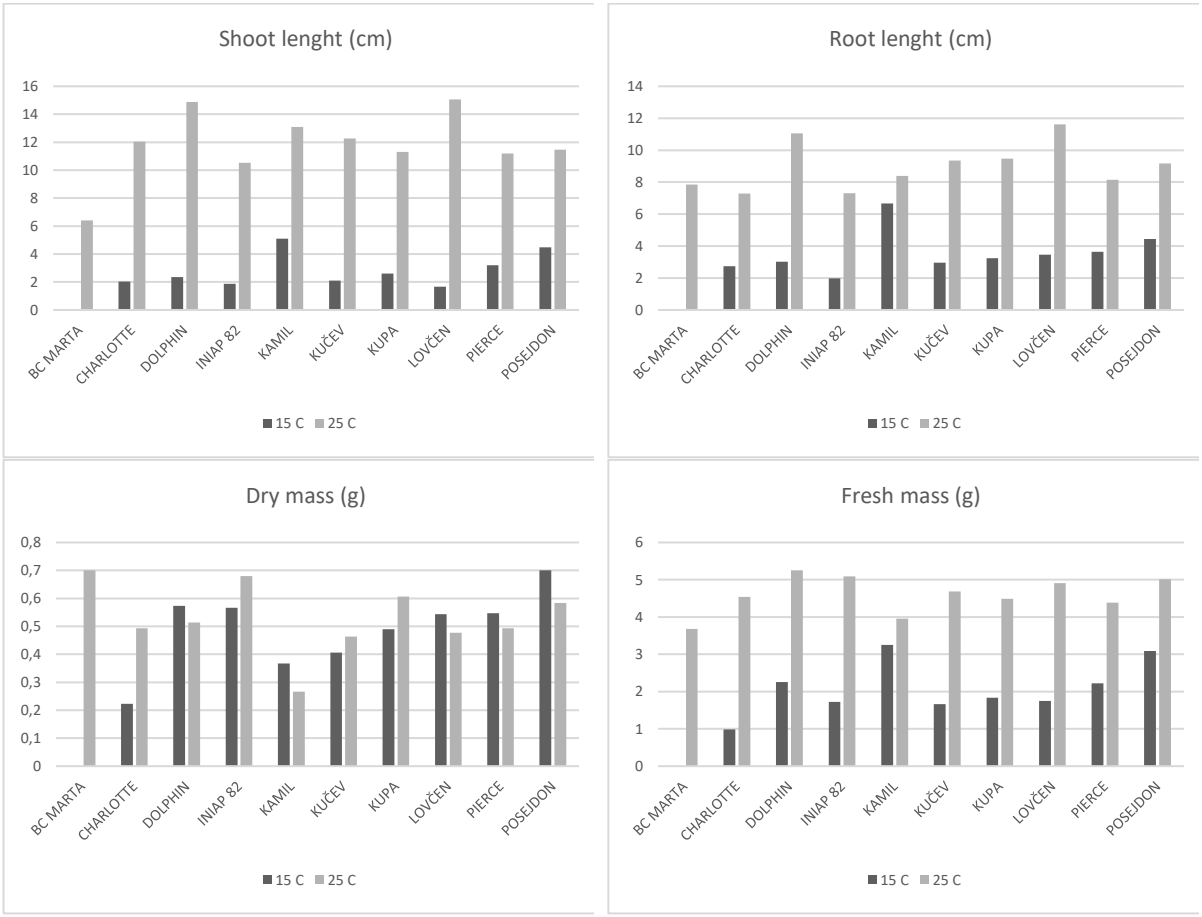
Graph 1. Germination energy and total germination (%)

Mean shoot length at 25 °C was 11.82 cm, compared to 2.54 cm at 15 °C. The longest shoots at the higher temperature were recorded in cultivars Lovčen (15.07 cm) and Dolphin (14.88 cm). At 15 °C, cultivar Kamil exhibited the longest shoot (5.11 cm), while BC Marta showed no germination. Root length followed a similar pattern, with means of 8.96 cm at 25 °C and 3.21 cm at 15 °C. Cultivars Lovčen and Dolphin developed the most extensive roots at the higher temperature, whereas Kamil again displayed relatively superior root elongation under cooler conditions compared to other cultivars. Fresh mass of seedlings exhibited a pronounced temperature dependence, with mean values of approximately 4.5 g at 25 °C compared to 2.4 g at 15 °C, representing nearly double the biomass accumulation under warmer conditions. The highest fresh masses at 25 °C were achieved by cultivars Lovčen (~5.5 g), Dolphin (~5.0 g), Kučev (~4.8 g), and Pierce (~4.7 g), while the lowest were recorded for Charlotte (~3.5 g) and Iniap 82 (~4.0 g). At 15 °C, Lovčen led (~3.0 g), followed by Kučev (~2.8 g) and Pierce (~2.6 g), with the lowest values for Charlotte (~1.8 g) and Iniap 82 (~2.0 g). These differences, ranging from 1.7–2.5 g per sample, clearly highlight the superior effect of 25 °C on seedling growth and fresh biomass accumulation. Dry mass of seedlings displayed a clear temperature effect, with mean values of approximately 0.65 g at 25 °C versus 0.37 g at 15 °C, indicating substantially greater dry matter accumulation under warmer conditions. The highest dry masses at 25 °C were observed in Lovčen (~0.80 g), Dolphin (~0.75 g), Pierce (~0.72 g), and Kučev (~0.70 g), while the lowest were in Charlotte (~0.45 g) and Kamil (~0.55 g). At 15 °C, Lovčen (~0.50 g), Kučev (~0.45 g), and Pierce (~0.42 g) led, with Charlotte showing the minimum (~0.22 g). These disparities, spanning 0.25–0.35 g per sample, underscore the pronounced positive influence of 25 °C on dry biomass production in oat seedlings.

Bagarinao et al. (2026) provide complementary biochemical evidence, showing enhanced lipid/carbohydrate degradation, protein digestibility (22% higher at gastric phase), and reductions in anti-nutritional factors (phytic acid by 23%, trypsin inhibitors by 14%) at 25 °C versus 15–20 °C. These metabolic shifts align with our observed increases in biomass, suggesting accelerated reserve mobilization at the optimal temperature.

The preference for 25 °C over 15 °C in promoting elongation and biomass contrasts with Radford and Key (1993), who found 15 °C optimal for mesocotyl/coleoptile elongation (113–252 mm) in dark-grown oat genotypes, with declines above 20 °C. This discrepancy likely arises from trait-specific responses (mesocotyl/coleoptile vs. shoot/root) and light conditions (darkness vs. standard laboratory lighting). Similarly, Sarić-Krsmanović et al. (2015) reported peak germination in wild oat (Avena fatua) at 15 °C (41–44%), lower at constant 25/30 °C,

differing from our cultivated oat results where 25 °C doubled biomass (e.g., Lovčen fresh mass ~5.5 g vs. ~3.0 g) and some cultivars like BC Marta failed at 15 °C. Species differences and experimental conditions (storage effects, darkness) likely explain these opposing trends.
These genotypic variations and temperature × genotype interactions highlight opportunities for breeding cultivars with tailored thermal adaptation, such as cold-tolerant Kamil or heat-responsive Lovčen, to address variable sowing conditions. All values (shoot and root length (cm), dry and fresh mass (g)), are shown in graph 2.



Graph 2. Shoot and root length (cm), dry and fresh mass (g)

Conclusion

This study demonstrates that 25 °C markedly enhances germination energy, total germination (58.46% vs. 67.4%), shoot/root elongation, and fresh/dry biomass accumulation in oat seedlings compared to 15 °C across tested cultivars. Cultivars Lovčen and Dolphin excelled at warmer temperatures, while Kamil showed superior cold tolerance. These genotypic differences, supported by literature alignments and contrasts, underscore opportunities for breeding temperature-adapted oats to optimize establishment under variable climate conditions.

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ORIGINAL SCIENTIFIC PAPER

Phenolic content and antioxidant activity (DPPH) of winter wheat under different fertilization treatments

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Summary
The aim of this study was to assess the effects of NPK fertilization regimes and phenophases on antioxidant activity and total phenolic content in wheat, and their relationship with agronomic traits. The results showed that fertilization regime, phenophase, and their interaction significantly influenced antioxidant parameters. Low and moderate nutrient input enhanced antioxidant activity, while high inputs reduced responses, with peak values observed at stem elongation growth stage. These findings indicate that fertilization strategy and growing stage strongly influence antioxidant potential, suggesting their relevance as biochemical indicators for optimizing nutrient management to improve wheat productivity.

Keywords: NPK fertilization, antioxidant activity, phenolic compounds, agronomic traits

Introduction

Wheat (*Triticum* spp.) is a key staple crop, providing a major share of global protein and caloric intake and playing a crucial role in food security. With the growing global population, wheat demand is expected to increase substantially, requiring improvements in yield per unit area rather than expansion of arable land. However, wheat production is increasingly constrained by biotic and abiotic stresses, particularly drought and high temperatures, which significantly reduce grain yield (Brković et al., 2025, Pešić et al., 2026).

Under abiotic stress conditions, wheat plants accumulate reactive oxygen species, which may cause oxidative damage and reduce productivity, while antioxidant compounds, particularly phenolics, play a key role in mitigating these effects (Hossain et al., 2021, Rao, and Zheng, (2025). Therefore, assessing antioxidant activity across different developmental stages provides knowledge and evidence on plant stress responses and their relationship with yield performance (Zhu et al., 2020). However, a comprehensive understanding of plant responses to environmental conditions requires the integration of physiological and biochemical parameters with agromorphological traits, which are widely used as indicators of crop performance and yield potential.

The present study aimed to evaluate the effects of NPK fertilization regimes and phenophases on antioxidant activity and total phenolic content in wheat, and to examine their relationships with key agronomic traits using a multivariate approach.

Material and Methods

Experimental Design

The experiment was arranged as a randomized complete block design with three replications. Six NPK regimes were included in the experiment, with each experimental plot measures 10 ×

10 m (20 rows). An examination was carried out on a winter variety of common wheat, Apilco, using different N–P–K fertilization rates applied manually, as described in Pešić et al. (2026): T1 – (0–0–0); T2: 50–50–50; T3: 100–50–50; T4: 100–100–100; T5: 130–50–50; and T6: 130–130–130 (kg ha^{-1}).To assess antioxidant activity and evaluate plant responses to prevailing environmental conditions, leaf samples were collected from each plot during three key phenological stages of wheat development, according to the BBCH scale: tillering (BBCH 25), stem elongation (BBCH 35), and heading (BBCH 59). Agronomic parameters were measusre and described in Pešić at el. (2026).

Biochemical Parameters: DPPH Radical Scavenging Activity and Total Phenolic Content
Leaf samples were collected at three key phenological stages of wheat development (BBCH scale), and biochemical analyses of antioxidant activity (DPPH radical scavenging activity) and total phenolic content were performed on methanolic extracts of dried plant material using spectrophotometric methods. The analyses were conducted following established protocols, as described in Brković et al. (2025).

Statistical analysis
A two-way analysis of variance (ANOVA) was performed to evaluate the effects of fertilization treatments, phenophases, and their interaction on the variation of the studied parameters. In addition, a one-way ANOVA was applied to assess the effect of treatments on the variation of the analyzed parameters within each phenophase separately. Comparisons among treatment means were conducted using Tukey's HSD test at a significance level of 5% (agricolae package, de Mendiburu, 2023). All statistical analyses were performed using R software, version 4.3.2 (https://www.R-project.org/).
To examine the relationships among the investigated variables (traits) in wheat grown under different NPK fertilization treatments, principal component analysis (PCA) was conducted. All variables were standardized by centering to the mean and scaling to unit variance to eliminate the effect of different measurement units. The results were visualized using a biplot (PC1 vs. PC2) with the factoextra package (Kassambara and Mundt, 2020) in R. Since three principal components contributed significantly to the total variation, a three-dimensional plot was also generated using the Plotly library (version 4.11.0) in R version 4.3.2 (Sievert, 2020).

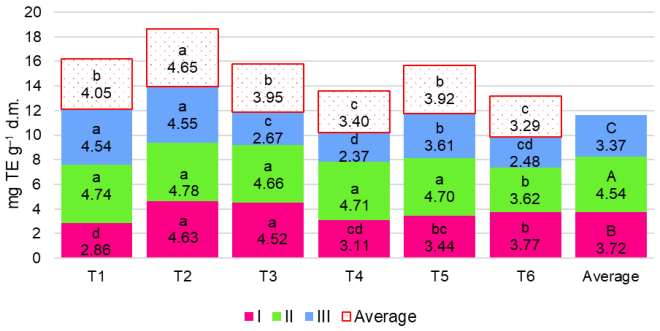
Results and discussion
DPPH Radical Scavenging Activity
A two-way analysis of variance revealed that fertilization treatments, phenophases, and their interaction had a highly significant effect ($p < 0.01$) on the DPPH radical scavenging activity in wheat. The significant interaction indicates that the effect of fertilization treatments on antioxidant activity depends on the phenophase (Table 1).

Table 1. Two-way analysis of variance of DPPH radical scavenging activity in wheat leaves at different phenophases under various NPK fertilization treatments

Source of variation	Df	SS	MS	F value	p value
Treatment	5	10.892	2.178	142.7	0.000**
Phenophase	2	12.809	6.404	419.7	0.000**
Treatment × Phenophase	10	15.455	1.546	101.3	0.000**
Error	36	0.549	0.015	–	–
Total	53	39.702	–	–	–

**p<0.01

On average, the highest DPPH radical scavenging activity was recorded under treatment T2 (4.65 mg TE g^{-1} d.m.), while the lowest was observed under treatment T6 (3.29 mg TE g^{-1} d.m.). However, changes in the ranking of treatments were observed depending on the phenophase, indicating variable treatment effects across growing stages. Therefore, treatments were compared within each phenophase separately. At the level of individual phenophases, treatment T2 consistently showed the highest antioxidant activity. In the tillering stage, the lowest value was observed under treatment T1, whereas in stem elongation the lowest activity was recorded under treatment T6 (3.62 mg TE g^{-1} d.m.). In phenophase heading, the lowest DPPH radical scavenging activity was observed in wheat grown under treatment T4 (2.67 mg TE g^{-1} d.m.). Comparison among phenophases showed that the highest antioxidant activity, averaged across all treatments, was recorded in stem elongation (4.54 mg TE g^{-1} d.m.), followed by a significantly lower value in tillering (3.72 mg TE g^{-1} d.m.), while the lowest activity was observed in heading (3.37 mg TE g^{-1} d.m.) (Figure 1).



Note: Different lowercase letters indicate treatment differences (overall and within phenophases), while uppercase letters indicate phenophase (I – tillering, II – stem elongation, III – heading) differences (Tukey's HSD test, $p < 0.05$).

Figure 1. Mean DPPH radical scavenging activity in wheat leaves depending on fertilization treatments and phenophases

Total Phenolic Content

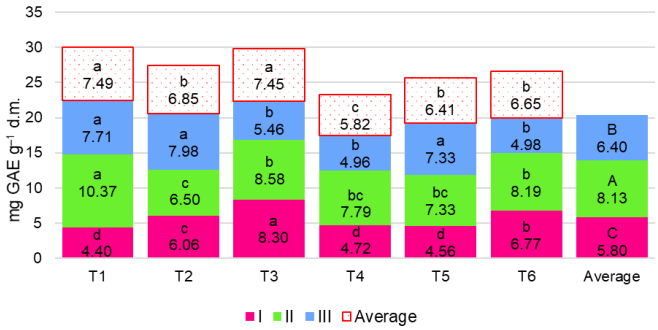
A two-way analysis of variance revealed a highly significant effect ($p < 0.01$) of all analyzed factors on the variation of total phenolic content in wheat leaves. The most pronounced effect was observed for the treatment × phenophase interaction, indicating that wheat responded differently in phenolic content depending on the phenophase under different treatments. In addition, phenophase accounted for a substantial proportion of the total sum of squares (35.0%), while the effect of fertilization treatment was considerably lower (12.23%) (Table 2).

Table 2. Two-way analysis of variance of total phenolic content in wheat leaves at different phenophases under various NPK fertilization treatments

Source of variation	Df	SS	MS	F value	p value
Treatment	5	18.32	3.664	23.54	0.000**
Phenophase	2	52.43	26.216	168.43	0.000**
Treatment × Phenophase	10	73.44	7.344	47.19	0.000**
Error	36	5.60	0.156		
Total	53	149.79	–	–	–

**p<0.01

Averaged across all phenophases, the highest total phenolic content was recorded under treatment T1 (7.49 mg GAE g^{-1} d.m.), followed by treatment T3 (7.45 mg GAE g^{-1} d.m.), while the lowest value was observed in wheat grown under treatment T4 (5.82 mg GAE g^{-1} d.m.). Due to the significant treatment × phenophase interaction, treatments were compared within each phenophase separately. In tillering, the highest phenolic content was recorded under treatment T3 (8.30 mg GAE g^{-1} d.m.), in stem elongation under treatment T1 (10.37 mg GAE g^{-1} d.m.), and in heading under treatment T2 (7.98 mg GAE g^{-1} d.m.). Conversely, the lowest phenolic content in tillering was observed under treatments T1 (4.40 mg GAE g^{-1} d.m.) and T5 (4.56 mg GAE g^{-1} d.m.), in stem elongation under treatment T2 (6.50 mg GAE g^{-1} d.m.), and in heading under treatments T4 (4.96 mg GAE g^{-1} d.m.) and T6 (4.98 mg GAE g^{-1} d.m.). Across phenophases, the highest total phenolic content, averaged over all fertilization treatments, was recorded in stem elongation (8.13 mg GAE g^{-1} d.m.), followed by heading (6.40 mg GAE g^{-1} d.m.), while the lowest value was observed in tillering (5.80 mg GAE g^{-1} d.m.) (Figure 2).



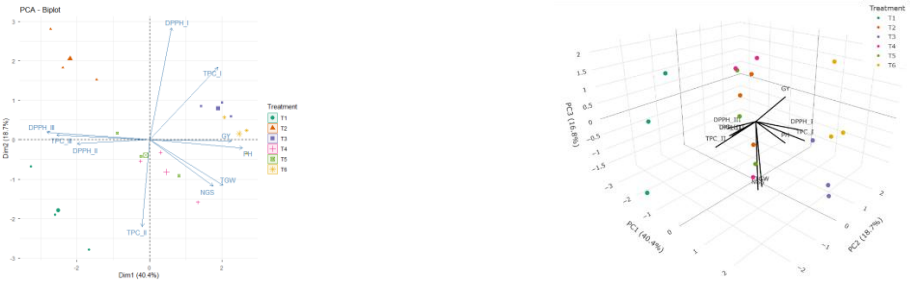
Note: Different lowercase letters indicate treatment differences (overall and within phenophases), while uppercase letters indicate phenophase (I – tillering, II – stem elongation, III – heading) differences (Tukey's HSD test, $p < 0.05$).

Figure 2. Mean total phenolic content in wheat leaves depending on fertilization treatments and phenophases

Relationship between agronomic traits and antioxidant activity parameters

Principal component analysis (PCA) was performed to examine the relationships between antioxidant activity parameters and agronomic traits in wheat. The first two components (Dim1 and Dim2) explained 59.1% of the total variation in the data (Figure 3a). Since the third component contributed significantly to the variation (16.8%), a 3D PCA plot was also generated to provide a more detailed visualization of relationships among the studied variables (Figure 3b). PCA has been previously applied by Matković Stojšin et al. (2022) and Brković et al. (2025) to explain the relationships between agronomic traits and antioxidant parameters. Similarly, Falcinelli et al. (2022) used PCA to assess the relationship between antioxidant activity and physiological parameters in wheatgrass under different nitrogen fertilization treatments. The values of agronomic traits (plant height, number of grains per spike, thousand grain weight, and grain yield) were presented in Pešić et al. (2026) and were here analyzed in relation to antioxidant parameters. The grain yield vector formed acute angles with the vectors of plant height, thousand grain weight, and number of grains per spike, indicating positive correlations among these traits. Grain yield showed a strong association with plant height, with data points corresponding to treatment T6, suggesting that higher NPK input resulted in increased grain yield and plant height (Figure 3). Positive correlations between grain yield and plant height were also reported by Javed et al. (2024) and Brković et al. (2025), particularly under conditions of reduced precipitation, where higher-yielding genotypes exhibited greater plant height. These authors also emphasized that trait relationships are largely influenced by

environmental factors. The grain yield vector was positioned in the same quadrant of the biplot as the vectors representing antioxidant parameters measured in tillering (Figure 3a). These vectors were associated with treatment T3, which showed the highest total phenolic content and high DPPH values in this phenophase. Positive relationships between grain yield and antioxidant parameters at early growth stages have also been reported by Matković Stojšin et al. (2022) and Brković et al. (2025), highlighting their potential as markers in wheat selection and breeding. In this study, DPPH radical scavenging activity and total phenolic content were positively correlated within each phenophase, indicating that phenolic compounds significantly contribute to antioxidant activity in wheat (Figure 3a, b). Similar positive correlations were reported by Niroula et al. (2019) and Lacko-Bartošová et al. (2023). In the growth stages stem elongation and heading, data points corresponding to treatment T1 (control) were positioned near the vectors representing antioxidant parameters, which may be associated with the higher values of antioxidant activity observed under this treatment (stress conditions) in these phenophases (Figures 1 and 2).



a) b)

Note: PH – plant height; NGS – number of grains per spike; TGW – thousand grain weight; GY – grain yield; DPPH – free DPPH radical scavenging capacity; TPC – total phenolic content

Figure 3. PCA biplot (PC1 vs. PC2, a) and 3D PCA plot (PC1 vs. PC2 vs. PC3, b) of agronomic traits and antioxidant activity measured at different phenophases (I – tillering, II – stem elongation, III – heading) in wheat grown under different NPK treatments.

Conclusion

The integration of antioxidant profiling with agronomic trait analysis provides understanding of wheat responses to different fertilization regimes. The results demonstrated that fertilization strategy and phenophase significantly influence antioxidant activity and phenolic content. The highest antioxidant activity was recorded during stem elongation, particularly under the control treatment and the low N–P–K regime, reflecting the plant's physiological adaptations to nutrient availability and stress. Positive associations between antioxidant parameters and key agronomic traits, particularly grain yield and plant height, highlight their functional linkage and potential use as biochemical indicators of crop performance. The observed variability in responses across fertilization regimes and phenophases emphasizes the importance of optimizing nutrient management in relation to plant growing stage.

Acknowledgment

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ORIGINAL SCIENTIFIC PAPER

Air–Soil Temperature Decoupling and Early Spring Risk in Estonia

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Summary
The study analysed changes in the onset of the thermal growing season in Estonia using daily air temperature data (1965–2024). Results show a significant advancement of climatic spring, especially in southern inland regions. However, early exceedance of the +5 °C threshold may precede soil warming, creating a mismatch between aboveground and root activity and increasing the risk of premature management decisions. The findings highlight the need to consider soil temperature in early spring management of winter cereals.

Keywords: thermal growing season, air–soil temperature lag, winter cereals, early spring risk

Introduction

Temperature is a key environmental factor regulating crop phenology and seasonal development. In agronomic practice across Northern and Central Europe, the onset of the growing season is commonly defined by the persistent exceedance of a +5 °C mean daily air temperature threshold. This indicator is widely used to describe vegetation activity and to guide early spring management operations in winter cereals.

Long-term climate observations (Jaagus, 2006) indicate a clear lengthening of the thermal growing season in northern regions, including in Estonia (Saue, 2020), primarily driven by an earlier onset of spring. While this trend may suggest improved growing conditions, increasing climatic variability raises concerns regarding the agronomic interpretation of air-temperature-based thresholds. In cool-temperate regions, soil warming often lags behind atmospheric warming due to high soil moisture and thermal inertia (Jury & Horton, 2004), while root activity and nutrient uptake remain constrained (Marschner, 2012). As a result, management decisions based solely on air temperature indicators may not coincide with the actual physiological readiness of crops. Early spring operations such as fertilisation may therefore be poorly synchronised with plant physiological processes. This potential mismatch is of particular relevance for winter cereals, which resume growth rapidly after winter dormancy, increasing their sensitivity to subsequent frost events (Gu et al., 2008).

The aim of this study is to analyse recent changes in the onset of the thermal growing season in Estonia and to discuss their implications for early spring management of winter cereals, with a focus on the risk arising from air–soil temperature decoupling.

Material and Methods

Air temperature data

Daily mean air temperature data were obtained from the Estonian Environment Agency for 21 meteorological stations distributed across Estonia. The analysis covers the period from 1965 to 2024, providing a long-term basis for assessing changes in spring onset.

Definition of the thermal growing season

The onset of the thermal growing season was defined based on the sustained transition of mean daily air temperature above 5 °C. This definition follows established agrometeorological practice in Estonia and neighbouring regions. A transition was considered sustained when the

cumulative sum of positive temperature deviations above the threshold (5 °C) following the initial exceedance was greater than the cumulative sum of subsequent negative deviations below the threshold. In cases where the sum of negative deviations exceeded the positive deviations, the onset of spring was assigned to the end date of the cold spell preceding the renewed and sustained warming. This approach has been proposed by Raik (1963) and further developed by Tarand et al. (2013). The approach ensures that short-lived temperature fluctuations around the threshold do not lead to spurious identification of spring onset and allows for a consistent determination of the climatological transition to spring conditions. This temperature-based definition of spring onset follows established climatological and phenological practice (e.g. Schwartz et al., 2006).

Trend and period comparison analysis

Linear trends in the onset of the thermal growing season were calculated for each station over the full study period. In addition, mean onset dates were compared between a historical reference period (1965–1990) and a more recent period (1991–2024). The statistical significance of differences between periods was assessed using paired t-tests. Data processing and visualisation were conducted using the R statistical software environment.

Results and Discussion

Changes in the onset of the thermal growing season

The analysis reveals a clear advancement in the onset of the thermal growing season across Estonia during the study period. On average, climatic spring now begins approximately one week earlier than during the historical reference period.

The advancement of spring onset shows considerable variability among stations. The largest negative shifts were observed at stations such as Tartu-Tõravere, Viljandi, and Võru, representing southern inland locations, whereas smaller changes were recorded at northern and coastal stations such as Narva and Pakri. The most pronounced advancement was observed in southern and southeastern inland regions, whereas northern coastal areas exhibited smaller changes (Fig.1). This spatial pattern reflects regional differences in continentality and the moderating influence of large water bodies on spring warming.

These results are consistent with previous studies across Europe (Cornes et al., 2018), reporting a lengthening of the growing season in Northern Europe. In Estonia, the observed changes are primarily driven by earlier spring onset rather than delayed autumn cooling.

Agronomic interpretation of early spring warming

In early spring, soil temperatures in the root zone often remain low, particularly in wet mineral and organic soils (Jury & Horton, 2004). This air–soil temperature lag may persist for several days during periods of rapid atmospheric warming. Although soil temperature dynamics were not directly analysed in this study, their behaviour is interpreted based on established soil physical principles and previous empirical studies under comparable early spring conditions (Jury & Horton, 2004).

Under such conditions, aboveground plant parts may respond to increasing air temperatures, while root activity and nutrient uptake remain constrained (Marschner, 2012). This mismatch may increase the risk of inefficient early spring management, especially when fertilisation is applied based on air temperature indicators alone.

Early spring risk concept

The concept of early spring risk emerges from the interaction between advancing atmospheric signals and delayed soil thermal responses. In addition to potential nutrient uptake limitations, early spring warming may induce partial de-hardening of winter cereals, increasing their

sensitivity to subsequent frost events. In this study, early spring risk refers to the mismatch between atmospheric warming and delayed soil thermal response affecting crop functioning. Rather than viewing the +5 °C threshold as a strict biological boundary, it should be interpreted as an indicator requiring contextual evaluation. Integrating information on soil thermal conditions could improve the timing of early spring management operations and reduce uncertainty during the transition from winter dormancy to active growth.

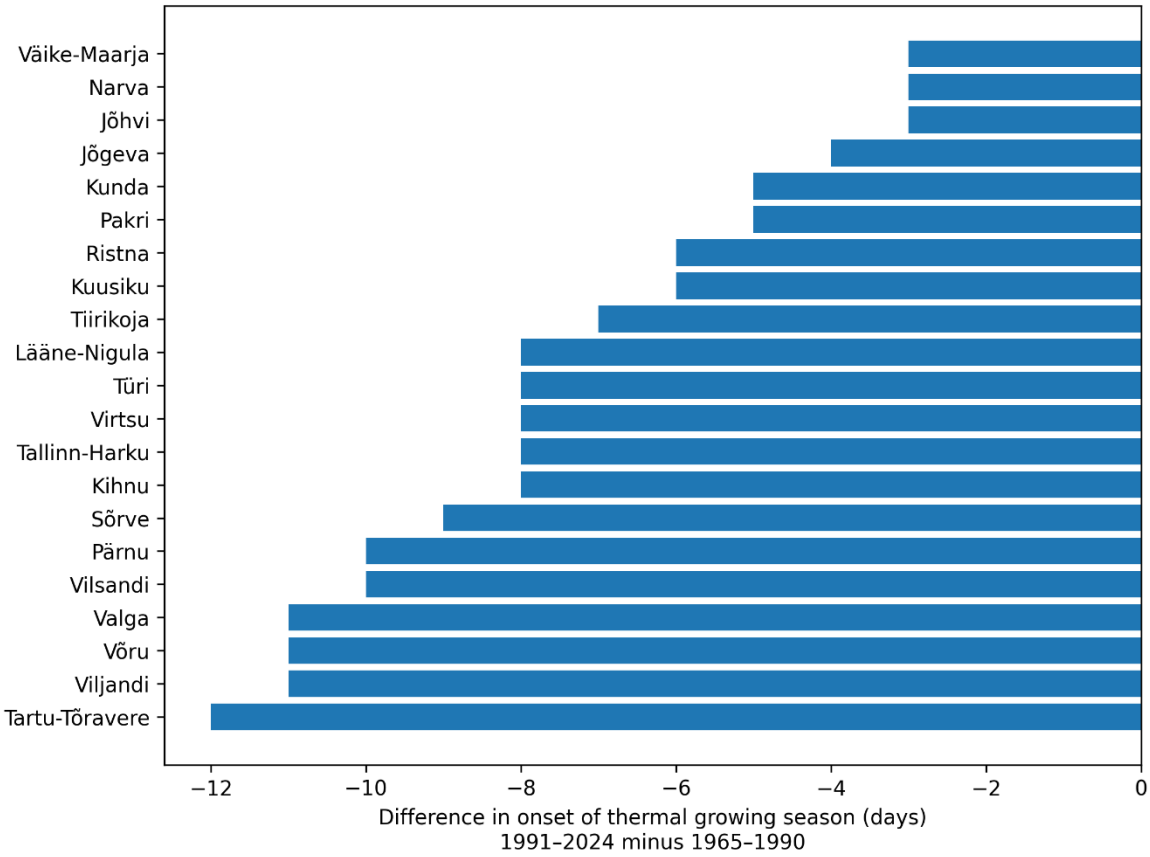

Figure 1. Change in the onset of the thermal growing season at Estonian meteorological stations between 1965–1990 and 1991–2024. Negative values indicate earlier onset.

While the advancement of the thermal growing season provides clear evidence of earlier atmospheric warming, its agronomic interpretation requires caution. The exceedance of the +5 °C air temperature threshold does not necessarily indicate favourable conditions for root activity and nutrient uptake, particularly under early spring conditions characterised by cold and wet soils. To support the interpretation of early spring conditions, a functional framework linking air temperature signals with expected soil thermal states and agronomic implications is presented in Table 1. Rather than representing strict physiological thresholds, the temperature ranges shown summarise typical early spring situations observed under Estonian conditions. The results in Table 1 highlight a transitional risk window between +5 and +8 °C, during which aboveground activity may resume while root-zone processes remain constrained. This functional interpretation emphasises that early spring management decisions based solely on air temperature indicators may be premature. Integrating information on soil thermal status can help reduce uncertainty during the transition from winter dormancy to active growth and improve the timing of early spring operations in winter cereals. The magnitude of air–soil temperature decoupling may vary depending on soil type, moisture conditions, and snow cover, which were not explicitly analysed in this study.

Table 1. Agronomic interpretation of early spring temperature conditions for winter cereals

Air temperature, °C	Expected soil thermal state	Dominant limitation	Agronomic implication
< +5	Cold, often near 0–2 °C	Root inactivity	No active growth, avoid management
+5…+8	Slowly warming, thermally lagging	Limited root uptake	Risk of premature fertilisation
+8…+10	Functionally active root zone	Improving uptake	More suitable timing for N application
> +10	Stable soil warming	Low thermal limitation	Effective nutrient uptake

*Note: Temperature ranges represent generalized early spring conditions and should be interpreted as agronomic guidance rather than fixed physiological thresholds.

Conclusion

The onset of the thermal growing season in Estonia has advanced significantly over the past decades, with the largest changes observed in southern inland regions. While the +5 °C air temperature threshold remains a useful climatic indicator, its agronomic interpretation requires caution. Early exceedance of this threshold may precede sufficient soil warming, creating an air–soil temperature mismatch that increases early spring management risks. To improve the effectiveness of early spring operations in winter cereals, management decisions should consider both atmospheric and soil thermal conditions rather than relying solely on air temperature indicators.

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ORIGINAL SCIENTIFIC PAPER

Mineral content of microgreens grown in climate chamber

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Summary
The study evaluated the effects of lighting (LED 6500 K; FLUO 6000 K, and LED 4R:1B) on yield, dry matter, and mineral composition of microgreens (red cabbage, peas, radish, broccoli, coriander, beetroot and arugula). Among the tested lighting treatments, LED 6500 K yielded the highest overall across microgreen species while maintaining a comparable dry matter content to FLUO 6000 K. In contrast, LED 4R:1B showed consistently lower yield and dry matter values. Mineral composition varied depending on light treatment. LED 6500 K ensured the most balanced and consistent mineral profile across species. FLUO 6000 K enhanced the accumulation of specific elements such as Ca and Zn in selected species, while LED 4R:1B significantly increased Fe content in pea, beetroot, and arugula. However, these increases were accompanied by greater variability and, in the case of LED 4R:1B, lower yield performance.

Keywords: Microgreens, Lighting systems, Yield, Dry matter, Mineral composition

Introduction

Commercial urban farms are increasingly relying on microgreens, young plants harvested at early stages of growth, for their high nutritional value and bioactive compounds (Vaštakaitė-Kairienė et al., 2023; Lone et al., 2024). Microgreens are rich in antioxidants, phenolics, flavonoids and essential minerals, contributing to human health and providing protective roles to plants (Bajwa et al., 2023). Light is a key factor affecting photosynthesis, plant growth and the accumulation of these compounds and minerals (Samuolienė et al., 2019; Zhang et al., 2020). Red and blue light specifically regulate plant morphology and metabolic pathways, affecting the content of pigments, antioxidants and minerals (Samuolienė et al., 2012). Controlled artificial lighting, especially LEDs with tunable spectral quality and intensity, can optimize microgreen growth and enhance the accumulation of bioactive compounds and minerals (Paradiso et al., 2024). Manipulating the light spectrum and exposure allows for targeted stimulation of antioxidants, phenolics, and nutrients, offering a strategy for producing microgreens with superior nutritional value. Understanding these interactions is key to optimizing controlled environment farming and enhancing the health benefits of microgreens. Although numerous studies have examined the effects of light intensity and spectral composition on microgreen growth, there remains a lack of comprehensive understanding of how different lighting systems influence the accumulation of specific mineral elements, such as K, Ca, Zn, Mn, and Fe, across multiple species. Therfore, this study aimed to determine the effect of different lighting conditions on yield, dry matter content, and the content of mineral elements (K, Ca, Zn, Mn, and Fe) in the investigated microgreen species.

Material and Methods

The experiment was conducted in the controlled indoor conditions at the University of Sarajevo - Faculty of Agriculture and Food Sciences. Microgreens were grown under controlled conditions simulating day and night cycles, at a temperature of 20±2°C during a 16-hour light period and 17±2°C during an 8-hour dark period with relative humidity maintained at 50-60 %.

Three different lighting systems intended for microgreens production were used: LED 6500 K; FLUO 6000 K, and LED 4R:1B. The light source was positioned at a height of 25–30 cm above the plants. The study was carried out on seven plant species commonly cultivated as microgreens: red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*); peas (*Pisum sativum* L.); radish (*Raphanus sativus* L.); broccoli (*Brassica oleracea* var. *italica*); coriander (*Coriandrum sativum* L.); beetroot (*Beta vulgaris* L.) amd arugula (*Eruca sativa* Mill.). Organic seeds intended for sprout and microgreens production were used. Seed rates per tray were set as follows: red cabbage 60 g/m^2; peas 250 g/m^2; radish 50 g/m^2; broccoli 25 g/m^2; coriander 20 g/m^2; beetroot 80 g/m^2, and arugula 30 g/m^2, following supplier recommendations and common production practice. Sowing was performed in plastic tray (25.4 × 50.8 cm) with perforated bottoms for drainage, filled with a growing substrate (Klasmann Potgrond H). Microgreens were harvested at the stage of the first pair of true leaves. Red cabbage, radish, broccoli, beetroot, and arugula were harvested 12 days after sowing, while peas and coriander were harvested 15 days after sowing.
Immediately after harvest, the following parameters were determined:

- Yield was measured using an analytical balance (0.001 g) by weighing freshly harvested plant material, including the entire aboveground biomass (stems and leaves), and expressed in g/m^2.
- Dry matter content was determined gravimetrically by oven drying at 55 °C until constant mass, following procedures commonly applied in microgreens research (Kyriacou et al., 2021).
- The contents of K, Ca, Fe, Zn, and Mn were determined by atomic absorption spectrophotometry (AAS, ISO 11047) using the Shimadzu AA-7000 device (Shimadzu Instruments, Tokyo, JP).
- All assays were performed in triplicate, and results are presented as means ± standard deviation. Statistical differences among treatments were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test. All analyses were conducted using the SPSS software package (version 22.0; IBM SPSS Inc., Chicago, IL, USA).

Results and Discussion

The data in Table 1 illustrate the differences in yield and dry matter content among the tested microgreens across the applied light treatments. The highest yield was observed in radish grown under LED 6500 (3472.0 g/m^2), while the lowest was recorded in broccoli under LED 4R:1B (548.6 g/m^2).

Table 1 Yield and Dry Matter Content of Microgreens (Red Cabbage, Peas, Radish, Broccoli, Coriander, Beetroot, Arugula) under Different Light Treatments (LED 6500 K, Fluorescent 6000 K, LED 4R:1B).

Species	**Lighting type***	**Yield [g/m^2]**	**Dry matter [%]**
Red Cabbage	LED 6500	2312.6 ± 85.1^{a}	6.2 ± 0.2^{ab}
	FLUO 6000 K	1522.3 ± 45.9^{b}	5.4 ± 0.4^{bc}
	LED 4R:1B	1328.5 ± 73.2^{c}	6.5 ± 0.5^{abc}
Peas	LED 6500	1180.3 ± 85.3^{a}	8.9 ± 0.1^{a}
	FLUO 6000 K	681.5 ± 55.2^{c}	9.1 ± 0.2^{a}
	LED 4R:1B	1945.9 ± 81.3^{b}	7.5 ± 0.2^{b}

Radish	LED 6500	3472.0 ± 77.5[a]	5.1 ± 0.1[abc]
	FLUO 6000 K	3421.8 ± 75.6[a]	5.4 ± 0.1[ab]
	LED 4R:1B	1956.4 ± 967.7[b]	4.7 ± 0.3[bc]
Broccoli	LED 6500	1333.5 ± 81.9[a]	4.6 ± 0.1[b]
	FLUO 6000 K	852.5 ± 77.5[b]	5.7 ± 0.1[a]
	LED 4R:1B	548.6 ± 7.9[c]	5.7 ± 0.2[a]
Coriander	LED 6500	1175.7 ± 30.9[a]	11.6 ± 0.6[b]
	FLUO 6000 K	825.0 ± 27.1[c]	13.5 ± 0.1[a]
	LED 4R:1B	1022.9 ± 27.7[b]	10.9 ± 0.2[b]
Beetroot	LED 6500	918.6 ± 59.6[a]	8.6 ± 0.6[a]
	FLUO 6000 K	733.5 ± 32.1[b]	5.1 ± 0.2[b]
	LED 4R:1B	568.5 ± 20.9[c]	3.9 ± 0.1[c]
Arugula	LED 6500	2990.2 ± 40.8[a]	5.7 ± 0.1[a]
	FLUO 6000 K	3036.4 ± 80.7[a]	5.3 ± 0.1[b]
	LED 4R:1B	1686.3 ± 66.8[b]	5.7 ± 0.1[a]

*The means followed by different letters in the same column indicate significant differences at $P < 0.05$.

In terms of dry matter content, coriander under FLUO 6000 K showed the highest value (13.5 %), whereas beetroot under LED 4R:1B had the lowest (3.9 %). LED 6500 K lighting resulted in the highest yields in most species, particularly in radish. In contrast, LED (4R:1B) lighting increased yield in specific cases, such as pea, but generally produced lower biomass compared to LED 6500 K and FLUO 6000 K. Dry matter content was highest in coriander and pea, especially under FLUO 6000 K, suggesting that light spectrum plays an important role in dry matter accumulation (Table 1). Supplemental lighting significantly affected both yield and dry matter content in all tested species. LED 6500 generally resulted in higher yields compared to FLUO 6000 K and LED 4R:1B in most species, while responses in dry matter content varied depending on the species and lighting treatment.

Mineral composition varied depending on light treatment. LED 6500 K ensured the most balanced and consistent mineral profile across species (Figure 1).

FLUO 6000 K enhanced the accumulation of specific elements such as Ca and Zn in selected species, while LED 4R:1B significantly increased Fe content in pea, beetroot, and arugula. However, these increases were accompanied by greater variability and, in the case of LED 4R:1B, lower yield performance. Among the species, arugula, beetroot, and red cabbage stood out for their higher mineral content, while radish and arugula combined high yield with good nutritional quality (Figure 1).

These findings are consistent with previous studies indicating that white LED light (around 6500 K) promotes higher biomass production in microgreens due to a more balanced light spectrum that supports photosynthesis and plant development (Kyriacou et al., 2017). In contrast, red and blue light combinations, such as LED 4R:1B, have been reported to enhance the accumulation of specific nutrients, particularly minerals like Fe, although often at the expense of total yield (Samuolienė et al., 2012).

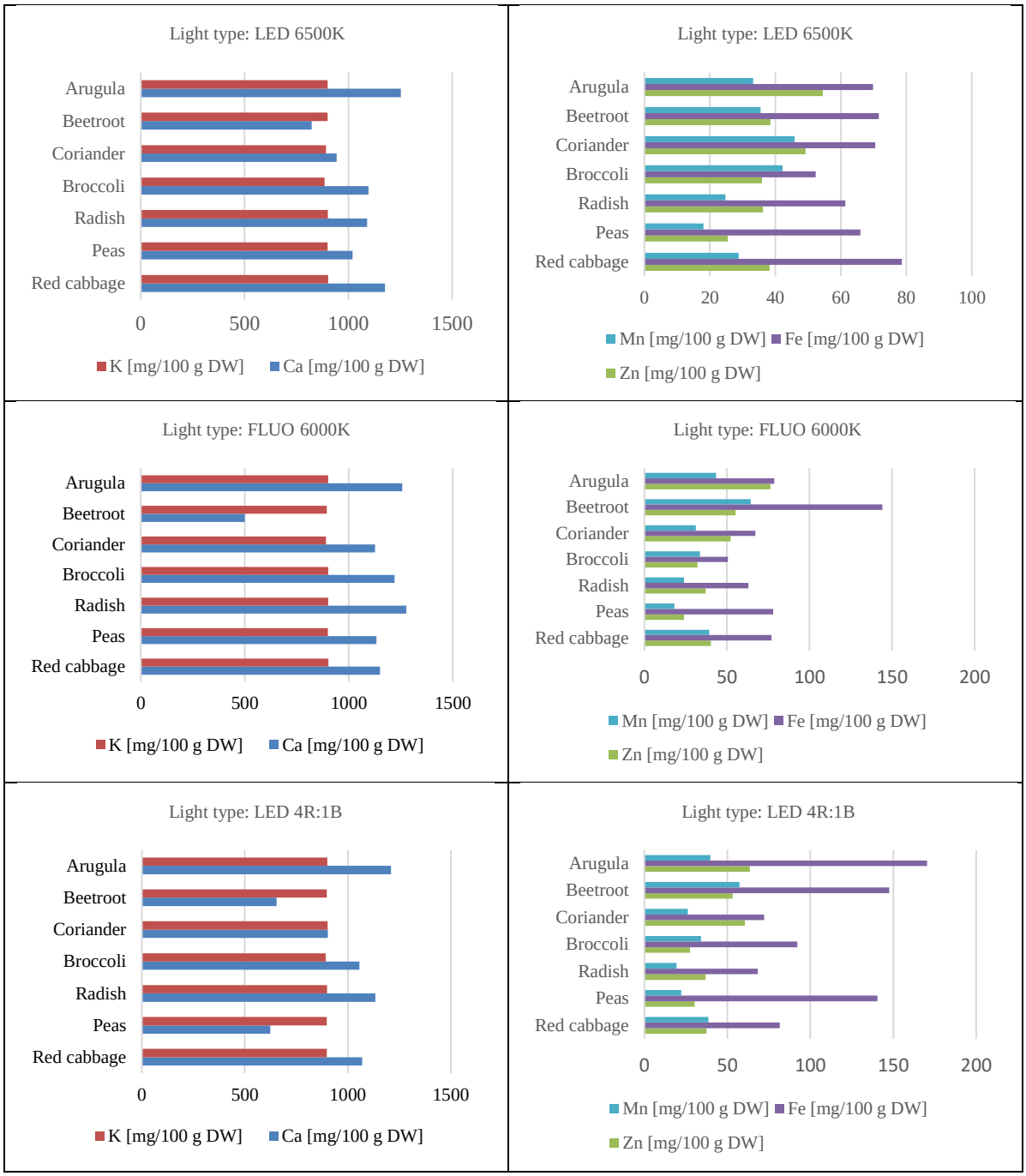

Figure 1 Mineral composition of microgreens (red cabbage, peas, radish, broccoli, coriander, beetroot, arugula) under different light treatments (LED 6500 K, Fluorescent 6000 K, LED 4R:1B). Values expressed in mg/100 g DW.

Different plant species respond differently to light in terms of their mineral composition (Figure 1). This can be explained by variable responses of plants to light quality because they possess distinct sets of photoreceptors and associated signaling pathways that perceive and transduce spectral information into physiological changes (Paik and Huq, 2019). Furthermore, species differ in their photosynthetic pigment composition and efficiency of converting light energy into biomass, which affects how light is used for growth and metabolic processes (Zhang et al., 2020), and these genetic and physiological differences result in species specific regulation of gene expression and metabolic allocation under different light spectra, leading to variation in mineral uptake and biochemical profiles among species (Teixeira, 2020).

Conclusion

The study demonstrates that lighting conditions critically influence both the productivity and nutritional quality of microgreens. While lighting LED 6500 K, effectively maximizes biomass yield, LED (4R:1B) enhances the accumulation of essential micronutrients such as iron and manganese. These findings have important implications for urban agriculture and microgreen production. Adjusting light conditions can optimize nutritional compounds such as, providing practical guidance for producers. However, the results may be limited to the species and light treatments tested. Future studies should explore additional light spectra and intensities across a wider range of microgreens to refine cultivation strategies.

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ORIGINAL SCIENTIFIC PAPER

Cadmium in Potato Crops: Differences Among Varieties

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Summary
Cadmium (Cd) is considered as one of the most ecotoxic metals, posing risks to humans, animals, and plants. Vegetables such as turnips, carrots, and potatoes represent major dietary sources of Cd. Cd accumulation varies among different vegetable species and varieties. This study aimed to evaluate Cd concentration in 19 potato varieties grown under identical field conditions in 2024 and 2025. Results showed varietal differences; KIS Kokra had the highest Cd levels (mean of both years: 0.1307 mg/kg FM, exceeding the EU maximum level of 0.1 mg/kg FM), while Messi, Peter Pan, Elland, Jelly had the lowest (mean of both years: 0.0567–0.0670 mg/kg FM). Cd concentrations were consistently higher in 2024, indicating environmental influence. These results highlight the importance of variety selection to reduce Cd intake.

Keywords: *Solanum tuberosum*, concentration, environmental conditions, Cd intake

Introduction

Cadmium (Cd) is one of the most ecotoxic metals, with harmful effects on humans, animals and plants. The International Agency for Research on Cancer (IARC) classifies Cd as a carcinogen (IARC, 2025). The presence of Cd in food is due to soil and water contamination. Cd is transferred from soil and water to plants and aquatic organisms and accumulates in the food chain (NIJZ, 2023). European Commission Regulation 2023/915 (OJ EU L 119/103, 2023) sets maximum levels for certain contaminants in food, including Cd, with the maximum level for Cd in fresh and peeled potatoes set at 0.1 mg/kg FM. Cd is not an essential element for plants, but it could be highly available in soils, and some plant species have a greater affinity to adsorb Cd (Kabata-Pendias and Pendias, 2001; Kabata-Pendias and Mukherjee, 2007). Globally, the average Cd content in soils ranges between 0.06 and 1.1 mg/kg, and it is a relatively mobile and bioavailable element (Kabata-Pendias and Mukherjee, 2007).

Plant species differ in their affinity for Cd uptake (Kabata-Pendias and Pendias, 2001; Alexander et al., 2006; Fang et al., 2019). Vegetables belonging to the Brassicaceae, Asteraceae, Apiaceae, and Convolvulaceae families generally exhibit higher Cd uptake, whereas those from the Liliaceae and Amaranthaceae families tend to accumulate lower amounts of Cd (Fang et al., 2019). Leafy vegetables, as well as root and tuber vegetables such as turnips, carrots, and potatoes, represent major dietary sources of Cd (Kabata-Pendias and Pendias, 2001). Cd accumulation varies significantly not only among different vegetable species but also among varieties within, particularly in leafy vegetables such as spinach (Kong et al., 2024). Differences in Cd uptake among plant varieties have been widely reported. For example, Alexander et al. (2006) observed variability among carrot and pea varieties, Zhu et al. (2007) reported differences among asparagus bean varieties, and Chen et al. (2012) and Wang et al. (2014) identified significant variation among pakchoi cultivars. Similarly, Brengi et al. (2021) found considerable differences in Cd concentrations in tubers among potato cultivars, Smolnikar et al. (2024) reported differences in Cd accumulation among garlic varieties, while Sana et al. (2024) reported variable responses of chili varieties grown under Cd stress.

To evaluate Cd concentration in potato tubers of different varieties grown under identical field conditions, we analysed 19 varieties and present the results in this paper.

Material and Methods

In 2024 and 2025, within the framework of potato variety trials conducted at the experimental fields of the Agricultural Institute of Slovenia (IC Komenda), samples were collected to determine Cd content and assess differences among varieties. The soil characteristics of the experimental field before the setup of the trials in 2024 (sampled in March 2024) were: pH 5.8 in KCl, 3.7% organic matter, 13 mg P_2O_5 100/g of available phosphorus, and 21 mg K_2O 100/g of available potassium. In both years, the same 19 varieties were analysed. All varieties were grown in the same field under identical agronomic conditions. Planting was carried out at the end of April and harvesting at the end of October in both years. Potatoes from year 2024 were sampled in mid-March 2025, whereas from 2025 they were sampled in mid-February 2026, after the storage. A representative sample of tubers (1 kg) was collected for analysis, and the potatoes were peeled before further processing. For the determination of Cd, homogenised potato tubers (weight 0.250 g) were digested using a high-pressure microwave oven (Milestone ETHOS 1600) with 5 ml 65 % nitric acid (HNO_3, SUPRAPUR, Merck) and 1 ml 30 % hydrogen peroxide (H_2O_2, SUPRAPUR, Merck). Cd in digested samples was analyzed with ICP-MS (Agilent 7900) in helium (He) collision mode to eliminate potential interferences. Results are presented as two-year mean Cd concentrations (2024–2025) calculated for each variety.

Results and Discussion

Cd concentrations varied among potato varieties and between the environments (fields-soils + year). KIS Kokra showed the highest Cd concentrations in both years, varieties such as Messi and Peter Pan showed lower values. These observations suggest potential varietal differences in Cd accumulation. In 2024, 9 varieties exceeded the European Commission Regulation 2023/915 limit set for Cd at 0.1 mg/kg FM, while in 2025 no varieties exceeded the limit. KIS Kokra had the highest Cd concentration in tubers 0.1775 mg/kg FM in 2024 (Table 1). In 2024 Cd concentrations in tubers ranged from 0.255 to 0.789 mg/kg DW, with KIS Kokra showing the highest value, followed by Twister, KIS Sora, and Marabel. In 2025 Cd concentrations were generally lower, ranging from 0.173 to 0.435 mg/kg DW, with the highest values observed in Labella, KIS Mangart, and KIS Slavnik, while Messi and Desiree showed the lowest concentrations. Our results align with findings from Brengi et al. (2021), who reported significant differences in Cd accumulation among peeled tubers of three potato cultivars, with Cd contents ranging from 0.08 to 0.21 mg/kg DW across field sites and seasons, and a mean value of 0.145 mg/kg DW. Öztürk et al. (2011) reported that 16 potato cultivars showed considerable variation in heavy metal concentrations, including Cd (0.08-0.32 mg/kg). Analysis of tubers from 10 potato cultivars grown in the same field revealed a significant difference in mean Cd concentrations among the cultivars, concentrations varied from 0.05 to 0.21 mg/kg DW (Ashrafzadeh et al., 2017).

Crops of 19 potato varieties accumulated Cd at different levels. For all varieties, Cd concentrations were consistently higher in 2024 than in 2025 on a fresh weight (FW) basis. The variety KIS Kokra had the highest Cd concentration in both years, particularly in 2024, while varieties such as Messi, Peter Pan, and Elland showed relatively lower Cd concentrations. When comparing Cd concentrations on a dry matter (DM) basis, the differences between years are smaller but still noticeable for some varieties (Table 1). This suggests that part of the observed decrease in Cd from 2024 to 2025 on a FW basis may be attributed to longer storage of the 2024 crop, which likely caused greater water loss and a concentration effect, rather than reflecting just differences in uptake from the soil.

Table 1. Cadmium concentrations (in mg/kg fresh matter and dry matter) in potato tubers of 19 varieties grown in 2024 and 2025.

VARIETY	Cd 2024 (mg/kg FM)	Cd 2024 (mg/kg DM)	Cd 2025 (mg/kg FM)	Cd 2025 (mg/kg DM)	Cd mean of 2024 and 2025 (mg/kg FM)
Messi	0.0719	0.341	0.0416	0.196	0.0567
Peter Pan	0.0599	0.255	0.0565	0.276	0.0582
Elland	0.0730	0.304	0.0567	0.291	0.0648
Jelly	0.0801	0.424	0.0538	0.268	0.0670
Desiree	0.0992	0.430	0.0355	0.173	0.0674
Alouette	0.0783	0.351	0.0578	0.301	0.0680
Maris Bard	0.0959	0.398	0.0482	0.211	0.0721
KIS Tamar	0.0875	0.431	0.0592	0.238	0.0733
Labella	0.0740	0.376	0.0761	0.435	0.0750
Soraya	0.1027	0.650	0.0556	0.304	0.0791
Levante	0.1039	0.550	0.0570	0.294	0.0805
KIS Blegoš	0.0869	0.383	0.0827	0.369	0.0848
KIS Slavnik	0.1005	0.529	0.0744	0.404	0.0874
Agria	0.1238	0.604	0.0554	0.281	0.0896
KIS Mangart	0.1112	0.601	0.0852	0.412	0.0982
Marabel	0.1215	0.710	0.0754	0.406	0.0984
Twister	0.1213	0.783	0.0802	0.399	0.1007
KIS Sora	0.1370	0.678	0.0765	0.361	0.1068
KIS Kokra	0.1775	0.789	0.0840	0.370	0.1307

Environmental conditions also likely contributed to the observed differences. The 2024 crop was grown at Lahovče and the 2025 crop at Zgornji Brnik. Average temperature and precipitation were higher in 2024, with mean temperatures 0.7 °C higher than in 2025, creating warmer and wetter conditions that may have promoted higher Cd accumulation. Yi et al. (2020) reported that temperature is a key climatic factor affecting crop Cd uptake, as elevated temperatures accelerate chemical reactions and mineral weathering, increasing Cd availability, while also enhancing plant transpiration. This effect is most pronounced in spring and summer, when temperatures exceed 20 °C (Drabesch et al., 2024). In contrast, 2025 experienced more heat waves and drought stress, which may have reduced Cd uptake, consistent with findings by Xiao et al. (2023) showing that drought stress can limit Cd accumulation in wheat seedlings through altered root morphology and decreased Cd in roots. The combination of storage duration and environmental factors likely explains the observed year-to-year differences in Cd concentrations in potato tubers.

Conclusion

The results of this study confirm that Cd concentration in potato tubers is strongly influenced by varietal differences, even when grown under identical agronomic conditions. Among the 19 analyzed varieties, KIS Kokra consistently exhibited the highest Cd concentrations and can therefore be considered a high-risk variety in terms of Cd accumulation. In contrast, varieties such as Messi, Peter Pan, Elland and Jelly showed lower Cd levels and may represent more suitable choices for minimizing Cd intake. In addition to genetic factors, environmental conditions also played an important role, as evidenced by the consistently lower Cd

concentrations observed in 2025 compared to 2024 across all varieties. This suggests that year-to-year variability, likely related to soil and climatic conditions, can significantly influence Cd uptake. Overall, the findings highlight the importance of selecting low-accumulating potato varieties as a practical strategy to mitigate Cd entry into the food chain, particularly in regions with elevated soil Cd levels.

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ORIGINAL SCIENTIFIC PAPER

The influence of protective treatments on the intensity of black spot (*Phomopsis viticola* Sacc.) infection in the Kutjevo vineyard.

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Summary
Black spot of grapevine is a disease that has become established in almost all vineyards in Croatia, caused by a fungus from the genus *Diaporthe/Phomopsis*. The disease affects all above-ground parts of the grapevine, especially the bark of the grapevine, where it is most noticeable. The aim of the study is to investigate the intensity of infection with black spot of grapevine during the growing season at different locations with the application of different protection measures. In both vineyards studied, as expected, the most significant infection was on the first basal internodes due to infected old grapevines from the previous growing season. The difference in the intensity of infection in the studied vineyards is primarily due to the application of protection measures, which is reflected in the number of treatments and the choice of preparations.

Keywords: vineyard, infection intensity, internode, black spot, protection

Introduction

The Kutjevo vineyard is located in the Požega-Slavonia County and is characterized as one of the most famous vineyards in Croatia. It covers 1243 hectares. It is one of the ten vineyards of the Slavonia Subregion, and includes grape and wine production from the area of the city of Kutjevo and the municipalities of Čaglin, Kaptol and Velika (Mesić et al., 2023). Black spot of grapevine is a typical fungal disease and represents one of the most economically significant diseases resulting from modern cultivation methods. It belongs to the group of diseases known as grapevine trunk diseases (Urbes-Torres et al., 2012), which also includes several other diseases caused by phytopathogenic fungi from the genera *Botryosphaeria, Diplodia, Neofusicoccum, Cylindrocarpon, Eutypa, Phaemoniella, Phaeoacremonium, Fomitiporia* and others (Bertsch et al., 2013; Niekerk et al., 2011). Symptoms of black spot are recognizable throughout the growing season, especially in winter and early spring. Black spot gradually weakens the host plant and reduces the yield year after year (Cvjetković, 2010). Infections are present to a greater or lesser extent in all vineyards in Croatia, as well as in the Kutjevo vineyard (Kovačević, 2017). The disease is particularly noticeable during winter pruning with characteristic symptoms of bark bleaching and the appearance of pycnidia on the surface. Some of the aforementioned symptoms may be absent, because there are isolates that do not parasitize the petiole and berries (Schilder et al., 2005). With the onset of growing season, pycnospores are released from the pycnidia and spread to susceptible green tissue. With sufficient moisture, the first primary infections occur, and later the disease spreads. There are two types of spores in Phomopsis viticola: α spores, which are oval or elliptical, transparent and contain two small vacuoles at the ends, and β spores, which are longer, filamentous and curved at the ends. Pycnidiospores infect grapevines over a wide temperature range from 1 to 37º C, and the

optimum for disease development is 23º C with 98.99% relative humidity (Kišpatić and Maceljski, 1979). The reason for the difficulty in controlling this disease is that the mycelium is located in the bark tissue and is inaccessible to the applied fungicides. For this reason, fungicides can only act on the fruiting body of the fungus, which are located on the surface (Vrandečić et al., 2010). Great attention should be paid to the control of black spot in vineyards, as if it occurs with greater intensity and for a long period of time, it is later very difficult to eradicate the disease, even with frequent use of fungicides (Miličević, 2021). The fungus can enter the trunk via the mycelium, which gradually causes rotting (Kaliterna et al., 2012). The pathogenic fungus belongs to the family *Diaporthaceae*, within which other species of the genus *Diaporthe/Phomopsis* have been described as pathogens on grapevines worldwide, such as: *Diaporthe ambigua, Diaporthe amygdali, Diaporthe australafricana, Diaporthe foeniculacea, Phomopsis fukushii*, etc. (Miličević, 2021). Black spot is also transmitted by infected planting material, and planting healthy grafts significantly facilitates protection against this disease, while in infected productive vineyards, as many diseased canes as possible should be removed by pruning.

Material and Methods

The assessment of infection intensity was conducted by monitoring the spread of the disease during the growing season at two selected sites within the Kutjevo vineyard region, in order to gain insight into the intensity of disease presence depending on the applied protection measures during 2024. The experiments were carried out at two locations in the Kutjevo vineyard region, specifically in the vineyards of the Kaptol and Kutjevo municipalities. The selected vineyards were in full production, of similar age (15 to 18 years), and planted with the Graševina variety. Two agricultural holdings were included in the research:

-OPG Ivan Mitrović: vineyard area 3.3 ha, variety Graševina, age 15 years, location Brdine, Municipality of Kutjevo;

-PG Dalibor Mikić: area 2.6 ha, variety Graševina, age 18 years, location Crniševac, Municipality of Kaptol.

The assessment of infection intensity was performed on 4 selected shoots per vine during the growing season in July and August 2024, on 10 randomly selected vines in each vineyard. The vines and shoots were marked with red ribbons to ensure that the infection across treatments was monitored on the exact vines and shoots under investigation. The research was conducted according to the Desaymard method, by monitoring disease progression on the 4 basal internodes at different inspection intervals, with the results being recorded.

Table 1. Infection ratings (Desaymard, P; 1968)

Infection grade / Rating	Affected internode surface area (%)
0	No infection
2	5% infection zaraze
3	15%
4	30%
5	50%
6	70%
7	85%
8	95%
9	97.5%
10	100%

In the assessment of the infection, 5 of the 10 rating levels were selected, namely: 0, 3, 5, 7, and 10. The assessments were carried out using a hand lens across four repetitions on dates 15 days apart, adapted to the specific research locations.

Table 2. Chemical control measures against black spot at the Kaptol

Agricultural farm	Treatment (date)	Variety	Diseases, pests, weeds	Plant Protection Product (PPP)	Dosage (per ha)	Active substance
Agricultural farm (PG): Dalibor Mikić, Municipality of Kaptol, location Crniševac	02.04 2024.	Graševina	black spotting, mites, scale insects	Blue oil, oil concentrate for suspension	3 L	Copper 100 g/l (from copper II hydroxide) Mineral oil 550 g/l
	15.04.2024.		black spotting, mites, scale insects	Nordox 75-WG	1,50 kg	Copper from cupric oxide (75%)
	06.05.2024.		black spotting, powdery mildew, mites	Sulfur SC	6 L	800 g/kg sulfur
	06.05.2024.		grapevine fire, black spotting	Folpan 80 WDG	2 kg	folpet 80 %

Table 3. Chemical control measures against black spot at the Kutjevo

Agricultural farm	Treatment (date)	Variety	Diseases, pests, weeds	Plant Protection Product (PPP)	Dosage (per ha)	Active substance
Agricultural farm (OPG): Ivan Mitrović, Municipality of Kutjevo, location Brdine	10.04.2024	Graševina	black spotting, mites, scale insects	Nordox 75-WG	1,50 kg	Copper from cupric oxide (75%)
	02.05.2024.		grapevine fire, black spotting	Folpan 80 WDG	2 kg	folpet 80 %

In Tables 2 and 3, the chemical agents listed were used specifically for protection against black spot, without considering the additional significant effects of chemical products used for protection against downy mildew and gray mold during the growing season. IBM SPSS Statistics 26.0 was used for data processing and statistical analysis. Statistical analysis was performed using the general linear model - GLM model for repeated measurements. The assumption of sphericity was checked using the Mauchly test, and if sphericity was not met, the Greenhouse-Geisser correction was used. For multiple comparisons, the Bonferroni correction was used. The significance level was set at $\alpha=0.05$.

Results and Discussion

The difference in the intensity of black spot infection between internodes within a single assessment date was analyzed using a GLM model for repeated measures.

The results obtained indicate that there is a statistically significant difference in the intensity of infection between internodes ($p < 0.001$) in both observed vineyards. Analysis of data from vineyards at the Kutjevo and Kaptol sites revealed a significant linear decrease in the intensity of infection ($p < 0.001$) from the first to the fourth internode. The mean values of infection intensity from the Kutjevo location in the first assessment were I-1 = 5.90, I-2 = 4.55, I-3 = 3.90 and I-4 = 2.65, in the second assessment they were I-1 = 6.75, I-2 = 5.30, I-3 = 4.55 and I-4 = 3.30, in the third assessment I-1 = 6.48, I-2 = 5.25, I-3 = 4.30 and I-4 = 3.33, and in the fourth assessment I-1 = 7.58, I-2 = 6.02, I-3 = 4.93 and I-4 = 3.53. The mean values of infection intensity from the Kaptol location in the first assessment were I-1 = 2.85, I-2 = 1.95, I-3 = 0.97, I-4 = 0.37, in the second assessment I-1 = 3.60, I-2 = 2.80, I-3 = 1.85, I-4 = 0.6, in the third assessment I-1 = 3.17, I-2 = 2.63, I-3 = 2.30, I-4 = 1.20 and in the fourth assessment I-1 = 4.15, I-2 = 3.50, I-3 = 2.90, I-4 = 1.55.

Post-hoc analysis with Bonferroni correction found that the intensity of infection in the Kutjevo vineyard is statistically significantly different between all pairs of internodes, with the intensity of black spot infection being statistically significantly higher on the internodes closer to the base of the vine. The estimated decrease in infection intensity indicates a decrease in the infected surface of the internodes in the first assessment from approximately 70% infection of the first internode to 15% infection of the fourth internode, and in the fourth assessment from approximately 95% infection of the first internode to 30% infection of the fourth internode. Using the same analysis in the Kaptol vineyard, it was determined that the intensity of the infection is statistically significantly different between all pairs of internodes in the second assessment, while in the other evaluations there are differences only in individual pairs. Thus, in the first evaluation, significant differences were found between internodes I-1 and I-2, I-1 and I-3, I-1 and I-4, I-2 and I-3 and I-2 and I-4, in the third evaluation between internodes I-1 and I-3, I-1 and I-4, I-2 and I-4 and I-3 and I-4, and in the fourth evaluation between internodes I-1 and I-3, I-1 and I-4, I-2 and I-3, I-2 and I-4 and I-3 and I-4. In the Kaptol vineyard, the intensity of black spot infection is also statistically significantly higher on internodes closer to the base of the vine. The estimated decrease in the intensity of the infection indicates a decrease in the infected surface of the internodes, and this in the first evaluation from approximately 30% infection of the first internode to 0% infection of the fourth internode, and in the fourth evaluation from approximately 30% infection of the first internode to 5% infection of the fourth internode. Multivariate tests indicated the existence of statistically significant differences in the intensity of black spot infection between the two observed vineyards regardless of the internode, and for each assessment date (Phillai's Trace $p < 0.001$). The univariate test confirms the obtained results ($p < 0.001$). In all four assessments, a statistically significantly stronger infection was determined in the Kutjevo vineyard. The mean values of the intensity of infection regardless of the internode are shown in Table 1.

Table 4. Intensity of black spot infection in Kutjevo and Kaptol vineyards

	Intensity of infection			
	Grading 1	Grading 2	Grading 3	Grading 4
Kutjevo	4.25	4.98	4.84	5.51
Kaptol	1.54	2.21	2.32	3.03

Conclusion

The choice of applied preparations and the number of treatments significantly influenced the intensity of the infection, which is visible in the results obtained through all four assessment periods. At PG Dalibor Mikić, the infection with black spot of selected vines and shoots is significantly lower and is within the expected infection, which represents a better condition of the vine in the following years of cultivation, as well as the assumption that the protection and care measures of the plantations were adequately implemented in previous years, although the vineyard is 16 years old. In the vineyard of OPG Ivan Mitrović, the infection with black spot of selected vines and shoots according to the results obtained is significantly higher compared to the vineyard of PG Dalibor Mikić. Considering that the vineyard is 18 years old and only 2 targeted treatments against black spot were carried out in the period 10.04. to 02.05. 2024. a more significant infection with black spot on shoots during the vegetation period is expected. Analyzing the results, the difference in the intensity of infection in the studied vineyards is evident primarily due to the application of protection measures, which is reflected in the number of treatments and the choice of preparations. The use of mineral oil in the first winter-spring protection significantly influenced the reduction of infection, as did the application of sulfur preparations against fungal diseases in addition to the applied preparations, which had a targeted effect on black spot.

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ORIGINAL SCIENTIFIC PAPER

Influence of selenite and selenate seed priming duration on the mitigation of drought stress in industrial hemp

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Summary
This study evaluated the effect of different durations of seed priming with selenite and selenate on germination and early sprout growth of industrial hemp (*Cannabis sativa* L.) cv. Felina 32 under drought stress conditions. Seeds were primed with distilled water and Se solutions: sodium selenite and sodium selenate (40 μM Se L^{-1}) for 0, 6, and 12 h. Polietilen glycol (PEG 6000) was used to induce drought stress. Germination was tested under PEG 0% and PEG 5%. The average germination rate was 95%, and seed priming significantly affected germination, while PEG had no significant effect. The lowest germination was found in the non-primed control (92%), and the highest in sodium selenate treatments after 6 h and 12 h (97%). The average total sprout length was 7.9 cm, root length 4.1 cm, shoot length 3.85 cm, and root-to-shoot ratio 1.08. The highest total sprout length (10.0 cm) and root length (5.4 cm) were recorded after hydropriming for 6 h under PEG 0%, while the lowest total sprout length (5.9 cm) and shoot length (2.68 cm) were found in the non-primed treatment under PEG 5%. The highest shoot length (4.86 cm) was observed in the sodium selenate 6 h treatment under PEG 0%. PEG-induced stress significantly reduced total sprout length, root length, shoot length, and fresh mass, while dry mass was not significantly affected. In general, seed priming improved the early growth of industrial hemp sprouts. Sodium selenite showed more stable performance under stress, while sodium selenate was more effective in improving germination.

Keywords: seed priming duration, sodium selenite, sodium selenate, PEG 6000, germination, early growth, Felina 32

Introduction

Industrial hemp (*Cannabis sativa* L.) is a multipurpose crop that is used for fibre, food, oil, and other industrial products (Visković et al., 2023; Ranogajec et al., 2024). It is also valued for its fast growth and good adaptability, but early growth and germination can be limited by drought stress (Kwiatkowska et al., 2024). According to FAOStat (2026), the largest producers of hempseed are Canada, Australia, the Russian Federation, Chile, and the USA (Figure 1). Except for seed, interest in industrial hemp is increasing because it is a useful crop for many industries, including textiles, food, medicine, and construction (Campiglia et al., 2020). However, drought has become an important problem in hemp production because it can reduce yield, affect fibre quality, and change the chemical composition of the plant.

Drought stress during early growth can strongly affect germination and sprout development, leading to weaker plant establishment. Under climate change conditions, the increasing frequency and intensity of drought make this stage especially important for crop production (Ahluwalia et al., 2021; Müller et al., 2022).

In recent years, seed priming with selenium has been recognized as a promising approach for improving germination and early plant growth under stress conditions, as it may increase stress tolerance through better antioxidant protection, improved metabolic activity, and more stable

seedling development during drought (Bayat and Aminifard, 2021; Hu et al. 2022, Sekhurwane et al., 2025). Although studies on selenium (Se) seed priming in industrial hemp are still limited, available findings suggest promising results. Stonehouse et al. (2020) reported that industrial hemp shows good tolerance to Se, indicating strong potential for both selenium biofortification and phytoremediation



Figure 1. Production quantities in tons of hempseed by country, average 2020 – 2024 (FAOStat, 2026)

The aim of this study was to evaluate the influence of different durations of seed priming with selenite and selenate on germination and early sprout growth of industrial hemp under drought stress conditions. In particular, the study examined whether selenium priming could mitigate the adverse effects of PEG-induced osmotic stress and improve growth parameters such as root length, shoot length, and total sprout length.

Material and Methods

Seeds of industrial hemp (*Cannabis sativa* L.) cultivar 'Felina 32' were used in this study. Selenium seed priming was performed using distilled water (hydropriming) and solutions containing sodium selenite and sodium selenate at a concentration of 40 µM Se L^{-1} with different forms of Se source – Na_2SeO_3 and Na_2SeO_4. The seeds were primed for 0 h (dry seeds, control), 6 h, and 12 h. After priming, the seeds were dried and used for a germination test.

Germination was carried out using Sartorius Stedim Biotech filter paper (size of 290 × 290 mm, 84 g m^{-2}, grade 391). For each replicate, 50 seeds were placed between layers of filter paper moistened with 20 mL of distilled water or PEG 6000 solutions prepared at 0 % (control) and 5 %. The rolled filter paper method (between paper rolls) was used. The rolls were placed in polyethylene (PE) bags, kept in a vertical position, and incubated in a germination chamber (Memmert, Germany) at 25 °C and 60 % relative humidity. Each treatment was conducted in four replications.

Total germination was evaluated on the 7th day, while germination energy in hemp was recorded on the 3rd day according to ISTA guidelines (2026). In addition, seedling morphology was evaluated by measuring root length, shoot length, and total seedling length.

Results and Discussion

In industrial hemp sprouts, the average germination rate of 95 % was the same as germination energy; thus, only germination rate is presented (Figure 2). Seed priming had a significant effect on total germination, while PEG treatment had no significant effect, as both PEG 0% and PEG 5% showed the same value (94%). The lowest germination was recorded in the no-priming

treatment (92%), whereas the highest values were observed after sodium selenate priming for 6 h and 12 h (97%). Hydropriming for 6 h also improved germination (96%) compared with the control. These results suggest that seed priming, especially with sodium selenate, had a positive effect on the germination of industrial hemp sprouts.

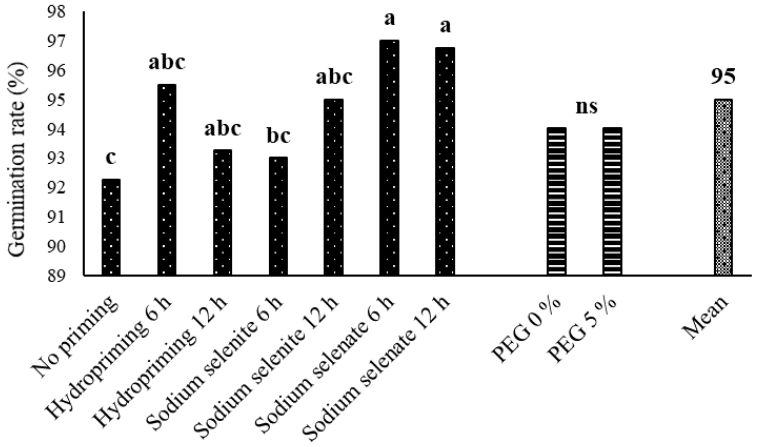

Figure 2. Germination rate of industrial hemp. Different letters indicate significant differences at p≤0.05

Drought stress significantly reduced all measured length parameters of Felina 32 sprouts. Total length of industrial hemp sprout significantly decreased ($p < 0.05$) from 8.9 (PEG 0 %), to 6.9 cm (PEG 5 %), root length from 4.7 to 3.5 cm, and stem length from 4.3 to 3.4 cm (Figure 3), which confirms the negative effect of osmotic stress on early sprout growth (Herppich et al., 2020;). Moreover, PEG treatment reduced fresh mass, with a value of 0.13 g at PEG 0% and 0.11 g at PEG 5%, indicating a negative effect of osmotic stress on sprout fresh biomass. In contrast, dry mass was very similar between treatments, ranging from 0.53 g to 0.54 g, and the difference was not significant (ns). This suggests that PEG stress affected water accumulation more strongly than dry matter formation.

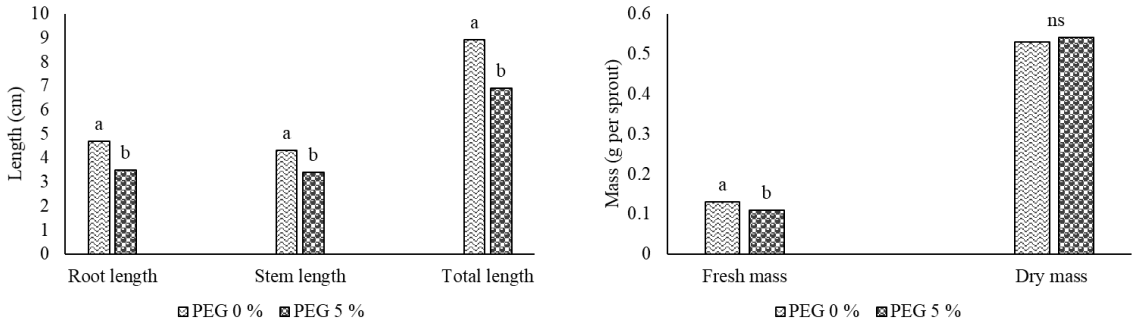

Figure 3. Influence of drought induces stress on the morphology of industrial hemp sprouts and fresh and dry mass per sprout. Different letters indicate significant differences at p≤0.05

The results showed that seed priming had a positive effect on the growth of Felina 32 industrial hemp sprouts, while PEG-induced stress reduced all growth parameters (Figure 4). In general, the best values for total sprout length, root length, and stem length were recorded under PEG 0%, especially after hydropriming for 6 h (10.0, 5.4, and 4.58 cm, respectively). Under PEG 5%, growth was reduced, and the lowest values were mostly found in the no-priming treatment and in the sodium selenate 6 h treatment. Among selenium treatments, sodium selenite generally showed more stable results under stress than sodium selenate, suggesting a better

protective effect on sprout growth. The average values in the experiment were 7.9 cm for total sprout length, 4.1 cm for root length, 3.85 cm for stem length, and 1.08 for root-to-shoot ratio.

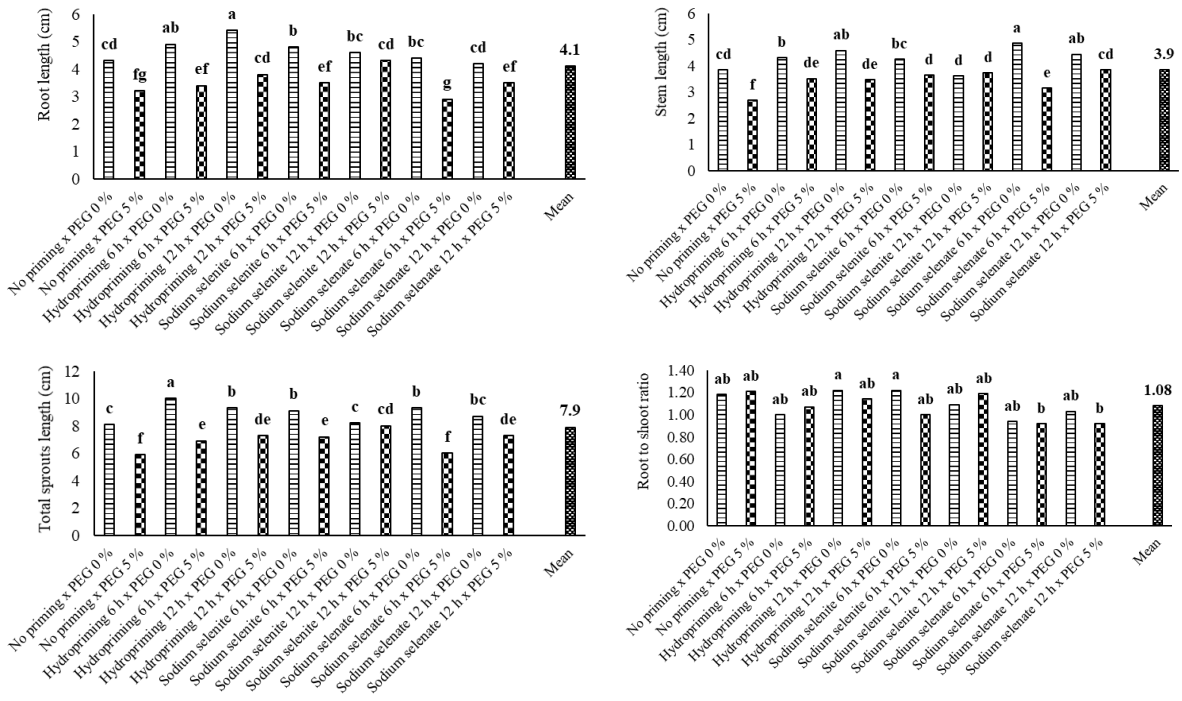

Figure 4. Effects of industrial hemp seed priming and PEG-induced stress on root length, stem length, total sprout length, and root-to-shoot ratio of industrial hemp sprouts. Different letters indicate significant differences at $p \leq 0.05$

The positive effect of Se seed priming to mitigate drought stress was confirmed in other studies. García-Locascio et al. (2024) in tomato found that Se nanoparticles (NPs) had a positive effect on tomato seed germination and early growth, but the response depended on concentration. The best germination was recorded at 10 ppm in Petri dishes and at 1 ppm in trays, where germination increased by 10% and 32.5%. Ishtiaq et al. (2023) found that seed priming with Se-NPs improved tomato tolerance to drought stress, with the best results at 75 ppm, where carotenoids increased by 13.5%, α-tocopherols by 22.8%, flavonoids by 25.2%, anthocyanins by 19.6%, and ascorbic acid by 26.4%, showing that SeNPs improved antioxidant defence and helped maintain tomato production under drought conditions.

Conclusion

Seed priming improved the germination and early growth of industrial hemp sprouts of the cultivar Felina 32. The best germination was found after sodium selenate priming for 6 h and 12 h, while hydropriming for 6 h gave the best results for total sprout length and root length. PEG-induced drought stress reduced total length, root length, stem length, and fresh mass, but it did not significantly affect dry mass. In general, primed seeds performed better than non-primed seeds under stress conditions. Among selenium treatments, sodium selenite showed more stable growth under PEG stress, while sodium selenate was more effective for improving germination. These results show that seed priming with Se can help reduce the negative effects of drought stress during early growth of industrial hemp.

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ORIGINAL SCIENTIFIC PAPER

Mitigation of abiotic stress effects on maize using different nutrient management strategies

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Summary
A 2025 field experiment assessed nutrient and soil conditioning treatments on maize (*Zea mays* L.) grown under acidic and low-nitrate soil as well as extreme weather conditions (cold May, drought in June). The 81 kg N + vermicompost solution gave the highest yield (8690.5 kg ha^{-1}), followed by vermicompost solution + pelleted vermicompost (8672.3 kg ha^{-1}). During the June drought, photosynthetic rates declined significantly in all treatment groups; however, they were lower in the plots treated with nitrogen (24.82 µmol m^{-2} s^{-1}), while the soil conditioner alone mitigated stress (36.87 µmol m^{-2} s^{-1}). Findings suggest combined nutrient and conditioner use may alleviate stress and improve yields.

Keywords: maize, stress, vermicompost, biostimulants

Introduction

Rehaman et al. (2023) demonstrated that the combined application of vermicompost (VC) and a nitric oxide donor enhanced photosynthesis, antioxidant defense, and nitrogen assimilation in maize under drought conditions. Complementing these findings, Hernández-Campos et al. (2024) showed that humic acids (HA) extracted from VC increased root and shoot biomass, as well as relative water and chlorophyll content in maize, even under water deficit. Both studies emphasize the stress-mitigating role of vermicompost-derived substances.
In a field experiment, Skliar et al. (2024) found that the combination of seaweed extract and HA was the most effective in maize, achieving a yield of 6100 kg ha^{-1} compared to 4150 kg ha^{-1} in the untreated control, highlighting the synergistic potential of combined biostimulant treatments. Lelesz et al. (2024), working in an aquaponic system, confirmed that HA significantly reduces heavy metal stress (mercury decreasing from 310.65 to 196.32 µg kg^{-1}) while increasing iron uptake, pointing to the broad stress-modulating capacity of HA. Csajbók et al. (2025) further investigated biostimulants' stress-mitigating effects in maize under combined drought and herbicide stress, reinforcing that biostimulants can be effective even under complex abiotic stress conditions. The results of the present study align well with these findings.

Material and Methods

The experiment was conducted in Újfehértó, Hungary, in 2025. During the maize growing season (April-September) the total precipitation (197 mm) was lower than the 1990-2020 reference period average (306 mm), while the mean air temperature (15.6 °C) was slightly above the reference value (14.9 °C). March was exceptionally wet (72 mm compared to the 30-year average of 30 mm). Subsequently, April and May were warmer than average but also experienced precipitation deficits for 56% (April) and 32% (May) in comparison with long term average. June was exceptionally warm and dry, with a mean temperature of 22.0 °C (above the 20.3 °C average) and only 11 mm of precipitation (compared to the usual 67 mm).

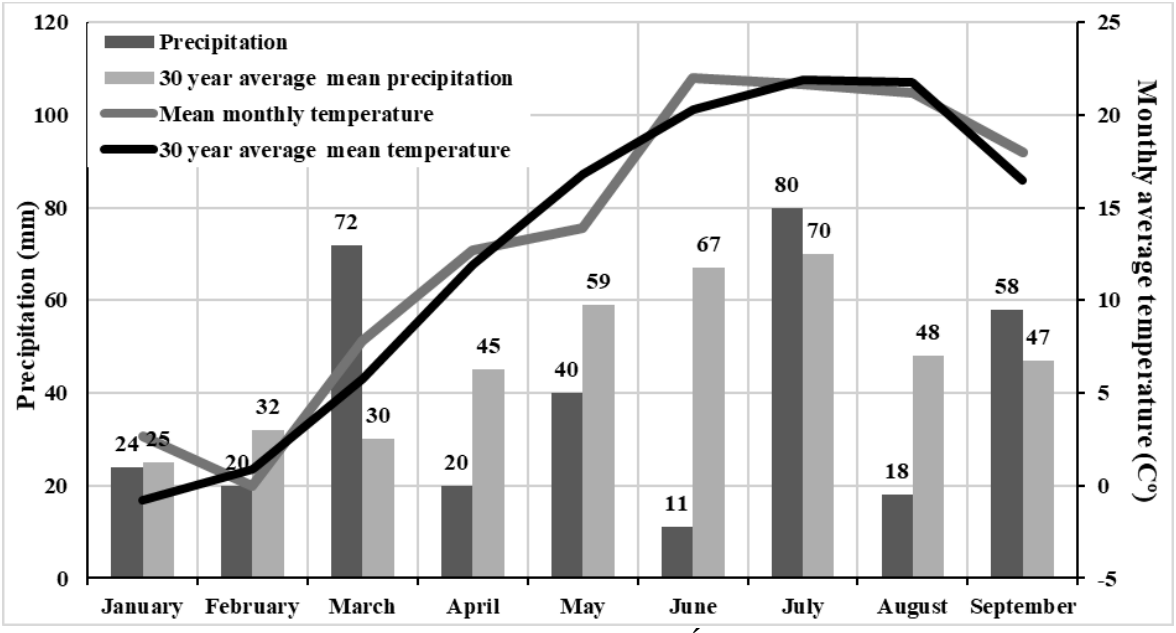

Figure 1. Temperature and precipitation trends in the Újfehértó region in 2025 compared to the 30-year average (1991-2020) (Újfehértó, 2025)

In July, precipitation reached 80 mm, although temperatures remained above average while August was again drier and warmer than usual (18 mm of precipitation compared to the 48 mm average). In September, rainfall (58 mm) slightly exceeded the average (47 mm), and the temperature was 1.5 °C higher than the long-term mean (Figure 1).

Soil characteristics

We collected 25 soil cores from the upper 0-30 cm layer across the 4500 m^2 area following an X-shaped sampling pattern as described Stefanovics et al. (1999). The cores were thoroughly mixed, and the composite sample was repeatedly halved until two 1 kg subsamples were obtained.The maize was grown on acidic, rusty-brown forest soil. The soil pH_{KCl} ranged from 4.97 to 5.01, indicating an acidic reaction. The plasticity index (KA) values of 33 to 39 suggest a moderately heavy, loam texture while total salt content was very low (0.03-0.04 m/m%). Humus content ranged from 2.33 to 2.47 m/m%, indicating a moderate level of soil organic matter. Phosphorus (P_2O_5) supply ranged from 81.7 to 91.4 mg kg^{-1}, and potassium (K) from 215 to 254 mg kg^{-1}, representing moderate to good supply levels. Nitrate content was very low (<0.100 mg kg^{-1}) in both soil samples. Magnesium (44.4-70.3 mg kg^{-1}) and calcium (308-347 mg kg^{-1}) concentrations were considered moderate, consistent with the acidic soil pH. The analytical procedures were carried out in accordance with the Hungarian standards MSZ-20135:1999, MSZ-08-0206-2:1978, MSZ-08-0205:1978, and MSZ-08-0210:1977. The soil analysis was performed at the Agricultural Laboratory Centre of the University of Debrecen, which is accredited by the National Accreditation Board of Hungary.

Applied products and treatments

The experiment included the following treatments: control (0 N), liquid biostimulant (BS) (vermicompost solution, 5 l ha^{-1}), pelleted soil conditioner (SC) (solid vermicompost granules, 200 kg ha^{-1}), as well as the combinations of 300 kg CAN + liquid biostimulant (81 kg N+BS) and liquid biostimulant + pelleted soil conditioner (BS+SC) . We applied the biostimulant twice: first after weed control (May 15 2025) at the 3-4 leaf stage (BBCH 13-14) (May 16

2025), and second at the 4th node detectable stage (BBCH 34) (June 13 2025). We incorporated SC and CAN fertilizers into the soil prior to sowing during seedbed preparation.
The liquid biostimulant had a potassium (expressed as K_2O) content of 0.88 m/m% (8.810 mg kg^{-1}); nitrogen, phosphorus, calcium, and magnesium were present at or below the detection limit. The product had a pH (in aqueous solution) of 9.18 and a dry matter content of 2.453 m/m%. The pelleted soil conditioner contained nitrogen 0.54 m/m%, phosphorus pentoxide 1.64 m/m% (16.400 mg kg^{-1}), potassium oxide 2.57 m/m% (25.700 mg kg^{-1}), calcium 3.16 m/m% (31.600 mg kg^{-1}), and magnesium 1.16 m/m% (11.600 mg kg^{-1}). Its pH (in aqueous solution) was 9.52, and its dry matter content was 88.07 m/m%.
We sowed on 21 April 2025 in plots measuring 4.5 m x 50 m (six rows) with four replications under field conditions. We harvested on 8 September 2025 using a combine harvester equipped with a maize header, and we determined grain yield by weighing the harvested grain on an MP-RW axle-load weighbridge upon discharge into a trailer.
Physiological measurements were conducted using a Minolta 502+ (SPAD) and a Licor Li-6800 portable photosynthesis system. Statistical analysis was performed using IBM SPSS 22, and graphical representations were created with MS Excel 2016. An ANOVA test was used to analyze variations in yield data and photosynthetic activity.

Results and Discussion
Photosynthetic activity (A, µmol m^{-2} s^{-1}) was measured on four dates: 16 May, 30 May, 13 June, and 26 June 2025 (Figure 2.).
On 16 May, the highest value was observed in the soil conditioner (SC) treatment (32.99 µmol m^{-2} s^{-1}), followed by the biostimulant (BS) treatment (29.26 µmol m^{-2} s^{-1}) and the combined BS + SC treatment (28.80 µmol m^{-2} s^{-1}). The 81 kg N + BS combination recorded 25.72 µmol m^{-2} s^{-1}, while the unfertilized control (0 N) exhibited the lowest value (22.03 µmol m^{-2} s^{-1}).

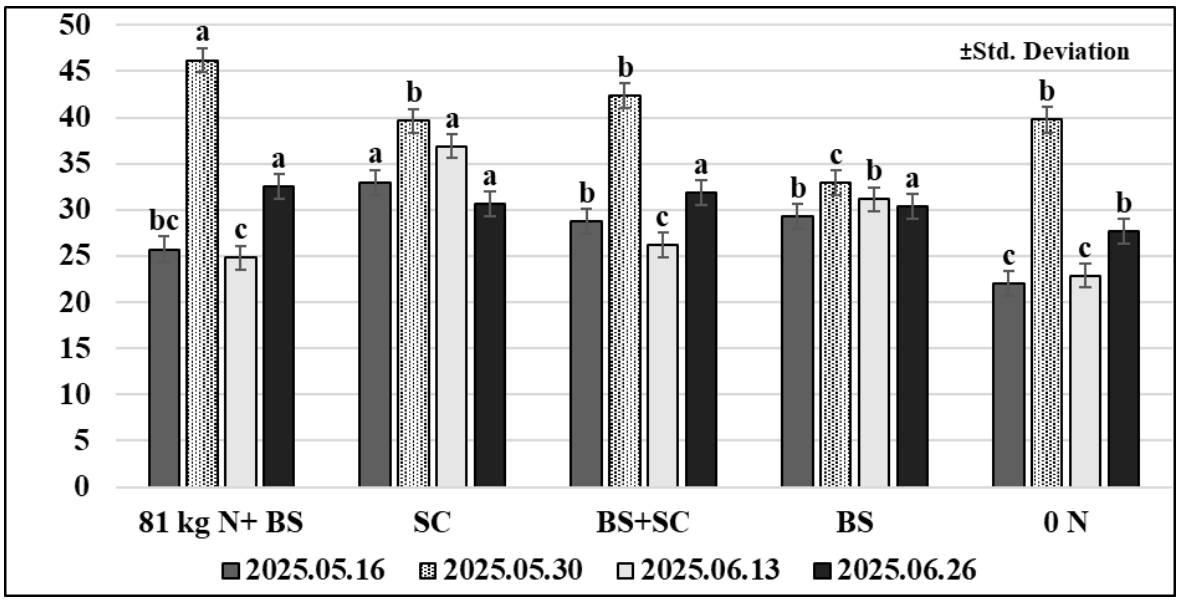

Figure 2. Measurement of photosynthetic activity in maize stands by treatment
(Note: The letters (a-c) indicate significant differences within treatments at different time points.)

By 30 May, a marked increase was evident in the treatments receiving nitrogen. The 81 kg N + BS combination attained the highest photosynthetic rate (46.19 µmol m^{-2} s^{-1}), followed closely by the BS + SC treatment (42.39 µmol m^{-2} s^{-1}). The SC treatment alone measured 39.61 µmol m^{-2} s^{-1}, whereas the BS treatment and the 0 N control recorded lower values of 32.98 µmol m^{-2} s^{-1} and 39.77 µmol m^{-2} s^{-1}, respectively.

On 13 June, coinciding with a drought period characterized by only 11 mm of monthly rainfall, photosynthetic rates declined across all treatments relative to the previous measurement. The SC treatment maintained the highest activity (36.87 µmol m^{-2} s^{-1}), whereas the BS treatment recorded 31.13 µmol m^{-2} s^{-1}. The 81 kg N + BS and BS + SC combinations decreased to 24.82 µmol m^{-2} s^{-1} and 26.22 µmol m^{-2} s^{-1}, respectively, and the 0 N control fell to 22.89 µmol m^{-2} s^{-1}. The ability of the pelleted vermicompost (SC) to moderate this drought-induced decline-evidenced by the retention of 36.87 µmol m^{-2} s^{-1} compared to the more pronounced reductions in the nitrogen-containing combinations-supports the stress-mitigating role of vermicompost-derived substances. By 26 June, photosynthetic activity showed signs of recovery. The 81 kg N + BS combination increased to 32.50 µmol m^{-2} s^{-1}, the BS + SC combination to 31.79 µmol m^{-2} s^{-1}, and the SC treatment measured 30.62 µmol m^{-2} s^{-1}. The BS treatment recorded 30.40 µmol m^{-2} s^{-1}, while the 0 N control remained the lowest at 27.66 µmol m^{-2} s^{-1}(Figure 2). Zayani et al. (2023) foliar fertilization treatments also showed that nutrient treatments increase photosynthesis.
Their synergistic effect, consistent with the findings of Skliar et al. (2024), Csajbók et al. (2025) and Csajbók (2026), suggests that the combined application of nutrient supply and biostimulants exerts beneficial effects through mechanisms analogous to those observed with humic acid.

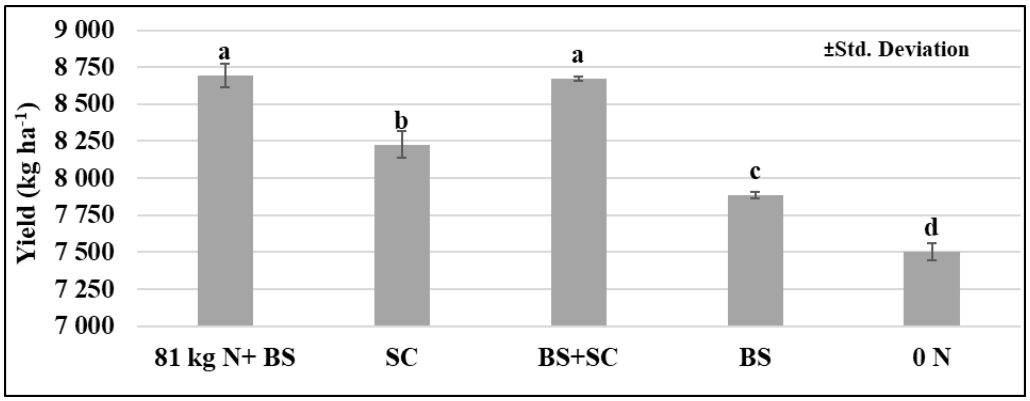

Figure 3. Grain yields by treatment calculated at 13.5% moisture content, kg ha^{-1}
(Note: N: Nitrogen fertilization, BS: liquid biostimulant, SC: pelleted soil conditioner, 0N: control; The letters (a-d) indicate significant differences within treatments.)

The highest yield was achieved with the 81 kg N + liquid biostimulant combination (8690.46 ± 79.41 kg ha^{-1}) (Figure 3). This was closely followed by the liquid biostimulant + pelleted soil conditioner combination (8672.25 ± 13.43 kg ha^{-1}). The pelleted soil conditioner applied alone resulted in 8227.31 ± 88.76 kg ha^{-1}, and the liquid biostimulant applied alone yielded 7887.15 ± 22.46 kg ha^{-1}. The control (0 N) treatment produced 7501.27 ± 59.39 kg ha^{-1} (Figure 3.).

Conclusion

The results of this study suggest that despite unfavorable soil properties (acidic pH, low nitrate content) and extreme weather conditions in 2025 (severe drought in June), the combined treatments may have provided significant yield increases. The 81 kg N + liquid biostimulant treatment produced the highest yield, raising the possibility that nitrogen supply may have enhanced assimilatory performance during the more favorable water supply period in July. The liquid biostimulant + pelleted soil conditioner combination also resulted in a notable yield, which is consistent with the stability observed in the photosynthesis measurements, suggesting that the soil conditioner may have mitigated drought stress. Based on the findings of this study, the investigated products (especially when applied in combination) may prove to be effective tools for improving yield security under extreme weather conditions and unfavorable soil properties. However, further multi-year studies are needed to confirm these conclusions.

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ORIGINAL SCIENTIFIC PAPER

Maceration length and fermentation protocol as modulators of *cv.* Trnjak red wine chemical composition

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Summary
This study evaluated the impact of sequential alcoholic fermentation with *Lachancea thermotolerans* and *Saccharomyces cerevisiae* and different maceration lengths on the physicochemical and polyphenolic composition of *cv.* Trnjak red wines across two vintages. Sequential fermentation resulted in significantly lower alcohol concentrations, higher concentrations of total acidity and lower pH values. Although vintage had the strongest influence on phenolic composition, extended maceration increased anthocyanin and flavan-3-ol concentrations. The results confirmed the potential of sequential fermentation for improving wine balance under warm-climate conditions.

Keywords: Trnjak, *Lachancea thermotolerans*, sequential fermentation, maceration, polyphenolic compounds

Introduction

Alcoholic fermentation in winemaking is primarily conducted by S*accharomyces cerevisiae* (*Sc*), which ensures efficient sugar conversion into ethanol. However, recent studies have emphasized the importance of non-*Saccharomyces* yeasts due to their ability to enhance wine complexity and modulate chemical composition (Ciani et al., 2016; Padilla et al., 2016). Among them, *Lachancea thermotolerans* (*Lt*) has gained considerable attention because of its capacity to produce lactic acid, thereby increasing total acidity and lowering pH (Benito, 2018; Morata et al., 2019). This property is particularly relevant in warm-climate viticulture, where rising temperatures lead to higher sugar accumulation and reduced acidity in grapes, resulting in unbalanced wines with high alcohol content (Mira de Orduña, 2010). Sequential fermentation involving *Lt* and *Sc* has therefore been proposed as an effective strategy to improve wine freshness and stability (Comitini et al., 2011; Jagatić Korenika et al., 2021). Maceration is another key technological factor influencing red wine composition. The duration of skin contact directly affects the extraction of phenolic compounds, including anthocyanins and tannins, which contribute to wine color, structure and stability (Cheynier et al., 2006; Rossi et al., 2022). Trnjak is an autochthonous grape variety from the Imotski region and Western Herzegovina, characterized by high sugar content, moderate acidity and rich phenolic potential (Maletić et al., 2015). Despite its enological potential, it remains insufficiently studied, particularly regarding modern fermentation strategies.
Therefore, the aim of this study was to evaluate the combined effect of sequential fermentation (*Lt+Sc*) and maceration length on the physicochemical and phenolic composition of Trnjak wines.

Material and Methods

Trnjak variety grapes from Potpolje vineyard site (Mostar wine region, Bosnia and Herzegovina), vintage 2019 and 2021 were used in this study. A total of 300 kg of grape was processed, homogenized, and evenly distributed into 50L-tanks, sulfited with 5%-H_2SO_3. Treatment K (control) underwent spontaneous fermentation with epiphytic yeast. Treatment *Lt+Sc* was inoculated with *Lt* yeast (Laktia™, Lallemand; 15 g/50 L), followed by *Sc* (Uvaferm BDX™, Lallemand; 15 g/50 L) after 24 hours. Treatment *Sc* was inoculated with *Sc* (Fermol Premier Cru™, AEB; 15 g/50 L). Maceration lasted for 8 days and 12 days, for each treatment with regular punch-downs every 12 hours.

After 8 days of maceration and alcoholic fermentation, the must from the treatments K, *Sc* and *Lt+Sc* were pressed using a hydraulic press, and the fermenting must was transferred into three 10L-glass containers per treatment (in duplicate) with airlocks, allowing fermentation to continue. The same procedure was repeated after 12 days of maceration for treatments K, *Sc* and *Lt+Sc*.

Basic physicochemical parameters were determined using standard oenological methods (OIV, 2020). The concentration of individual phenolic compounds in wines was determined by using high-performance reversed-phase chromatography using an Agilent 1100 Series liquid chromatograph (Agilent, USA) on a phenyl-hexyl column (Phenomenex, USA). The chromatograms were simultaneously recorded with a diode array detector and a fluorescence detector. The identification of wine components was performed by comparing their retention times on the chromatographic column and the corresponding UV-Vis spectra and fluorescence at characteristic excitation and emission wavelengths with the retention times, spectra and fluorescence of standard compounds. The mass concentrations of the identified compounds were determined using calibration curves made by analyzing an external standard (Tomaz and Maslov, 2016). Data were analyzed using ANOVA ($p \leq 0.05$), and principal component analysis (PCA) was applied to evaluate overall variability (XLSTAT software).

Results and Discussion

Sequential alcoholic fermentation (*Lt+Sc*) resulted in significantly lower alcohol concentrations and higher total acidity with lower pH values compared with K and *Sc* treatments. This confirms the acidifying effect of *Lt*, as previously reported (Comitini et al., 2011; Morata et al., 2019).

Table 1. Physicochemical parameters of *cv*. Trnjak red wines, vintage 2019.

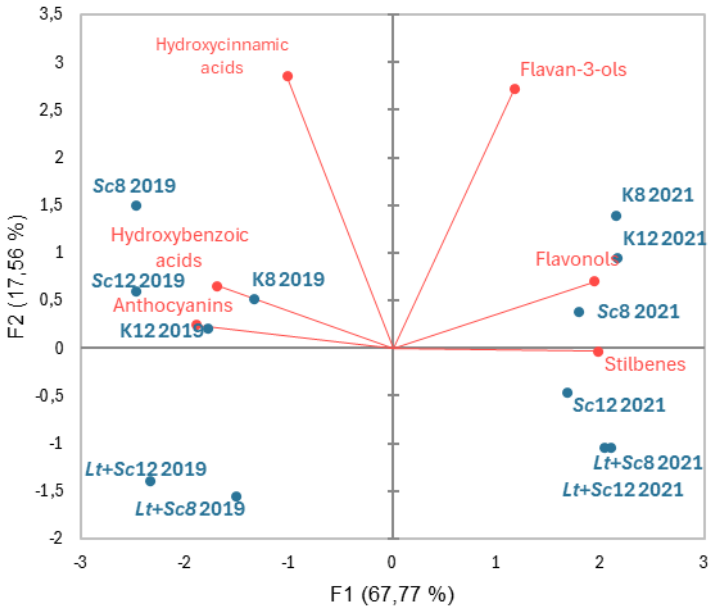
Parameter	Maceration length (days)	K	*Lt+Sc*	*Sc*
Alcohol (% *v/v*)	8	11.80 c	11.70 d	11.24 e
	12	12.17 b	11.71 d	12.54 a
Dry extract (g/L)	8	23.73 c	29.10 a	25.60 b
	12	28.06 b	29.43 a	29.60 a
Ash (g/L)	8	3.41 b	3.88 a	3.82 a
	12	3.82 b	3.85 b	4.61 a
Total acidity, as tartaric (g/L)	8	4.82 b	6.90 a	4.25 c
	12	5.52 c	6.47 a	5.10 cd
Volatile acidity, as acetic (g/L)	8	0.37 a	0.35 a	0.32 a
	12	0.32 a	0.29 a	0,35 a
pH	8	3.86 b	3.76 c	4.02 a
	12	3.85 b	3.77 c	4.02 a

Table 2. Physicochemical parameters of *cv*. Trnjak red wines, vintage 2021.

Parameter	Maceration length (days)	K	*Lt+Sc*	*Sc*
Alcohol (% *v/v*)	8	12.10 a	11.03 c	12.04 ab
	12	12.06 ab	11.74 b	12.14 a
Dry extract (g/L)	8	26.13 b	25.67 b	24.60
	12	25.76 b	27.23 a	24.25
Ash (g/L)	8	3.34 b	3.53 a	3.36 b
	12	3.18 c	3.41 ab	3.47 ab
Total acidity, as tartaric (g/L)	8	6.30 c	7.22 b	6.09 d
	12	6.20 cd	7.42 a	5.81 e
Volatile acidity, as acetic (g/L)	8	0.40 c	0.38 c	0.62 a
	12	0.48 b	0.36 c	0.50 b
pH	8	3.43	3.47	3.58
	12	3.53	3.55	3.63

K-control, spontaneous fermentation; *Sc*.- *Saccharomyces cerevisiae*; *Lt.+Sc*.- *Lachancea thermotolerans*+ *Saccharomyces cerevisiae*. Results are expressed as mean value (n=2); different letters (a, b, c, d, e) in each row indicate a statistical difference between treatments (length of maceration and combination of yeasts) with $p \leq 0.05$, based on Duncan's multiple range test.

Vintage significantly influenced these parameters, with generally lower pH values observed in 2021, indicating the strong effect of climatic conditions (Tab. 1 and 2).



K– spontaneous fermentation; *Sc*– *Saccharomyces cerevisiae*; *Lt+Sc*– *sequential fermentation* with *Lachancea thermotolerans* and *Saccharomyces cerevisiae*; 8 and 12- days of maceration

Graph 1. PCA separation of *cv*. Trnjak wines (2019 and 2021) according to fermentation treatments based on phenolic compounds groups

Extended maceration (12 days) significantly increased anthocyanin concentrations, confirming the positive effect of prolonged skin contact on pigment extraction (Fulcrand et al., 2004). Flavan-3-ols generally increased with maceration, although sequential fermentation resulted in lower concentrations compared with *Sc* and K treatments. Sequential fermentation also resulted

in lower concentrations of phenolic acids, indicating that yeast metabolism influences phenolic extraction and transformation processes (Hranilović et al., 2021; Mucalo et al., 2023).

The PCA biplot (Graph 1) explained 85.33% of total variance (F1 = 67.77%, F2 = 17.56%), indicating strong data representation. F1 clearly separated wines according to vintage, confirming that vintage was the dominant factor affecting phenolic composition. Wines from 2021 were associated with higher concentrations of stilbenes, flavan-3-ols and flavonols, whereas 2019 wines were related to hydroxycinnamic acids, hydroxybenzoic acids and anthocyanins. This reflects the strong influence of climatic conditions and grape maturity on phenolic accumulation. F2 further differentiated wines according to fermentation treatment and maceration length. Sequential fermentation (*Lt+Sc*) was associated with stilbenes, while control and *Sc* treatments were more closely related to anthocyanins and phenolic acids. Extended maceration shifted samples toward higher phenolic content. These results emphasized the dominant role of vintage, while fermentation strategy and maceration length acted as secondary but important modulators of Trnjak red wine phenolic composition.

Conclusion

Sequential alcoholic fermentation with *Lachancea thermotolerans+Saccharomyces cerevisiae* significantly influenced the physicochemical and phenolic composition of Trnjak red wines, resulting in lower alcohol concentrations, higher total acidity and reduced pH values. Vintage was identified as the main factor affecting phenolic composition, while extended maceration increased anthocyanin and flavan-3-ol concentrations. Overall, the combined application of sequential fermentation and controlled maceration represents a promising approach for modulating wine composition and improving balance in Trnjak red wines under warm-climate conditions.

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ORIGINAL SCIENTIFIC PAPER

From waste to yield: Can sewage sludge biochar enhance crop production and carbon storage?

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Summary
Municipal sewage sludge represents a challenging waste stream but also contains valuable nutrients such as phosphorus (P). This study evaluated whether sewage sludge biochar can enhance crop production while contributing to soil carbon (C) storage. Results from a three-year maize field experiment showed higher yields in the biochar treatment than in the control, indicating its potential as a recycled P fertilizer. In an incubation experiment, increasing biochar additions raised soil C contents while respiration indicated limited mineralization of the C added. These findings suggest that sewage sludge biochar may support nutrient recycling, crop productivity and soil C storage.

Keywords: sewage sludge biochar, phosphorus recycling, crop yield, soil carbon, circular nutrient management

Introduction

Municipal sewage sludge is an unavoidable by-product of wastewater treatment and its sustainable management remains a major environmental challenge. At the same time, sewage sludge contains considerable amounts of phosphorus (P), an essential and finite nutrient element for crop production (van Dijk et al., 2016). This creates a need for treatment strategies that reduce disposal problems while enabling nutrient recycling. In Germany, for example, recent legislation increasingly requires P recovery from sewage sludge, which is expected to promote thermal treatment pathways.
Among these, pyrolysis represents a promising option because it converts sewage sludge into biochar. Such biochars may serve as recycled P sources and improve soil properties while adding relatively stable carbon compounds to soil organic matter (Lehmann et al., 2011; Zhao et al., 2023). In addition, biochar application has been suggested as a potential strategy for climate change mitigation through soil carbon sequestration (Woolf et al., 2010). However, the combined effects of sewage sludge biochar on crop production and soil carbon dynamics still require further evaluation under practical conditions.
Therefore, this study evaluated sewage sludge biochar as a sustainability-oriented valorization pathway by combining results from a three-year maize field experiment with an incubation study on soil carbon dynamics.

Material and Methods

Sewage sludge biochar was produced from municipal sewage sludge pellets by pyrolysis at approximately 800 °C in an industrial countercurrent screw reactor. The resulting biochar, containing considerable amounts of phosphorus and stable carbon, was subsequently mixed with shredded wheat straw, water, and a liquid by-product from a nearby cabbage fermentation

facility to produce a biochar mixture suitable for field application. The biochar mixture was stored under field-moist conditions for several months prior to application in order to reduce dust formation and improve handling.

A three-year field experiment (2021–2023) was conducted on a sandy Gleysol in Brandenburg, northeastern Germany (52°43′N, 12°17′E). The experiment followed a randomized block design with six replicates and plot sizes of 36 × 40 m. Three phosphorus (P) fertilization treatments were compared: (i) unfertilized control (control), (ii) triple superphosphate (TSP; 40 kg P ha^{-1} yr^{-1}), and (iii) sewage sludge biochar mixture (BC; applied once in 2021 at a rate equivalent to 120 kg total P ha^{-1}). Further details on fertilization management and soil properties are reported in Zacher et al. (2025). Maize (*Zea mays* L., hybrid P8329, Pioneer) was grown as the test crop during all experimental years. Soil samples were collected from 0–20 cm depth at several maize growth stages to determine total and plant-available P concentrations, and crop yields were recorded at harvest.

To assess the effects of sewage sludge biochar on soil carbon dynamics, a laboratory incubation experiment was conducted using soils from three sites differing in texture and organic carbon content (Gülpe, Knockbek, and Swojec). The same sewage sludge biochar mixture used in the field experiment was applied at different rates (0, 0.1, 1, 5, and 10 g per 10 g soil) and incubated for 100 days under controlled conditions.

Results and Discussion

Application of sewage sludge biochar mixture (BC) increased maize yields compared with the unfertilized control in all experimental years (Figure 1), with increases of approximately 10–25% depending on the year. Yield responses varied between years but consistently showed higher values in the BC treatment. In 2022 and 2023, BC produced the highest yields among the tested fertilization treatments. These findings indicate that sewage sludge biochar can contribute to improved crop productivity under field conditions.

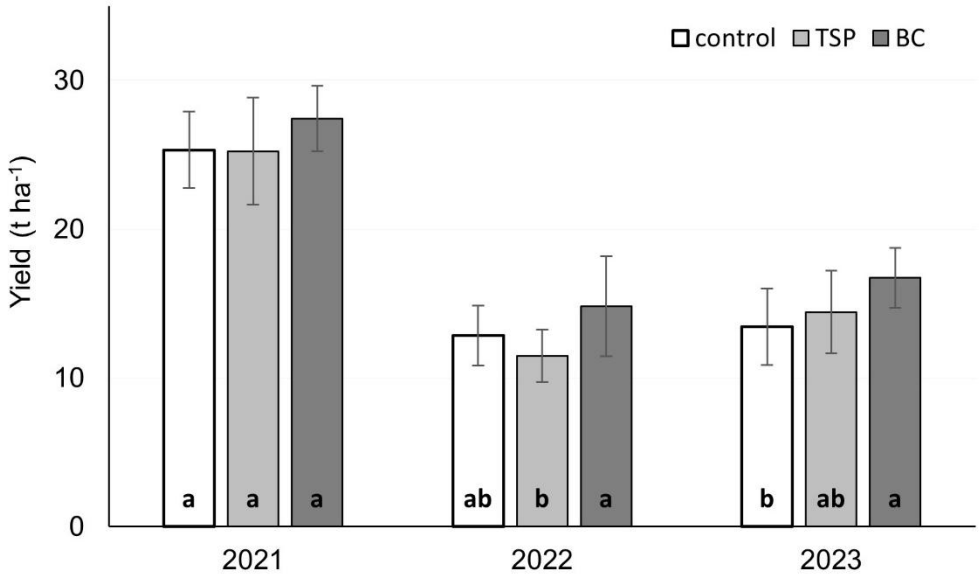

Figure 1. Maize aboveground biomass yield (fresh matter basis) under different phosphorus fertilization treatments (Control, TSP, and sewage sludge biochar mixture, BC) during three experimental years (2021–2023). Error bars represent standard deviation (n = 6). Different letters indicate significant differences between treatments ($p \leq 0.1$). Adapted from Zacher et al. (2025).

The observed yield increase is likely related to the phosphorus (P) fertilization effect of sewage sludge biochar. Municipal sewage sludge contains considerable amounts of P, and pyrolysis

converts this resource into a recyclable fertilizer material. In the field experiment underlying this contribution, BC increased both total and plant-available soil P concentrations as well as plant P uptake compared with the unfertilized control (Zacher et al., 2025), demonstrating its potential as a recycled P fertilizer. Recycling P from sewage sludge is increasingly important because phosphate rock is a finite resource essential for global food production (van Dijk et al., 2016). The results therefore support the concept of sewage sludge biochar as a strategy for closing nutrient cycles in agricultural systems.

In addition to its fertilization effect, sewage sludge biochar also influenced soil carbon dynamics. In the incubation experiment, soil carbon contents after 100 days (C_T100) increased consistently with increasing biochar additions in all three investigated soils (Figure 2), ranging from approximately 10–25 g kg^{-1} in the control treatments to about 120–130 g kg^{-1} at the highest biochar addition. Although the soils differed in texture and initial carbon contents, the response to biochar addition followed a similar pattern across sites. Respiration measurements during the incubation indicated only limited mineralization of the added biochar carbon, suggesting a high short-term stability of the material (data not shown). The observed increase in soil carbon therefore reflects the accumulation of relatively stable pyrogenic carbon derived from the biochar material.

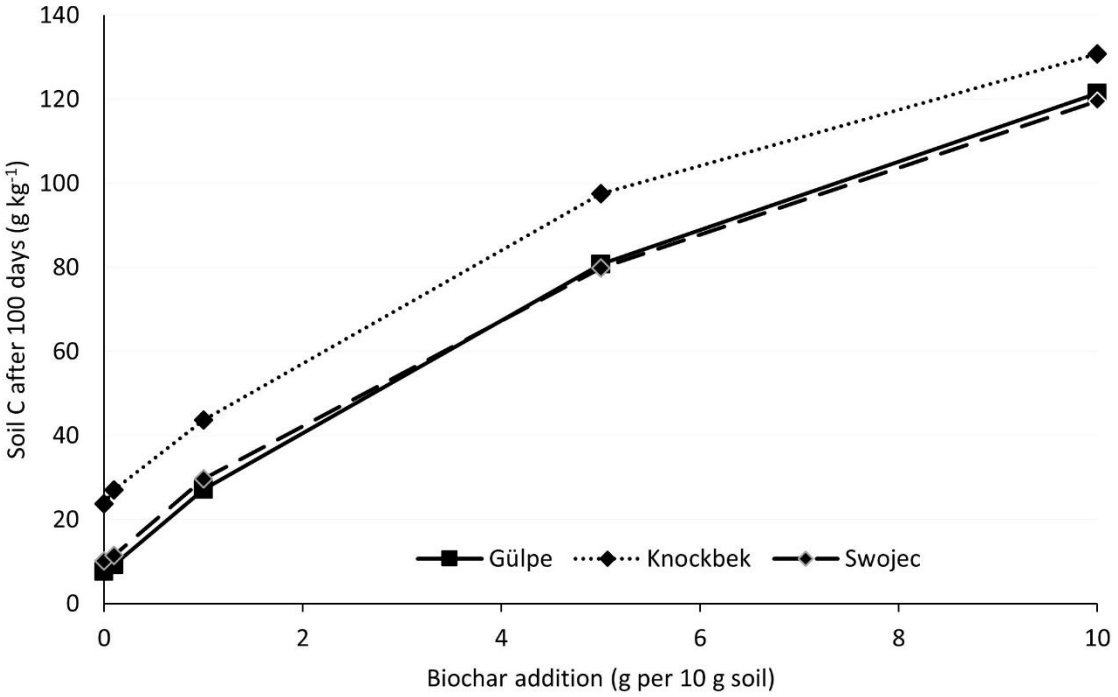

Figure 2. Soil carbon content after 100 days of incubation (C_T100) in soils amended with increasing amounts of sewage sludge biochar. Results are shown for three soils differing in texture and organic carbon content (Gülpe, Knockbek, and Swojec).

Biochar is known to contain relatively stable aromatic carbon structures formed during pyrolysis that can persist in soils for extended periods (Lehmann et al., 2011). Consequently, biochar application has been proposed as a potential strategy for enhancing soil carbon storage and contributing to climate change mitigation (Woolf et al., 2010). The results of the incubation experiment support this concept by demonstrating a clear increase in soil carbon contents with increasing biochar additions.

Conclusion

The results demonstrate that sewage sludge biochar can represent a promising pathway for the sustainable utilization of municipal waste streams. In the field experiment, the application of sewage sludge biochar mixture increased maize yields compared with the unfertilized control, indicating its potential as a recycled phosphorus fertilizer and general soil amendment. The incubation experiment further showed a consistent increase in soil carbon contents with increasing biochar additions across different soils. These findings suggest that converting sewage sludge into biochar may simultaneously contribute to nutrient recycling, improved crop production, and enhanced soil carbon storage in agricultural soils. This approach may become increasingly relevant where regulatory frameworks promote sustainable recycling pathways for municipal sewage sludge.

Acknowledgement

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Session 2

Animal Production and Food Quality

EXPERT PAPER

Milk productions in Bosnia and Herzegovina - challenges in the future

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Summary
Milk production in Bosnia and Herzegovina faces numerous challenges. The number of milk producers is decreasing, as well as the number of dairy cows. In the last 25 years, the number of dairy cows has decreased by 70 and currently there are around 170,000 of them. On the other hand, production per head has increased and now amounts to around 3,500 kg of milk, but this is still insufficient if we look at the European average. Unfortunately, faced with a number of challenges, manufacturers struggle with it more and more. On the other hand, the import of dairy products is growing year by year and exceed 100 million KM, because domestic producers are less and less competitive on the market. One of the possibilities in the future would be to start a small-scale cheese production and produce traditional products. In the future, more importance should be given to longer-lasting dairy products and value-added products. So traditional production should be preserved, and measures should be taken to standardize and protect the geographical origin and originality of some types of cheese.

Keywords: milk productions, cheese, cows

Introduction

One of the important branches of agricultural production in Bosnia and Herzegovina is milk productions, with significant potential for increasing the country's economic stability. The tradition in this sector is quite long whit many "domestic" brands, which are poorly used.
According to research by Krilić et al. (2022), although dairying is a strategically important sector for BiH, the sector suffers from low efficiency and weak coordination among actors in the value chain. A similar conclusion was reached by Vuković (2019), who in his work analyzes the competitiveness of the milk sector in BiH and points to very low competitiveness, which is reflected in low efficiency and poor organization. According to USDA, the average milk production per cow in BiH is 2,612 liters, which is significantly below the EU average, while more than 70% of farms in BiH are engaged in production with less than 5 cows (USDA, 2020). These data show the low productivity of dairy farms, the small herd size is limiting factors in use and application of modern technologies (Krstić, 2018). Many milk producers sell their products on local markets, while they are unable to increase the production and export of dairy products (Jovanović 2020). In addition, the informal sector in BiH has a large share in total production, which further complicates the monitoring of food quality and safety (FAS, 2020). Similar situation is also in the milk market in the region of developing country, were informal markets still play a dominant role in the distribution of dairy products (Kanire et al., 2024). In addition, the informal sector in BiH has a large share in the total production, which further complicates the monitoring of food quality and safety (FAS, 2020). There are 35 dairies in BiH and all of them work below their capacity, with only a third of the maximum capacity of 2 million liters per day (SEE Industry, 2021). Milk and milk products are placed on the European market by 11 dairies (Ministry of Foreign Affairs and Economic Relations, 2024). According to the available data, in 2020 there were 35 entities registered in Bosnia and Herzegovina that deal with the

processing of raw milk and with a processing capacity of 450 million liters (Komora BiH, 2024). Most of the products that are placed on the market are of low added value, such as UHT milk, while products with a higher added value, such as aged cheese, are mostly imported (EBRD, 2018).

Petrović (2021) stated that the fragmentation of the milk market in Bosnia and Herzegovina and weaknesses in product distribution make it difficult to position domestic products on the wider market, while informal markets, additionally negatively affect product quality and safety. Miljković et al. (2022) suggest that the development of institutional mechanisms could enable better connection and coordination of actors within the value chain, which would lead to cost reduction and improvement. According to the analysis of Vuković (2019) and also Savić (2020), the sector faces problems in organization and the lack of associations and clusters that could improve cooperation between actors. In recent years, the dairy sector in Bosnia and Herzegovina has also faced specific challenges related to the labor market, where high unemployment people, but also the migration of young people to cities, make the availability of labor on farms a problem (Gajić, 2021). In addition, dairies struggle with financial problems, because many cannot invest in the modernization of production, which limits their competitiveness compared to dairies from EU countries and the surrounding area. (Matić and Kolega, 2021). This approach can contribute to better coordination and strengthening the competitiveness of the dairy sector in BiH (Stojanović, 2021). Despite all the challenges, dairy farming in Bosnia and Herzegovina has potential for growth, and proper management of the value chain could significantly contribute to strengthening the sector's competitiveness and improving economic performance (Budimir, 2025). With the modernization and improvement of existing capacities, there is an opportunity to increase production, better product quality and strengthen the market position (Padjeni, 2018). This paper analyzes the current structure of the dairy industry in Bosnia and Herzegovina and trends in dairy cow number and milk production.

Material and Methods

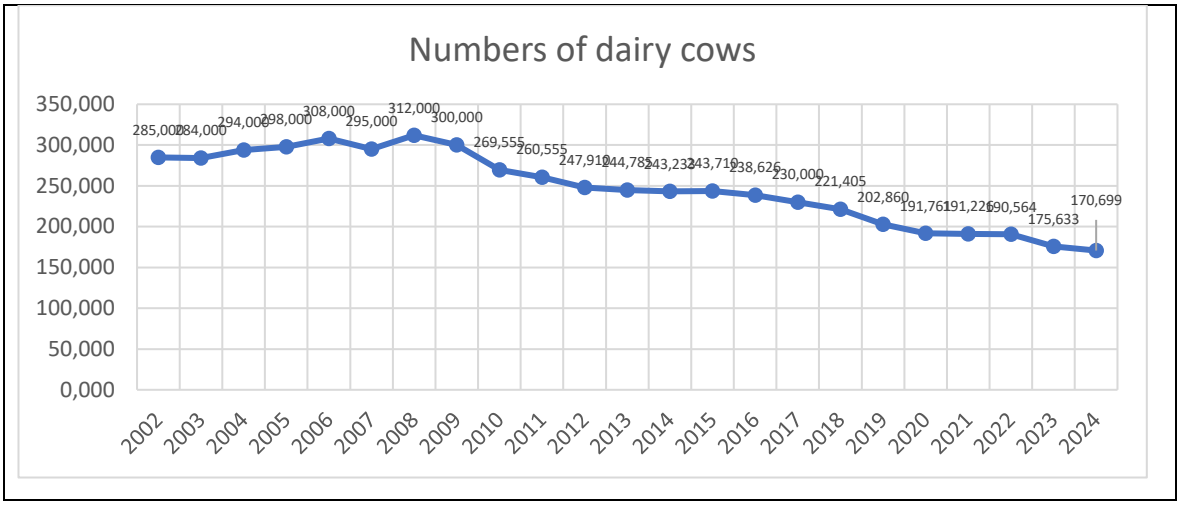
The research is based on the analysis of secondary data from available sources, including reports of international organizations, academic papers and official statistics. One of the problem here is the complexity of the country's organization, so the sources of data are different, and therefore sometimes contradictory, which should all be considered. Bosnia and Herzegovina was supposed to have a list of agricultural holdings in 2023, which was not implemented, which would certainly give a much clearer picture of the state of agricultural production. Available data from the Ministry of Foreign Trade and Economic Relations, the Agency for Statistics of Bosnia and Herzegovina, the Federal Institute for Statistics of the FBiH, the Institute of Statistics RS were used for the analysis and preparation of the report. In creating this report, we used the recommendations from the Guide for consultation and dialogue of civil society members to strengthen the position of farmers in the value chain, which was created as part of the Farm to Fork Academy project. A total of five consultative meetings were held with producers, representatives, producers, representatives of cooperatives working in the dairy sector, buyers (large and small dairies). Interviews were also held with representatives of advisory services and the academic community. After interviews and consultations, data processing was done.

Results and Discussion

The production of raw fresh milk in Bosnia and Herzegovina represents one of the most important livestock production branches and is one of the leaders in the food industry.

According to available data, before the civil war, the number of dairy cows in BiH in 1990 was 623,000. After that, there was a decrease in the number of dairy cows due to the war. In

the period from 2002 to 2008, according to the data of the Statistical Office, there was a continuous increase in the number of milking cows. So that the largest number of milking cows in Bosnia and Herzegovina was registered in 2008 (312,000) (Graph 1.). Since 2008, the number of dairy cows has been in constant decline, so according to the data, the total number of dairy cows in 2024 in BiH is 170,669.



Graph 1. Numbers of dairy cows in BiH from 2002 till 2024
(Source: Federal Statistical Office (2025), Republican Statistical Office of Republika Srpska (2025))

At the beginning of 2026, several farms in the republic Srpska entity were closed due to reduced milk purchases from the dairy industry. Reduced purchase of fresh milk and increased production costs, a further decrease in the number of milk producers, and thus the number of dairy cows, is expected this year (Table 1.).
The production of raw milk in the last couple of years has been more or less stable at 600 million liters, about 60 percent self-sufficiency in milk needs. 7,000 producers in Bosnia and Herzegovina produce milk for the market. The number of milk producers has decreased by 80 percent in the last 20 years.

Table 1. Total milk production and average milk production per cow in Bosnia and Herzegovina in period between 2020 and 2025

Year	2020	2021	2022	2023	2024	2025
Cow milk (million kg)	609.63	522.78	542.9	562.22	581.87	597.58
Average production per head	3179	2734	2849	3201	3409	3524

Unfortunately, milk production in Bosnia and Herzegovina is still underdeveloped, unorganized and there is a lack of capacities in the processing industry, related to products of added value. In addition to this with increased investments, individual milk producers invest additional funds in the development of their farms and their modernization. It is positive that, especially the correction criteria in accordance with the requirements of international standards and quality regulations.
The dairy industry, which has about 23 dairies (data on the number of processing capacities varies up to 20-35), of which 11 export products to the European market, generates an annual income of more than 500 million KM and employs about 2,000 people. It is estimated that during the year 2025, around 597 million liters of raw cow's milk were produced, and the

production recorded a slight increase of 2%. In the year 2026, we can expect a decrease in the purchase of milk by some 15-20%, because there are big problems with surplus milk in the dairy industry. In parallel with that, a decrease in the number of livestock and the number of farms that deliver fresh milk can be expected.
The foreign trade balance of milk and milk products was negative during the observed period it amounted to about 100 million KM and is 10% lower, given that the growth of exports was recorded while imports remained at almost the same value. Milk is the most important product of the dairy industry in BiH and an export product that achieves a surplus in foreign trade. The share of milk in the total export of milk and milk products from Bosnia and Herzegovina to the global market is about 55%, while on the import side, this share is much smaller, amounting to only 8%.

Conclusion

The raw milk production development program in Bosnia and Herzegovina should be based on four categories of producers: small dairy herds with an average of up to 5 heads in the herd have no prerequisites or motivation for business advancement. Modern conventional production systems on adapted smaller farms with an average of 15 to 20 dairy cows in the herd. It is estimated that an average production of 4,500 to 5,500 liters of milk per cow per year could be achieved on these farms. A system of specialized family dairy farms with 40 to 50 cows in the herd. On these farms, the production of 5,500 to 6,500 liters of milk per cow per year could be achieved. Large milking cow farms (with more than 100 cows in the herd), which could ensure milk production of more than 6,000 liters per cow per year.
Milk processing should be primarily focused on cheaper and better-quality products. This is possible with new technologies, especially in the way of keeping and treating animals, nutrition, hygiene, etc. Preference should be given to systems that maximize the use of cheaper inputs - fodder from lawns and arable crop rotation. The intensity of milk production per head should be contrasted with intensity, or profit per unit of area, or invested capital.
Cheese production is mainly in smaller processing facilities. In the future, more importance should be given to longer-lasting dairy products and value-added products. So traditional production should be preserved, and measures should be taken to standardize and protect the geographical origin and originality of some types of cheese.
In the markets, cheese and cream are mainly sold as domestic traditional products

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ORIGINAL SCIENTIFIC PAPER

Correlation of somatic cell count and casein and whey protein content in milk

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Summary
Mastitis affects the udder synthesis ability, and somatic cell count (SCC) in milk is one of the key indicators of it. This research aimed to determine the correlation between the SCC and the content of milk proteins. A total of 10127 milk samples from four farms were analyzed on Combifoss. The research determined that the average protein content of milk samples was 3.45%, with the average SCC of 464,820/ml. A positive statistically significant correlation was found between SCC and total protein content, as well as casein and whey protein. While only the correlation between SCC and the proportion of casein in total protein was negative and statistically significant.

Keywords: somatic cells, total proteins, casein, whey protein

Introduction

Milk is a complex biological system primarily composed of water, macronutrients, micronutrients, and bioactive components, which together determine its nutritional value and suitability for dairy product production such as cheese, yogurt and butter (Acquavia et al., 2025; Górska-Warsewicz et al., 2019).

Milk proteins are complex molecules where casein represents about 80% of total milk proteins, while whey proteins represent the remaining 20%. Casein is the primary protein in milk, essential for cheese production because it forms the structural matrix of curds. Through coagulation (induced by acid or rennet), casein micelles aggregate to trap fat and moisture, determining cheese yield, texture, and meltability. Its breakdown during ripening determines flavor and texture development (Glantz et al., 2010).

In the milk of a healthy udder, the number of somatic cells varies from 20,000-200,000/ml of milk. According to Vishnoi et al. (2007) and Dang et al. (2008), in the milk of cows and buffaloes, the maximum permissible number of somatic cells in milk is up to 200,000/ml of milk. In inflammatory processes of the udder, the number of cells may increase to a lesser or greater extent. Increased SCC leads to significant changes in the physicochemical and microbiological properties of milk. First, there is an increase in the protein content of milk, especially serum proteins and enzymes released from lysed cells, such as lactoperoxidase and lysozyme. Also, the activity of ribonucleases and proteases can cause partial degradation of milk proteins, which affects the quality and stability of milk in further processing (Alhussien and Dang, 2018).

Moradi et al. (2021) point out that the enzymatic activity accompanying the increased SCC leads to the degradation of milk proteins and a deterioration in the ability of milk to coagulate, which results in lower yield and poorer quality of cheese. Similarly, Ahmed et al. (2021) indicate that milk with increased SCC exhibits impaired technological properties due to changes in the ratio of casein to whey proteins, as well as a tendency towards a weaker texture and instability during fermentation. Danieli et al. (2025) also state that milk with

high SCC has a reduced suitability for industrial processing, especially in the production of cheese and products with a longer shelf life.
This research aimed to determine the correlations between the somatic cell count and the content of total protein, as well as casein and whey protein in the analysed milk samples.

Material and Methods

Milk samples were taken as part of regular milk control (ICAR, 2019), from four Holstein-Friesian cow farms in Vojvodina. A total of 10,127 milk samples were collected. The milk samples were collected between October 2023 and September 2024. After collection, the milk samples were preserved with bronopol and stored in a refrigerator at a temperature of 4 °C, and then transported cold (up to 8 °C) to the Laboratory for Milk Quality Control, where they were analyzed.
Milk samples were analyzed on a MilcoScan™ FT + 6000 instrument using Fourier transform infrared spectrophotometry (ISO 9622:2020) in the accredited laboratory in accordance with the international standard ISO 17025:2017 and ICAR guidelines (2020). Whey protein content (WP %) was obtained as the difference between total protein and casein content.
Somatic cell count (SCC) was determined using a FOSSOMATIC FC instrument using flow cytometry (ISO 13366-2:2008). For the statistical analysis of SCC data the absolute values were transformed into somatic cell linear scores (Log 2 SCC) by applying the following equation (Sant' Anna and Paranhos da Costa, 2011): Log 2 SCC = log2 (SCC/100.000) + 3. Logarithmic transformations are the most appropriate for the SCC data because they yield normality and homogeneity of the variances, enabling the execution of statistical analysis taking into account the above assumptions (Ali and Shook, 1980).
Based on the SCC, the samples were divided into 11 groups in order to better understand the impact of SCC on the observed milk parameters There were differences of 100,000 SCC/ml between the groups, where the first group had less than 100,000 SCC/ml, while the last group had more than 1,000,000 SCC/ml.
The average values and variability of examined traits as well as the effect of the factor on mentioned traits were studied by means of the PROC UNIVARIATE and PROC GLM procedures within the Statistic software package (ver. 14 Stat-Soft Company, 2020). Post-hoc analysis (Duncan test) was used to determine the statistically significant differences between the mean values of different groups, with a significance level at $P < 0.05$.
Finally, the correlation between SCC and content of total proteins, casein and whey proteins, and between casein proportion in total proteins, and whey proteins proportion in total proteins, was performed using the correlation procedure (Statistic. 14). For all parameters, model effects were declared significant at $P<0.01$.

Results and Discussion

The average values of the analyzed milk samples are shown in Table 1. The following statistical indicators were calculated for the tested parameters: mean, minimum, maximum, standard deviation, and coefficient of variation.

Table 1. Average values of the analyzed milk samples

Parameter	N	Mean	Min	Max	SD	CV
Fat (%)	10127	3.78	2.01	7.93	0.92	24.23
Proteins (%)	10127	3.45	2.15	4.98	0.45	12.97
Lactose (%)	10127	4.78	4.01	5.50	0.23	4.91
SNF (%)	10127	8.94	7.22	10.57	0.47	5.23

SCC (*1000/ml)	10127	464.82	10.00	9903.00	911.14	196.02
Log SCC	1027	3.87	-0.32	9.63	1.88	48.53
Urea (mg/dl)	10127	24.80	1.10	63.00	9.04	36.46
Casein (%)	10127	2.66	1.55	3.97	0.37	14.00
Whey proteins (%)	10127	0.78	0.520	1.38	0.11	13.90
Casein proportion in TP (%)	10127	77.19	67.44	82.18	2.18	2.83
WP proportion in TP (%)	10127	22.80	17.82	32.56	2.18	9.57

SD-Standard deviation, CV-Coefficient of variation, SCC – somatic cell count, SNF - solids non-fat, *TP – total protein, WP – whey protein*

In Table 1, it can be seen that the average milk fat content (3.78%) is within the range for the Holstein-Friesian breed (3.5–4.2%) (Glantz et al., 2009; Palladino et al., 2010), while the high value of the coefficient of variability (> 20%) is due to differences between farms, diet, lactation period, and health status of the herds. The average protein content (3.45%) is in line with the average for Holstein-Friesian cow milk (3.1–3.5%) (Glantz et al., 2009; Lim et al., 2020). Milk obtained from healthy cows contains about 4.6–4.9% lactose (Yoo et al., 2019), and the average lactose content (4.78%) in this study indicates an average good health status of the herd. The average solids non-fat (SNF) content (8.94%) is in accordance with the standard for cow's milk according to Codex Alimentarius and according to the Regulation of the Republic of Serbia on the quality of raw milk (2017), the minimum SNF is 8.5%. The average SCC in this study was 464,820/ml. This value is higher than the results reported by Litwińczuk et al. (2011), who recorded average values in the range of about 200,000 to 300,000/ml in healthy animals. The high coefficient of variation of SCC (196%) and also log SCC (48.53%) indicates large differences in somatic cell counts between samples/animals. The average urea content in milk was 24.80 mg/dl, which is within the optimal content of 20–30 mg/dl (Yoo et al., 2019; Lim et al., 2020). Values below 10 or above 40 mg/dl may indicate an unbalanced protein-energy ratio in the diet. The proportion of casein in total protein was 77.19%, and the proportion of whey protein in total protein was 22.80%. Maurmayr et al. (2018) reported a proportion of casein in total protein of 76–80%.

Table 2 shows the average values of the proteins, by SCC groups. Analysis of variance showed a statistically significant difference (* $p<0.05$; ** $p<0.01$) in the content of observed milk components by SCC groups, while the Duncan test determined statistically significant differences ($p<0.05$) between individual groups.

Table 2. Average content and proportion of proteins by SCC groups

SCC group	N	Proteins (%)	Casein (%)	WP (%)	Casein proportion in TP (%)	WP proportion in TP (%)
1	3423	3.31^{a}	2.56^{a}	0.75^{a}	77.21^{ab}	22.79^{ab}
2	1993	3.44^{b}	2.66^{b}	0.78^{b}	77.33^{b}	22.67^{b}
3	1243	3.47^{bc}	2.68^{bc}	0.79^{bc}	77.16^{ab}	22.84^{ab}
4	718	3.48^{bc}	2.69^{bc}	0.79^{b}	77.12^{ab}	22.88^{ab}
5	419	3.59^{d}	2.77^{d}	0.82^{d}	77.12^{ab}	22.88^{ab}
6	366	3.52^{bd}	2.72^{bd}	0.80^{be}	77.31^{b}	22.69^{b}
7	229	3.59^{d}	2.77^{d}	0.82^{d}	77.13^{ab}	22.87^{ab}
8	198	3.55^{bcd}	2.75^{cd}	0.81^{cde}	77.26^{b}	22.74^{b}
9	159	3.61^{d}	2.78^{d}	0.83^{d}	76.99^{ab}	23.01^{ab}
10	136	3.57^{d}	2.76^{d}	0.81^{cde}	77.33^{b}	22.67^{b}
11	1067	3.60^{d}	2.77^{d}	0.83^{d}	76.87^{a}	23.13^{a}
F		**59,70****	**48,1****	**62,8****	**4****	**3.6****

SCC –somatic cell count, TP – total proteins, WP – whey proteins; $^{a, b, c, d}$ *– Mean values within the same column with different labels differ significantly (P<0,05); Significant differences: * P<0.05; ** P<0.01; NS, P>0,05= Insignificant*

In Table 2, the results indicate that with the increase in SCC, the content of total proteins increases, primarily as a result of the increased proportion of serum (non-casein) proteins. However, these changes do not indicate an improvement in milk quality, since the proportion of casein does not increase. The results in Table 2 are in accordance with the data of other authors (Litwińczuk et al., 2011; Moradi et al., 2021; Ahmed et al., 2021) who have researched this topic. Litwińczuk et al. (2011) state that an increase in SCC leads to a change in the protein fraction, especially a decrease in casein and an increase in serum proteins, which directly affects the technological quality of milk.
The coefficient of correlation between the SCC and Log SCC with the content of milk proteins, casein, and whey proteins is presented in Table 3

Table 3. Correlation of SCC per ml and Log SCC with the content of individual milk components

Component	SCC per ml	Log SCC
Proteins (%)	0.1443**	0.2523**
Casein (%)	0.1225**	0.2269**
Whey proteins (%)	0.1716**	0.2577**
Casein proportion in TP (%)	-0.0516**	-0.0345**
WP proportion in TP (%)	0.0516**	0.0345**

SCC –somatic cell count, TP – total proteins, WP – whey proteins,
*Significant differences: * P<0.05; ** P<0.01*

Protein content and SCC show a statistically significant positive correlation (0.1443*), which means that the increase in SCC (Log SCC) is associated with an increase in total protein content. However, this is a consequence of the increase in the proportion of whey proteins, as shown by the positive statistically significant correlation with SCC (0.0516*) and also with Log SCC (0.0345*), whilst the correlation between SCC and Log SCC with the proportion of casein is negative and statistically significant (-0.0516*; -0.0345*). Similar results have been obtained by other authors (Bochniarz et al., 2023; Stanek et al., 2024; Sviridenko et al., 2024; Costa et al., 2025). A weak positive statistically significant correlation (0.1225*) was found between casein content and SCC, which indicates that casein content may increase, but the percentage of casein in total protein decreases.

Conclusion

Based on the results of this study, it can be concluded that there is a positive correlation between SCC and the content of total proteins, casein, and whey proteins. Also, the proportion of whey proteins in total proteins had a statistically significant positive correlation with SCC. A statistically significant negative correlation was found, only between SCC and the proportion of casein in total proteins.
This study highlights the importance of monitoring SCC, as elevated levels impair the technological properties of milk—specifically its coagulability, protein stability, and yield in cheese and dairy production.

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ORIGINAL SCIENTIFIC PAPER

Morphological variability and population structure of *Apis mellifera carnica* in the Republic of Srpska

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Summary
Geometric morphometric analysis of honey bee forewings was used to assess morphological variability and population structure of *Apis mellifera carnica* in the Republic of Srpska. Samples were collected from 15 breeding centers, including 8 colonies per location and 348 worker bees (696 forewings). A total of 19 homologous landmarks, defined as intersection points of forewing veins, were used for shape analysis. The results confirm the dominance of *A. m. carnica* and indicate varying levels of similarity with A. m. cecropia, suggesting introgression. A gradient of introgression was observed among populations. Continuous monitoring and controlled breeding are recommended.

Keywords: *Apis mellifera carnica*, geometric morphometrics, forewing, population structure, introgression

Introduction

The western honey bee (*Apis mellifera*) is one of the most economically and ecologically important insect species due to its essential role in pollination and the production of high-value apicultural products (Southwick and Southwick, 1992; Khalifa et al., 2021; Feketéné Ferenczi et al., 2023). Among its numerous subspecies, the Carniolan honey bee (*Apis mellifera carnica*) is native to southeastern Europe, with its distribution traditionally limited to the region between the Alps, Carpathians, and Dinaric mountains (Ruttner, 1988; Lukić et al., 2024), and is well adapted to continental and subcontinental climatic conditions (Kovačić et al., 2020; Zelenina et al., 2025). It is characterized by favorable behavioral and physiological traits, such as low aggressiveness, rapid spring population development, and efficient foraging, although these may vary depending on genetic background and environmental conditions (Ruttner, 1988; Uzunov et al., 2014; Kovačić et al., 2020). Reliable identification of honey bee subspecies requires robust analytical methods. Traditional morphometric approaches have long been used to distinguish subspecies based on measurable traits and multivariate statistics (Ruttner, 1988; Meixner et al., 2013). However, geometric morphometrics has emerged as a more advanced method, capturing shape variation through the spatial configuration of homologous landmarks (Bookstein, 1991; Zelditch et al., 2012). In honey bee research, this approach is commonly based on wing venation patterns, where vein intersection points serve as landmarks for describing morphological variation (Francoy et al., 2008). The forewing is particularly suitable for such analyses due to its stability and taxonomic relevance (Ruttner, 1988; Francoy et al., 2008). Typically, 18–20 homologous landmarks are used, enabling precise discrimination among subspecies and detection of subtle morphological differences, including those associated with hybridization (Francoy et al., 2008; Oleksa and Tofilski, 2015; Nawrocka et al., 2018). Geometric morphometric methods are often supported by specialized software such as TPSdig, DrawWing, and more recently IdentiFly, which allow efficient and standardized landmark digitization and classification (Tofilski, 2004; Nawrocka et al., 2018). These

approaches have become important tools not only in taxonomy, but also in studies of biodiversity, phylogeny, and evolutionary processes (Tan et al., 2026). Moreover, both traditional and geometric morphometric methods have proven effective and comparable in subspecies identification (Meixner et al., 2013; Oleksa and Tofilski, 2015). In addition to taxonomic applications, accurate identification of honey bee subspecies is crucial for biodiversity conservation, breeding programs, and the prevention of uncontrolled hybridization (De la Rúa et al., 2009; Meixner et al., 2013). Despite the widespread use of geometric morphometrics, data on the population structure and potential hybridization of *Apis mellifera carnica* remain limited in specific regional contexts, particularly in areas influenced by intensive beekeeping practices and queen trade.

The aim of this study is to evaluate morphological variability and population structure of Apis mellifera carnica, with a focus on detecting potential hybridization and assessing differentiation among sampled populations.

Material and Methods

Honeybee samples were collected from 15 registered queen breeding and selection centres in the Republic of Srpska and analyzed at the Entomology Laboratory of Genetic Resources Institute, University of Banja Luka. At each location, 8 colonies were included in the study. Specimens were preserved in 70% ethanol and stored at −5°C until analysis.

Worker bees were sampled from each location for morphometric analysis. For the majority of locations, 20 worker bees were analyzed, while for selected locations (Kozarska Dubica, Kotor Varoš, Milići, Zvornik 2, Zvornik 5, and Bratunac), 28 individuals were included. In total, 348 bees were analyzed.

From each individual, both forewings were removed and analyzed, resulting in a total of 696 forewings. Forewings were air-dried and mounted between microscope slides. Images were captured using a 25 mm lens camera (RICOH FL-CC2514-2M), stored in PNG format, and processed using a standardized protocol.

Geometric morphometric analysis was performed based on forewing venation patterns using 19 homologous landmarks, defined as vein intersection points on the forewing. Landmark digitization and analysis were conducted in IdentiFly software, with each image processed individually.

Canonical Variate Analysis (CVA) was performed in IdentiFly to assess morphological differentiation and maximize separation between subspecies groups.

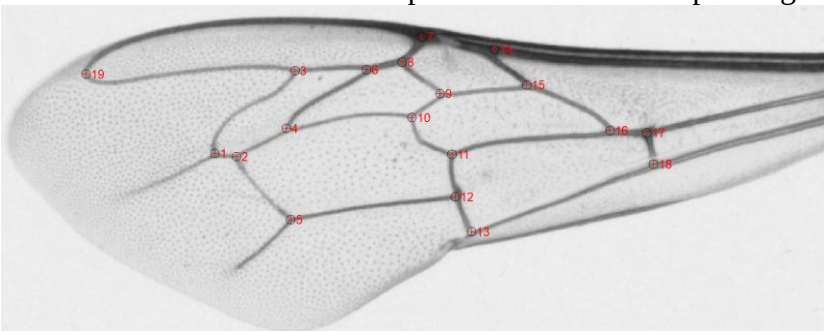

Figure 1. Forewing of *Apis mellifera* with 19 homologous landmarks used in geometric morphometric analysis.

Results and Discussion

The results of the geometric morphometric analysis of forewing venation, based on 19 homologous landmarks, indicate that *Apis mellifera carnica* represents the dominant subspecies in the analyzed populations, as reflected by consistently high classification probabilities across most samples. However, variation among localities was observed, with

certain populations showing lower conformity to the reference carnica profile and increased similarity with *Apis mellifera cecropia*. These findings, suggest the presence of morphological variability and potential introgression within the studied populations.

Table 1. Classification probabilities of honeybee subspecies across breeding centres based on geometric morphometric analysis of forewing venation using 19 homologous landmarks.

Location of the breeding centre	*Apis mellifera carnica*	*Apis mellifera cecropia*	*Apis mellifera ligustica*
Kozarska Dubica	0.008977	**0.036487**	0.000831
Srbac	**0.084172**	0.000358	0.001156
Banja Luka	**0.000300**	0.000179	1.83E-05
Kotor Varoš	**0.160370**	0.000277	0.004193
Petrovo	**0.000824**	6.91E-05	5.85E-05
Milići	**0.003790**	4.19E-17	0.000183
Zvornik 1	0.000322	**0.025270**	3.27E-05
Zvornik 2	**0.006781**	4.11E-17	0.000241
Zvornik 3	2.72E-05	**3.83E-11**	3.44E-05
Zvornik 4	8.80E-06	**0.000424**	1.3536E-05
Zvornik 5	**7.71E-05**	4.42E-10	4.75E-05
Zvornik 6	2.14E-06	**0.355751**	3.49E-05
Bratunac	0.001340	**0.713533**	0.000651
Rudo	**5.02E-06**	3.90E-16	3.05E-07
Trebinje	**2.23E-06**	6.12E-17	1.24E-06

Bold values indicate the highest classification probability for each population.

Table 1 presents the classification probabilities of honey bee subspecies across breeding centres. Although *A. m. carnica* is predominant, several populations show notable probabilities of belonging to *A. m. cecropia*. The highest values are observed in Bratunac (0.7135) and Zvornik 6 (0.3557), indicating a higher level of introgression. Moderate values are recorded in Kozarska Dubica (0.0365) and Zvornik 1 (0.0253), while lower values in locations such as Zvornik 4 indicate low levels of introgression. In contrast, populations such as Rudo, Trebinje and Zvornik 5 exhibit negligible values, indicating high conformity with *A. m. carnica*.

These findings indicate a gradient of introgression, where populations are distributed along a continuum of increasing influence of *A. m. cecropia*. This pattern suggests ongoing gene flow and gradual incorporation of morphological traits from neighboring subspecies, as previously reported in European honey bee populations (De la Rúa et al., 2009; Meixner et al., 2013).

No notable influence of *Apis mellifera ligustica* was detected, as all recorded probabilities for this subspecies remain extremely low across all locations.

The Canonical Variate Analysis (CVA) supports these findings (Figure 2.). Most samples cluster within or near the *A. m. carnica* group, while a smaller number shift toward *A. m. cecropia*, indicating partial overlap. The clear separation between major subspecies confirms the reliability of geometric morphometric analysis for subspecies discrimination (Oleksa and Tofilski, 2015; Francoy et al., 2008).

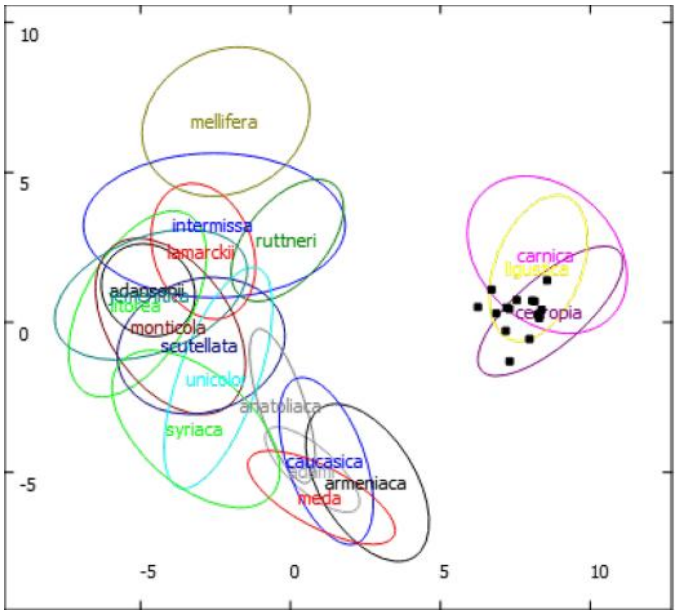

Figure 2. Canonical Variate Analysis (CVA) of honeybee populations based on forewing geometric morphometric data derived from 19 homologous landmarks.

From an ecological perspective, the observed introgression pattern may be associated with environmental and climatic factors, particularly in eastern and warmer regions, indicating increased contact between subspecies (Kovačić et al., 2020; Zelenina et al., 2025).
Overall, the results, based on geometric morphometric analysis of forewing venation using landmark configuration, highlight the presence of ongoing gene flow and emphasize the importance of continuous monitoring and controlled breeding strategies to preserve the genetic integrity of Apis mellifera carnica. Similar patterns of introgression and population structure have been documented both in central and southeastern Europe and in the studied region, confirming gene flow among subspecies influenced by beekeeping practices (Muñoz and De la Rúa, 2021; Péntek-Zakar et al., 2015; Ilić et al., 2025).

Conclusion

The results of this study confirm that *Apis mellifera carnica* is the dominant subspecies in the analyzed populations from the Republic of Srpska. However, varying classification probabilities for *Apis mellifera cecropia* indicate the presence of introgression and a gradient of morphological variability among populations. Populations differ in their degree of conformity, ranging from highly consistent to those with moderate and strong influence of other subspecies, suggesting ongoing gene flow. The negligible presence of *Apis mellifera ligustica* indicates that its influence is currently limited in the studied area. The applied geometric morphometric approach proved to be a reliable tool for detecting subtle morphological differences and assessing population structure. These findings highlight the need for continuous monitoring and the implementation of controlled breeding strategies to preserve the genetic integrity of *Apis mellifera carnica*.

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ORIGINAL SCIENTIFIC PAPER

Morphometric characteristics and body development indices of Lika Pramenka sheep in semi-intensive breeding

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Summary
This research investigates morphometric characteristics and body development indices of Lika Pramenka sheep and compares them with researches performed with similar production lines. The research was carried out on 30 selected adult Lika Pramenka sheep aged 5 years at the Kučan family farm in the region of Lika in Croatia. Morphometric measurements performed for this study were: height at withers, back height, cross height, trunk length, chest width and girth, chest depth, shin circumference, thigh length and width, and head length and width. The following body development indices were also calculated: indices of body condition, anamorphosis, body proportions, chest, chest width, body compactness, muscularity and leg length. There Some of the results of physical measurements were significant positive correlations. Based on the conducted research into morphometric characteristics and body development indices of Lika Pramenka sheep and their comparison with similar sheep breeds, it is concluded that sheep were in good physical condition, which indicated satisfactory nutrition.

Key words: Lika Pramenka sheep, body measurement, body development indices

Introduction

In the Republic of Croatia, there are 482,667 sheep. In the sheep population, there are 23 sheep breeds, 9 of which are indigenous and 14 are conventional. According to the data published by HAPIH (2025), at present there are 64,849 Lika Pramenka sheep bred in Croatia, and 9,428 heads are registered for breeding. The registered Lika Pramenka breeding population consists of 7,825 ewes, 1,409 ewe lambs and 194 breeding rams kept by 74 farmers with an average herd size of 127 heads (HAPIH, 2025). The Lika Pramenka sheep is a Croatian indigenous sheep breed originating from the mountainous areas of Lika and Gorski Kotar. It belongs to a group of combined, medium-developed, resistant and highly adaptable sheep (Mioč et al., 2007a). Their body constitution is solid and strong. The body weight of ewes is 45-55 kg, and of rams 60-70 kg. They have an open fleece of mixed wool, composed of pointed strands. Rams have usually strong horns, ewes are polled. Their fleece is usually white, dotted with larger and smaller black spots. The average annual wool yield is 1.5-3.5 kg. Their fertility is 120-150%. The lambing index is 1.03, and the litter size is 1.06. Milk production in lactation is 120-180 liters. At the age of 3-4 months, lambs reach a body weight of 25-30 kg. The birth weight of male lambs in the performance test is 3.7 kg, the average daily gain is 0.26 kg, and the final body weight at the end of the test is 31.21 kg (HAPIH, 2025). Lika Pramenka sheep is traditionally kept outdoor on pastures as long as weather conditions allow. Previous research into Lika Pramenka sheep indicates an increase in body measurements compared to the older recorded research (Mioč et al., 1998). This was influenced by numerous attempts to improve Lika Pramenka sheep, from the breeding with

numerous meat types of sheep, to the process of merinization with imported French and German merinos of a dual production line (meat-wool; Mioč et al., 2007b). Efforts put into breeding of Lika Pramenka sheep have led to significant investments in the promotion of its products, especially lamb meat. It is important to note that Lika lamb is a protected product in the EU. Furthermore, additional research is required to obtain knowledge about the production potential of Lika Pramenka, and this includes determination of morphometric characteristics and body development indices. The aim of this research is to determine the morphometric characteristics and body development indices of Lika Pramenka sheep and compare them with those of a similar production line.

Material and methods

The research was carried out on 30 selected adult Lika Pramenka sheep kept at the Kučan family farm in the Croatian region of Lika. The sheep were on average 5 years old. The sheep for this research were selected from a herd of almost 800 sheep in semi-intensive breeding. The sheep were about fed 1.8 kg/day of meadow hay and 800 g/day of oat. Body mass was determined using an electronic scale, and body measurements (height at withers-HAW, back height-BH, cross height-CH, trunk length-TRL, chest width-CW, chest depth-CD) were taken using a Lydtin rod. Chest circumference (CC), tibia circumference (TC), thigh length (THL), thigh width (THC), head length (HL) and head width (HW) were taken using a cattle measuring tape. The body condition score (BCS) of sheep was determined according to Russell (1991), with scores ranging from 1 to 5. The anamorphosis index-AI (chest circumference x chest circumference / height at withers) and the body proportion index-BPI ((body mass / height at withers) x 100) were determined according to Chiofalo et al. (2004). Chest index-CI ((chest width / chest depth) × 100), chest width index CWI ((chest width / height at withers) × 100), body compactness index-BCI ((chest circumference / trunk length) × 100), muscularity index-MI ((chest circumference / height at withers) × 100) and leg length index-LLI ((height at the withers – chest depth / height at withers) × 100) were determined according to Ćinkulova et al. (2003). Statistical data processing was performed in SAS® 9.4 (SAS Institute Inc., Cary, NC, USA). The results were obtained using the MEANS procedure and are expressed as arithmetic mean (Mean), standard deviation (Sd), minimum and maximum values, and coefficient of variation (CV). Pearson's correlation coefficients were used to determine the association between certain variables.

Results and discussion

Table 1 overviews the morphometric body measurements of Lika Pramenka sheep expressed through body measurements.

Table 1. Descriptive statistics of body measurements of Lika Pramenka sheep

Body measures, cm	Mean	Sd	Minimum	Maximum	CV (%)
Height at withers (HAW)	69.77	1.26	67.00	73.00	1.80
Back height (BH)	67.77	1.84	64.00	72.00	2.71
Trunk length (TRL)	70.67	2.40	65.00	75.00	3.39
Cross height (CH)	68.77	1.76	65.00	72.00	2.57
Chest width (CW)	22.27	1.15	20.00	24.00	5.18
Chest circumference (CC)	102.80	1.66	100.00	106.00	1.62
Tibia circumference (TC)	8.33	0.75	8.00	10.00	8.94
Chest depth (CD)	30.90	2.15	28.00	35.00	6.96
Thigh length (THL)	35.73	1.31	34.00	38.00	3.68
Thigh width (THC)	45.17	2.98	39.00	51.00	6.59
Head length (HL)	24.27	0.77	22.00	26.00	3.18
Head width (HW)	13.70	0.74	12.00	15.00	5.38

Mean- arithmetic mean; Sd- standard deviation; CV – coefficient of variation

Analysis of data obtained on body measurements of Lika Pramenka (Table 1) shows that the highest arithmetic mean value was obtained for chest circumference and trunk length (102.80 and 70.67 cm, respectively), and the least value was obtained for shin circumference and head width (8.33 and 13.70 cm, respectively). The highest coefficient of variation was for tibia circumference and chest depth (8.94 and 6.96 %, respectively), and the smallest coefficient of variation was obtained for chest circumference and height at withers (1.62 and 1.80 %, respectively). In the research by Mioč et al. (1998), adult Lika Pramenka sheep had lower mean value for height at withers, length of body, width of chest, depth of chest, chest circumference and tibia circumference (60.75, 67.35, 16.64, 29.28, 83.38 and 7.48 cm, respectively). Mikulec et al. (1984) also measured Lika Pramenka sheep to determine lower average height at withers (63.10 cm) than the measure established in this research. Adult lactating Dubrovnik Ruda sheep had lower body mass (44.22 and 50.17 kg; Antunović et al., 2019). Novoselec et al. (2020) conducted research on adult Travnik Pramenka sheep and published similar values for the height at withers (69.63 cm) and chest depth (31.40 cm), slightly higher values for the trunk length (74.78 cm), and smaller values for chest circumference (93.61 cm) and chest width (20.15 cm). In comparison to this research, Mioč et al. (1998) also determined lower average body mass of adult Lika Pramenka sheep (49.25 kg). In their research on Tsigai sheep, Antunović et al. (2008) found that adult sheep had higher average body mass (76.60 kg), height at whiters (82.40 cm) and trunk length (93.40 cm) if compared to the results obtained in this research. The results of morphometric body measurements obtained in this research can be linked to the satisfactory nutrition of investigated sheep and to the continuous efforts put into sheep production to obtain better meat quality. According to the Sheep Breeding Program in the Republic of Croatia (Mioč et al. 2011), desirable body measurements and production characteristics of Lika Pramenka sheep range from 45 to 55 kg for body weight, and from 60 to 65 cm for the height at withers.

Table 2. Descriptive statistics of body mass and body development index (BDI) of Lika Pramenka sheep

Indicator	Mean	Sd	Min	Max	CV (%)
Body weight (BW), kg	49.93	3.94	42.00	58.00	7.89
Body condition score (BCS)	3.67	0.37	3.00	4.00	10.16
Anamorphosis index (AI)	151.50	3.71	144.93	160.51	2.45
Body proportion index (BPI)	71.54	5.11	60.87	81.69	7.14
Chest index (CI)	72.33	5.18	58.82	82.14	7.16
Chest width index (CWI)	31.91	1.49	28.99	34.78	4.67
Body compactness index (BCI)	145.65	5.69	133.33	156.92	3.91
Muscularity index (MI)	147.37	2.09	143.66	152.24	1.42
Leg length index (LLI)	55.72	2.78	50.00	60.00	4.98

Mean- arithmetic mean; Sd- standard deviation; CV – coefficient of variation

Analysis of BDI of Lika Pramenka sheep (Table 2) confirmed that the highest indices were noted for AI, MI and BCI (151.50; 147.37 and 145.65). Established BCS of Lika Pramenka sheep pointed out satisfactory body condition. In the research into Tsigai sheep, Antunović et al. (2008) determined higher AI and BPI (166.71 and 92.99) if compared to the established indices of Lika Pramenka in this research. Dubrovnik Ruda sheep had lower BDI (Antunović et al., 2019), as follows: AI (109.92 and 114.55), BCI (128.28 and 131.53) and BCS (2.76 and 3.03), while BPI was similar or slightly higher (68.74 and 77.03). The sheep body condition score is an indisputable indicator of feeding quality and it is a reliable indicator of the nutritional status of sheep (Antunović et al., 2019; Kenyon et al., 2014).

Table 3. Correlations between body measurements of Lika Pramenka sheep

	BW	HAW	BH	TRL	CH	CW	CC	SC	CD	THL	TC	HL	HW
HAW	0.66 <0.001	1.00											
BH	0.61 <0.001	0.61 <0.001	1.00										
TRL	0.30 <0.001	0.29 0.002	0.09 0.329	1.00									
CH	0.27 0.003	0.20 0.025	0.01 0.873	0.06 0.494	1.00								
CW	0.48 <0.001	0.40 <0.001	0.28 0.002	0.47 <0.001	0.158 0.085	1.00							
CC	0.68 <0.001	0.67 <0.001	0.48 <0.001	0.21 0.019	0.06 0.486	0.55 <0.001	1.00						
SC	0.41 <0.001	0.52 <0.001	0.43 <0.001	0.09 0.350	-0.07 0.418	0.38 <0.001	0.48 <0.001	1.00					
CD	0.60 <0.001	0.53 <0.001	0.65 <0.001	0.05 0.619	0.06 0.519	0.36 <0.001	0.53 <0.001	0.38 <0.001	1.00				
THL	0.63 <0.001	0.69 <0.001	0.55 <0.001	0.16 0.087	0.29 0.002	0.43 <0.001	0.54 <0.001	0.49 <0.001	0.52 <0.001	1.00			
TC	0.72 <0.001	0.64 <0.001	0.61 <0.001	0.26 0.004	0.03 0.775	0.46 <0.001	0.57 <0.001	0.54 <0.001	0.62 <0.001	0.70 <0.001	1.00		
HL	0.39 <0.001	0.45 <0.001	0.54 <0.001	-0.09 0.306	- 0.004 0.969	0.18 0.050	0.37 <0.001	0.54 <0.001	0.59 <0.001	0.46 <0.001	0.50 <0.001	1.00	
HW	0.37 <0.001	0.25 0.005	0.41 <0.001	0.02 0.803	-0.20 0.032	0.36 <0.001	0.40 <0.001	0.42 <0.001	0.48 <0.001	0.36 <0.001	0.64 <0.001	0.55 <0.001	1.00

The optimal BCS of sheep is from 2.5 to 3.5 (Kenyon et al., 2014). It is known that BDI and body mass indicate the development of the respiratory and digestive systems, while BPI indicates the development of skeleton in the longitudinal direction (Chiofalo et al. 2004). The stated BDI of Lika Pramenka sheep indicates satisfactory nutrition and body development. Significant positive correlations were established between the most body measurements of sheep (Table 3). The above indicates a significant interdependence in the development of sheep's body, which is understandable given the fact that the research was conducted on adult sheep.

Conclusion

Based on the morphometric characteristics and body development index of Lika Pramenka sheep and their comparison with similar sheep breeds, this research concludes that investigated sheep were in good body condition, which indicated satisfactory nutrition.

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REVIEW ARTICLE

From genotype to product: a phenomic perspective on premium pork products from Croatian indigenous breeds

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Summary
Croatian indigenous pig breeds represent a valuable genetic source for production of premium quality food products. Due to their ability to accumulate fat and their particular lipid metabolism, they are well suited for producing high-quality products derived not only from meat but also from fat, such as dry-cured bacon. However, this potential remains underexplored, especially in the case of the recently recognized Banija spotted pig breed. In this context, a phenomic approach offers a valuable framework for linking the genetic background of these breeds with product quality traits. The aim of this paper is to review the phenomic approach as a basis for establishing this link, while addressing current limitations in the integration of phenomic data into product development and market valorization, and highlighting the need for further research to support the effective positioning of underexplored breeds in premium market segments.

Keywords: pig, genotype, phenomics, meat quality, fat quality, dry-cured bacon

Introduction

Modern food systems are witnessing increasing demand for products that are authentic, traceable and linked to specific geographical origin. This trend is not only driven by consumer preferences, but also by sustainability, animal welfare and environmental impact. In this context, local breeds play a key role in the production of niche products, as their unique production and metabolic characteristics enable products with distinctive composition and sensory quality. Studies have shown that muscle and adipose tissue of indigineous pig breeds exhibit distinctive traits, such as high intramuscular fat content, higher water holding capacity, more pronounced colour, enhanced sensory properties and more favourable fatty acid composition, with a higher proportion of monounsaturated fatty acids compared to modern breeds (Čandek-Potokar et al., 2019, Poklukar et al., 2020). These characteristics, especially the favourable composition of adipose tissue, make local pig breeds particularly suitable for the production of premium products ranging from dry-cured ham to fat-rich products such as dry-cured bacon.

Fat-rich products of animal origin have long been associated with a higher risk of cardiovascular disease due to the presence of a higher proportion of saturated fats (Siri-Tarino et al., 2010). In recent years, however, this view has become more refined and it is today recognized that the health impact of these products depends on factors such as dietary pattern, portion size and frequency of consumption. In addition, elements like traditional processing methods and the specific fatty acid profile may influence their nutritional and health relevance. Nevertheless, fat-rich premium products are primarily appreciated for their distinctive sensory characteristics and cultural or gastronomic value.

High-quality niche products are typically associated with traditional production systems where genotype, feeding and processing technology together shape final quality. In this context, policy and market frameworks play an important role in supporting their development. The European Union emphasizes the importance of conserving animal genetic resources, while quality schemes such as PDO and PGI provide opportunities to differentiate these products and increase their market value. However, despite the positive policy and market framework, scientific and practical work related to the development of products from local pig breeds still has many gaps.

In Croatia research has been mainly focused on established local breeds such as the Black Slavonian and Turopolje pig, including studies on characteristics of their meat, fat and different products (Kušec et al., 2022; Kušec et al., 2025; Latin et al., 2022; Đikić et al., 2003; Karolyi et al., 2024). Nevertheless, these breeds are still not fully understood, especially in relation to production systems and traditional fat-based products. The Banija spotted pig, recognised only in 2018, remains even less explored, especially in terms of its potential for the production of traditional meat products. To date, research on this breed has primarily addressed reproductive traits and pedigree structure (Škorput et al., 2018; Škorput et al., 2023) with only limited studies focusing on carcass composition and meat quality (Luković et al., 2023). In this context, phenomics offers a promising approach for linking biological traits with product quality, thereby providing a scientific basis for the valorisation of products derived from underexplored local pig breeds.

Phenomics and the phenomic approach to product quality

Phenomics can be defined as the systematic measurement of phenotypes at a high resolution, where phenotypic traits are defined as a result of genotype and environmental interactions (Houle et al., 2010). In meat science, the phenomic approach represents a theoretical model for linking biological processes with technological and sensory characteristics of the product, which allows us to shift from descriptive to explanatory and predictive assessment of the quality of meat products (Picard and Gaganua, 2020). In this context, product quality is no longer seen as an isolated phenomenon but rather as a complex structure that emerges from multiple biological layers.

Genomics enables the identification of genetic markers and supports the selection of animals with desirable traits such as growth, stress resistance and fat deposition (Rothschild and Ruvinsky, 2011). However, genomics alone cannot fully explain phenotypic variation, which highlights the importance of functional regulation. Transcriptomics provides insight into gene expression patterns and metabolic regulation, explaining phenotypic differences. Studies on local pig breeds have shown differential expression of genes involved in lipid metabolism and energy regulation, such as SCD, PPARD, ACLY, ADIPOQ and LPIN1, which are associated with intramuscular fat content and fatty acid composition (Muñoz et al., 2018; Poklukar et al., 2020). These molecular mechanisms are further reflected at the lipid level.

Lipidomics provides quantitative analysis of lipid molecules, which are regulated at the transcriptional level. Lipids and fatty acid composition together with their distribution in the carcass are responsible for several meat quality traits, such as texture, water-holding capacity, oxidative stability and potential aroma formation during technological processing. For example, a higher proportion of monounsaturated fatty acids, particularly oleic acid (C18:1n-9), is associated with more favourable texture and sensory properties in dry-cured bacon (Wood et al., 2008; Pugliese and Sirtori, 2012). Advances in analytical techniques, including high-resolution mass spectrometry and nuclear magnetic resonance, allow detailed lipid profiling and improved understanding of underlying biological processes (Li et al., 2022).

Finally, volatilomics translates the chemical composition of the product into its sensory perception. Volatile compounds formed during processing, particularly through lipid oxidation, amino acid degradation and Maillard reactions, contribute to the characteristic aroma of dry-cured products (Toldra, 1998). Their formation reflects interactions between raw material properties, processing conditions and microbial activity.
Therefore, the combination of genomic, transcriptomic, lipidomic and volatilomic data enables the construction of a "genotype-phenotype-product" structure, forming the basis of of the phenomic approach. This integration supports the identification of quality biomarkers and the development of predictive models for meat quality and authenticity. However, despite recent advances, such integrated phenomic approaches are still rarely applied in studies on local breeds and traditional meat products, mainly due to variability in production systems and the lack of standardized methodologies

Role of breed in production of premium products
Traditional premium products rely heavily on the quality of the raw material. Within the framework of phenomics, this dependency can be considered as a direct manifestation of biological processes, where genetic characteristics and regulation of metabolism affect the composition of lipids, which then determines technological characteristics and sensory properties.
Locally adapted pig breeds generally have a high fat content and unique composition of fat tissue, making them suitable for production of dry-cured smoked bacon. Studies on the Black Slavonian pig have shown differences in chemical composition and fatty acid composition compared to commercial breeds, with a higher proportion of oleic acid and more favourable sensory properties of bacon (Latin et al., 2022). Similar results have been obtained for other European local breeds, such as Iberian and Alentejano pigs, supporting the connection between breed characteristics and product quality (Albuquerque et al., 2021; Pugliese and Sirtori, 2012). However, translating these characteristics into consistent product quality requires a better understanding of the underlying biological variation, as well as reliable analytical approaches. At present, the lack of detailed phenomic data represents a major limitation, particularly for local breeds and traditional products. The Banija spotted pig remains insufficiently studied, especially with regard to its lipid and volatile profiles, which are essential for evaluating its potential and current knowledge is still limited.

Authentication and market valorisation
Translating biological uniqueness into market value highlights the need for authentication and traceability. To develop premium products, it is important to have reliable methods that link product characteristics to breed, feeding and processing. Lipidomic and volatilomic profiles can serve this purpose because they make it possible to classify and differentiate meat products based on their biological and technological characteristics. This approach has already been implemented in the Iberian ham industry, where lipid and volatile fingerprints are used to differentiate quality categories and provide certification for these products (Pajuelo et al., 2023; Rodríguez-Hernández et al., 2024). However, application of volatilomics and lipidomics to Croatian local breeds remains limited, reflecting the still fragmented integration of phenomic research with product development. While these approaches provide detailed insight into measurable product traits, their relationship with consumer perception and product value remains insufficiently explored.
Neuromarketing offers tools to better understand consumer responses by capturing subconscious reactions related to attention, emotion and decision-making (Plassmann et al., 2015). However, to date, such approaches have not been applied in research on traditional and dry-cured meat products, particularly those derived from local pig breeds. In contrast, existing studies have largely relied on stated preference methods, which do not fully capture

underlying drivers of consumer behaviour, especially in the case of fat-rich products where health perceptions and sensory expectations may conflict (Font-i-Furnols and Guerrero, 2014; Jelić Milković et al., 2021). Integrating phenomic data with consumer-oriented approaches could therefore improve understanding of how measurable product traits translate into consumer perception and purchasing decisions, which is particularly relevant for products from local and underexplored breeds.

Conclusion

Local Croatian pig breeds have strong potential for the production of premium products such as dry-cured bacon. However, fully realising this potential requires a better understanding of how genetic background and external factors, including production systems, interact to shape product quality. In this context, phenomics provides a useful framework, as it allows traits to be studied across multiple levels, from genomic and transcriptional responses to the characteristics of the final product, although its application is still limited. Further research is therefore needed, particularly to integrate molecular, lipidomic and volatilomic data and to better understand their relationship with technological and sensory properties. Such efforts could support the more effective use and market positioning of underexplored breeds and contribute to unlocking their potential in high-value food systems.

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REVIEW ARTICLE

Robotic milking among new technological trends in the development of dairy farms in Croatia

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Summary
In today's working conditions, the milking robot is not only a technological innovation, but also represents an organizational transformation of the farm. Producers who are ready to embrace digitalization and adapt their operations to new technologies can achieve greater control over production, reduce dependence on labour, and improve their quality of life. The key question is no longer whether automation will become part of the dairy sector, but at what speed and to what extent it will be implemented on Croatian farms to reach the European average of livestock-developed countries. Timely adaptation will be a decisive factor for the survival and development of cattle production in the Republic of Croatia in the coming years. However, without strong and clear support from the state, success will be limited.

Keywords: robotic milking, technological trends, dairy farms, Croatia

Introduction

Milk production in Croatia, as in most European Union member states, is experiencing a long-term and increasingly pronounced labour shortage. According to official data (Statista, 2026), a total of about 1.75 million people are employed in Croatia. Although total employment has remained relatively stable in recent years, the structure of the workforce is changing significantly. The relatively small proportion of employees in agriculture is particularly notable (only 4.68% in 2023), with a constant downward trend. One reasons for this decline is the average net salary of workers, which in the agricultural sector in 2023 was 17% lower than the average net salary of other employees in the Republic of Croatia (MPŠR, 2025).

Robotic milking, or automatic milking systems (AMS), were developed specifically in response to these challenges. Their use enables continuous milking 24 hours a day, reduces the need for the constant presence of workers in the milking parlour, and transforms the role of the farmer from a contractor to a manager of a production system based on concrete data (Mijić et al., 2021). Numerous studies confirm that automation brings not only technological change, but also a deep organizational transformation of the farm. Hogeveen et al. (2010) state that automated milking systems significantly affect the structure of labour costs and the economics of production, while de Koning (2011) emphasises that one of the key motives for introducing robots is precisely to reduce dependence on labour and improve the quality of life of farmers. Research by Tse et al. (2018) shows that automation can increase work efficiency per unit of milk produced, while Svennersten-Sjaunja et al. (2008) emphasize the importance of adapting herd management in conditions of voluntary milking. Also, Rasmussen et al. (2002) also analysed changes in work organisation and cow behaviour after the introduction of the AMS system, and found positive production results. These few statements clearly indicate that robotic milking is not just a technological innovation, but a

strategic response to long-term changes in the structure of the workforce and the organisation of agricultural production. In the context of the Croatian dairy sector, where farms are often family-owned and face limited human resources, automation is increasingly becoming a question of farm and milk production sustainability.
The aim of this article was to analyse possible technological directions for the development of the primary dairy sector in Croatia, such as robotic cow milking. We also aim to show, through the results of other research on this issue, how such changes would affect the production results and work organisation of Croatian farmers.

Key characteristics of the transition from conventional to robotic milking
The first notable change with the introduction of an AMS is the reduction in interaction between the farmer and the cow. This is also demonstrated by a study that analysed the transition from conventional milking to AMS (Wildridge et al., 2020). The authors concluded that AMS changes the nature of daily tasks and interactions with cows. After switching to AMS, farmers spent less time in direct interaction with cows because milking-related tasks were automated, but this "saved" labour time was often reinvested in other activities such as animal care or herd management.
The results in Table 1 show that the time spent on feeding-related activities (such as feed distribution, feeder checks, etc.) in AMS is often similar to conventional milking system (CMS) or slightly reduced. This suggests that feeding-related tasks remain a regular activity regardless of the milking system. The time spent bringing cows to be milked indicates that in CMS it is part of the milking routine because cows are driven to the parlour. In AMS, cows come on their own (voluntarily), but the farmer has to bring those that did not come on their own to be milked. This task is usually the main difference between the two systems. The introduction of AMS brings significant changes in farm management: from feeding to health management. For example, a large Canadian study (Tse et al., 2018) showed that the transition from CMS to AMS not only affects milking, but also affects broader farm management factors including changes in the feeding system, cow health and management of cow stay in the waiting area. The frequency of using free systems, reassignment of work, and changes in work tasks among employees are key elements of a successful transition.

Table 1. Estimated time (min/day) spent interacting with cows during lactation in CMS and AMS during daily tasks (Wildridge et al., 2020).

Task	**CMS**	**AMS**	***P- value***	**SED***
Feed	6.3	3.4	0.48	0.65
Fetch	82.5	73.8	0.60	0.19
Milk	245.7	1.2	<0,01	0.80
Near	0	59.8	<0,01	0.44
Contact	3.3	17.6	0.03	0.44

* SED = standard error of difference of means.

The results indicating that in the study by of Tse et al. (2018), a higher proportion of farmers (83%) experienced an increase in milk production, particularly among those who used AMS for more than 24 months (Table 2). Based on this result, it can be said that the positive effects of AMS often stabilize and are expressed only after a period of adaptation. Regarding milk quality, the data are inconsistent. Some producers report improvements, such as better control of somatic cells counts, while others observe no change or encounter initial difficulties. In the longer period after the transition, stabilization or improvement in quality is more often reported (Mijić and Bobić, 2021). The impact of the length of the transition period indicates that, in the short term, certain challenges in adaptation (work organization,

cow adaptation) may occur, while in the long term, positive effects on production are more often reported.

Table 2. Proportion of farmers (%) who experienced changes in milk quantity and quality, after switching from CMS to AMS, grouped into two-time categories (⩽24 and >24 months) since the switch. (Tse et al., 2018)

	Share of farmers with changes (%)				
Interval since transition[1]	**Increased**	**Decreased**	**Stayed the same**	**Respondents (n)**	***P- value***
Milk yield					
⩽24 months	79	6	15	87	
>24 months	83	3	14	118	
Overall[2]	81[a]	4[b]	14[c]	205	0.001
Bulk tank SCC[3]					
⩽24 months	24	41	35	86	
>24 months	16	45	39	116	
Overall	20[a]	43[b]	37[b]	202	0.001

[a,b]Within a row, values without a common superscript differ (P <0.05). [1]Distributions of perceived change for each item did not differ by time since transition. [2]Overall value includes three respondents that did not provide information on time since transition. [3]Somatic cell count.

In addition to changes in farm operations when switching to AMS, a new approach to herd management also plays an important role. Studies, such as Gislon et al. (2024), show that the switch to AMS also implies a new approach to herd management, as robotic milking requires adaptation not only of physical equipment but also of the control of movement and turnover of cattle in the barn, the motivation of cows to enter the system, and the feeding and health strategies (Figure 1). Such changes often include the reorganization of work tasks and a greater focus on herd optimisation. The eight most common topics mentioned by these authors in the scientific literature are: cow turnover in the barn and milking time, farm management, udder health, comparison with conventional milking, milk production, AMS data analysis, disease detection, and feeding management.

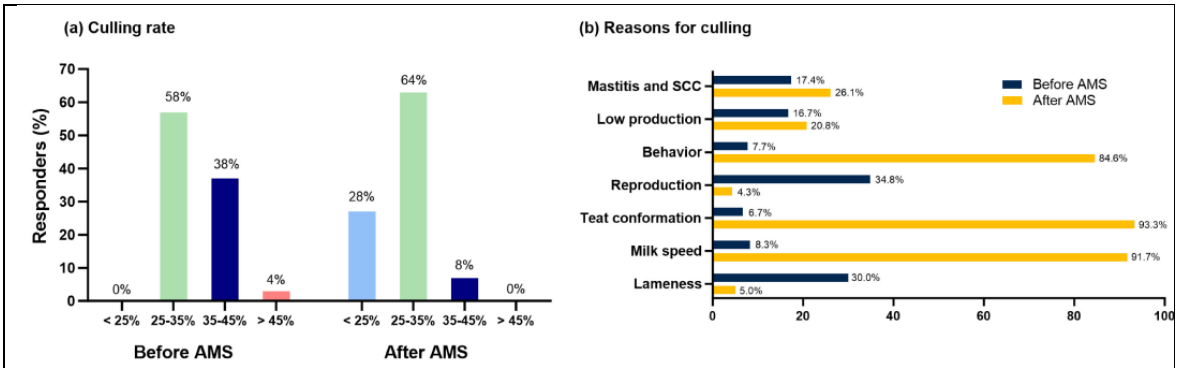

Figure 1. Culling rates (**a**) and main reasons for culling cows before and after transitioning to AMS (**b**). (Lage et al., 2024)

Organizational changes on the farm

The introduction of AMS represents a profound transformation of the organisation of work on dairy farms. The traditional routine, in which farmers spend many hours of physical work with cows every day, is replaced by a system in which robots take over most of the routine

milking tasks, while the role of human role shifts to supervision, system maintenance and data analysis (Mathijs, 2004).

Table 3. Reasons for installing AMS on the farm (Mathijs, 2004)

Parameter	***Country**				
	B	**NL**	**D**	**DK**	**Total**
Reduced work	7.7	33.3	34.7	21.4	28.9
More flexible work	35.5	19.3	34.7	35.7	27.1
Solving the problem of hired labour	7.7	17.5	8.7	21.4	14.9
Improving technical features	7.7	14.0	4.3	21.4	12.1
Keeping up with the future and challenges	7.7	12.3	0	0	7.5
Other activities	30.7	3.5	17.4	0	9.3

* Country: B= Belgium; NL= Netherlands; D= Germany; DK= Denmark

Although the robot reduces the need for manual labour, it does not reduce the need for professional supervision, but works in a completely different way, requiring not only time but also a much higher level of expertise and continuous education. In family farms, this often means transferring knowledge to younger members who are more inclined to digital technologies. Dairy farms that have milking robots require less labour than those that have conventional milking, however, this factor will only come into play if they also have skilled farmers who can perform such tasks (Rodenburg, 2016). Research conducted by Vik et al. (2019) shows that the introduction of AMS on farms in Norway has caused widespread structural changes in the dairy sector. Although the introduction of AMS into production was actually a technological response to the desire for greater flexibility and a reduced in manual labour, its application has had much broader consequences for the entire organisational structure of farms (Rasmussen et al., 2002; Bijl et al., 2007).

Conclusion

Robotic milking of cows is one of the most significant innovations in modern livestock production. The introduction of AMS increases the precision, consistency and efficiency of the process, while reducing the physical burden on farmers and the need for intensive manual labour. This technology enables timely detection of changes in milk production in each cow and a rapid response to possible health problems. In the long term, investing in this technology contributes to the stability and competitiveness of dairy farms, especially in conditions of increasing market demands and labour shortages. Therefore, robotic milking can be seen as a strategic step towards more sustainable, efficient and economically profitable milk production in the Republic of Croatia. Its application confirms that modern agriculture, relying on innovation and digitalization, has strong potential for further development and adaptation to the challenges of the future, but with strong support from the state.

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REVIEW ARTICLE

Predicting body weight from morphometric traits: a comparative modelling approach across livestock species

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Summary
Body weight estimation based on body measurements represents a practical alternative to weighing in systems where livestock scales are not available. This review synthesises existing research on the use of morphometric indicators, such as heart girth, height at withers, and body length, for predicting body weight in domestic animals. Emphasis is placed on models developed under different production conditions, including mainly extensive systems. Both statistical and more recent machine learning approaches are considered, with evaluation of their accuracy and applicability. In conclusion, morphometric models provide a reliable, simple, and economically feasible method for estimating body weight in practice.

Keywords: body weight, morphometric measurements, predictive models, extensive production systems, regression analysis

Introduction

In extensive and small-scale production systems, particularly in rural areas, weighing animals is often logistically and financially unfeasible (no fixed scales, high transport costs, animal handling increases stress and injury risk) (Oroz et al., 2022). However, body weight (BW) is essential for: accurate dosing of veterinary drugs including antiparasitic treatments, estimating nutritional requirements, monitoring growth, assessing market value, and making selection decisions.

Numerous methods have been developed for indirect estimation of BW. The most widely used approaches rely on morphometric traits: heart girth (HG), body length (BL), height at withers (WH), hip/rump height (RH), and occasionally body widths, chest depth, and body condition score (BCS) (Sowande and Sobola, 2008). Often, the strongest predictor is HG, because it reflects overall body "volume," while BL and height measures capture additional variation in conformation (Heinrichs et al., 1992).

A particular challenge in some livestock species is diversity of production conditions, with substantial differences between intensive commercial systems and smallholder farms with local breeds, crossbreds, and variable feeding regimes. Studies comparing models across such systems indicate that adequate internal calibration (high coefficient of determination - R^2 in development and internal validation) is necessary to ensure reliable predictions within the target population (Sungirai et al., 2014).

Types of statistical models and representation of body conformation

The most commonly used models in the literature can be grouped into several categories.

Simple linear regression (SLR) is the most practical approach for field use (single measurement), often achieving high R^2 within the analysed population, for example in market-weight pigs in smallholder systems (Kabululu, 2023).

Multiple linear regression (MLR) is used when improved accuracy is required by incorporating additional measurements such as length, height, or width. An example is pig model in intensive systems including age, BL, and HG (Sungirai et al., 2014).

Polynomial and quadratic regressions include terms such as HG^2 or combinations of variables to capture non-linear relationships, particularly in conformationally heterogeneous populations or over time (e.g., during sow gestation) (O'Connell et al., 2007).
Allometric and transformed models involve logarithmic transformations or cube-root transformations of BW, reflecting the volumetric nature of body mass (mass ~ dimension^3). In practice, this can yield equations such as: $BW = (\alpha + \beta \cdot HG)^3$), which are suitable for implementation in weigh bands (Marshall et al., 2023).
Mixed-effects models are useful when data are clustered (e.g., multiple pigs per farm), incorporating random effects (farm/household) to improve generalisation and obtain unbiased coefficients (Marshall et al., 2023).
Machine learning (ML) methods include random forest (RF), classification and regression trees (CART), support vector regression (SVR), multivariate adaptive regression splines (MARS), and polynomial models with validation. These are particularly relevant for capturing non-linearities and interactions but require larger datasets and careful validation.
For instance, in goats, the MARS algorithm achieved high R^2 in the training set (Rashijane et al., 2023). In sheep, recent work on indigenous breeds explicitly compares RF, SVR, and CART models, highlighting HG as the key predictor with a clear field-oriented objective (replacement of scales) (Megersa et al., 2025).

Studies across livestock species

In pig studies conducted in smallholder systems, HG is typically measured as the circumference of the chest just behind the front legs, as this anatomical location is relatively easy to standardise and does not require precise head positioning. In pigs, BL is often defined from the midpoint between the ears to the base of the tail, although variations in definition are common and represent a frequent source of inconsistency among studies.
In cattle smallholder systems, the standard set of variables often includes HG, BL, WH, sometimes complemented by BCS as an additional predictor (Kabululu, 2023). In goats (e.g., meat breeds under field conditions), HG, BL, WH, and RH are commonly used, with variable combinations depending on age and production system (Rashijane et al., 2023).
In horses, BW is typically estimated using weight tapes or formulas based on HG and BL, with literature emphasising differences across breeds and conformations (Ellis and Hollands, 1998). A key practical insight across these studies is that measurement error is not solely due to subjective judgement, but also systematic factors: tape tension, positioning, animal posture, and variables such as body condition and conformation all influence measurements and the HG-BW relationship.
In table 1. overview of studies on body weight estimation among different livestock species is shown.

Table 1. Comparative overview of studies on body weight estimation using morphometric traits

Species	Sample Size	Analysed Measurements	Model Type	R^2 / Accuracy	Reference
Pig	140	Heart girth, body length	Linear regression	$R^2 = 0.92$	Mutua et al., (2011).
Horse	200	Heart girth, body length	Regression model	$R^2 = 0.95$	Carroll and Huntington, (1988).
Cattle	250	Heart girth, body length, height at withers	Linear regression	$R^2 = 0.96$	Heinrichs et al., (1992).

Sheep	152	Heart girth, body length	Multiple regression	$R^2 = 0.90$	Yakubu, (2010).
Goat	150	Heart girth, height, body length	Regression model	$R^2 = 0.85$	Chitra et al., (2012).

Practical and robust predictor of pig BW in extensive systems is HG, with high accuracy reported in Tanzania ($R^2 = 0.94$) (Kabululu, 2023) and Uganda ($R^2 = 0.93$) (Marshall et al., 2023). In Black Slavonian pigs field-oriented studies, conducted in smallholder systems, high predictive performance ($R^2 \approx 0.95$) is reported for HG (Oroz et al., 2022).

Adding measurements such as BL can improve precision in heterogeneous or young populations, but models developed in intensive systems often perform poorly in these settings, highlighting the need for local validation. Tools like weight bands can help standardise BW estimation in practice.

In horses, research focuses on predictive equations and weight tape reliability. Accuracy varies with horse height and tape type, often expressed as percentage error (Ellis and Hollands, 1998). Systematic bias, especially underestimation in heavier horses is common and influenced by body condition and breed (Grimwood et al., 2023). Predictive formulas support management decisions but require local calibration due to breed differences (Górniak et al., 2020). Advanced methods, including ML, can achieve high accuracy when measurements are consistent (Satola et al., 2022). Overall, weight tapes are useful for tracking changes over time but less reliable for precise estimates.

In cattle, HG shows a strong relationship with BW ($R^2 = 0.78$ and adjusted $R^2 = 0.71$), with moderate prediction error, while adding BCS offers limited practical benefit (Lukuyu et al., 2016). Similar patterns are observed in zebu cattle and small ruminants, where HG remains the key variable.

In goats and sheep, advanced models (e.g., MARS, random forest, SVR) achieve high accuracy, though validation is sometimes incomplete. Despite its practicality, prediction errors can still be significant, and advanced models require proper validation for field use (Lukuyu et al., 2016).

A major limitation across studies is limited transferability, as models are often population-specific. Inconsistent reporting of prediction error and lack of measurement standardisation further reduce comparability and applicability. Future research should prioritise external validation, transparent error metrics (e.g., root mean square error - RMSE and mean absolute error - MAE), and simple, field-applicable protocols that balance accuracy with feasibility.

Conclusion

Evidence from this review consistently indicates that heart girth is the most practical and often the most accurate single predictor of body weight in the absence of weighing scales, particularly in pigs and ruminants. These findings are supported by robust validation procedures, validation procedures, which enable the development of simple, reliable, and user-friendly tools such as weight bands.

However, transferring models between intensive and extensive systems remains problematic without local validation. The largest practical errors often arise from a lack of measurement standardisation and from structural differences between populations, including breed, body condition, and age. For future research, a key priority is to establish a minimal standard framework: standardised measurement protocols, models with transparent validation, and error reporting in user-relevant units (e.g., kg or percentage), with clearly defined applicability ranges.

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REVIEW ARTICLE

Guidelines for assessing the working capacity of horses in equestrian sport

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Summary
Equestrian sport constitutes a complex interdisciplinary system in which performance is realised through the integration of biomechanical, physiological, veterinary, and psychological factors within the human–horse interaction. Contemporary developments in this sector are increasingly shaped by demands for ethical legitimacy, particularly in relation to equine welfare, thereby necessitating the establishment of scientifically grounded and standardised frameworks for assessing working capacity. In this context, the evaluation of a horse's working capacity extends beyond performance-based criteria and incorporates objective indicators of physiological condition, biomechanical efficiency, and behavioural responses. The advancement of equestrian sport thus requires the integration of regulatory standards, performance management, technological innovation, and stakeholder coordination within a coherent multi-level governance framework. The aim of this study is to systematise and critically analyse guidelines for assessing the working capacity of horses across different equestrian sport disciplines in the Republic of Croatia, with particular emphasis on their role in ensuring both performance efficiency and animal welfare. The findings highlight the need for further methodological standardisation and the implementation of objective indicators of equine physical and psychological condition as a prerequisite for the sustainable development of equestrian sport and the equine sector.

Keywords: equestrian sport, guidelines, working capacity, horse training, Republic of Croatia

Introduction

Equestrian sports, together with recreation and breeding, constitute the core activities within the equine industry, while secondary activities include services associated with these primary sectors, such as veterinary services, farriery, and the production and distribution of horse feed (Čačić, 2010). Equestrian sports represent a specific mode of horse utilisation in which the fundamental principle is the assurance of animal welfare. The establishment and maintenance of good practices in breeding, care, and training are contingent upon a high level of expertise and skills among professionals, as well as the active involvement of relevant stakeholders. The breeding of sport horses constitutes a key driver of the equine industry in more developed European Union countries and represents a significant source of financial resources directly linked to equine breeding and equestrian sport (MPŠR, 2021). Breeding and sport form mutually complementary systems, whereby the quality and breeding value of breeding stock and their offspring are evaluated through competitive performance, with the involvement of specialised professionals, including trainers, riders,

judges, and other stakeholders. The sustainability of equestrian sport and the quality of sport horse breeding largely depend on the efficient management of financial, land, and production resources, as well as on the availability of qualified human resources. Sport horse breeding further contributes to the advancement of veterinary medicine through increased demand for specialisation and the application of complementary therapeutic and rehabilitation methods, such as physiotherapy, chiropractic care, osteopathy, and equine massage. The production of feed for sport horses represents an important component in the valorisation of agricultural and land resources. Continuous professional development and the recognition of acquired competencies, together with interdisciplinary collaboration between agronomic and veterinary sciences, constitute key prerequisites for the development of standards and the preservation of the credibility of equine breeding in the Republic of Croatia (RC). Sport horse breeding also promotes participation in organised competitive activities and enhances the social visibility of equestrian sport. The aim of this paper is to analyse the current state of sport horse breeding in RC, with a comparative perspective on practices in other countries, focusing on the interrelationship between breeding and sporting activities and the role of professional expertise in ensuring system sustainability and efficiency.

Institutional Structure and Organisation of Equestrian Sports in the Republic of Croatia

In the RC, the Croatian Equestrian Federation has been operating since 2004 with the aim of promoting, regulating, and developing equestrian sports. The Federation is organised and functions in accordance with the provisions of the Sports Act, the Associations Act, the Statute of the Croatian Olympic Committee, and other relevant regulations (HKS, 2024). It is one of 135 member organisations of the Fédération Equestre Internationale (FEI), established in 1921 to ensure internationally harmonised rules and qualification systems, with the objective of preserving the integrity of equestrian sports while safeguarding horse welfare. The FEI governs both Olympic disciplines - dressage, show jumping, eventing, and para-dressage and non-Olympic disciplines, including endurance riding, driving, and vaulting (FEI, n.d.). Equestrian activities not regulated by the FEI, such as flat racing and harness racing, fall under the jurisdiction of the Croatian Galloping Federation and the Croatian Trotting Federation, the latter currently inactive. The Croatian Galloping Federation is affiliated with the International Federation of Horseracing Authorities (IFHA) (Baban et al., 2025). Comparatively, the roles and organisational structures of national equestrian federations vary across countries. In Germany, for example, the German Equestrian Federation (FN) comprises 17 regional umbrella organisations encompassing 403 regional associations. Within this system, approximately 7,700 equestrian clubs operate alongside around 58,000 individual members, 4,000 private or commercial equestrian centres, and 25 breeding organisations. Breeding organisations are closely aligned with the sport system, with breeding objectives and competition results serving as key inputs for selection and breeding improvement. Annually, Germany hosts approximately 3,600 events comprising around 68,000 competitions, with about 1,450,000 entries, 80,000 competitors, and 138,000 competing horses (WBFSH, 2022). The RC operates within a comparatively smaller, yet structured institutional framework that provides opportunities for improving standards and strengthening the integration of sporting and breeding activities. According to available data, the Croatian Equestrian Federation comprises 71 equestrian clubs (HKS, n.d.), supported by 131 licensed professionals, including 50 trainers, 44 judges and stewards, 10 course designers, and 27 veterinarians (MPŠR, 2026). In 2025, a total of 65 competitions were organised at the national level, including 35 in show jumping (53.84%), 17 in dressage (26.15%), 7 in endurance riding (10.8%), and 6 in driving (9.23%), with this distribution remaining relatively stable over time (HKS, n.d.). Driving consistently records the lowest

number of competitions, despite maintaining a relatively stable number of competitors, with the most represented category being two-in-hand driving. Croatian competitors in this discipline regularly participate in international events, including world championships. Over the past four years, an increase in participation has been particularly evident in dressage and endurance riding (Baban et al., 2025). Dressage, in particular, has shown continuous development, with Croatian national teams achieving notable results at the international level, especially in junior and young rider categories. Changes in competition requirements and classification systems introduced since 2020 have further contributed to standardisation and alignment with international practices. Endurance riding has also experienced sustained growth in both participation and licensing, culminating in the organisation of the Balkan Championship in 2025 in Barban, a key centre for the development of this discipline in RC (MPŠR, 2026). The spatial distribution of competitions reveals significant regional disparities. In 2025, the lowest share of competitions was recorded in Dalmatia (4.6%), Slavonia (9.2%), and Istria (18%), while the highest concentration was observed in Zagreb and its surrounding area (HKS, n.d.). This centralisation reflects the greater availability of infrastructure, capital, and market demand in the central region, whereas in other parts of Croatia equestrian activities are more commonly associated with cultural and traditional events. This indicates the need for strategic investment in infrastructure and the promotion of competition organisation in less represented regions to support balanced sectoral development. Equestrian sports exhibit considerable potential for attracting sponsorship, international funding, and partnerships with the private sector, local communities, and tourism, although financial stability remains subject to fluctuations (SKSS, 2025). The Ministry of Agriculture, Forestry and Fisheries has supported equestrian competitions for over a decade through the National Programme for the Development of Warmblood Horse Breeds. In the 2026–2030 programming period, €205,000 annually is allocated for competition organisation, of which €115,000 is designated for FEI disciplines and €90,000 for flat and harness racing. Flat racing was reintroduced in 2024 after a five-year hiatus, with an international event held over three days, featuring up to five horses per category and a prize fund of €20,000 per race day (Crogallop, n.d.). In contrast, harness racing, although historically significant within Croatian equestrian sport, has experienced a decline in popularity and participation at the national level in recent years. The enhancement of the international competitiveness of Croatian riders can be based on both the import and breeding of high-quality sport horses, with particular emphasis placed on the development of domestic breeding. Horses bred or trained within professional breeding and training systems achieve high market value, reflecting long-term investment and systematically structured training supported by qualified personnel. According to Čačić (2010), the ownership of sport horses is associated with the highest cost levels within the equine industry. Sport horse breeding represents a more financially accessible alternative, particularly due to the availability of high-quality genetic material through artificial insemination. At the same time, its development requires continuous investment in human capital through the education and training of riders, trainers, farriers, and veterinarians, as well as the development of appropriate infrastructure that ensures optimal conditions for breeding and training. One of the key directions for strengthening equestrian sport in Croatia involves encouraging the participation of younger generations and supporting their professional development, while simultaneously addressing constraints related to employment opportunities and career advancement within the sector.

Licensed Competitors and Horses in the Republic of Croatia

The total number of licensed horses within the Croatian Equestrian Federation amounts to 762, alongside 585 licensed competitors, including riders and carriage drivers (HKS, n.d.).

Show jumping and dressage dominate in terms of the number of licensed participants. In show jumping, a total of 304 riders are registered, including 83 juniors, 41 cadets, and 132 seniors. Dressage accounts for 142 licensed competitors, comprising 8 pony riders, 28 cadets, 36 juniors, 3 young riders, 17 seniors, 49 amateur seniors, and 1 para-equestrian athlete. In driving, 21 licensed competitors are recorded, including 1 cadet and 20 seniors, while endurance riding includes a total of 141 licensed participants, with 86 seniors, 35 juniors, and 20 cadets (HKS, n.d.). Participation in international competitions under the Fédération Equestre Internationale (FEI) is conditional upon the registration of both horses and competitors through the Croatian Equestrian Federation. According to data for 2025, Croatia has a total of 111 registered FEI horses, including 68 in show jumping, 5 in dressage, 19 in endurance riding, 18 in driving, and 1 in para-dressage. The total number of FEI-licensed competitors is 71, comprising 38 show jumping riders, 8 dressage riders, 18 endurance riders, 7 drivers, and 1 para-dressage athlete. Compared to 2022, when 52 FEI-licensed competitors and 82 registered horses were recorded, a positive upward trend in international participation is evident. This trend can be associated with improvements in training standards, increased investment in equestrian sport, including the acquisition of high-quality sport horses, and collaboration with international experts. In addition to competitors and horses, the FEI also licenses technical personnel, with RC currently having 10 show jumping officials, 7 dressage officials, 1 eventing official, 5 driving officials, 3 endurance officials, and 1 para-dressage official (FEI, 2025). Further enhancement of the international competitiveness of Croatian riders may be achieved through systematic investment in the breeding of high-quality sport horses, complemented by the selective import of superior equine stock. In this context, increasing emphasis is being placed on the development of domestic breeding as a more sustainable long-term model. Horses bred or trained in professional breeding and training systems achieve high market value, which implies long-term investment and the systematic implementation of training processes with the involvement of qualified professionals. According to Čačić (2010), ownership of sport horses is associated with some of the highest cost within the equine industry. In this regard, sport horse breeding represents a more financially accessible alternative, particularly due to the possibility of utilizing genetically valuable reproductive material through artificial insemination. At the same time, its development requires continuous investment in human capital through the education and professional development of riders, trainers, farriers, and veterinarians, as well as the development of appropriate infrastructure that ensures high-quality conditions for breeding and training. One of the key strategic directions for strengthening equestrian sports in the RC is to encourage the involvement of younger generations in equestrian disciplines and to support their further professional development, while simultaneously addressing the limitations related to employment opportunities and career advancement within the sector.

Conclusions

Equestrian sports in the RC exhibit considerable development potential at both professional and amateur levels, with their long-term sustainability largely dependent on the efficient management of financial, land, and production resources, as well as the quality of human capital. The development of the amateur segment carries additional social value by promoting active lifestyles, social interaction, and the cultivation of responsibility and ethical attitudes towards animals. A key prerequisite for the broader popularisation of equestrian sports lies in the systematic promotion of elite athletes, clubs, and professionals through contemporary communication channels and public events, thereby stimulating interest among younger generations and encouraging potential career pathways within the equine sector. At the same time, limited employment opportunities and restricted career

advancement remain significant constraints affecting youth engagement in equine-related professions. Accordingly, there is a need for continuous investment in the development and standardisation of educational programmes aimed at aligning professional competencies with sectoral requirements, alongside ensuring the recognition of acquired qualifications within the labour market. In this context, the concept of accessible sport horse breeding should focus on enabling structured and financially sustainable participation in equestrian activities, irrespective of horse ownership, supported by professionally guided training and adherence to safety standards. Further sectoral development requires the stimulation of employment opportunities in management, horse breeding, event organisation, and related service activities. Given the existing base of horses and competitors, RC possesses a solid foundation for enhancing international visibility and competitiveness. In this process, sport horse breeding plays a particularly important role as a financially more sustainable alternative to the import of high-value horses, while simultaneously strengthening the domestic breeding base. Institutional support, particularly through programmes implemented by the Ministry of Agriculture, Forestry and Fisheries aimed at the development of warmblood breeds and the promotion of equestrian competitions, represents a key mechanism for increasing both the number and quality of horses. However, the formulation of effective development strategies requires reliance on scientifically grounded evidence derived from the analysis of market indicators and long-term trends, thereby ensuring the sustainable and competitive advancement of the equestrian sector.

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ORIGINAL SCIENTIFIC PAPER

Litter size of the Croatian White goat

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Summary
This study aimed to determine litter size and evaluate factors affecting prolificacy in the Croatian White goat. Data from 626 does from the Šibenik-Knin and Zadar counties, collected over a three-year period (2022–2024), were analysed. A total of 906 goat kids were obtained during the research period, with the average litter size being 1.45 goat kids, or an average prolificacy of 144.73%. Most litters consisted of single goat kids (56.71% of does). The year and month of kidding had a significant effect on litter size. An increasing trend in litter size with parity was observed, although the effect was not statistically significant. The results indicate that environmental and management conditions play a key role in determining reproductive performance in this indigenous breed.

Keywords: indigenous breed, reproductive traits, year, kidding season, parity

Introduction

Reproductive traits of goats, such as litter size, are considered key indicators of reproductive efficiency and largely determine the productivity and profitability of goat production (Mellado et al., 2006). Litter size is a key indicator of goat prolificacy, that is, reproductive efficiency, particularly in breeds and flocks with a restricted breeding season and reared predominantly under extensive production conditions, in which kidding therefore occurs once a year (which is especially true for indigenous Croatian goat breeds).
The Croatian White goat is an indigenous Croatian breed developed in the 20th century through the improvement of native goats by crossbreeding with imported white (Saanen) bucks, intending to increase milk production. Historically, it was traditionally reared in small flocks, primarily for household milk production in areas with limited conditions for cattle farming (Mioč et al., 2012). The Croatian White goat is classified as an early-maturing breed, and doelings may be first mated as early as the year of their birth. Nowadays, it is increasingly oriented towards meat production, particularly goat kid meat (National Gene Bank of Domestic Animals, 2025). According to data from the Croatian Agency for Agriculture and Food (2025), the average birth weight of male kids of the Croatian White goat is 2.6 kg, while the average daily weight gain is 220 grams. Their average body weight at the end of the performance test is 25.25 kg.
Since reproductive traits are the basis of profitable goat meat production, and regular and successful reproduction is a prerequisite for lactation (milk production), this paper aims to determine the litter size of the Croatian White goat.

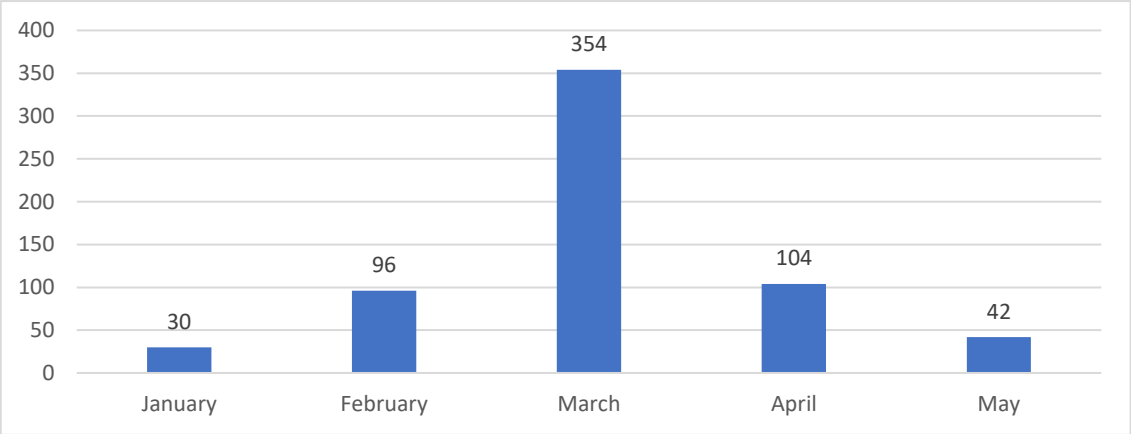
Material and Methods

For this study, data on reproductive traits of the breeding population of the Croatian White goat from the Šibenik-Knin and Zadar counties were used, obtained from the central database of the Croatian Agency for Agriculture and Food (HAPIH). The study included records of confirmed kidding events for a total of 626 goats over three years (2022–2024),

comprising the following parameters: date of kidding, litter size, sex of goat kids, parity order of the dam, lifetime identification number of the dam and sire, and county.
The representation of goats included in the study, according to the analysed sources of variability of the reproductive traits, is shown in Table 1. The distribution of kidding in the Croatian White goat is presented in Graph 1.

Table 1. Representation of Croatian White goats included in the research according to the analysed sources of variability

Sources of variability		Number of does
Year of kidding	2022	222
	2023	166
	2024	238
Parity	First kidding	162
	Second kidding	128
	Third kidding	98
	Fourth kidding	77
	Fifth and later kiddings	161



Graph 1. Distribution of kiddings in the studied Croatian White goat population by month (number of does kidding per month)

Litter size and sex of goat kids were determined individually immediately after kidding. The calculated average prolificacy (%) was defined as the ratio of the total number of goat kids born to the total number of goats included in the study.
Descriptive statistical indicators of reproductive traits were determined using the MEANS procedure, while the effects of parity order, year and month of kidding on the reproductive traits of goats were analysed using the GLM procedure of the SAS statistical software package, version 9.4 (SAS Institute Inc., 2013). The analysis also included possible interactions between the aforementioned effects. When significant effects were detected, differences among least squares means were compared using the Tukey–Kramer *post hoc* test.

Results and Discussion

Out of a total of 626 kidded does of the Croatian White goat included in the study over the specified three-year period, the majority (56.71%) consisted of does with a single goat kid per litter (Table 2). A total of 906 goat kids were obtained from 626 does, so the average litter size was 1.45 goat kids, with the breed's average prolificacy at 144.73% (Table 3). The majority of goat kids originated from more prolific litters (twins and triplets), while

singletons accounted for 39.18% of all goat kids. In the previously studied population of the Croatian Spotted goat (Beran et al., 2010), the average recorded prolificacy was lower (130%) than in the Croatian White goat. These differences in litter size between Croatian indigenous breeds are expected, given that the Croatian White goat was developed by breeding native goats with imported Saanen bucks to increase milk production. Namely, it is well known that high-yielding dairy goat breeds are characterised by relatively high prolificacy. For instance, the average litter size of the German fawn goats (also reared under Mediterranean conditions) was 1.85 goat kids per litter (Prpić et al., 2015).

Table 2. Proportion of Croatian White goats according to the number of goat kids per litter

Number of goats	n	Total number of goats (%)
Goats with one kid per litter	355	56.71
Goats with two kids per litter	262	41.85
Goats with three kids per litter	9	1.44
Goats totally	626	100.00

Table 3. Number of goat kids in litters of the Croatian White goat

Trait	Number of kids	Total number of goat kids obtained (%)
Singles	355	39.18
Twins	524	57.84
Triplets	27	2.98
Total number of goat kids obtained	906	100.00
Average litter size*	1.45	-
Prolificacy (%)	144.73	-

*number of kids obtained/number of goats.

The kidding season of the Croatian White goat lasted from January to May (Graph 1), with the highest number of kiddings occurring in March (56% of all kiddings). Nearly 90% of all kiddings took place between February and April. Accordingly, the main mating season of the studied goats was from September to November. The month of kidding had a significant effect ($P<0.01$) on litter size (Table 4). The highest average litter size was recorded in does that kidded in February (1.58 ± 0.05) and March (1.48 ± 0.03), while the lowest was observed in does that kidded in January (1.07 ± 0.08), i.e. at the beginning of the kidding season. However, fewer than 5% of all studied goats kidded in January. Thus, goats kidding in late winter and early spring achieve better reproductive performance, which may be associated with improved nutritional conditions and a more favourable physiological status before and during the mating season.

In the context of these results, it should be emphasised that goats are seasonally polyoestrous animals whose reproductive activity is primarily regulated by photoperiod (sexual activity and ovulatory dynamics increase during periods of shortening daylight). Increased melatonin secretion during the autumn and winter months stimulates ovarian activity, resulting in improved conception rates and a higher number of ovulated ova during the optimal part of the reproductive season (Stewart, 2024; Šavorić et al., 2024). In line with this, does kidding in February and March achieved a higher prolificacy compared to those kidding in January (i.e. does mated during August, or in summer). Elevated ambient temperatures often induce

heat stress in animals and may negatively affect reproductive traits in both does and bucks (such as reduced conception rate, disrupted ovulation, and decreased embryonic survival), which is consistent with the findings of Adjassin et al. (2022) in goats.

Table 4. Effect of month of kidding on the average litter size of the Croatian White goat (LSM ± SE)

	January	February	March	April	May	Level of significance
Litter size	1.07±0.08[a]	1.58±0.05[b]	1.48±0.03[ab]	1.40±0.05[a]	1.24±0.07[a]	P<0.01

[a, b] Values marked with different letters differ significantly ([a:b] – P<0.01).

The year of kidding had a significant effect (P<0.001) on the prolificacy of the Croatian White goat (Table 5), with the highest average litter size of the studied animals recorded in 2024 (1.62 ± 0.04 goat kids per litter), while the lowest was in 2023 (1.19±0.04). This may indicate a pronounced influence of varying environmental conditions and management regimes between years on the reproductive performance of goats. However, caution should be exercised when interpreting differences in prolificacy of goats of this breed between years, since a significant interaction (P<0.05) was found between the influence of year and month of kidding on litter size. Namely, the vast majority of the January kiddings occurred in 2023.

Table 5. Effect of year of kidding on litter size in the Croatian White goat (LSM±SE)

	2022	2023	2024	Level of significance
Litter size	1.45±0.04[a]	1.19±0.04[b]	1.62±0.04[c]	P<0.001

[a, b, c] Values marked with different letters differ significantly ([a:b] - P<0.01; [a:c] – P<0.01; [b:c] – P<0.001).

Although the study did not reveal a significant effect of parity on goat litter size (Table 6), an increase in average litter size was observed with increasing parity (from the first to the fourth kidding), rising from 1.37 to 1.60 goat kids per litter, depending on the parity. Hamed et al. (2009), in their study of Zaraibi goats, also found a significant effect of parity on litter size, which the authors attribute to increased ovulatory capacity and a more fully developed reproductive system compared to younger does.

Table 6. Effect of parity on litter size in the Croatian White goat (LSM ± SE)

	First kidding	Second kidding	Third kidding	Fourth kidding	Fifth and later kidding	Level of significance
Litter size	1.37±0.04	1.46±0.05	1.46±0.05	1.60±0.06	1.43±0.04	NS

NS: not significant.

Conclusion

As a breed shaped within extensive production systems, the Croatian White goat demonstrates moderate litter size under environmental constraints. Factors such as year and month of kidding significantly influenced litter size, highlighting the importance of management and production conditions.

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REVIEW ARTICLE

Population and landscape genomic approaches to genetic structure and connectivity in wild boar (*Sus scrofa*)

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Summary
This review evaluates the most current developments in the use of both population and landscape genomics for European Wild Boar (*Sus scrofa*). The application of genomic techniques such as SNP arrays; RADseq; and whole genome sequencing provides greater detail on population structure, gene flow and hybridization than previous methodologies. Landscape genomics integrates genetic and environmental data to examine connectivity among subpopulations and to evaluate habitat features that function as barriers or conduits for dispersal. In addition, these methodologies will be useful in informing a science-based approach to managing European Wild Boar populations, specifically with respect to control of infectious diseases and mitigation of negative impacts from agricultural activities.

Keywords: wild boar, population genomics, landscape genomics, genetic structure, connectivity

Introduction

Wild Boar (*Sus scrofa*) is an extremely adaptable species that is able to reproduce quickly. As a result, it is capable of a very rapid increase in population size. Data regarding the amount of wild boar hunted across Europe clearly indicate that wild boar populations have significantly increased over time. Several factors have contributed to this expansion of wild boar populations. Land use changes (such as conversion of natural habitat to cropland) have provided wild boars with additional sources of food. Management strategies have also likely played a role. Massei et al. (2015) reported that wild boars are having significant economic impacts on agriculture. Those impacts include crop loss and replanting expenses. They also report that the presence of wild boars increases costs related to protecting crops through means such as fencing. From a biosecurity standpoint, wild boars pose a major risk. Wild boars act as both a reservoir and a vector for African Swine Fever (ASF). Containing the spread of ASF will depend on reducing both wild boar population numbers and the number of areas wild boars can travel through. Therefore, understanding landscape connectivity and travel patterns among wild boar populations is crucial. Genetic studies of wild boar suggest that wild boar genetic structures are influenced by distance between populations, past events, and human activities (including translocations and hybridizations). Due to the complexity of the forces influencing the genetic structure of wild boar populations, defining meaningful management units is difficult. However, genomic and landscape genomic approaches offer tools for addressing this challenge more efficiently than were available previously (Šprem et al., 2016; de Jong et al., 2023).

Methods of population genomics analysis of structure and connectivity

Studies of wild boar typically utilize one of four categories of genetic markers: microsatellites, single nucleotide polymorphisms (SNPs), reduced representation DNA sequencing (RADSeq), and whole genome sequencing (WGS). Each category provides different levels of resolution for detecting various aspects of genetic structure and connectivity (Table 1). One advantage of microsatellite markers is that they provide continuous results that can be compared to previous studies that used similar types of markers. However, they tend to lack sufficient power to detect subtle barriers or fine scale structure within populations. Another advantage of SNP arrays is that they can identify whether individuals carry domestic pig ancestry. Although SNP arrays may suffer from ascertainment bias if the array does not contain adequate representations of local wild boar alleles, SNP arrays have proven to be effective in detecting introgression into wild boar populations (Ramos et al., 2009; Iacolina et al., 2018). RADSeq technologies have emerged as an intermediate solution for many studies. RADSeq generates tens-of-thousands to hundreds-of-thousands of SNPs per individual at relatively low cost per individual. Like SNP arrays, RADSeq are well-suited for studies requiring extensive spatial sampling because they are less costlythan WGS while providing more information than microsatellites. While WGS generates the largest amount of information about an organism's genotype, WGS is expensive and requires significant computational capabilities to process large amounts of sequence data. As a result, WGS is often used in conjunction with smaller marker sets for monitoring purposes (Groenen et al., 2012; Wang et al., 2025).

Table 1. Genomic methods for wild boar structure and connectivity studies - practical comparison

Method	Typical outputs	Pros/cons
Microsatellites (STR)	FST, clustering/assignment, relatedness	Pros: cheap, fast, legacy continuity. Cons: limited fine-scale power; cross-lab comparability issues.
SNP arrays	PCA/ADMIXTURE, ROH/inbreeding, introgression, F_{ST}	Pros: standardized, strong for introgression and monitoring. Cons: ascertainment bias; fixed content.
RADseq/ ddRAD/ GBS	Fine-scale structure, IBD/IBR, GEA, assignment	Pros: flexible, good cost–resolution, dense spatial sampling feasible. Cons: batch effects, missing data, lower cross-study comparability.
WGS	Demography, fine-scale gene flow, selection scans	Pros: maximal information, supports panel design. Cons: high cost/compute, may reduce spatial coverage.

Sampling design

Sampling design often determines the quality of inference regarding genetic structure and connectivity. Wild boars exhibit a strong pattern of isolation by distance, so uneven or opportunistic sampling can create artificial clusters. Robust study designs for exploring genetic structure and connectivity typically involve balanced spatial sampling (grid-based or stratified by ecoregion), replicated samples across hypothesized barriers and corridors, high-quality spatial metadata including coordinates and date collected, and managing close kinship relationships (e.g. excluding first-degree relatives) to minimize artificially elevated measures of local differentiation resulting from family group sampling (Lecis et al., 2022; Rutten et al., 2019; de Jong et al., 2023). Hybridization assessment requires samples from known hybrids and reference domestic pigs. Lack of suitable reference samples can introduce biases into estimates of admixture proportions particularly in regions of secondary contact (Iacolina et al., 2018).

Population structure and differentiation analyses

Typically, a well-developed analytical workflow will combine several supplementary approaches. Rather than depending upon one approach, PCA is an assumption-free method that can show the greatest genetic gradients. ADMIXTURE estimates ancestry components and can find evidence of admixture and introgression given appropriate reference populations (Alexander et al., 2009; Iacolina et al., 2018). Pairwise F_{ST} is a commonly used measure of genetic differentiation and is useful for comparison within different regions and assisting in the determination of management units. Pairwise F_{ST} should however always be evaluated in combination with isolation-by-distance patterns since moderate levels of F_{ST} can result in continuous populations sampled over long spatial scales (de Jong et al., 2023). Discriminant Analysis of Principal Components (DAPC) offers an alternative multivariate approach to describing and categorizing genetic clusters. DAPC is a popular choice for use in management contexts because of its stability and ability to be interpreted (Jombart et al., 2010).

IBD and IBR analyses

The process of conducting a Landscape Genetics analysis typically begins with evaluating the presence of isolation-by-distance (IBD) to determine if genetic distance increases in conjunction with increasing geographic distance. Wild boar exhibit IBD very frequently, and therefore, it is often important to investigate if there exist other landscape attributes that have explanatory value above and beyond distance (Rutten et al., 2019; de Jong et al., 2023). An extension of IBD is represented by Isolation-By-Resistance (IBR), which considers landscapes as resistance surfaces, where each environmental feature imposes varying costs to the movement of individuals and gene flow. The resultant resistance distances can then be compared with genetic distances to identify potential barriers to movement and/or functional corridors. For example, recent research has demonstrated that roadways and land cover types can have significant impacts on population structure in wild boars providing empirical support for the utility of IBR modelling in both management planning and disease control (Lecis et al., 2022).

Circuit Theory and resistance-surface optimization

In Circuit Theory applications, landscapes are defined as electric circuits, allowing gene flow to occur via multiple routes rather than just a single most efficient pathway. This type of circuitry is thought to more accurately represent the complex movement dynamics found in highly migratory animals like wild boars (McRae et al., 2008). One of the primary challenges associated with applying these models is defining the parameters used to assign resistance values. Through the utilization of genetic algorithms, it is possible to optimize resistance surfaces based on observed genetic patterns, thus enhancing reproducibility and minimizing subjectivity (Peterman, 2018).

Connectivity metrics

Effective Migration Surfaces (EEMS) offer a direct means to visualize differences in effective migration rate. EEMS highlight areas that experience greater or lesser migration than would be predicted using a simple IBD model (Petkova et al., 2016). EEMS results can be utilized in conjunction with resistance-based models. For example, areas experiencing decreased migration may be attributed to physical barriers such as roads or urban development. Conversely, areas exhibiting increased migration can serve as indicators of functionally relevant corridors.

GEA methods for environment-associated genomic variation (LFMM and RDA)

Genotype-Environment Association (GEA) methods are another component of Landscape Genomics. The objective of GEA methods is to evaluate statistical associations between allele frequency and environmental gradient while controlling for population structure. Latent Factor Mixed Models (LFMM) utilize unobserved structure through the employment of latent factors making them a preferred option for large-scale SNP datasets possessing spatial structure (Frichot et al., 2013). Redundancy Analysis (RDA) is a multivariate alternative capable of identifying polygenic signatures and subtle associations among multiple loci (Forester et al., 2018). Studies examining wild boars in Sardinia illustrate how combinations of SNP data with LFMM and RDA can identify candidate genes/loci associated with environmental variation (Fabbri et al., 2022). Results derived from GEA analyses must be viewed carefully as population structure, admixture and historical demography can create false positives if they are not adequately accounted for (Forester et al., 2018; de Jong et al., 2023).

European studies of wild boars, focusing on Croatia and the Adriatic region

Wild boar studies based on genomics across Europe show that there is a strong relationship between geographic distance and genetic diversity, and these relationships were influenced by historical events and how the animals have been managed locally (De Jong et al., 2023). Studies using SNP-arrays have demonstrated clusters of hybridization that exist at different spatial scales. Studies like Iacolina et al. (2018) found it necessary to screen for introgression in domestic pig populations because they had previously identified hybridization hotspot areas. Landscape genetic studies carried out in locations such as Flanders and Sardinia showed significant influences from landscape features (such as forests, roads, and land-use patterns) on gene-flow and population structure (Rutten et al., 2019; Lecis et al., 2022). Genotype-environment association studies in island ecosystems, similar to those studied in Sardinia by Fabbri et al. (2022), have clearly demonstrated the role of the environment on genetic variation. In Croatia, previous studies suggest that while the Dinaric Mountains may act as an impediment to wild boar migration, this mountain range does not completely block gene flow among wild boar subpopulations. Thus, suggesting that wild boar in Croatia exhibit an isolation-by-distance pattern of population differentiation and indicating that a connectivity-based approach would be appropriate to develop effective wildlife management strategies. The prior work of Cubric-Curik et al. (2011) used mitochondrial DNA to describe wild boar population structure in Croatia and suggested that mtDNA studies could be valuable in describing broad scale patterns of population structure. However, Cubric-Curik et al. (2011) also emphasized that nuclear markers should be employed to analyze connectivity.

Conclusion

The use of population and landscape genomics as a tool will aid in defining the genetic structure and connectivity of wild boar. In general, these approaches demonstrate that there is primarily continuous gene flow through the populations due to a variety of factors; including natural occurrences and anthropogenically-related events. Additionally, combining data from genome space with data from geographic space allows researchers to better identify areas of barriers and corridors for potential management decisions. In particular, this is useful when considering the impact of agriculture and the spread of diseases. Therefore, future research efforts would be best directed toward developing management practices that incorporate all three types of data (genomic, ecological, and epidemiologic) and are aware of connectivity.

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REVIEW ARTICLE

Genetic diversity and structure of European wild boar (*Sus scrofa*): a review

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Summary
Studies show that there is a large amount of genetic diversity and a complex population structure of wild boars in Europe, due to the influence of both postglacial recolonization and translocation caused by humans. The process of recolonizing after the last ice age has had an impact on the population dynamics and has led to gene flow. Additionally, many researchers have reported evidence of hybridization between European wild boar and domestic pig as well as continued movement of animals which will affect the genetic makeup. Therefore, future studies need to include genomics for a complete understanding of the genetic composition of European wild boar. Furthermore, it would be beneficial if the genetic changes from hybridization could be monitored in real time.

Keywords: wild boar, phylogeography, genomics, hybridization, gene flow.

Introduction

Wild boars (*Sus scrofa*) are one of the most widely distributed large mammals worldwide. Their global distribution spans from Western Europe to Eastern Asia and they inhabit numerous different types of habitats. One reason for the wide distribution of wild boars is their ability to thrive in a number of environments. They have adapted well to various forms of land use and have proven themselves capable of surviving in nearly every type of environment.

Wild boars are present in almost all regions of Croatia. They live in both forested systems and agricultural-natural mosaics. Over the past few decades, the numbers of wild boars have grown significantly in terms of both density and geographical extent. Several countries reported two to threefold increases in abundance since the 1980s. In some regions, population densities exceed 5-10 individuals per km^2, reflecting both range expansion and local population growth (Massei et al., 2015; Linnell et al., 2020). Wild boar population increases are generally caused by a combination of different factors, including decreased predator activity, altered hunting regulations and/or increased food availability. Additionally, their spread across Europe has been influenced by both historic and contemporary processes. During periods of glacial-interglacial fluctuations, populations retreated to refugia. Later, after the end of the last Ice Age, Europe was repopulated (Scandura et al., 2008). In addition, the spread of wild boars has been influenced by human actions during recent times (Apollonio et al., 2010; Linnell et al., 2020). Increased reproduction rates of wild boars, lack of large carnivores, intensive agriculture, climate change and less hunting pressure (Massei et al., 2015) contribute to their rapid population increase. Although wild boars are abundant and economically important, research on genetics of wild boar populations in Croatia is limited compared to other countries in Europe. Previous studies have mostly used microsatellites or mtDNA (mitochondrial DNA) as genetic markers and analyzed small sample sizes or studied populations in geographically

limited regions (Šprem, 2009; Cubric-Curik et al., 2011), limiting the evaluation of population structures, management units and hybridization with domestic pigs. In comparison to those limitations in Croatia, a lot of progress has been made in the rest of Europe regarding wild boars using SNP genotypes and WGS (whole genome sequences). These methods have provided detailed insights into genetic clusters, gene flow, admixtures, demographic histories and adaptive variations (Veličković et al., 2016; Iacolina et al., 2018; Niedziałkowska et al., 2021; Wang et al., 2025). These studies have enabled distinguishing clearly between natural genetic variation and hybridization from domestic pigs. Nonetheless, no data have yet been generated for Croatia and thus limit the use of scientific information for management decisions as well as identifying hybridization zones, isolated populations or barriers to gene flow. Considering the rising issues related to urban development, habitat fragmentation and hybridization with domestic pigs, further investigations using new genomics techniques will be necessary.
Therefore, this review aims at summarizing current literature about genetic diversity and population structure, phylogenetics and demography as well as hybridization and gene flow for wild boar populations with special emphasis on Croatia.

Population genetic diversity

A wealth of evidence suggests that most European wild boar populations exhibit high genetic diversity. Heterozygosities and allelic richness are particularly high in most studies examining both microsatellite- and genomic-derived markers. Observed heterozygosity (Ho) in European wild boar populations typically ranges from approximately 0.60 to 0.75, depending on the marker type and geographic region, indicating generally high levels of genetic diversity across populations (Veličković et al., 2016; Mihalik et al., 2020). Studies focusing on the Carpathians (Mihalik et al., 2020) indicated high levels of allelic diversity and heterozygosity. Although Lithuanian populations showed high variability with very little differentiation amongst subpopulations (Griciuvienė et al., 2021). While European wild boar populations show slightly lower genetic diversity when compared to East Asian wild boar populations (Scandura et al., 2011), possibly resulting from post-glacial bottleneck effects (Scandura et al., 2008), the current level of diversity is nonetheless substantial. Also, average heterozygosities are high and inbreeding coefficients are usually low indicating that no severe bottleneck occurred recently.
In contrast to other European countries, however, available studies on Croatian wild boars are limited. Available data indicate comparable results though. Cubric-Curik et al. (2011) investigated mitochondrial DNA in wild boars sampled from Istria and Cres Island. All analyzed samples displayed multiple haplotypes belonging to haplogroups E1. Thus, they concluded that Croatian wild boars display a high mitochondrial diversity. Since most samples were assigned to commonly found European haplotypes, it indicates that these Croatian populations reflect typical European genetic backgrounds. Furthermore, a study using microsatellites carried out by Šprem (2009) demonstrated that Croatian wild boar populations show high genetic diversification, i.e. high heterozygosities. Furthermore, most genetic variation occurs within populations in conformity with data from other parts of Europe.

Population structure and phylogeography

In spite of its overall high genetic diversity at all levels (i.e. within each individual and among individuals), European wild boars are not entirely panmictic. Several investigations have demonstrated that the European wild boar is structured into separate populations, which were formed historically by geographic separation in different refuges and later on by physical obstacles (Table 1). The main mitochondrial haplogroups found are E1, E2 and A.

These three haplogroups are broadly distributed throughout Europe for E1 and Eastern Asia for A, whereas the E2 haplogroup indicates another, second European haplogroup divergent from E1 by one or more fixed nucleotide substitutions (Scandura et al., 2008; Alexandri et al., 2012). While there are no sharp limits between these haplogroups, their boundaries are subject to overlap in central Europe. Recent work using mitogenomes to analyze the distribution of mtDNA in European wild boar has identified an area of overlap or "contact zone" spanning northwest Croatia eastward to western Hungary and then continuing north-eastward through Poland to the Baltic States, where the highest level of mtDNA diversity was detected (Niedziałkowska et al., 2021) due to overlapping of mtDNA sequences derived from two distinct refuge areas.

Table 1. Summary of key studies on wild boar population genetics in Europe

Study	Marker type	Main findings
Scandura et al. 2011	mtDNA	Identified distinct genetic clusters, including a cohesive Balkan genetic group
Alexandri et al. 2012	mtDNA	Identified major post-glacial refugia and confirmed the Balkans as an important source of recolonisation in Europe
Veličković et al. 2016	mtDNA + microsatellites	Revealed high genetic diversity and gene flow, with the Balkans acting as a genetic hotspot
Frantz et al. 2019	whole-genome sequencing	Identified introgression between wild boar and domestic pigs and complex demographic history
Mihalik et al. 2020	microsatellites	Reported high heterozygosity and moderate population structuring
Niedziałkowska et al. 2021	SNPs	Showed long-distance dispersal and significant gene flow across populations
Wang et al. 2025	whole-genome SNPs	Provided high-resolution insights into population structure and admixture patterns

Although less intense than those of mtDNA, population structure can be seen in analyses conducted using nuclear markers such as microsatellites and genomic-wide single nucleotide polymorphism (SNP) data. Male mediated gene flow is thought to contribute to the weaker signal of population structure seen in analyses of nuclear markers compared to mtDNA (Wang et al., 2025). A large-scale microsatellite analysis determined that multiple genetically independent populations exist across Europe. Isolated "hot spots" and groups of refugiums can be distinguished, with a cohesive genetic group existing in the Balkans, but a more mixed origin due to post-glacial migration and recent translocation events present in populations from Central and Western Europe (Scandura et al., 2011; Veličković et al., 2016).

Croatian populations display partial genetic differentiation. Those from Istria and Cres exhibit relationships with northern Italy and Slovenia (Šprem, 2009; Cubric-Curik et al., 2011), while populations in eastern regions demonstrate closer genetic ties to both the Pannonian and Carpathian gene pools. This distinction however, may simply be indicative of an isolation-by-distance pattern as opposed to an indication of a significant barrier imposed by the Dinaric Mountain range (Šprem et al., 2016). Therefore, the genetic structure of European wild boar reflects a mix of past divergence and subsequent admixture.

Hybridization with domestic pigs

Wild and domestic pigs belong to the same species (*Sus scrofa*), which allows interbreeding when contact occurs. In many parts of Europe, traditional outdoor pig farming or escape of domestic pigs has resulted in occasional introgression of domestic genetic material into wild boar populations. Nevertheless, contemporary genomic studies indicate that the extent of recent hybridisation remains relatively limited. A comprehensive SNP-based study showed that approximately 11% of individuals exhibited a significant proportion of domestic ancestry, with hybrids concentrated in certain regions such as Austria and parts of the Balkans (Iacolina et al., 2018). In most areas, introgression was lower or negligible, suggesting that wild and domestic pigs remain largely genetically distinct despite historical interbreeding (Scandura et al., 2011; Iacolina et al., 2018). Genetic markers also reveal signals of ancient admixture. Certain mitochondrial haplotypes are shared between wild boar and domestic pig breeds, indicating either common ancestry or historical introgression (Cubric-Curik et al., 2011; Giuffra et al., 2000). In Croatia, systematic studies of hybridisation are still lacking, although available data suggest occasional admixture. From a management perspective, hybridisation remains important due to the potential introduction of maladaptive traits or diseases.

Migration patterns and gene flow

High mobility and ecological adaptability of wild boars are reflected in genetic data, which indicate large distances of gene flow between populations. Postglacial colonisation of Europe originated from several refugia areas, including Iberian Peninsula, Apennine Peninsula and Balkans, followed by range expansion and widespread admixture (Scandura et al., 2008; Niedziałkowska et al., 2021). Genetic analyses support this scenario. For example, Veličković et al. (2016) found that there was gene flow from peripheral isolated populations towards neighbouring regions resulting in higher genetic diversity of central populations. Continuous migration patterns also exist today as individuals can disperse over long distances. Sex-biased dispersal contributes additional shaping of genetic structure. Males are more likely than females to disperse while females remain closer to their natal areas. Therefore, mitochondrial DNA has stronger signals of previous history structure, while nuclear markers have a stronger signal of later admixture (Scandura et al., 2008; Scandura et al., 2011). In Croatia, specific migration patterns have not been extensively studied, but available finding suggests continuous connectivity between populations, particularly along the Dinaric region and the Pannonian Plain (Šprem et al., 2016).

Impact of habitat fragmentation

In Europe, habitat fragmentation and man-made barriers such as roads and fencing can greatly affect wildlife by preventing gene exchange among fragmented groups. However, wild boars exhibit higher resistance to these effects compared to many other species; they are mobile and adaptable to different environments. As a result, genetic studies have shown low levels of population subdivision when analyzed across regions with human made boundaries.

For example, studies in Lithuania found no significant genetic differences between wild boar living on opposite sides of highways (Griciuvienė et al., 2021). Similar results were found in Hungary where large highways had little effect on genetic structure of local populations (Mihalik et al., 2020). Effects of fragmentation appear to be greatest under conditions of complete or long-term separation of groups. Examples of this include island populations or areas completely enclosed by fencing for hunting purposes. These areas restrict movement within them and create unique genetic patterns. Wild boars on Sardinia are an example of the former where populations have been isolated for extended periods and developed distinct

genetic characteristics (Schleimer et al., 2022). The influence of domestic pig genes into wild boar may have played a larger or at least equal role than loss of genetic variation. On the other hand, there was evidence of possible contact or recent separation between the island population on Cres and the one on the Istrian peninsula in Croatia (Cubric-Curik et al., 2011). Overall, the lack of widespread genetic structuring associated with the fragmentation of mainland habitats suggests that wild boar continue to utilize patches of habitat throughout the landscape mosaic and move over both agricultural land and forested land.

Conclusion

Genetic research in European wild boars has given new insight into how their populations have developed in recent decades. The genetic diversity is currently maintained at a level that reflects both historical events and an increase in population size. Phylogenetic patterns can still be seen as well; however, they have been diminished by the amount of interbreeding between these different populations. The Croatian populations belong to the Balkan genetic complex; although there may be local differences (e.g., genetic variation), the general connectivity of all the Croatian populations is relatively high. Future research on Croatian wild boar populations should prioritise genome-wide approaches, including whole-genome sequencing and high-density SNP panels, to improve the resolution of population structure and detect hybridisation with domestic pigs. In particular, the development of reliable genomic tools for identifying introgression is essential for wildlife management and conservation. These research directions are aligned with the objectives of the GENSUS project, which aims to provide a comprehensive assessment of genetic diversity and population structure in Croatia.

Acknowledgement

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ORIGINAL SCIENTIFIC PAPER

Morphological and physiological parameters of honey bee (*Apis mellifera carnica*) queens from Croatian breeding program

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Summary
The aim of this study was to investigate the morphological and physiological parameters of Carniolan honey bee queens (*Apis mellifera carnica*) from Croatia. A total of 71 queens from the Adriatic, Mountain, and Continental regions were analyzed. The average body mass of the queens was 0.189 g, ovary mass 0.0398 g, number of ovarioles 138, spermatheca diameter 1.16 mm, and sperm count 3.55×10^6. Ovary mass and spermatheca diameter differed significantly among regions ($p < 0.05$), while the other parameters did not show significant differences. These results indicate the influence of regional conditions on specific queen traits and highlight the importance of monitoring their health status.

Keywords: *Apis mellifera carnica*, honey bee queens, reproductive parameters, geographic regions

Introduction

The health and reproductive quality of honey bee queens (*Apis mellifera* L.) are crucial factors for the survival, development, and productivity of a colony (Amiri et al., 2025). Both abiotic and biotic factors, including diseases, nutrition, and environmental stressors, can cause morphological and physiological abnormalities, thereby reducing colony stability. Queens mate only once in their lifetime, typically between the sixth and tenth day of age during the so-called nuptial flight. On average, they mate with 12 drones, although the number can exceed 30 (Rhodes, 2002). After mating, approximately 5 million spermatozoa are stored in the spermatheca, while the total number in the oviducts reaches around 80 million spermatozoa (Woyke, 1988). The quality of drone semen directly affects the queen's reproductive success and the colony's productivity (Pettis et al., 2016). The reproductive potential of a queen can be assessed through various morphological and physiological parameters, including body mass, ovary mass, number of ovarioles, spermatheca diameter, sperm count and sperm viability. Abnormalities in these parameters can result from genetic factors, environmental stressors, or diseases and can reduce reproductive performance. Previous studies have shown that queen reproductive parameters vary depending on region, genetic background, and breeding practices, although standardized rearing conditions often ensure consistent quality (Dražić et al., 2015; Gregorc and Smodiš Škerl, 2015; Prešern and Smodiš Škerl, 2019). Research conducted in Croatia, Slovenia, and Italy has highlighted significant regional differences in queen body mass, number of ovarioles, and spermatheca quality; however, adequate nutrition and controlled mating conditions generally contribute to high reproductive quality (Facchini et al., 2021; Prešern and Smodiš Škerl, 2019).
The aim of this study was to evaluate the morphological and physiological parameters with a focus on reproductive characteristics of honey bee queens across different regions of

Croatia. Understanding these traits and their interrelationships can help queen breeders reduce losses and increase colony productivity.

Material and Methods

A total of 71 queens were collected in June of 2025. from registered queen breeders in Croatia. The queens were delivered in transport cages accompanied by attendant workers. Upon arrival, they were anesthetized with CO_2, weighed, and visually inspected. Queen dissections were performed according to standard methods (Carreck et al., 2013) in order to isolate reproductive organs and determine ovary mass, number of ovarioles, spermatheca diameter, as well as sperm count and viability. The spermatheca diameter was measured from photographs taken after removal of the tracheal network. Sperm count and viability were determined using a Neubauer hemocytometer under 400× magnification with an inverted microscope. The collected data were statistically analyzed using the software package R version 4.5.2 (R Development Core Team, 2014).

Results and Discussion

The average values of reproductive parameters by region are presented in Table 1.

Table 1. Average values of reproductive parameters of queens by region (n = 71)

Parameter	Mountain (n = 22)	Lowland (n = 34)	Coastal (n = 15)
Queen mass (g)	0.186	0.194	0.181
Ovary mass (g)	0.0396	0.0435	0.0318
Number of ovarioles	144	135	136
Number of spermatozoa (millions)	3.35	3.87	3.15
Spermatozoa viability	83.1	88.9	84.6
Spermatheca diameter (mm)	1.18	1.17	1.12

Queen body mass did not differ significantly among regions ($F_{2,68} = 1.226$, $p > 0.05$), although queens from the Continental region showed the highest mean value (0.194 g), while those from the Adriatic region had the lowest (0.181 g). Similar results were reported by Dražić et al. (2015), who also found no significant effect of region on queen quality. In contrast to body mass, ovary mass differed significantly among regions ($F_{2,68} = 9.571$, $p < 0.001$). Queens from the Adriatic region had the lowest average ovary mass (0.0318 g) (Figure 1), whereas the highest values were recorded in the Continental region (0.0435 g). A similar correlation between these traits was reported by Gregorc and Smodiš Škerl (2015), who found a significant correlation between queen body mass and ovary mass.

The number of ovarioles did not differ significantly among regions ($F_{2,68} = 1.759$, $p=0.18$), although queens from the Mountain region showed a slightly higher average (144) compared to the other regions. Similar values were reported by Facchini et al. (2021), where the average number of ovarioles was approximately 141, confirming the relative stability of this parameter. The number of spermatozoa showed a trend toward regional differences ($F_{2,68} = 3.045$, $p=0.054$). Queens from the Adriatic region had the lowest average spermatozoa count (3.15 million), while the highest values were recorded in the Continental region (3.87 million).

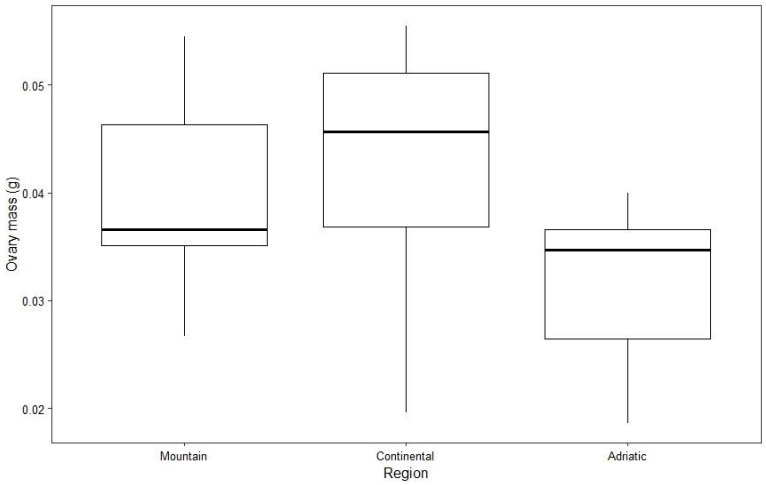

Figure 1. Ovary mass of dissected honey bee queens from three different regions in Croatia.

Although these differences were not statistically significant, the observed trend may indicate the influence of environmental factors or mating success. It is possible that hot and dry weather in June in Adriatic region is having a negative impact on drone production, and consequently queen was not mated as well as in Continental region. Similar number of stored spermatozoa (approximately 3–4 million) were reported by Al-Lawati et al. (2009) and Kovačić et al. (2016). Spermatozoa viability was high across all regions (83.1–88.9%) with no significant differences ($F_{2,68}$ = 0.722, p=0.489), indicating stable spermatozoa quality regardless of geographic origin. In contrast, spermatheca diameter differed significantly among regions ($F_{2,59}$ = 3.933, p=0.025) (Figure 2), with queens from the Adriatic region showing the smallest diameter (1.12 mm). The obtained values are slightly lower than those reported by Hatjina et al. (2014) and Amiri et al. (2017), where a spermatheca diameter above 1.2 mm is considered an indicator of high-quality queens.

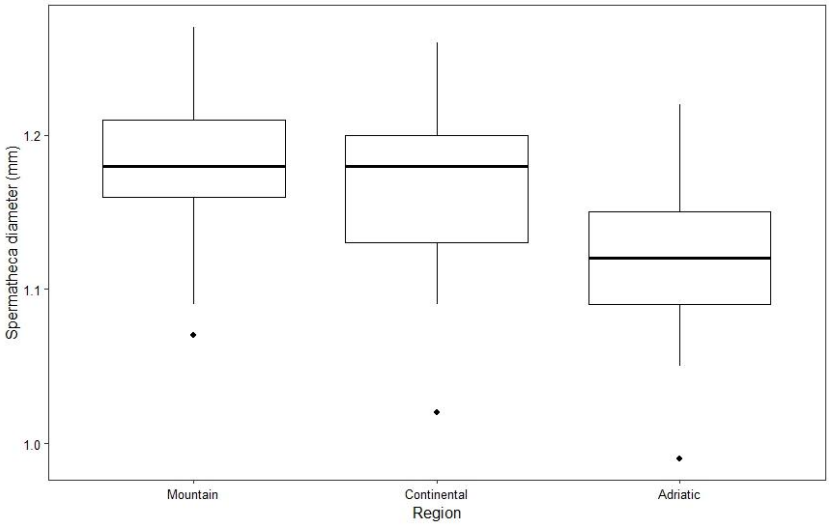

Figure 2. Value of spermatheca diameter by region.

A significant positive correlation was found between queen body mass and ovary mass (ρ = 0.485; $p < 0.001$) (Figure 3), as well as between queen body mass and the number of ovarioles (ρ = 0.335; p = 0.004). Additionally, a positive correlation was observed between ovary mass and the number of ovarioles (ρ = 0.288; p = 0.015). Other correlations, including those involving spermatozoa count and spermatozoa viability, were not statistically significant ($p > 0.05$).

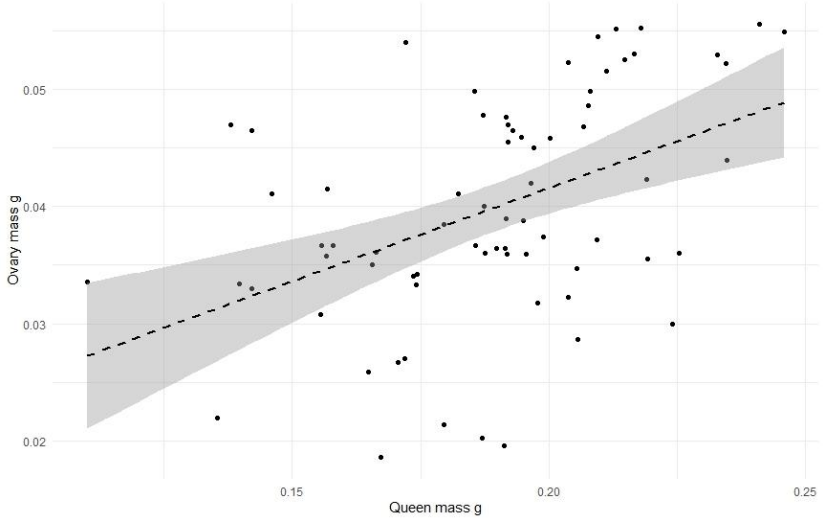

Figure 3. Correlation between queen mass and ovary mass.

Conclusion

The results of this study indicate that most reproductive parameters of honey bee queens in Croatia do not vary significantly among regions, suggesting relatively uniform queen quality. However, ovary mass and spermatheca diameter showed significant regional differences, with queens from the Adriatic region exhibiting lower values for these parameters. The identified positive correlations between queen body mass, ovary mass, and the number of ovarioles indicate a correlation among key morphological traits. In contrast, spermatozoa count and spermatozoa viability showed high stability and were not significantly correlated with other variables. In conclusion, queens from Croatian breeding program of *Apis mellifera carnica* showed generally stable morphological traits across regions, with significant regional differences observed only in ovary mass and spermatheca diameter, while other parameters remained consistent and demonstrated postive correlation between queen body mass and reproductive potential.

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ORIGINAL SCIENTIFIC PAPER

Overview of pollen analyses of honey from the Požega Valley in a period 2018–2024

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Summary

The geographical and botanical origin and as well as the proportion of pollen grains from particular plant species in honey, can be determined by microscopic analysis of pollen grains in honey. The aim of this research was to analyse and compare honey types from the Požega Basin in the period 2018–2024 based on the results of pollen analysis, and to determine which type of honey was dominant during that period. A total of 261 honey samples from the Požega Basin were analysed over seven years, as part of the county honey evaluation, prior to sensory evaluation. During this period, the largest number of samples was identified as black locust honey (118 samples, 45.2%) and flower honey (101 samples, 38.7%). Most black locust and flower honey samples contained pollen from the Rosaceae family.

Keywords: honey, pollen analysis, Požega Valley

Introduction

In different habitats in continental Croatia, the following bee pastures are distinguished: black locust, chestnut pasture, linden, amorphous pasture, rapeseed pasture, lowland meadow pasture, mountain meadow pasture, sunflower pasture, etc. (Bučar, 2008). The main pasture begins when the flowering of honey-bearing plants that grow on larger areas begins around the apiary. Most bee pastures in Croatia take place during May and June. Thanks to the large number of plant species, Croatia is rich in various types of honey. The most common are black locust, chestnut pasture, linden, sage, honeydew and flower (meadow) honey. Vahčić and Matković, 2009 state that the most important and most common types of honey in Croatia are: black locust, chestnut pasture, linden, amorphous honey, blossom honey, sunflower, sage, lavender, rosemary and heather honey. Honey differs from each other in color, taste and medicinal properties. Sabo et al. (2010) state that honey-bearing plant families are those from whose flowers bees take the most nectar and pollen, and that the type of honey can be monofloral if the honey in the insoluble sediment contains at least 45% of pollen grains of the same plant species.The geographical and botanical origin as well as the proportion of pollen grains from particular plant species in honey, can be determined by microscopic analysis of pollen grains in honey. Many plant species in the Požega Valley are honey-bearing and contribute to the diversity of the pollen spectrum of honey types in this region (Štefanić et al., 2012). Numerous authors have conducted pollen analysis of honey from specific areas, among whom the works of Špoljarić Maronić et al. (2017), Rašić et al. (2018), and Pašagić (2018) are noteworthy. The aim of this research was to analyse and compare honey types from the Požega Valley between 2018 and 2024 based on pollen analysis results, and to determine which type of honey was dominant during that period.

Material and Methods

A total of 261 honey samples were collected from the Požega Basin for pollen analysis over seven years, as part of the county honey evaluation, prior to sensory assessment (Figure 1). The honey samples were analysed between 2018 and 2024 at the Institute of Public Health of Požega-Slavonia County, Department of Health Ecology. Qualitative melissopalynological analysis was conducted by counting 300 pollen grains using an Olympus BX41 microscope at 400x magnification. Pollen grains in the insoluble sediment were identified according to the literature (Martin and Harvey, 2017; Von der Ohe and Von der Ohe, 2003). The results of the melissopalynological analysis are expressed as percentages relative to the total number of pollen grains. The quantity of pollen grains is classified as follows: pollen exceeding 45% is considered main pollen (>45%), followed by accompanying pollen (16–45%), other pollen (3–15%), and rare pollen (<3%) (Kaya et al., 2005).

Results and Discussion

Over seven years (2018–2024), a total of 261 honey samples were analysed. The highest number of samples (65) was collected in 2024, while the lowest (8) was in 2021. During this period, the most frequently identified types after analysis were black locust honey (118 samples, 45.2%) and multifloralhoney (101 samples, 38.7%). There were 18 samples of chestnut pasture honey (6.9%), 9 samples (3.4%) identified as linden honey, 3 samples (1,1%) as blossom honey, 2 samples (0.8%) as honeydew honey, and 1 sample (0.4%) as sunflower honey (Table 1, Figure 1). The largest number of black locust honey samples were collected in 2024 and 2018 (29 samples each). The highest number of flower honey samples was collected in 2020 (26 samples) and 2024 (25 samples) (Table 1).

Table 1. Number of samples and types of honey during the period 2018–2024.

A type of honey	2024	2023	2022	2021	2020	2019	2018	Total/%
black locust	29	23	22	2	8	9	25	**118 (45.2 %)**
multiflorel	24	12	12	3	26	18	6	**101 (38.7 %)**
chestnut pasture	3	1	5	2	1	5	1	**18 (6.9 %)**
linden	2	3	1	-	2	1	-	**9 (3.4 %)**
dew honey	4	1	3	1	-	-	-	**9 (3.4 %)**
blossom	1	1	1	-	-	-	-	**3 (1.1 %)**
honeydew	2	-	-	-	-	-	-	**2 (0.8 %)**
sunflower	-	-	-	-	1	-	-	**1 (0.4 %)**
Total	**65**	**41**	**44**	**8**	**38**	**33**	**32**	**261**

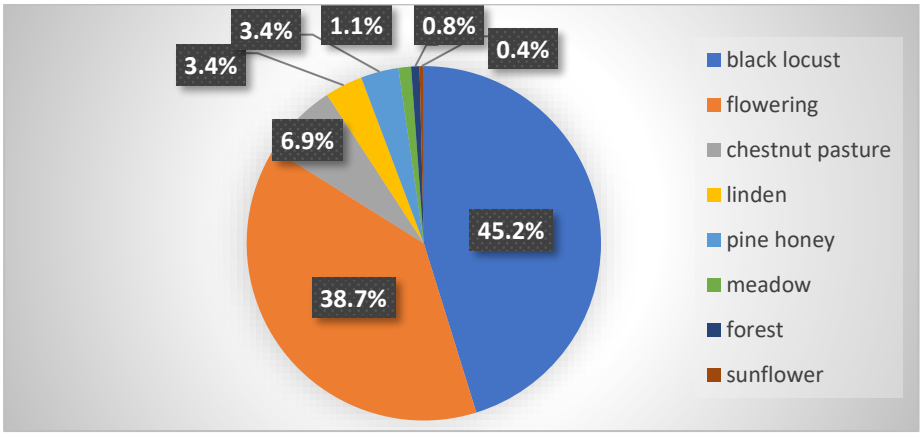

Figure 1. Types of honey analysed during the period 2018–2024.

In black locust honey, the percentage of black locust pollen grains ranges from 20 % to 51%. Pollen from at least 24 plant species was found in all samples of black locust honey. Sixty-seven samples (56.78%) of black locust honey contain pollen from the Rosaceae family, with the proportion of pollen grains in these samples ranging from 9% to 54%. Among the genera and families with a significant presence in black locust honey, plants from the genus *Brassica* sp. are notable, with their pollen found in 57 samples (48.3%), and plants from the genus *Trifolium* sp., whose pollen is found in 53 samples (44.9%). All represented families, genera, or species in the analysed samples of black locust honey from the Požega basin from 2018 to 2024 are shown in Table 2.

Table 2. Prevalence of pollen grains in black locust honey from the Požega Valley, 2018–2024.

Family, genus, species	Representation in black locust honey	Pollen percentage
Rosaceae	67 (56.78%)	9-54%.
Brassica sp.	57 (48.3%)	1–41 %
Trifolium sp.	53 (44.9%)	3-38%.
Fabaceae	38 (32.20)	4 -28%
Salix sp.	32 (27.1%)	5-30%
Sambucus sp.	23 (19.5%)	4-22%
Castanea sp.	17 (14.4%)	7-52 %
Amorpha sp.	17 (14.4%)	1-24 %
Prunus sp.	15 (12.7%)	8-40%
Asteraceae	11 (9.3%)	5-38 %
Cornus sp.	11 (9.3%)	5–33%
Fagus sp.	8 (6.8%)	2-30%
Phacelia sp.	8 (6.8%)	6-38%
Aceraceae	6 (5.1%)	1-13 %
Tilia sp.	3 (2.5%)	1-3%
Plantago sp.	3 (2.5%)	3-8%
Lythrum sp.	2 (1.7%)	2-10%
Salvia sp.	2 (1.7%)	1-2%
Poaceae	1 (0.8%)	1%
Solidago sp.	1 (0.8%)	1%,
Taraxacum sp.	1 (0.8%)	1%
Vitis sp.	1 (0.8%)	5%
Fragaria sp.	1 (0.8%)	16%

In all multifloral honey samples, pollen from at least 26 plant species was identified. Among the 101 samples, pollen from the *Rosaceae* family was present in 83 samples (82.2%). Among the genera and families with a high presence in flower honey, plants from the Brassica genus were notable, with their pollen found in 55 samples (54.5%), and plants from the *Fabaceae* family, with their pollen detected in 48 samples (47.5%). Pollen from the Castanea genus was also significantly present, appearing in 46 samples (45.5%). All families, genera, or species identified in the analysed flower honey samples from the Požega Valley between 2018 and 2024 are listed in Table 3.

Table 3 Prevalence of pollen grains in blossom honey from the Požega Valley, 2018–2024

Family, genus, species	Representation in blossom honey	Pollen percentage
Rosaceae	83 (82.2%)	1-73%
Brassica sp.	55 (54.5%)	3-87%
Fabacea	48 (47.5%)	1-38%
Castanea sp.	46 (45.5%)	3-88%
Robinia sp.	38 (37.6%)	2-19%
Salix sp.	33 (32.7%)	5-42 %
Asteraceae	33 (32.7%)	3-35%
Trifolium sp.	22 (21.8%)	2- 35%
Amorpha sp.	15 (14.9%)	6-43%
Helianthus sp.	14 (13.9%)	5-31%
Tilia sp.	12 (11.9%)	2-14%
Sambucus sp.	11 (10.9%)	3-26%
Phacelia sp.	10 (9.9%)	5-23%
Aceraceae	8 (7.92%)	1-14%
Fagus sp.	5 (4.95%)	3-28%
Plantago sp.	5 (4.95%)	3-17%
Cornus sp.	4 (3.96%)	5 -28%
Taraxacum officinale	3 (2.97%)	4%
Apiaceae	2 (1.98%)	6%
Artemisia sp.	2 (1.98%)	8-12%
Lythrum sp.	2 (1.98%)	19-34%
Quercus sp	2 (1.98%)	3 - 13 %
Loranthus sp	1 (0.99%)	14%
Prunus sp	1 (0.99%)	16%
Salvia sp.	1 (0.99%)	9%

In chestnut pasture honey, the percentage of pollen grains ranges from 85% to 98%. In all chestnut pasture honey samples, pollen from at least 13 plant species was found. The highest percentage in chestnut pasture honey samples was pollen from the genus Tilia (27.8% of samples) and from the families *Brassicaceae* and *Rosaceae*, each found in 22.2% of samples. In linden honey, the percentage of linden pollen grains ranges from 25% to 78%. In all linden honey samples, pollen from at least 15 plant species was found. In 66.7% of linden honey samples, pollen from the genus Castanea was present. Blossom honey was represented by only three samples during the research period and contained pollen from the families *Asteraceae*, *Rosaceae,* and *Fabaceae*; the genera Lythrum, Phacelia, Plantago, and Trifolium; and the species *Castanea sativa* and *Helianthus annuus*. Forest honey was represented by only two samples during the research period and contained pollen from the families *Fabaceae* and *Rosaceae*, the species *Castanea sativa*, and elements of honeydew.

Conclusion

A total of 261 honey samples were analysed in the pollen analysis of honey from the Požega Valley during the period 2018–2024. The highest number of samples (65) was analysed in 2024. During this period, most samples were classified as black locust honey (118 samples, 45.2%) and flower honey (101 samples, 38,7%). In black locust honey, pollen from at least 24 plant species was detected, and pollen from the *Rosaceae* family was present in 56.78% of these samples. In flower honey, pollen from at least 26 plant species was detected, with pollen from the *Rosaceae* family found in 82.2% of the samples.

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Session 3

Agroecology and Regenerative Agriculture

ORIGINAL SCIENTIFIC PAPER

Impact of Short Rotation Coppice Windbreak Systems on Soil Wind Erosion: A Case Study

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Summary
Soil quality plays a crucial role in maintaining sustainable agriculture and stable food production. Earlier studies indicate that Short Rotation Coppice (SRC) may contribute to soil improvement, especially in the rehabilitation of degraded farmland due to wind erosion. This research aimed to assess the effects of introducing SRC on agricultural soils, focusing on minimizing wind erosion. Wind erosion was evaluated by measuring airborne soil particles and recording wind speed and direction. The findings revealed statistically significant differences in wind erosion, which was markedly reduced within the alley cropping system compared to the open field. The study concludes that alley cropping systems with SRC play an important role in protecting soil from wind erosion, although long-term research is necessary to determine their full impact on soil quality.

Keywords: agroforestry, black locust, poplar, willow

Introduction

Soil is a non-renewable natural resource that is subject to degradation processes such as soil erosion and loss of organic matter (Martinović, 1997). In addition to natural and climatic factors, human activity has significantly contributed to soil degradation through irresponsible use and nutrient depletion. In the past, practices such as growing multiple crops together (intercropping) helped maintain soil health. However, during the 20th century, agricultural modernization and the development of mineral fertilizers led to intensive agriculture becoming dominant, replacing traditional intercropping systems.

Intensive agriculture, based mainly on mineral fertilization, frequent machinery passes and without green manure, increases the risk of wind erosion. Wind erosion removes the most fertile topsoil layer, reduces productivity, contributes to desertification, damages soil structure and water retention, worsens air quality, harms infrastructure, reduces crop growth, and causes economic losses (Jug et al., 2022).

Intercropping (growing two or more crops simultaneously on the same land) is now recognized as a method for protecting natural resources (Špoljar, 2019). It improves nutrient and light use efficiency, increases resistance to abiotic stress, enhances soil fertility, improves yield quality, and increases production stability. One of such intercropped systems is the agroforestry practice of alley cropping, where crops are grown within the tree alleys. Alley cropping integrates trees and shrubs with crops, combining forestry and agriculture. In such a system trees have protective functions, such as windbreaks against erosion, while also providing additional income (timber, firewood, fruit) (Tsonkova et al. 2012; Žalac et al. 2022).

The most common silvoarable agroforestry practice of windbreaks is established with fast-growing short-rotation tree species, such as black locust, willow, and poplar. These species grow quickly and can also be used for biomass energy production. Short-rotation coppice (SRC)

significantly benefits the environment by increasing biodiversity and providing habitats for many plant and animal species (Stolarski et al., 2014; Dimitriou & Rutz, 2015). In Croatia, the cultivation of short-rotation woody crops is regulated by the Act on Short-Rotation Woody Crops (NN 15/18, 111/18) and the Ordinance on the List of Plant Species for Establishing Short-Rotation Woody Crops and the Conditions for Their Cultivation (NN 16/19). Although this legal framework represents a positive step toward soil conservation and potential recovery of degraded land, it does not explicitly mention terms such as "agroforestry" or "alley cropping." While establishing agroforestry and alley cropping systems is possible, the absence of these terms in legislation, insufficient ministerial support, and limited farmer education restrict the wider adoption of these practices in Croatian agriculture. Research shows that introducing trees into agricultural land improves soil quality, although measurable changes often become visible only after 10–20 years (Ivezić et al., 2022). One of the most important effects is the reduction of soil erosion. Since soil erosion reduces fertility and nutrient availability, understanding how short-rotation tree species can reduce erosion is crucial for developing sustainable agricultural practices. This research aims to determine the impact of establishied short-rotation coppice on agricultural land to reduce the wind erosion.

Material and Methods

Experimental site

The experiment with short-rotation crops (SRC) was set up on 2021 at the experimental site of the Faculty of Agrobiotechnical Sciences Osijek. The selected species were black locust, willow, and poplar. The trees were planted in four strips, each 8 meters wide, with each strip consisting of six rows of trees (spacing between tree rows within a strip was 1–2 m). The distance between two tree strips (crop alley between strips) was 24 meters wide to allow undisturbed passage of agricultural machinery (Figure 1). The length of each strip was 150 m, while individual species occupied 50 m sections. In 2024, experiment was conducted observing the wind erosion within the crop alleys when trees were between 5 and 8 meters tall (depending on the species). Figure 1 shows a satellite image of the experiment, with orange-marked locations representing the sites where airborne particles were collected to determine wind erosion intensity. On the western side of the experimental field, there is a canal excavated in the mid-20th century. During excavation, the removed soil was piled along the canal edge, causing soil degradation extending from the canal inward, particularly affecting the first strip and the first inter-row area.



Figure 1. Experimental design – orange dots indicate the location of collecting wind erosion residue. Yellow rectangles show the location of meto stations

Wind

The wind direction and speed were measured using anemometers (Davis Vantage Pro2) installed as part of weather stations. One weather station was placed in an open area to record meteorological data outside the alley cropping system, while the second station was located within the system in the first inter-row between two tree strips. The aim was to determine the impact of short-rotation crops on wind direction, wind speed, and wind run (distance travelled by wind). Wind speed was measured in meters per second (m/s), and wind direction was recorded relative to cardinal directions.

Erosion

Soil wind erosion was measured using the "dust catcher" method, where devices were installed in the field to capture airborne soil particles. Although commercial measuring stations are available, improvised devices made from plastic bottles were used in this experiment. Each station had openings facing all cardinal directions (north, south, west, east) to determine the dominant direction of erosion. All stations were placed and measured at heights of 15 cm while four stations were also measured at 50 cm. Erosion was monitored from February 26, to April 9, 2024, the period without any vegetation. Measuring stations were installed at seven locations: two in the control area (away from the short-rotation crops) and the others within the intercropping system (Figure 1). After removing the measuring stations from the field, the collected material was rinsed with water and transferred into Petri dishes. The dishes were then placed in a drying oven to allow the remaining water used during rinsing and sample transfer to evaporate. After drying, the dry mass of the material collected by each station was measured with a precise digital scale.

Results and Discussion

Soil erosion is considered the leading soil degradation process in Europe. According to Kohnke (1972), surface soil layers contain higher concentrations of calcium, phosphorus, and other materials. Since erosion primarily removes the topsoil layer, it leads to nutrient depletion. Plants grown on eroded soils are not necessarily lower-yielding, but they may contain fewer minerals and higher levels of nitrogen and carbohydrates, which can negatively affect human health.

The results of the experiment provided insight into the effect of SRC on wind direction, speed, wind run, and transport of soil particles. Based on the obtained data, it is possible to assess whether SRC significantly influenced soil changes and in what way.

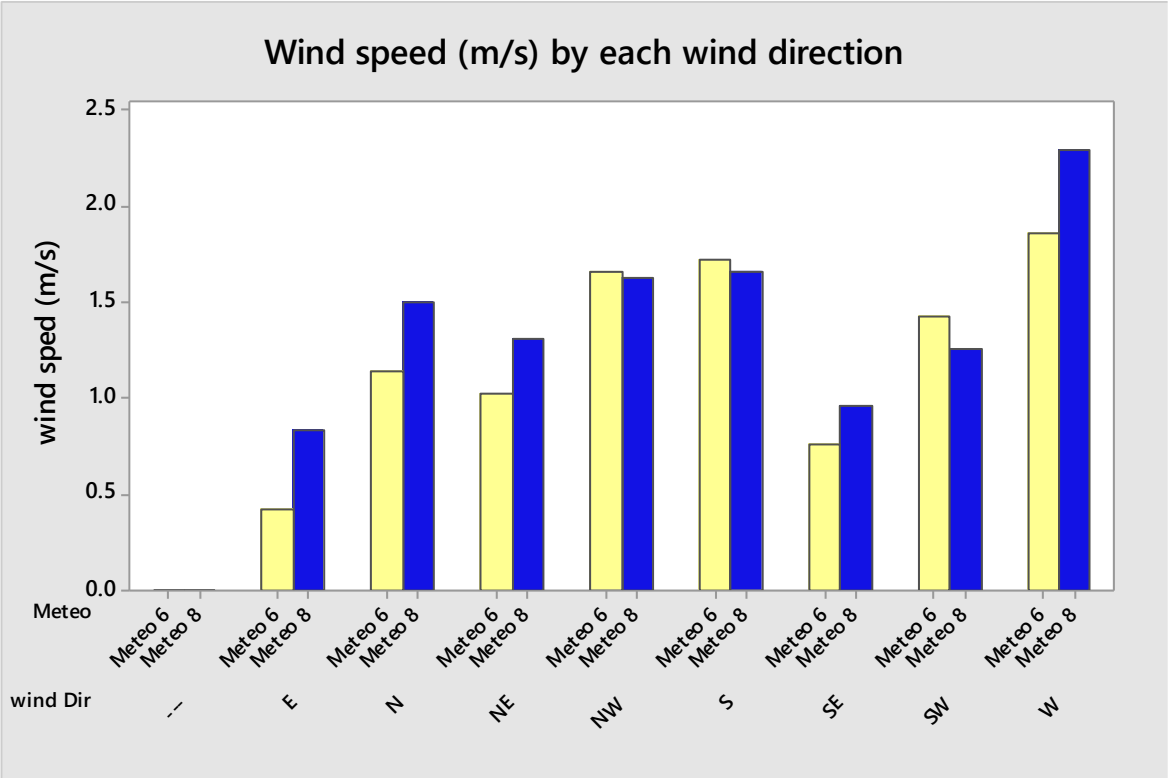
Analysis of variance of the data collected during March from the weather stations (one located in the open field and the other within the intercropping system) showed that wind speed outside the intercropping system was statistically significantly higher than inside (Table 1). Descriptive statistics indicate that the wind most frequently blew from the east, while the strongest winds came from the west.

Table 1. Wind speed and captured soil particles

	Wind speed		Airborne particles (15 cm)		Airborne particles (50 cm)		Airborne particles (Total)	
	n	m/s	n	g	n	g	n	g
Open field	259	0.0160^{A}	8	0.027^{A}	8	0.004^{A}	16	0.015^{A}
Within the alley	271	0.0046^{B}	20	0.005^{B}	8	0.002^{A}	28	0.005^{B}

Data analysis shows that wind coming from the east was 50% weaker within the alley cropping system, while winds from the west, north, northeast, and southeast were about 20% weaker compared to wind strength in the open field (Graph 1). Statistical analysis also showed similar

results when observing wind run (the distance traveled by wind – not shown here). The distance traveled by wind was reduced by about 20% compared to conditions without protection or barriers. In other words, establishing alley cropping systems or windbreak strips reduces the distance over which wind has an impact. Thus, intercropping with woody species plays an important role in wind protection (Jug et al., 2022), which is confirmed by the results of this study.



Graph 1. Wind speed and direction (Meteo 6 - inside the alley, Meteo 8 – open field)

The erosion results (Table 1) show that the mass of airborne particles was statistically significantly lower within the alley cropping system—three times lower—than in open areas. Based on these results, it can be concluded that SRC intercropping has a important wind-protective role. For a more detailed analysis, the data were categorized according to measurement height, which shows that more statistically significant differences were recorded at 15 cm height compared to 50 cm, although at 50 cm the mass of airborne particles within the intercropping system was nearly half that measured in the open field (Table 1). In addition to measuring particle mass at different heights, analysis was also conducted according to cardinal directions (not shown here). The largest amount of airborne particles was collected from the west. This amount was so significant that the mass of particles collected from the east was ten times lower than that from the west. These results align with wind speed data (Graph 1), which showed that the strongest winds statistically came from the west, explaining the higher particle mass from that direction.

Even when data were divided according to wind direction, the mass of collected particles within the intercropping system remained significantly lower compared to the open field. Regardless of measurement station location, the highest amount of airborne particles was collected from the west, and the lowest from the east.

Conclusion

This research confirmed the influence of short-rotation crops (SRC) on soil quality in agroecosystems. SRC stood out particularly as windbreak strips, effectively reducing soil erosion. This is crucial for preserving soil fertility and ensuring the long-term sustainability of agricultural land. The results suggest that the use of SRC can reduce the negative effects of soil erosion and improve soil fertility, creating new opportunities for integrating these practices into conventional agriculture. In addition to existing research, further studies should examine the impact of SRC on soil moisture and long-term crop yields to determine whether intercropping with SRC is more productive than sole cropping. The implementation of SRC species such as black locust, willow, and poplar has proven to be an effective wind protection strategy, improving soil quality and preserving natural resources. This research lays the foundation for further studies and broader application of SRC, aiming to increase the resilience of agricultural systems to degradation processes and to create sustainable agroecosystems.

Although further investigation and more detailed analysis of the obtained results are needed, this study confirms a positive—or at least neutral—impact of SRC on soil. However, it unequivocally confirms their important role in protecting soil from erosion.

Acknowledgement

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ORIGINAL SCIENTIFIC PAPER

The effect of lime application on garlic (*Allium sativum* L.) growth

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Summary
Garlic (*Allium sativum* L.) is an annual or biennial plant in the onion family (Alliaceae). It contains the essential oil allicin, which gives it an intense aroma and taste. As a crop, garlic is considered medium-demanding, and its cultivation in crop rotation is required. The aim of the research was to determine the influence of lime on the growth and development of garlic. The research was conducted in spring 2022 in Brodski Zdenci, Brodsko-Posavina County. The results showed that garlic grown on a plot without lime had higher values of leaf length, pseudo steam length, bulb diameter, steam diameter and plant weight except for root length, indicating that the calcium in lime is beneficial only for the growth of garlic roots.

Keywords: garlic, soil analysis, lime material

Introduction

Garlic (*Allium sativum* L.) is an annual or biennial plant belonging to the onion family (Alliaceae). Along with onions, garlic is the most important representative of the bulbous vegetable group and is one of the oldest vegetable crops. Its importance as a food and medicinal plant has been known for centuries (Addis and Abebaw, 2017; Al-Karaki et al., 2024), and today the economic value of garlic lies in its application in the food industry, medicine and the production of dietary supplements (Anđelini et al., 2025). Garlic extract has antibacterial, antifungal, antiviral and antiparasitic properties and plays a significant role in traditional and modern medicine (Stanway, 2013; Sharma, 2019; Al-Karaki et al., 2024). Garlic contains the essential oil allicin, which gives it intensive aroma and taste; therefore, garlic is not used as a vegetable in the strict sense, but as a dietary supplement (Parađiković, 2009). It originates from Central Asia, but is now cultivated worldwide, due to its adaptability to various climatic conditions and relatively simple cultivation technology (Anđelini et al., 2025). Garlic grows best on sunny location with medium-heavy and well-drained soils (Parađiković, 2009). Calcium as one of the macroelements is essential for plant growth, is important for good root development and rooting, disease resistance, affects the availability of molybdenum, improves soil structure, stimulates the decomposition of organic matter and has a positive effect on better storage (Lončarić et al., 2015). Many agricultural soils are poor in calcium, either due to their natural poverty in calcium, or due to its loss from the soil through the removal of agricultural products and erosion or leaching into lower soil layers (Znaor, 1996). Research indicates that garlic cultivation is most influenced by soil characteristics, climatic conditions, fertilization and cultivar (Al-Karaki et al., 2024). In addition, successful cultivation has been reported with the use of organic material such as vermicompost (Paczka et al., 2021) the application of zinc and iron (Alam et al., 2019), phosphorus (Majumdar et al., 2003; Jitarwal et al., 2018; Al-Karaki et al., 2024) and potassium (Jiku et al., 2020). The aim of this research was to determined the impact of lime application on the growth and development of garlic.

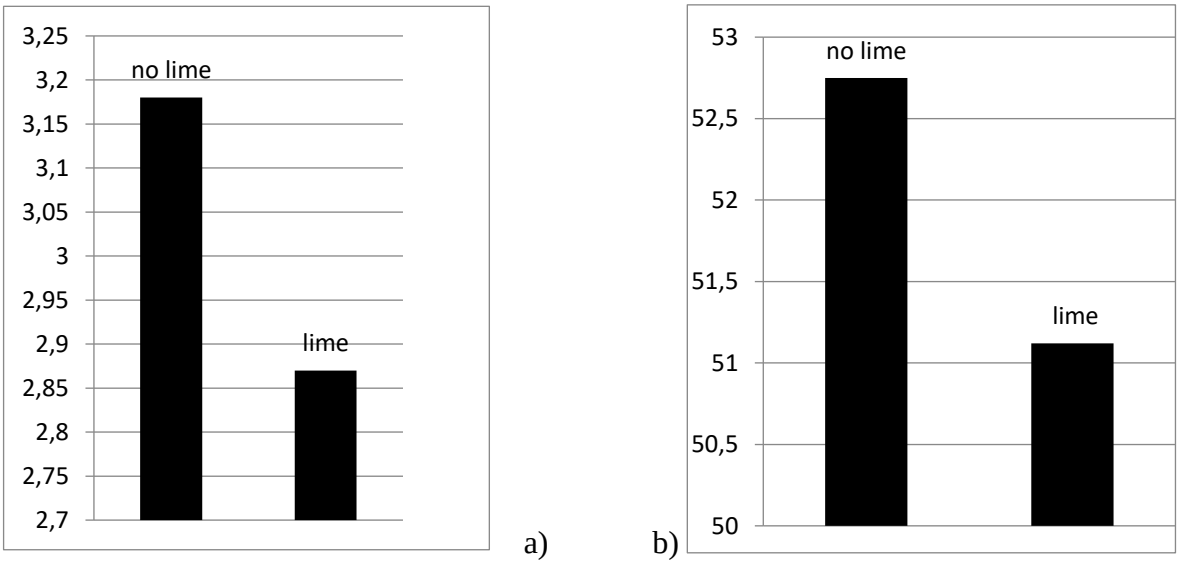
Material and Methods

The trial was established in March 28th 2022 on a family farm in Brodski Zdenci, Brodsko-Posavina County. The Harpek variety was used as planting material. Before setting up the experiment, soil samples were taken for the chemical analysis. In location of Brodski Zdenci is a former limestone quarry where natural limestone was mined. For the purposes of the experiment, 1 kg of powder lime was used, which was applied 20 days before planting. The experiment was set up on two plots measuring 1 m^2, the row spacing was 30 cm, and the planting site spacing was 10 cm. In May, daily temperatures reached 18°C, which had a favorable effect on the further development of garlic. On June 4th, 33 garlic bulbs were sampled from each plot and the lengths of the roots, thickened part, i.e. pseudo stem and leaves were measured, while other measurements were carried out in the Practicum for Pedology and Plant Nutrition at UNISB.

Results and Discussion

The effect of lime application on the growth and development of garlic was determined using 33 garlic samples from each plot. The chemical analysis revealed that experiment was set up on a soil with a neutral reaction (pH-KCl 6.59; pH-H_2O 7.74), highly humus-rich (5.4% humus), slightly carbonated (0.42%), poorly supplied with phosphorus (AL-P_2O_5 3.68 mg 100 g^{-1} soil), moderately supplied with potassium (AL-K_2O 15.97 mg 100 g^{-1} soil). The measurement results of garlic growth were expressed as average values for each measured parameter and are presented graphically.

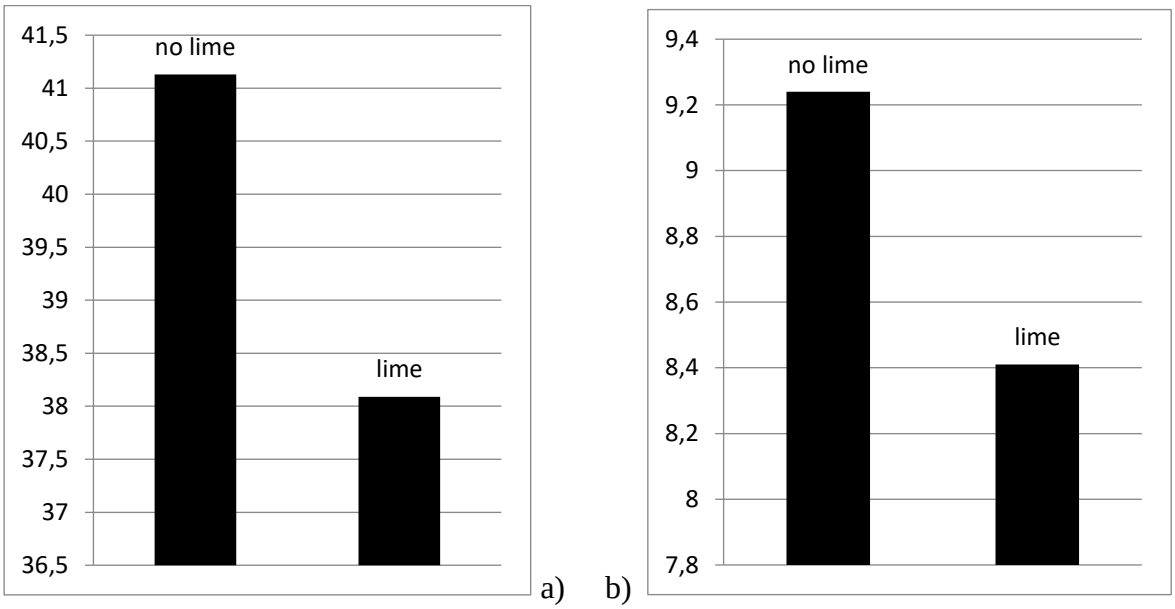
Graph 1 shows measurements of the lengths of leaves and pseudo stems of garlic. Leaf lengths ranged from 50 to 53 cm for garlic grown on a plot without lime, with an average leaf length of 52.75 cm. Garlic grown on a plot with lime had leaf lengths ranging from 50 to 52 cm, with an average length of 51.12 cm. The length of the pseudo stem from the plot without lime ranged from 2.7 to 4 cm, with an average length of 3.18 cm. On the plot with lime, the range was 2.7 to 3 cm, with an average length of 2.87 cm.



Graph 1. Leaf length (a) and pseudo stem length of garlic (b) without lime and with lime (cm)

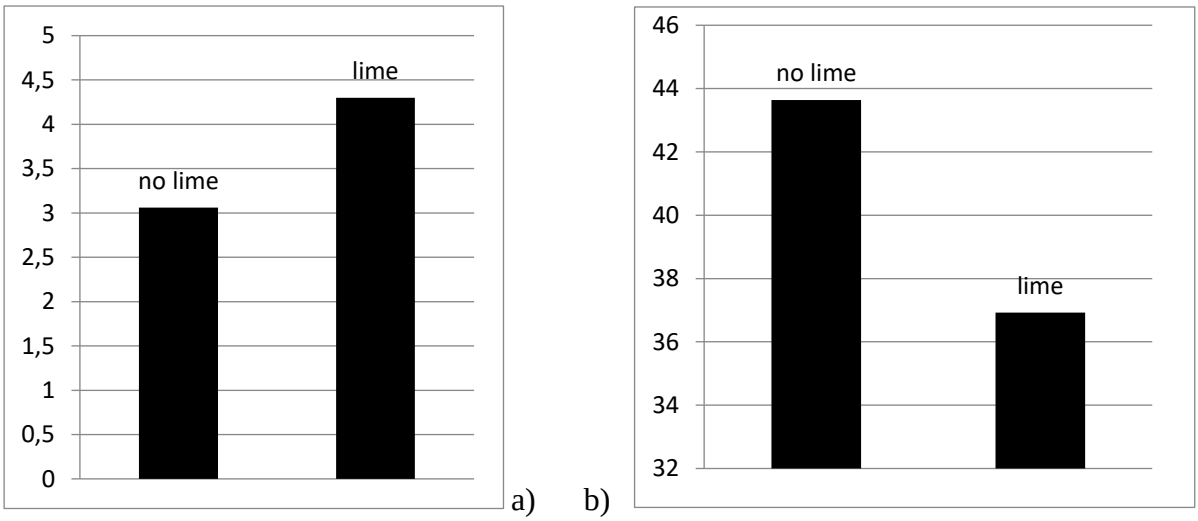
Bulb and stem diameter measurements were taken with slide role and the results are shown in graph 2. In the plot without lime addition, the measured bulb diameter ranged from 36.5 mm to 42 mm, with an average of 41.13 mm. In the plot with lime addition, the bulb diameter ranged from 36.5 mm to 39 mm, with an average of 38.09 mm. The pseudo stem diameter in the plot without lime ranged from 7.8 mm to 9.3 mm, with an average of 9.24 mm. In the plot with lime

addition, the pseudo stem diameter ranged from 7.8 mm to 8.4 mm, with an average of 8.41 mm.



Graph 2. Bulb diameter (a) and garlic stem diameter (b) without and with lime addition (mm)

Root length (graph 3a) ranged from 0 to 3 cm, with an average length of 3.06 cm. In plots with lime addition, root length ranged from 0 to 4.5 cm, with an average length of 4.3 cm. Lime addition had a positive effect on root elongation and garlic root growth.
The plant weight (graph 3b) from the plot without lime addition ranged from 32 g to 44 g, with an average weight of 43.46 g. With lime addition, the weight ranged from 32 g to 38 g, with an average of 36.92 g.



Graph 3. Root length (cm) (3a) and plant weight (g) (3b) without lime and with lime

In terms of soil characteristics, the optimal pH for garlic cultivation is 6.5 pH on mineral soils and 5.3 pH on very organic soils. In the experimental field, garlic was grown on a high humus content soil with a neutral reaction. Research indicates that the influence of humic acids is stimulating for growth because it increases the vegetative growth of garlic, the number of leaves/plant, stem diameter, chlorophyll content (Hassan Yousif, 2019), plant height, bulb

diameter, stem dry matter, bulb weight and total garlic yield (Al-Mharib et al., 2025, Afghan et al., 2025). In the study by Al-Mharib et al. (2025), it was found that humic acids play a role in increasing calcium assimilation products in the development of heads and garlic yield. Given that it is slightly carbonated soil and the availability of carbonate material in the study area, many producers apply it for vegetable cultivation. In this study, the addition of lime proved to be an unnecessary measure in this particular soil with neutral pH and low content of phosphorus. In the work of the author Kažimir et al. (2020), it was found that the influence of location along with the choice of garlic genotype has a significant impact on garlic cultivation. The study states that a location with a neutral soil reaction, low carbonate content and high humus content recorded a higher yield and percentage of garlic bulbs compared to a location with the same soil reaction and humus content, but with a high carbonate content. In the same study, a good supply of available phosphorus was determined at a location with a low carbonate content. In this study, the experiment was set up on soil with a low phosphorus supply. Phosphorus availability in soils may be limited in alkaline and carbonate soils, and lime application could limit phosphorus uptake by the plant and limit garlic cultivation (Al-Karaki et al., 2024). The significant role of available phosphorus in garlic growth and development, mineral nutrient uptake, and the formation of secondary metabolites (allicin, phenols, flavonoids) in garlic has been noted in research by other authors (Majumdar et al., 2003; Jitarwal et al., 2018; Divyasree et al., 2021. Paczka et al., 2021).

Conclsions

The results of the research indicated that the additional application of lime on neutral soils had no effect on the growth and development of garlic in most of the measured parameters. The only exception was an increase in root length in garlic grown on plots with lime addition. This research highlights a case of inadequate agricultural practice, characterized by cultivating vegetables without prior soil analysis or consultation with specialists, as well as the unnecessary application of amendments. In this study, the addition of lime in garlic cultivation resulted in reduced growth and development performance. For the agronomic profession, such cases of inappropriate cultivation practices remain relatively common, and the aim of this research is to encourage growers to conduct soil analysis and to select vegetable crops suitable for efficient and productive cultivation.

Acknowledgement

The results presented in this paper are part of the results from the graduate thesis of Anita Petrović.

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ORIGINAL SCIENTIFIC PAPER

Projected Irrigation Requirements for Fruit and Vegetable Crops under Climate Change in Krapina-Zagorje County

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Summary
Climate change is increasingly affecting agricultural production in northwestern Croatia through altered precipitation patterns and more frequent drought events. This study assesses current and future irrigation water requirements for four economically important crops (apple, plum, potato, oil pumpkin) in Krapina-Zagorje County using the FAO CROPWAT model and a 30-year climatic dataset (1995-2024). Agricultural land use was analyzed using the ARKOD database. Results show significant baseline irrigation demands ranging from 53 mm (oil pumpkin) to 126 mm (potato). Under the RCP4.5 scenario (2041-2070), irrigation requirements increase by 45-51% across all crops due to higher evapotranspiration and reduced summer precipitation. These findings highlight the urgent need for irrigation infrastructure development and climate-adaptive water management strategies in this region.

Keywords: irrigation, climate change, CROPWAT, RCP4.5, northwestern Croatia

Introduction

Climate change poses significant challenges to agricultural water management in Europe, with increasing irrigation demands driven by rising temperatures and changing precipitation patterns (Döll, 2002; IPCC, 2021). Croatia, situated in the transitional zone between Mediterranean and continental climates, is particularly vulnerable to these changes. Recent projections indicate substantial reductions in summer precipitation and increased evapotranspiration for southeastern Europe, including the Carpathian-Dinaric region (Jacob et al., 2014; Torma et al., 2020). Krapina-Zagorje County in northwestern Croatia represents a typical example of fragmented agricultural land with limited irrigation infrastructure. According to the latest data, the county has 9,039 registered agricultural holdings cultivating approximately 21,645 hectares (Krapinsko-zagorska županija, 2025). While irrigation development has expanded in Croatia, with over 30,000 hectares of irrigated land by 2020 (Romic et al., 2021), most irrigation infrastructure is concentrated in eastern regions, leaving northwestern areas underserved. Accurate quantification of crop water requirements is essential for sustainable water resource management and long-term agricultural planning. The FAO CROPWAT model, based on the Penman-Monteith method (Allen et al., 1998), provides a standardized approach for estimating crop water demands and has been widely applied in climate change impact assessments globally.

This study aims to: (1) analyze agricultural land use structure in Krapina-Zagorje County using the ARKOD database; (2) quantify current irrigation requirements for four selected crops using CROPWAT 8.0; and (3) project future irrigation demands under the RCP4.5 climate scenario for the 2041-2070 period.

Material and Methods

The study area covers Krapina-Zagorje County in northwestern Croatia (46°10'N, 15°52'E), characterized by a moderate continental climate (Cfb according to Köppen-Geiger classification) with moderately warm summers and cold, humid winters. Agricultural land use data were obtained from the ARKOD database (2024), which records all registered agricultural parcels and their land use categories. Based on the prevalence and economic importance identified through ARKOD analysis, four crops were selected: apple (*Malus domestica* (Suckow) Borkh.), plum (*Prunus domestica* L.), potato (*Solanum tuberosum* L.), and oil pumpkin (*Cucurbita pepo* L.).

Reference evapotranspiration (ETo) was calculated using the FAO Penman-Monteith equation (Allen et al., 1998) implemented in CROPWAT 8.0 software. Climatic input data for the baseline period (1995-2024) were obtained from the Krapina meteorological station, including monthly averages of temperature (°C), relative humidity (%), wind speed (m s^{-1}), and insolation (hours). Effective rainfall was estimated using the USDA Soil Conservation Service method. Crop evapotranspiration (ETc) was calculated as ETc = Kc × ETo, where Kc represents the crop coefficient varying across growth stages according to FAO guidelines (Allen et al., 1998; Doorenbos and Kassam, 1979).

For future climate projections, the delta-change methodology was applied using factors derived from published regional climate studies covering the broader continental Croatia and southeastern Europe region (Jacob et al., 2014; Torma et al., 2020; Seneviratne et al., 2021). The RCP4.5 scenario (equivalent to SSP2-4.5; O'Neill et al., 2016) was selected as a moderate emissions pathway, projecting global warming of approximately 2.7°C above pre-industrial levels by 2100. For the 2041-2070 period, the following delta factors were applied to the baseline climatology: temperature increase of +1.6°C (growing season average), annual precipitation decrease of 8% with a 12% reduction during summer months (June-August), and a 2.5% decrease in relative humidity.

Results and Discussion

Analysis of the ARKOD database revealed that arable land (53.6%), grasslands (33.3%), and fruit orchards (6.2%) dominate the agricultural landscape of Krapina-Zagorje County (Table 1). Among individual crops, maize covers the largest area (6,537 ha), followed by grasslands (6,610 ha) and mixed vegetable crops (1,015 ha). The dominance of maize reflects the traditional agricultural structure of the region, while the significant presence of mixed fruit and vegetable cultivation highlights the importance of high-value crops. Detailed crop analysis revealed that mixed orchards occupy 862 ha (6,061 individual parcels) and mixed vegetable crops cover 1,015 ha (4,705 individual parcels) in Krapina-Zagorje County. While mixed cultivation systems represent the dominant production model, specific crops were selected for water requirement modeling based on their economic importance and prevalence. For fruit crops, apple (*Malus domestica*) and plum (*Prunus domestica*) were chosen as representative species commonly grown in mixed orchards across the region. Similarly, for vegetable crops, potato (*Solanum tuberosum*) and oil pumpkin (*Cucurbita pepo*) were selected as typical components of mixed vegetable production systems.

Table 1. Agricultural land use structure in Krapina-Zagorje County (ARKOD, 2024)

ARKOD Code	Land use category	Area (ha)	Share (%)
200	Arable land	11,598	53.6
310	Grasslands	7,209	33.3
422	Fruit orchards	1,350	6.2
410	Vineyards	600	2.8
	Other	888	4.1

Baseline irrigation requirements (1995-2024) showed considerable variation among the four selected crops (Table 2). Oil pumpkin demonstrated the lowest irrigation demand (53 mm), primarily due to its shorter growing season and relatively low crop coefficient during critical growth stages. Potato required the highest irrigation input (126 mm), consistent with its sensitivity to water stress during tuber formation (Doorenbos and Kassam, 1979; Ierna and Mauromicale, 2022). Apple and plum showed intermediate requirements of 105 mm and 95 mm, respectively. These baseline values align well with findings from similar climatic regions in Central Europe, where irrigation demands for fruit crops typically range between 80-120 mm in humid continental climates (Stachowski et al., 2021).

Table 2. Baseline and projected irrigation requirements for selected crops

Crop	Baseline (1995-2024)			RCP4.5 (2041-2070)		
	ETc (mm)	Peff (mm)	Irr. Req. (mm)	ETc (mm)	Peff (mm)	Irr. Req. (mm)
Apple	476.7	463.5	105.0	513.3	429.1	158.3
Plum	385.5	352.3	95.0	414.0	325.1	138.1
Potato	458.1	401.4	126.4	490.8	415.4	184.5
Oil pumpkin	413.6	445.5	53.3	440.1	405.0	79.8

ETc – crop evapotranspiration; Peff – effective precipitation; Irr. req – irrigation requirement

Under the RCP4.5 scenario for 2041-2070, irrigation requirements increased substantially for all crops (Table 2). The relative increase ranged from 45 % for apple (from 105 to 158 mm) to 51 % for plum (from 95 to 138 mm), 47 % potato (from 126 to 185 mm), and 51 % oil pumpkin (from 53 to 80 mm). These projections are consistent with regional climate model ensembles for the Carpathian-Dinaric region, which project increases in irrigation water demand of 15-40% by mid-century under moderate emission scenarios (Torma et al., 2020). The pronounced summer precipitation deficit projected for this region (-12% during June-August) coincides with critical growth stages for all analyzed crops, exacerbating water stress and irrigation requirements. Global analyses of climate change impacts on irrigation demonstrate similar trends, with estimated increases of 5-20% in irrigation water demand by 2050 in temperate regions (Döll, 2002). The IPCC Sixth Assessment Report confirms these patterns for continental Europe, projecting significant increases in agricultural water stress under both moderate and high emission scenarios (IPCC, 2021; Seneviratne et al., 2021). For Croatia specifically, the absence of regional irrigation development plans in northwestern counties represents a critical vulnerability, as evidenced by the projected 46% average increase in irrigation demands across all analyzed crops. Several limitations should be acknowledged. The delta-change methodology, while widely accepted for agricultural impact assessments, assumes constant relative variability in climate parameters and does not capture potential changes in extreme events or intra-seasonal variability. Additionally, the delta factors employed were derived from regional projections covering the broader southeastern European region rather than site-specific downscaling for Krapina-Zagorje County. Future studies should consider ensemble approaches using multiple climate models and emission scenarios to better characterize uncertainty in projected irrigation requirements.

Conclusion

This study provides the comprehensive assessment of agricultural land use and irrigation water requirements for high-value crops in Krapina-Zagorje County (Croatia). ARKOD database analysis revealed that arable land dominates the county (11,598 ha, 53.6%), followed by grasslands (7,209 ha, 33.3%) and fruit orchards (1,350 ha, 6.2%). Within these categories, mixed cultivation systems represent the primary production model, with mixed fruit orchards covering 6,061 ha and mixed vegetable crops occupying 4,705 ha. Based on economic

importance and regional prevalence, four representative crops were selected for detailed irrigation analysis: apple and plum (fruit crops), and potato and oil pumpkin (vegetable crops). Under baseline climatic conditions (1995-2024), irrigation requirements varied considerably among crops: oil pumpkin 53 mm, plum 95 mm, apple 105 mm, and potato 126 mm. Climate change projections under the RCP4.5 scenario (2041-2070) indicate substantial increases across all crops, with absolute requirements reaching 80 mm for oil pumpkin (+51%), 138 mm for plum (+45%), 158 mm for apple (+51%), and 185 mm for potato (+47%). Modeled increases, averaging 46% across all analyzed crops, are driven by elevated evapotranspiration rates (temperature increase of +1.6°C during the growing season) combined with reduced summer precipitation (-12% during June-August critical growth stages). These findings underscore the urgent need for irrigation infrastructure development and climate-adaptive water management strategies in northwestern Croatia, particularly in counties currently lacking regional irrigation development plans. Implementation of efficient irrigation technologies—particularly drip systems with 85-90% application efficiency combined with county-level irrigation plans should be prioritized to ensure the sustainability and economic viability of high-value fruit and vegetable production under projected climate change conditions.

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ORIGINAL SCIENTIFIC PAPER

Effects of Conservation Tillage and Fertilization on Weed Infestation in Winter Wheat

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Summary
The aim of this study was to evaluate the effects of conservation tillage and fertilization on weed occurrence and winter wheat yield. Field experiment in 2022/2023 included three tillage systems (ST - conventional, CTD – deep conservation, CTS – shallow conservation) and two fertilization levels (F - recommended, FR - reduced). Conservation tillage increased weed density, biomass, and number of perennial species. Fertilization increased weed infestation, but resulted in higher wheat yield. Despite variable weed infestation, yield differences among tillage systems were not pronounced, indicating stable productivity of conservation tillage systems under optimal fertilization.

Keywords: soil disturbance, nutrient management, weed occurence, *Triticum aestivum* L.

Introduction

Conservation tillage is increasingly adopted in Croatia as a key component of conservation agriculture, contributing to climate change adaptation and reducing the environmental impact of agricultural production (Jug et al., 2025). This approach in soil management improves soil properties by enhancing fertility, increasing water retention, stabilizing yields, reducing production costs, and supporting biodiversity (Lal, 2015; Pittelkow et al., 2015). However, its wider adoption is still partly limited by concerns about potential increases in weed infestation in cropping systems (Nichols et al., 2015). In addition to tillage, fertilization is an important factor influencing the sustainability of crop production systems and can also affect weed infestation (Brozović et al., 2021). Winter wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, playing a key role in human nutrition and global food security (Shewry and Hey, 2015). Its production is influenced by the interaction of several factors, including climatic conditions, soil properties, and management practices. Among these, nutrient management plays a crucial role, as weeds often have a competitive advantage in nutrient uptake and respond rapidly to increased nutrient availability (Cheimona et al., 2016; Savić et al., 2025). In contrast, optimal fertilization can enhance crop competitiveness and suppress weed growth, whereas suboptimal nutrient supply may favor weeds due to their lower nutrient requirements (Blackshaw et al., 2005). Although winter wheat is a dense crop with relatively good competitive ability, it can still be affected by numerous annual and perennial broadleaf and grass weeds, which may reduce yield. Therefore, it is important to better understand how conservation tillage systems combined with fertilization practices influence weed communities and, finaly, crop yield.

Material and Methods

A field trial examining different soil management practices was carried out in 2022/2023 in Križevci (16°33′32″ E, 46°01′38″ N, 141 m) representing the third year of a long-term study.

Three tillage systems were evaluated: conventional tillage (ST), deep conservation tillage (CTD), and shallow conservation tillage (CTS). In the conventional system (ST), soil was plowed to a depth of 30 cm. Deep conservation tillage (CTD) involved loosening the soil to 30 cm, maintaining at least 30% residue cover, while in the shallow system (CTS), the same implement was used at 5–10 cm depth with a minimum of 50% surface cover. Fertilization treatments included recommended (F), and 50% reduced amount of fertilizers (FR). Seedbed preparation was completed in a single pass with a spike-tooth harrow and hollow rollers. Fertilizers were applied using a spreader, sowing was carried out with a no-till seeder, and crop protection treatments were applied using a sprayer. Fertilization was carried out according to recommendations, applying (NPK) in 0:20:30 ratio at a rate of 615 kg ha^{-1} and in the half amount in autumn 2022 prior to primary tillage, along with an additional 388 kg ha^{-1} of calcium ammonium nitrate (CAN) applied in two split dressings (full ang half amount). Winter wheat (cultivar Indira) was sown on October 21, 2022, at a seeding rate of 250 kg ha^{-1} and the preceding crop was soybean. Crop protection included several treatments, uniform for all tillage treatments and fertilization subtreatments. On March 15, 2023, herbicides were applied at a rate of 50 g ha^{-1} (halauxifen-methyl 104.2 g kg^{-1} and florasulam 100 g kg^{-1}), along with pinoxaden (50 g L^{-1}). On April 17, 2023, a fungicide (benzovindiflupyr 75 g L^{-1} and prothioconazole 150 g L^{-1}) was applied at 1 L ha^{-1}, together with an insecticide (cypermethrin 500 g L^{-1}) at 50 mL ha^{-1}. A second fungicide treatment (tebuconazole 125 g L^{-1} and prothioconazole 125 g L^{-1}) at 1 L ha^{-1}, along with an insecticide (lambda-cyhalothrin) at 0.15 L ha^{-1}, was applied on May 10, 2023. After manual harvesting of the subplots, the remaining experimental area was harvested using a combine with a residue chopping implement. Wheat was harvested on July 10, 2023. Grain yield was expressed per hectare (ha^{-1}) at 14% moisture content. Weed sampling was conducted at the BBCH 25 growth stage (tillering). Weed density and biomass were assessed using a 0.25 m^2 quadrat in four replicates per plot. Weeds were cut at ground level, identified, counted, dried at 65 °C for 24 hours, and weighed. Results were expressed as number of plants per m^2 and biomass in g m^{-2}. The effects of different tillage systems and fertilization on weed abundance, biomass, and wheat yield were evaluated with analysis of variance (ANOVA) using the Statistica software package, version 14.0.0 (TIBCO Software Inc., Palo Alto, CA, USA). The model included tillage (T) as the main factor and fertilization (F) as a sub-factor. Mean comparisons were performed at a significance level of $p = 0.05$ using Fisher's protected LSD test.

Results and Discussion

A total of 11 weed species were identified in the winter wheat, including seven perennial and four annual species. The most abundant species was *Symphytum officinale* L. followed by *Taraxacum officinale* Web probably due to the specific characteristics of the experimental site, which had been a long-term meadow prior to the first establishment of the trial. The other recorded weed species represented the typical weed flora associated with winter wheat, including *Lamium purpureum* L., *Stellaria media* L., and *Sonchus arvensis* L. The observed weather conditions were characterized by above-average precipitation and increasing temperatures, which likely influenced crop growth and weed development. September had high precipitation (almost 155 mm), while October was much drier (26.4 mm), with both months warmer than average. In the first half of 2023, marked variations from long-term averages (1991–2020) were observed. January recorded very high precipitation (almost 150 mm), followed by a dry February (35 mm). From March to July, precipitation remained above average, while temperatures increased from April (10 °C) to July (23 °C). Weed density (WD) and weed biomass (WB) were significantly affected by the tillage system (T) (Table 1). The highest average values of both WD and WB were recorded under deep conservation tillage (CTD), followed by shallow conservation tillage (CTS), while the lowest average values were observed in conventional tillage (ST) similar to Dolijanović et al. (2025). This indicates that

conservation tillage promotes higher weed infestation compared to conventional systems, due to reduced soil disturbance and the accumulation of weed seeds near the soil surface which is consistent with Feledyn-Szewczyk et al., 2020. and Chauhan et al., 2012. Fertilization (F) also influenced weed occurrence, with higher average values of WD and WB observed under optimal fertilitation. This suggests that increased nutrient availability can stimulate weed growth, as many weed species efficiently utilize available nutrients (Ryan et al., 2010; Rehling et al., 2021). The interaction among tillage and fertilization was not significant for WD but was highly significant for WB, indicating that combined management practices mainly affected weed biomass rather than weed density.

Table 1. Weed density and biomass in winter wheat (BBCH 25 – tillering)

Tillage (T)	WD (m^{-2})		Av (T)	WB (g m^{-2})		Av (T)
	F	FR		F	FR	
ST	8.33	7.00	7.66^{C}	1.93^{e}	1.16^{e}	1.54^{C}
CTD	20.33	17.66	19.00^{A}	32.22^{a}	20.63^{d}	26.43^{A}
CTS	16.00	13.33	14.66^{B}	26.97^{b}	23.08^{c}	25.03^{B}
Av (F)	14.89^{A}	12.66^{B}		20.37^{A}	14.96^{B}	
$F_{(T)}$	**			**		
$F_{(F)}$	*			**		
$F_{(TxF)}$	n.s.			**		

WD-weed density, WB-weed biomass, ST-conventional tillage, CTD-deep conservation tillage, CTS-shallow conservation tillage, F-recommended fertilization, FR-reduced fertilitazion, F(T)-F test for tillage, F(F)-F test for fertilization, F(TxF)-F test for tillage and fertilization interaction, n.s.-non significant, Av (T)-average value for tillage, Av (F)-average value for fertilization. Values within the same column/row followed by different letters are statistically different- (LSD) test at $p<0.05$.

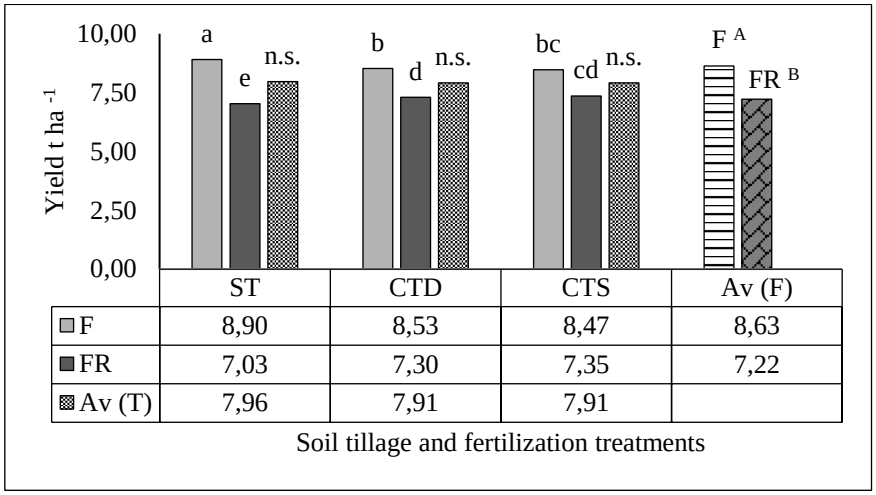
Table 2. Total number of perennial and annual weeds in winter wheat (BBCH 25 – tillering)

Tillage (T)	P (m^{-2})		Av (T)	A (g m^{-2})		Av (T)
	F	FR		F	FR	
ST	2.66	2.66	2.66^{C}	5.66	4.33	5.00^{B}
CTD	12.33	10.66	11.50^{A}	8.00	7.00	7.50^{A}
CTS	9.33	8.33	8.83^{B}	6.66	5.00	5.83^{B}
Av (F/FR)	8.11	7.22		6.77^{A}	5.44^{B}	
$F_{(T)}$	**			**		
$F_{(F)}$	n.s.			*		
$F_{(TxF)}$	n.s.			n.s.		

P- perennial weed density, A-annual weed density, ST-conventional tillage, CTD-deep conservation tillage, CTS-shallow conservation tillage, F-recommended fertilization, FR-reduced fertilitazion, F(T)-F test for tillage, F(F)-F test for fertilization, F(TxF)-F test for tillage and fertilization interaction, Av (T)-average value for tillage, Av (F)-average value for fertilization. Values within the same column/row followed by different letters are statistically different- (LSD)test at $p<0.05$.

The total number of perennial weeds (P) and annual weeds (A) was significantly influenced by tillage system (T). Perennial weeds were more abundant under conservation tillage systems, with the highest average values recorded in CTD, followed by CTS, while the lowest average number was observed in conventional tillage which is also supported by Håkansson, (2003). This indicates that reduced soil disturbance in conservation systems favors the establishment and persistence of perennial species. In contrast, although annual weeds (A) also showed higher values under conservation tillage, the differences were less pronounced compared to perennials. The highest average number of annual weeds was recorded in CTD, while ST and CTS had lower and similar values. Fertilization had no significant effect on the number of perennial weeds, but it significantly affected the number of annuals, suggesting that annual species respond more strongly to increased nutrient availability which is also reported by Chetan et al.,

2025. Overall, conservation tillage systems promoted a greater presence of perennial weeds compared to annuals, likely due to limited soil disturbance, which allows perennial species to survive and spread more easily.



Graph 1. Winter wheat yield (t ha^{-1})

Winter wheat yield was significantly affected by fertilization while tillage systems had no significant effect in average. The highest yield was recorded under conventional tillage (ST), followed by conservation systems, CTD and CTS when recommended fertilization was applied which is similar with Dolijanović et al., 2025. Optimal fertilization showed higher average yields compared to reduced fertilization with statistically significant differences ($p<0.05$). The higher average yield observed in conventional tillage can be associated with lower weed density and biomass recorded in this system. Conservation tillage systems, particularly CTD, had higher weed infestation but yield differences compared to conventional tillage were minimal. This indicates that conservation tillage, although associated with higher weed infestation, may still ensure stable yields, especially when combined with optimal fertilization although several studies have indicated that wheat yields in conservation tillage systems can be lower compared to those achieved under conventional tillage (Seepamore et al., 2020, Liliane et al., 2020).

Conclusion

Tillage system and fertilization significantly affected weed occurrence in winter wheat. Conservation tillage increased weed density, biomass, and the number of perennial species compared to conventional tillage. Fertilization increased weed infestation but also improved winter wheat grain yield. Despite higher weed infestation under conservation systems, yield differences were not pronounced, indicating stable productivity under optimal fertilization. These findings highlight that conservation tillage can be effectively implemented when supported by recommended fertilization and weed management practices.

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ORIGINAL SCIENTIFIC PAPER

Evaluation of Heavy Metal Contamination and Ecological Risk in Urban Garden Soils of Zagreb

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Summary

Urban gardening in Zagreb exposes soils to heavy metal accumulation from anthropogenic sources like traffic emissions and historical pesticide use. This study evaluated contamination and ecological risk in nine public urban gardens across 2015-2020 using geoaccumulation index (I_{geo}), enrichment factor (E_r), and potential ecological risk index (RI). While Cd, Cr, Ni, Pb, and Zn concentrations remained below Croatian geochemical baselines (practically unpolluted, Class 0), mercury (Hg) emerged as the primary concern. Hg's E_r exceeded 40 at most sites, peaking at 160 (Prečko, 2019), with RI values often surpassing 100, indicating moderate-high ecological risk driven by Hg's high toxicity factor (Tr=40). Cu showed slight enrichment (Pi 1.0-2.0) at Savica and Klara from fungicide applications. Recent anthropogenic Hg inputs require systematic monitoring and mitigation for safe urban agriculture.

Keywords: geoaccumulation index, enrichment factor, ecological risk index, mercury, copper

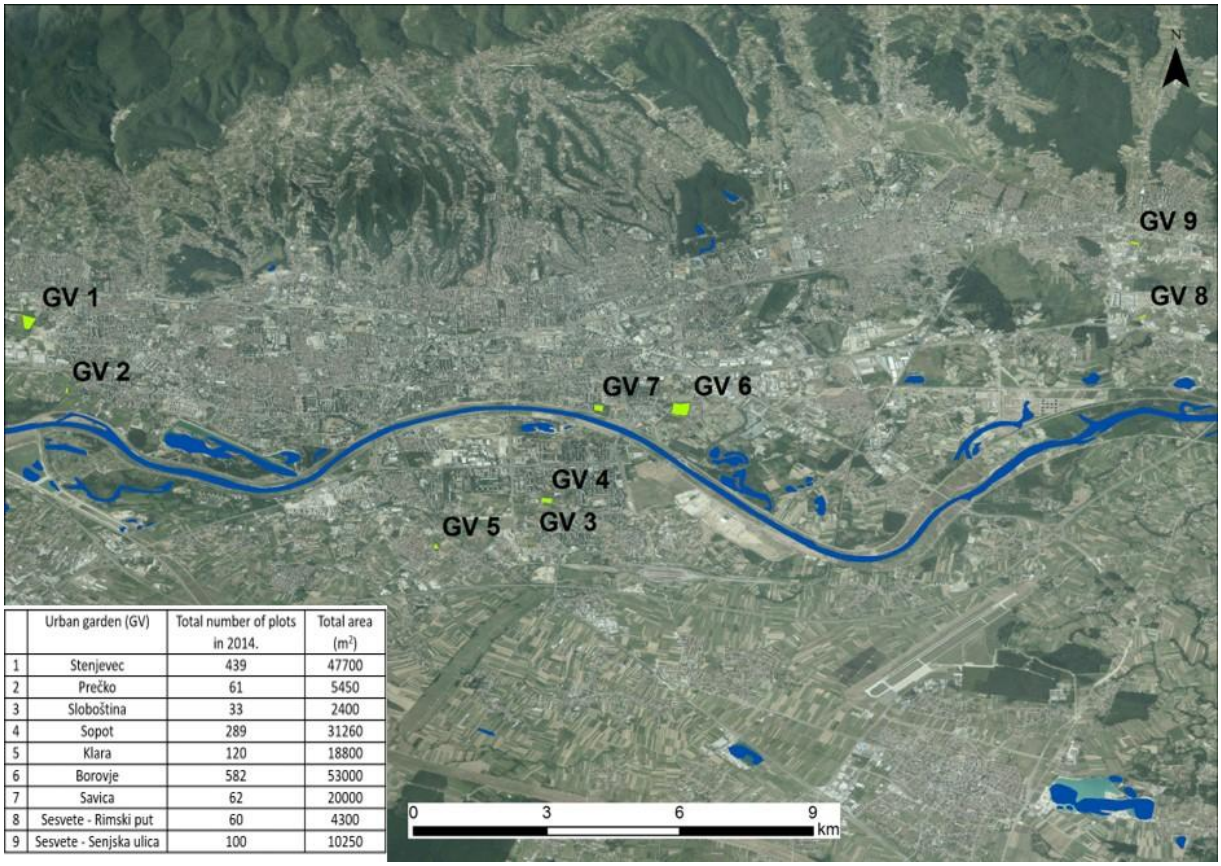
Introduction

Soil serves as a multifunctional resource playing key roles in ecological regulation, water filtration, climate moderation, biodiversity support, and most critically providing plants with water, air, and nutrients to fuel photosynthesis and organic compound production. All heavy metals occur naturally in soils, but elevated concentrations typically arise from contamination of either anthropogenic or natural origins. Key anthropogenic activities driving soil pollution include industry, mining, waste disposal, traffic, agriculture, synthetic fertilizers, metal-based pesticides, municipal sewage sludge, and irrigation (Kisić, 2011; Van Camp et al., 2004). In urban areas like Zagreb, where urban agriculture is intensively developing, garden soils are exposed to numerous above mentioned anthropogenic influences, leading to the accumulation of heavy metals. These contaminants, such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), pose a significant threat to human health and the ecosystem as they easily accumulate in plants and enter the food chain (EFSA, 2026).

Research results show that elevated levels of heavy metals already exist in soils of vegetable gardens in Zagreb's periurban zones, highlighting the need for systematic monitoring (Puntarić et al., 2013). The ecological risk from heavy metals in Zagreb's urban gardens has been assessed through several studies that identified links between soil contamination and content in leafy greens (Puntarić et al., 2013), often exceeding permissible limits for Pb and Hg in some samples. Factors such as wind direction and proximity to traffic routes further exacerbate pollutant distribution, potentially leading to chronic health risks for the local population growing food in these areas (EPA, 2014). These findings call for the implementation of ecological risk assessments to define mitigation measures. This paper evaluates the level of heavy metal contamination and ecological risk in the soils of Zagreb's urban gardens, providing empirical data that complements existing research and offers recommendations for sustainable urban agriculture.

Material and Methods

Soil sampling in 9 public urban gardens in Zagreb was conducted once a year in two sampling campaigns consisting of two consecutive years (2015-2016, 2019-2020), following HRN ISO 10381-(1-5) standards. Samples were collected from a depth of 30 cm, with 2 or 4 representative composite samples per urban garden (depending on total garden area, as shown in Figure 1), each comprising 5 soil sub-samples. All samples were labeled and stored in plastic bags.



	Urban garden (GV)	Total number of plots in 2014.	Total area (m^2)
1	Stenjevec	439	47700
2	Prečko	61	5450
3	Sloboština	33	2400
4	Sopot	289	31260
5	Klara	120	18800
6	Borovje	582	53000
7	Savica	62	20000
8	Sesvete - Rimski put	60	4300
9	Sesvete - Senjska ulica	100	10250

Figure 1. Urban garden locations and basic information

In the laboratory, samples were homogenized and air-dried at room temperature. After crushing larger aggregates, the soil was sieved through a 2 mm φ mesh. Sample preparation followed HRN ISO 11464:2004 protocols for physical and chemical soil analysis. Concentrations of Cd, Cr, Cu, Hg, Ni, P, Pb, and Zn were determined in first two years by inductively coupled plasma optical emission spectrometry (ICP-OES) (HRN ISO 22036:2011) on a Varian Vista MPX AX instrument, following microwave digestion with aqua regia in closed TFM vessels using a MARS Xpress system (CEM, USA) with automated pressure/temperature control (HRN ISO 11466:2004).

The contamination level of metal(loid)s was evaluated using the geoaccumulation index (I_{geo}):

$$I_{geo} = \log_2\left(\frac{C_i}{1.5 \times B_i}\right) \quad \text{Eq. 1}$$

where: C_i is the measured concentration of element i in soil samples (mg/kg dry weight – d.w.),
B_i is the background concentration of the respective element in reference soil (mg/kg d.w.),
1.5 is a correction factor accounting for natural variability in the background values.

B_i concentrations were defined using national geochemical baseline values for Croatia, calculated as mean concentrations from 2,521 topsoil samples (0–25 cm) collected on a systematic 5 × 5 km grid (Halamić and Miko, 2009), with values (mg kg⁻¹ d.w.) of: Cr 97, Cu 30, Zn 99, Ni 55, Pb 38, Cd 0.74, Hg 0.093.

The potential ecological risk factor for each examined element was calculated as:

$$E_r^i = T_r^i \times \frac{C_i}{B_i} \quad \text{Eq. 2}$$

where: $T_r^i$is the toxic response factor for element *i*,
$C_i$is the measured concentration in sewage sludge (mg/kg d.w.),
$B_i$is the background concentration (mg/kg d.w.).

The overall ecological risk index (RI) was calculated as the sum of individual risk factors:

$$RI = \sum E_r^i \quad \text{Eq. 3}$$

Results and Discussion

Even though above described methodology was used to assess environmental risks, table 1 shows only partial results that indicate potential pollution. The results indicate that the concentrations of Cd, Cr, Ni, Pb, and Zn across most locations remain within or below the natural background levels. The geoaccumulation index (I_{geo}) for these elements is consistently negative and according to Müller's scale (1969), this places the soil in the "practically unpolluted" category (Class 0)(not shown). The negative I_{geo} values suggest that any slight fluctuations in concentration are negligible relative to the natural variability of the soil, even when accounting for the 1.5 correction factor used to mitigate lithogenic differences.

Hg stands out as the primary element of ecological concern in the investigated gardens. Its pollution index E_r exceeds 1.0 at most locations, indicating an accumulation above the natural background levels. Prečko and Borovje are the locations where the highest pollution factors are recorded. For instance, in Prečko in 2020 and Borovje in 2016, the E_r values suggest "moderate to heavy pollution."

Table 1. Partial results of environmental risk assessment within urban garden soils

Urban garden	Year	Er (Hg)	RI	Urban garden	Year	Er (Hg)	RI
Stenjevec	2015	52.73	87.76	Borovje	2015	94.69	115.05
	2016	48.91	80.62		2016	93.52	112.28
	2019	65.38	93.45		2019	60.22	74.07
	2020	49.89	78.84		2020	107.53	130.61
Prečko	2015	121.47	148.90	Savica	2015	84.95	105.59
	2016	128.19	151.87		2016	78.99	102.70
	2019	160.00	184.62		2019	59.35	80.70
	2020	132.04	159.95		2020	50.32	73.95
Sloboština	2015	40.53	61.77	Sesvete - Rimski put	2015	33.45	78.42
	2016	40.08	68.56		2016	34.01	63.55
	2019	36.13	54.48		2019	24.52	38.26
	2020	38.28	57.03		2020	31.40	55.65
Sopot	2015	74.97	96.25	Sesvete - Senjska ulica	2015	25.10	44.88
	2016	60.20	81.93		2016	109.36	163.38
	2019	65.38	82.11		2019	46.88	103.90
	2020	74.84	101.18		2020	30.97	74.27
Klara	2015	93.80	116.29				
	2016	81.79	119.92				
	2019	72.26	99.95				
	2020	59.78	88.28				

Due to its high toxicity factor (Tr=40; Håkanson, 1980), even minor increases in mercury concentration result in a significant ecological risk. While other metals remain in the low-risk category ($E_r < 40$), mercury at locations like Prečko reaches levels classified as "considerable ecological risk." Given that the I_{geo} for mercury is generally in the "unpolluted to moderately polluted" category, it can be concluded that the contamination is of recent, anthropogenic origin (likely atmospheric deposition from urban sources or historical fertilizer residues) but has not yet reached critical levels that would permanently alter the geochemical profile of the site (Loska et al., 1997).

Apart Hg, Cu shows slightly elevated values (Pi between 1.0 and 2.0) at the Savica and Klara locations. The I_{geo} value for copper remains near zero or negative, confirming that this represents slight enrichment rather than systemic pollution. In the context of urban gardening, elevated Cu is often associated with the long-term application of Cu-based fungicides in horticulture (Kabata-Pendias, 2011). Locations like Savica, which have a longer history of cultivation, are more susceptible to this type of accumulation in the topsoil.

Conclusion

This research confirms Zagreb's urban garden soils remain largely unpolluted for Cd, Cr, Ni, Pb, and Zn per Müller's I_{geo} scale, reflecting natural geochemical baselines rather than contamination. However, mercury consistently poses moderate to considerable ecological risk across monitoring years, with Prečko and Borovje exhibiting the highest E_r and RI values, driven by Hg's exceptional toxicity and accumulation above background levels (0.093 mg kg^{-1}). These findings indicate recent anthropogenic Hg inputs. Mild Cu enrichment at longer-cultivated sites aligns with copper-based fungicide use in horticulture. There are few key implications for urban agriculture policy: (a) to prioritize Hg monitoring in high-risk gardens (Prečko, Borovje, Sesvete-Senjska ulica), (b) to implement phytoremediation trials and soil amendments, (c) to educate urban gardeners about appropriate application of pesticides and fertilizers, (d) to develop urban gardening guidelines integrating ecological risk assessment. Regular monitoring ensures sustainable urban agriculture while protecting public health from chronic heavy metal exposure via homegrown produce. These results provide baseline data supporting Croatia's soil protection strategy within EU frameworks.

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EXPERT PAPER

Challenges and perspectives of aligning water reuse legislation in Bosnia and Herzegovina with the European Union Water Policy

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Summary
Water scarcity, exacerbated by climate change and shifting hydrological regimes, is placing increasing pressure on agricultural systems in Bosnia and Herzegovina (BiH), where agriculture remains the largest water consumer. In this context, water reuse, particularly the use of treated wastewater, represents a key strategy for improving resource efficiency and strengthening climate change resilience. This paper analyzes the current legislative framework in the Republic of Srpska (RS), with a focus on its alignment with European Union (EU) water policy, including Directive 91/271/EEC on Urban Wastewater Treatment and Regulation 2020/741 on minimum requirements for water reuse.
A qualitative comparative analysis was conducted on principal regulatory documents, including the National Water Laws and existing environmental strategies of the RS. The findings indicate that while a solid basis for water protection and classification exists, significant gaps remain regarding the transposition of EU Regulation 2020/741 on minimum requirements for water reuse (Voulvoulis et al., 2017; Mianzan et al., 2022).
Current legislation does not explicitly regulate reclaimed water quality standards for agricultural applications, creating legal uncertainty for operators and investors. Furthermore, irrigation is restricted to high-quality water classes, while the use of treated wastewater remains largely unaddressed in legal terms. The study concludes that for BiH to achieve sustainable water management, it must introduce risk-based water reuse standards and strengthen regulatory coherence between water protection, agriculture, and climate adaptation strategies. Progressive harmonization with the EU environmental acquis is not only a requirement for EU accession but a strategic necessity for enhancing resource efficiency and regional cooperation in the face of increasing drought frequency.

Keywords: water legislation, water reuse, circular economy, irrigation, climate change

Introduction

Climate change is reshaping hydrological regimes worldwide as well as in Bosnia and Herzegovina (BiH), increasing drought frequency and intensifying competition among urban and rural water users, consequently making an impact on agricultural production as the largest water consumer (Srdić et al., 2023; Cerekovic et al., 2024; Playán et al., 2024; Čereković et al., 2025).
Therefore, primary in the sense of use of treated wastewater, water reuse is the beneficial and widely recognized as a strategic tool for climate resilience (Salgot and Folch, 2018; Florides et al., 2024; Berbel et al., 2024). Within the European Union (EU), the Water Framework Directive (2000/60/EC) and Regulation 2020/741 on minimum requirements for water reuse provide the legal cornerstone for risk-based water management. Although BiH is not yet an EU Member State, gradual alignment with the EU environmental acquis is a key requirement for

accession and an important driver of policy reform. However, the extent to which current national legislation supports water reuse remains unclear.
The objective of this paper is therefore to analyze the existing legislative framework in the Republic of Srpska (RS) and to identify key gaps in relation to EU water reuse policy, with a particular focus on agricultural applications.

Material and Methods

This study is based on a qualitative comparative legal analysis of the principal regulatory and strategic documents governing water management in RS. The analysis includes the Water Law, the Regulation on Wastewater Discharges into Surface Waters, the Regulation on Discharge of Wastewater to Public Sewage, and relevant environmental and water management strategies.
These documents were systematically reviewed to assess their scope, institutional arrangements, monitoring requirements, and degree of alignment with EU directives and regulations. Particular attention was given to identifying provisions related to wastewater treatment, discharge standards, and potential reuse applications.
The analysis was complemented by a comparison with key EU policy instruments, notably Directive 91/271/EEC and Regulation 2020/741, in order to evaluate the level of regulatory harmonization and identify gaps relevant to sustainable water management.

Results and Discussion

The legislative framework of RS establishes a structured approach to water protection and wastewater management. The Water Law provides the legal basis for integrated water management, including permitting systems, institutional responsibilities, and pollution control measures. Strategic documents further support these objectives by emphasizing the need for balancing water use with environmental protection.
The existing regulatory system is largely aligned with the principles of Directive 91/271/EEC, particularly in relation to wastewater collection, treatment, and discharge control. The Directive introduces requirements based on the size of agglomerations and the sensitivity of receiving waters, mandating secondary or more advanced treatment and defining limits for key pollutants.
At the entity level, wastewater discharges are regulated through a combination of legal and technical instruments. These include emission limit values, monitoring obligations, and requirements for industrial pre-treatment. Discharges are assessed both at the point of emission and in terms of their impact on receiving water bodies, reflecting a dual compliance approach that integrates emission standards and environmental quality objectives.
The regulatory framework is underpinned by the “polluter pays” principle, which assigns responsibility for monitoring, reporting, and financing pollution control measures to the discharger. Public utilities play a central role in overseeing compliance within sewer systems, while industrial operators are required to conduct self-monitoring and ensure that their effluents meet prescribed standards.
A key component of the system is the definition of limit values for wastewater discharged into public sewage systems. These limits are designed to protect infrastructure, maintain the efficiency of wastewater treatment plants, and prevent environmental contamination. The regulation specifies thresholds for a wide range of physical, chemical, and hazardous parameters, including suspended solids, nutrients, heavy metals, and organic pollutants according to the Regulation on Discharge of Wastewater to Public Sewage, Official Journal of the RS, no. 44/01 (Table 1).

Table 1. Regulatory limit values for wastewater discharge to public sewer systems in the Republic of Srpska.

Parameter	Unit	Limit value RS
pH		6.5-9
Temperature	°C	40
Sedimentation during ½ h	ml/l	5
Suspended solids	g/m^3	500
COD	mg /l O_2	*
BOD	mg /l O_2	*
Ammonia-N	g/ m^3	40
Nitrate-N	g/ m^3	-
Nitrite-N	g/ m^3	10
Total N	g/ m^3	100
Total P	g/ m^3	5
Carbon tetrachloride	mg/ m^3	3 000
DDT	mg/ m^3	400
Pentaclorophenol	mg/ m^3	2 000
Aldrin	mg/ m^3	10
Dieldrin	mg/ m^3	10
Endrin	mg/ m^3	10
Isodrin	mg/ m^3	10
Hexaclorobenzene	mg/ m^3	2 000
Hexachlorobutadien	mg/ m^3	3 000
Chloroform	mg/ m^3	1 000
1.2-dichlorethane	mg/ m^3	200
Trichloroethylene	mg/ m^3	200
Tetrachlorethylene	mg/ m^3	200
Hexachlorocyclohexane	mg/ m^3	4 000
Trichlorobenzene	mg/ m^3	100
PAN sum	mg/ m^3	200
PCB sum	mg/ m^3	20
Phenol index	mg/ m^3	100 000
Benzole	mg/ m^3	100 000
Toluol	mg/ m^3	100 000
Xylene	mg/ m^3	7 000
Formaldehyde	mg/ m^3	25 000
Mineral oils	g/ m^3	100 000
Detergent	mg/ m^3	10 000
Ag	mg/ m^3	100
Al	mg/ m^3	**
As	mg/ m^3	100
Cd***	mg/ m^3	50
Co	mg/ m^3	1 000
Cr total	mg/ m^3	1000
Cr^{6+}	mg/ m^3	200
Cu	mg/ m^3	1000
Fe	g/ m^3	-
Hg***	mg/ m^3	10

Mn	mg/ m^3	500
Ni***	mg/ m^3	50
Pb	mg/ m^3	500
Se	mg/ m^3	2 000
Sb	mg/ m^3	2 000
Sn	mg/ m^3	2 000
Zn	mg/ m^3	2000
Fluoride	g/ m^3	50
Cyanides	g/ m^3	1
Sulphides	g/ m^3	2
Sulphates	g/ m^3	200
Sulphites	g/ m^3	10
Chlorides	g/ m^3	250

*COD and BOD are limited by permits considering technical and economic factors influencing joint WWTP and inflow from groundwater to sewage resulting low concentration of organic matter in inflow to WWTP
**Al and Fe are not limited
***Priority substances by EU 2000/0035

Monitoring requirements, including sampling frequency and analytical methods, are defined based on the characteristics and pollution load of the discharger. While these standards provide a solid basis for pollution control and environmental protection, they are primarily focused on ensuring safe discharge rather than enabling resource recovery.

Despite the existence of a comprehensive framework for wastewater management, current legislation does not explicitly address the reuse of treated wastewater, particularly for agricultural purposes. Irrigation practices are restricted to water of relatively high quality, and there are no defined standards or guidelines for the use of reclaimed water. This represents a significant gap when compared to Regulation 2020/741, which establishes minimum quality requirements and risk management procedures for water reuse in agriculture.

The EU approach is based on a fit-for-purpose principle, where water quality is matched to the intended use, supported by monitoring, risk assessment, and management plans. In contrast, the RS regulatory framework treats wastewater exclusively as a waste stream to be disposed of, rather than as a potential resource. As a result, there is no legal basis for permitting reuse, no defined responsibilities for operators, and no quality standards to ensure safe application in agriculture. This lack of regulatory clarity creates barriers to investment and innovation in water reuse systems and limits the ability of the agricultural sector to adapt to increasing water scarcity.

Conclusion

The Republic of Srpska has established a relatively robust legislative framework for wastewater management, with clear provisions for pollution control, monitoring, and environmental protection. However, this framework remains predominantly oriented toward wastewater disposal and does not address the growing need for resource-efficient water management. The absence of specific regulations for water reuse, particularly in agriculture, represents a critical gap in the context of climate change and increasing water scarcity. Aligning national legislation with EU Regulation 2020/741 will require the introduction of risk-based quality standards, the definition of institutional responsibilities, and the integration of water reuse into broader water and agricultural policies. Such a transition from a disposal-oriented to a resource-oriented approach is essential for enhancing resilience, supporting sustainable agricultural production, and advancing the process of EU integration.

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ORIGINAL SCIENTIFIC PAPER

Soil CO_2 Flux Responses to Tillage Intensity: Associations with Soil Chemical Properties and Agroecological Factors

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Summary
This study aimed to evaluate the effects of different tillage systems on soil C-CO_2 emissions and to examine their associations with selected soil chemical properties and agroecological factors in a soybean cropping system. Three tillage systems were: conventional tillage (CT), conservation tillage system deep (CTD), and conservation tillage system shallow (CTS). Soil samples were collected from each treatment to determine total carbon content, humus, and soil pH, while soil CO_2 emissions (flux) was also measured. Average annual soil C-CO_2 emissions varied among treatments, ranging from 11.07 kg ha^{-1} day^{-1} under CT to 12.63 kg ha^{-1} day^{-1} under CTD. Seasonal soil CO_2 emissions were not significantly correlated with total carbon, humus, or soil pH. The study contributes to understanding soil CO_2 dynamics in the context of sustainable soil management.

Keywords: soil respiration, conventional tillage, conservation tillage, total carbon content

Introduction

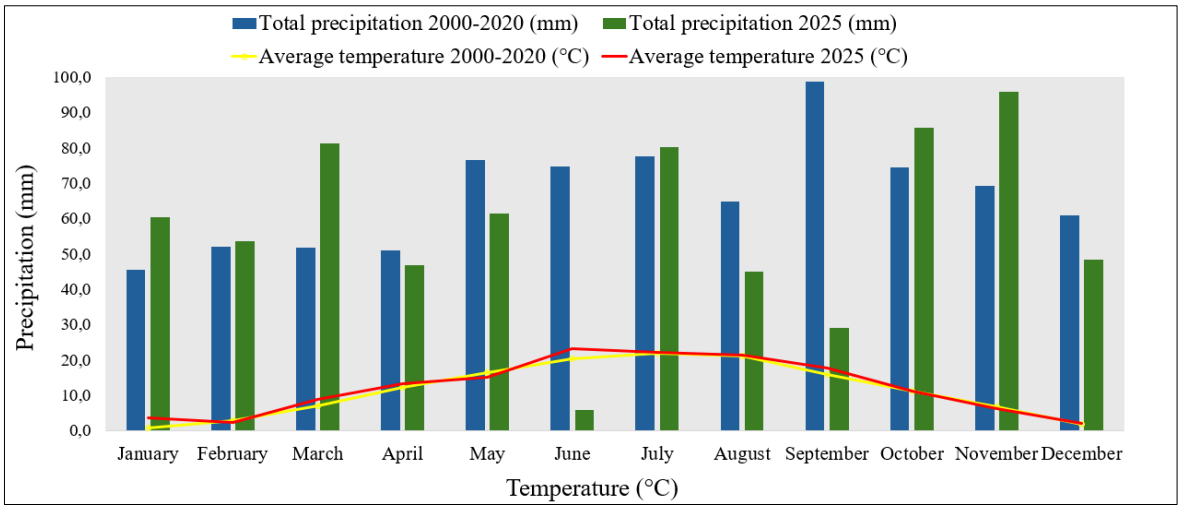
Agriculture emits around 10-15% of all global emissions, mostly from enteric fermentation (CH_4), manure management (CH_4, N_2O), synthetic fertilizers (N_2O), and soil emissions (CO_2). Therefore, understanding the relationship between agriculture and climate change is paramount for making informed mitigation and adaptation strategies (IPCC, 2022). Soil in particular is very important since it plays a dual role of being both a source and a sink for CO_2 (Benbi, 2013). This highlights the need for good soil management practices to achieve global sustainability in food production and climate change mitigation (Lai, 2007). Soil tillage, a common practice worldwide, creates soil disturbances that potentially promote CO_2 flux through organic matter decomposition (Srivastava, 2025). The amount of soil disturbed, causing soil carbon loss, is largely dependent on the intensity and type of tillage employed (West and Marland, 2002). Therefore, changes in tillage practice may reduce soil carbon losses and, under certain conditions, contribute to carbon in agricultural soils (Kern and Johnson, 1993). Identifying tillage systems that enhance soil integrity while incurring the least negative biogeochemical impacts is thus necessary for climate-smart agriculture. Different tillage systems induce different physical, chemical, and biological soil properties. Among these, chemical properties are particularly important since they represent the strength and quality of a soil to support plant production (Huang et al., 2018). The key indicators of soil chemical properties include soil organic matter, organic carbon, total carbon, nutrients, metal cations, cation exchange capacity, and pH (Malik et al., 2018). Overall, conservation tillage practices are expected to improve soil fertility by reducing soil disturbance while maintaining or increasing crop residue retention (Sithole et al., 2016). Exploring the effects of different tillage systems on soil chemical properties can therefore provide a reference for enhancing ecosystem services provided by the soil. Different tillage systems also alter agroecological factors, especially soil moisture and soil

temperature. Soil moisture and temperature directly influence soil respiration, which is a large pathway for soil carbon loss (Srivastava et al., 2016). Hence, there is a need to understand the relationship between these agroecological factors, tillage, and carbon fluxes. Therefore, the aim of this study was to evaluate the effects of different tillage systems on soil C-CO_2 emissions in a soybean agroecosystem and to examine the associations between seasonal soil C-CO_2 emissions, selected soil chemical properties and agroecological factors.

Material and Methods

Site Description and Treatments

The experiment was performed in 2025 in northwestern Croatia in Križevci (16°33′32″ E; 46°01′38″ N). The soil type is classified as Gleysol (FAO, 2015). The experiment was established as a randomized complete block design (RCBD), including three tillage systems, each with three replicates (n = 3), and was carried out over a total area of 15.000 m^2. Individual plots measured 160 m^2, with a 2 m distance separating the tillage treatments on each side and a 1 m distance between replicates. The tillage systems were: CT-conventional tillage (based on plowing up to 30 cm); CTD-conservation tillage system deep (soil loosening up to 30 cm with a minimum crop residue cover of 30% on the soil surface) and CTS-conservation tillage system shallow (soil loosening up to 10 cm depth with a minimum crop residue cover of 50% on the soil surface). The main crop at the experimental plot was soybean (Glycine max. L), seeded on April 30, 2025 and harvested on September 29, 2025. The experimental site is characterized by a typical moderate continental climate, with hot summers and cold winters. The mean annual temperature and total precipitation for the investigated 2025 and the reference period (2000-2020) are shown in Graph 1. In 2025, total precipitation amounted to 692.7 mm, with the highest value recorded in November (95.9 mm) and the lowest in June (5.7 mm). The average annual temperature was 12.2 °C, with December being the coldest month (2.0 °C) and June the warmest (23.2 °C). In comparison with the reference period (2000–2020), characterized by a mean annual temperature of 11.5 °C and annual precipitation of 796.4 mm, 2025 was slightly warmer and drier, with a 0.7 °C higher average temperature and 103.7 mm less precipitation than the long-term mean (DHMZ, 2025). Pronounced monthly variability in precipitation was observed in 2025, in June and September, when rainfall amounts were well below the long-term average.



Graph 1. Monthly precipitation amount (mm) and mean temperature (°C) in the reference 2000-2020 and the investigated 2025

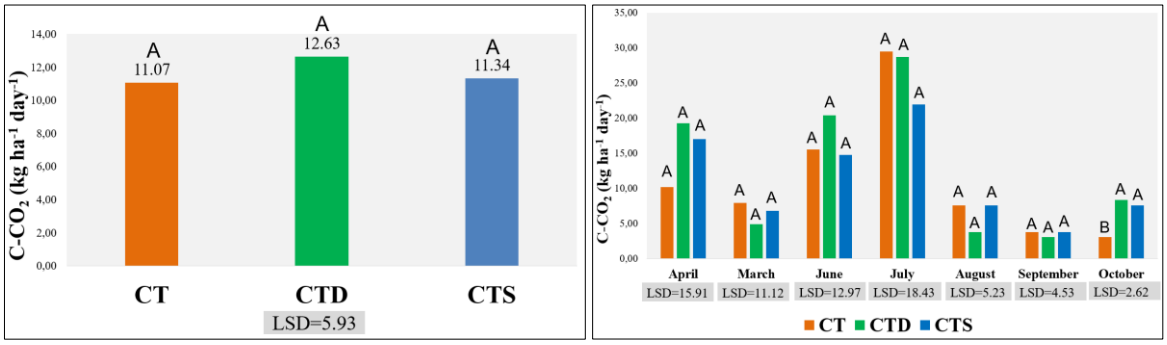
Measurement of carbon dioxide emissions and agro-ecological factors
The measurements of soil CO_2 concentration were made seven times in the following months: April, May, June, July, August, September, and October, in three replicates for each treatment. The soil CO_2 concentrations were measured by the in situ closed static chamber method. At the beginning of measurements, the circular frames were inserted about 10 cm into the soil, and initial CO_2 concentrations were measured. Before each measurement, all vegetation in the chamber area was removed. After closing the chamber, the accumulation of CO_2 emitted from the soil was measured, and the incubation period of 30 min began. After an incubation period, the CO_2 concentration in ppm was measured using a portable infrared CO_2 GasAlertMicro5 IR (2011) and BW Ultra CO_2 IR (2025) detector. The calculation of soil CO_2 flux was described in Galić et al. (2025). For each measurement of the CO_2 concentration, agroecological factors (soil temperature (°C), soil moisture (%), and electrical conductivity (mS m^{-1})) were measured using the IMKO HD2 device with the Trime Pico64 (2011) probe.

Soil Sampling and Laboratory Analysis
Soil samples (0–30 cm) were taken at the beginning of the research, in three repetitions on each tillage treatment, to determine the chemical soil properties. Soil sampling was performed using an Eijkelkamp soil probe. Soil preparation, including air-drying, homogenization, and sieving (<2 mm) were performed according to the protocol HRN ISO 11464 (ISO 11464, 2009). Soil reaction was determined by potentiometric measurement in a suspension of soil and extraction agent (1 mol dm^{-3} KCl and H_2O) in a ratio of 1:2.5 according to the modified HRN ISO 10390 protocol (ISO 10390, 2005). Soil organic matter (SOM) content was determined by the sulfochromic oxidation method, which involves wet combustion and titration (modified ISO 14235, 2004). The total carbon content in the soil was determined simultaneously using the dry combustion method with the Vario Macro CHNS analyzer (Elementar, 2006), according to HRN ISO 10694 (ISO 10694, 1995) protocol.

Data analysis
Statistical analysis was conducted using SAS software (version 9.1; SAS Institute Inc., 2002–2004, Cary, NC, USA). Differences among tillage treatments were evaluated by analysis of variance (ANOVA), followed by Fisher's LSD post hoc test. All tests were performed at a 5% significance level. Linear relationships between seasonal soil C-CO_2 emissions and selected soil properties, as well as agroecological factors, were assessed using Pearson correlation analysis, and the strength of the correlations was interpreted according to the Roemer-Orphal scale (Vasilj, 2000).

Results and Discussion
Soil C-CO_2 emissions for the three tillage treatments (CT, CTD, and CTS) are shown in Graph 2. The highest emissions were recorded under the CTD treatment (12.63 kg ha^{-1} day^{-1}), followed by CTS (11.34 kg ha^{-1} day^{-1}) and CT (11.07 kg ha^{-1} day^{-1}). Differences among treatments, on annual basis, were not statistically significant. The average daily C-CO_2 emissions for the three tillage treatments are presented in Graph 3. Emissions varied from 3.02 kg ha^{-1} day^{-1} in September (CTD) and October (CT) to a maximum of 29.48 kg ha^{-1} day^{-1} in July (CT). The emission peak observed in July coincided with the warmest part of the growing season, when increased plant activity and microbial processes likely stimulated soil respiration. Although statistically significant differences between treatments were observed only in October, treatment-related variations in emissions were evident throughout the study period. Similar findings have been reported by Gelybó et al. (2022), who observed higher soil respiration under conservation tillage compared to conventional tillage. Increased CO_2 emissions in conservation systems are often associated with improved soil moisture conditions and residue accumulation

in the surface layer, which may stimulate microbial activity and enhance organic matter decomposition (Praharaj et al., 2025). In contrast, Srivastava (2025) reported higher CO_2 emissions under conventional tillage, primarily due to enhanced soil disturbance and aeration. These contrasting findings suggest that the effect of tillage on soil CO_2 emissions depends on the interaction between residue retention and soil disturbance, as well as site-specific climatic and soil conditions (Jug et al., 2017; Bozóki et al., 2025).



Graphs 2 and 3. Average annual soil C-CO_2 emission values between three tillage treatments (left - Graph 3); Average daily C-CO_2 emissions per month for three investigated treatments (right - Graph 4). Statistically significant differences between different treatments are indicated by different letters (A, B) ($p < 0.05$).

Pearson correlation analysis showed no statistically significant associations between seasonal soil C-CO_2 emissions and investigated soil properties (Table 1). A negative correlation was observed between C-CO_2 emissions and humus content (r=-0.576); however, this relationship was not statistically significant (p=0.104). Weak correlations were also found for soil pH (r=-0.359, p=0.342) and total carbon content (r=0.002, p=0.995). The observed negative trend with humus content may suggest a potential role of soil organic matter in stabilizing soil carbon; however, given the lack of statistical significance, this relationship should be interpreted with caution (Guo et al., 2025; Piccolo and Drosos, 2025). The non and weak relationships observed for soil pH and total carbon content suggest that these properties had a limited influence on seasonal emission variability under the studied conditions. In contrast, soil temperature exhibited a weak but statistically significant positive correlation with soil C-CO_2 emissions (r=0.258, p=0.042) (Table 2), suggesting that short-term environmental conditions may have played a more prominent role in regulating soil respiration dynamics (Della Lunga et al., 2025). Previous studies have also reported similar relationships between soil temperature and CO_2 emissions (Galić et al., 2019; Wang and Yang, 2018), while other studies have observed contrasting results (Yerli et al., 2022). Soil moisture showed a very weak and non-significant association with C-CO_2 emissions (r=0.214, p=0.093), suggesting a limited influence under the conditions of this study.

Table 1. Correlation between soil C-CO_2 emissions and total C (%), SOM (%) and pH

Soil property	p-value	Coefficient of correlation (r)	Strength of correlation
Total C, %	0.995 ns	0.002	None
SOM, %	0.104 ns	-0.576	Strong
pH	0.342 ns	-0.359	Weak

*Level of Statistical Significance: *-p≤0.05; **-p≤0.01; ***-p<0.001; ns-not significant*

Table 2. Correlation between soil C-CO_2 emissions and agroecological factors

Agroecological factors	p-value	Coefficient of correlation (r)	Strength of correlation
Soil temperature (°C)	0.042*	0.258	Weak
Soil moisture (%)	0.093 ns	0.214	Very weak

*Level of Statistical Significance: *-p≤0.05; **-p≤0.01; ***-p<0.001; ns-not significant*

Conclusion

Although the investigated tillage systems did not result in statistically significant differences in soil C-CO_2 emissions, variations in emission patterns were recorded. The absence of significant relationships between emissions and most soil chemical properties suggests that relatively stable soil characteristics had a limited influence on seasonal CO_2 fluxes under the studied field conditions. In contrast, soil temperature showed a statistically significant positive association with emissions, indicating that short-term environmental variability played a more prominent role in regulating soil respiration dynamics. However, the limited number of replicates and the lack of statistically significant differences among treatments indicate that the experimental design may have constrained the detection of treatment effects. The findings highlight the complexity of soil C-CO_2 emissions and emphasize the need to consider multiple interacting factors when developing strategies aimed at reducing emissions and promoting sustainable soil management.

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ORIGINAL SCIENTIFIC PAPER

Incidence of wilting and dieback of olive trees in Croatia, Bosnia and Herzegovina and Italy

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Summary
Samples of symptomatic olive trees were collected in three different countries from 2016 to 2024 in a total of 116 locations within 12 olive-growing areas. From 255 samples collected from 24 olive varieties, using molecular and morphological identification, sequencing, and pathogenicity tests, 25 fungal pathogens have been identified. The objective of this work is to identify the causal agents of the diseases observed in the orchards in three investigated countries, and to study the levels of susceptibility of different olive varieties to these pathogens. No pathogenic fungi have been found in autochthonous varieties Črnica, Moražola, Bjankera, and Bova, which emphasizes the importance of these varieties. The variety Buža seems, on the other hand, the most susceptible to the investigated fungal infections.

Keywords: *Olea europaea* L., phytopathogenic fungi, varietal susceptibility, *Verticillium dahliae*

Introduction

Olive is the most important crop in the Mediterranean. Different fungal pathogens have been documented on olive trees in the world so far, especially in Spain, and in California, and the number is increasing (Moral et al., 2008, Úrbez-Torres et al., 2013). Like this, in Italy many species have been found (Nigro, et al., 2005, Lazzizera et al., 2008, Carlucci et al., 2013; Frisullo et al., 2015). Recently, a number of new olive fungal pathogens has been described in Croatia, too (Ivić, et. al. 2010, Kaliterna et al., 2012, Kaliterna et al., 2016, Ivić et al., 2018) and also in Bosnia and Herzegovina (Crnogorac et al, 2023). The aims of this work are to identify the causal agents of the diseases observed in the orchards in three different countries using morphological and molecular methods, and to study the levels of susceptibility of different olive varieties to these fungal pathogens.

Material and Methods

The field study covered more than 380 ha in three countries and a total of 116 locations and 12 growing areas (51 locations in Croatia within four olive-growing areas, 59 in Bosnia and Herzegovina within five areas, and six in Italy within three areas). Samples of symptomatic olive branches were collected in three investigation periods, I (from 2016 to 2018); II (2021-

2022), and III (2023-2024). Immediately after collection, the samples were stored in plastic bags, labeled, and stored at +4 ºC. In total, there were 255 samples i. e. 215 samples collected in Croatia in periods I (103) and II (112), 34 in Bosnia and Herzegovina collected in periods II (4) and III (30), and six in Italy in period III. The age of the investigated olive trees ranged from two to over 300 years old. For the identification of species, classical (cultivation, analysis of isolate morphology, and microscopic identification) like molecular methods (PCR and sequencing) have been performed. Total DNA was extracted with Maxwell® RSC Plant DNA Kit (Promega, Madison, WI, USA). PCR was conducted using ITS1/ITS4 (White et al.,1990), Bt2a/Bt2b (Glass and Donaldson, 1995), and EF1-728F/EF1-986R pairs of primers (Carbone and Kohn, 1995). Sequencing was carried out by Macrogen Europe BV® (Amsterdam, The Netherlands). Sequences were edited in Sequencher® (Gene Codes Corporation, USA) and compared with sequences retrieved from GenBank® database. In order to confirm the pathogenicity of the isolated species, pathogenicity tests have been done. Inoculation was performed on olive seedlings of different cultivars and ages in a greenhouse. Branches were inoculated by placing a mycelium plug from a PDA culture of isolates in a wound made with a cork-borer and protected with parafilm. In an attempt to fulfill Koch's postulates, small pieces of necrotic tissue were placed on PDA to recover inoculated fungi. In pathogenicity tests several phytopathological parameters were also determined like resistance level (RL), according to García-Ruiz et al. (2015). Varietal susceptibility was determined on olive seedlings of different cultivars according to the total necrotic lesion diameter.

Results and Discussion

During field research, a wide range of symptoms were observed on infected olive trees, including wilting and dieback of branches and shoots, rotting and falling of fruits, cracking and reddish-brown discoloration of the bark, the appearance of necrotic lesions, cankers, and tree dieback. Different pathogenic fungi, 25 in number, have been isolated in three studied countries (Table 1). Specifically, *Verticillium dahliae* Kleb. was confirmed as the most widespread species. It was detected in all three countries (Table 1 and 2). This species has been identified in Bosnia and Herzegovina for the first time (Table 2). *V. dahliae* was confirmed in Croatia recently (Kaliterna et al., 2016; Godena et al., 2022). It has been detected in almost all regions where olive is cultivated (Nigro et al., 2005, López-Escudero and Mercado-Blanco, 2011). New species of fungi have been recorded on olive trees that have not been identified in Croatia and in Bosnia and Herzegovina yet, and/or the world so far. In Croatia the presence of species *Diplodia mutila, Dothiorella iberica, Do. sarmentorum, Biscogniauxia mediterranea* and *Cytospora pruinosa* on olive trees has been documented recently (Petrović, et al., 2023a, Petrović et. al., 2024a, Petrović et. al., 2024b). Furthermore, for the first time *B. nummularia, Sordaria fimicola, Phaeoacremonium iranianum, Nigrospora gorlenkoana, N. osmanthi* and *N. philosophiae-doctoris* were identified as causative agents of olive diseases (Petrović, et al., 2022; Petrović, et al., 2023b, Petrović, et al., 2024a). In Bosnia and Herzegovina, the species *Alternaria alternata* has been documented for the first time (Crnogorac et al., 2023). Besides that, in tested autochthonous varieties Črnica, Moražola, Bjankera (synonym Istarska Bjelica), and Bova, no pathogenic fungi have been found (Table 1.), which emphasizes the importance of these varieties. The variety Buža, on the other hand, seems to be the most susceptible to the investigated fungal infections.

Table 1. Pathogenic fungi identified by morphological and molecular methods in natural infections and susceptibility of olive varieties to different fungal pathogens in induced artificial infection intermittently in I, II and III investigated periods (from 2016 to 2024) in three studied countries.

Olive varieties (24)	**Fungal species (25) and their abbreviation** 1. *Pleurostomophora richardsiae* (Nannf.) L. Mostert, W. Gams and Crous (***P.r.***)*;* 2.*Verticillium dahliae* Klebahn (***V.d.***); 3. *Phaeoacremonium iranianum* Mostert (***Ph.i.***)*;* 4.*Diplodia seriata* de Notaris (***D.s.***); 5. *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & Phillips (***N.p.***)*;* 6. *Pseudophaeomoniella oleae* Crous (***P.o***.)**;* 7.*Diatrype stigma* (Hoffmann) Fries (***Di.s.***)**;* 8. *Diaporthe* sp. (***D.* sp.**); 9. *Geosmithia* sp. (***G.*sp.**)*; 10. *Cytospora pruinosa* (Fries) Saccardo (***C.p.***); 11. *Nigrospora gorlenkoana* Novobr. (***N.g.***); 12. *Nigrospora philosophiae-doctoris* M. Raza, Qian Chen & L. Cai ***(N.p-d)****;* 13. *Nigrospora osmanthi* Mei Wang & L. Cai (***N.o***.)*;* 14. *Biscogniauxia mediterranea* (de Notaris) Kuntze (***B.m.***); 15. *Biscogniauxia nummularia* (Bulliard) Kuntze (***B.n.***); 16.*Sordaria fimicola* (Roberge ex Desmazières) Cesati & de Notaris (***S.f.***); 17.*Botryosphaeria dothidea* (Mougeot) Cesati & de Notaris (***B.d.***); 18. *Diplodia mutila* (Fries) Montagne (***D.m.***); 19. *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves (***Do.i.***); 20. *Dothiorella sarmentorum* (Fries) A.J.L. Phillips, Alves & Luque (***Do.s.***); 21. *Armillaria mellea* (Vahl) Kummer (***A.m.***); 22. *Alternaria alternata* (Fries) Keissler (***A.a.***); 23. *Fusarium* sp. (***F.*sp**.) or *F. solani* species complex (***F.s.***); 24. *Pythium* sp. (***P.* sp.**); 25. *Phoma* sp. (***Ph.* sp.**)
Buža	*V.d.* (ES)**§**; *Ph.i.; D.s.; Di.s.***; P.o.***; D.* sp.*; G.* sp******.; C.p.; N.p-d.; B.m.; B.n.; D.m.; Do.i.; N.p.; A.m.*
Istarska bjelica	*V.d.* (ES); *B.m.; B.n.; N.p.; B.d.; A.m.; D.s*[2]
Rosinjola	*V.d.* (S); *Ph.* sp.
Črnica	-
Karbonaca	*V.d.* (MR); *N.p.; D.s.*
Buža puntoža	*B.m.*
Črna	*V.d.*
Plominka	*V.d.*
Porečka rosulja	*V.d.; C.p.; B.m.*
Moražola	-
Karbonera	*N.p.*
Bjankera	-
Bova	-
Unknown**	*N.o.; B.d.; A.m.*
Oblica***	*A.m.; V.d.*[2]*; A.a.*[2]*; P.* sp.[2]*; Ph.* sp.[2]*; F. s.*[2]
Drobnica***	*A.m.*
Frantoio	*B.d.*
Picholine	*V.d.*
Leccino	*V.d.* (ES); *N.g.; S. f,; B.m.; A.m.; Ph.* sp.*; F. s.*[2]*; P.* sp.[2]*; D.s.*[2]*; V.d.*[3]
Pendolino	*S.f.; Do.s.; A.m.; D.s.*[2]
Leccio del corno	*B.m.*
Coratina	*A.m.*
Ascolana tenera	*A.a.*[3]
Gentile di Chieti	*F.*sp.[3]*; A.a.*[3]

***** No visible lesions formed on inoculated plants in the pathogenicity test; ****** Collected in Istria, and Kvarner; *** Collected in Dalmatia; **§** RLc = resistance level in pathogenicity test; HR –highly resistant; R – resistant; MR - moderately resistant; MS – moderately susceptible; S – susceptible; ES – extremely susceptible; [2] Areas of Neum, Ljubuški, Stolac and Mostar in Bosnia and Herzegovina; [3] Regions of Puglia, Marche and Abruzzo in Italy.

Table 2. Identified fungal species, number of identified species, isolates and olive growing areas in which pathogens have been found in northwestern Croatia (Istria), Bosnia and Herzegovina and Italy.

Identifed species	No. of identified species/ genus	No. of isolates	Olive growing areas	Investigation period (I, II, III) and incidence in the referred period (no. of identified isolates/ total no. of samples) (%)
CROATIA (300 ha)				
Verticillium dahliae nondefoliating pathotype (ND) race 1	1	28	R; P-V; B-U; V-P	I-27.2
Sordariomycetes (*Geosmithia* sp., *Diaporthe* sp., *Diatrype stigma*); Botryosphaeriacae (*Diplodia seriata*‡, *Neofusicoccum parvum*‡), and Eurotiomycetes (*Pseudophaeomoniella oleae*)	6	25	V-P; R; P-V; B-U	I-24.3
Armillaria mellea sensu lato*	1	12	R; P-V; B-U; V-P	I-11.7
Botryosphaeriaceae (*Diplodia seriata*‡, *D. mutila, Neofusicoccum parvum*‡, *Botryosphaeria dothidea, Dothiorella iberica, Do. sarmentorum*)	4 (+ *D.s.*‡ and *N.p.*‡)	13	R; P-V; V-P	II-11.6
Biscogniauxia mediterranea, B. nummularia, Sordaria fimicola	3	12	B-U; R; P-V; V-P	II-10.7
Pleurostomophora richardsiae	1	10	B-U; V-P	I-9.7
Cytospora pruinosa	1	10	P-V, V-P	II-8.9
Phaeoacremonium iranianum	1	4	R	II-3.6
*Nigrospora gorlenkoana, N. philosophiae-doctoris, N. osmanthi***	3	3	R; P-V	II-2.7
Total in Croatia	**21**	**117**		
BOSNIA AND HERZEGOVINA (77 ha)				
Verticillium dahliae	1	1	LJ	II-25.0
Alternaria alternata	1	2	M, LJ	II-50.0
Fusarium solani species complex	1	3	S, LJ	III-10.0
Pythium sp.	1	2	S, N	III-6.7
Diplodia seriata	1	2	Č, M	II-25.0, III-3.3
Phoma sp.	1	1	S	III-3.3
Total in Bosnia and Herzegovina	**6**	**11**		
ITALY (4 ha)				
Verticillium dahliae	1	3	Ce, Ca	III-50.0
Alternaria alternata	1	2	Ca, Pe	III-33.3
Fusarium sp.	1	1	Pe	III-16.7
Total in Italy	**3**	**6**		

I – from 2016 to 2018; II – from 2021-2022: III – from 2023 to 2024; *Identified also in Dalmatia; **Identified also in Kvarner; R - Rovinj; P-V - Poreč–Vrsar; V-P - Vodnjan–Pula; B-U - Buje–Umag; N - Neum, Lj - Ljubuški, S – Stolac, M – Mostar, and Č - Čapljina; Ce – Cerignola, Puglia, Ca – Carassai, Marche, and Pe – Pescara, Abruzzo; ‡ has been identified in the I and in the II investigation period.

Pathogenicity tests showed that all fungal isolates were pathogenic to olive trees, except *Geosmithia* sp., *Diatrype stigma*, and *Pseudophaeomoniella oleae* which did not develop necrotic lesions on olive seedlings (Ivić et al., 2023). Average necrotic lesion (mm) ranged from 2.8±0.1 recorded on Buža infected by *Diaporthe* sp. to 320.75±87.39 recorded by *Neofusicoccum parvum* on Istarska bjelica (Ivić et al., 2023; Petrović et al., 2024b). When evaluating the resistance of varieties to *N. parvum*, Buža was the most resistant (16.15±5.32) (Petrović et al., 2024b).

Conclusions

The scientific contribution of this work is reflected in identifying new and less known olive fungal pathogens in three countries, and studying the levels of resistance in different olive varieties. In these countries, 25 fungal pathogens have been identified using morphological and molecular diagnostic methods. Of all investigated varieties, Istrian autochthonous variety Buža seems to be relatively susceptible, extremely susceptible to *V. dahliae,* as the most widespread

fungal species. No pathogenic fungi in natural infections have been found in autochthonous varieties Črnica, Moražola, Bjankera, and Bova, which emphasizes the importance of these varieties. In the preventive control of olive diseases, choosing resistant varieties serve as an appropriate tool to limit the negative effects of infection, especially nowadays when the global market and climate changes are challenging agricultural production.

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REVIEW ARTICLE

Energy utilization of biomass grown on contaminated soils: a review

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Summary
Soil degradation and contamination presents significant global problem that limits conventional agricultural production and requires the development of alternative production systems. At the same time, the growing need for renewable energy sources encourages research into the possibilities of growing non-food biomass on marginal/contaminated soils. The aim of this review is to systematically analyze the conditions for biomass production on different types of contaminated soils and to assess their energy utilization. Special emphasis is placed on the interaction of soil, plant and production system and on the safety and sustainability of energy approaches.

Keywords: contaminated soils, marginal lands, energy crops, biomass, phytoremediation

Introduction

Soil degradation represents one of the most significant limiting factors of modern agricultural production, with more than one third of the world's soils affected by various forms of degradation driven by industrialization, intensive agriculture, mining activities, and urbanization (FAO, 2021). According to the European Commission (n.d.), 60–70% of soils in the EU are unhealthy. Contamination with heavy metals, organic pollutants, and secondary degradation processes such as salinization or soil acidification reduce land productivity and increase pressure on high-quality agricultural areas. Conventional remediation methods are often economically unsustainable and energy-intensive (Kisic et al., 2017). In this context, the functional repurposing of contaminated soils within the circular bioeconomy has emerged as a relevant approach. The use of such soils for biomass production can reduce pressure on productive land and support the development of bioenergy systems (Pidlisnyuk et al., 2021; FAO, 2021). The integration of phytoremediation and energy crop cultivation enables simultaneous soil stabilization and the production of lignocellulosic biomass (Pidlisnyuk et al., 2021), with species such as Miscanthus, willow, and poplar showing high tolerance to abiotic stress (Clifton-Brown et al., 2017; Zgorelec et al., 2020). However, biomass management after phytoremediation remains a critical issue, particularly for non-degradable contaminants such as heavy metals, where harvested biomass may act as a pathway for secondary contaminant transfer. In contrast, organic pollutants are largely degraded through rhizosphere activity and plant metabolism, reducing associated risks. Therefore, the assessment of post-harvest utilization must be contaminant-specific. In this context, phytoextraction systems may lead to the accumulation of persistent contaminants in aboveground biomass, whereas phytostabilization primarily retains them within the soil matrix (Kisic et al., 2017). Accordingly, this paper assumes that the sustainability of biomass production on contaminated soils depends on the effective management of biomass beyond the cultivation phase, particularly in the post-phytoremediation stage.

Hierarchy of terms of land degradation

The terms marginal soils, degraded soils, contaminated soils and post-industrial land are often used interchangeably in the literature, although they represent different levels of land use restrictions. The following conceptual hierarchy is proposed: marginal soils → degraded soils → contaminated soils → post-industrial land. Marginal soils have limited productivity, degraded soils show impaired physical, chemical or biological properties, contaminated soils contain toxic substances that limit agricultural production, while post-industrial land denotes former industrial areas with combined environmental stresses (FAO, 2021; Kisic et al., 2017).

Agronomic classification of contaminated soils

Agronomic classification of contaminated soils is based on production limitations that determine plant growth, nutrient availability and yield stability, and not solely on the chemical nature of the contaminant (Nagajyoti et al., 2010).

Soils contaminated with heavy metals

Heavy metals such as Cd, Pb, Zn and Ni cause plant growth inhibition, reduced photosynthetic activity and disturbances in nutrient metabolism, especially in acidic soils where metal mobility is increased (Nagajyoti et al., 2010). Agronomic acceptability of cultivation depends on soil pH, organic matter content and cation adsorption capacity which regulate metal bioavailability (Kisic et al., 2017).

Perennial energy crops such as Miscanthus, willow and poplar show limited translocation of metals into aboveground biomass, thus reducing the risk of secondary contamination and enabling stable biomass production (Zgorelec et al., 2020).

Organically contaminated soils

Organic contaminants include PAH compounds, pesticides and petroleum derivates that affects primarily the microbiological activity of the soil (Pidlisnyuk et al., 2021). Rhizosphere interactions of energy plants stimulate microorganisms capable of breaking down organic pollutants, which leads to gradual biological stabilization of the soil (Pidlisnyuk et al., 2021). Such systems enable simultaneous production of biomass and phytoremediation, which represents a significant agronomic advantage compared to classical remediation methods (Pidlisnyuk et al., 2021).

Saline and alkaline soils

Soil salinization reduces water potential and causes ionic stress in plants, which limits conventional food production (Pidlisnyuk et al., 2021). Energy grasses and lignocellulosic species show increased tolerance due to the ability to regulate Na^+ and K^+ balance and more efficient use of water (Pidlisnyuk et al., 2021). In agronomic terms, key challenges include irrigation management and maintenance of soil structure to preserve long-term productivity (Lewandowski et al., 2016).

Acidic mining and degraded soils

Mining soils are characterized by high acidity, low fertility and unfavorable soil structure, which significantly limits root development and microbial activity (Kisic et al., 2017). Perennial woody crops show the greatest adaptability due to their deep root systems and low fertilizer requirements (Pidlisnyuk et al., 2020).

Soils with multiple stress factors

In real agroecosystems, contamination is often associated with erosion, soil compaction and nutrient deficiency, which requires extensive production systems with minimal inputs (Zgorelec et al., 2025). Energy crops in such conditions allow the maintenance of vegetation cover and the prevention of further soil degradation (Lewandowski et al., 2016).

Biomass cultivation systems on contaminated soils

The success of biomass production depends on the stability of the production system, not only on the selection of a particular plant species (Clifton-Brown et al., 2017).

Perennial energy crops

Perennial energy crops, particularly *Miscanthus x giganteus*, are among the most extensively studied options for biomass production on contaminated soils due to their high resource-use efficiency, low input requirements, robust rhizome-based persistence, and generally limited translocation of contaminants to aboveground harvestable biomass (Pidlisnyuk et al., 2020). Addition, their long-term cultivation contributes to increased soil organic matter and improved soil stability (Lewandowski et al., 2016), while rapid canopy establishment reduces erosion and minimizes the need for intensive agronomic inputs, making these crops suitable for marginal and contaminated sites (Pidlisnyuk et al., 2021).

Short rotation coppice (SRC)

Willows and poplars enable high biomass production with short rotations and effective stabilization of contaminated soils through their root systems (Pidlisnyuk et al., 2020). Such systems are particularly suitable for metal-contaminated and mining soils.

Extensive systems and spontaneous vegetation

In extremely degraded locations, spontaneous vegetation can represent a functional source of biomass with a very favorable energy input-output ratio (Zgorelec et al., 2025).

The influence of contamination on biomass yield and quality

Soil contamination affects plant physiological processes including photosynthesis, respiration and nitrogen metabolism, which can lead to an initial reduction in yield (Nagajyoti et al., 2010). In the long term, stabilization of yields is achieved by adaptation of plants and improvement of soil structure (Lewandowski et al., 2016).

Changes in the chemical composition of biomass include increased ash content, changes in lignocellulosic ratio and accumulation of mineral elements that affect the energy value of biomass (Pidlisnyuk et al., 2021).

Regulation and legal framework

Management of biomass produced on contaminated soils is associated with regulatory constraints. The Waste Framework Directive (2008/98/EC) defines the classification of waste and by-products, while the Renewable Energy Directive (EU) 2018/2001 determines the sustainability criteria of biomass. The Industrial Emissions Directive (2010/75/EU) regulates emissions from biomass energy conversion plants. The lack of specific regulatory guidelines for phytoremediation biomass represents a key obstacle to the wider implementation of energy systems.

Production management and agrotechnical measures

Adaptation of agrotechnical measures is essential for stable biomass production on contaminated soils (Pidlisnyuk et al., 2020). Maintaining neutral pH by liming reduces metal mobility and improves nutrient availability (Kisic et al., 2017). Minimal tillage reduces the redistribution of contaminants, while selective fertilization prevents their mobilization into the plant (Nagajyoti et al., 2010). Control of biomass harvesting and storage is important to prevent secondary spread of contamination (Pidlisnyuk et al., 2021).

Energy valorization of biomass

Biomass obtained from phytoremediation systems represents a specific category of material whose value depends on type of contaminant and the conversion technology (Pidlisnyuk et al., 2021). Energy conversion pathways for biomass produced on contaminated soils can be broadly

divided into thermochemical processes (combustion, pyrolysis, and gasification) and biochemical processes, primarily anaerobic digestion. Among available utilization pathways, thermochemical conversion is generally considered the most suitable option for contaminated lignocellulosic biomass because it enables efficient energy recovery, significant volume reduction, and controlled management of contaminant-enriched ash fractions (Obernberger et al., 2006). Biochemical processes such as anaerobic digestion require careful management due to the possible inhibition of microorganisms by metals. The increased content of mineral elements can affect ash melting and the operational efficiency of combustion systems (Obernberger et al., 2006). Pyrolysis allows the production of biochar that can immobilize metals and potentially return to the soil as a stabilization material (Pidlisnyuk et al., 2021). Anaerobic digestion is applicable to grass biomass with a lower lignin content (Lewandowski et al., 2016).

A synthetic overview of biomass production systems on contaminated soils

Table 1. Relationship between contaminated soil types, suitable energy crops, agronomic constraints and preferred biomass utilization pathways

Soil type	Main contaminant	Recommended crops	Agronomic constraints	Preferred conversio route
Heavy metal-contaminated soils	Cd, Pb, Zn, Ni	Miscanthus × giganteus, Salix spp., Populus spp.	Phytotoxicity, metal mobility, dependence on pH and organic matter	Combustion, pyrolysis
Organically contaminated soils	PAHs, pesticides, petroleum derivatives	Grasses, Miscanthus × giganteus, Salix spp., Populus spp.	Microbial activity dependence, degradation efficiency, rhizosphere interactions	Anaerobic digestion, combustion
Saline and alkaline soils	Na^+, Cl^-	Energy grasses, Miscanthus spp.	Osmotic stress, ionic imbalance, water availability	Combustion
Mining and acidic soils	Low pH, low fertility, metal presence	Salix spp., Populus spp.	Nutrient deficiency, poor soil structure, limited microbial activity	Combustion, pyrolysis
Multi-stress soils	Combined stresses (contamination, erosion, compaction)	Extensive grasslands, mixed perennial systems	Low productivity, system instability, minimal input conditions	Small-scale decentralized energy use

A synthetic review shows that the choice of production system must be primarily determined by agronomic soil constraints, while energy technology represents a secondary optimization criterion (Lewandowski et al., 2016).

Contaminated Miscanthus biomass as a solid biofuel

Recent studies have shifted focus from biomass production feasibility on contaminated soils towards the usability of the harvested biomass. *Miscanthus x giganteus* is particularly relevant due to its combination of phytoremediation potential and favorable lignocellulosic fuel properties. Biomass cultivated on Cd and Hg contaminated soils has been shown to retain key solid biofuel characteristics, with contamination and soil amendments not significantly affecting its energy-related properties (Bilandžija et al., 2022). These findings indicate that contaminated biomass can be considered a viable bioenergy feedstock, provided that appropriate conversion pathways are applied to ensure controlled management of contaminant-enriched by-products.

Conclusion

The analysis of available literature indicates that biomass production on contaminated soils has clear conceptual potential, but also significant practical limitations. Biomass yield and composition remain highly dependent on local soil conditions, and most systems are still at the experimental or demonstration stage. More importantly, the sustainability of such systems is not determined solely by biomass production, but by the feasibility of its safe and technically appropriate utilization. In this context, contaminated biomass should not be regarded merely as a by-product of phytoremediation, but as a resource whose use requires carefully selected conversion pathways and controlled management of contaminant-related risks. Accordingly, future development should focus on long-term field validation, as well as on establishing clear technical and regulatory frameworks for the safe utilization of biomass produced on contaminated soils.

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ORIGINAL SCIENTIFIC PAPER

Assessing the Economic Sustainability of Organic and Conventional Wheat Production Systems from an Agroecological Perspective

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Summary

The paper analyzes the economic viability of organic and conventional wheat production systems by calculating gross margin indicators under field conditions in Serbia. The research is based on a case study conducted at the experimental field of the Research and Development Institute "Tamiš" in Pančevo during the 2024/2025 agricultural season.

Results show that organic wheat production achieved a significantly higher gross margin of 2,287.17 EUR ha^{-1}, compared to 483.71 EUR ha^{-1} in conventional production. Variable costs accounted for approximately 15% of total income in the organic system, while in the conventional system they represented about 62%. Yield levels were recorded at 6 t ha^{-1} (organic) and 6.5 t ha^{-1} (conventional), indicating lower physical output in the organic system but substantially higher economic efficiency. The findings suggest that higher market prices and lower input dependency enable organic wheat production to achieve superior economic performance despite yield differences.

Keywords: organic production, wheat, gross margin, agroecology, Serbia, farm economics, cost-benefit analysis

Introduction

The definition and application of the principles of sustainable agriculture include economic, ecological and social aspects of production, with the aim of maximizing yields while minimizing negative impacts on soil, biodiversity and ecosystem services (Velten et al., 2015). In modern agriculture, there is a growing interest in the integration of economic profitability and agro-ecological sustainability, which is particularly reflected in grain production systems that combine low input costs, reduction in the use of chemical agents and preservation of soil fertility (Reganold & Wachter, 2016). In this context, agroecology is increasingly recognized as a holistic approach that integrates ecological principles into agricultural production, aiming to enhance resilience, resource-use efficiency, and long-term sustainability of farming systems (Wezel et al., 2009).

The growing demand for environmentally friendly and health-safe food has contributed to the expansion of organic agriculture worldwide, including in Europe. Organic farming systems are based on the principles of ecological balance, biodiversity conservation, and restricted use of synthetic inputs, which distinguishes them significantly from conventional production systems (Seufert & Ramankutty, 2017). Although organic production is often associated with lower yields compared to conventional systems, it frequently achieves higher market prices and may benefit from policy support mechanisms, which together can positively influence its economic performance (Ponisio et al., 2015). Therefore, the assessment of economic sustainability in organic agriculture requires a comprehensive approach that takes into account both yield variability and price premiums.

In Serbia, organic agriculture is supported through specific agricultural policy measures that include significantly higher direct payments per hectare compared to conventional production

systems. According to national regulations, subsidy levels for organic production can be up to 2.5 times higher than those for conventional agriculture, reflecting the policy objective of encouraging environmentally sustainable farming practices. These payments represent an important component of farm income and can substantially influence the economic performance of organic production systems.

Conventional wheat production is characterized by a high yield, but also a higher use of synthetic inputs, which can increase variable costs and environmental pressure (Bavec et al., 2011). Wheat yield is largely determined by the application of appropriate mineral fertilizers and plant protection products, which is also characteristic of most field crops. According to the results of some research, in European countries the level of wheat yield is influenced by several interrelated factors, among which the level of applied production technology, as well as the type and intensity of fertilizer and pesticide treatment stand out (Tudor et al., 2023). However, such input-intensive systems are increasingly being questioned due to their environmental externalities, including soil degradation, water pollution, and greenhouse gas emissions (Foley et al., 2011).

Economic goals, measured by gross margin, are used as key indicators of profitability and enable comparison of the economic efficiency of different crops and production systems (Filipović et al., 2015). In addition to its simplicity and practical applicability, gross margin analysis provides valuable insights into the relationship between input use and financial outcomes at the farm level. Previous studies indicate that while conventional systems may achieve higher physical yields, organic systems can reach comparable or even higher profitability due to lower input costs and price premiums on the market (Seufert & Ramankutty, 2017). This highlights the importance of analyzing not only production outputs but also the structure of costs and revenues when evaluating the sustainability of agricultural systems.

Within this work, the focus is on the comparative analysis of the economic and agroecological sustainability of wheat production in the conventional production system and wheat in the organic production system. The analysis includes an assessment of the gross margin, variable cost structure and yield, to identify the strengths and limitations of each production system and provide insight into the possibilities of sustainable and profitable grain production in modern agriculture.

To achieve the research goal, the paper focuses on the following research questions:

- Does wheat production in the organic production system achieve a competitive gross margin compared to conventional wheat production?
- How does the cost structure differ between the two production systems?
- Does the higher market price of the organic product compensate for the lower yields?

Answers to the above questions will enable a better understanding of the economic and agroecological sustainability of the observed wheat production systems.

Material and Methods

The study is based on a comparative case study design involving two wheat production systems: organic and conventional. Both systems were analyzed under field conditions at the experimental field of the Research and Development Institute “Tamiš” in Pančevo during the 2024/2025 agricultural season. The analysis was conducted on a per-hectare basis to ensure comparability of economic indicators between the two systems with the same wheat variety.

The research is based on the assessment of the economic effects of wheat production in two different systems - organic and conventional production. The data used in the analysis were provided by the Research and Development Institute "Tamiš" from Pančevo and served as the basis for making analytical calculations of wheat production according to the observed systems. Only income, applied agrotechnical measures and costs related to their production were included, and the obtained results cannot be generalized to other agricultural crops or to

different production conditions. The economic analysis was conducted for one production cycle, i.e. the agro-economic year 2024/2025, where all indicators were calculated on the surface of one hectare of arable land and expressed in national currency (RSD). For the sake of easier comparison of the results with international research, all financial indicators expressed in the national currency were converted into euros using the average exchange rate for the observed period (1 EUR = 117.1666 RSD on the reference date July 11, 2025).

Within the analytical calculation, the inclusion of certain cost categories was omitted. The costs of purchasing mechanization were not considered, considering that agrotechnical operations were realized through service use of equipment. Depreciation of available machinery is not included due to its average age and assessment that it does not have a relevant impact on the gross margin. Also, labor costs were not recorded because there were no hiring of seasonal workers in the observed period.

For the purposes of the analysis, data on fuel prices (Eurodiesel) and mechanization services were used, taken from the publication *Price list of machinery services in agriculture 2024*, published by the Cooperative Union of Vojvodina. All stated costs per unit of measure are shown without added value tax (VAT). Data on the purchase price of wheat for the year 2025 were collected from the official website of the Novi Sad Comodity Exchange, in the period from July 7 to 11, 2025. The yields of wheat in the organic production system and wheat produced in the conventional production system reflect the actual situation achieved at the end of the agro-economic year 2024/2025, that is, the final results of the production cycle on the observed land.

Total income includes both market income from wheat sales and direct agricultural subsidies received per hectare. Total income is calculated as the product of the realized yield and the purchase price at a given time. The total variable costs were calculated based on the actual activities in the wheat production process, namely: seeds, fertilizers, pesticides, diesel fuel, paid machinery services and other costs.

Finally, gross margin (GM) was calculated as the difference between total revenue and total variable costs, according to the following formula (equation 1):

$$\mathbf{GM = Total\ income - Total\ variable\ costs} \quad \mathbf{(1)}$$

Gross margin indicates whether the generated revenue is sufficient to cover variable costs and potentially contribute to covering fixed costs and achieving profit.

Finally, the difference between realized income and total variable costs represents the gross margin, which shows whether the realized income can cover variable costs, and possibly fixed costs.

Results and Discussion

This part shows the results of the realized gross margin in the production of wheat in the system of organic and conventional production on an area of 1 ha. The achieved wheat yields in the observed production systems were 6 t ha^{-1} for the organic system, and 6.5 t ha^{-1} for the conventional system. These yield levels form the basis for the calculation of total income presented in the following tables.

Accordingly, in Table 1. the calculation of the gross margin in the production of wheat in the organic production system on 1 ha of arable land is presented.

Table 1. Calculation of gross margin of wheat production in the organic production system per 1 ha of arable land

Category	Value, RSD ha^{-1}	Value, EUR ha^{-1}	Share, in %
I Total income	**315,000**	**2,688.48**	**-**
II Variable costs	**47,020**	**401.31**	**100**
Seeds	14,000	119.49	29.8
Paid services by machinery	13,020	111.12	27.7
Other variable costs - control costs	20,000	170.70	42.5
III Gross margin	**267,980**	**2,287.17**	

Source: Author's calculation based on internal data of „Tamiš“ Research and Development Institute, Pančevo, Serbia.

Data shown in Table 1 indicate that the variable costs of wheat production amount to EUR 401.31 ha^{-1} and participate with approximately 15% in the total income, which amounts to EUR 2,688.48. The achieved gross margin in the amount of EUR 2,287.17 ha^{-1} indicates the significant economic potential of this production, as it provides space for covering fixed costs and achieving a positive financial result (profit).

The structure of variable costs incurred in the production of wheat in the organic production system shows a distinct dominance of other costs - control costs (42.5%) in total production costs. The costs of mechanization services and seeds have a significantly smaller share (27.7% and 29.8%, respectively), while there were no other costs. Such a pronounced structure of costs indicates that the profitability of production largely depends on the cost of control costs, which can represent an important factor in the economic stability of production.

The value of the gross margin shows us a high level of profitability of wheat production in the organic production system, which is the result of a high market price. Subsidies from the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia contributed to such a high income, as they are 250% higher for organic compared to conventional production per 1 ha of cultivated land (Regulation on the distribution of incentives in agriculture and rural development in 2025).

In the Table 2. the calculation of the gross margin in the production of wheat in the system of conventional production and classic tillage on 1 ha of arable land is presented

Table 2. Calculation of the gross margin of conventional wheat production and in the classical tillage system on 1 ha of arable land

Category	Value, RSD ha^{-1}	Value, EUR ha^{-1}	Share, in %
I Total income	**148,000**	**1,263.16**	**-**
II Variable costs	**91,326**	779.45	**100**
Seeds	9,900	84.5	10.8
Fertilizer	26,288	224.36	28.8
Pesticides	6,598	56.32	7.2
Diesel fuel	6,060	51.72	6.6
Paid services by machinery	37,160	317.16	40.7
Other variable costs	5,320	45.41	5.8
III Gross margin	**56,674**	**483.71**	**-**

Source: Author's calculation based on internal data of „Tamiš“ Research and Development Institute, Pančevo, Serbia.

Data shown in Table 2 indicate that the variable costs of wheat production in the system of conventional production and classic tillage amount to EUR 779.45 ha^{-1} and participate with approximately 62% in the total income, which amounts to EUR 1,263.16 ha^{-1}. After covering variable costs, the gross margin was realized in the amount of EUR 483.71 ha^{-1}, which indicates a limited space for covering fixed costs and making a profit in the observed production system.
Analysis of the structure of variable costs shows that the largest share is the cost of machinery services, which make up 40.7% of total variable costs. Fertilizer costs also have a significant share with 28.8%, while the costs of seeds, pesticides and fuel take part with 10.8%, 7.2% and 6.6%, respectively. Other variable costs (machine maintenance and transport costs) account for 5.8% of total costs. This cost structure indicates a high participation of agrotechnical operations that require the use of mechanization, but also the necessary inputs for wheat feeding.

Table 3. Comparative summary of key economic indicators (organic vs conventional wheat production per 1 ha)

Category	Organic system	Conventional system
Total income (EUR ha^{-1})	2,688.48	1,263.16
Variable costs (EUR ha^{-1})	401.31	779.45
Gross margin (EUR ha^{-1})	2,287.17	483.71

Source: Author's calculation based on internal data of „Tamiš“ Research and Development Institute, Pančevo, Serbia.

It should be noted that a significant portion of the profitability of organic wheat production is influenced by higher subsidy levels for organic production, which partially explains the observed differences between the two systems.
In addition to production costs and yields, subsidies and market price fluctuations represent key determinants of the economic performance of wheat production systems. In the case of organic production, higher direct payments per hectare significantly enhance profitability and partially offset lower physical productivity. At the same time, wheat prices are subject to market volatility, which can influence the stability of farm income in both production systems. Therefore, economic results should be interpreted as a combined effect of production efficiency, market conditions, and agricultural policy support rather than isolated production indicators.
These findings are in line with recent empirical studies highlighting the importance of price premiums and policy support in determining the profitability of organic farming systems. For example, Reganold and Wachter (2016) emphasize that the economic viability of organic agriculture depends heavily on institutional and market support mechanisms, while Seufert and Ramankutty (2017) demonstrate that organic systems can achieve competitive profitability despite yield gaps when price and policy conditions are favorable. Similar conclusions are reported by Ponisio et al. (2015), who underline the role of market incentives in closing the economic gap between organic and conventional production systems.
These findings are consistent with recent literature emphasizing that the economic performance of organic agriculture is strongly influenced by policy support and market price premiums. Recent studies highlight that while organic systems often exhibit yield gaps compared to conventional agriculture, they can remain economically competitive under favorable policy conditions and market incentives (Acs et al., 2018; Müller et al., 2020). Furthermore, evidence from European agricultural systems confirms that direct payments and subsidy schemes play a critical role in maintaining the profitability of organic farms (European Commission, 2021; FiBL & IFOAM, 2023). These results support the view that the economic viability of organic wheat production is context-dependent and shaped by both market dynamics and agricultural policy frameworks.

The value of the gross margin shows us the low level of profitability of wheat production, and this result may indicate the necessary optimization of certain inputs in those segments where there are opportunities for greater savings or efficiency.

Conclusion

A comparative analysis of conventional and organic wheat production showed significant differences in the economic and agroecological characteristics of the observed production systems. Wheat production in the organic production system achieved a higher gross margin per hectare, which indicates a higher potential for covering fixed costs and making a profit. The results show that organic wheat production achieved a gross margin of 2,287.17 EUR/ha, compared to 483.71 EUR/ha in conventional production. At the same time, variable costs were lower in the organic system (401.31 EUR/ha) than in the conventional system (779.45 EUR/ha), despite differences in yield and production structure. The obtained results confirm that a higher market price of an organic product can compensate for lower yields, thus organic production can represent an economically viable alternative to conventional production systems. The findings provide clear answers to the research questions formulated in the Introduction. Also, the results indicate that subsidies play a decisive role in the economic performance of organic wheat production and represent a key factor in its competitiveness compared to conventional systems.

From an agroecological aspect, the production of organic wheat is characterized by reduced use of synthetic inputs and greater tolerance of old varieties to diseases and pests, which contributes to the preservation of the agroecosystem and the sustainability of production. In contrast, conventional wheat production is more dependent on mechanization and chemical inputs, which can increase production yields, but also pressure on the environment.

These findings suggest that, under current market conditions and subsidy schemes, organic wheat production can be economically more attractive for farmers, while agricultural policy support remains a key factor in enhancing the competitiveness of organic production systems.

Bearing in mind that the research was conducted on one sample field and during one production year, the results represent indicative indicators of economic and agroecological sustainability of production systems. Longer-term research in different agroecological conditions is necessary to make more widely applicable conclusions.

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ORIGINAL SCIENTIFIC PAPER

Sowing date and variety effect on winter wheat traits in Eastern Croatia

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Summary
The main objective of this study was to evaluate the effects of different sowing times and varieties on wheat agronomic traits. A field experiment was set up during the 2018/2019 season in eastern Croatia using three wheat varieties (Maja, Viktorija and Sofru) and three sowing dates (optimal, late and very late) in four replicates. A total of ten wheat traits were analyzed, including yield, yield components, harvest index (HI), hectoliter weight, and morphological traits. The variety x sowing date interaction had a significant effect on nine out of ten traits, following the variety effect on five and sowing date on four wheat traits. The average values were: 8.13 t ha^{-1} of grain yield, 750 spike density, 30.9 grains per spike, 42.2 g1000 grain weight, 71.7 kg hl^{-1} hectoliter weight, 31.5 harvest index, plant height of 78.4 cm, stem weight of 32.5 g, ear length of 7.17 cm and ear weight of 45.0 g. In conducted research with applied agricultural techniques and weather conditions of the 2018/2019 season, the best results were achieved in optimal sowing time, with Maja and Sofru varieties.

Keywords: wheat varieties, sowing date, yield, agronomic parameters, weather characteristics

Introduction

In today's world agricultural production, wheat plays an irreplaceable role. It is a staple food for a large part of the worldwide population and is the most important source of carbohydrates in temperate countries (Singh Meena et al., 2025). Wheat is an important raw material for many industries and generally has a strategic importance for every country. Wheat is an eurytropic plant and has many species and cultivars, which are divided into winter and spring types, and it adapts well to different climatic and soil conditions. However, despite these advantages, wheat production is often affected by adverse weather conditions (Harkness et al., 2020). It is well known that the climate is changing, which has led to increasingly frequent extreme fluctuations in precipitation, air temperature, heat waves or late spring frosts (Dixit et al., 2025). Under such conditions, it is necessary to provide the best possible growing conditions by applying appropriate agricultural practices and/or selecting suitable varieties in order to achieve the best possible results. One of the important agronomic measures is the selection of the right wheat variety. The selection should be made according to the climatic conditions prevailing in a particular region. Although the choice of varieties is indeed wide, it is desirable to sow several different varieties because this contributes to greater yield stability. Another important agronomic measure is sowing time, which can regulate the duration of the growing season and/or reduce unfavorable growing conditions.

Although the optimal sowing period in Croatia is well established, sometimes late or very late sowing is equal to or better than the optimal sowing date (Španić et al., 2023). So, the aim of this study was to determine the yield, agronomic and some morphological traits of three winter wheat varieties sown at three different sowing dates during the 2018/2019 growing season.

Material and Methods

The field trial was set up during the 2018/2019 growing season using three winter wheat varieties (Maja and Viktorija originating from Agrigenetics and Sofru originating from RWA company) in three sowing dates. The first was October 15, 2018 (optimal), the second October 30, 2018 (late) and the third November 15, 2018 (very late). The trial was conducted on the Vidaković Marina family farm in Strošinci in Vukovar-Srijem County (44°54'49.76" N 19°4'30.68" E). The experiment consisted of 9 treatment combinations (3 varieties × 3 sowing dates) arranged in three replications, resulting in a total of 27 plots. The size of each plot was 654 m², giving a total experimental area of approximately 1.76 ha. The preceding crop was sunflower, after which only discing was carried out in three passes and one pass with a seedbed conditioner. Sowing was carried out with an Amazone D8 (Amazone, Germany) precision wheat seeder. Before sowing, 100 kg ha^{-1} of urea and 480 kg ha^{-1} of NPK 7:20:30 were applied. During the growing season, four top dressings were carried out: the first on February 20, 2019, with 150 kg ha^{-1} of KAN, the second on March 18 with 120 kg ha^{-1} of KAN, the third on April 4, 2019, foliarly with the liquid fertilizer Fertilader elite and the fourth on May 4 with KAN in the amount of 90 kg ha^{-1}. Weed control was performed on March 23, 2019, with Sekator in the amount of 150 mL ha^{-1} and disease control with Prosaro in the amount of 1 L ha^{-1}.

A total of ten parameters were analyzed, i.e. yield, yield components (number of spikes per m^2, number of grains per spike and 1000 grain weight), agronomic traits (hectoliter weight in kg hl^{-1} and harvest index), and morphological characteristics (plant height in cm, ear length in cm, ear weight in g and stem weight in g). Grain yield was determined by harvesting each basic plot, and the 1000-grain weight and hectoliter weight were measured from the harvested samples. Spike density was determined by counting spikes from 1 m^2, and other measurements were based on a sample of 30 plants per basic plot.

Meteorological data were obtained from the State Hydrometeorological Institute of the Republic of Croatia from the meteorological station Gradište near Županja. Data included mean monthly air temperatures (°C) and total monthly precipitation (mm) during the wheat growing season 2018/2019, as well as multi-year data for the period from 1961 to 1990, for the purpose of comparing the examined year with the multi-year average. The results obtained in this experiment were processed using Microsoft Excel and SAS Software 9.1.4. (SAS Institute Inc., 2003). Statistical analysis of data was carried out by individual analysis of variance (ANOVA) using the F test and $LSD_{0.05}$.

Results and Discussion

Weather conditions have a significant, often negative, impact on wheat properties. In the conducted research, the total amount of precipitation and average air temperature during the vegetation period was 3.2% and 14.9% higher than the multi-year average (MYA), respectively (Table 1). In October, when wheat sowing began, as well as in November and December, there was less precipitation, with deviations of 59.3%, 30.5% and 20.0% less, while the same trend was recorded during February (33.3% less) and March (52.3% less) in comparison with MYA. In contrast, in April, May and June, when wheat has an intensive need for water, a large excess of precipitation was recorded compared to MYA, with a deviation of 83.0%, 71.2% and 16.0%. However, unlike precipitation, air temperature throughout the growing season was above MYA, except in May.

Table 1. Monthly precipitation (mm) and average air temperature (°C) during 2018/2019 and multi-year average values (MYA) from 1971-1990 for the Gradiste meteorological station

Year/Month	X	XI	XII	I	II	III	IV	V	VI	VII*	
					Precipitation (mm)						Total
2018/2019	24	41	40	43	24	20	97	113	94	81	577
MYA	59	59	50	41	36	42	53	66	81	72	559
					Air temperature (°C)						Average
2018/2019	14.5	7.8	2.2	0.7	5.0	10.2	13.0	14.4	23.4	22.5	11.4
MYA	11.3	5.6	1.7	0.3	2.4	6.9	11.5	16.7	19.6	21.2	9.7

* data from July 1-25, 2019

Analysis of variance showed significant effects of variety for five traits, while sowing date was significant for four. The biggest effect was their interactions. The average yield was 8.13 t ha^{-1} and was significantly influenced by sowing date and the interaction with variety, while there was no statistical significance between varieties. Based on the above-mentioned applied agricultural practice, fertilization and weather conditions in 2018/2019, the highest average yield was determined in the first sowing date, and the lowest at the third sowing date, with a variation of 26% (Table 2). As stated by Berski et al. (2025), a delayed sowing date affected spike biometry, i.e. the length of the spike rachis, the number of spikelets per spike, and spikelet density. Furthermore, the highest grain yield was achieved by the Maja and Sofru varieties, both at the first sowing date (October 15) with 11.5 t ha^{-1} and 10.3 t ha^{-1}, while the lowest yield was recorded for the variety Viktorija, also at the first sowing date, with 6.76 t ha^{-1}. The main reasons for high yield can be related to high spike density (for the Maja variety) or grain number per spike (for the Sofru variety).

Table 2. Significance of the tested parameters for agronomic properties

Sowing date (B)	Variety (A)			Average (B)	Variety (A)			Average (B)
	Maja	Viktorija	Sofru		Maja	Viktorija	Sofru	
	Yield (t ha^{-1})				Spike density			
Optimal	11.5 ^{a}	6.76 ^{c}	10.3 ^{ab}	9.52 ^{A}	998 ^{a}	653 ^{b}	670 ^{b}	773
Late	7.46 ^{c}	7.41 ^{c}	8.65 ^{bc}	7.84 ^{AB}	711 ^{b}	790 ^{ab}	775 ^{ab}	759
Very late	7.08 ^{c}	7.10 ^{c}	6.92 ^{c}	7.04 ^{B}	803 ^{ab}	778 ^{ab}	573 ^{b}	717
Average (A)	8.67	7.09	8.63	8.13	838 ^{A}	740 ^{AB}	673 ^{B}	750
	$LSD_{0.05}$ (A) = ns; $LSD_{0.05}$ (B) = 1.82; $LSD_{0.05}$ (AB) = 2.71				$LSD_{0.05}$ (A)=153; $LSD_{0.05}$ (B)=ns; $LSD_{0.05}$ (AB)= 246			
	Grain number per spike				1000-grain weight (g)			
Optimal	33.7 ^{ab}	27.1 ^{c}	36.8 ^{a}	32.5 ^{A}	43.5 ^{ab}	40.2 ^{c}	41.0 ^{bc}	41.6
Late	30.3 ^{bc}	29.9 ^{bc}	34.4 ^{a}	31.6 ^{AB}	43.6 ^{ab}	41.9 ^{bc}	41.7 ^{bc}	42.4
Very late	29.1 ^{c}	29.6 ^{c}	27.7 ^{c}	28.8 ^{B}	39.3 ^{c}	43.6 ^{ab}	45.2 ^{a}	42.7
Average (A)	31.0 ^{AB}	28.9 ^{B}	32.9 ^{A}	30.9	42.1	41.9	42.6	42.2
	$LSD_{0.05}$ (A) = 3.26; $LSD_{0.05}$ (B) = 3.31; $LSD_{0.05}$ (AB) = 3.79				$LSD_{0.05}$ (A)=ns; $LSD_{0.05}$ (B)= ns; $LSD_{0.05}$ (AB)= 3.02			
	Hectoliter weight (kg ha^{-1})				Harvest index (HI)			
Optimal	71.9 ^{abc}	72.8 ^{abc}	69.9 ^{bcd}	71.5	31.0 ^{b}	28.6 ^{b}	43.2 ^{a}	34.3
Late	73.6 ^{ab}	73.7 ^{ab}	67.6 ^{d}	71.6	30.8 ^{b}	29.1 ^{b}	31.7 ^{b}	30.6
Very late	72.1 ^{abc}	74.7 ^{a}	68.9 ^{cd}	71.9	25.7 ^{b}	30.5 ^{b}	32.9 ^{ab}	29.7
Average (A)	72.5	73.7	68.8	71.7	29.2 ^{B}	29.4 ^{B}	35.9 ^{A}	31.5
	$LSD_{0.05}$ (A) = ns; $LSD_{0.05}$ (B) = ns; $LSD_{0.05}$ (AB) = 3.94				$LSD_{0.05}$(A)=6.25; $LSD_{0.05}$(B)=ns; $LSD_{0.05}$ (AB)=10.6			

All values in the table section marked with different superscripts are statistically different (α = 0.05)
Capital letters indicate statistical significance between average Variety (A) and Sowing date (B)

The positive effect of yield components on yield has been reported by other authors emphasizing that these traits are influenced by both genetic and environmental factors (Li et al., 2016; Chu et al., 2023). Generally, delayed sowing time in this research resulted in lower grain yield, spike density, number of grains per spike and lower HI, probably due to a shortened growing season and faster passage through phenological stages. Only the 1000-grain weight was increased under delayed sowing time.

The number of grains per spike was significantly affected by variety and sowing date, as well as their interaction, while the 1000-grain weight influenced only the interaction of variety × sowing date (Table 2). The highest number of grains per spike was recorded in the Sofru variety at the first sowing date (36.8 grains), and the highest 1000-grain weight was achieved by the same variety at the third sowing date (45.2 g). Using the Sofru variety as an example, it can be underlined that one yield component complements the other, because a lower number of grains per spike was connected with a higher 1000-grain weight. Among the other examined parameters, the hectoliter weight should also be highlighted, which was extremely low in this study. Generally, the average hectoliter weight was 71.7 kg hl^{-1}, which could be a consequence of stormy weather with abundant precipitation occurring just before sampling and harvesting. The harvest index (HI) averaged 31.5, and it was significantly influenced by variety and the interaction, while the effect of sowing date was not statistically significant.

Morphological traits do not have a direct influence on grain yield but can have a positive effect (Ashfaq et al., 2003). For example, too tall plants have an increased risk of lodging, which can consequently reduce yield, while spike length is positively correlated with the number of grains per spike.

In the present research, sowing date had a significant effect only on plant height and stem weight, while variety significantly influenced ear length and ear weight (Table 3). However, the optimal sowing date resulted in higher values for all four morphological parameters, with increases of 2.4% in plant height, 2.4% in ear length, 6.6% in ear weight and 13.4% in stem weight. In the context of variety, better results were shown by Maja and Sofru since they achieved longer and heavier spikes, the most important yield related traits, as well as shorter stems.

Table 3. Significance of the tested parameters for morphological properties

Sowing date (B)	**Variety (A)**			**Average (B)**	**Variety (A)**			**Average (B)**
	Maja	Viktorija	Sofru		Maja	Viktorija	Sofru	
	Plant height (cm)				**Ear length (cm)**			
Optimal	78.9	82.1	77.4	79.5 ^{A}	7.42 ^{ab}	6.68 ^{d}	7.59 ^{ab}	7.23
Late	78.6	77.6	78.4	78.2 ^{AB}	7.20 ^{bc}	6.90 ^{cd}	7.58 ^{ab}	7.23
Very late	77.3	77.7	77.9	77.6 ^{B}	7.76 ^{a}	6.89 ^{cd}	6.54 ^{d}	7.06
Average (A)	78.3	79.1	77.9	78.4	7.46 ^{A}	6.82 ^{B}	7.24 ^{A}	7.17
$LSD_{0.05}$ (A) = ns; $LSD_{0.05}$ (B) = 1.80; $LSD_{0.05}$ (AB) = ns					$LSD_{0.05}$ (A)=0.41; $LSD_{0.05}$ (B)=ns; $LSD_{0.05}$ (AB)=0.48			
	Ear weight (g)				**Stem weight (g)**			
Optimal	48.5 ^{ab}	39.7 ^{c}	53.3 ^{a}	47.1	35.4 ^{ab}	31.8 ^{c}	37.0 ^{a}	34.7 ^{A}
Late	48.3 ^{ab}	39.1 ^{c}	43.8 ^{bc}	43.7	35.7 ^{a}	31.9 ^{bc}	28.3 ^{d}	32.0 ^{AB}
Very late	41.5 ^{c}	42.5 ^{bc}	48.6 ^{ab}	44.2	31.7 ^{cd}	31.3 ^{cd}	28.9 ^{cd}	30.6 ^{B}
Average (A)	46.1 ^{A}	40.4 ^{B}	48.6 ^{A}	45.03	34.3	31.7	31.4	32.5
$LSD_{0.05}$ (A) = 4.36; $LSD_{0.05}$ (B) = ns; $LSD_{0.05}$ (AB) = 6.18					$LSD_{0.05}$(A)=ns; $LSD_{0.05}$ (B)=2.91; $LSD_{0.05}$ (AB)=3.49			

All values in the table section marked with different superscripts are statistically different (α = 0.05)
Capital letters indicate statistical significance between average Variety (A) and Sowing date (B)

Conclusion

Based on the conducted research, some features can be underlined. According to the analysis of variance, the variety × sowing date interaction had a significant effect on nine out of ten observed traits, followed by the variety effect on five traits and sowing date on four traits. However, grain yield, as the main trait, was significantly influenced by sowing date, while the variety effect alone was not statistically significant. In the 2018/2019 growing season, the best variety for producers in the eastern part of Croatia, according to most of the evaluated traits, was the Maja and Sofru varieties sown in the optimal time. The Viktorija variety achieved some better results in the second and third sowing periods, which is a recommendation for those producers who are late with sowing beyond the optimal agrotechnical terms. The obtained results indicate the possibility of increasing yields through the appropriate selection of variety combined with optimal sowing dates. In the conditions of a delayed sowing, there is a greater risk of reducing the yield and other relevant parameters of wheat.

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REVIEW ARTICLE

From Degradation to Regeneration: Agronomic Practices Supporting Soil Health and Biodiversity Recovery

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Summary
This paper examines regenerative and conservation agriculture as approaches to mitigate soil degradation, biodiversity loss, and climate change. Both systems rely on minimal soil disturbance, permanent soil cover, and crop diversification, improving soil organic carbon, structure, and biological activity. These practices enhance biodiversity and ecosystem services while supporting climate mitigation through carbon sequestration and increased resilience. Key limitations include the lack of standardized indicators and insufficient long-term field studies. Future research should focus on integrative, region-specific approaches to better quantify impacts and support wider implementation.

Keywords: regenerative agriculture, conservation agriculture, carbon sequestration, climate change, ecosystem services

Introduction

Soil degradation and biodiversity loss are among the most critical challenges confronting modern agricultural systems, threatening ecosystem stability, climate resilience, and long-term food security (Jug et al., 2022). Intensive agricultural practices, such as frequent tillage, simplified crop rotations, excessive use of mineral fertilizers, and insufficient organic matter inputs, have led to declines in soil organic carbon (SOC), deterioration of soil structure, increased erosion, and disruption of soil biological communities (Montgomery, 2007; Lal, 2015). These processes are particularly evident in European agroecosystems where long-term conventional management has increased the vulnerability of structurally sensitive soils and contributed to widespread soil degradation (Orgiazzi et al., 2017). At the same time, agricultural intensification has accelerated biodiversity loss, weakening key ecosystem services such as nutrient cycling, pollination, and biological pest control (Bardgett and van der Putten, 2014; IPBES, 2019). In response, regenerative agriculture (RA) and conservation agriculture (CA) have emerged as complementary, systems-based approaches aimed at restoring soil health, enhancing biodiversity, and improving ecosystem services. CA is founded on minimal soil disturbance, permanent soil cover, and crop diversification, while RA builds upon these principles by integrating additional practices such as organic amendments and, in some cases, livestock inclusion (FAO, 2021). Together, these practices improve soil structure, increase SOC sequestration, enhance water retention, and stimulate biological activity (Paustian et al., 2016; Lal, 2020). Recent studies confirm that RA and CA practices can significantly improve soil health and promote biodiversity recovery (Colombi et al., 2025; Hawes et al., 2025), while also contributing to climate change mitigation and resilience (Bossio et al., 2020; Berthon et al., 2025). However, their effectiveness remains highly context-dependent, varying with soil type, climate, and management history (Lemke et al., 2024; Kumar et al., 2025).
Therefore, there is a need for integrative and region-specific research linking agronomic practices with measurable changes in soil health and biodiversity. The objective of this review

is to evaluate the agronomic principles of RA, in relation to CA, and their role in reversing soil degradation and biodiversity loss.

Agronomic Principles of Regenerative Agriculture in relation to Conservation Agriculture

RA is based on interrelated agronomic principles aimed at restoring soil functionality, enhancing ecosystem resilience, and ensuring long-term agricultural sustainability. CA represents a key foundation of this approach, as both systems share core principles: minimal soil disturbance, permanent soil cover, and crop diversification (Hobbs et al., 2008; FAO, 2021). While CA focuses on these three pillars, RA expands them by incorporating livestock, organic amendments, and broader ecosystem restoration (Palm et al., 2014). Minimal soil disturbance, achieved through conservation tillage, is fundamental to both systems. Conventional plowing disrupts soil structure and accelerates organic matter mineralization (Lal, 2015), whereas conservation tillage improves aggregate stability and promotes carbon retention (Paustian et al., 2016). However, its benefits are maximized when combined with soil cover and crop diversification (Pittelkow et al., 2015; Nunes et al., 2018). Permanent soil cover, maintained through residues or cover crops, protects soil from erosion, regulates temperature, and reduces water loss, while increasing soil organic carbon inputs (Lal, 2020). Cover crops also enhance microbial activity, support biodiversity, and suppress weeds (Kumar et al., 2025). Crop diversification further strengthens system resilience by improving nutrient cycling, reducing pest pressure, and promoting biodiversity (Bardgett and van der Putten, 2014; Palm et al., 2014). RA extends these benefits through livestock integration, which enhances nutrient cycling and carbon sequestration (Teague et al., 2016), and through organic amendments that improve soil fertility and structure. Overall, CA provides the structural basis for RA, while regenerative practices broaden its ecological and functional scope. Their relationship is complementary, combining agronomic stability with enhanced ecosystem restoration and climate resilience.

Regenerative and Conservation Agriculture and Soil Health

Soil health is a multidimensional concept encompassing physical, chemical, and biological properties, all of which are strongly influenced by regenerative and conservation agricultural practices. One of the most significant effects of these practices is the increase in SOC, which represents a key indicator of soil quality and functionality. Higher SOC levels improve nutrient cycling efficiency by supporting microbial-mediated processes and increasing nutrient availability, thereby reducing the dependence on external inputs. In addition to chemical improvements, regenerative and conservation practices positively affect soil physical properties. Enhanced soil structure, increased porosity, and improved aggregate stability contribute to better water infiltration and retention. These changes reduce the susceptibility of soil to erosion and improve its capacity to store plant-available water, which is especially important under conditions of increasing climate variability and drought stress (Lal, 2020). Improved physical conditions also facilitate root growth and enhance overall plant performance. Soil biological activity represents another critical dimension of soil health influenced by regenerative and CA. Soil organisms, including microorganisms and macrofauna, play a central role in decomposition processes, nutrient mineralization, and the formation of stable soil aggregates. Regenerative systems promote higher microbial biomass, increased enzymatic activity, and greater diversity of soil fauna, all of which contribute to improved soil functionality and resilience (Bardgett and van der Putten, 2014; Hawes et al., 2025). Collectively, these physical, chemical, and biological improvements demonstrate the integrative impact of regenerative and conservation practices on soil health and their potential to support sustainable agricultural systems.

Regenerative and Conservation Agriculture and Biodiversity Recovery

Biodiversity is a key indicator of agroecosystem sustainability, reflecting the capacity of agricultural systems to maintain ecological balance and provide essential ecosystem services. RA enhances biodiversity through increased habitat heterogeneity and reduced disturbance, and is closely linked to CA, which is based on minimal soil disturbance, permanent soil cover, and crop diversification (Hobbs et al., 2008; FAO, 2021). Together, these approaches create favorable conditions for diverse biological communities. In the soil environment, conservation tillage and increased organic inputs, such as crop residues and manure, support a wide range of organisms, including bacteria, fungi, and soil fauna. These organisms play a central role in nutrient cycling, organic matter decomposition, and soil aggregation, thereby improving soil functionality and resilience (Palm et al., 2014). Increased soil biodiversity also enhances ecosystem stability through functional redundancy. Aboveground biodiversity is similarly influenced by these practices. Crop diversification, cover crops, and reduced chemical inputs increase habitat availability for pollinators and beneficial organisms. Long-term studies confirm higher species richness and functional diversity under regenerative systems (Hawes et al., 2025), while diverse plant communities strengthen above–belowground interactions. Enhanced biodiversity directly contributes to improved ecosystem services, including nutrient cycling, pollination, and natural pest control. These processes reduce dependency on external inputs and increase system resilience to environmental stress (IPBES, 2019; Colombi et al., 2025). Consequently, RA and CA are increasingly recognized as nature-based solutions for climate change mitigation and adaptation.

Regenerative and Conservation Agriculture and Climate Change Mitigation

RA is increasingly recognized as a nature-based solution for climate change mitigation and adaptation due to its capacity to enhance soil carbon storage, improve system resilience, and reduce greenhouse gas emissions. It is closely linked with CA, which is based on minimal soil disturbance, permanent soil cover, and crop diversification - principles that support carbon sequestration and climate resilience (Hobbs et al., 2008). Soils represent one of the largest terrestrial carbon sinks, and practices such as reduced tillage, cover cropping, and the application of organic amendments can significantly increase SOC. The accumulation of SOC improves soil fertility and structure while contributing to the reduction of atmospheric CO_2 concentrations (Bossio et al., 2020). In addition, improved soil structure, increased porosity, and higher water-holding capacity enhance the resilience of agroecosystems to climate variability and extreme weather events, such as drought and heavy rainfall (Kumar et al., 2025). Core elements of CA, particularly reduced soil disturbance and continuous soil cover, play a crucial role in stabilizing soil carbon and minimizing losses (Pittelkow et al., 2015). Regenerative systems further contribute to climate mitigation by reducing dependence on synthetic inputs and improving nutrient-use efficiency. Enhanced biological activity supports more efficient nitrogen cycling and can reduce nitrous oxide emissions, although outcomes remain highly site-specific (Berthon et al., 2025). Overall, CA provides the agronomic foundation for regenerative systems, while RA expands its role by integrating additional practices aimed at maximizing climate mitigation and adaptation benefits.

Limitations and Challenges of Regenerative and Conservation Agriculture

Despite their considerable potential, RA and CA face several limitations and challenges that may affect their adoption and long-term effectiveness. One of the primary constraints is strong context dependency, as the outcomes of these practices vary significantly depending on soil type, climatic conditions, and previous management history. This variability makes it difficult to generalize results and requires site-specific adaptation to achieve optimal benefits (Lemke et al., 2024). Similar challenges have been reported in CA, where the effectiveness of practices

such as no-till and crop diversification depends on local agroecological conditions (Hobbs et al., 2008). Another important limitation is the transition period from conventional to regenerative or conservation systems. During this phase, farmers may experience temporary yield reductions and increased economic uncertainty, particularly in the absence of technical support or financial incentives. The time required for improvements in soil properties and biological communities may delay the realization of full system benefits. A further challenge is the lack of standardization in defining and assessing RA. While CA is clearly defined through its three core principles (FAO, 2021), RA lacks a universally accepted framework and consistent indicators for evaluating its impacts. This complicates comparisons across studies and hinders the development of policies and certification systems. Finally, significant knowledge gaps remain, particularly regarding long-term and region-specific effects of both regenerative and conservation systems. Limited availability of long-term field experiments and integrative studies restricts the understanding of system performance under diverse conditions. Addressing these gaps is essential for developing robust, science-based recommendations and supporting the wider adoption of sustainable agricultural practices.

Conclusions

RA, closely linked to CA, represents an effective approach for addressing soil degradation, biodiversity loss, and climate change. By applying key principles such as reduced soil disturbance, permanent soil cover, and crop diversification, these systems improve soil health, enhance biodiversity, and strengthen ecosystem services. However, their effectiveness is highly context-dependent, and further long-term, region-specific research and standardized assessment methods are needed to support wider adoption and implementation.

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PRELIMINARY COMMUNICATION

Development of smart biofertilizers to enhance bioactive compounds of *Capparis spinosa* L.

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Summary
The first stage of the research aimed to study the rooting properties of Capparis spinosa L. growing in Uzbekistan in vitro. C.spinosa L. is a medicinal plant, and since the stem contains lignin, the rooting process is difficult and takes a long time. Therefore, we studied the effect of BERES organic fertilizer, which is rich in IAA, IBA, humic and fulvic acids, as growth factors in vitro. According to the results obtained, the effect of organic fertilizer on the rooting indicators of C. spinosa L. was close to the indicators of IAA stimulants. The data prove that the use of organic fertilizers in in vitro cultivation of plants is of great importance in creating environmentally friendly cultures.

Keywords: *Capparis spinosa*, micropropagation, humic fertilizers, smart biofertilizer, medicinal plants

Introduction

Capparis spinosa L. is a perennial shrub with a woody stem, a major medicinal plant, widely distributed in arid and semi-arid regions. This plant is rich in biologically active substances with pronounced antioxidant, anti-inflammatory, and other medicinal properties [Isagaliev M. et al., 2022; Khalilova S.A. et al., 2024; Khojimatov O.K. et al., 2015]. The arid climate and saline soils of Uzbekistan create favorable conditions for the growth of C. spinosa L. under stressful conditions. However, with traditional propagation methods, the seeds of this species are characterized by low germination and rooting capacity [Koufan M., 2022; Mahmodi N., 2022]. In vitro micropropagation enables the rapid production of highly productive genotypes. However, synthetic auxins are often required to ensure high plant productivity, resulting in low plant survival [Parvin S. et al., 2023]. In recent years, increasing research has focused on the minimal yet effective use of fertilizers in agriculture, making the development of targeted fertilizers a pressing issue. The use of the innovative humic fertilizer BERES Universal stimulates plant morphogenesis, enhances root formation, and improves soil microflora and plant adaptation to stressful conditions. In the second stage, "smart" fertilizers based on chitosan biopolymer and consortia of beneficial local soil microorganisms are developed, enhancing the biological value of medicinal plants and improving the composition of the soil microbiota. These technologies are particularly relevant in Uzbekistan, and their use in drought- and salinized soils is essential for increasing plant productivity. Our research focuses on the development of "smart" fertilizers for optimizing the arid and saline soils of Uzbekistan. The first stage involved studying the effect of organic fertilizers rich in humic and fulvic acids on optimizing the rooting of Capparis spinosa L. regenerates in vitro.
The aim of this study was to optimize the micropropagation of C. spinosa L. in vitro using BERES Universal, as well as to develop "smart" biofertilizers aimed at increasing the content of biologically active substances.

Materials and Methods

Young vegetative shoots of Capparis spinosa L. were collected in the mountainous areas of the Urgut district of the Samarkand region. The plant material was divided into 10-cm-long segments in the laboratory of the Department of Biotechnology at Sharof Rashidov Samarkand State University. The explants were then surface sterilized in a 0.5% sodium hypochlorite (NaClO) solution for 10 minutes and rinsed several times with sterile distilled water. After sterilization, the explants underwent a three-stage process of subculturing and propagation. Explants that exhibited high proliferative activity during the propagation stage were transferred to the next stage.

Murashige and Skoog medium supplemented with 3% sucrose and 0.7% agar was used as the basal culture medium. After autoclaving the nutrient medium, the BERES biostimulant (produced by BERES Research and Production Company, RF) was sterilized through a membrane filter (0.22 μm) and added to the medium under aseptic conditions. Experiments were conducted using the following conditions: Murashige and Skoog medium (MS)+indole-3-acetic acid (IAA, 1.0 *mg/L*), Murashige and Skoog medium (MS)+indole-3-butyric acid (IBA, 1.0 *mg/L*), Murashige and Skoog medium (MS)+BERES. BERES solutions were prepared at a ratio of 500 *ml*/1.25 g and proportionally added to 2 liters of nutrient medium. Twenty explants were used for each condition, and the experiment was repeated three times independently (n=60 for each condition). The crops were grown at a temperature of 25±1°C, a photoperiod of 16 hours light/8 hours dark, a light intensity of 40 $\mu mol\ m^{-2}\ s^{-1}$, and a relative humidity of 50-60%. During growth, germination, shoot formation, and rooting were regularly recorded on days 15, 50, 65, and 75. Rooted plants were treated with a 0.1% Bavistin® solution, transplanted into a sterile mixture of peat, compost, and perlite (1:1:1), and adapted to greenhouse conditions in one or two stages.

Field experiments and adaptation work were conducted on a dedicated plot (100 *cm* row spacing, 60 *cm* row length) allocated in the Dargom State Forestry in the Samarkand Region. During the growing season, plants were watered with a BERES solution (500 *ml*/1.25 g), and their viability, growth, and stress resistance were assessed. All experiments were performed in triplicate. Data are presented as mean±standard error. Statistical analysis was performed using one-way analysis of variance (ANOVA), and Tukey's test ($p<0.05$) was used to identify significant differences between treatments.

Results and Discussion

When IAA, IBA, and BERES organic fertilizer were added to the rooting medium of C. spinosa in vitro at a concentration of 1 *mg/L*, the rooting rate (%), root length (*mm*), and shoot height of the plants were higher in the IAA-treated group (Table 1).

Table 1. Rooting parameters of *Capparis spinosa* regenerants after 65 days

Medium	Concentration (mg/L)	Rooting (%)	Root length (mm)	Shoot height (mm)
MS + IAA	1.0	69±1.5 ***	21±0.2 ***	25±1.4 ***
MS + IBA	1.0	60±1.2 **	17±0.3 **	23±1.1 **
MS + BERES	1.0	64±1.1 ***	18±0.2 ***	22±1.3 ***
MS + BERES	0.5	61±1.1 **	17±1.2 **	21±1.3 **
Hormone-free	-	47±1.0	12±1.0	18±1.2

*** Significant at $p<0.001$ compared with control, ** Significant at $p<0.01$ compared with control

Table 1 presents the results of in vitro microclonal growth, root formation percentage, root length and shoot height of Capparis spinosa L. regenerants over a 65-day period. According to the results, among the media, MS+IAA (1.0 *mg/L*) showed the highest root formation percentage (69±1.5%), the longest roots (21±0.2 *mm*) and the highest shoots (25±1.4 *mm*) compared to the control without hormone ($p<0.001$), and the results were found to be highly reliable. At 1.0 mg/L, MS+BERES had root growth (64±1.1%) and shoot growth (22±1.3 *mm*), which were slightly lower than IAA but higher than the control ($p<0.001$). The results showed that *BERES universal* effectively stimulates plant root growth and morphophysiological development, increases plant viability and resistance to infectious microorganisms. *BERES universal* had an average effect at a concentration of 0.5 *mg/l*, with root growth of 61±1.1% and root length of 17±1.2 *mm* ($p<0.01$). Although effective growth was observed at lower concentrations, the indicators were slightly lower than at a concentration of 1.0 *mg/l*. In MS+IBA (1.0 *mg/l*), root growth was 60±1.2%, root length was 17±0.3 *mm*, and shoot height was 23±1.1 *mm* ($p<0.01$) compared to the control group. The MS medium without hormones showed the lowest root formation percentage (47±1.0%), root length (12±1.0 *mm*) and shoot height (18±1.2 *mm*). These experiments confirm the importance of synthetic auxins and biostimulants in the effective in vitro propagation of *C. spinosa L*. The results showed that *BERES Universal* at a concentration of 1.0 *mg/l* is effective in stimulating root growth and shoot development and is of great importance as a sustainable and environmentally safe alternative.

The next stage of the in vitro rooted regenerants is their adaptation to external environmental conditions, i.e. acclimatization. The success of this process directly depends on the quality indicators of the regenerants, and the initial selection stage is of great importance. The selection criteria were a root length of at least 25-30 *mm*, a regenerant height of not less than 20-25 *mm*, and the presence of at least 3-4 leaves.

In total, 75-90 regenerants were selected for the experiment. Of these, 30 were isolated from MS+ISK 1.0 *mg/L*, 20-25 from MS+BERES 1.0 *mg/L*, 12-15 from MS+BERES 0.5, 8-10 from MS+IBK 1.0 *mg/L*, and 5 from GM (harmon-free) medium. In addition, 10-15 reserve regenerants were also selected. The selected regenerants were subjected to sanitary treatment, rinsed in warm distilled water, cleaned of agar residues on the roots, and treated for 1-2 minutes in a 0.1% fungicide (Bavistin®) solution to reduce the risk of infection. Then the regenerants were planted in a sterile prepared mixture of peat, compost: perlite (1:1:1). Each plant was placed in individual cups with a capacity of 250 *ml*, and the substrate was lightly watered with a 0.15% solution of *BERES*®. The explants were kept in a microgreenhouse for the first 7 days at high humidity of 90-95% and a temperature of 20-22 °C. In the following stages, the lid was opened every 1-2 hours, and after 14-21 days it was completely removed. The light regime was 16 hours a day, and the light intensity was maintained at a level of approximately ~40 *mE* $m^{-2} \cdot s^{-1}$. Watering was carried out every 2-3 days with a *BERES*® solution. Regenerants adapted to greenhouse conditions were kept for 30-45 days at a temperature of 24-30°C and a relative humidity of 60-65%. During this period, their biometric indicators were recorded. At the next stage, the plants were adapted to external conditions for 7 days, and then transferred to field conditions.

According to the analysis of the experimental results, the regenerants grown in the MS+ISK 1.0 *mg/L* medium showed the highest survival rate, which was 76%. This is explained by the well-developed root system in this medium. The survival rate of plants in the MS+IBK 1.0 *mg/L* medium was slightly lower, 72%, and despite the stable root formation process, the adaptation process was slower than in the MS+ISK 1.0 *mg/L* medium. In the MS+GM medium without hormones, the lowest result was observed, 47%, which is associated with the slowness of root formation and difficulties in the acclimatization stage. The best result was recorded in the MS+BERES 1.0 *mg/L* Universal combination, the survival rate of regenerants was 84%.

In comparison, the control group without BERES Universal showed a rate of 47% in MS+GM medium, while the application of BERES 1.0 *mg/L* increased this rate by 37% to 84%. The results obtained show that the nutrient medium used during the rooting stage has a significant impact on the survival rate of regenerants during the subsequent acclimatization process.

Conclusion

These studies serve as the basis for creating complex and sustainable technologies for optimizing microclonal propagation conditions of Capparis spinosa L., increasing the synthesis of bioactive substances, and adapting it to arid soil conditions. It will allow selecting the most effective local microorganisms from the soil microbiota and using them in the production of fertilizers based on biopolymers.

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PRELIMINARY COMMUNICATION

Herbicide residues in irrigation water: assessment and environmental implications

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Summary
Herbicide residues in irrigation water are an emerging concern in intensively cultivated regions. This study assessed their occurrence and environmental implications in canals in Vojvodina (Serbia). A total of 41 herbicides were analyzed using SPE and LC/MS and GC/MS. Multiple active substances were detected, with concentrations varying by location and time. The most frequent were s a-metolachlor (0.031 µg/L) and terbuthylazine (0.011 µg/L), followed by nicosulfuron, clomazone, and amidosulfuron. Based on PNEC values, smetolachlor poses low acute risk, while terbuthylazine indicates potential chronic risk. Overall, residues may affect aquatic organisms under prolonged exposure.

Keywords: herbicides, irrigation water, environment, risk assessment

Introduction

Water resources are increasingly exposed to degradation processes, particularly in regions with intensive agricultural activity, where diffuse pollution has become a major environmental concern. Agriculture is recognized as one of the dominant anthropogenic sources of water contamination, primarily due to the widespread and repeated application of plant protection products. According to projections of the United Nations, the global population is expected to grow significantly in the coming decades, necessitating a corresponding increase in food production. Achieving this goal relies on sustainable agricultural practices that balance high productivity with the preservation of natural resources, particularly water. In this context, the rational use of pesticides is essential, although contemporary agricultural systems continue to depend heavily on their application (Kapsi et al., 2019).

The use of chemical pesticides has substantially contributed to reducing crop losses and ensuring food security. It is estimated that a significant proportion of global agricultural production depends on plant protection products, with millions of tons of active substances applied annually. Despite these benefits, the extensive use of pesticides has raised serious concerns regarding their environmental fate and potential impacts on non-target organisms. After application, pesticides can be transported from treated surfaces through runoff, drainage, and leaching processes, leading to their presence in surface waters, groundwater, and irrigation systems.

Water bodies, including rivers, canals, and irrigation networks, act as recipients and conduits for pesticide residues, reflecting both current and historical agricultural practices. The occurrence of these compounds in aquatic environments is influenced by multiple factors, including physicochemical properties of the active substances, climatic conditions, soil characteristics, and application patterns (Reichenberger et al., 2007). Once present in water,

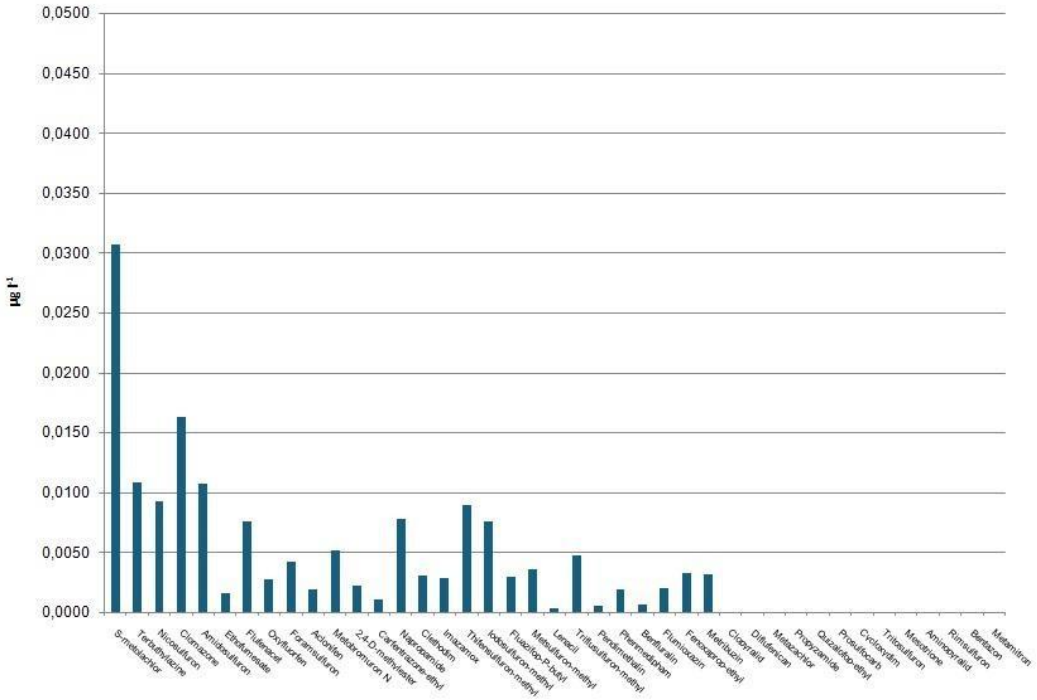
pesticides may exert adverse effects on aquatic organisms, even at low concentrations, particularly under conditions of chronic exposure or in the presence of complex mixtures.
The ecological risks associated with pesticide contamination of water are of particular concern for sensitive groups such as phytoplankton, macrophytes, and aquatic microorganisms, which play essential roles in ecosystem functioning. In response to these challenges, water quality monitoring has been formally regulated within the European Union through the Water Framework Directive (2000), which establishes a comprehensive framework for the protection of aquatic environments. Nevertheless, the occurrence of pesticide mixtures and their combined effects remain insufficiently understood, highlighting the need for continued monitoring and research. Thus, the aim of this study was to assess the potential ecological risks of herbicide residues founded in irrigation water.

Material and Methods

The monitoring conducted in our previous study (Mitić et al., 2025) covered the Danube–Tisza–Danube (DTD) canal system, an irrigation network located in northern Serbia. A total of 100 water samples were analyzed. Herbicides were extracted from water samples using solid-phase extraction (SPE) with Empore™ C-18 disks (47 mm; Cat. No. 2215, CDC Analytical), while the determination was performed using GC-MS/MS and LC-MS/MS, depending on their physicochemical properties. In total, 41 herbicides were analyzed. Afterward, considering the Predicted No Effect Concentration (PNEC) values for aquatic organisms, environmental risk was assessed by calculating risk quotients (RQ), defined as the ratio between the measured environmental concentration (MEC) and the corresponding PNEC (RQ=MEC/PNEC) (Li et al., 2022). PNEC values were obtained from available ecotoxicological data, applying appropriate assessment factors where necessary. Risk was classified as low ($RQ<0.1$), moderate ($0.1 \leq RQ<1$), or high ($RQ \geq 1$), indicating a potential adverse effect on aquatic organisms.

Results and Discussion

The results revealed the presence of multiple herbicide active substances, with concentrations varying depending on sampling location, season, and proximity to treated agricultural fields (Graph 1) ((Mitić et al., 2025). This variability reflects differences in application practices, hydrological conditions, and potential runoff events. The most frequently detected herbicides were S-metolachlor and terbuthylazine, with average concentrations of 0.031 µg l^{-1} and 0.011 µg l^{-1}, respectively, indicating their widespread use and persistence in the aquatic environment. In addition, nicosulfuron, clomazone, and amidosulfuron were commonly detected, with average concentrations of 0.009, 0.002, and 0.011 µg l^{-1}, respectively. Although detected concentrations were generally low, the frequent occurrence of these compounds suggests continuous input into the canal system and highlights the importance of ongoing monitoring, particularly considering potential mixture effects and long-term exposure risks for aquatic organisms.
Based on the obtained concentration data, environmental risk was further assessed by calculating risk quotients (RQ). The RQ is defined as the ratio between the measured environmental concentration (MEC) and the corresponding Predicted No Effect Concentration (PNEC). This approach enables the evaluation of potential adverse effects of detected herbicides on aquatic organisms. Predicted No Effect Concentration (PNEC) values used for risk assessment were obtained from the European Chemicals Agency (ECHA) database.



Graph 1. Herbicide residues in irrigation water from the Danube–Tisza–Danube (DTD) canal system (Mitić et al., 2025)

Table 1. Risk quotients (RQ) of detected herbicides in irrigation water

Herbicide	Risk quotients (RQ)	Measured environmental concentration (MEC)	PNEC value
S-metolachlor	0.09	0.031	0.34
terbuthylazine	0.12	0.011	0.09
nicosulfuron	0.75	0.009	0.012
clomazone	0.04	0.016	0.4
amidosulfuron	0.11	0.011	0.1

The calculated risk quotients (RQ) indicate varying levels of potential risk to aquatic organisms among the detected herbicides. Considering the obtained values, the presence of S-metolachlor and terbuthylazine indicates a low acute risk on aquatic organisms. Although nicosulfuron, clomazone, and amidosulfuron were detected in low concentrations, their presence may still contribute to chronic effects on algae and aquatic plants.

Overall, the detection of herbicides in irrigation water of the Danube–Tisza–Danube (DTD) canal system raises important concerns regarding their potential impact on non-target organisms, including aquatic biota and soil microorganisms. Even though most detected concentrations were below established regulatory thresholds, the presence of multiple active substances, often simultaneously, suggests that chronic exposure could have cumulative effects. Prolonged exposure, even at low concentrations, may lead to bioaccumulation, sublethal toxicity, and disruption of physiological and reproductive processes in sensitive species. Moreover, the calculated risk quotients (RQ) indicate that certain herbicides, particularly nicosulfuron, approach levels that may pose moderate ecological risk, highlighting the need for

continuous monitoring and management. The frequent occurrence of herbicides near treated fields emphasizes the role of agricultural runoff and irrigation practices in contaminant transport.

Conclusion

These findings underscore the importance of implementing integrated risk assessment and mitigation strategies to protect aquatic ecosystems. They also suggest that regulatory frameworks should consider mixture effects and long-term exposure scenarios to better safeguard biodiversity and the ecological integrity of irrigation networks.

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ORIGINAL SCIENTIFIC PAPER

Spatial Distribution and Diversity of Landscape Elements in Intensive Agricultural Regions of Slovenia

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Summary
This study analyzes the spatial distribution and diversity of landscape elements across five major intensive agricultural regions in Slovenia: the Ljubljana Basin, Celje Basin, Dravsko–Ptuj Field, Mura Plain, and Krka Basin. Less Favoured Areas (LFA), as defined under Directive 75/268/EEC (1975), were excluded from research. Land-use and landscape element data were derived from national datasets and analyzed using ArcGIS. Landscape diversity was assessed using Shannon's index, Simpson's index, and Pielou's evenness. The results show clear regional differences in both the abundance and spatial distribution of landscape elements. The Mura Plain exhibited the highest number of elements and the greatest diversity, indicating a well-balanced landscape structure. In contrast, the Celje Basin showed the lowest diversity and a more fragmented distribution pattern. Hedgerows, solitary trees, and riparian vegetation were the dominant elements across all regions. Overall, the findings highlight spatial variability in landscape structure, reflecting differences in agricultural intensity and land management practices.

Keywords: landscape elements, agricultural area, GIS, spatial analysis, Slovenia

Introduction

In recent decades, the European Union has increasingly emphasized the importance of landscape elements in addressing biodiversity loss and ecosystem degradation in agricultural environments (Willemen et al., 2008). The EU Biodiversity Strategy for 2030 sets a target that at least 10% of agricultural land should consist of high-diversity landscape elements, underlining their importance for ecological functioning and landscape connectivity (Robuchon et al., 2025). However, agricultural intensification across European plains, valleys, and lowland areas has led to increasingly homogeneous land-use patterns dominated by intensive production systems. In this process, many small landscape elements have been removed or converted into arable land, resulting in simplified landscape structure and reduced ecological functionality (Ivanjšič et al., 2024). In Slovenia, these elements are systematically recorded and monitored through the national register of landscape features, which is primarily maintained in selected, more intensively used agricultural regions where landscape features important for biodiversity conservation and landscape diversity are supported, maintained, or established through agricultural policy measures (MKGP, 2023). Also, a large proportion of agricultural land in Slovenia is classified as areas with natural constraints (Less Favoured Areas, LFA), where agricultural production is limited by geomorphological and climatic conditions. These areas are typically characterized by more extensive land use and often retain a higher level of landscape heterogeneity, and are further classified into three main categories: mountain areas (MA), areas with other natural constraints (ANC), and areas with specific constraints (SC) (MKGP, 2015). In contrast, areas outside LFA are mainly located in lowlands and basins, where agricultural production is more intensive (Cunder et al., 2007). Although the importance of landscape elements is widely acknowledged, their spatial distribution and diversity in intensively used

agricultural areas remain insufficiently documented. This is particularly evident in Slovenia's lowland regions, where agricultural intensification has led to increasingly simplified landscape structures. The aim of this study was to analyze the composition, spatial distribution, and diversity of landscape elements in the intensive agricultural regions of Slovenia, focusing specifically on areas outside LFA constraints. For this purpose, landscape diversity indices (*Shannon index, Simpson index, and Pielou's evenness*) were calculated for the five largest contiguous agricultural areas outside LFA: the Ljubljana Basin, Celje Basin, Dravsko–Ptuj Field, Mura Plain, and Krka Basin.

Material and methods

The spatial analysis was conducted in Slovenia using ArcGIS Pro. To focus the analysis on productive agricultural land, LFA were first excluded. By removing these constrained areas, the remaining agricultural land represented the most intensive production regions in the country. Based on this approach, five major lowland agricultural regions, along with their surrounding areas, were selected for further analysis: *the Ljubljana Basin, the Celje Basin, the Dravsko–Ptuj Field, the Mura Plain, and the Krka Basin with surrounding agricultural area* (Figure 1). The analysis was based on the official agricultural land-use dataset (*RABA*) for 2025, downloaded from the MKGP geoportal in March 2026. The analysis used vector-based land-use data with parcel-level spatial detail. The minimum mapping unit depended on land-use category and ranged from 25 m² (greenhouses) and 500 m² (vineyards, olive groves, hop plantations, and other permanent crops) to 1000 m² for most agricultural land-use categories. Although the dataset provides high spatial detail, some discrepancies between recorded and actual land use may occur due to periodic database updates. Only selected agricultural land-use categories representing active agricultural production were retained: *arable land, temporary grassland, permanent grassland, overgrown agricultural land, vineyards, intensive orchards, extensive orchards, olive groves, other permanent crops, hop plantations, agricultural land in mosaic structure, and agricultural land with natural elements.*

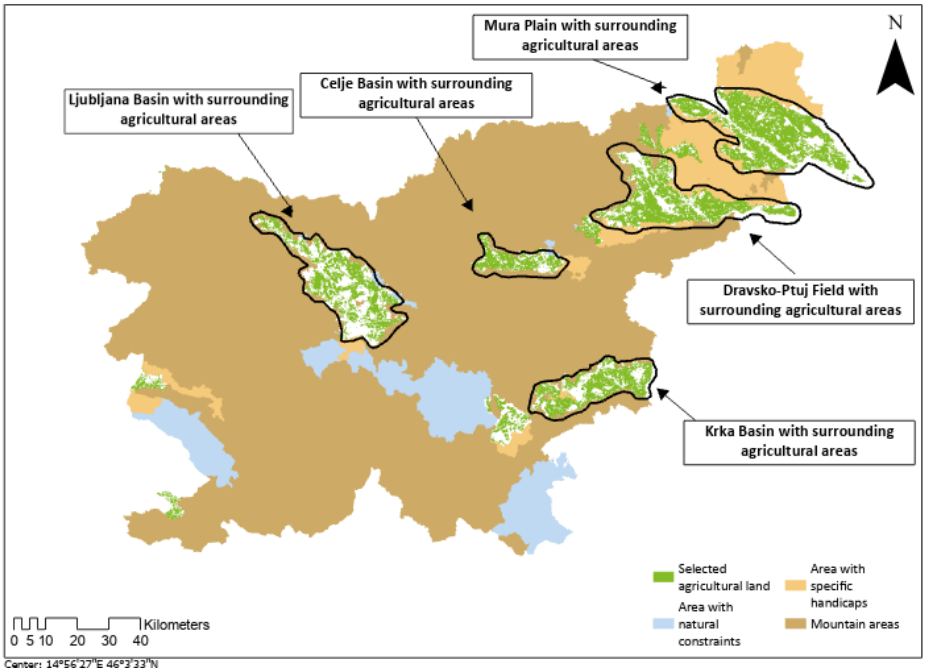

Figure 1. Selected lowland agricultural study areas in Slovenia outside areas with natural constraints.

Landscape elements were obtained from the national Register of Landscape Features (EKRZ), maintained by the Ministry of Agriculture, Forestry and Food of Slovenia. The dataset was accessed in March 2026. The national database includes semi-natural elements embedded within agricultural land, such as hedgerows, tree lines, solitary trees, riparian vegetation, groups of trees or shrubs, and water-related features. The analysis used vector-based spatial data with great spatial detail, where the minimum dimensions and mapping criteria depend on landscape feature type (e.g., hedgerows must be at least 10 m long and cover at least 25 m²). The number of landscape elements within each study area was calculated using a spatial join in ArcGIS Pro, which intersected landscape elements with the delineated study areas. Landscape diversity was assessed using the *Shannon diversity index* (H'), *Simpson's diversity index* (1–D), and *Pielou's evenness* (E). These indices account for both the number of landscape element types and their relative abundance. Higher values of the Shannon and Simpson indices indicate greater diversity, while Pielou's evenness ranges from 0 to 1, where values closer to 1 indicate a more uniform distribution of landscape elements (Maisyaroh et al., 2021).

Results and discussion

Figure 2 highlights the spatial differences in the density and clustering of landscape elements across the study areas, with the highest concentration observed in the Mura Plain and Dravsko–Ptuj Field, whereas the Celje Basin exhibits a more fragmented, less dense distribution. The results presented in Table 1 reveal clear differences in both the composition and diversity of landscape elements across the selected agricultural regions. In terms of absolute numbers, the Mura Plain contains the highest total number of landscape elements (17,910), followed by the Dravsko–Ptuj Field (15,313) and the Ljubljana Basin (10,445). In contrast, the Celje Basin (8,313) and the Krka Basin (8,492) exhibit considerably lower totals, indicating a less dense distribution of landscape elements. These differences suggest that landscape structure varies substantially between regions, particularly in terms of element abundance and spatial presence. Across all regions, the most represented landscape elements are hedgerows, solitary trees, and riparian vegetation, which together account for the majority of recorded features.

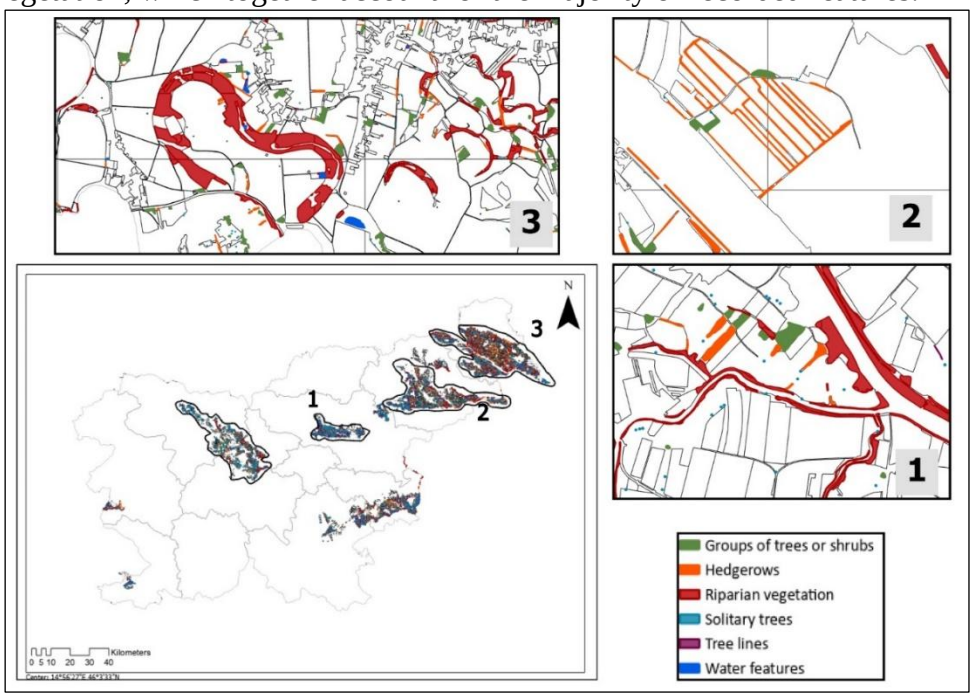

Figure 2. Spatial distribution and typological composition of landscape elements in selected lowland agricultural regions of Slovenia, with detailed examples of representative landscape structures.

A more detailed comparison reveals notable regional differences in the composition of landscape elements. Average number of landscape elements by type and corresponding diversity indices (Shannon, Simpson, and Pielou's evenness) across the selected agricultural regions, as presented in Table 1. The observed diversity patterns indicate moderate but consistent regional differences in the structure of agricultural landscapes across the selected lowland regions of Slovenia. Similar applications of Shannon, Simpson, and Pielou's evenness indices have been widely used in landscape ecology studies to evaluate landscape heterogeneity, structural complexity, and the distribution of semi-natural elements within agricultural areas (Ramezani, 2019; Dušek and Popelková, 2017). As presented in Table 1, the Mura Plain exhibited the highest diversity and evenness values (H' = 1.50; Simpson = 0.76; E = 0.84), indicating a more balanced and heterogeneous landscape structure. Comparable relationships between higher diversity values and increased landscape heterogeneity were also reported in studies of Central European agricultural landscapes, where high density and heterogenity of landscape elements was associated with greater ecological complexity and connectivity (Fahrig et al., 2011; Lange et al., 2023). In contrast, the Celje Basin showed the lowest diversity and evenness values (H' = 1.38; Simpson = 0.71; E = 0.77), suggesting stronger dominance of individual landscape element types and a less heterogeneous landscape structure. Similar patterns have been identified in intensively managed agricultural regions, where landscape simplification and reduced diversity are often linked to agricultural intensification and the removal of semi-natural elements (Benton et al., 2003).The Krka Basin and the Dravsko–Ptuj Field exhibited intermediate to relatively high diversity, indicating moderately heterogeneous agricultural landscapes with balanced distributions of landscape elements. The Ljubljana Basin showed similar patterns, though with slightly lower diversity values than the leading regions. Overall, the obtained diversity indices are comparable to values reported in other European agricultural landscape studies assessing the structural diversity and composition of semi-natural landscape elements (Batáry et al., 2010; Garratt et al., 2017).

Table 1. Number of landscape elements by type and corresponding diversity indices (Shannon, Simpson, and Pielou's evenness) across the selected agricultural regions.

Type of landscape element/ Area	*Dravsko-Ptuj Field**	*Mura Plain**	*Celje Basin**	*Krka Basin**	*Ljubljana Basin**
Groups of trees or shrubs	1536	3051	597	1122	1493
Hedgerows	4585	5156	1503	2246	3213
Riparian vegetation	4058	5398	3156	2884	2708
Solitary trees	4471	3622	2695	1901	2838
Tree lines	537	362	334	197	158
Water features	126	321	28	142	35
Total number of landscape elements	**15313**	**17910**	**8313**	**8492**	**10445**
Shannon diversity index (H')	1.46	1.50	1.38	1.48	1.43
Simpson's diversity index (1–D)	0.74	0.76	0.71	0.75	0.74
Pielou's evenness (E)	0.81	0.84	0.77	0.83	0.80

***with the surrounding agricultural area*

Conclusion

This study revealed clear regional differences in the composition and diversity of landscape elements across the selected intensive agricultural regions of Slovenia. The calculated Shannon, Simpson, and Pielou's evenness indices indicated moderate but consistent variation in landscape heterogeneity among the study areas. The Mura Plain exhibited the highest diversity and evenness values, reflecting a more balanced and structurally heterogeneous agricultural landscape, while

the Celje Basin showed lower diversity and greater dominance of individual landscape element types, indicating a simpler landscape structure. Hedgerows, riparian vegetation, and solitary trees were the most abundant landscape elements across all regions, underscoring their important role in the structural composition of agricultural landscapes. The obtained diversity values were comparable to findings from other European agricultural landscape studies, in which higher landscape diversity is associated with greater ecological complexity and improved landscape connectivity. The results emphasize the importance of preserving semi-natural landscape elements in intensively used agricultural areas, as they contribute to landscape heterogeneity and ecological stability. The applied diversity indices proved to be a useful approach for evaluating structural differences among agricultural landscapes and may support future spatial planning and agri-environmental management measures in Slovenia.

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ORIGINAL SCIENTIFIC PAPER

The influence of seaweed treatment on the morphological characteristics of cucumber seedlings

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Summary
Seaweeds are a promising renewable biomass resource, with global production that has tripled over the past two decades. They can be used as fertilizers, soil improvers, and biostimulators. Here, the influence of *Ascophyllum nodosum* byproducts on morphological properties of the cucumber seedlings was tested. The results showed that the tested seaweed materials were mature or very mature soil amendments and mostly safe to use when mixed with soil or quartz sand at adequate concentrations. Most treatments had a positive influence on observed properties, but the results were not statistically significant. Additional tests are needed to optimize seaweed as an effective soil management practice.

Key words: biostimulators, fertilizers, macroalgae, phytotoxicity, sustainable resources

Introduction

Providing adequate, high-quality food for the growing world population is a major challenge, and it's necessary to prevent or mitigate further soil degradation. Seaweeds are a promising multi-sector renewable resource, rich in nutrients and bioactive compounds. Their production requires minimal cultivation conditions (DeAngelo et al., 2023.), and UN Global Seaweed Initiative (UNGSI) shows that global seaweed production has tripled over the past two decades, while exports have quadrupled to reach $3.9 billion in 2022. Initially, seaweeds were used traditionally and empirically in the crop production in coastal areas, and today they can be used as fertilizer - due to the content of macro and micro elements of plant nutrition, or as soil quality improver - due to the high content of phycocolloids, and as biostimulators - due to the content of vitamins and plant hormones, especially auxins and cytokinins (Illera-Vives et al. 2020). In addition to beneficial elements and nutrients, seaweeds also accumulate heavy metals from waters, such as arsenic, cadmium, lead, and mercury, and its use can affect human health and the environment when transferred to soil and the food chain. That's why seaweed is also included in the European Union regulation 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food. In some seaweed processing industries, such as biostimulators' production, there are biomass leftovers from harvest or the production process that can be reused in agriculture (Ivezić et al., 2025). The purpose of this paper was to examine the impact of seaweed byproducts of *Ascophyllum nodosum* from the biostimulators' production on cucumber seedlings' morphological properties, when used as a soil fertilizer or conditioner at certain concentrations.

Material and methods

Seaweed properties

Two types of seaweed material were used in the analysis, the seaweed material from *Ascophyllum nodosum*: a) granulated fertilizer available on the market (Asco. granulate = AG), and b) a byproduct from the production of liquid fertilizer derived from Ascophyllum sp. where nitric acid was used in the production process (Algae fiber = AF+), as a digestion agent to

extract bioactive compounds. The seaweed content of total carbon (TC) and total nitrogen (TN) was analysed on Skalar C/N analyzer, and the concentration of nutrients (P and K), toxic elements (As and Cd) (ISO 2009), EC (FAO 2021) and pH value (pH ISO 1994) were analyzed at the Faculty of Agrobiotechnical Sciences Osijek.

Soil properties

Two soils from Croatia were used in the biological assay of testing the influence of seaweed treatment on the morphological characteristics of cucumber seedlings. Soil 1 was from the Podravlje site (alluvial soil), and Soil 2 was from the Zelčin site (luvisol). Soil properties determined at the Faculty of Agrobiotechnical Sciences Osijek, were: soil texture (ISO 2020), pH (ISO 1994), EC (FAO 2021), available P and K (Egner et al. 1960), and the content of total carbon (TC) and total nitrogen (TN) analysed on C/N Skalar analyzer.

Biological Assays

The influence of seaweed treatment on the morphological characteristics of cucumber seedlings was tested by Test Methods for the Examination of Composting and Compost, 05.05-A Seedling emergence and relative growth. Instead of vermiculite, quartz sand (granulation 0,1 – 0,6 mm) was used as a negative control, because its inertness and high compaction caused by its accumulation over time make quartz sand a great aggregate material (Qaidi et al. 2022), and it prevents unwanted chemical interactions or nutrient buffering in chemical testing of organic matter in the experiments. A preliminary experimental design consisted of 2 soils (alluvial soil – Soil 1, and luvisol – Soil 2) x 2 seaweed products (AG, AF+) x 2 concentrations (1% and 3%) + 2 positive controls (soil only) + 5 negative controls (quartz sand only; quartz sand + seaweed at 1% and 3% concentrations) - a total of 15 treatments in 3 repetitions. The basic information on soil and seaweed materials are presented for a better understanding of their influence on the observed seedlings' properties and development (Table 1). Top line Standard selected certified cucumber seed Green Garden - Franchi Sementi, Sunčani potok (Sunny Stream) was used for the experiment, and a total of 450 plants were expected to be grown within 12-14 days. Prepared trays were put in transparent plastic bags with a supporting structure, to prevent the bag from collapsing, and a zipper seal to prevent the treatments from drying out.

Table 1. Basic properties of soil and seaweed material used in experiment

Soil	**Text.**	**pH KCl**	**EC µS/cm**	**TC %**	**TOC %**	**TN %**	**AL-P_2O_5 mg $100g^{-1}$**	**AL-K_2O mg $100g^{-1}$**
Soil 1 (alluvial)	Sandy loam	7,12	140,80	2,63	1,28	0,12	176,8	15,66
Soil 2 (luvisol)	Silt loam	4,65	38,10	0,96	0,96	0,10	19,82	18,55

Seaweed	**pH H_2O**	**EC mS cm^{-1}**	**TC %**	**TN %**	**K mg kg^{-1}**	**P mg kg^{-1}**	**As µg kg^{-1}**	**Cd µg kg^{-1}**
Asco. granulate (AG)	5,58	6,51	34,13	1,03	27770	896	22230	155,72
Algae fiber (AF+)	8,86	26,7	34,92	1,29	84402	4360	26400	1066,29

They were placed in a walk-through chamber for controlled plant growth, with the following conditions: 14 hours of constant light, 10 hours of constant darkness, at a constant temperature of 25°C and air humidity of 50% for 13 days. The trays were removed from the plastic bags daily for visual observation and occasional irrigation. On the 13th day, evaluation of relative

emergence rate and relative seedling vigor was conducted, and photographs of the plants were taken to determine their heights with ImageJ software (version v1.53k) - a versatile and open source software package designed for scientific image processing and analysis.

Statistical analysis

Descriptive statistics and analysis of variance (One-way ANOVA) were performed using Minitab® Statistical Software, version 17. Descriptive statistics were performed to determine the influence of the different seaweed treatments on plant morphological properties (plant height and weight). Analysis of variance with Tukey pairwise comparison was performed to determine whether there was a significant difference among treatments/controls (which include different soils, different seaweed products applied in different concentrations) and plants' height and weight. Pearson correlation was performed to determine whether there was a correlation between plants' height, weight, relative emergence rate, and relative seedling vigor of the examined plants.

Results and discussion

After 12 days, morphological characteristics were examined and parameters for determining the effect of treatment on the plant emergence and seedling vigor were reviewed. The number of plants in every tray cell was recorded, and all plants were photographed at the end of the experiment, for the purpose of height measuring with the ImageJ software. Emergence rate was determined using following formula:

$$E = ES \div C \times 100$$

where E is relative percent emergence, % of control; ES is the number of cucumber seedlings with exposed cotyledons and hypocotyl, and C is the number of emerged control seedlings. Relative Seedling Vigor was determined using following formula:

$$V = VS \div C \times 100$$

where V is relative percentage of healthy, vigorous, % of control; C is the number of healthy, vigorous control seedlings, and VS is the number of cucumber seedlings with well formed, undeformed cotyledons, and turgid hypocotyl with a length equal to or greater than the positive control average. The values of the relative emergence rate ranged from 83% - 130%, and those of relative seedling vigor from 80 – 119% (Table 2). The highest value of relative emergence rate was determined in the treatment with AG seaweed material added at a concentration of 3% to Soil 2, and the highest value of relative seedling vigor was determined in the treatments with 3% of AG seaweed material added to quartz sand. Seaweeds in all treatments could be rated as mature or very mature organic matter, except AF+ seaweed material added at a concentration of 3% to quartz sand, which showed signs of phytotoxicity on the tested plants (dead or stunted). A determined value of relative emergence rate and relative seedling vigor higher than 100% in a biological assay indicates that the tested sample outperforms the control group. Regarding the plants' height, each plant in every repetition of every treatment and control was measured 3 times (triplicate measurement) to ensure data consistency by identifying measurement errors and reducing variability, and it was done manually in ImageJ software. Finally, the total weight of the above-ground parts of all plants for each treatment and its respective control were measured. When comparing the height and fresh weight of the plants in treatments with their respective controls, it has been determined that most of the treatments had a positive effect on observed morphological properties (achieved values were higher than in respective controls), but the results showed no statistically significant difference (Table 3). It is established that some treatments had statistically significant differences in the electrical conductivity (EC) and in the pH (KCl) value of the growing media, and the highest value of the EC, as well as pH value, was established precisely in the treatment were all the plants died or were stunted (AF+ 3% on quartz sand). Although the EC value was still within the safe EC range for the development of

young cucumber plants (874,67 µS cm^{-1}), the pH value (9,60) was considered toxic and highly stressful for cucumber plants that thrive in soils with a pH range of 5.5–6.5 (Mohammadnia et al., 2025). In pure quartz sand, unfavorable hydrogen-ion concentrations may develop, owing to the nonequivalent absorption of cations and anions, and the importance of considering the hydrogen-ion concentration of the zone contiguous to the root, rather than that of the whole medium, is emphasized (Gile and Feustel, 1943).

Table 2. Evaluation of Emergence and Relative Growth and Vigor of cucumber seedlings, treated with different seaweed material (AF+, AG), added in concentrations of 1% and 3%

Quartz sand and seaweed	**Q_AF+ 1%**	**Q_AG 1%**	**Q_AF+ 3%**	**Q_AG 3%**
Emergence, %	110	101	0	126
Seedling vigor, %	80	98	0	119
Soil 1 and seaweed	**T1_AF+ 1%**	**T1_AG 1%**	**T1_AF+ 3%**	**T1_AG 3%**
Emergence, %	108	83	93	96
Seedling vigor, %	108	82	81	97
Soil 2 and seaweed	**T2_AF+ 1%**	**T2_AG 1%**	**T2_AF+ 3%**	**T2_AG 3%**
Emergence, %	107	107	100	130
Seedling vigor, %	117	100	110	110

Interpretation

	Very mature	**Mature**	**Immature**
Emergence, %	> 90	90 -80	< 80
Seedling vigor, %	> 95	85-95	< 85

Q_AG (Asco. granulate mixed with quartz sand), Q_AF+ (Algae fiber mixed with quartz sand), S1_AG (Asco. granulate mixed with quartz sand and alluvial soil), S1_AF+ (Algae fiber mixed with quartz sand and alluvial soil), S2_AG (Asco. granulate mixed with quartz sand and luvisol), S2_AF+ (Algae fiber mixed with quartz sand and luvisol)

The correlations between observed morphological properties, relative emergence rate and relative seedling vigor were also tested. The results showed that the correlation between plants' height and relative emergence rate was very strong and positive, statistically significant (= 0,895, P-Value = 0,000), as well as between relative emergence rate and plants' fresh weight (= 0,703, P-Value = 0,005), and also between plants' height and fresh weight (= 0,739, P-Value = 0,003). The correlation between vigor and plants' height was strong, positive and statistically significant (= 0,688, P-Value = 0,006). Strong, negative, statistically significant correlation is noticed between relative emergence rate and electrical conductivity (EC) in treatments (= -0,705, P-Value = 0,003), and also relative seedling vigor and EC (= -0,618, P-Value = 0,014). The correlation was similar, but not as strong between EC and observed morphological properties of the plants. EC analysis of the seaweed material showed that AF+ seaweed material had 4 times higher EC value than AG seaweed material (26,70 mS cm^{-1}; 6,51 mS cm^{-1}). Later, EC and pH (KCl) analysis of seaweed-treated soils indicated that soils with AF+ seaweed material also had higher EC than soils with AG seaweed material, but all values were in the non-toxic range for cucumber plants. Some studies show that soil electrical conductivity, potassium (K^{+}), sulfate (SO_4^{2-}), and active carbon (C) increased with seaweed addition relative to the organic fertilizer (Possinger and Amador, 2016). When it comes to pH (KCl), a significant moderate-to-strong negative relationship between pH and relative emergence rate in treatments was determined (= -0,636, P-Value = 0,011), and also statistically significant, moderate negative correlation with relative emergence rate (= -0,553, P-Value = 0,033). The high pH value in some treatments could cause

lower relative emergence rate and relative seedling vigor, due to the strong negative correlations between pH and micronutrient availability, which is primarily the result of precipitation and hydroxide formation under alkaline conditions (Ricci et al., 2021). As the pH increases, the surface of plant root becomes increasingly negative, meaning that the concentration of cations near the surface will decrease, and the concentration of anions will increase (Lu et. al, 2020). Therefore, it's justified to say that soil pH is a master soil variable that has a bidirectional relationship with soil biogeochemical processes (Neina, 2019).

Table 3. Influence of the different seaweed treatment, at a concentration of 1% and 3%, on plant height and fresh weight

		plant height (cm)			plant fresh weight (g)		
Treatment	n	1%	3%	control	1%	3%	control
Q_AG	3	2,19^{a}	2,91^{a}	1,97^{ab}	3,20^{ab}	3,87^{ab}	2,46^{ab}
Q_AF+	3	2,14^{a}	0,00^{b}		3,69^{ab}	0,00^{c}	
S1_AG	3	1,50^{ab}	2,27^{a}	2,11^{a}	1,92^{bc}	2,87^{ab}	2,53^{ab}
S1_AF+	3	2,46^{a}	1,90^{ab}		4,11^{a}	2,68^{ab}	
S2_AG	3	2,77^{a}	2,95^{a}	3,28^{a}	3,29^{ab}	3,33^{ab}	2,07^{b}
S2_AF+	3	2,39^{a}	2,17^{a}		3,04^{ab}	3,04^{ab}	

Q_AG (Asco. granulate mixed with quartz sand), Q_AF+ (Algae fiber mixed with quartz sand), S1_AG (Asco. granulate mixed with quartz sand and alluvial soil), S1_AF+ (Algae fiber mixed with quartz sand and alluvial soil), S2_AG (Asco. granulate mixed with quartz sand and luvisol), S2_AF+ (Algaefiber mixed with quartz sand and luvisol)

Conclusion

The results of the biological assay conducted in this study showed that the applied seaweed materials can be rated as mature or very mature soil amendments, and in most cases safe to use when mixed with soil or quartz sand at adequate concentrations. Only one treatment showed phytotoxic effect, probably due to high pH value, reducing nutrient availability, but it is necessary to further examine the impact of potentially toxic elements. Strong, positive, and statistically significant correlation was determined between morphological properties of the plants (fresh weight and height) and relative emergence rate in the performed treatments. Although the tested seaweed materials mostly had a positive effect on the plants' morphological properties, the results were not statistically significant. Therefore, it is to be concluded that further tests, involving plant examination across different stages of growth and development, should be conducted to create a better insight into the usage of these seaweed materials as a soil fertilizer or conditioner, and to test their effect on other plant properties, e.g., nutrient uptake and nutrient content of the plants, Including the other plant species in the experiment should also be considered. Overall, seaweed has the potential to promote plant growth, but further research is needed to optimize it as a sustainable resource and safe soil management practice, because of the potential phytotoxic effects when applied at higher doses, and to different soil types or plant growing media.

Acknowledgment

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Test Methods for the Examination of Composting and Compost, 05.05-A Seedling emergence and relative growth (2001)

ORIGINAL SCIENTIFIC PAPER

Allelopathic effect of elderberry (*Sambucus nigra* L.) extracts on lettuce (*Lactuca sativa* L.) growth

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Summary
Elderberry (*Sambucus nigra* L.) is a medicinal species with a high content of biologically active substances. The study evaluated the allelopathic effects of water extracts from *S. nigra* aboveground biomass on lettuce (*Lactuca sativa* L.), as well as the total phenolic (TPC) and flavonoid (TFC) contents in *S. nigra*. The inhibitory effect of *S. nigra* water extracts increased with extract concentration. Lower concentrations showed varied effects on lettuce germination and growth, while concentrations of 5% and above completely inhibited all measured parameters. Phytochemical analysis of *S. nigra* biomass showed a TPC of 26.6 mg GAE g^{-1} and a TFC of 5.2 mg QE g^{-1} of dry weight. The significant allelopathic effect of *S. nigra* highlights its potential for further research.

Keywords: allelopathy, elderberry, phytotoxic potential, phenolic compounds, biocontrol

Introduction

Sambucus nigra L., also known as elderberry or black elder, is a deciduous shrub or small tree in the family Adoxaceae. The species is characterized by small, fragrant, white to yellowish flowers which are arranged into umbel inflorescences and black-purple drupes gathered in grape-like clusters that ripen in late summer. It is commonly found in Europe, Western Asia, North Africa, and North America and typically occurs in forests, forest edges, and clearings. Elderberry flowers and fruits are utilized in diverse food products, such as desserts, juices, syrups, jams, infusions, and alcoholic beverages. In addition, all plant parts are used in folk medicine to treat different medical conditions (Grlić, 2005; Turker and Usta, 2006; Enescu et al., 2016; Corrado et al., 2023). The aerial biomass of *S. nigra* is rich in secondary metabolites, including cyanogenins, phenolic acids, flavonoids, flavanols and terpenoids (D'Abrosca et al., 2001; Viapiana and Wesolowski, 2017; Basas-Jaumandreu and de las Heras, 2019; Studzińska-Sroka et al., 2024).

These secondary metabolites are central to the phenomenon of allelopathy, where plants release allelochemicals into their surroundings that can positively or negatively influence the growth of other species (Rice, 1984; Bhadoria, 2011). In agroecosystems, these interactions can provide eco-friendly weed control measures, potentially reducing reliance on synthetic herbicides, or exert stimulatory effects on crop growth (Bhadoria, 2011; Findura et al., 2020). The allelopathic activity and allelochemical content vary depending on factors such as plant part, plant development stage, genotype, extraction method, and environmental conditions (Parafiniuk and Czerwińska, 2019; Ravlić et al., 2022; Studzińska-Sroka et al., 2024; Talmot et al., 2025). Previous research has indicated allelopathic activity in *S. nigra* (Turker and Usta, 2006; Findura et al., 2020; Lam-Gutiérrez et al., 2025), supporting further investigation of its effects in the present study.

The aim of this research was to: (i) investigate the allelopathic effects of water extracts prepared from the aboveground biomass of *S. nigra* on germination and growth of lettuce (*Lactuca sativa* L.), and (ii) assess total phenolic (TPC) and flavonoid (TFC) contents in *S. nigra*.

Material and Methods

The study was performed at the Central Agrobiotechnical Analytical Unit of the Faculty of Agrobiotechnical Sciences Osijek, in the Laboratory for Phytopharmacy, to assess the allelopathic potential of *S. nigra* on lettuce.

Flowers and leaves of *S. nigra* were collected at full flowering (phenological stage 6/63, Hack et al., 1992) in May 2024 from the left bank of the Drava River in Osijek. The plant material was air-dried, then oven-dried at 40 °C, milled into a fine powder, stored in bags, and kept frozen until use.

Water extracts were prepared following a modified protocol of Norsworthy (2003): 10 g of dry plant biomass was combined with 100 ml of distilled water, and the mixture was incubated for 24 h at 21 ± 2 °C. After incubation, the mixture was first filtered through cloth and then twice through filter paper, yielding 10% extract concentration. Dilutions of 7.5%, 5%, 2.5%, and 1% were prepared with distilled water.

Lettuce seeds ('Majska kraljica') were disinfected using 1% NaOCl and washed with distilled water. Bioassay was performed in Petri dishes and consisted of six treatments with four replications. Each Petri dish contained 30 lettuce seeds placed on filter paper, to which 3 ml of the respective extract or distilled water for the control was applied. To maintain moisture, 2 ml of extract or distilled water was added on day four. The seeds were maintained at 21 ± 2 °C for a six-day period. Allelopathic effects were assessed by measuring: (i) total seed germination, (ii) root length, (iii) shoot length (cm), (iv) fresh seedling weight, and (v) seedling vigour index (SVI = germination × seedling length (Abdul-Baki and Anderson, 1973)).

Total phenolic content (TPC) in *S. nigra* plant material was assessed using the Folin–Ciocalteu method (Singleton and Rossi, 1965), with gallic acid as the standard. A total of 100 µL of the diluted extract was combined with 1500 µL of distilled water and 100 µL of the Folin–Ciocalteu reagent. After incubating for 5 minutes, 300 µL of sodium carbonate (Na_2CO_3) solution was added, and the mixture was vortexed. The mixture was then kept in the dark at 37 °C for 60 minutes. Absorbance was subsequently recorded at 765 nm. Total phenolic content was determined using a calibration curve of gallic acid and expressed as gallic acid equivalents (GAE) in mg g^{-1} of dry plant biomass.

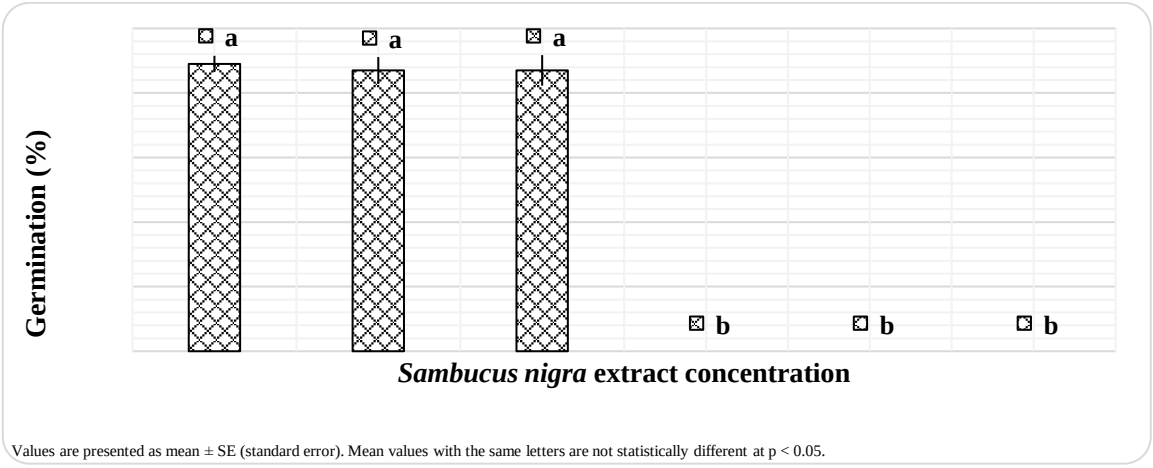
Total flavonoid content (TFC) in *S. nigra* plant material was assessed using aluminium chloride colorimetric assay (Ordonez et al., 2006) with quercetin as a standard. For the assay, 200 µL of extract was combined with 100 µL of 4% aluminium chloride (AlCl3) solution and 1700 µL of 96% ethanol. The mixture was kept in the dark at room temperature for 60 minutes, followed by absorbance measurement at 415 nm. Total flavonoid content was determined using a calibration curve of quercetin and expressed as quercetin equivalents (QE) in mg g^{-1} of dry plant biomass.

The data were statistically analysed with one-way analysis of variance (ANOVA), and differences between treatment means were evaluated using the LSD test at a significance level of 0.05, while results are presented as mean ± standard error (SE, n=4).

Results and Discussion

The application of *S. nigra* water extracts significantly influenced lettuce germination (Graph 1), with lower concentrations showing no noticeable effect, while concentrations of 5% and above completely inhibited seed germination (100%). Similarly, Lam- Gutiérrez et al. (2025) reported that low concentration of *S. nigra* root extract stimulated tomato (*Solanum lycopersicum* L.) seed germination, whereas higher concentrations had an inhibitory effect.

According to Turker and Usta (2006), a concentration of *S. nigra* flower extract as low as 1% significantly reduced the germination of radish (*Raphanus sativus* L.), while a higher concentration (7.5%) caused up to 90% inhibition. In addition to concentration and plant part used, the method of extraction can also influence allelopathic activity of plant species. *S. nigra* extracts prepared as macerates, infusions, or decoctions differ significantly in their impact on target species (Parafiniuk and Czerwińska, 2019; Findura et al., 2020).



Graph 1. Allelopathic effect of elderberry (*Sambucus nigra* L.) water extracts on lettuce germination

The extract of *S. nigra* exhibited a statistically significant allelopathic effect on seedling growth of lettuce (Table 1). Root and shoot lengths and fresh weight of seedlings were progressively reduced with increasing extract concentration. At 2.5%, root and shoot length were reduced by 79.2% and 71.3%, respectively, and fresh weight declined by 51.1%. At concentrations of 5% and above, growth parameters were completely suppressed. An exception was observed at 1% concentration, where fresh weight was significantly stimulated by 17%. SVI followed a similar pattern, decreasing as extract concentration increased.

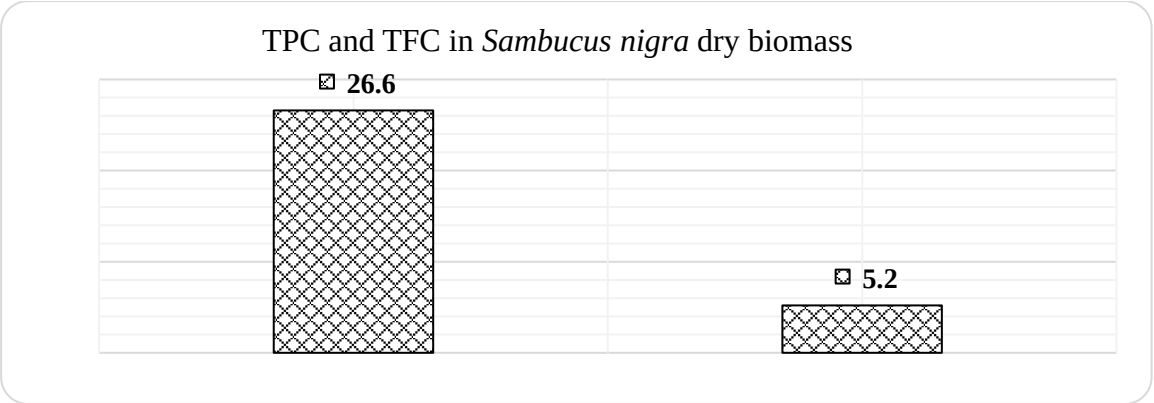
Table 1. Allelopathic effect of elderberry (*Sambucus nigra* L.) water extracts on lettuce growth

Sambucus nigra extract concentration	root length (cm)	shoot length (cm)	fresh weight (g)	SVI (Seedling vigour index)
Control	3.66 ± 0.22 a	1.71 ± 0.07 a	14.00 ± 0.90 b	477.3
1%	2.09 ± 0.27 b	1.57 ± 0.14 a	16.38 ± 0.64 a	318.4
2.5%	0.76 ± 0.09 c	0.49 ± 0.07 b	6.84 ± 0.24 c	108.5
5%	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 d	0.0
7.5%	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 d	0.0
10%	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 d	0.0

Values are presented as mean ± SE (standard error). Mean values with the same letters are not statistically different at p < 0.05.

These results are in line with Turker and Usta (2006), who observed a strong reduction in radish root length (>95%) at both low and high concentrations of *S. nigra* flower extract. Similarly, Mardani et al. (2016) reported that a higher dose (50 mg) of dry *S. nigra* leaves reduced lettuce shoot length, whereas a lower dose (10 mg) had a stimulatory effect.

On average, *S. nigra* extracts reduced germination by 60.9%, root length by 84.4%, shoot length by 75.9%, and fresh weight by 66.8%. Among the growth parameters, seedling root length is generally the most sensitive, due to the direct root exposure to allelochemicals released from the plant material (Turker and Usta, 2006; Mardani et al., 2016).



Graph 2. Total phenolic content (TPC) and total flavonoid content (TFC) in elderberry (*Sambucus nigra* L.) dry biomass

The total phenolic content (TPC) and total flavonoid content (TFC) were determined in *S. nigra* aboveground biomass (Graph 2). The results revealed a TPC of 26.6 mg GAE g^{-1} and a TFC of 5.2 mg QE g^{-1} of dry plant biomass. According to Studzińska-Sroka et al. (2024), TPC ranged from 41.36 to 62.53 mg GAE g^{-1}, while TFC ranged from 5.52 to 10.79 mg QE g^{-1} in leaves of twelve *S. nigra* varieties collected at two growth stages, the beginning of vegetation and flowering. Similarly, TPC in *S. nigra* berry and flower infusions varied from 19.81 to 23.90 mg GAE g^{-1} and 15.23 to 35.57 mg GAE g^{-1} of dry weight, respectively (Viapiana and Wesolowski, 2017). In *S. nigra*, 154 compounds were identified in leaves and 194 in inflorescences, including alkanes, acids, and terpenoids, with mandelonitrile being the predominant compound in leaf extracts (Basas-Jaumandreu and de las Heras, 2019). D'Abrosca et al. (2001) tested the allelopathic activity of 19 compounds isolated from *S. nigra*, including cyanogenins, flavonoids, lignans, and phenolic glycosides, and reported both inhibitory and stimulatory effects on germination and root length of monocotyledonous and dicotyledonous species.

Conclusion

Sambucus nigra extracts strongly inhibited lettuce growth, with high concentrations completely suppressing germination, seedling development and seedling vigour index. The biomass showed high TPC and TFC, highlighting its chemical richness and potential role in allelopathic activity. *S. nigra* is a promising species for further allelopathic research on various target species, including weeds.

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ORIGINAL SCIENTIFIC PAPER

Soil health monitoring in a coastal polder affected by salinization

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Summary
Soil salinization represents a risk to agricultural productivity in coastal polders. This study assessed soil health indicators in the Vidrice polder in the Neretva River Delta (Croatia), an agricultural area exposed to salinization risk. Electrical conductivity (EC), pH, soil organic matter, nutrients (N and P), and particle size distribution were analyzed. Soil samples were collected from two depths (0-20 cm, 20-50 cm) at 12 sampling points across the polder. The results showed low EC values in the topsoil (mean 0.26 dS m^{-1}), indicating non-saline conditions, while higher values in the subsurface layer reached 0.97 dS m^{-1} suggesting possible salt accumulation. Correlation analysis indicated that EC was positively correlated, with clay content, SOM, and total nitrogen, while a negative relationship was observed between EC and pH. In addition, EC measurements at one location in April and September 2024 suggested seasonal differences in salt distribution along the soil profile, with lower topsoil EC after the rainy period and higher topsoil EC after the dry summer period.

Keywords: soil salinity, soil health, delta, coastal areas, climate change

Introduction

Soil health is increasingly recognized as a key factor for sustainable agricultural production and ecosystem stability (European Commission, 2021). The concept refers to the capacity of soil to function as a living system that supports plant growth, maintains environmental quality, and promotes biological activity. Soil degradation processes such as erosion, compaction, and salinization threaten this capacity and can significantly reduce agricultural productivity.

Soil salinization is considered one of the most serious global threats to soil quality, particularly in coastal and deltaic environments. It occurs due to the accumulation of soluble salts in the soil profile as a result of natural processes and human activities, including irrigation practices, seawater intrusion, and shallow groundwater tables (Lovrinović et al., 2021; Romić et al., 2012). High salinity levels disrupt plant-water relations, limit nutrient availability and negatively affect soil structure and biological processes. River deltas often contain fertile soils that support intensive agriculture but are highly sensitive to salinization. The Neretva River Delta in southern Croatia is one of the most important agricultural regions in the country and represents a typical Mediterranean coastal agroecosystem exposed to salinity risk.

Within the delta, agricultural production is organized in hydromeliorated polders where drainage canals and pumping systems regulate groundwater levels. However, despite these management measures, seawater intrusion and saline groundwater continue to pose a threat to soil quality and long-term agricultural productivity.

The aim of this study was to evaluate properties that are related to the soil health in the Vidrice polder and investigate their spatial and vertical variability under conditions of potential salinization. Particular attention was given to the relationships between electrical conductivity (EC), soil pH, organic matter content, texture and nutrient composition (N and P).

Material and Methods

Study area

The research was conducted in the Vidrice polder located in the Neretva River Delta in southern Croatia (fig. 1). The delta represents the largest cultivated lowland in the Croatian coastal region and covers approximately 12,000 ha, with about 5,200 ha used for agriculture (Zovko et al., 2013; Racetin et al., 2020). The area is characterized by hydromorphic soils formed from alluvial and marine sediments (Zovko et al., 2013). According to the WRB classification, the dominant soil types include Calcic Fluvisols, Molic Fluvisols and Gleysols. These soils are influenced by shallow groundwater and are sensitive to salinization processes due to seawater intrusion and irrigation practices. Agriculture is the dominant land use in the delta and includes extensive tangerine plantations and vegetable production. However, the sustainability of these agricultural systems is increasingly threatened by salinity processes affecting both soil and groundwater.

Soil sampling and laboratory analysis

Soil sampling was conducted in October 2024 within the framework of the AI4SoilHealth project. A total of 12 sampling points were selected across the Vidrice polder (fig. 1) using a project-developed sampling algorithm that considered the soil map, distance from the sea, and the drainage canal network. At each location, soil samples were collected from two depths, 0–20 cm and 20–50 cm, resulting in 24 samples in total. In addition to the spatial survey, seasonal variability of EC in the Vidrice area was assessed using data from an established soil monitoring station. At this soil monitoring station, samples were collected in April and September 2024 at four depths (0–25, 25–50, 50–75 and 75–100 cm) in order to evaluate vertical changes in EC within the soil profile.



Figure 1. Soil sampling points in the Vidrice polder.

Samples were collected using a hand auger and transported to the laboratory for further preparation and analysis. After air-drying, samples were sieved and homogenized before laboratory measurements. The following soil properties were analyzed according to the LUCAS methodology:

- electrical conductivity (EC)
- soil pH
- soil organic matter (SOM)
- particle size distribution (soil texture)
- total nitrogen (N)
- available phosphorus (P)

Statistical analysis was performed to evaluate relationships between soil parameters.
GIS techniques were used to map and visualize the spatial distribution of selected soil properties. Spatial interpolation was performed using the inverse distance weighted (IDW) method, which estimates values at unsampled locations based on the distance from known sampling points (ESRI, 2024).

Results and Discussion

The results indicate that soils in the Vidrice polder currently show relatively low levels of salinity in the surface layer. The mean EC value was 0.26 dS m^{-1}, which falls within the range typical for non-saline soils. However, higher EC values were observed in the subsurface layer, where values reached up to 0.97 dS m^{-1}.
The vertical distribution of EC suggests that salts may accumulate at deeper layers due to the influence of saline groundwater and limited drainage capacity (fig. 2). Such conditions indicate a potential risk of salinization under prolonged dry periods or insufficient leaching (Romić et al., 2008, Srzić et al., 2020).

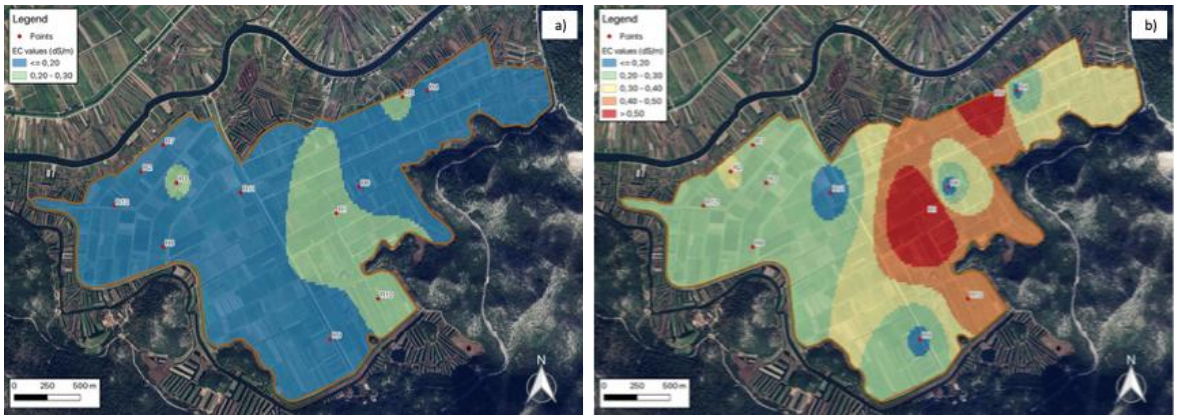

Figure 2. Spatial distribution of soil electrical conductivity (EC) (a) in the topsoil (0-20 cm) (b) in the subsoil (20-50 cm), Vidrice polder (sampled october, 2024.)

To assess seasonal variability of salinity conditions in the Vidrice area, EC values from an established soil monitoring station were analyzed for two climatically contrasting periods, April and September 2024 (Table 1).

Table 1. Seasonal variation of EC (dS/m) at different depths in the Vidrice polder

Depth, cm	EC, dS m^{-1}		Difference between EC in April and in September
	Apr 2024	Sep 2024	
0-25	1.7	2.3	+35.3 %
25-50	3.7	4.1	+10.8 %
50-75	6.8	5.9	-13.2 %
75-100	5.5	4.9	-10.9 %

In April, at the end of the rainy season, EC values were lower in the topsoil (1.7 dS m^{-1} at 0-25 cm), indicating salt leaching due to rainfall. The highest EC value was observed at 50-75 cm (6.8 dS m^{-1}), suggesting that soluble salts moved to deeper layers with percolating water.
In September, after the dry summer period, EC increased in the topsoil (2.3 dS m^{-1}), indicating salt accumulation near the surface. This increase may be related to irrigation with more saline water and to capillary rise and evaporation that transport salts toward the soil surface. In deeper layers (50-100 cm), EC values were lower than in April (5.9 and 4.9 dS m^{-1}), indicating upward salt movement.
Soil pH values ranged from 6.6 to 8.4, with most soils classified as slightly to moderately alkaline. These values are typical for soils developed on carbonate sediments, which dominate in the Neretva River Delta.
The analysis also revealed significant relationships between soil properties (fig. 3). EC showed positive correlations with clay content, soil organic matter and total nitrogen. This suggests that fine-textured soils tend to retain higher amounts of salts and nutrients due to their greater surface area and adsorption capacity.
In contrast, negative correlations were found between soil pH and parameters such as organic matter and total nitrogen. Soils with higher pH values generally contained lower levels of organic matter and nutrients.

R	EC (dS/m)	pH (H2O)	pH(KCl)	Humus, %	Olsen Phosphorus (mg/100g)	Total nitrogen (%)	Clay (<0.002 mm)	Silt (0.002-0.06 mm)	Sand (0.06-2.0 mm)
EC (dS/m)	1.0								
pH (H2O)	-0.4	1.0							
pH(KCl)	-0.2	0.9	1.0						
Humus, %	0.3	-0.8	-0.9	1.0					
Available phosphorus (mg/100g) 0-20	-0.1	-0.2	-0.4	0.9	1.0				
Total nitrogen (%) 0-20	0.6	-0.8	-0.9	1.0	0.6	1.0			
Clay (<0.002 mm)	0.6	-0.8	-0.8	0.7	0.6	0.8	1.0		
Silt (0.002-0.06 mm)	-0.2	0.1	0.1	0.1	-0.2	-0.1	-0.1	1.0	
Sand (0.06-2.0 mm)	-0.4	0.6	0.6	-0.7	-0.4	-0.6	-0.8	-0.5	1.0

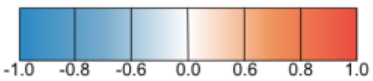

Figure 3. Correlation matrix of soil properties.

Conclusion

The results of this study indicate that soils in the Vidrice polder are currently classified as non-saline, although signs of salt accumulation in the subsurface layer suggest a potential future risk of salinization. Seasonal analysis of EC showed variability between sampling periods. After the rainy season, salts tend to leach into deeper soil layers, reducing EC values in the surface horizon. During dry periods, evaporation and irrigation may promote upward salt movement, increasing EC values in the upper soil layer. Electrical conductivity showed strong relationships with soil texture, organic matter and nutrient content, indicating that fine-textured soils are more prone to salt accumulation. These findings highlight the importance of continuous soil

monitoring in coastal agricultural regions. Monitoring soil health indicators provides valuable information for early detection of salinity risks and supports the development of sustainable soil management strategies in coastal polders.

Acknowledgment
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ORIGINAL SCIENTIFIC PAPER

Effect of aromatic plants aqueous extracts on phytopathogenic fungi of the genus *Fusarium* spp.

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Summary

The *Fusarium* genus consists of economically important phytopathogens that cause various diseases such as rots and wilts, which significantly reduce crop yield and quality. This study evaluated the effect of aqueous extracts of basil (*Ocimum basilicum*) and marjoram (*Origanum majorana*) on the mycelial growth and sporulation of the pathogens *Fusarium culmorum* and *Fusarium oxysporum*. The extracts were applied at 10% and 20% concentrations, and their effect were assessed after 48, 96, and 144 hours. All treatments increased mycelial growth, with a stronger effect observed in the early phases of incubation and at higher extract concentrations. In contrast, the extracts caused a significant reduction in sporulation in both pathogens. The greatest reduction was observed with 10% marjoram extract. In addition, *F. oxysporum* was more sensitive to the treatments than *F. culmorum*. These results indicate a selective antifungal effect, with a stronger impact on sporulation while promoting mycelial growth. The reduction in sporulation suggests a potential role in limiting pathogen spread and disease development.

Keywords: marjoram, basil, *Fusarium culmorum*, *Fusarium oxysporum*, sporulation

Introduction

The genus *Fusarium* is a cosmopolitan group of fungi (Esmaili & Sharifnabi, 2023; Bakker et al., 2018) and is among the most important phytopathogenic fungal genera (Dean et al., 2012). It was first described in 1809 (Chandra et al., 2011). Species of this genus can infect more than 150 plant species (Bahadur, 2021), causing diseases such as wilt, rot, and blight, and representing a significant problem in agricultural and natural ecosystems due to their diverse infection strategies (Ma et al., 2013). Among the most important species, *Fusarium culmorum* (W.G. Sm.) Sacc. and *Fusarium oxysporum* Schltdl. are notable because they cause diseases such as root rot and wilt, and can significantly affect both the quantity and quality of yields, especially in cereals.

Their presence in the soil increases with continuous cultivation without crop rotation, and they can also be transmitted by infected seeds (Yanashkov et al., 2016). Disease control is most often based on the application of fungicides, but this approach can lead to the development of pathogen resistance and, more importantly, negatively affect the environment and human health (Wang et al., 2014; Naqvi et al., 2025). Antifungal agents of plant origin are increasingly being investigated and are divided into essential oils and plant extracts (Fan et al., 2018). Previous studies have reported that essential oils show strong activity against various phytopathogenic fungi (Parikh et al., 2021; Petrović et al., 2025; ElSakhawy et al., 2025), but their wider application is often limited by high production costs. Plant extracts, including aqueous extracts, also demonstrate antifungal potential against various phytopathogenic fungi (Singh et al., 2024; Upadhayay et al., 2024). Therefore, they are increasingly emerging as a simpler and more accessible alternative. The aim of this study was to evaluate the antifungal effect of aqueous

extracts of basil and marjoram at different concentrations on the growth and sporulation of *F. culmorum* and *F. oxysporum*.

Material and Methods

The investigation of the inhibitory effect of aqueous extracts from the above-ground parts of basil (*Ocimum basilicum* L.) and marjoram (*Origanum majorana* L.) on the pathogens *F. culmorum* and *F. oxysporum* was conducted at the Central Analytical Unit of the Faculty of Agrobiotechnical Sciences in Osijek, Department of Phytopathology. Pure cultures of both pathogens were selected from the isolate collection of the Department of Phytopathology.

Preparation of plant aqueous extracts

Aromatic herbs, basil (*O. basilicum*) and marjoram (*O. majorana*), were grown in 2024 without fertilisers or pesticides and collected at the flowering stage at the experimental site of the Faculty of Agrobiotechnical Sciences Osijek. The above-ground parts of the plants were dried at 30 °C and ground into powder using a mill. For extract preparation, 20 g of ground, dry plant material was added to 100 mL of distilled water. The samples were incubated for 24 hours. After incubation, the mixtures were filtered through filter paper to obtain 20% (200 mg/mL) extracts. Part of the extract was diluted by adding an equal volume of distilled water to obtain a 10% (100 mg/mL) extract.

Antifungal test

The pathogens *F. culmorum* and *F. oxysporum* were grown on PDA medium under controlled conditions at 22 ± 2 °C with a 12-hour light /12-hour dark regime. Ten-day-old cultures were used for inoculation. A total of five treatments for each pathogen were included in the study: aqueous extracts of basil at 10% and 20% concentrations, aqueous extracts of marjoram at 10% and 20%, and a negative control (distilled water). Each treatment consisted of four replicates. The obtained extracts were first centrifuged at 600 rpm for 5 minutes. The supernatant was then decanted, further filtered using a 0.22 µm syringe filter, and the filtrate was collected in sterile laboratory beakers. According to the Anžlovar (2020) method, a 1 mL aliquot of the extract at a specified concentration from each laboratory beaker was evenly spread onto the surface of the PDA medium using a sterile laboratory stick. The Petri dishes were then left in a laminar flow chamber until the surface of the medium was completely dry. Pure culture isolates of *F. culmorum* and *F. oxysporum* were cut with a sterile circular cutter (4 mm diameter) from the active edge of the colony and placed at the centre of pretreated Petri dishes. Inoculation was carried out under sterile conditions in a laminar flow hood, after which the dishes were incubated at 22 ± 2 °C and 70% relative humidity. The antifungal effect of all treatments was assessed by measuring the mycelial growth diameter at 48, 96, and 144 hours of incubation. Based on the values obtained, the percentage of growth inhibition was calculated using the following formula:

$$I(\%) = \frac{C-T}{C-4} \times 100$$

Where:
C – diameter of mycelial growth in control (distilled water)
T – diameter of mycelial growth in extract treatment (4 mm
- diameter of mycelial inoculation disk)

After measuring mycelial growth on the seventh day after inoculation, the effect of the extracts on pathogen sporulation was assessed. Conidia were washed from the colony surface and suspended in 100 mL of sterile distilled water. The concentration of conidia for each treatment

was determined using a Neubauer hemocytometer and expressed as the number of conidia per mL of suspension.

Statistical analysis of data

Statistical analysis was conducted using SAS Software 9.1.4 (SAS Institute Inc., 2003) with analysis of variance (ANOVA). Differences between means were determined by Tukey's HSD test at a significance level of $p \leq 0.05$. Results are presented as mean ± SD (n = 4).

Results and Discussion

According to the results obtained, basil and marjoram extracts at both concentrations increased mycelial growth of *F. culmorum* (Table 1). This response may indicate a nutrient-related or hormetic effect, where compounds from the plant extracts promote fungal growth (Barreto et al., 2002; Anžlovar et al., 2020). In the early phase of incubation (48 and 96 h), the higher concentration of both extracts had a statistically significantly stronger effect. At the final measurement (144 h), no significant differences between concentrations were observed, and the increase in growth for both extracts was −39.06%. These results are consistent with the findings of Ozcan et al. (2011), who reported increased fungal growth following the application of basil extracts, as well as sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.), on the pathogens *Alternaria alternata* and *Aspergillus parasiticus*.

Table 1. Effect of aromatic plant extracts and concentration on mycelial growth inhibition (%) of *F. culmorum* at different incubation times

Aromatic extract	Incubation time (h)	Concentration (mg/mL)	
		100	200
Basil (*Ocimum basilicum* L.)	48	-20.70 ± 4.49 a	-49.41 ± 5.20 b
	96	-4.00 ± 10.06 a	-9.74 ± 5.91 a
	144	-36.91 ± 2.58 a	-39.06 ± 0.00 a
Marjoram (*Origanum majorana* L.)	48	-21.29 ± 10.55 a	-48.36 ± 7.97 b
	96	-7.13 ± 4.40 a	-12.70 ± 0.00 b
	144	-39.06 ± 0.00 a	-39.06 ± 0.00 a

Values are presented as mean ± standard deviation (n = 4). Different letters within the same row indicate significant differences between concentrations (Tukey's HSD test, p < 0.05). Negative values indicate a stimulating effect on mycelial growth.

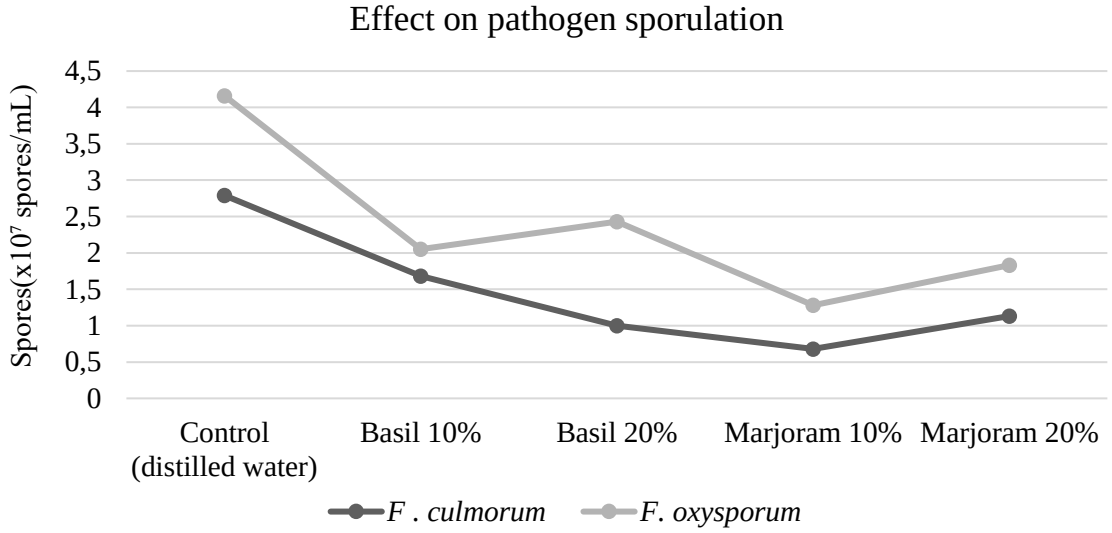
The application of both extracts to the pathogen *F. oxysporum* produced different effects on mycelial growth (Table 2). Basil extract exhibited a promoting mycelial growth on the pathogen at both concentrations, with the most pronounced effect at 100 mg/mL (−7.25%). In contrast, Kocic-Tanackov et al. (2011) reported that basil extracts at higher concentrations completely inhibited various *Fusarium* species, including *F. oxysporum*. Plant extracts from different species of the *Lamiaceae* family, such as thyme (*Thymus vulgaris* L.), summer savory (*Satureja hortensis* L.), spearmint (*Mentha spicata* L.), and rosemary (*R. officinalis*), demonstrate strong antifungal activity against various pathogens (Muna et al., 2025; Ozcan et al., 2011; Abkhoo & Jahani, 2017).

Statistically significant differences were found between the concentrations of marjoram extract throughout the incubation period (Table 2). The lower concentration of marjoram extract did not increase the mycelial growth, but showed a very weak inhibition, ranging from 0.68% to 3.65%. At higher concentrations, marjoram extract showed a promoting effect on mycelial growth, with the highest value being −11.88%. Similar results were reported by Muna et al. (2025), who found that marjoram extract had only a minimal inhibitory effect on *F. oxysporum* even at higher concentrations.

Table 2. Effect of aromatic plant extracts and concentration on mycelial growth inhibition (%) of *F. oxysporum* at different incubation times

Aromatic extract	Incubation time (h)	Concentration (mg/mL)	
		100	200
Basil (*Ocimum basilicum* L.)	48	-4.05 ± 1.56 a	-5.41 ± 3.12 a
	96	-7.25 ± 2.92 a	-3.19 ± 10.58 a
	144	-3.00 ± 3.43 a	-0.86 ± 6.63 a
Marjoram (*Origanum majorana* L.)	48	0.68 ± 4.05 a	-6.76 ± 2.70 b
	96	1.74 ± 1.11 a	-11.88 ± 2.22 b
	144	3.65 ± 2.56 a	-5.15 ± 4.35 b

Values are presented as mean ± standard deviation (n = 4). Different letters within the same row indicate significant differences between concentrations (Tukey's HSD test, $p < 0.05$). Negative values indicate a stimulating effect on mycelial growth, while positive values indicate inhibition.



Graph 1. Effect of aromatic plant extracts on *Fusarium* spp. sporulation

Although basil and marjoram extracts promoted mycelial growth in both pathogens, their effect on sporulation was more pronounced, as all treatments significantly reduced spore production compared to the control (Graph 1). These results are particularly significant, as sporulation is essential for infection and disease spread. Reducing the number of spores can significantly limit the spread of the disease, even when mycelial growth is not inhibited. For the pathogen *F. culmorum*, the number of spores in the control was 2.79×10^7 spores/mL, while in all treatments it was reduced to $0.68–1.68 \times 10^7$ spores/mL. Similar results were observed with the pathogen *F. oxysporum*, in which the control had 4.16×10^7 spores/mL, whereas the treatments reduced sporulation to $1.28–2.43 \times 10^7$ spores/mL. The greatest reduction in sporulation for both pathogens was observed when marjoram extract was applied at 10%. According to Silva et al. (2023), the application of various plant extracts reduced sporulation of *F. oxysporum* spores by 55–90%.

Conclusion

Basil and marjoram extracts exhibited a selective antifungal effect, inhibiting sporulation while promoting mycelial growth. They significantly reduced sporulation, which is important for limiting infections and the spread of diseases caused by these pathogens. It was also observed that the reduction in sporulation was more pronounced in *F. oxysporum* than in *F. culmorum*. These findings suggest that the tested extracts may have potential in plant protection strategies

aimed at reducing disease spread rather than directly inhibiting fungal growth. The observed promoting effect on mycelium growth opens the possibility of research in another direction, namely, examining the effect of these extracts on beneficial microorganisms, such as antagonistic species of the genus *Trichoderma*. Such an approach could contribute to the development of alternative strategies for the biological control of plant diseases.

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ORIGINAL SCIENTIFIC PAPER

The potential of intercropping of maize and chickpeas in the agroecological conditions of Slavonia in 2025

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Summary
In conditions of elevated global temperatures, maize is subject to numerous stresses, which leads to lower soil water, increased use of fertilizers and a higher risk of weeding in production, all of which can be reduced by the biodiversity on fields through joint sowing ("Intercropping"), in this case in combination with chickpeas. Chickpeas is a crop not so adopted in the continental part of Croatia, but with better growing characteristics in warmer and dryer conditions than other legumes. In order to test the potential of intercropping of maize and chickpeas, an experiment was started in 2025, with main treatments of standard sowing and twin row sowing for sole crops and their intercropping. Results are showing higher grain yield for maize in both intercropping sowings (in standard seeding +1100 kg ha^{-1}, in twin row +470 kg ha^{-1}), whereas chickpeas showed in both seeding treatments same level of grain yield, both in sole sowing and intercropping (362 vs, 438 kg ha^{-1} in standard, and 551 vs. 605 kg ha^{-1} in twin row).

Keywords: intercropping, maize, chickpea, standard sowing, twin row sowing

Introduction

Growing maize and chickpeas in intercropping is one of the most effective ways of regenerative agriculture. This combination of grain and legume creates a synergy that directly addresses the challenges of modern climate change. Since maize grows vertically and tall, and chickpeas occupy the lower "floor" of the vegetation, this spatial organization enables better layered absorption and use of light. Maize leaves capture direct sunlight, while chickpeas efficiently use diffused light that passes through the maize canopy. Also, maize serves as a natural shield from the strongest midday sun, providing a reduction of heat stress to more sensitive stages of chickpea development (Mobh et al., 2023). Lower growing chickpeas act as "living mulch" with its dense near-ground canopy, thus reducing the direct evaporation of water from the soil (Das et al., 2017). Due to the shade cast by both crops, the temperature of the surface layer of the soil remains lower, which preserves precious moisture for the root systems of both crops during the critical stages of grain filling. Additionally, there are indications that the deep roots of maize can pull up water from deeper layers (hydraulic lifting), which also benefits the shallow roots of chickpeas on a micro-scale (Santosh et al., 2022). Furthermore, chickpeas in symbiosis with rhizobia fix atmospheric nitrogen (Mirdoraghi et al., 2025). Part of this nitrogen becomes available to the maize immediately, and the greater part remains in the soil after harvest through crop residues. Both crops, due to different root types and higher total biomass per unit area are providing more nutrients for soil microorganisms. The result is a faster formation of stable humus that improves the soil structure (Zhang and Li, 2003). Regarding soil resource competition maize root goes deeper, while the chickpea root is branched in the upper and middle layers (Jat et al., 2025), thus uniformly using resources from the entire soil profile.

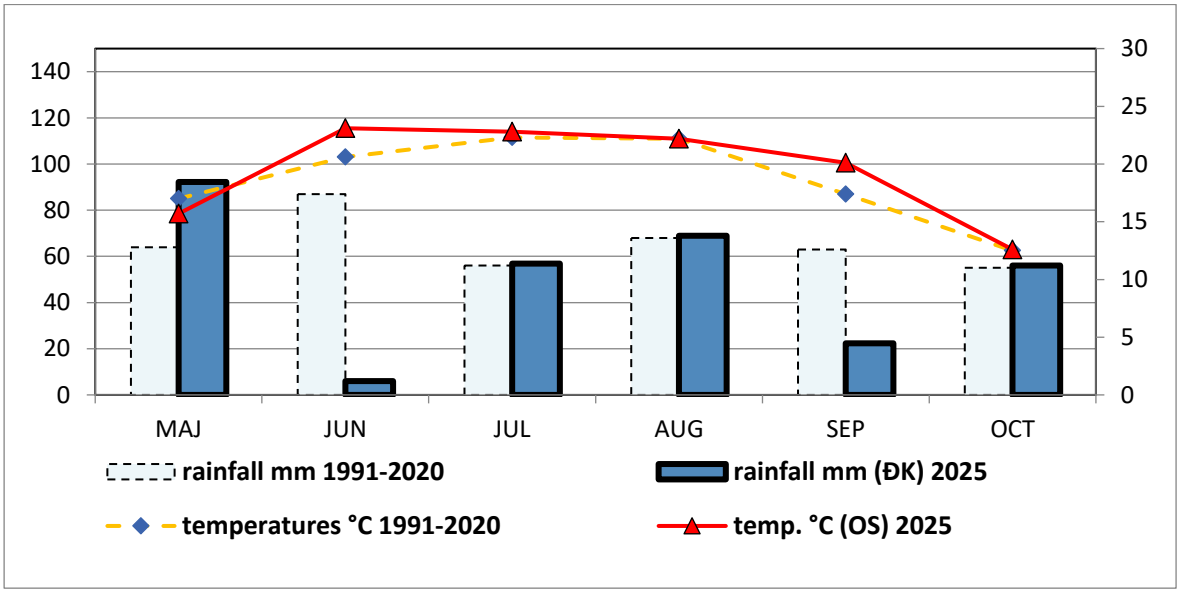
Regarding system resistance to global warming induced more frequent summer droughts and heat waves, combined crops are providing more stable biosystem through biodiversity, especially due to chickpeas extreme resistance to drought. In extremely dry years, where maize can fail, chickpeas ensure a stable yield, thus reducing the economic risk for farmers (Sharma and Banik, 2015). Given the recent weather pattern in northeast Croatia, also known as Slavonia, the research has started in the year 2025, in order to test intercropping of maize and chickpeas in different seeding patterns, plant populations, and nitrogen fertilization.

Material and Methods

The experiment has been set up at three sites in Slavonia (Koritna, Mrzović, and Gorjani) near the town of Đakovo, Croatia. In each experimental field the sowing scheme was a split-split-strip-plot design in three repetitions, with treatments as follow: A) Treatment of the sowing method; STANDARD-standard sowing, with 70 cm distance between rows, one crop over the entire area; TWIN-twin row, zigzag layout of one crop, double row 20 cm apart and 50 cm between rows; ST-inter-intercropping/combined sowing with standard sowing, crop belts containing 6 rows are alternating with chickpeas belts of 6 rows; TW-inter-combined sowing, with a zigzag arrangement of rows, crops belts, comprising of 4 twin rows, are alternating; B) type of crop: S-chickpeas (Bori and Katalin in *desi* type, Twist in *kabuli* type); M-maize (hybrid Hypolito); C) plant density (chickpeas only); 300K-300,000 plants per ha; 500K-500,000 plants per ha, and 650K-650,000 plants per ha; D) fertilization: N-without top dressing (0 kg N ha^{-1}), L-low dose (up to 30 kg N ha^{-1}) and H-high dose (up to 60 kg N ha^{-1}). Statistical analysis has been procured through anova analysis adopted for split-split-strip-plot field setup, and treatments significant by F-test have been compared by LSD test at 0.05 probability level.

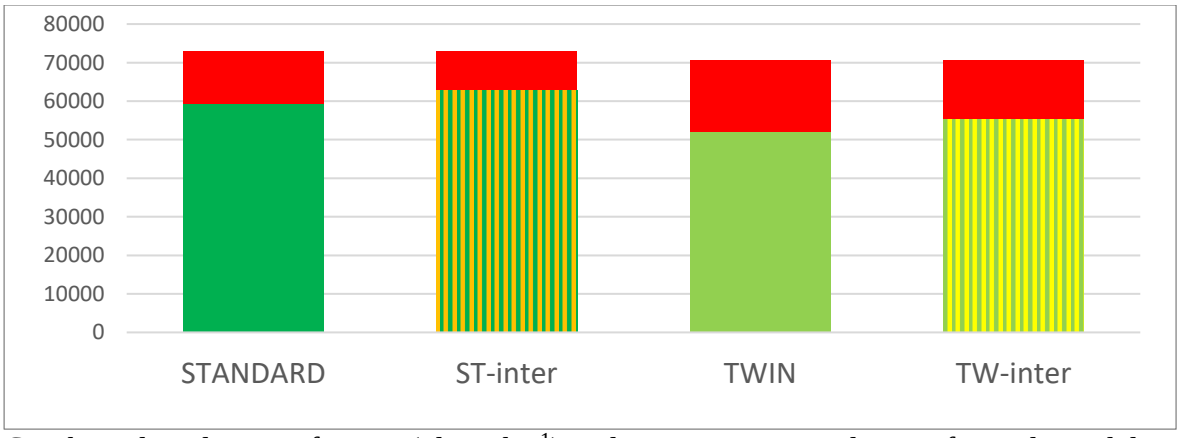
Results and Discussion

For both crops, it was determined that there were no statistically significant differences due to different levels of fertilization, the culprit of which in this vegetation year was the lack of precipitation and high temperature during the vegetation (Graph 1), which made mineral nitrogen from granular fertilizers (KAN) poorly absorbable, as reported by Bista et al. (2025).

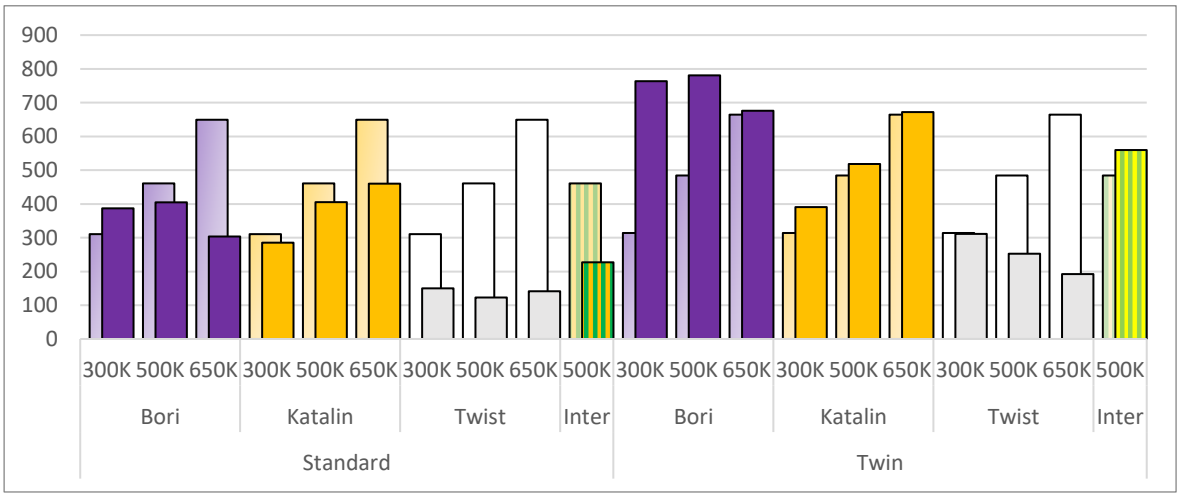


Graph 1. Weather conditions in maize and chickpeas production period, May-October 2025.

This is supported by the experience with the same hybrid from previous years, where it used to produce up to 12 t ha^{-1}, and in this case, the average yield was only 6.95 t ha^{-1} in the STANDARD treatment (Graph 4). Part of the problem was a large reduction of the crop population compared to the planned density due to heavy precipitation immediately after sowing in two of the three locations, followed by a severe drought (almost 60 days without precipitation), which reduced the crop stand from the sown 70 thousand plants to only 59 thousand plants ha^{-1} in the STANDARD treatment. Similar plant stand reduction was observed also by Banaj et al. (2023). The reduction of the stand was an even more pronounced factor influencing the yield in the TWIN treatment, where the combination of soil crust and drought reduced the stand to an even lower 52 thousand plants ha^{-1}. Somewhat better microclimatic conditions, as well as more available direct light in the maize canopy, created for intercropping of maize and chickpeas, resulted in higher yields compared to sole crops, both in standard and twin row sowing, which is observed earlier by Ogrizovic (2015) and Banaj et al. (2024). In the case of maize, this difference amounted to a significant 1100 kg ha^{-1}. In the case of twin row sowing, combining yielded about 470 kg ha^{-1} higher yield, also a significant difference, but not so impressive, because the twin row arrangement in itself provides maize with better initial growth conditions, a larger vegetation area per individual plant, less competition with neighboring crops, greater shading of the total area under the crop due to the zig zag arrangement, thus better water conservation and stronger competition against weeds. In this case, the decisive factor for the lower yield of twin row maize was a more strongly reduced assembly due to a stronger bowing at the very beginning of the growing season.

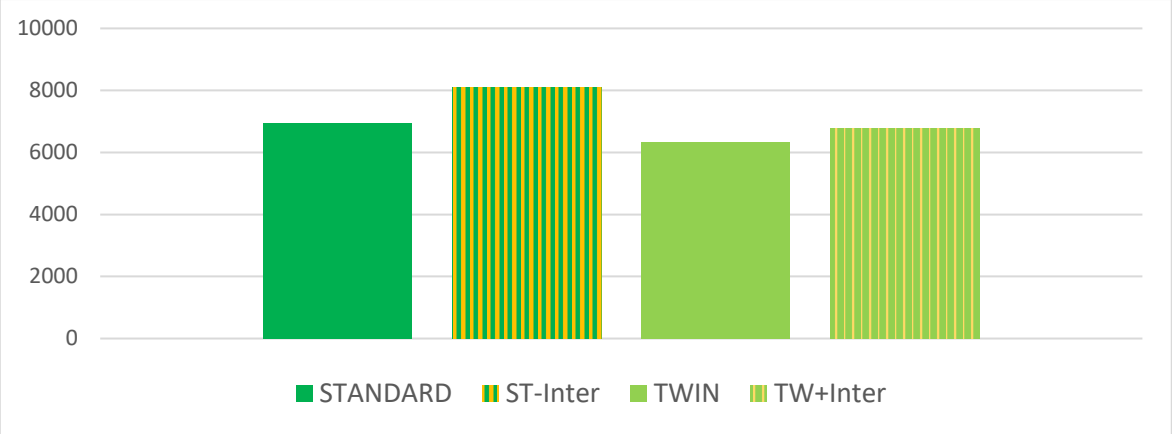


Graph 2. Plant density of maize (plants ha^{-1}); red part represents reduction from planned density



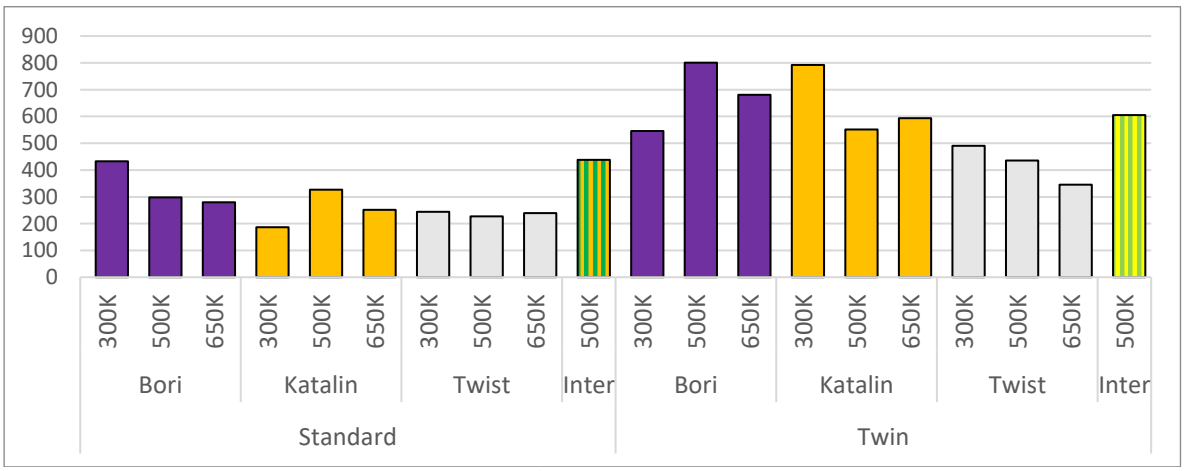
Graph 3. Plant density reduction of chickpeas (plants ha^{-1}) from planned densities

For chickpeas cultivars, yields of only 200 to slightly over 800 kg ha^{-1} were recorded (Graph 5), which is a rather low yield (the literature average is around 2000 kg ha^{-1}, with irrigation up to 5-6 t ha^{-1}), certainly caused by the torrent rainfall immediately after sowing, forming soil crust and reducing plant density (Graph 3), and the long-term drought afterwards. The differences in yields are a combination of different assortment (smaller, *desi* type of chickpeas in cultivars Bori and Katlin, against larger, *kabuli* type of cultivar Twist), grain shape and impact on sowing (more irregular *desi* types with more edges, weaker adhesion to the sowing plate of the pneumatic seeder), stronger branching (cultivar Bori) and better compensation of the assembly (cultivar Katalin).



Graph 4. Grain yield of maize (kg ha^{-1}, standardized grain moisture of 14%).

In most cases, the most favorable yield in 2025 turned out to be a set of 300,000 plants ha^{-1}, while in a few cases, increasing the set to 650,000 plants ha^{-1} significantly reduced the yield (from 150 to 200 kg ha^{-1}). It is also visible that all chickpea cultivars significantly increased the yield when using the twin row layout, in some cases by more than 100% (e.g. cultivar Bori in a group of 500 thousand plants ha^{-1}).



Graph 5. Grain yield of chickpeas (kg ha^{-1}, standardized grain moisture of 14%).

It is also noticeable that by combining chickpeas with maize in the standard sowing method, the yield of the cultivar Katalin in the same plot increased by 1/4 of the yield, while in the twin row sowing method it recorded a slightly higher level of yield in the same plot, and that the twin row method of combining was more favorable for the same cultivar than the standard

method of combining, by almost 160 kg ha^{-1}. Similar positive effects of intercropping of maize and chickpeas were observed by Hasan et al. (2021) and Maitra et al. (2024).

Conclusion

Based on the results of the research on the intercropping of maize and chickpeas during 2025 in Slavonia conditions, it can be recommended that intercropping is more favorable for achieving higher yields of maize, while the same cultivation method records the same or slightly higher yield of chickpeas in comparison with sole crops. Also, the use of the twin row method is recommended, especially for the establishment of the chickpea crop, with the need for more detailed research and settings of the seeding devices of the pneumatic seeders, chickpeas cultivars and their plant density.

Acknowledgment

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PRELIMINARY COMMUNICATION

Occurrence and environmental risk assessment of pesticide residues in the Danube-Tisa-Danube canal system

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Summary
This study investigates pesticide contamination in the Danube–Tisa–Danube canal system (DTD), a key hydrological network in northern Serbia. While agricultural sources are well studied, urban wastewater remains an underexplored pathway of pesticide input. In autumn 2025, 40 water samples were collected from 14 locations and analyzed using a validated LC–MS/MS method for the determination of 350 pesticides, revealing the presence of multiple pesticide residues. Due to the system's connection with the Danube River, these contaminants may pose risks to aquatic ecosystems. Detected pesticide concentrations were used to perform an environmental risk assessment and evaluate potential impacts on aquatic organisms.

Keywords: pesticide residues, irrigation canal, risk assessment, aquatic organisms

Introduction

The Danube–Tisa–Danube (DTD) hydrosystem's primary canal network comprises 20 canals with a total length of 929 km. Although its full organisational structure is not described in detail, the system includes the Karaš river as an integral component. The DTD canals serve multiple functions: draining excess water from approximately 760,000 ha, discharging surplus water into the Danube and Tisa rivers, providing irrigation, and supplying water for fisheries, industry, and settlements, while also supporting navigation. Overall, the hydrosystem drains 1,060,000 ha of land in Serbia, 159,000 ha in Hungary, and 285,000 ha in Romania, handling a water flow of 512 $m^3 s^{-1}$, with irrigation possible on 510,000 ha. The navigable portion of the canals extends for 600.6 km (Milovanov, 1972; Horvat et al., 2021).
Previous studies (Agarski et al., 2024) have shown that the DTD hydrosystem, designed for drainage and irrigation, contains residues of various pesticides. As such, it may present an ecological burden for aquatic organisms within the system, as well as for rivers receiving its waters, particularly the Danube and Tisa. The continuous presence of pesticides highlights the need for monitoring their concentrations and assessing potential risks to local aquatic ecosystems before the water is used for irrigation or other purposes.
The risk assessment framework consists of four key steps (Hristozov et al., 2016):

1. Hazard identification: evaluating whether a substance causes harmful effects based on *in vivo*, *in vitro*, and *in silico* studies, epidemiological data, or other sources, in accordance with good laboratory practice principles.
2. Dose–response assessment: establishing the relationship between exposure levels and adverse effects, i.e. the doses that cause such effects.
3. Exposure assessment: estimating population exposure by considering demographic characteristics, exposure pathways, sources, and exposure duration.
4. Risk characterization: integrating hazard and exposure information to determine whether the risk as acceptable or unacceptable.

The research presented in this study is based on the detection of pesticide residues in the DTD canal system, with the primary aim of assessing the ecological risk to aquatic organisms. The detected concentrations were used to calculate Toxicity Exposure Ratios (TERs), providing insight into potential risks for representative species from different trophic levels, including fish, invertebrates, algae, and aquatic plants.

Material and Methods

Sample collection: water sampling from the DTD canal system was conducted during October and November 2025., following the guidelines for the sampling of rivers and streams outlined in SRPS EN ISO 5667-6:2017. Samples were collected from multiple locations (Bečej, Senta, Novi Sad, Sombor, Vrbas, Bačka Palanka, Novi Kneževac, Grabovci, Erdevik, Adaševci, Višnjićevo, Žarkovac, Inđija and Ašanja) within the DTD canal network. The sampling depth was 50 cm. All samples were collected using a telescopic sampler and stored in dark plastic bottles (0.5 L). They were immediately transported to the Laboratory for Biological Research and Pesticides, Faculty of Agriculture, University of Novi Sad, and kept in a freezer until the analysis. The total number of samples was 40.

Pesticide extraction and chromatographyc analysis: The HLB cartridge was conditioned with 5 mL of methanol followed by 5 mL of water. Then, 250 mL of the water sample (spiked with 100 µL mL^{-1} of the internal standard carbofuran-D3) was passed through the cartridge over approximately 30 minutes. The cartridge was rinsed with 5 mL of deionized water and dried with an air stream for 15 minutes. The analytes were eluted with 5 mL of methanol, and the eluate was evaporated under a nitrogen stream. The residue was reconstituted in the mobile phase, filtered through a 0.45 µm filter into an autosampler vial, and subjected to chromatographic analysis. The samples were analysed using a validated liquid chromatography-tandem mass spectrometry (LC–MS/MS) method. Carbofuran-D3 was used as an internal standard. The determination of 280 pesticides was carried out in positive electrospray ionization (ESI+) mode. Method validation was conducted in accordance with SANTE/11312/2021 V2. In total, 40 pesticide residues were detected above the LOQ (0.10 µg L^{-1}).

Risk assessment: the Toxicity Exposure Ratio (TER) approach was applied (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013). Acute and chronic toxicity endpoints, including LC_{50} (or EC_{50}/IC_{50}) and NOEC (No Observed Effect Concentration), were obtained from relevant databases and EFSA systematic reviews. Measured pesticide concentrations at monitoring sites within the DTD hydrosystem were used to calculate TER values. TER values below the defined thresholds were considered indicative of potential unacceptable risk. For fish, such values were interpreted in relation to possible scenario refinements, whereas for algae and crustaceans, any TER below the threshold was considered to indicate unacceptable risk to the aquatic organisms in the DTD hydrosystem. The thresholds were defined as follows: acute toxicity (TER = LC_{50}/PEC, threshold = 100) and chronic toxicity (TER = NOEC/PEC, threshold = 10), where LC_{50} denotes the median lethal concentration (acute toxicity), and PEC the predicted environmental concentration.

Results and Discussion

Using a validated LC-MS/MS multi-residue method covering 280 pesticides, 40 pesticide active supstances were detected above the limit of quantification (LOQ = 0.10 µg L^{-1}), of which 15 were insecticides, 15 fungicides, and 10 herbicides. Many of the analysed samples contained multiple pesticides, highlighting the complexity and extent of contamination in the studied water sources.

A scientifically grounded approach that integrates substance toxicity with exposure levels is referred to as toxicological risk assessment. This process provides insight into population exposure and determines whether these concentrations exceed the reference values, allowing the

risk to be classified as acceptable or unacceptable (Agarski et al., 2024; Woutersen et al., 2020). The application of a validated multi-residue analytical method enabled the detection of a wide range of pesticide residues in the analysed samples. However, a substantial proportion of these detections were below the maximum residue limits (MRLs), indicating generally low contamination levels for many compounds. Within the scope of this study, the environmental risk assessment for aquatic organisms was therefore focused on pesticides detected at higher concentrations, as these are more likely to pose ecological risks. Accordingly, only the most relevant compounds, based on their elevated levels, were considered in the risk characterisation process (Table 1).

Table 1. Minimum and maximum concentrations of the pesticides detected in water samples

	Insecticides		**Fungicides**			**Herbicides**	
Station	**ACT**	**HTP**	**AZX**	**BUP**	**IPC**	**ACL**	**NIC**
Number of samples (n)	40	40	40	40	40	40	40
Positive samples (%)	100	95.0	100	42.5	32.5	32.5	30.0
Minimum concentrations (µg L^{-1})	0.033	0.078	0.016	0.011	0.010	0.013	0.013
Maximum concentrations (µg L^{-1})	2.403	0.802	7.928	3.426	1.078	0.148	0.082
SD	0.52	0.21	1.27	0.88	0.32	0.04	0.02

*ACT - acetamiprid; HTP - chlorantraniliprole; AZX - azoxystrobin; BUP - bupirimate; IPC – iprovalicarb; ACL - aclonifen; NIC – nicosulfuron; SD – standard deviation.

For acute and chronic toxicity assessment, LC_{50} (EC_{50} or IC_{50}) and NOEC values for all the investigated pesticides were obrained from the relevant databases and EFSA reports (EFSA, 2022; Lewis et al., 2016; RAC, 2018; Extoxnet, 1997; Horani and Dogan, 2025; SANCO/2034/2000) (Table 2).

Table 2. Aquatic toxicity endpoints of the investigated pesticides

	Species	*Pimephales promelas*	*Daphnia magna*	*Anabaena flos-aquae*	*Lemna gibba*
	Pesticide				
I	ACT	NOEC = 9.4 mg L^{-1}	EC_{50} = 49.8 mg L^{-1}	EC_{50} > 1.3 mg L^{-1}	EC_{50} > 1.3 mg L^{-1}
	HTP	NOEC = 5 mg L^{-1}	EC_{50} = 0.05 mg L^{-1}	EC_{50} > 100 mg L^{-1}	EC_{50} > 100 mg L^{-1}
F	AZX	LC_{50} = 0.47 mg L^{-1}	EC_{50} = 0.06 mg L^{-1}	EC_{50} = 0.15 mg L^{-1}	EC_{50} = 3.2 mg L^{-1}
	BUP	/	/	/	/
	IPC	LC_{50} > 22.7 mg L^{-1}	EC_{50} > 19.8 mg L^{-1}	EC_{50} > 10 mg L^{-1}	/
H	ACL	LC_{50} > 1.27 mg L^{-1}	LC_{50} > 2.4 mg L^{-1}	/	EC_{50} = 0.06 mg L^{-1}
	NIC	LC_{50} > 1000 mg L^{-1}	EC_{50} > 1000 mg L^{-1}	EC_{50} > 100 mg L^{-1}	/
	TER	**>100 (>10 for NOEC)**	**>100**	**>100**	**>100**

*ACT - acetamiprid; HTP - chlorantraniliprole; AZX - azoxystrobin; BUP – bupirimate; IPC - iprovalicarb; ACL – aclonifen; NIC - nicosulfuron; / - value are not available; I - insecticides, F - fungicides, H - herbicides.

The concentrations of selected pesticides (acetamiprid, chlorantraniliprole, azoxystrobin, bupirimate, iprovalicarb, aclonifen and nicosulfuron) in the aquatic environment, used as input values for the calculation of TERs, indicated a consistently low risk to the tested aquatic organisms. Specifically, the calculated TER values suggest that *Pimephales promelas* (fathead minnow), *Daphnia magna* (water flea), *Anabaena flos-aquae* (cyanobacterium) and *Lemna gibba* (duckweed) are unlikely to experience adverse effects under the measured environmental concentrations. These findings support the conclusion that, at the observed levels, the tested pesticides pose minimal acute or chronic risk to these representative species from different trophic levels within the aquatic ecosystem.

Conclusion

All the calculated TER values were substantially higher than the regulatory trigger value of 100, indicating a low risk of acute toxic effects. Even under this conservative mixture toxicity assessment, no concern regarding acute toxicity to fish species inhabiting the DTD canal system was identified. Overall, the findings suggest that, at the concentrations detected in October-November 2025, acetamiprid, bupirimate, azoxystrobin chlorantraniliprole, iprovalicarb, aclonifen, and nicosulfuron do not pose an acute toxicological risk to aquatic organisms in the studied environment. Nevertheless, continuous monitoring and periodic risk assessment remain essential to ensure long-term ecological safety.

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Growth, antibiotic resistance, and antibacterial potential of *Bacillus* spp. isolated from a karst cave

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Summary
Karst caves are extreme, nutrient-poor ecosystems hosting microorganisms with unique biotechnological potential. This study evaluated growth characteristics, antibiotic resistance, and antibacterial activity of *Bacillus* isolates from Stara Jametina cave (Croatia). Isolates showed broad tolerance to salinity (up to 10% NaCl), pH 6–9, and temperatures of 30–42 °C, indicating ecological flexibility. Most isolates were susceptible to clinically relevant antibiotics, though resistance to macrolides and some glycopeptides was detected. All strains exhibited varying degrees of inhibition against several human and plant pathogens, highlighting cave-derived *Bacillus* as promising sources of antimicrobial agents.

Keywords: carst cave sediment, *Bacillus* spp, growth characteristics, antibacterial potential, antibiotic resistance

Introduction

Karst caves represent extreme and nutrient-limited environments characterized by stable low temperatures, high humidity, limited light, and oligotrophic conditions (Ghosh et al., 2017). Microorganisms inhabiting these ecosystems have evolved unique physiological and metabolic adaptations that enable them to survive under environmental stress. As a result, cave microbiota are increasingly recognized as promising sources of novel bioactive compounds, including antimicrobial agents. Among cave-associated bacteria, the genus *Bacillus* is of particular interest due to its metabolic versatility, spore-forming ability, and capacity to thrive under diverse environmental conditions (Dame et al., 2021). *Bacillus* species are well known for producing a wide range of antimicrobial compounds, such as bacteriocins, lipopeptides, and polyketides, which play an important role in microbial competition and survival. These properties make *Bacillus* isolates valuable candidates for biotechnological applications (Wu et al., 2025). In addition, examining the antibiotic resistance patterns of environmental *Bacillus* strains is important for understanding their ecological roles, intrinsic resistance mechanisms, and potential risks associated with the spread of resistance genes. The growth of *Bacillus* species is strongly influenced by environmental factors such as temperature, pH, salinity, and nutrient availability. Studying these parameters is essential for understanding their ecological adaptability and optimizing conditions for antimicrobial production. However, information on *Bacillus* species isolated from karst cave environments remains limited (Zhu et al., 2021), particularly regarding their growth behavior, resistance profiles, and antibacterial potential under varying environmental conditions. Therefore, this study aims to evaluate the antibiotic resistance patterns, antibacterial activity, and growth characteristics of *Bacillus* species isolated from a karst cave. Understanding these traits will contribute to knowledge of cave microbial ecology and support the exploration of cave-derived *Bacillus* strains as potential sources of novel antibacterial agents.

Material and Methods

Sampling and identification of isolates

Samples were collected in the Stara Jametina cave located in the Dinaric karst region of Croatia, in the vicinity of the hamlet Gornji Krnići (Konjevrate). Sediment samples were collected aseptically from 0-3 cm depth at four sites as pooled samples in 2021 and 2022. All samples were transported to the laboratory at 4 °C. Isolation of bacteria was performed immediately upon arrival on R2A agar (HiMedia, India), and isolates were identified by MALDI-TOF mass spectrometer (Bruker Daltonics, Germany). Mass spectra were processed using the Biotyper software (version 4.1, Bruker Daltonics, Germany). Isolate identification was based on the similarity of the mass spectrum of each isolate to a reference spectrum and is expressed in numerical categories with the log(score) ranging from 0.00 to 3.00. All isolates belonging to *Bacillus* spp. (n= 8) were further analyzed in this study.

Growth in different abiotic conditions

The *Bacillus* spp. were cultivated in BHI broth (Biolife, Italy) under varying conditions of salinity (NaCl 1%, 3%, 5%, 7%, and 10%), pH (5–10), and temperature (30, 35, 37, 40, and 42 °C) for 7 days. Growth was assessed daily based on changes in turbidity. A control culture, grown without NaCl and under optimal growth conditions (pH 7, T=30 °C), was included for comparison.

Antibiotic susceptibility testing

Antibiotic susceptibility to five antibiotic classes (carbapenems, fluoroquinolones, glycopeptides, macrolides, and oxazolidinones) was assessed using the disk diffusion method (CLSI, 2020). Isolates were streaked onto BHI agar to obtain monocultures and incubated at 30 °C for 24 h. Bacterial suspensions were prepared by transferring one to three colonies into 5 mL sterile saline (0.85% NaCl) to match the 0.5 McFarland standard, verified with a DEN-1 densitometer (Biosan, Latvia), corresponding to 1.5×10^8 CFU mL^{-1}. Suspensions were evenly inoculated onto Mueller–Hinton agar (Biolife, Italy) using sterile swabs. After absorption, antibiotic disks (BD BBL™ Sensi-Disc™, USA) were applied, and plates were incubated at 37 °C for 24 h. Tests were performed in duplicate. Inhibition zones were measured with a caliper, expressed as mean ± standard deviation (mm), and interpreted as resistant (R), intermediate (I), or susceptible (S) according to EUCAST guidelines (2022).

Antibacterial analysis

The antibacterial activity of *Bacillus* spp. against potential pathogens (*E. coli* ATCC 25922, *B. cereus* DSM 6791, *S. aureus* DSM 20231, *L. innocua* ATCC 33090, *A. tumefaciens* DSM 30205, *E. amylovora* DSM 50901, *P. syringae* DSM 10604, *S. enterica* subsp. *enterica* DSM 14221, and *B. cinerea* DSM 877) was evaluated as described by Domig et al. (2014). In brief, pathogens were spotted in the center and at the edge of BHI agar, and overnight *Bacillus* spp. cultures were streaked as two parallel lines (2 cm apart) on BHI agar plates using sterile swabs (in duplicate) and incubated at 30 °C for 24 h. After incubation, antibacterial activity of *Bacillus* spp. was evaluated by comparing the diameters of pathogen colonies grown between the streak lines with those of control colonies at the plate edge. Variations in colony density between treated and control samples were also considered.

Results and Discussion

Bacillus isolates obtained from the karst cave exhibited considerable physiological diversity and adaptability to varying environmental conditions (Table 1). Most strains grew in the presence of 1–5% NaCl, while *Bacillus licheniformis*, *B. pumilus*, *B. megaterium*, and *B. mycoides* tolerated concentrations up to 10%, indicating moderate halotolerance. Such tolerance may reflect adaptation to mineral-rich cave environments where osmotic stress can occur. Growth was predominantly observed at pH 6–9, suggesting a preference for neutral to slightly alkaline conditions typical of karst ecosystems. Limited growth at pH 5 indicates that

acidic environments may restrict the proliferation of these isolates. All strains grew within the temperature range of 30–42 °C, confirming their mesophilic nature and demonstrating resilience to moderately elevated temperatures. This broad tolerance to environmental factors highlights the ecological flexibility of cave-derived *Bacillus* species and supports their survival in oligotrophic habitats (Dame et al., 2021). Antibiotic susceptibility testing showed that all isolates were sensitive to carbapenems (imipenem and meropenem) and fluoroquinolones (ciprofloxacin) (Table 2), suggesting the absence of resistance to these clinically important antibiotics. However, resistance to macrolides, particularly erythromycin, was common among several isolates, and some strains also exhibited resistance to glycopeptides and lincosamides. Notably, *B. megaterium* showed resistance to linezolid, whereas *B. mycoides* remained susceptible to all tested antibiotics. The presence of antibiotic resistance in environmental isolates is not unexpected, as resistance genes can naturally occur in microbial communities as part of competitive survival strategies rather than solely as a consequence of clinical antibiotic use (Ahn et al., 2025). These findings emphasize the importance of monitoring environmental reservoirs for resistance traits while also suggesting that the isolates generally possess low multidrug resistance. The antibacterial assays revealed that the cave-derived *Bacillus* strains inhibited a broad spectrum of both human and plant pathogens (Table 3). Consistent activity against *Staphylococcus aureus* and *Pseudomonas syringae* was observed in most isolates, while several strains demonstrated strong inhibition of *Listeria innocua* and *Escherichia coli*. Additionally, activity against important phytopathogens such as *Erwinia amylovora* and *Agrobacterium tumefaciens* indicates potential applications in agricultural biocontrol. The variability in inhibitory effects among isolates likely reflects differences in the production of antimicrobial metabolites, including lipopeptides and bacteriocins, which are commonly associated with *Bacillus* species (Markelova et al., 2025). The combination of environmental tolerance and antibacterial activity suggests that karst cave *Bacillus* strains are well adapted to competitive and resource-limited ecosystems, where the production of antimicrobial compounds provides a selective advantage. Importantly, their ability to inhibit diverse pathogens while maintaining relatively low levels of antibiotic resistance enhances their potential for biotechnological exploitation. Overall, these results support the growing recognition of cave environments as valuable reservoirs of microorganisms with promising antimicrobial properties and reinforce the need for further studies focused on the identification and characterization of their bioactive compounds.

Table 1.Growth of *Bacillus* spp. in varying range of NaCl, pH and temperature

Isolate	**NaCl (%)**					**pH**					**Temperature (°C)**				
	1	**3**	**5**	**7**	**10**	**5**	**6**	**7**	**8**	**9**	**30**	**35**	**37**	**40**	**42**
Bacillus sp.	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+
Bacillus sp.	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
Bacillus sp.	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+
B. licheniformis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. pumilus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. cereus	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+
B. megaterium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. mycoides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = visible growth, - = no visible growth

Table 2. Suscpectibilty of *Bacillus* spp. to diffrent classes of antibiotics

Isolate	Carbapenems		Fluoroquinolones		Glycopeptides	Macrolides & lincosamides		Oxazolidinones
	IPM-10	MEM-10	CIP-5	LVL-5	VA-5	E-15	CC-2	LZD-10
Bacillus sp.	S	S	S	R	S	S	S	S
Bacillus sp.	S	S	S	R	S	S	S	S
Bacillus sp.	S	S	S	R	S	R	S	S
Bacillus licheniformis	S	S	S	R	R	R	S	S
Bacillus pumilus	S	S	S	R	S	R	S	S
Bacillus cereus	S	S	S	R	S	R	S	S
Bacillus megaterium	S	S	S	S	S	S	S	R
Bacillus mycoides	S	S	S	S	S	S	S	S

S = susceptible, R = resistant; IPM-10 = imipenem (10 µg), MEM-10 = meropenem (10 µg), CIP-5 = ciprofloxacin (5 µg), LVL-5 = levofloxacin (5 µg), VA = vancomycin (5 µg), E-15 = erythromycin (15 µg), CC-2 = clindamycin (2 µg), LZD-10 = linezolid (10 µg)

Table 3. Antagonist activty of *Bacillus* spp. to human and plant pathogens

Isolate	*Escherichia coli*	*Salmonella enterica* subsp. *enterica*	*Staphylococcus aureus*	*Bacillus cereus*	*Listeria innocua*	*Pseudomonas syringae*	*Erwinia amylovora*	*Agrobacterium tumefaciens*
Bacillus sp.	++	-	+	+	-	+	-	+
Bacillus sp.	-	+	+	+	+	+	-	+
Bacillus sp.	+	+	+	+	-	+	+	+
Bacillus licheniformis	+	-	+	-	+	+	+	+
Bacillus pumilus	+	+	+	-	++	+	+	+
Bacillus cereus	+	+	+	+	+	+	-	-
Bacillus megaterium	-	-	+	-	++	+	+	+
Bacillus mycoides	-	+	+	+	++	+	-	+

++ = pronounced inhibition (difference in bacterial colony diameter between streak lines and control colonies at the plate edge=2-4 mm); + = weak inhibition (difference in bacterial colony diameter between streak lines and control colonies at the plate edge=1-2 mm); - = no inhibition (difference in bacterial colony diameter between streak lines and control colonies at the plate edge < 1 mm)

Conclusion

Karst cave *Bacillus* isolates showed strong tolerance to varying salinity, pH, and temperature, reflecting their ecological adaptability. While some antibiotic resistance was observed, most strains were susceptible to key antibiotics. The isolates exhibited notable antibacterial activity against human and plant pathogens, highlighting their potential as sources of bioactive compounds for biotechnological and agricultural applications.

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A preliminary study of antibacterial activity of plant extracts against *Xanthomonas arboricola* pv. *pruni*

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Summary
Some plants have been recognized as a valuable source of compounds for treating infectious diseases. Leaves and aerial parts were collected from three plant species that were examined: *Hypericum perforatum* L., *Centaurium erythraea* Rafn, and *Helichrysum italicum* (Roth) G. Don to prepare 8 different plant extracts for *in vitro* testing. Plant extracts were used to test the antibacterial activity against reference bacteria *Xanthomonas arboricola* pv. *pruni* CFBP 2535 (producer CIRM, France) using the disk diffusion method. Preliminary results showed that extracts of *Helichrysum italicum*, both cultivated and wild, derived from leaves and aerial parts, exhibited a positive antibacterial response at the highest concentrations against sample Gram-negative bacteria Xap. The leaf extracts of wild *H. italicum*, with a total phenolic content of 63.804 mgGAE/mL, demonstrated the highest antibacterial activity, with an inhibition zone of 11.0 ± 0.3 mm. However, *Centaurium erythraea* and *Helichrysum perforatum* plant extracts showed no antibacterial activity against the tested bacteria.

Keywords: ethanolic extracts, reference bacteria, phenolic compounds

Introduction

Xanthomonas arboricola pv. *pruni* (*Xap*) is classified among Gram-negative bacteria (EPPO/CABI 1997), recognized as an A2 quarantine organism by the EPPO. *Xanthomonas colonies appear convex and glistening, with a yellow pigment that is not soluble in water (Hayward et al., 1965¸Osdaghi, 2022).* Additionally, entry can take place through stomata in the following growing season (Zaccardelli *et al.*, 1995; Lolić *et al* 2024). This pathogen poses a significant threat to fruit production and vitality of the trees. The control of *Xap* mainly relies on the appropriate cultural practices and the selection of resistant cultivars. The use of chemicals plays an important role, streptomycin and oxytetracycline have been shown to effectively suppress *Xap* (Dhanvantari *et al.*, 1978). The increasing resistance of bacteria to antibiotics has emerged as a significant concern in recent years, primarily due to the widespread and unnecessary usage antibiotics. Since the use of antibiotics for plant protection is prohibited in the EU, there is a growing need for alternative treatments such as plant extracts. Medicinal plants possess their biological properties due to the presence of phenolic compounds. Antibacterial mechanism of phenolic compounds can be explained as interaction with membrane proteins, changing their physicochemical and biological properties (Brudzynski *et al.*, 2015), changes in membrane function and permeability by interaction with lipids and inhibition of enzyme synthesis (Reygaert, 2014), inhibition of cell energy production and cell wall damage (Lobiuc *et al.*, 2023). The aim of this study was to conduct an analysis of medicinal plants to determine their biological effects.

Material and Methods

Plant extracts

Different extracts were prepared from three plant species: *Hypericum perforatum* L., *Centaurium erythraea* Rafn, and *Helichrysum italicum* (Roth) G. Don. according to Kukavica *et al.* (2025) (Table 1). Leaves and aerial parts were collected separately for each plant species. Well air-dried plants were grounded to powder (2.5 g) and homogenized with 50 mL of 80% ethanol, then sonicated for 5 min. and stirred for 30 min. on a magnetic stirrer. Finally, the homogenate was filtered through filter paper and designated as E1. The remaining pellet on filter paper went under the same protocol one more time and was designated as E2. Two homogenates were put together (E1+E2) and used as a stock sample in further analyses. The stock solution was diluted with 80% ethanol to achieve solutions with concentrations of phenolic compound of 500 μg/mL, 1000 μg/mL, and 1500 μg/mL. All plant extracts were freshly prepared and stored in a fridge at 4°C until use.

Table 1. Plant extracts used for antimicrobial test

1. Cultivated *H. italicum* (leaf)	5. Cultivated *H. italicum* (aerial part)
2. Wild *C. erythraea* (leaf)	6. Wild *H. perforatum* (leaf)
3. Wild *H. perforatum* (aerial part)	7. Wild *H. italicum* (aerial part)
4. Wild *H. italicum* (leaf)	8. Wild *C. erythraea* (aerial part)

TPC – Total phenolic content

Total phenolic content (TPC) was determined according to Singleton and Rossi (1965). Reaction mixture contained 100 μL of plant extract and 450 μL of Folin-Ciocâlteu reagent (in ratio 1:3, Folin-Ciocâlteu: dH_2O). After incubation for 5 min at room temperature, 1 M 450 μL of Na_2CO_3 was added to the reaction mixture, vortexed, and incubated in a dark place for 60 min. Absorbance was measured at 724 nm. Quantification was done using a standard curve for gallic acid and expressed as mg GAE/mL (gallic acid equivalent).

Bacterial culturing

Reference bacteria *X.a.*pv. *pruni* CFBP 2535 (producer CIRM, France) was used to test the antibacterial activity of plant extracts. An amount of 100 μL of reference bacteria was spread onto a freshly prepared agar plate to be cultivated for further analysis. Bacterial cultures were grown on Phyto Yeast Extract Glucose Agar (HiMedia Laboratories Pvt. Ltd.), a non-selective medium recommended for subculturing suspected *Xanthomonas* spp. Preparation of nutrient media was done according to the manufacturer's recommendation (32.5 g into 500 mL dH_2O, autoclaved for 15 min. at 121ºC). The nutrient medium in a Petri dish was divided into 8 fields for different plant extracts, as well as for their respective solutions in different concentrations (500 μg/mL, 1000 μg/mL, and 1500 μg/mL). After inoculation using bacterial suspensions, sterile filter paper discs (10 mm) were placed and plant extracts (15-20 μL) added according to Costa *et al.* (2025). Another Petri dish, also divided into 8 fields, was for control: antibiotic amoxicillin, 80% ethanol, and a positive control. Commercially available antibiotic amoxicillin was used as the control, prepared by dissolving 10 mg of amoxicillin (Amoksicilin Hemofarm) in 1 mL of 80% ethanol. Since the plant extracts were diluted in ethanol, 80% ethanol was used as a control to confirm that the observed effects were attributable to the extracts themselves rather than to the ethanol. Every cultivation was repeated in 3 dish and read after 2-3 days.

Results and Discussion

TPC – Total phenolic content

Figure 1 displays the total phenolic content (TPC) for all eight ethanolic extracts. TPC decreases in the following order: aerial parts of wild *H. italicum* (746.287 mg GAE/g DW), wild *H.*

perforatum (513.165 mg GAE/g DW), cultivated *H. italicum* leaves (345.098 mg GAE/g DW), wild *H. perforatum* leaves (328.207 mg GAE/g DW), wild *H. italicum* leaves (290.885 mg GAE/g DW), cultivated *H. italicum* aerial parts (210.033 mg GAE/g DW), wild *C. erythraea* aerial parts (132.475 mg GAE/g DW), and wild *C. erythraea* leaves (119.168 mg GAE/g DW). Literature indicates that TPC content varies depending on the analysed plant part: for methanolic extracts of *H. italicum*, it is 271.36 ± 5.3 mg GAE/g DE in aerial parts, 221.04 ± 6.7 mg GAE/g DE in flowers, and 153.12 ± 4.6 mg GAE/g DE in leaves (Talić et al., 2019). Additionally, the vegetative season influences phenolic content (Makarova et al., 2021; Piatti et al., 2023). Ethanolic extracts of *H. perforatum* show TPC values for flowers ranging from 99.6 to 298.2 mg GAE/g DW, and for aerial parts, 64.4 ± 1.5 mg GAE/g DW, plus 10.366 ± 0.966 mg CTE/g extract (Makarova et al., 2021; Alahmad et al., 2022; Ayvvaz et al., 2023). Methanolic extracts vary between 37.91 ± 0.13 and 68.16 ± 1.85 mg GAE/g extract (Piatti et al., 2023). TPC for ethanolic extracts of *C. erythraea* (aerial parts) was 13.17 ± 1.14 mg GAE/g (Ivanišová et al., 2017). According to Šiler et al. (2014), methanolic extracts of the same plant species contained 5.18 ± 0.6 mmol GAE/100 mg DW in the aerial part and 1.82 ± 0.13 mmol GAE/100 mg DW in the root.

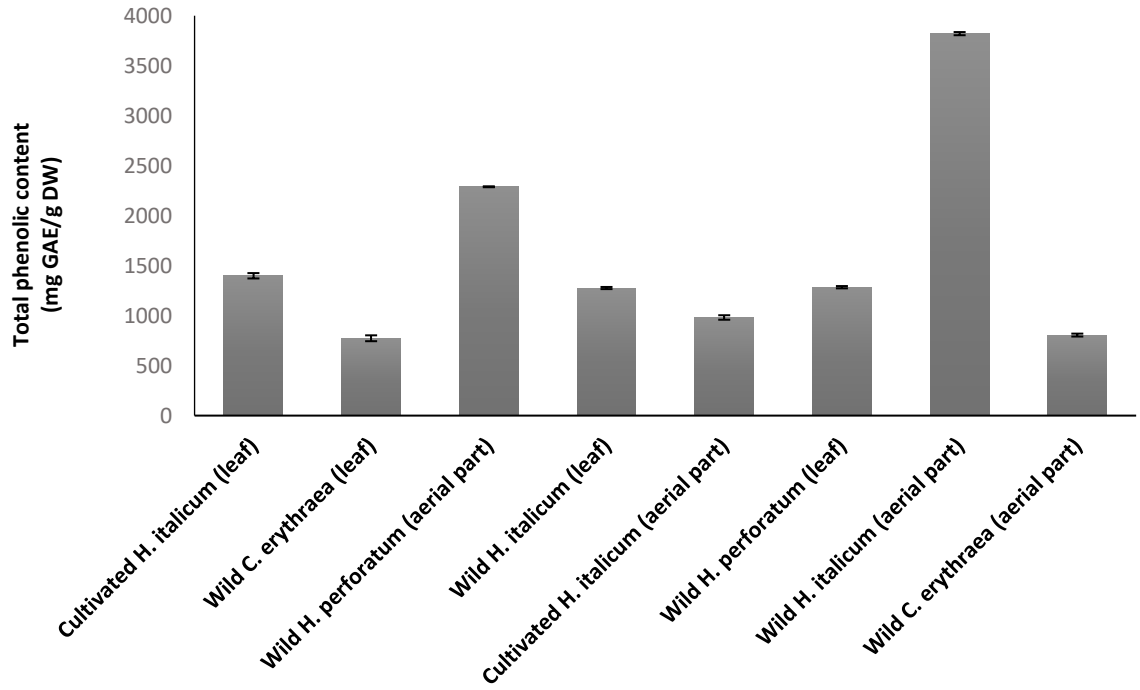

Figure 1. Total phenolic content of eight ethanolic extracts.

Antimicrobial activity

Although the experiment was done with stock solutions and dilutions of different plant extracts (500 µg/mL, 1000 µg/mL, and 1500 µg/mL), the results were obtained with stock solutions. The leaf of wild *H. italicum* (extract 4) demonstrated antibacterial activity against *Xap* at a TPC content of 290.885 mg GAE/g DW. The zone of inhibition around the filter paper was 11.0 ± 0.3 mm. Also, stock solution of leaf extract of cultivated *H. italicum* (1), aerial part of cultivated *H. italicum* (5), and aerial part of wild *H. italicum* (7) show a similar potential antibacterial effect. Leaf extract of cultivated *H. italicum* (1) had a zone of inhibition of approximately 6.0 ± 0.3 mm, extract of aerial part of cultivated *H. italicum* (5) had approximately 4.0 ± 0.3 mm and extracts of aerial part of wild *H. italicum* (7) had approximately 7.0 ± 0.3 mm. However, an inhibition zone was not detected in wild *C. erythraea* (leaf and aerial part) and wild *H. perforatum* (leaf) against *Xap*. The experiment was considered successful since 80% ethanol

and positive control both had no inhibition zone detected, while antibiotics amoxicillin had significant inhibition zone detected. (Figure 2).



Figure 2. The inhibition zones of (1) cultivated *H. italicum* (leaf), (4) wild *H. italicum* (leaf), (5) cultivated *H. italicum* (aerial part) and (7) wild *H. italicum* (aerial part). Petry dish with controls: AB- antibiotics amoxicillin, Et- 80% ethanol, K-positive control.

The study demonstrated that *H. italicum*, cultivated and wild, leaf and aerial part of the plant, had a positive antibacterial response at the highest concentrations (stock) tested against the sample Gram-negative bacteria Xap CFBP 2535. However, lower concentrations of plant extracts (500 µg/mL, 1000 µg/mL, and 1500 µg/mL) did not show adequate antibacterial activity. Additionally, the study's findings indicated that *C. erythraea* and *H. perforatum* plant extracts showed no antibacterial activity against representative Gram-negative bacteria Xap at the experimented concentrations. The results also revealed considerable variability in total phenolic content among the analyzed extracts, with the highest values recorded in aerial parts of wild *H. italicum*, suggesting a possible relationship between phenolic compounds and antibacterial activity. However, since not all extracts with high phenolic content demonstrated inhibitory effects, it is likely that specific compounds or synergistic interactions, rather than total phenolic content alone, contribute to antibacterial activity.

Conclusion

Ethanolic extracts of selected medicinal plants differ significantly in their biochemical composition and antibacterial potential against the phytopathogenic bacterium *Xap*. Among the tested plants, *H. italicum* showed pronounced antibacterial activity, particularly in both cultivated and wild forms, and in extracts obtained from leaves and aerial parts. The observed inhibitory effects were evident only at the highest (stock) concentrations, indicating a concentration-dependent response. In contrast, extracts of *C. erythraea* and *H. perforatum* did not exhibit antibacterial activity under the tested conditions. These results suggest that H. italicum represents a promising source of natural antibacterial compounds with potential application in the control of Xap and related phytopathogenic bacteria.

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ORIGINAL SCIENTIFIC PAPER

The influence of essential oils on mycelial growth, sporulation, and conidial germination of *Fusarium oxysporum* Schltdl.

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Summary

The fungus *Fusarium oxysporum* is a complex of pathogenic species that cause disease in many cultivated plants worldwide, with yield and quality losses often being economically significant. Finding alternative methods of protection against pathogens is a research priority today, as efforts to reduce the use of chemical fungicides are increasingly evident. The aim of the study was to examine the volatile effects of essential oils of anise, pine, cinnamon bark, clove, sweet orange, thyme, fennel, rosemary, and eucalyptus on mycelial growth, conidia production, and germination. The essential oils were applied at concentrations corresponding to oil such that the volume fraction of oil in the air was 0.02%, 0.1%, 0.2%, and 0.3%, while sterile distilled water was used as a negative control. Essential oils of thyme, clove, and cinnamon bark completely inhibited fungal growth at all applied quantities. Pine oil and sweet orange oil had the weakest effect on mycelial inhibition, stimulated conidial production, and their germination ranged from 35.48% to 61.29%.

Keywords: essential oils, volatile effect, *Fusarium oxysporum*, antifungal activity

Introduction

The *Fusarium oxysporum* species complex represents a group of genetically different, but morphologically very similar species that cause diseases in more than 120 economically important plant species. Consequently, the damage it causes ranks fifth among the most significant plant pathogens worldwide (Dean et. al., 2012; Eddel-Hermann & Lecomte, 2019). Reducing the use of plant protection products by 50% by 2030 is one of the EU's key objectives. To achieve this, given the substantial losses caused by harmful organisms in plant production, it is necessary to research and identify new ecologically acceptable but sufficiently effective alternatives. Essential oils, whose antifungal activity has been recognised for centuries, and confirmed by numerous studies (Chouhan et al., 2017; Zulu et al., 2023; Abhijeeta & Panigrahy, 2025; Siber et al., 2025).

The aim of this research was to determine the influence of nine essential oils on the growth of the fungus *Fusarium oxysporum* Schltdl., as well as on the production and germination of its conidia. The results obtained may serve as a starting point for further research and the development of effective and environmentally friendly biofungicides.

Material and Methods

The study of the volatile effects of essential oils on the growth of mycelium of the phytopathogenic fungus *F. oxysporum* was conducted at the Central Agrobiotechnical Analytical Unit of the Faculty of Agrobiotechnical Sciences Osijek. The experiment was set up in three replicates using the disk diffusion method (Edris & Farrag, 2003). Nine essential oils

were used: cinnamon bark (*Cinnamomum zeylanicum* Blume), fennel (*Foeniculum vulgare* Mill.), sweet orange (*Citrus aurantium* L. var. *dulcis* L.), clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry), mountain pine (*Pinus mugo* Turra.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), eucalyptus (*Eucalyptus globulus* Labill.), and star anise (*Illicum verum* Hook. f.), in quantities such that the volume fraction of oil in the air was 0.02%, 0.1%, 0.2% and 0.3%.

A 4 mm-diameter 7-day-old mycelial slice is placed in the centre of 65 mL Petri dishes containing 15 mL of PDA (Potato-dextrose agar). The selected essential oil is applied to the lid on sterile 7 mm filter paper to achieve the planned oil volume fraction in the air, as specified in the research plan. The Petri dishes are incubated in a climatic chamber at 22°C ± 1°C, with 70% relative humidity and a light regime of 12 hours light and 12 hours dark. Mycelial growth is measured 168 hours after inoculation.

After measuring mycelial growth, the strength of sporulation was assessed by preparing a conidial suspension in 100 mL of sterile distilled water and determining the concentration of conidia per mL of suspension for all experimental variants using a haemocytometer. Conidia germination was determined by taking 20 µL of the prepared suspension for each experimental treatment and spreading it onto PDA medium in Petri dishes. The Petri dishes were placed in a climatic chamber at 22 °C with a light regime of 12 hours light and 12 hours dark. After 24 hours, the number of germinated conidia was determined from a sample of 3 x 20 conidia. Conidia with germ tubes equal to or longer than the length of the conidia were considered germinated.

Statistical analysis of the data was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The strongest inhibitory effect on the growth of *F. oxysporum* mycelium after 168 hours of incubation, regardless of the amount applied, was observed with the essential oils of cinnamon bark, cloves, and thyme, which completely inhibited mycelium growth at all tested amounts. Previous studies also indicate a strong inhibitory effect of these essential oils on various phytopathogenic fungi (Parikh et al., 2021; Siber et al., 2025). The weakest antifungal effect was observed with the oils of sweet orange (inhibition of mycelial growth between 8.11% and 21.64%) and pine (inhibition between 11.27% and 49.14%). The results are shown in Table 1. The weak antifungal effect of these oils on some phytopathogenic fungi i salso reported by Tanović et al. (2005), Švitek et al. (2024), and Siber et al. (2025). On the other hand, Velázquez-Nuñez et al. (2013) found a strong antifungal effect of sweet orange oil on the growth of *Aspergillus flavus* L. Further analysis of the results showed that, in most cases, as the amount of essential oils applied increased, mycelial growth significantly decreased. This finding is consistent with the research of Slim et al. (2017), Zulu et al. (2023), Brajković et al. (2024), and Siber et al. (2025).

The concentration of spores was determined for all experimental variants in which mycelium developed (Table 2). Pine essential oil had a highly stimulating effect on conidia production, with the number of conidia ranging from 10.5 x 10^7 (volume fraction of oil in the air 0.1%) to 15.7 x 10^7 (0.02% and 0.3%), compared to 4.4 x 10^7/mL in the control variant. Conidia production was 50% or more lower than in the control when fennel oil was applied at a volume fraction of 0.3%, and for anise and rosemary oils at volume fractions of 0.2% and 0.3%. A strong antifungal effect of various essential oils on the spore production of some phytopathogenic fungi was also reported by Ferreira et al. (2013), Zulu et al. (2023), Grgić et al. (2024), and Siber et al. (2025).

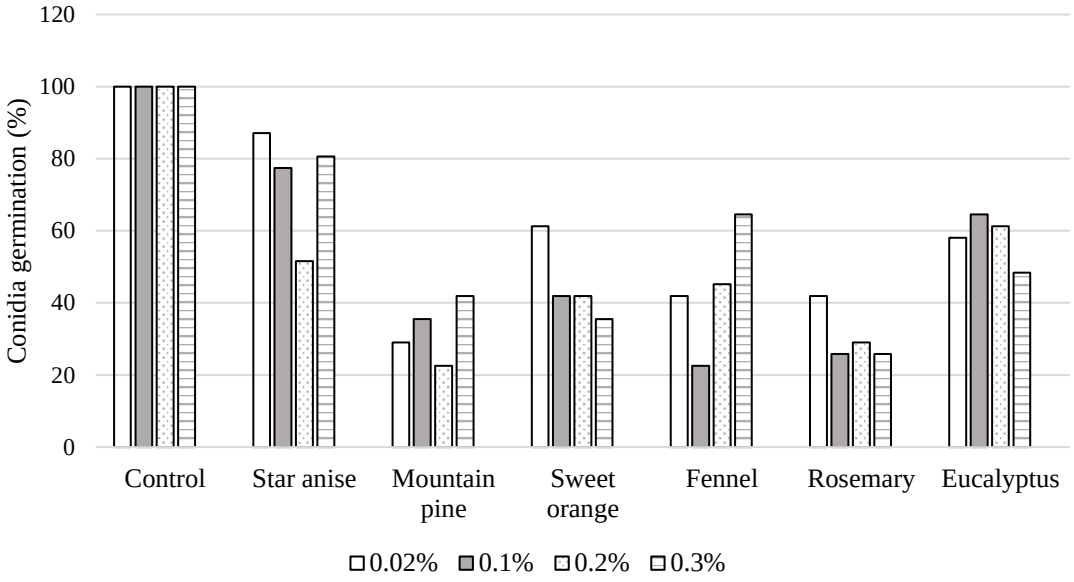
Table 1. Inhibitory effect of essential oils on the growth of *F. oxysporum* mycelium

Inhibition (%)					
Essential oil	Volume fraction of essential oil in air (%)				**LSD 0.05**
	0.02	0.1	0.2	0.3	
Star anis	26.60 ± 10.33	54.55 ± 0.00	66.73 ± 0.00	70.33 ± 2.07	**9.92**
Mountain pine	11.27 ± 4.69	21.64 ± 2.07	49.14 ± 38.66	36.52 ± 4.13	**36.92**
Cinnamon bark	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	**0.00**
Clove	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	**0.00**
Sweet orange	8.11 ± 2.07	7.21 ±1.35	16.68 ± 2.34	21.64 ± 2.07	**3.75**
Thyme	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	**0.00**
Fennel	33.81 ± 5.47	40.57 ± 3.12	52.75 ± 4.75	60.41 ± 3.12	**7.99**
Rosemary	14.87 ± 2.07	38.77 ± 2.82	49.14 ± 3.58	54.55 ± 14.12	**14.22**
Eucalyptus	10.82 ± 2.82	14.87 ± 5.12	28.4 ± 2.82	30.66 ± 1.56	**6.28**
Control (pure PDA)	0.00 ± 1.56	0.00 ± 1.56	0.00 ± 1.56	0.00 ± 1.56	
LSD 0.05	**7.18**	**3.90**	**21.18**	**8.40**	

Table 2. Spore concentration (x10^7/ml) of *F. oxysporum* depending on the applied essential oil and the volume fraction of oil in the air

Volume fraction of oil in the air (%)	**Essential oil**				
	Star anise	Mountain pine	Fennel	Sweet orange	Rosmary
0.02	8	15.7	5.3	6.7	4.6
0.1	2.8	10.5	5.9	3.6	2.9
0.2	1.7	12.1	3.5	6.5	1.8
0.3	1.3	15.7	1.6	5.4	2.2
Control (pure PDA)	4.4				

The ability of the fungus to cause infection depends, among other factors, on the number of spores formed and their germination. The average percentage of germinating conidia compared to the control (100%) ranged from 22.58% (fennel, oil volume fraction in air 0.1%) to 87.10% (star anise, oil volume fraction in air 0.02%) (Graph 1). The negative impact of essential oils on spore germination has also been reported by Gemeda et al. (2014), Karaca et al. (2017), and Zulu et al. (2023). According to these authors, spore germination can be reduced by a few per cent up to 100%, depending on the type of fungus and the type and amount of essential oil applied.



Graph 1. Influence of essential oils on *F. oxysporum* conidia germination

Conclusion

By testing the antifungal effect of essential oils on the fungus *F. oxyspoum*, it was found that the effect depends on both the type of essential oil and the amount applied. The strongest antifungal effect was observed with thyme, clove, and cinnamon bark oils, which completely inhibited fungal growth, while pine and sweet orange oils had the weakest effect. Compared with the negative control (pure PDA), pine oil stimulated spore production, whereas anise, rosemary, and fennel oils reduced conidial production by more than 50 %. Conidia formed in the presence of essential oils showed a lower germination rate, ranging from 12.90% to 77.42%.

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Session 4

Biodiversity Conservation

ORIGINAL SCIENTIFIC PAPER

Biochemical characterization of pepper (*Capsicum annuum* L.) accessions from the Gene Bank of the Republic of Srpska

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Summary
Pepper is an important vegetable crop, with numerous landraces in the Balkans adapted to local conditions. This study analysed 13 accessions from the Gene Bank of the Republic of Srpska to evaluate their nutritional value and breeding potential. Field trials in 2022 assessed pH, soluble solids, phenolics and flavonoids in ripe fruits. Significant variation was found in all traits except pH. Accession GB00863 showed the highest soluble solids and phenolic content, while GB00026 had the highest flavonoids. Results highlight the nutritional potential and genetic diversity of local landraces, supporting their conservation and use in breeding for improved food quality and sustainability.

Keywords: Capsicum spp., pH, soluble solids, phenolics, flavonoids

Introduction

Pepper (*Capsicum spp*.) is one of the most important vegetable crops worldwide, valued for its culinary and nutritional qualities as well as its significant agro-economic role. In the Balkan region, pepper cultivation has a long-standing tradition, with numerous local landraces developed through decades of adaptation to specific regional agroecological conditions. The pepper fruit is primarily consumed as fresh vegetable or dried as a spice, but its range of applications is very broad, including the food, pharmaceutical, and cosmetic industries. (Milenkovic et al., 2009)

Most cultivated varieties today belong to the species *Capsicum annuum*, which, according to genetic and archaeobotanical evidence, was first domesticated approximately 6,000 years ago in present-day Mexico (Taitano et al., 2019). Archaeological findings of fruit and seed remains from the Tehuacán Valley indicate that peppers were part of the human diet and were subjected to selection during the early Preclassic period of Mesoamerica. Following domestication, pepper spread globally, adapting to diverse environments and developing considerable morphological and genetic variability.

Autochthonous Balkan pepper varieties represent a valuable genetic resource. Todorova et al. (2024) analysed 21 accessions of *Capsicum annuum* from this region, identifying "kapia" and "pumpkin-shaped" types characterized by high productivity (up to 0.74 kg per plant), resistance to aphids and thrips, as well as elevated levels of total polyphenols and antioxidant compounds. These findings underscore the potential of local varieties for both conventional and organic production systems, as well as their importance for the conservation of regional agrobiodiversity.

Pepper holds significant nutritional value, its fruits are rich in bioactive compounds, including carotenoids (capsanthin, β-carotene, lutein), phenolic compounds, organic acids, and vitamin C. A recent study by Kolašinac et al. (2025) analysed carotenoid content in fresh fruits and in ajvar, a traditional pepper-based product, across four genotypes. The results confirmed that

substantial levels of beneficial bioactive compounds are retained even after thermal processing, highlighting the nutritional benefits of these products.
The aim of our research was to evaluate the biochemical characteristics of selected pepper (*Capsicum annuum*) accessions conserved in the Gene Bank of the Republic of Srpska. Specifically, the research focused on assessing the variability in key fruit quality parameters, including pH, total soluble solids, total phenolic content, and total flavonoid content. By analysing these biochemical traits, the study sought to identify accessions with superior nutritional and functional properties and to highlight their potential value for breeding programs.

Material and Methods

The research was carried out on 13 pepper accessions obtained from the Gene Bank of the Republic of Srpska (Table 1.) These accessions were originally collected from various locations, including Romanovci (Gradiška), Donje Crnjalovo (Bijeljina), Petrovo, Kostajnica, Vrela (Petrovo), Karanovac (Petrovo), Konopljišta (Petrovo), Dugo Polje (Srbac), Vakuf (Gradiška), and Bistrica (Banja Luka). One accession lacked collection location data. The studied accessions exhibited diverse fruit shapes, including elongate, triangular, almost round, blocky, and campanulate forms. Fruit colors varied widely among the accessions, ranging from dark red, red, lemon-yellow to orange-yellow, and lemon-yellow to red hues. This diversity in morphological traits highlights the rich genetic variability preserved within the Gene Bank and offers valuable material for breeding and nutritional studies.
The experiment was conducted during the 2022 growing season. Containerized seedlings were produced under standard agricultural technology in an unheated glass greenhouse at the Faculty of Agriculture. Seedlings were transplanted 50 days after germination into the open field at the Institute of Genetic Resources, with 10 plants per accession, using a planting distance of 90 × 45 cm. To prevent cross-pollination, different accessions were planted with a 20 m isolation distance between them according to McCormack (2012). A drip irrigation system was installed, and fertilization with organic and mineral fertilizers was applied prior to planting. Plants were maintained following standard horticultural practices.
Fruits from 10 different plants per accession were harvested at full ripeness. Seeds were extracted and stored in the Gene Bank of the Republic of Srpska, while fruit samples were homogenized using a Tefal HB853A38 homogenizer. Approximately 200 ml of homogenate per accession was divided into four replicates for four different parameters to be analysed and stored at −20°C. The following biochemical parameters were analysed: pH, total soluble solids, total phenolic content, and total flavonoid content.
The pH was measured using a handheld digital pH meter (WTW Vario pH meter). Total soluble solids were determined with a digital refractometer (Hanna Instruments, HI 96801, operating range 0–85 °Brix), with three replications per sample. Total phenolic content was analysed by the Folin-Ciocalteu colorimetric method as described by Singleton and Rossi (1965). Each sample was prepared in triplicate, and absorbance was measured on a VWR UV-3100PC spectrophotometer at 765 nm. Total phenolic content was calculated from a gallic acid standard curve and expressed as mg gallic acid equivalents per gram of fresh weight. Total flavonoid content was determined by a colorimetric method using aluminium chloride, according to Tehrani et al. (2011). Samples were prepared in triplicate and absorbance was measured at 510 nm using the same spectrophotometer. Flavonoid content was calculated using a catechin standard curve and expressed as µg catechin equivalents per gram of fresh weight. The coefficient of variation among replicates within samples was below 2% for both phenolic and flavonoid content.

All data were analysed using standard descriptive statistical methods, including calculation of means and standard deviations. Statistical significance was assessed at the 0.01 probability level using Fisher's F-test (ANOVA).

Results and Discussion

Biochemical characterization of the pepper accessions from the Gene Bank of the Republic of Srpska revealed considerable variability across all analysed parameters (Table 1).

Table 1. Average and standard deviation for biochemical characteristics for pepper accessions

Sample *(Gene Bank code)*	pH	Total soluble solids (°Brix)	Total phenolic content (mg GAE/ g FW)	Total flavonoid content (µg catechin/ g FW)
GB00026	6.6	4.5 ± 0.4	11.18 ± 0.19	937.47 ± 12.7
GB00028	6.46	4.53 ± 0.06	13.36 ± 0.17	45.92 ± 0.4
GB00863	6.41	7.1 ± 0.26	17.49 ± 0.32	610.88 ± 2.08
GB00869	6.14	5.63 ± 0.06	10.86 ± 0.09	369.08 ± 6.75
GB01091	6.33	6.9 ± 0.1	12.24 ± 0.22	507.34 ± 8.79
GB01093	6.13	5.47 ± 0.06	12.31 ± 0.11	360.92 ± 6.52
GB01111	6.16	6.4 ± 0	16.77 ± 0.32	422.58 ± 1.35
GB01112	6.16	4.2 ± 0.2	8.47 ± 0.16	379.17 ± 6.78
GB01115	6.17	4.1 ± 0	10.91 ± 0.03	133.8 ± 2.57
GB01130	6.77	4.93 ± 0.06	12.48 ± 0.13	496.24 ± 2.91
GB01133	6.07	5.57 ± 0.06	13.29 ± 0.14	98.4 ± 0.19
GB01350	6.33	4.13 ± 0.12	11.3 ± 0.18	928.78 ± 12.84
GB01351	6.84	5.7 ± 0	5.06 ± 0.09	24.74 ± 0.28
min	6.07	4.1	5.06	24.74
max	6.84	7.1	17.49	928.78
Average	**6.35**	**5.4**	**11.98**	**408.87**
Fisher's F-test		**	**	**

** - statistical significance between different accessions at the 0.01 probability level

The pH values of pepper fruits were relatively stable among the accessions, ranging narrowly between slightly acidic values of 6.07 and 6.84, with an average of 6.35. These results are consistent with those reported by Petek (2021) for pepper fruits. Fruits with lower pH values indicate higher citric acid content, which is beneficial for human consumption (Wang and Lin, 2002) and is associated with longer shelf life (Hernandez-Perez et al., 2005).

Total soluble solids (TSS) varied significantly among the accessions, ranging from approximately 4.1 to 7.1 °Brix, with an average of 5.4 °Brix. These findings align with previous research by Tadesse et al. (2002), who reported TSS values between 5 and 6 °Brix in pepper varieties. Higher TSS values are generally correlated with better taste quality, and accession GB00863, exhibiting TSS values above 7 °Brix, stands out as a promising candidate for consumer preference. The analyses of the significance of the differences shows that there is highly significant difference in total soluble solids between different accessions (F_{exp} = 126.82 > $F_{0.01}$ = 2.96).

Total phenolic content also showed considerable variation, with an average value of 11.98 mg GAE/g FW. The lowest phenolic content was recorded in accession GB01351 (5.06 mg GAE/g FW), while the highest was found in accession GB00863 (17.49 mg GAE/g FW), indicating notable diversity in antioxidant potential among the accessions. These values are comparable to those reported by Chávez-Mendoza et al. (2013), who observed average total phenolic

content of 10.54 and 9.95 mg GAE/g FW in *Capsicum annuum*. The same authors noted that phenolic and flavonoid accumulation largely depends on pepper variety and fruit maturity stage. The analyses of the significance of the differences shows that there is highly significant difference in total phenolic content between different accessions ($F_{exp} = 887.35 > F_{0.01} = 2.96$). Similarly, total flavonoid content varied widely, ranging from 24.74 µg catechin/g FW in accession GB01351 to 937.47 µg catechin/g FW in accession GB00026, with an average of 408.87 µg catechin/g FW. Flavonoids contribute significantly to antioxidant activity, and the highest values observed in accessions GB00026, GB01350, and GB00863 suggest their potential utility in breeding programs aimed at nutritional enhancement. Olatunji and Afolayan (2019) reported that total flavonoid content is strongly influenced by pepper species and variety, with the highest levels found in *Capsicum frutescens* var. *baccatum* and the lowest in *Capsicum annuum* var. *grossum*. The analyses of the significance of the differences shows that there is highly significant difference in total flavonoid content between different accessions ($F_{exp} = 6187.90 > F_{0.01} = 2.96$).

Conclusion

This study presents the first comprehensive biochemical characterization of pepper accessions conserved in the Gene Bank of the Republic of Srpska. The results reveal considerable variability among accessions in terms of total soluble solids, total phenolic content, and total flavonoid content, while pH values remained relatively stable. Notably, accession GB00863 stood out with exceptionally high levels of total soluble solids as well as phenolic and flavonoid compounds, highlighting its potential as a valuable source of health-promoting traits.

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REVIEW ARTICLE

Centuries-old Pastures in Croatia: Then and Now

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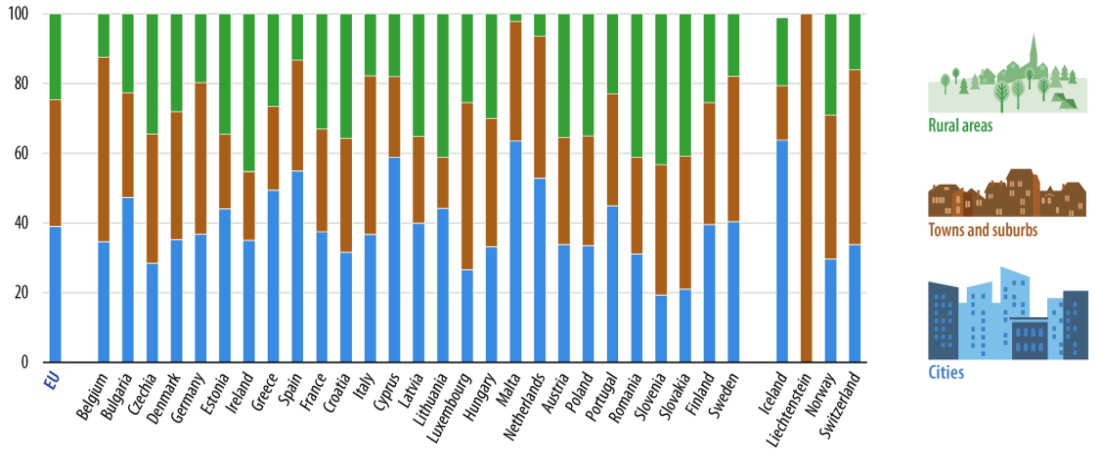
Summary
Traditional floodplain and lowland pastures are among the most valuable semi-natural grassland habitats in Croatia, shaped by long-term extensive grazing and seasonal flooding. A comparison of historical data and the current state of centuries-old pastures in Croatia, with an emphasis on the Gajna and Čistine pastures, revealed a significant reduction in the area of the Čistine pasture, as well as a decrease in the species of domestic animals present on the pasture, while the Gajna pasture maintained its historical area and the number of domestic animals present. The results show that the maintenance of these habitats depends on active grazing, legal protection, spatial planning and control of invasive species. Although protected landscapes preserve key ecological values, abandonment of grazing and changes in land use threaten biodiversity and landscape identity. Continuous monitoring and maintenance of regular grazing is essential for keeping them in a favourable conservation status.

Keywords: Gajna, Čistine, biodiversity, spatial planning

Introduction

The area of agricultural land in the European Union (EU) was 38.8% of the total area in 2022 (Eurostat, 2025). Croatia, with its relief and regional characteristics, is suitable for the development of agriculture, which has historically been present as a fundamental branch of the economy. Croatia's centuries-old pastures preserve indigenous breeds of cattle, sheep and pigs, and are also home to various species of plants, fungi, insects and animals, and are thus important for the preservation of biodiversity. As an EU member state, Croatia follows its common agricultural policy, which establishes a "*New Agenda for Sustainable Prosperity and Competitiveness of Europe intending to strengthen the attractiveness of the agri-food sector and improve its long-term sustainable competitiveness*" (European Commission, 2026). As location is a key feature of almost all official statistics, the maintenance of traditional pastures as a determinant of landscape value determines a specific territorial dimension, which in turn shows most of the economic, social and environmental development. In Croatia, the population living in rural areas (Graph 1) is approximately equal to the population living in cities and smaller towns. Through the "Zero Hunger" program, and within the framework of monitoring the Sustainable Development Goal, the EU is investing heavily in sustainable agriculture and its impact on the environment. It is increasing investments in the agricultural sector, but also in organic agricultural production, in order to achieve its goal of covering 25% of total agricultural land with organic production by 2030. Economic losses related to climate change in Croatia increased from 2018 to 2023, according to the document Sustainable development in the European Union (European Commission, 2025). A decline in the number of birds on agricultural land has been observed, and Pannonian Croatia records an above-average share of crop production of 64.6%, as well as the highest share of livestock farming in Croatia, which amounts to 244,000 heads and 636,000 pigs, which is a share of 31% of total livestock production in Croatia (Eurostat, 2024).



Graph 1. Distribution of national populations by degree of urbanisation in 2021 (Eurostat, 2024)

Rural development is an important topic of the European Commission for the EU budget for the period 2028-2034. Legislative proposals were made and published on 16 July 2025, which introduce measures of the common agricultural policies within the common National and Regional Partnership Plan per Member State, financed from the new single fund - the European Fund for Economic, Social and Territorial Cohesion, Agriculture and Rural Development, Fisheries and Maritime Affairs, Prosperity and Security (EU Agenda, 2026). By preserving centuries-old pastures in Croatia, we preserve significant landscape values, biodiversity and traditional breeding of Croatian breeds, and their monitoring is necessary, as well as the formation of a special relationship and law within agricultural policy.

Material and Methods

The research was conducted at two locations, on the Čistine pasture (located in Vukovar-Srijem County) and the Gajna pasture (located in Brod-Posavina country), in the form of an overview and comparison of the state of centuries-old pastures then and now, analyzing their area according to old maps and spatial plans, as well as the numerical state of domestic breeds and the total number of animals that are currently on the observed pastures. The information is based on a detailed review of scientific and professional articles, strategic documents of the European Union and documents of spatial plans of the given areas of the Ministry of Agriculture, Forestry and Fisheries, and other sources. Through field research and analysis of the floristic characteristics of the pastures, conclusions were drawn, and a possible model of sustainability and level of protection was set. Regarding the nature conservation status, Gajna pasture was protected in the significant landscape category in 1990, and an EU Natura 2000 SAC site under the Habitat Directive HR2000427 Gajna, under the Regulation on Ecological Network and Competence of Public Institutions for Managing Ecological Network Sites (Official Gazette 80/2019, 119/2023, 87/2025).

Results and Discussion

For centuries, old pastures in the lowland areas of Croatia near the Sava, Drava and Danube rivers have represented a large part of the rural economy and everyday life of people (Andreata-Koren, 2021; Tomac and Čižek, 1992). According to the Ordinance on the List of Habitat Types and Habitat Map (Official Gazette 27/2021, 101/2022), and valid National Classification of the Republic of Croatia (State Institute for Nature Protection, 2018), both observed pastures belong to the group of habitat types designated as C.2.4. Wet, nitrophilous pastures (Order AGROSTIDETALIA STOLONIFERAE Oberd. 1967, syn *POTENTILLOPOLY-GONETALIA AVICULARIS Tx. 1947), and subtype C.2.4.1. Nitrophilous pastures of the

lowland vegetatinal belt (Alliance *Agropyro-Rumicion crispi* Nordhagen, 1940). The aforementioned community includes wet, nitrophilous grasslands and pastures of the lowland vegetation zone. On the maps of the first military survey of the Habsburg Monarchy from the 18th century (fig. 1), the Čistine pasture occupied an area five times larger than it occupies today. Throughout history, sheep, horses and cows grazed here, and there were also geese due to the floodplain of a large part of the pasture. During the floristic survey in 2012, 72 plant species were determined, and the presence of European waterclover (*Marsilea quadrifolia* L.) was recorded, which is listed in the Red List of the vascular flora of Croatia under the Near Threatened category, and in Appendix I of the Bern Convention (Čosić, 2013). Six invasive plant species were also found. A comparison of the state of the Čistine pasture from 2012 to 2025 indicates an increase in plant species from 72 to 152, while 14 species were not re-determined (Peranović et al., 2025). At the Gajna pasture, *Marsilea quadrifolia* L., is also present, and is listed as a target species of the EU Natura 2000 SAC site HR2000427 Gajna.



Figure 1. Comparison of the approximate spatial extent of the Čistine pasture between the historical view (around 1780, First Military Survey) and the current. (Arcanum Maps, 2026)



Figure 2. Gajna once (Gaich) has not changed its surface, but only the shape of the forest part and the water surface (Arcanum Maps, 2026; Google Maps, 2026)

Continuous grazing on these pastures is important for maintaining biodiversity, because when grazing ceases, weeds and invasive plant species appear (Hulina, 1984; Antunović et al., 2022). The importance of preserving traditional Croatian breeds is very important because of the possible organisation of organic livestock farming, which implies the preservation of parent populations. The Slavonian-Srijem Podolac breed was most common in Croatia in the late 19th and early 20th centuries, serving mainly for agricultural land cultivation. While the Slavonian-Srijem Podolac disappeared from the Čistine pasture, there are Podolac herds in the Gajna protected landscape that are included in the breeding program and maintain the pasture through grazing. In Croatia, there is an "Association of Slavonian-Srijem Podolac Breeders" that protects this breed (Gavran et al., 2022). This native breed is over 2000 years old, has very modest requirements, can remain on pasture all year round if there is no snow, and calving

mortality is low. Cows produce 800 to 1000 litres of milk during lactation. In the past, the Čistine pasture was grazed by autochthonous breeds such as the Croatian Coldblood horse and the Cigaja sheep, which, according to Čačić (2009), are well adapted to grazing on pastures. In the parent population of Slavonian-Srijem Podolc, a gradual increase in the number of breeding individuals is visible from 134 heads in 2009 to 392 heads in 2024 (HAPIH, 2025; MP, 2026), which represents a positive trend in the preservation of this autochthonous and endangered breed, which plays an important role in the extensive management of floodplain pastures, including the Čistine and Gajna pastures. The Čistine pasture, which borders the Spačva Forest area, located within two EU Natura 2000 sites: SPA HR1000006 Spačvanski bazen (under the EU Birds Directive), and SAC HR2001414 Spačvanski bazen (under the Habitat Directive, is now overrun with invasive plant species (Table 1), including the species *Dipsacus laciniatus* L. Cattle grazing is currently carried out on approximately 26.5 ha. Gajna is an example of a centuries-old pasture in Croatia, preserved on an area of approximately 280 ha, and falls into the category of protected landscapes. It is located near the Sava River, and the pasture area itself is also under protection. Research conducted by Antunović et al. (2022) showed that grazing by animals such as Podolian cattle helps to control plants such as *Amorpha fruticosa* that can harm the environment.

Table 1. Invasive plant species recorded at the Gajna site and assessment of the possibility of their suppression by extensive grazing (Antunović et al., 2022; Živković, 2019; Galzina et al., 2010)

Species	**Status**	**Grazing effect**	**Removal difficulty**	**Recommended measure**
Amorpha fruticosa	confirmed	proven positive effect of grazing by Podolian cattle	medium	continuous extensive grazing + mechanical removal where density is high
Ambrosia artemisiifolia	confirmed	limited effect	high	early mowing / pulling before flowering
Conyza canadensis	confirmed	weak to moderate effect in early stages	high	mowing before seed set + grassland maintenance
Erigeron annuus	confirmed	moderate in early stages	medium to high	early mowing + maintaining grass cover
Veronica persica	confirmed	secondary/indirect effect	medium	maintaining competitive grassland cover
Xanthium strumarium subsp. *italicum*	confirmed	unreliable and potentially risky	very high	mechanical removal before seed set; avoid relying on grazing
Marsilea quadrifolia	confirmed	secondary/indirect effect	high	no removal; habitat conservation (maintaining hydrological regime + controlled extensive grazing)

The required structure and number of animals depend on the biological structure of the pasture and the forage yield. The annual forage yield on the pasture could be estimated based on the assumption that by mid-July, the grasslands build up about ¾ of the annual yield, while the remaining quarter is built up during the autumn growth (Gantner et al., 2021), so that the annual potential dry matter yield of the pasture could be between 2,900 and 3,000 kg/ha. The area of pasture currently used is 26.5 ha, while a total of 60 ha is available, which gives a potential production of 174,000 kg of dry matter per year. If this production is evenly distributed between cattle, horse and sheep farming, the number of animals can be estimated according to their dry matter needs during the grazing season. A horse weighing 500 kg consumes about 10 kg of dry matter per day (1,830 kg per season), a cattle of the same weight about 12.5 kg (2,288 kg per season), and a sheep weighing 70 kg about 2.1 kg (384 kg per season). Based on the projected dry matter needs of pasture during the grazing season, and based on an equal allocation of the produced pasture to the three livestock branches (1/3 horse breeding, 1/3 cattle breeding and 1/3 sheep breeding), the carrying capacity of the pasture was calculated for 32 horses, 25 cattle and 151 sheep on 60 ha of the Čistine pasture.

The Croatian legislation that protects these pastures is based on numerous EU directives, regulations, and legal acts that link environmental protection and agricultural management. The Nature Protection Act, management plans, protected areas / spatial plans, are also very important for the preservation of these pastures. The proposal for the protection of the Čistine pasture was presented in Vukovar (Turalija, 2008), where the level of protection was proposed, and the need for an inventory of the Čistine pasture was presented, with an emphasis on the need for the development of rural tourism. Within the protected area of Spačva Forest, according to the Spačva Forest Destination Management Plan (2025), the construction of the "Josip Lovretić" ethno park with an interpretation centre is planned. The project is outlined in the Spatial Development Plan of the City of Otok (see amendments), on Map 5 - Čistine, and the scope of the intervention refers to a part of the Čistine pasture, namely, cadastral plots 4130/1, 4130/2 and 4130/3 (Figure 3).

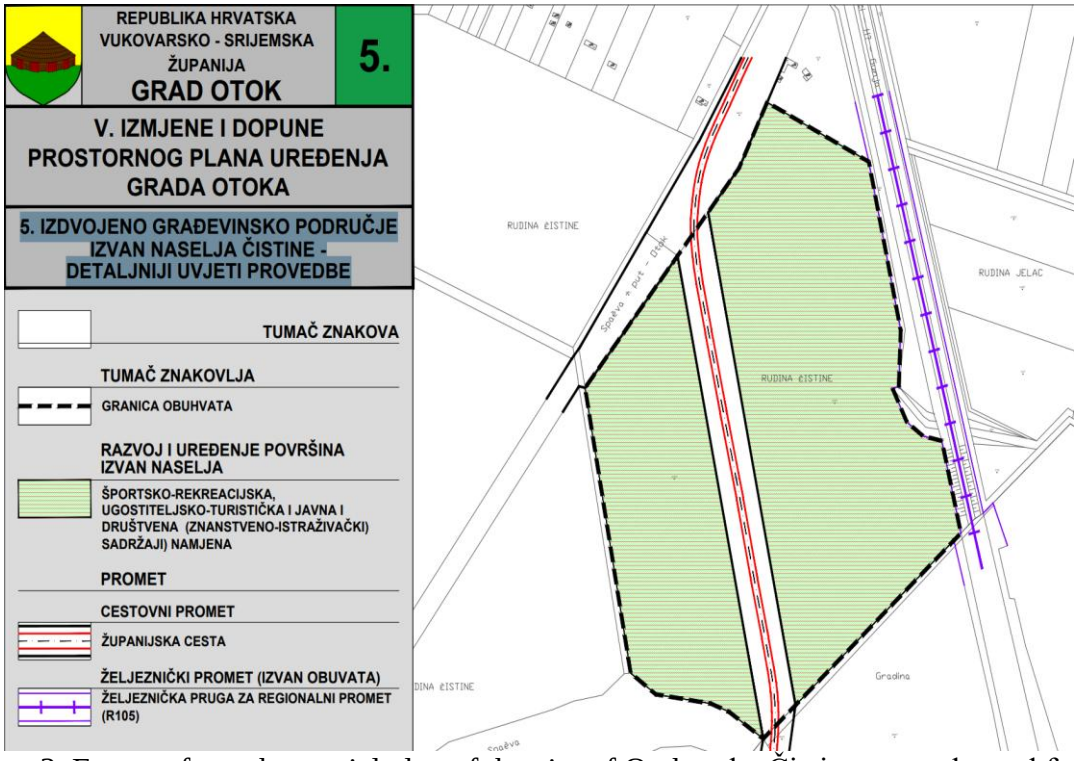


Figure 3. Excerpt from the spatial plan of the city of Otok – the Čistine area, planned for the development of an ethno park (City of Otok, 2023)

Conclusion

Centuries-old Croatian pastures deserve to be preserved and included in organic milk and meat production as a source of healthy food, but also for the sake of preserving the landscape, traditional values and biodiversity. With proper agricultural policies, spatial planning, and the involvement of all state and local government structures, it is possible to identify a model that ensures sustainability. Connecting and involving the profession is essential in this.

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PRELIMINARY COMMUNICATION

Diversity and Composition of Ichthyofauna in Čambina

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Summary
This study aimed to assess the ichthyofauna composition of side channel Čambina located in Drava River floodplain. A total of 402 fish from 19 species and 7 families were recorded, with five non-native species identified. The most abundant species were roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*) and, bleak (*Alburnus alburnus*). Only two protected species, Crucian carp (*Carassius carassius*) and Cactus roach (*Rutilus virgo*), were identified. Biodiversity indices indicate high species richness and low evenness with one species dominance suggesting that the community could be under influence of ecological stress. Based on that, there is a need for active management to preserve this key biodiversity hotspot.

Keywords: ecological status, quantitative analysis, biodiverity, protected area

Introduction

Oxbow lakes as a essential components of large river floodplains, represent crutial habitats for wide variety of aquatic taxa. These habitats play role in ecological integrity of large river systems, by providing diverse environmental conditions that differ from main river watercourse (Aarts & Nienhuis, 2003). In such habitats fish rely on hydrologic connectivity among water bodies as they can mitigate to such systems for spawning, feeding or shelter during extended floods (Terrazas-Villarroel et al., 2026). Water level fluctuations and flood events enable the exchange of nutrients and organisms, that reflects on (Moyle & Leidy, 1992). However, the absence of the water exchange can lead to isolation and shift in community structure. According to (Zhang et al., 2019), fish diversty strongly responds to changes in water quality and habitat fragmentation, which can result in the dominace of eurytopic species. Accordingly, such changes can also create favorable conditions for invasive alien species, by opening the new niches (Cambray, 2003). Fruthermore, Błońska et al. (2016), showed how opportunistic invaders can have a significant competitive pressure of native communities. The side channel Čambina is a protected area in Croatia, situated in Podravina region. Location is known fot its diverse microhabitats, that contain numerous protected plants and bird. Althought it is separated from the main Drava River watercourse, it remains connected via natural channel that provides fresh water during high water levels. Because ot this it serves as a valuable ichthyological habitat for the spawning and development of species such as pike (*Esox lucius*), carp (*Carassius carassius*) and zander (*Sander lucioperca*). It consists of two main parts: Mala Čambina and Velika Čambina. It is an integral part of the Mura-Drava Regional Park and Natura 2000 ecological network ("Značajni krajobraz Čambina," n.d.). The aim of this study was to analyze ichthyfauna of the Čambina location and based on the collected data assess the habitat status.

Material and Methods

Sampling was preformed at the location Čambina, a side channel located in the Drava River flooplain, during June, 2023. This microlocation provides insight into the fish community of an oxbow, which is currently separated from the main river channel but remains under the influence of rivers water level fluctuations. Sampling was performed by electrofishing from the boat using a single-pass method with the AGK EL65 II electrofisher, connected to a round

stainless-steel anode with a diameter of 50 cm and a mesh size of 10 mm. All caught individuals were identified to the species level based on external morphological traits (Kottelat & Freyhof, 2007), and counted. The fish caught during electrofishing was kept in aerated water tank. Upon completion of the measuring process, native species were returned to the sampling location. Non-native fish species from the catch were not returned to the habitat in accordance with the current legislation Prevention of the Introduction and Spread of Alien and Invasive Alien Species and Their Management Act, 2018; Amendments to Prevention of the Introduction and Spread of Alien and Invasive Alien Species and Their Management Act, 2019. and were euthanized using clove oil, following the instructions of Woody et al., (2002). The Simpson diversity Index, Shannon-Weiner Index and Margalef Index were used to analyze biodiversity. Strictly protected species were listed in accordance with the Strictly Protected Species Act (2013) and the Amendments to the Strictly Protected Species Act (2016).

Results and Discussion

At the Čambina site, sampling resulted in the capture of 402 specimens (Table 1). Qantitative analysis of the speciment showed a total of 19 species belonging to 7 families. The most abundant family was Leuciscidae, represented by eight species, Cyrinidae and Percidae with three species, Centrarchidae were represented by two species, while Acheilognathidae, Ictaluridae, and Esocidae each had a single species.

Table 1. Fish composition during samping on site

Speciec name	Number of cought individuals
Europian biteling, *Rhodeus amarus*	16
Crucian carp,*Carassius carassius*	2
Prussian carp, *Carassius gibelio*	21
Common carp, *Cyprinus carpio*	1
Bream, *Abramis brama*	9
Bleak, *Alburnus alburnus*	27
White bream, *Blicca bjoerkna*	5
Asp, *Leuciscus aspius*	7
Roach, *Rutilus rutilus*	166
Cactus roach, *Rutilus virgo*	1
Rudd, *Scardinius erythrophthalmus*	68
European chub, *Squalius cephalus*	2
Black bullhead, *Ameiurus melas*	5
Northren pike, *Esox lucius*	8
Pumpkinseed, *Lepomis gibbosus*	25
Largemouth bass, *Micropterus salmoides*	6
Danube ruffe, *Gymnocephalus baloni*	1
Perch, *Perca fluviatilis*	31
Zander, *Sander lucioperca*	1
TOTAL	402

Regarding ecological preference and habitat type, eurytopic species were dominant, An analysis of spawning guilds revealed a dominance of phytophilic species. That is in consistent with the habitat characteristics, which include stagnant or slow-moving water rich in aquatic veteration. Also, there was one ostracophilic species (*Rhodeus amarus*) present at Čambina site (Table 2). The most abundant fish caught during sampeling were roach (*Rutilus rutilus*)(41.29%), rudd (*Scardinius erythrophthalmus*)(16.92%), perch (*Perca fluviatilis*)(7.71%), bleak (*Alburnus*

alburnus)(6.72%), pumpkinseed (*Lepomis gibbosus*)(6.22%), prussian carp (*Carassius gibelio*)(5.22%), and european bitterling (*Rhodeus amarus*)(3.98%). All other species were represented with a proportion less than 3% (Table 2).
The ichthyofaunal composition at the Čambina site was dominated by eurytopic and ominivorous species. The abundance of roach can have a significant influence on ecosystem nutrient cycling and energy flow. By providing connection between bottom sediment and water column, the species enhances nutrient recycling. Fruthermore, the high abundance of this species can influence the community structure through competition with other omnivore species. Taking all this into account, the roach can represent a reliable indicator of moderately eutrophic conditions and habaitat stability (Jeppesen et al., 2010; Kawamura, 2008).

Table 2. Number of species, individuals, species richness and biodiversity indices for the sampling site (according to Aarts & Nienhuis, 2003; Kottelat & Freyhof, 2007)

Species	Ratio in sample (%)	Ecological habitat preference	Water column	Spawing substrate	Feeding strategy
European bitterling	3.98	Eurytopic	Water column	OSTR	OMNI
Crucian carp	0.50	Limnophilic	Bentic	FITO	OMNI
Prussian carp	5.22	Eurytopic	Bentic	FITO	OMNI
Common carp	0.25	Eurytopic	Bentic	FITO	OMNI
Bream	2.24	Eurytopic	Bentic	FITO/LITO	OMNI
Bleak	6.72	Eurytopic	Water column	FITO/LITO	OMNI
White bream	1.24	Eurytopic	Bentic	FITO	OMNI
Asp	1.74	Rheophilic	Water column	LITO	PISC
Roach	41.29	Eurytopic	Water column	FITO/LITO	OMNI
Danube roach	0.25	Rheophilic	Bentic	FITO	INS/INV
Rudd	16.92	Limnophilic	Water column	FITO	OMNI
Chub	0.50	Eurytopic	Water column	LITO	OMNI
Black bullhead	1.24	Limnophilic	Bentic	LITO	OMNI
Northern pike	1.99	Eurytopic	Water column	FITO	PISC
Pumpkinseed	6.22	Limnophilic	Water column	LITO	INS
Largemouth bass	1.49	Limnophilic	Water column	FITO	PISC
Balon's ruffe	0.25	Rheophilic	Bentic	FITO	INS/INV
Perch	7.71	Eurytopic	Water column	FITO/LITO	INV/PISC
Pike-perch	0.25	Eurytopic	Water column	FITO	PISC

Abbrevations: LITO – lithophile, FITO – phytophile, OSTR – ostracophile, PEL – pelagophile, INS – insectivore, INV – invertivore, OMNI – omnivore, PISC - piscivore

The presence of pumpkinseed, prussian carp, black bullhead (*Ameiurus melas*), Largemouth bass (*Micropterus salmoides*) which are invasive species can indicate a disturbance in natural balance of the research site. Generally, they represent a threat to natural habitat and biodiverity. The Prussian carp is a direct competitor to the native crucian carp, which was found in significantly lower numbers (only two individuals). Black bullhead and largemouth bass are highly predatory species that feed on native fish eggs and larvae (Costantini et al., 2023). Pumpkinseed, besides being agressive food competitor, thrives in antropogenic altered enviroment that can be highly degraded and poluted (Almeida et al., 2014). Fruther monitoring is needed to track changes in the ecosystem. A minor predator/prey proportion is recorded, with higher number of northren pike and perch. Based on their ecology as a phytophilic species, confirms that Čambina site has highly developed submerged vegetation with low water flow

(Miguet et al., 2024). Presence of cactus roach (*Rutilus virgo*), species that usually prefers flowing waters, indicates that habitat is under an influence or connected to a larger river. That is in accordance with the geographic position of the Čambina site, which is located in close proximity to the Drava river.

Table 3. Summary of Species Richness, Composition, and Diversity Indices for the Čambina Site

	Čambina (cumulative)
Species number	19
Number of individuals	402
Number of native species	14
Number of alien species	5
% of alien species	14.43%
Number of protected species	2
Margalef index	3.002
Shannon_Wiener index	0.218
Simpson index	2.018

Analysing the biodiversity indices, a specific structure of the ichthyofauna is observed (Table 3.). Although the Margalef index indicates satisfactory species richness with high value (d = 3.002), Shannon-Wiener and Simpson indces (H' = 0.218, D = 2.018) reveals a distinct dominance of a small number of species, primarily roach and rudd. On the Čambina locality, high species richness and low evenness with one species dominance suggest that the community could be under influence of environmental stressors (Larentis et al., 2022). The presence of alien species (14.43%) confirms this observation. According to that, natural balance is disturbed and under competitive pressure on native limnophilic species. All this, makes this system ecologically sensitive and susceptible to fruther changes.

Conclusion

The ichthyofauna analysis of the Čambina side channel shows a high species richness, suggesting a complex habitat. However, the community is dominated by a single species. The significant presence of invasive species indicates environmental pressure and eutrophication. Nevertheless, the presence of crucian carp and cactus roach, confirms the sites importance for conservation and manangment.

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ORIGINAL SCIENTIFIC PAPER

Evaluation of *Beauveria bassiana* virulence, feeding suppression and horizontal transmission in *Leptinotarsa decemlineata* larvae

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Summary
The Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say) is among the most destructive pests of potato crops. Entomopathogenic fungi (EPF) are considered as sustainable alternative to insecticides. We evaluated the EPF *Beauveria bassiana* virulence, feeding suppression, and horizontal transmission in CPB larvae. Tested concentrations of *B. bassiana* caused significant larval mortality compared to the control and reduced insect feeding activity. Horizontal transmission of the fungus was observed, indicating its ability to spread to live insects. These findings highlight the potential of *B. bassiana* as an effective biological control agent against CPB.

Keywords: the Colorado potato beetle, biological control, entomopathogenic fungi, mortality, mycelial growth

Introduction

The Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say) remains one of the most challenging insect pests in modern agriculture due to its exceptional adaptability and rapid development of insecticide resistance (Hare, 1990). It is a major pest of potato (*Solanum tuberosum* L.), capable of causing severe defoliation and significant yield loss. Its high reproductive capacity and ecological plasticity further complicate its management in agricultural systems (Hare, 1990). In response to these challenges, alternative approaches such as biological control have gained importance. Entomopathogenic fungi (EPF) represent a key group of biological agents, as they naturally infect insects through direct penetration of the cuticle, unlike many other pathogens that require ingestion (Shah and Pell, 2003). This characteristic makes them particularly suitable for controlling foliar-feeding pests such as the CPB. Among EPF, *Beauveria bassiana* is one of the most extensively studied species due to its wide host range and proven efficacy against numerous insect pests. Infection begins with conidial adhesion and germination on the insect cuticle, followed by enzymatic degradation and penetration, after which the fungus proliferates within the host and produces metabolites that contribute to insect mortality (Zimmermann, 2007).

The virulence of *B. bassiana* is strongly influenced by strain variability, as well as environmental conditions and host susceptibility (Zemek et al., 2021). Strains isolated directly from CPB cadavers can exhibit particularly high pathogenicity, emphasising the importance of selecting appropriate isolates for effective biological control (Zemek et al., 2021). Therefore, in the context of increasing insecticide resistance and the need for sustainable pest management, evaluating the virulence of *B. bassiana* against Croatian population of CPB is an important step in developing efficient and environmentally acceptable control strategies. This study aims to determine the virulence of *B. bassiana* against CPB larvae, its effect on their feeding activity, and its capacity for horizontal transmission.

Material and Methods

A natural population of the CPB was collected in May 2025 in Belišće (Croatia) (45.6761857° N, 18.4114069° E). The collected specimens were in the larval stage (L3rd and L4th) and processed the following day. Potato leaves used for feeding and treatment were freshly collected and prepared before the experiment from the same location. The stock solution of EPF used in the experiment was prepared immediately before the experimental setup using the commercial product Naturalis® (BIOGARD Division, Grassobbio, Italy), which contains the *B. bassiana* strain ATCC 74040. Two concentrations were prepared: 1% (1 mL of Naturalis in 100 mL of distilled water) and 0.5% (0.5 mL of Naturalis in 100 mL of distilled water), while distilled water was used as the control treatment. As the commercial suspension claims to contain a minimum of 2.3×10^7 viable spores per mL, we presumed the 1 mL stock solution contained at least 2.3×10^7 spores, while the 0.5 mL sample contained at least 1.15×10^7 spores. The experiments were conducted under laboratory conditions at room temperature (22-23 °C). The first experiment was conducted as an oral bioassay to evaluate the virulence of *B. bassiana* against the CPB, and its effects on larval feeding activity. The second experiment focused on the horizontal transmission of the EPF.

In the first experiment, three treatments were established in three replications with ten insect larvae per replication. Treatments were control (distilled water), 0.5%, and 1% concentration of the fungal suspension. For each treatment, healthy and active insect larvae were selected and placed in Petri dishes. Potato leaves were previously immersed in the EPF stock solution or distilled water for 10 seconds. Before placement in the Petri dishes, potato leaves were weighed to ensure equal amounts of food across all treatments and replicates.

Feeding was monitored daily by visually assessing and recording the percentage of leaf area consumed. Leaves were replaced with untreated leaves from the second day onwards. The impact of the EPF on feeding suppression was evaluated based on the observed reduction in leaf consumption. Larval mortality was recorded daily. Cadavers were removed and transferred to separate sterile Petri dishes lined with moist filter paper, and maintained at room temperature in closed containers to allow EPF development. The appearance and growth of fungal mycelium on cadavers were monitored over a period of 10 days.

Cadavers exhibiting visible mycelial growth of *Beauveria bassiana* were used as a source of infection in the second experiment testing horizontal transmission. A single infected CPB cadaver with well-developed mycelium was transferred into a sterile Petri dish containing ten live CPB larvae. Insect larvae were fed daily with untreated potato leaves. The Petri dishes were maintained under laboratory conditions at room temperature, with moist filter paper to ensure adequate humidity. Larval mortality and the appearance of mycelial growth on newly infected individuals were recorded daily over a period of 10 days. Throughout the experiment, any signs of contamination were also documented.

Statistical analyses were performed using a one-way ANOVA to evaluate the effect of treatment (1%, 0.5%, and control) on the measured response variables. When significant differences were detected, means were compared using Tukey's HSD test at $p < 0.05$.

Results and Discussion

The oral bioassay results demonstrated a clear effect of *B. bassiana* on the mortality and feeding activity of CPB larvae. Both tested concentrations resulted in higher mortality than the control, indicating the pathogenic effect of the EPF (Table 1). Both treatments caused 100% insect mortality, while mortality in the control group was 26.66%. The mortality dynamics differed between treatments, as mortality gradually increased over time, but it was more pronounced in the lower EPF concentration (0.5% Naturalis) treatment. The lower concentration proved to be

the most optimal in this experimental setup. “In the 1% treatment, mortality on the first day after exposure was lower than in the 0.5% treatment (23.33% vs. 56.67%). A statistically significant difference in mortality between the treatments and the control was observed from the third day onwards for both treatments, while in the lower concentration treatment, mortality was already statistically significant compared to both the higher concentration and the control on the first day after exposure.

Table 1. Virulence of *Beauveria bassiana* against *Leptinotarsa decemlineata* larvae

Treatment	Conc. *B. bassiana* strain ATCC 74040	Days after exposure				
		1	2	3	4	5
		Mortality (%)				
1%	2.3×10^7	23.33a	26.66a	36.66b	56.66b	100.00b
0.5%	1.15×10^7	56.67b	56.67b	63.34b	76.67b	100.00b
Control	Dis. Water	0.00 a	0.00 a	3.33a	3.33a	26.66a

Values in columns marked by different letters are significantly different ($p < 0.05$).

Feeding activity, expressed as the percentage of consumed leaf area, was reduced in treated groups compared to the control (Table 2). Leaf consumption was highest in the control, with larvae consuming up to 99.33% of leaf area on the first day and 58.33% on the fifth day. In contrast, feeding was markedly reduced in treated groups, particularly in the 0.5% treatment, where consumption dropped to 0% by the fourth and fifth day, indicating a strong effect of *B. bassiana* on larval feeding activity. Larvae in the 1% treatment consumed less leaf area on the first day compared to the 0.5% treatment (55.00% vs. 93.33%), indicating a negative effect of the fungus on feeding behaviour. The CPB may have detected the presence of the EPF on the leaves in the treatment with the higher EPF concentration and therefore reduced its feeding.

Table 2. Feeding activity of *Leptinotarsa decemlineata* larvae after the treatment with *Beauveria bassiana*

Treatment	Conc. *B. bassiana* strain ATCC 74040	Days after exposure				
		1	2	3	4	5
		Leaf consumption (%)				
1%	2.3×10^7	55.00	43.33	28.33	6.67	13.33
0.5%	1.15×10^7	93.33	16.67	5.00	0.00	0.00
Control	Dis. Water	99.33	93.33	91.67	83.33	58.33

The development of mycelium on cadavers was observed in both treatments, confirming successful fungal infection, and was followed by sporulation, indicating the production of infective conidia capable of initiating new infections. These conidia serve as a source of secondary infection and facilitate the spread of the pathogen within the pest population (Roy et al., 2006). The average number of larvae exhibiting mycelial growth was higher in the 1% EPF treatment compared to the 0.5% EPF treatment. The appearance of characteristic white mycelium is a key indicator of *B. bassiana* infection and confirms that mortality was caused by the pathogen (Ortiz-Urquiza and Keyhani, 2013).

In the horizontal transmission test, survival of the CPB larvae decreased over time in both treatments following exposure to infected cadavers (Figure 1). In the 1% treatment, survival declined more rapidly, dropping from 100% on the first day to 90% on the second day, 50% on the third day, 10% on the fourth day, and reaching 0% by the fifth day. In the 0.5% treatment, survival remained at 100% during the first two days, followed by a gradual decrease to 90% on the third day and 50% on the fourth day, with complete mortality observed on the fifth day.

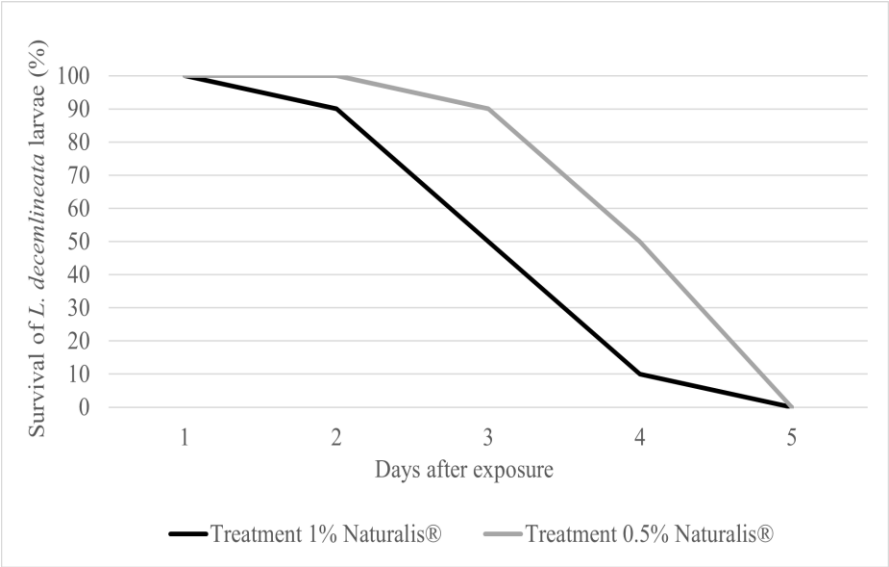

Figure 1. Survival of *Leptinotarsa decemlineata* larvae following horizontal transmission of *Beauveria bassiana* at two concentrations (1% and 0.5% Naturalis®)

Overall, survival declined more rapidly in the higher concentration treatment, although both treatments resulted in complete mortality by the end of the observation period. Healthy larvae exposed to infected cadavers developed signs of EPF infection, including mortality and subsequent mycelial growth. These results confirm that B. bassiana can spread through contact with infected cadavers, enabling secondary infections within pest populations (Quesada-Moraga et al., 2004).

The rapid decline in feeding activity indicates that *B. bassiana* exerts a strong sublethal effect before mortality, which may substantially reduce plant damage even before lethal outcomes occur (Kaur et al., 2011). Other studies confirm these sublethal effects on insect larvae, including reduced feeding, developmental delays, shorter larval periods, deformities, and decreased reproductive potential (e.g., lower egg hatching from survivors) (Moorthi et al., 2015; Kaur et al., 2011). The differences in mortality dynamics between concentrations, with higher early mortality in the 0.5% treatment, suggest that factors such as concentration or larval response may influence infection efficiency under laboratory conditions. However, most research indicates a typical dose-response pattern where higher concentrations (e.g., 0.8% or 8 g/100 mL, 2.03 × 10^8 spores/mL) cause faster and greater mortality (e.g., 40% by day 6, up to 100% within 10 days), contrasting our observation of earlier effects at lower doses (Kaur et al., 2011). This discrepancy may stem from strain-specific traits, host interactions, or lab variables like humidity and spore viability, which can alter toxin production and infection speed. Previous studies report variability in *B. bassiana* virulence and highlighting the importance of strain-host interactions in determining pathogenicity (Wang et al., 2022). Field isolates and different strains show biological heterogeneity, with environmental factors further modulating outcomes, underscoring the need for optimized formulations in applied settings. No clear evidence supports reversed mortality at low doses across studies, suggesting our results warrant further field validation.

Conclusion

Our study on CPB populations in eastern Croatia demonstrates that *B. bassiana* induces rapid sublethal effects, notably reduced feeding activity in treated larvae, offering substantial potential to mitigate plant damage before mortality. While mortality dynamics varied unexpectedly with higher early effects at 0.5% concentration, contrasting typical dose-response

patterns observed elsewhere, these findings underscore the critical role of strain-host interactions and laboratory conditions in modulating virulence against local CPB strains. Further field trials in eastern Croatian potato fields are recommended to validate these results and optimize *B. bassiana* formulations for integrated pest management.

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EXPERT PAPER

Attempt to conserve the plant biodiversity of hilly pastures *in-situ*: Breakage of bracken with slatted roller aimed to suppress the bracken

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Summary
Bracken has become an invasive species in hilly pastures of Croatia following the depopulation of hilly countryside. Invasion of bracken threatens plant biodiversity there, as well as diversity of insects and animal species. Bracken invasion also lowers pastures forage yield thus causing the forage shortages (except on heat- and drought-prone pastures where it acts protective during summer), and brings the risk of livestock poisoning. Various measures for bracken control were proposed and tested in other countries and some were in Croatia. In this research there was tested a slatted roller in breaking the young shoots of bracken with aim to suppress its growth. Testing shown that the roller was ineffective, but also revealed the required improvements of roller that are needed to make it more effective: there has to be increased weight of the roller, the density of slats, the sharpness of slats and diameter of the roller. Improved variants of the roller have to be tested in order to find the optimal one considering the maximum bracken suppression and minimum sward damage. To combat the bracken, other measures have to be employed too, including correction of excessive soil acidity, soil fertilization and overseeding pastures with forage grasses and legumes.

Keywords: plant biodiversity, bracken, grassland, invasive plant, weed control

Introduction

Bracken (*Pteridium aquilinum* L.) becomes increasingly important noxious plant species in hilly pastures of Croatia, especially when on deeper and acidic soils. It is considered invasive there (Jelinčić et al., 2022) due to its rapid spread and because it overshadows, suppresses and overcompetes the currently present grassland vegetation, thus leading to the loss of forage yield and of valuable biodiversity in Croatian hilly pastures. Hay prepared from bracken-infested grasslands is useless because livestock cannot select forage from poisonous bracken. Reputation of bracken that threats the grasslands biodiversity is also found in other parts of the world, including Italy (Argenti et al., 2012), United Kingdom (Marrs et al., 2007), and others. According to literature review by Argenti et al. (2012), main reasons for the contemporary spread of bracken on pastures in hilly regions are depopulation of hilly countryside, diminishment of pastoral activities and lack of extensive livestock farming. Historically, hilly countryside of Croatia was fairly populated with domination of small farms (imposed administrative upper limit was 20 ha per household). With many people and many livestock in these times, the land was very valuable resource, so many parts of land with deeper soil were cultivated for cereals and potatoes production, whilst the livestock was grazed on shallow soils, where bracken naturally does not thrive or thrives poorly. Thereafter the bracken wasn't serious problem in these times. Modern depopulation of hilly countryside led to abandonment of arables

there and to their conversion to pastures which are prone to bracken invasion since bracken prefers deeper soils. Contemporary attempt to fight the spread of bracken by flail-mowing/mulching three-times a year (begin of June, begin of July and end of August, near Primišlje village), didn't kill the bracken. Even worse, it caused that during the autumn lack of forage grasses, the young bracken shoots became the only available herbage to grazing livestock, which led to consumption of poisonous bracken and livestock poisoning. Otherwise, bracken would stop its growth at mid of August and would avoid the risk of livestock poisoning. Therefore mowing seems to be risky operation for livestock, while providing poor bracken control and poor weakening. Conversely, when the bracken wasn't mown, it didn't produce attractive young shoots in autumn. Researchers in Italy tested the efficiency of plowing followed by cuttings vs. harrowing, each followed by seeding of forage mixture in grassland restoration and conservation of open areas (Argenti et al., 2012). By using the tested methods they achieved certain success in recovery of pastures and botanical composition. Though, plowing in majority of Croatian pastures is not feasible due to sloped terrain and stony soil. Some sources propose chemical control of bracken by spraying glyphosate, asulam (Burge and Kirkwood, 1992) and dicamba herbicides, but vast of Croatian grasslands are managed in organic manner thus making the chemical control undesired there. Despite bracken contains toxic compounds in its herbage (Burge and Kirkwood, 1992), it can be useful in drought and heat prone grasslands since it provides shadow for the grassland understory, thus helping the survival of forage vegetation in summer, and accordingly provision of forage to grazing livestock. According to a grassland and cattle farmer Bariša Dejanović, bracken is more effectively suppressed by frequent breaking of young shoots. Some local farmers achieved a gradual success in bracken suppression by successive scrapping of bracken infested swards by old automobile tyres mounted below the tine harrows, pulled by small tractors. Although scrapping with tyres didn't much damage the grassland sward, there is a need to find a better implement that can be repeatedly applied from beginning of spring (when the sward height is very low), over the end of spring (when sward height would be high if bracken was suppressed) and summer, till the autumn. Historically, bracken on pastures was also suppressed by correction of excessive soil acidity, by spreading the ash from wood over the infested land area. Inspired by the farmers' experience that bracken is sensitive to frequent breakage of young shoots, this research was aimed to test the effectiveness of slatted roller in breaking these young shoots of bracken.

Material and Methods

The tested slatted roller (Figure 1) was made of steel and taken from an old seedbed preparation implement. Mass of the slatted roller was 27 kg. Working with was 1 m, and outer diameter was 0.31 m (Figure 2). Nine slats were fixed to the common axes via stars, and distance between slats was 0.1 m. The roller was pulled manualy by walking (about 4 km/h) and running (about 8 km/h) man, downhill and uphill over the bracken-infested pasture area on 22nd April 2026 near the village Primišlje, Kordun, Croatia. Density of bracken shoots was about 20 per m^2 and average height of the shoots was about 30 cm (Figure 3a and 3b). At the area of testing there was no significant litter of the dead leaves of last year bracken, so the roller could easily roll over the sward surface.



Figure 1. Slatted roller which was tested in hilly pasture



Figure 2. Diameter of the tested roller



Figure 3a. Young shoots of bracken



Figure 3b. Density of bracken in sward

Results and Discussion

Rolling the young shoots of bracken with the tested slatted roller could not break the bracken shoots. It caused bending of young shoots to the ground during the passage of the roller and afterwords straightening when the roller past the shoots. Rarely there could be found a broken bracken stem. Roller neither did any harm to the existing sward of the pasture. Measured pulling force downhill was about 50 N and uphill about 100 N, thus indicating that the roller requires very low pulling force.

Based on the results of this first testing of slatted roller, there was found that further improvements of the roller are necessary to accomplish the desired breakage:

1. The roller has to be heavier, probably at least twice,
2. Slats over the roller surface should be slightly sharpened to exert easier breaking of bracken shoots,
3. Slats should be denser spaced over the roller surface for better targeting the short shoots of young bracken (maybe the distance between slats should be 0.05 m),
4. Diameter of the roller should be greater to enable the rolling over the thicker litter of last year dead bracken residue.

The sharpness of the slats shoud be adequate (not too sharp) to avoid damaging the tuft or grass sward, but sharp enough to exert adequate breaking of the bracken. Namely, there is required selectivity of this rolling operation in order to suppress bracken but not to damage grassland vegetation. Fortunately, physical properties of young shoots of bracken differ significantly from the shoots and leaves of grasses. Namely, shoots of bracken are thicker and more fragile and

brittle than shoots of grasses, thus giving the room for fine adjusting to optimal sharpness. Much heavier alternative to the tested roller might be a conventional crosskill roller used in arable soil preparation, but authors deem that crosskill rollers' excessive mass (about 400 kg per meter of working width) might be an obstacle for their acceptance since they would require more powerful tractors, much more fuel consumption and greater purchase costs of the implement. Also, excessive soil compaction might impair the soil fertility which is required for achieving the high forage yields.

Combat with bracken does not end after its successfull suppression by frequent breaking during the vegetation period because the belowground plant parts will be still alive. According to Petrov and Mitev (1994), best prevention of bracken reestablishment in treated grasslands is combination of soil fertilization plus overseeding with forage species. Namely, vigorous forage vegetation can more easily outcompete the remnants of bracken than the present poor old sward.

Conclusion

Preliminary trial of breaking the young shoots of bracken by slatted roller indicated that the tested roller was ineffective. Though, this testing gave the insight into required improvements of the roller to become effective: the roller has to be havier, with denser spacing of slats over the roller's surface, slats have to be adequately sharpened and the roller diameter should be greater. New prototypes have to be tested to find the best variant regarding the success of breaking the bracken, minimum sward damage and with the least traction force required.

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PRELIMINARY COMMUNICATION

Fish Diversity and Distribution in the Vučica River

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Summary
Fish assemblage of the Vučica River was analyzed using BioAtlas data and two field samplings. A total of 27 fish species were recorded in the database, while field surveys confirmed 15 species of which three are IAS species. Field surveys indicates that native fish species represented 44.27% of total abundance and dominated biomass with 75.25%. Non-native species accounted for 22.09% of biomass, while invasive species reached 39.49% of total abundance. Two additional species - brown trout (*Salmo trutta*) and striped ruffe (*Gymnocephalus schraetser*), were recorded during field sampling but were absent from BioAtlas records. Diversity indices indicated low species richness and uneven distribution, with dominance of adaptable invasive species such as topmouth gudgeon (*Pseudorasbora parva*) and Prussian carp (*Carassius gibelio*).

Keywords: ichthyofauna assemblage, species diversity, IAS, Vučica River

Introduction

In the context of increasing anthropogenic pressures on freshwater ecosystems, a systematic and methodologically standardized assessment of the river's fish assemblages is necessary to establish a reliable reference dataset and to provide a scientifically sound foundation for future monitoring and management actions (Dudgeon et al., 2006; Pont et al., 2007). River Vučica is well known at the regional level and previous fish surveys have reportedly been conducted, but the results of those investigations have never been formally published and are not available in the peer-reviewed scientific literature. Consequently, there is no accessible, verifiable baseline on the composition, structure, and conservation status of its ichthyofauna. The absence of published data prevents robust temporal comparisons, limits the assessment of native, endemic, and non-native species, and constrains evidence-based management and conservation planning. There is several incorrectness concerning the Vučica River itself, including uncertainties regarding its exact length, where its starts and where it ends (for more information, see Nadilo, 2014). The primary reason for this confusion lies in the fact that the river does not retain a single name along its entire course. According to Nadilo (2014), the Vučica River originates from several streams from south part of Papuk; Cipalovac Stream, Žervanjski Stream, Kaptolački Jarak, Ninkovački Stream, and Hercegovac all converges to form the Radlovačka River. In the depression near town Orahovica, the Radlovačka River receives additional tributaries, Tisovac and Duzluk Stream, after which the watercourse is referred to as the Vučica River. The river then flows through the town of Orahovica (where it was formerly known as the Orahovica River), continues through the fishpond Grudnjak and alongside the fishpond Našice, and ultimately merges into the Karašica River near the settlement of Ladimirevci. The purpose of this work was to collect data on the presence of fish species and supplement them with the results of our own research.

Material and Methods

For the purposes of this paper, the Vučica River is defined as the section of the watercourse extending from the confluence of the Tisovac and Duzluk Streams with the Radlovačka River near the town of Orahovica, downstream to its mouth at the Karašica River near the settlement of Ladimirevci. Data on the presence of fish species in the Vučica River were obtained from the BioAtlas website (Bioatlas, 2026), which indicates that the datasets provided come from institutions, individual collectors, and communities. However, during the search, none of the sources included "Vučica River" in their name, therefore the authenticity of the data remains unclear. Data from field samplings are from two locations, booth in second part of river: location 1 – in near proximity of fishpond Grudnjak carried out on July 8, 2025 and location 2 – in near proximity of confluence to Karašica River, carried out on November 26, 2016 (Figure 1.). Sampling was conducted by electrofishing from a boat using a single-pass method with the AGK EL65 II electrofisher, connected to a round stainless-steel anode with a diameter of 50 cm and a mesh size of 10 mm. All caught individuals were identified to the species level based on external morphological traits (Kottelat & Freyhof, 2007), and all individuals were counted. The fish caught during electrofishing were kept in aerated water. Upon completion of the measuring process, native species were returned to the sampling location. The fish were released into the water near the shore and, after adapting to the conditions, swam away voluntarily. Non-native fish species from the catch were not returned to the water in accordance with the Prevention of the Introduction and Spread of Alien and Invasive Alien Species and Their Management Act (2018) and Amendment to the Act (2019) and were euthanized using clove oil, following the instructions of Woody et al. (2002).

A Simpson Index, Shannon-Weiner Index and Margalef Index were used to analyze biodiversity (Table 3.). Data on the classification of strictly protected species was prepared in accordance with the Strictly Protected Species Act (2013) and the Amendments to the Strictly Protected Species Act (2016).

Results and Discussion

Based on data from the Bioatlas, a total of 27 fish species have been recorded in the Vučica River (Table 1). Of these, five species: Vladykov's lamprey (*Eudontomyzon vladykovi*), stone loach (*Barbatula barbatula*), Danube barbel (*Barbus balcanicus*), Danube gudgeon (*Gobio obtusirostris*), and Eurasian minnow (*Phoxinus phoxinus*), were documented exclusively in the river section upstream of the Orahovica Fishpond.



Figure 1. Locations of sampling (1 – near fishpond Orahovica, 2 – near confluence to Karašica river

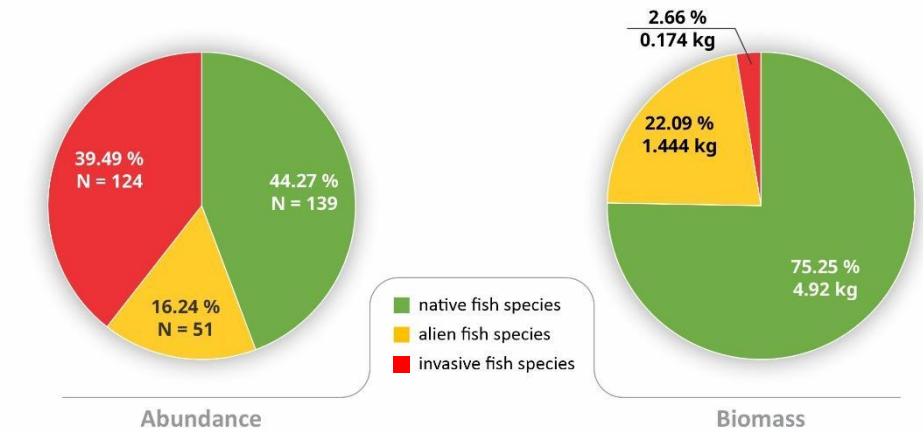
Two species, the European bitterling (*Rhodeus amarus*) and the chub (*Squalius cephalus*), occur in both river sections, whereas the remaining 20 species are present only in the downstream section of the river, beyond the fishpond.

Table 1. Presence of fish species according to BioAtlas data and two field samplings

Common name	**Scientific name**	**BioAtlas data**		**Field survey**	
		Before fishpond	After fishpond	Nov 26, 2016	July 8, 2025
Vladykov's lamprey	*Eudontomyzon vladykovi*	•			
Danube spined loach	*Cobitis elongatoides*		•		
stone loach	*Barbatula barbatula*	•			
European bitterling	*Rhodeus amarus*	•	•	74	5
Danube barbel	*Barbus balcanicus*	•			
Prussian carp	*Carassius gibelio*		•	6	45
common carp	*Cyprinus carpio*		•	2	1
Danube gudgeon	*Gobio obtusirostris*	•			
topmouth gudgeon	*Pseudorasbora parva*		•	111	4
Danube whitefin gudgeon	*Romanogobio vladykovi*		•		
bream	*Abramis brama*		•		1
bleak	*Alburnus alburnus*		•	24	6
white bream	*Blicca bjoerkna*		•		
asp	*Leuciscus aspius*		•		1
ide	*Leuciscus idus*		•		
Eurasian minnow	*Phoxinus phoxinus*	•			
roach	*Rutilus rutilus*		•	4	5
rudd	*Scardinius erythrophthalmus*		•	1	2
chub	*Squalius cephalus*	•	•	2	
grass carp	*Ctenopharyngodon idella*		•		
wels catfish	*Silurus glanis*		•		
black bullhead	*Ameiurus melas*		•		9
pike	*Esox lucius*		•	7	1
brown trout	*Salmo trutta*			1	
monkey goby	*Neogobius fluviatilis*		•		
pumpkinseed	*Lepomis gibbosus*		•		
striped ruffe	*Gymnocephalus schraetser*				1
perch	*Perca fluviatilis*		•	5	
pikeperch	*Sander lucioperca*		•		
	Number of species	**7**	**22**	**11**	**12**
TOTAL NUMBER OF SPECIES		**27**		**15**	

CONTEXT: Before fishpond – part of watercourse from confluence near Orahovica to fishpond Orahovica, **After fishpond** – part of watercourse from fishpond Orahovica to confluence with Karašica River, **Symbol •** – the species is present according to Bioatlas data, **Field survey** – number of individuals during field sampling

This pronounced difference in fish species distribution indicates a clear influence of the fishpond Orahovica and suggests the presence of a Barbel zone upstream of the fishpond and a Bream zone downstream, consistent with the longitudinal river zonation proposed by Huet (1959). Field sampling confirmed the presence of a lower number of species, with a total of 15 fish species recorded. It should be noted that these field surveys were conducted as inventory sampling rather than as part of a long-term monitoring program. Consequently, the number of detected species was lower and did not include all species listed in the Bioatlas. Nevertheless, field sampling identified two additional species: brown trout (*Salmo trutta*) and striped ruffe (*Gymnocephalus schraetser*), that are not included in the species list reported by Bioatlas.



Graph 1. Abundance and biomass of native, alien and invasive fish species during filed sampling on Vučica river

Of the 15 fish species captured during field sampling, 12 were native. One species Prussian carp (*Carassius gibelio*) was classified as a non-native species, while two species topmouth gudgeon (*Pseudorasbora parva*) and black bullhead (*Ameiurus melas*) were categorized as invasive (European Commission, 2025). In terms of species abundance, native species accounted for 44.27%, whereas the proportion of invasive species was also relatively high, reaching 39.49% (Graph 1.). In terms of total biomass, native species dominated with 75.25%, however, non-native species represented almost one quarter of the total catch (22.09%).

Table 2. Number of species, individuals, species richness and biodiversity indices for the Vučica River

	Field survey (cumulative)	**BioAtlas (cumulative)**
Species number	15	27
Number of individuals	318	-
Number of native species	12	21
Number of alien species	3	6
% of alien species	20%	22.2%
Number of protected species	1	2
Margalef index	2.43	-
Shannon-Wiener index	1.782	-
Simpson index	0.227	-

The proportion of invasive species in the total biomass was minimal (2.66%), although this may mask their actual ecological impact. According to data from the BioAtlas, two strictly protected fish species could be expected in u river: Vladykov's lamprey (*Eudontomyzon vladykovi*) and

Danube whitefin gudgeon (*Romanogobio vladykovi*). However, these species were not recorded during field surveys. Instead, the strictly protected species, striped ruffe (*Gymnocephalus schraetser*), was documented at the site. All three analyzed indices used to assess species diversity and species richness indicate low species richness, diversity, and uniformity of the fish population. The Shannon-Wiener index clearly shows that the abundances of different species are not similar, meaning that the population is dominated by easily adaptable species (topmouth gudgeon and Prussian carp). The high abundance of topmouth gudgeon and Prussian carp is likely a consequence of the influence of the fishpond Orahovica, where these species encounter more favorable habitat conditions than in the river itself, as well as reduced predation pressure from carnivorous fish, which are poorly represented in fishpond production. These species likely reach the Vučica River by escaping from production ponds through drainage channels. Such a high proportion of invasive species in terms of abundance, together with the substantial contribution of non-native species to total biomass, may negatively affect the survival of larvae of native fish species.

Conclusion

Field investigations indicate that the Vučica River hosts fish species that are not listed among the species records available on the Bioatlas. Therefore, systematic ichthyological field surveys should be conducted in order to accurately determine the full composition of fish species along the entire course of the river. In addition, the Vučica River is not included in the ecological network along its entire length. Consequently, certain sections of the river are not subject to the same level of protection, despite being part of the same watercourse.

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ORIGINAL SCIENTIFIC PAPER

Karlovac among Europe's Leading Urban Dendrofloras? Analysis of Species Composition in the Urban Area

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Summary
The aim of this study was to assess the dendrological diversity of the urban central area of Karlovac and to analyze the structure of urban trees. Diversity was evaluated using the Shannon- Wienner index (H') and the Simpson index (1-D), with a high H' index of 3.55 and a Simpson index of 0.95 recorded. The dominant species, *Acer platanoides*, accounts for 11.17% of the total tree composition, indicating an even distribution of abundance and low dominance of individual species. The results emphasize the importance of systematic maintenance and planned planting to preserve the stability, functionality, and aesthetic value of the urban dendroflora.

Keywords: biodiversity, Shannon-Wiener index, Simpson index, dominant species, tree

Introduction

Urban areas represent the dominant form of anthropogenic influence on landscapes in the modern world, with continuous urbanization processes significantly affecting the structure, function, and biodiversity of ecosystems. In this context, urban dendrology has emerged as an interdisciplinary scientific and professional field encompassing the planning, planting, management, and maintenance of woody vegetation in urban environments, aiming to optimize the ecological, social, and aesthetic functions of urban trees.

Contemporary approaches in urban arboriculture emphasize the importance of quantitative analysis of species composition and structure, where tree diversity represents a key factor in the resilience of urban ecosystems. Research indicates that higher species diversity reduces the vulnerability of urban trees to biotic and abiotic stressors (Roebuck et al., 2022), while other studies highlight that tree diversity directly contributes to the stability and adaptive capacity of urban systems (Jovanović et al., 2025).

Biodiversity of urban trees serves as an indicator of urban ecosystem functionality and is most often quantified using the Shannon diversity index, which integrates species richness and relative abundance. Empirical studies show that Shannon index values vary significantly within urban areas, typically being lower in city centers due to intense anthropogenic pressures and higher in peripheral or less urbanized zones (Jovanović et al., 2025). Additional studies confirm the importance of applying both Shannon and Simpson indices to assess urban biodiversity and compare different types of public green spaces (Fedor and Zvaríková, 2019). Investigations across diverse urban systems indicate that higher species diversity provides a broader range of ecosystem services and enhances resilience to climatic and anthropogenic stressors (Atchade et al., 2023), while specific Shannon index values in particular urban environments indicate relatively high diversity in well-managed systems (Muthulingam and Thangavel, 2012).

Urban trees play a critical role in microclimate regulation through shading, evapotranspiration, and air temperature reduction, directly contributing to urban heat island mitigation and air quality improvement. Studies confirm that vegetation structure and diversity significantly influence the cooling intensity of urban spaces and the stabilization of microclimatic conditions (Nowak et al., 2010; Gill et al., 2007; Tzoulas et al., 2007; Bowler et al., 2010). Furthermore, soil degradation and unfavorable urban conditions considerably limit tree growth and vitality (Muthulingam and Thangavel, 2012), while climate change exacerbates stress through extreme temperatures and altered precipitation patterns (Atchade et al., 2023). Inadequate management of urban green spaces can lead to reduced diversity and dominance of individual species, increasing the vulnerability of urban ecosystems (Fedor and Zvaríková, 2019).
Currently, no studies are known that comprehensively assess the quantitative and qualitative composition of dendroflora in Karlovac. The aim of this research is to determine the qualitative and quantitative diversity of woody species using the Shannon and Simpson indices.

Material and Methods

This study was conducted in the urban central area of Karlovac, in central Croatia, and did not include trees from rural or peripheral areas of the city. Karlovac is located in the continental biogeographical region, surrounded by four rivers, at an altitude of 112 m, with a population of 49,377 inhabitants, of which 41,869 reside in the urban central area according to the 2021 census.
Tree locations were recorded using a Garmin Oregon 650 GPS, the topographic map of Karlovac at 1:25,000 scale, and a GIS system. Species identification was carried out using a dichotomous key (Idžojtić, 2009). Trees were visually identified by walking through the study area and then determined based on bark, leaf, and fruit characteristics. After species identification, the location and coordinates of each tree were recorded using the map and GPS and subsequently entered into the GIS database.
Descriptive statistical analyses were performed in Microsoft Excel. Biodiversity was quantified using the Shannon-Wiener diversity index (H') and the Simpson index (D). The Shannon-Wiener index is calculated as $H = -\Sigma pi * \ln(pi)$, while the Simpson index is calculated as $D = \Sigma ni(ni-1)/N(N-1)$. These indices are commonly used to assess urban tree populations because they account for population size, species richness, and species evenness (Cowett and Bassuk, 2017).
The Shannon index (Shannon, 1948) is logarithmic and gives greater weight to less abundant species, making it less sensitive to sample size. Shannon diversity increases as both species richness and community evenness increase. Values range from 0 to 5, but in most ecological studies they typically fall between 1.5 and 3.5, rarely exceeding 4 (Magurran, 1988). The Simpson index (Simpson, 1949) gives greater weight to more abundant species and is more sensitive to sample size (Cowett and Bassuk, 2017).

Results and Discussion

Between 2015 and January 2026, a total of 9,968 trees were counted and identified. It is important to note that not all trees within the Karlovac urban area have been surveyed; counting and identification of the remaining trees will continue throughout 2026, and if necessary, later. Recorded trees acount for more than 83% of the total tree abudancein the urban area of Karlovac City.The survey recorded 9,968 trees, representing 119 different species (Table 1.) from 60 genera and 30 families. The most represented family is Rosaceae with 20 species, followed by Sapindaceae and Pinaceae with 16 species each. The least represented families are Taxaceae, Ginkgoaceae, Simaroubaceae, Cannabaceae, Cercidiphyllaceae, Ebenaceae, Elaeagnaceae, Altingiaceae, Paulowniaceae, Platanaceae, Anacardiaceae, and Adoxaceae, each represented by only one species.

The most species rich genera are Acer, Prunus, and Fagus, while in terms of tree numbers, the genera Acer (2,084 trees), Tilia (1,244 trees), and Aesculus (742 trees) dominate. The genus Populus comprises only 260 trees. By individual species abundance, *Acer platanoides* dominates with 1,096 trees (11.17%), followed by *Tilia cordata* with 841 trees (8.57%), *Aesculus hippocastanum* with 742 trees (7.56%), and *Betula pendula* with 702 trees (7.15%). Notably, 21 species are represented by only a single tree. The low dominance of the most abundant species, *A. platanoides*, contributes to a more stable and ecologically resilient urban dendroflora.

Six hybrids were identified, with the most abundant being *Magnolia* × *soulangeana* (88 trees), *Aesculus* × *carnea* (36 trees), and *Sorbus* × *intermedia* (27 trees), totaling 156 hybrid trees. Hybrid trees were not included in the calculation of biodiversity indices.

Using the Shannon-Wiener formula, the calculated diversity value is very high, H' = 3.5527. The Simpson index also indicates significant diversity, with 1-D = 0.95.

Table 1. Diversity of dendroflora species in the urban area of Karlovac City.

Species	**N**	**Species**	**N**	**Species**	**N**
Abies alba	10	*Betula pendula*	702	*Prunus cerasus*	68
Abies concolor	3	*Betula pubescens*	1	*Prunus domestica*	255
Abies nordmaniana	1	*Betula utilis*	3	*Prunus laurocerasus*	1
Cedrus atlantica	1	*Betula verrucosa*	4	*Prunus persica*	81
Cedrus deodara	15	*Carpinus betulus*	386	*Prunus serrulata*	66
Larix decidua	30	*Carpinus orientalis*	2	*Prunus spinosa*	3
Picea abies	474	*Corylus avellana*	19	*Pyrus communis*	64
Picea glauca	5	*Corylus colurna*	8	*Sorbus aria*	10
Picea omorica	45	*Ostrya carpinifolia*	6	*Sorbus aucuparia*	3
Picea pungens	38	*Caragana arborescens)*	2	*Dyospiros virginiana*	2
Pinus nigra	314	*Cercis siliquastrum*	17	*Elaeagnus angustifolia*	1
Pinus ponderosa	2	*Gleditsia triacanthos*	57	*Ficus carica*	7
Pinus strobus	49	*Laburnum anagyroides*	12	*Morus alba*	14
Pinus sylvestris	207	*Laburnum watereri*	2	*Morus nigra*	3
Pinus wallichiana	2	*Robinia pseudoacacia*	153	*Morus plantifolia*	4
Cryptomeria japonica	8	*Sophora japonica*	149	*Fraxinus americana*	1
Taxodium distichum	15	*Castanea sativa*	1	*Fraxinus angustifolia*	113
Sequiadendron giganteum	1	*Fagus silvatica*	23	*Fraxinus excelsior*	175
Taxus baccata	21	*Quercus rubra*	55	*Fraxinus ornus*	113
Cupressus sempervirens	4	*Quercus cerris*	1	*Juglans nigra*	20
Chamaecyparis lawsoniana	7	*Quercus petraea*	10	*Juglans regia*	211
Thuja occidentalis	60	*Quercus robur*	57	*Liquidambar styraciflua*	186
Juniperus communis	3	*Catalpa bignonioides*	134	*Liriodendron tulipifera*	193
Metasequoia glyptostroboides	1	*Cercidiphyllum japonicum*	23	*Magnolia grandiflora*	3
Ginkgo biloba	10	*Celtis australis*	4	*Magnolia kobusa*	2
Acer campestre	94	*Cornus alba*	2	*Parrotia persica*	3
Acer ginnala	6	*Cornus florida*	1	*Paulownia tomentosa*	7
Acer negundo	174	*Cornus kousa*	8	*Platanus acerifolia*	199
Acer palmatum	2	*Cornus mas*	10	*Populus alba*	7
Acer platanoides	1096	*Crataegus laevigata*	8	*Populus nigra*	252
Acer pseudoplatanus	585	*Crataegus monogyna*	3	*Populus tremula*	1
Acer rubrum	4	*Crataegus oxyacantha*	9	*Rhus typhina*	58

Acer saccharinum	90	*Malus domestica*	74	*Salix alba*	25
Acer tataricum	33	*Malus floribunda*	6	*Salix babylonica*	47
Aesculus hippocastanum	742	*Malus sylvestris*	6	*Salix integra*	6
Ailanthus altissima	2	*Prunus armeniaca*	5	*Salix matsudana*	4
Sanbucus nigra	5	*Prunus avium*	88	*Salix purpurea*	5
Tilia cordata	841	*Tilia platyphyllos*	312	*Ulmus carpinifolia*	6
Tilia tomentosa	91	*Zelkowa serrata*	2	*Ulmus glabra*	26
Alnus spaethii	*5*	*Prunus cerasifera*	106		

Table 2. Comparison of dendroflora diversity in cities worldwide.

City	**N**	**H'**	**Dominant species**	**%**	**1-D**
Karlovac	9812	3,55	*Acer platanoides*	11,17	0,95
Paris	13228	2,61	*Platanus acerifolia*	36,00	0,78
Bologna	3053	3,07	*Platanus acerifolia*	17,00	0,88
Oslo	828	2,00	*Tilia spp.*	56,00	0,68
Amsterdam	7828	3,08	*Ulmus hollandica*	31,00	0,75
Cambridge	1938	3,41	*Gleditsia triacanthos*	14,00	0,90
Niš	3998	1,37	*Platanus acerifolia*	22,00	NO DATA
Buenos Aries	39942	2,22	*Fraxinus pennsylvanica*	35,00	0,80
Vancouver	3631	3,20	*Acer rubrum*	21,00	0,93
Melbourne	3237	2,52	*Platanus ×acerifolia*	44,00	0,83

The Shannon index value for Karlovac falls within the upper range of typical values for urban dendroflora communities (Magurran, 1988), while the Simpson index confirms minimal dominance of a single species within the city's dendroflora composition. The dominant species in Karlovac is *A. platanoides*, with a relatively low relative abundance of 11.17%, which further contributes to ecosystem stability and reduces the risk of widespread disease or pest outbreaks specific to a single species.

Comparisons with other cities show that Karlovac (Table 2.) has a significantly higher diversity index than Paris, where the Shannon index is 2.61 with high dominance of *Platanus acerifolia* at 36%, and Oslo, with an index of 2.00 and dominance of *Tilia spp*. at 56%. Other cities, including Bologna, Amsterdam, Cambridge, and Vancouver, have Shannon indices ranging from 3.07 to 3.20, with dominant species comprising 17–31% of the composition, placing Karlovac at the upper range of biodiversity and species evenness. The only comparable Shannon-Wiener index is observed in Cambridge (3.41), where *Gleditsia triacanthos* represents 14% of the population, but the total tree sample is significantly smaller, with only 1,938 trees.

In Karlovac, the relatively low dominance of the dominant species implies greater complexity in urban tree maintenance, as multiple species require simultaneous care, necessitating ongoing dendrological monitoring and precise planning for planting and replacement. From an urban forestry management perspective, the results indicate the importance of preserving existing diversity and continuously monitoring tree conditions to maintain ecological stability and resilience to biological invasions of alien tree species, pests, and climate-related stressors.

The high dendrological diversity in Karlovac enables long-term ecosystem stability, resilience to extreme weather events, and preservation of the functional and aesthetic values of urban green spaces, while simultaneously requiring professional and systematic management of planting and maintenance for all species included in the urban tree composition. Comparisons with cities such as Niš, where the Shannon index is 1.37, or Buenos Aires, with an index of 2.22, clearly demonstrate Karlovac's superiority in terms of urban dendroflora diversity and interspecies balance.

Conclusion

The high dendrological diversity of Karlovac indicates an exceptionally even distribution of abundance among species and minimal dominance of individual species. The relatively low representation of the dominant species implies greater complexity in maintenance, requiring diverse care methods for multiple species, systematic monitoring of tree conditions, and careful planning of planting to preserve balance and high dendrological heterogeneity.

In the context of long-term urban tree management, these results suggest that Karlovac has significant potential for maintaining a sustainable urban dendroflora, resilient to climatic stressors and biological invasions. However, maintaining such diversity necessitates professional management and continuous interventions, including silvicultural practices and tree replacement.

Based on the biodiversity composition of the dendroflora in the urban central area, Karlovac leads compared to other European cities for which data are available.

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ORIGINAL SCIENTIFIC PAPER

Biodiversity of biofouling bivalve community on larval collectors in two Natura 2000 sites in Split-Dalmatia County

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Summary
A monitoring survey of bivalve communities using larval collectors was conducted in 2024 at two Natura 2000 sites in Split-Dalmatia County: Pantan (HR3000430) and the Cetina Estuary (HR3000126). The study aimed to assess biodiversity, detect alien species and evaluate the potential recruitment of the critically endangered noble pen shell *Pinna nobilis*. A total of 27 bivalve species from 12 families (N = 4688) were recorded, with *Musculus costulatus* (N = 1690), *Musculus subpictus* (N = 1159) and *Mytilaster minimus* (N = 383) being the most abundant. Multivariate analyses (Bray–Curtis similarity, MDS, PERMANOVA) revealed significant differences in community structure among locations. No juvenile *P. nobilis* were detected, indicating the absence of recruitment during the study period. The results confirm the usefulness of larval collectors as a tool for biodiversity monitoring and highlight the need for continued surveillance of endangered and alien species in Natura 2000 sites.

Keywords: fouling bivalve community, bivalve larval collectors, alien species, Natura 2000

Introduction

Marine biodiversity encompasses the species, habitats and ecological processes that sustain marine ecosystems and provide essential services to human society (EEA, 2020). However, pollution, habitat degradation and overexploitation are increasingly threatening it, reducing ecosystem resilience and negatively impacting coastal communities and economies (Lotze, 2021). As filter feeders, bivalves play an important ecological role in water purification and nutrient cycling, and are important in food webs and aquaculture (Helm et al., 2004; FAO, 2022). Fouling communities are mainly composed of filter-feeding invertebrates, including bivalves, that regulate coastal food webs and contribute to water quality, and often develop on artificial structures that can be hotspots for the spread of alien species (Leclerc et al., 2024). Native bivalve species can be further disadvantaged by competition for food and space with invasive fouling organisms, which may alter settlement success, community composition and recovery dynamics in degraded coastal ecosystems (Lord, 2017; Raabe and Gilg, 2020). Biological invasions represent a major driver of biodiversity loss in marine ecosystems worldwide (Katsanevakis et al., 2023). Invasive species are among the most significant drivers of global environmental change and biodiversity decline, one of the successful alien species is the ascidian *Styela plicata*, which is resistant to a wide range of environmental conditions (Galià-Camps et al., 2024). At the same time, the endemic *P. nobilis* has experienced mass extinctions caused by the pathogen *Haplosporidium pinnae*, making it critically endangered today (Catanese et al., 2018; IUCN, 2022). Monitoring its recovery can be carried out by setting up bivalve larval collectors that allow research on larval settlement and local biodiversity. However, data on bivalve recruitment and fouling community structure in transitional coastal systems of the south eastern Adriatic remain limited, particularly in the context of *P. nobilis* recovery following mass mortality events.

Material and Methods

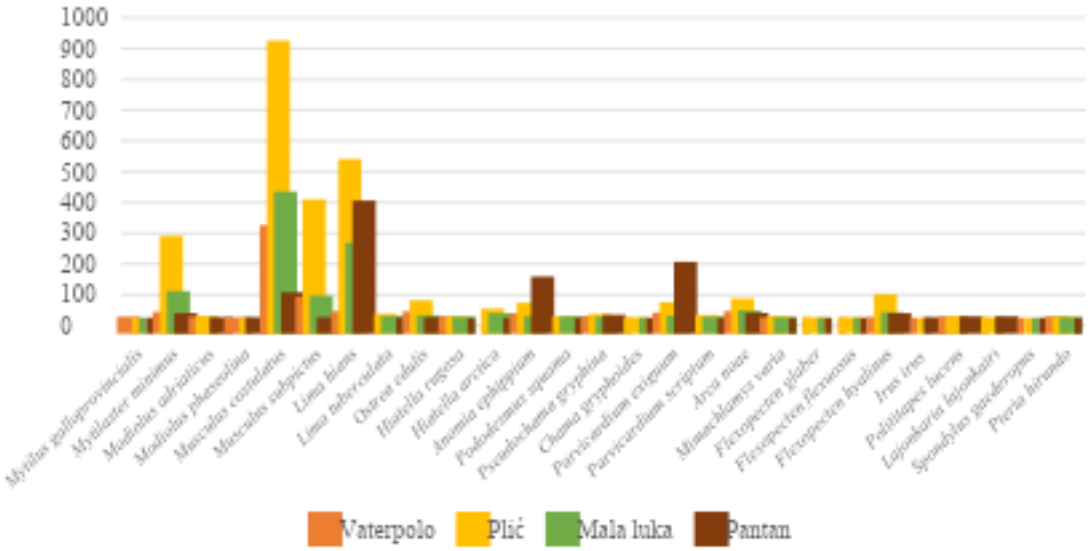
The study of the bivalve community was conducted in 2024 in the ecologically significant Natura 2000 areas of the Split-Dalmatia County: the Pantan wetland and the Cetina River estuary. These areas were selected due to their high biodiversity and previous presence of the protected *P. nobilis* (Direktiva 92/43/EEZ, 1992). Samples were collected using larval collectors made of polypropylene mesh bags that simulate natural larvae habitats.

The collectors were deployed at four stations (one in Pantan and three in the Cetina estuary: L1-Plić, L2-Vaterpolo, and L3-Mala luka). At Pantan and L2, collectors were placed at a depth of 2 m; at L1, they were deployed at 2 and 4 m; while at L3, they were placed at 2, 4, 6, 8 and 10 m. Due to field loss, two replicate collectors at the L3 station and one mesh bag at Pantan were missing, resulting in an unbalanced design. After approximately six months of immersion (from May/June to October/November), the remaining collectors were removed, and the material was filtered through a 500 µm sieve and preserved in 96% ethanol. In the laboratory, the specimens were classified according to morphological characteristics using relevant literature.

Data were analysed using PRIMER software. Bray–Curtis similarity was calculated on square-root transformed abundance data. Non-metric multidimensional scaling (nMDS) and PERMANOVA were applied to test differences in community structure among locations (fixed factor).

Results and Discussion

A total of 27 species of bivalves from 12 families (Anomiidae, Arcidae, Cardiidae, Chamidae, Hiatellidae, Limidae, Mytilidae, Ostreidae, Pectinidae, Pteriidae, Spondylidae and Veneridae) were determined on the installed collectors in 2024 (graph 1). Also, the alien ascidian *S. plicata* was determined at the Pantan station, with 53 individuals counted across all collectors. In addition, the invasive alien alga *Caulerpa cylindracea* was determined to be growing on the collectors at the L2 location. Of the bivalves found, commercially important species such as *Mytilus galloprovincialis*, *Ostrea edulis*, *Mimachlamys varia* and *Arca noae* were recorded. However, the most numerous was *Musculus costulatus* (1690), followed by *Musculus subpictus* (1159), *Mytilaster minimus* (383), *Parvicardium exiguum* (253), and *Anomia ephippium* (199) (graph 1). Unfortunately, not a single *Pinna nobilis* individual was found in the samples.



Graph 1. Representation of the total number of bivalves by species and locations.

The distribution of bivalve species among stations and depths showed distinct community patterns. The L2 location was dominated by *M. costulatus*, accounting for 55–71% of the total abundance. In contrast, the L1 location exhibited a more balanced community structure compared to other sites in the Cetina estuary. The highest biodiversity was recorded at station L1, 2 at a depth of 4 m. At L3, species richness decreased with increasing depth, and the community was mainly dominated by *M. costulatus* and *Lima hians*. Pantan differed from the other locations, with *L. hians* as the most abundant taxon and a noticeably lower presence of *M. costulatus*. The biodiversity indices showed that the largest number of species (17) and individuals (866) were recorded at the L1, 2 station (2 and 4 m), while the lowest values were recorded at the L3 and L2 1 locations. The index values indicate moderate biodiversity of the communities, with a pronounced dominance of certain species at some stations (table 1).

Table 1. Values of selected diversity indices distributed across stations and depths (*S* = number of species, *N* = number of individuals, *d* = Margalef index, *J′* = Pielou evenness, *H′* = Shannon-Wiener index, 1- *λ′* = Simpson index).

Station name and depth	***S***	***N***	***d***	***J′***	***H′***	**1- *λ′***
L1, 1, 2 m	13	754	1,811	0,6035	1,548	0,7146
L1, 1, 4 m	11	146	2,007	0,7579	1,817	0,7946
L1, 2, 2 m	14	866	1,922	0,6595	1,741	0,7733
L1, 2, 4 m	17	162	3,145	0,8016	2,271	0,8767
L1, 3, 2 m	16	406	2,497	0,618	1,714	0,7496
L1 3, 4 m	13	112	2,543	0,7548	1,936	0,8065
L2, 1, 2 m	8	70	1,648	0,4895	1,018	0,4687
L2, 2, 2 m	13	139	2,432	0,662	1,698	0,6742
L2, 3, 2 m	13	293	2,113	0,5661	1,452	0,6174
L3, 2 m	11	399	1,67	0,6443	1,545	0,7423
L3, 4 m	12	179	2,121	0,4784	1,189	0,5238
L3, 6 m	10	100	1,954	0,6149	1,416	0,6133
L3, 8 m	8	80	1,597	0,5688	1,183	0,6047
L3, 10 m	13	141	2,425	0,6567	1,684	0,7466
Pantan 1, 2 m	13	408	1,996	0,5943	1,524	0,7157
Pantan 3, 2 m	11	433	1,647	0,6255	1,5	0,6935

Bray–Curtis coefficients showed the highest similarity of bivalve communities between stations L3, 10 m and L1 1, 4 m (85.0%), while the lowest similarity was recorded between L2 1, 2 m and Pantan 3, 2 m (26.9%). MDS analysis (stress = 0.13) confirmed the patterns of similarity among stations (fig. 1). PERMANOVA revealed significant differences between locations, with L3 significantly different from L2 and Pantan ($p < 0.001$), while no significant differences in dispersion were detected (PERMDISP, $p = 0.089$). The dominance of *Musculus* species is consistent with previous studies of fouling communities in the Adriatic, where opportunistic mytilids rapidly colonise artificial substrates. The absence of *P. nobilis* recruitment further supports recent findings indicating a lack of recovery following mass mortality events.

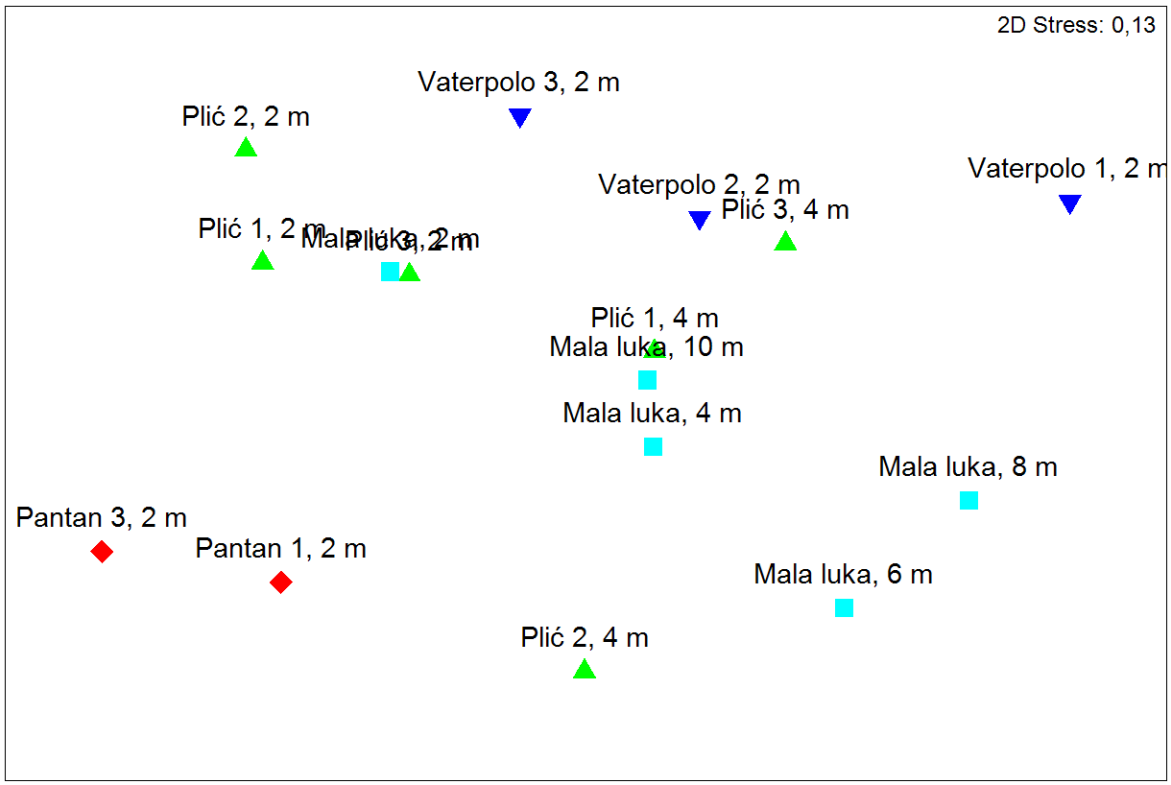


Figure 1. Two-dimensional representation of nMDS ordination of species distribution by location and depth based on Bray-Curtis similarity coefficients.

Conclusion

Biodiversity indices and multivariate analyses indicate moderate community diversity with pronounced differences between locations, which confirms the importance of continuous monitoring of biodiversity and populations of endangered and alien species. These findings suggest that, despite suitable habitats, recruitment of *P. nobilis* remains absent, emphasizing the urgency of conservation actions and long-term monitoring.

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REVIEW ARTICLE

Ragweed – allergenic plant

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Summary
Ragweed (*Ambrosia artemisiifolia* L.) is native to North America but has been introduced to Europe and many parts of the world. This plant is conquering Europe at a speed of 6 to 20 km per year, spreading from centers of "infection". Ragweed pollen is one of the most common environmental allergens, which can cause hay fever (sneezing, swelling of the eyelids, conjunctivitis, asthma), which impact the life quality. Between mid-August (late summer) and mid-September (early fall) is the pollen peak and the season typically lasts six to ten weeks. Climate change can affect pollen allergies in several ways. Many studies suggest that higer carbon dioxide levels and rising temperatures contribute to longer growth time and increase plant productivity and pollen production.

Keywords: ragweed, pollen, allergies, climate change

Introduction

Ragweed is an annual plant from the *Asteraceae* family. This plant prefers temperate climate, dry and sunny places, but it also tolerates shade. Outside its native habitat, ragweed occurs only occasionally. Northern geographic areas have low temperatures and this plant cannot reproduce properly there, while in southern geographic areas, drought is the main factor preventing ragweed invasion. It is assumed that climate change and warming will help ragweed spread to the north and northeast of Europe. Ragweed is an agricultural weed (in sunflower, soybean, corn crops), but it also occurs in stubble fields, along roads and railways. The lack of natural enemies and resistance to many herbicides increase the invasiveness of this plant (Knolmajer et al., 2025).

Global data shows an increase in allergic diseases regardless of age and social status in both developed and developing countries (Pawankar, 2014, Lake et al., 2017, Traidl-Hoffmann, 2017). The World Health Organization (WHO) states that allergic respiratory disease is the fourth most important chronic disease in the world and represents a major public health problem that affects the life quality. Allergic diseases of the respiratory system are caused by pollen from plants with high allergenic potential. In recent decades, there has been a significant increase in the number of people sensitive to pollen in Europe, especially in larger cities and industrial areas (Smith et al., 2014). The presence of pollen in the air is seasonal, and the spectrum of pollen grains reflects the floristic composition of the plant cover of the studied area. Pollen is a small and detachable part of a plant. One plant can release a billion pollen grains per year. Pollen grains are one of the most powerful allergens. The wind carries pollen grains over long distances because they are light (Prank et al., 2013).

Warmer spring weather triggers earlier pollen production, while warmer autumn weather extend the growing season for other plants, such as ragweed. Global warming facilitates a northward migration of invasive ragweed in Europe. Warmer weather as well as increased carbon dioxide concentrations enable ragweed and other plants to produce more allergenic pollen, in larger quantities (Ziska and Caulfield, 2000, Ziska et al., 2003). The results of these authors research support the existence of a link between rising CO_2 levels, global change and public health. Research from the Medical University of Vienna warns that the ozone increases

the severity of symptoms in people sensitive to birch, grass, and ragweed pollen (Berger et al., 2020). This means that in many locations, allergy season could be extended and pollen levels could increase as a result of climate change.
Due to the increasing problem of climate change and allergies related to it, more and more scientific and professional articles are published on this topic every year.

Pollen allergy and climate change

A small part of the total number of particles in the air is made up of pollen grains, which are the most common cause of allergic diseases and are considered the most powerful natural allergens. One of the most important impacts on allergens and human health is climate change, which is evident in the increase in research published on this topic (Beggs, 2015). Many clinical experts point out that allergic diseases will increase due to climate change (Lake et al., 2017). Climate change affects the timing and length of the pollen season, can increase plant productivity and pollen production as well as release atmospheric dispersion of pollen (Beggs, 2015, Bielory et al., 2012). The studies of the aforementioned authors, conducted mostly in the northern hemisphere (Europe, Japan and the USA), support this. The increase in airborne pollen depends on both plant species and region, so future research should focus on species and regions, especially regions of the Southern Hemisphere. The distribution of many plant species has shifted in response to climate change. Increasing temperatures and CO_2 concentrations in urban areas increase the allergenicity of pollen grains and greater allergic sensitization (Salo et al., 2014). All previous studies highlight the importance and need for continuous monitoring of allergenic pollen in the air. The prevalence of pollen allergy in the general population in Europe is estimated to be 40% (D'Amato et al. 2007). Many tree, grass, and weed species are anemophilous (the wind spreads pollen). These species are particularly prone to causing seasonal allergies or hay fever. Members of the *Poaceae* family (grass) are the main cause of allergic reactions caused by pollen in Europe due to their wide geographical range. Among trees, the most allergenic pollen is produced by birch in northern, central and eastern Europe, and by olive and cypress in Mediterranean regions (D'Amato et al., 2007). The role of pollen in the development and severity of allergic diseases depends on a number of factors, including duration and intensity of exposure, and allergenicity of the pollen. These factors have high geographical and temporal variability, resulting in differences in the prevalence of pollen-related allergic rhinitis between locations and periods (Bousquet et al., 2020).
The spread of invasive species to new parts of the world is also causing allergic reactions in younger populations. Ragweed has spread beyond North America to Europe, as well as Australia, Asia, and South America. Already, about 60% of population in Hungary, 20% in Denmark, and 15% in the Netherlands are allegedly sensitive to the pollen of this plant. It is estimated that ragweed pollen causes asthma approximately twice as often as pollen from other plant species (Skjøth et al., 2010). Research by El Kelish et al. (2014) indicates that ragweed allergenicity may increase under the influence of global changes (elevated CO_2, drought). Pollen allergen release can vary depending on the region, season, atmospheric pollutants, humidity and storm periods. Environmental factors, particularly atmospheric pollution, intensify the allergenicity of certain plant pollens. Ragweed pollen contains more than 60 different proteins (glycoproteins) that can cause hay fever and severe health problems (Liu et al., 2022). Two major allergens of ragweed pollen are Amb a I and Amb a II. Ragweed pollen is an extremely strong allergen – even very small concentrations (10–20 grains/m^3 of air) can cause symptoms in sensitive individuals (Pham et al., 2016).

Ragweed and climate change

The potential impact of climate change on the occurrence of allergic rhinitis has most often been studied in the case of ragweed. Ragweed (*A. artemisiifolia*) is invasive plant and very harmful for public health because of the large amount of pollen grains with high allergenic potential (Taramarcaz et al., 2005, Fumanal et al., 2007). The climate of an area is determined by its geographical location, geological background and terrain. Noteworthy is the wind, which is conducive to the distribution of ragweed pollen in any area. Pollen grains can be transported hundreds of kilometres by breezy conditions causing allergy symptoms where ragweed is not yet widespread (Zhang and Steiner, 2022). The highest concentrations of pollen grains are usually in the morning and lower in the evening or after rain. Ragweed has a higher biomass and pollen production at increased CO_2 concentrations. It is expected that future climate change will increase the amount of pollen in the air.

Ragweed is currently present in most European countries, especially in France, Italy, Hungary, Slovakia, Czechia, Poland, Bulgaria, Slovenia, Croatia, and Serbia. The research conducted by Rasmussen et al. (2017) warns that areas with a high risk of ragweed pollen allergies will expand in Europe by 27–100%, depending on the climate scenario, with new areas of expansion including Denmark, France, Germany, Russia, and Baltic countries. The same authors predict that by 2100, more and more people in Europe will suffer from severe ragweed allergies. To avoid this, they suggest developing management strategies that limit the spread of ragweed and the establishment of new populations. Lake et al. (2017) estimate that ragweed sensitivity will double in Europe, from 33 to 77 million people, between 2041 and 2060, and that the bigger problem will be in countries that have not been ragweed hotspots so far (like France and Germany). They propose establishing systematic control of the spreading of ragweed, which may be an important adaptation strategy in response to climate change. Changes in agricultural land use, urbanization and climate change are the main causes of the spread of ragweed in Europe. According to Hamaoui-Laguel et al. (2015), by 2050 the concentration of its pollen in the air could be four times higher than today.

French agency for food, environmental and occupational health and safety (ANSES) 2020, estimated that between 1,115,000 and 3,504,000 people were allergic to ragweed pollen in mainland France and Corsica. The plant is now widespread across the entire country by human activities. ANSES expects the costs of the health impacts associated with ragweed to increase in the future. Without controls, the influence of climate change will increase the medical and work productivity costs (ANSES).

Ragweed pollen can be found in every state in the United States and Canada. The research conducted between 1995 and 2015 in the central United States and Canada shows that the pollination season is longer, and the factors influencing this are changes in temperature and precipitation (EPA, 2016). Last frost in spring occurrs earlier, whereas the first frost in fall ocurrs later, which causes longer pollination season. Depending on latitude, the ragweed pollination season in North America has been extended. Ragweed pollen is estimated to affect more than 23 milion people in the USA, and more than 26% of the population is sensitized to ragweed (Arbes et al., 2005).

Conclusion

Many studies indicate that ragweed pollen is becoming a common health problem across Europe. Ragweed's rapid spread, adaptability, and high allergenicity make it one of the most dangerous weeds today. Because of that, control of ragweed spread is the most important for public health. It is necessary to continuously work on educating citizens and working together with local communities. Only with a responsible approach and persistent measures can we reduce the harmful effects of ragweed and protect both people and the environment.

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ORIGINAL SCIENTIFIC PAPER

Ampelographic characterization of newly discovered grapevine varieties ‘Stara belina aromatična’, ‘Modra batovka’ and ‘Gusonoška’

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Summary
According the project „Inventory of Indigenous Grape Varieties in the Ozalj-Vivodina Region“ the varieties ‘Gaensfuesser‘, ‘Batttraube‘ were confirmed for the first time in the Republic of Croatia. A review of historical documents determined vrieties under the names ‘Gusonoška’ and ‘Modra batovka’. The variety ‘Stara belina aromatična’ was unknown by name but widespread in this region. Historical documents mentions synonyms ‘Biela plavičina’ and ‘Muktovka’. Ampelographic characterization included “OIV” morphological descriptors, analysis of economic properties and chemical analysis of the must. The ‘Gusonoška’ variety is a red grape variety, very vigorous, low yield, small berries and good must quality parameters. The ‘Modra batovka’ variety is a variety, vigorous, with large cluster and berry size characteristic of table varieties, with lower must quality. The ‘Stara belina aromatična’ variety is a white variety, medium vigorous, lower yield and good must quality.

Keywords: autochtonous, varieties, Gaensfuesser blau, Batttraube, belina

Introduction

The most comprehensive period of research on the varieties of the Republic of Croatia began in 1998 and lasted until 2002 through the program "Genetic identification of Croatian indigenous grape varieties". The program was implemented by the Faculty of Agriculture and carried out a detailed inventory, morphological description and most of the genetic identification of varieties in the territory of the Republic of Croatia (Maletić et al., 2015a). The program ultimately identified and collected 130 different indigenous and domesticated varieties in the Republic of Croatia (Maletić et al., 2015b). The area of the Ozalj-Vivodina wine region was not covered by systematic research on traditional varieties at that time. In this small wine region, there are still traditional vineyards over 80 years old. By launching the project Identification and "Inventory of Indigenous Grape Varieties in the Ozalj-Vivodina Wine Region" in 2016, the goal was to identify a certain number of previously unknown varieties in such vineyards, in addition to the traditionally represented varieties. During the 5 years of research, 61 samples of plant material were collected for genetic identification. Of these, 18 samples did not match any known varieties from international databases. 23 known varieties recorded in databases were also confirmed, including Belina starohrvatska, Pinella, Dišeća ranina, Pršljivka, etc. Two varieties ‘Gaensfuesser‘, ‘Battraube‘ were confirmed for the first time in the Republic of Croatia. The ‘Stara belina aromatična‘ (sinonims: ‘Mehlweiss‘, ‘Lisztes feher‘) variety was confirmed as very represented in this area. A review of historical documents determined that the varieties were recorded in the area of northwestern Croatia as early as the 19th century under the names ‘Gusonoška’ (Gansfuessler), ‘Modra batovka’ (Battraube) and ‘Biela plavčina‘/ ‘Muktovka‘ (‘Stara belina aromatična’ (Stražimir, 1876). Meanwhile, ‘Modra batovka’ has been confirmed at another location near place Kosinj (Mance et. al., 2019). The plant material from old vines of these varieties have been propagated and planted in the national collection of indigenous varieties. There is interest among local producers in planting or

revitalizing them. With the aim of evaluating the varieties as best as possible, a standard ampelographic and economic evaluation was conducted within the National Collection of Indigenous Varieties in Zagreb.

Material and Methods

The genetic analysis was carried out by "SSR" marker technique (“Simple Sequence Repeat”). The analysis was performed on 9 standard gene-loci (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VRZAG62, VRZAG79) prescribed by the International Organization for Vine and Wine ("OIV") grapevine identification. Protocol of PCR amplification, electrophoretic separation and sizing were performed as described in Žulj Mihaljević et al. (2013). Standardization was taken by SSR profile of ‘Pinot Blanc‘. Genetic profiles were compared with the Vitis International Variety Catalog ("VIVC") and with results of identification based on international project “Southeast European Development Network” (SEEDNet, 2009-2010) . Morphological description of collected accessions was performed using 47 “OIV” descriptors for grapevine cultivars and Vitis species also prescribed by „OIV“. In this work we will present sample of 32 most common descriptors. Genetic profile and OIV description are compared with variety ‘Pinot blanc’. The analysis of economic properties was conducted on sample of six vines and include measuring the average clusters number, average yield per vine (kg), average bunch weight. Chemical analysiss of must determining the sugar content in the must (^{0}Oe), total acids (g/L, expressed as tartaric acid), and pH value of the must. The results of economic properties were compared with the ‘Kraljevina’ variety, which is the most common known indigenous variety in the research area. The results are presented as preliminary for two years of research in 2024 and 2025. A detailed statistical analysis will be conducted after three years of research, i.e. after the 2026 harvest.

Results and Discussion

The results of genetic identification of varieties using SSR markers are shown in Figure 1. In the case of samples V1.7/V4.5, SKV 18-1A/SKV 18-5A, it was confirmed that they were a match with the varieties ‘Stara belina aromatična’ (Croatia, SEEDNet), ‘Slavitza’ (Romania, SEEDNet), ‘Mehlweis’, ‘Lisztez feher’, ‘Topolovina’ (“VIVC”), According Stražimir (1876) historical synonyms for “Mehlweiss’ is ‘Biela plavičina’ and ‘Muktovka’. Due to inconsistencies in the historical descriptions of varieties as well as the synonym database within the "VIVC" database, the name of the variety has not yet been defined and is kept under the first recorded sample via SEEDNet project, which is ‘Stara belina aromatična’. Samples NN_GOR_C/SKV18-6A matched the ‘Batttraube’ (“VIVC”) variety, which is given the name ‘Modra batovka’ (Stražimir, 1876) in historical literature and later research in the Kosinje area confirmed it under the local name ‘Vingor’ (Mance et al., 2019). Sample F1.1 was confirmed to be the ‘Gaensfuesser blau’, ‘Argant’, ‘Margillien’, (“VIVC”) varieties. A review of historical literature determined that it is a descendant of the ‘Gusonoška’ (Stražimir, 1876) variety.

Table 1. Results of genetic identification for varieties ‘Stara belina aromatična’, ‘Modra batovka’ and ‘Gusonoška’.

Sample		V1.7, V4.5, SKV 18-1A, SKV 18-5A	NN_GOR_C, SKV 18-6A	F1.1	Control
SSR marker	*vvs2*	131	133	131	135
		143	141	141	151
	md7	245	247	251	237
		247	247	253	241
	md27	175	175	175	181
		177	181	190	185
	zag62	203	199	191	187
		203	199	203	193
	zag79	234	248	248	236
		256	256	248	242
	md5	228	222	222	224
		236	224	224	234
	md25	237	253	253	237
		239	253	253	247
	md28	232	247	247	216
		247	258	258	234
	md32	248	252	252	240
		258	272	264	272
Matching		Stara belina aromatična/Mehlweiss/Lisztez feher/Slavitza	Modra batovka/Batttraube	Gusonoška/Gaensfuesserblau/Argant/Margillien	Pinot bijeli/Pinot blanc

After identification and collection of cultivars, an ampelographic evaluation of cultivars was carried out, which included OIV description (Figure 2.) as a standard procedure when describing cultivars.

Table 2. Morphological description of varieties ‘Stara belina aromatična’, ‘Modra batovka’, ‘Gusonoška’ i ‘Pinot bijeli’.

OIV code	Characteristic	Stara belina aromatična	Modra batovka	Gusonoška	Pinot bijeli
OIV – 002	Distribution of anthocyanin coloration on prostate hairs of the shoot tip	1-Absent	2-Piping	1-Absent	2-Piping
OIV – 003	Intensity of anthocyanin coloration on prostate hairs of the shoot tip	1-None or very low	3-Low	1-None or very low	1-None or very low
OIV – 004	Density of prostate hairs of the shoot tip	5-Medium	1-None or very low	1	5-Medium
OIV – 007	Colour of the dorsal side of internodes	2-Green and red	1-Green	1-Green	2-Green and red
OIV – 008	Colour of the ventral side of internodes	2-Green and red	1-Green	2-Green and red	2-Green and red
OIV – 009	Colour of the dorsal side of nodes	2-Green and red	1-Green	2-Green and red	2-Green and red
OIV – 010	Colour of the ventral side of nodes	2-Green and red	1-Green	2-Green and red	2-Green and red
OIV – 051	Colour of upper side of blade (4th leaf)	2-yellow	3-bronze	3-bronze	3-bronze
OIV – 053	Density of prostrate hairs betwen main veins on lower side of blade (4th leaf)	7-High	5-Medium	1-None or very low	5-Medium
OIV – 065	Size of blade	7-Large	7-Large	7-Large	5-Medium
OIV – 067	Shape of blade	3-Pentagonal	2-Wedge shaped	3-Pentagonal	2-Wedge shaped
OIV - 068	Number of lobes	3-Five	3-Five	3-Five	2-One
OIV - 070	Area of anhocyanin coloration of main veins on upper side of blade	1-Absent	3-Up to the first bifurcation	1-Absent	1-Absent
OIV - 072	Goffering of blade	3-Weak	3-Weak	1-Absent or very veak	1-Absent or very veak
OIV - 075	Blistering ofupper side of blade	5-Medium	7-Strong	1-Absent or very vweak	5-Medium
OIV - 076	Shape of teeth	3-Both side convex	2-Both side straight	2-Both side straight	5-Both side convex, both side straight
OIV - 079	Degree of opening/overlapping of petiole sinus	3-Open	3-Open	1-Very wide open	5-Closed
OIV - 080	Shape of base of petiole sinus	3-V-shaped	2-Brace shaped	2-Brace shaped	3-V-shaped
OIV - 081	Teeth in petiole sinus	1-None	9-Present	1-None	1-None
OIV - 081	Petiole sinus base limited by vein	1-Not limited	1-Not limited	1-Not limited	1-Not limited
OIV - 082	Degree of opening/overlapping of upper lateral sinuses	2-Closed	3-Slightly overlaped	1-Open	2-Closed
OIV - 083	Shape of base of upper lateral sinuses	1-U shaped	2-Brace shaped	1-U shaped	1-U shaped
OIV - 083	Teeth in upper lateral sinuses	9-Present	1-None or very low	1-None or very low	1-None or very low
OIV - 084	Density of prostrate hairs betwen main veins on lower side of blade	3-Low	5-Medium	1-None or very low	3-Low
OIV - 094	Depth of upper lateral sinuses	5-Medium	5-Medium	7-Deep	Shalow
OIV - 151	Sexual organs	3-Fully developed	3-Fully developed	3-Fully developed	3-Fully developed
OIV - 202	Bunch length	4-Short/medium	7-Long	7-Long	3-Short
OIV - 204	Bunch density	3-Loose	7-Dense	5-Medium	7-Dense
OIV - 208	Bunch shape	1-Cilindrical	3-Funnel shaped	2-Conical	2-Conical
OIV - 220	Berry length	5-Medium	7-Long	4-Short to medium	5-Medium
OIV - 223	Berry shape	6-Obtuse ovoid	2-Globose	2-Globose	2-Globose
OIV - 225	Color of berry skin	1-Green yelow	5-Dark red violet	6-Blue black	1-Green yelow

Based on the OIV description, the varieties can be described according to their morphological characteristics: **'Stara belina aromatična'**- The tip of the shoot is without anthocyanin coloration. The density of prostrate hairs is medium. The color of the young apical leaf is yellow, with a medium density of woolly hairs. The adult leaf is large, pentagonal, with light wrinkling and medium blistering of the lamina. The petiole sinus is V-shaped. Low density of woolly hairs on the reverse side of the leaf. Teeth are present at the base of the upper lateral sinuses. The cluster is small, up to 120mm long, loose and cylindrical. The shape of the berry is slightly ovoid, with a yellow-green color. **'Modra batovka'**- The tip of the shoot has low anthocyanin coloration, and almost no woolly hairs. The color of the young apical leaf is bronze. The adult leaf is large and wedge-shaped with 5 lobes, and has a closed petiole sinus. Anthocyanin coloration of the leaf veins up to the first bifurcation. Teeth are present on the petiole sinus. The density of prostrate hairs on the reverse side is low. The cluster is large, funel shaped, dense. The mature berries are round, dark redd or violet in color**. 'Gusonoška'**- The tip of the shoot is without or with very little anthocyanin coloration, without the presence of woolly hairs. The young leaf is bronze in color without prostrate hairs on the lower side of young shoot leaf. The mature leaf is large, pentagonal in shape, with 5 lobes. There are no or very few woolly hairs on the reverse side. The shape of the base of the petiole sinus is very wide open. The size of the cluster is medium, with a length of about 160 mm, medium compact. The shape of the cluster is conical. The berries are small to medium large, globose shaped, medium length. Berry skin is dark blue in color. The results of measuring economic characteristics and must quality in 2024 and 2025 are shown in Table 2.

Table 2. Economic characteristics and must quality in 2024 and 2025 for 'Stara belina aromatična', 'Modra batovka' and 'Gusonoška'.

Variety	Year	Number of clusters per vine	Yield per vine (kg)	Average bunch weight (g)	Must sugar content (^{0}Oe)	Must total acid (g/L)	pH value of must
'Stara belina aromatična'	2024	13,5	2,27	168,15	72,5	5,27	3,31
	2025	20,3	2,25	110,82	74	6,3	3,12
'Modra batovka'	2024	13,4	4,65	347,16	69,8	7,52	2,96
	2025	13,0	4,97	382,56	61	10,37	2,96
'Gusonoška'	2024	9,5	1,49	157,19	106,4	4,42	3,45
	2025	16,3	2,96	182,31	70	5,59	3,22
'Kraljevina'	2024	14,8	3,99	269,46	67,6	4,56	3,29
	2025	20,3	5,64	277,83	61	4,42	3,36

The table shows large differences in the average number of clusters per vine for the two years of measurement for the 'Stara belina aromatična', 'Gusonoška' and 'Kraljevina' varieties. For the 'Modra batovka' variety, the number of clusters was almost the same in both years (13.4 and 13). The 'Modra batovka' variety also had the highest yield per vine (4.65 and 4.97 kg) and average cluster weight (347,16 and 382,56 g) in both years. The 'Kraljevina' variety also proved to be a high-yielding variety with a yield of 3.99 to 5.64 kg and high cluster weight (269,46 and 277,83 g). The 'Gusonoška' variety had deviations between 2024 and 2025. 'Stara belina aromatična' variety had a stable yield of 2.27 and 2.25 kg per vine in both years also as smallest bunch average weight (168,15 and 110,82 g). Both varieties show a yield acceptable for the production of quality wines with yeald approximately 2 kg per vine. The measurement results support the morphological observations and it can be concluded that 'Modra batovka' is a high-

yielding variety with large bunches and large berries that can be characterized as a table variety. According must quality results, 'Modra batovka' had highest total acidity and lowest pH value in both observed years. It also has a very low sugar content. The 'Kraljevina variety' was also found to have a lowest sugar content and the lowest total acidity in both years of research. The 'Stara belina aromatična' variety had the highest sugar content in 2025 and the 'Gusonoška' variety in 2024. As in previous measurements, a large deviation in the observed indicators was found for the 'Gusonoška' variety, and a better insight into the indicators of this variety will require several more years of measurements. A detailed statistical comparison between varieties will be conducted after three years of monitoring, i.e. after the 2026 harvest.

Conclusion

Ampelographic characterization confirmed that the varieties differ in the main morphological indicators (coloration of the shoot tip, leaf shape, size and shape of the bunch, size and colour of the berry), and economic indicators (yield, number of clusters, sugar and acid content in the must). The 'Modra batovka' variety, due to the size of the bunch and berries and the lower sugar content and higher total acid in the must, can even be characterized as a table variety. 'Stara belina aromatična' and 'Gusonoška' varieties can be classified as wine varieties based on yield indicators, size of the bunch and berries, and indicators of the chemical composition of the must, especially since the must quality indicators are better than those of the 'Kraljevina' variety, which is a traditional indigenous wine variety in the area.

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ORIGINAL SCIENTIFIC PAPER

Environmental contaminant exposure assessment for *Mauremys rivulata* nests on agricultural land in Croatia

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Summary
Mauremys rivulata habitat in southern Croatia includes 40% of agricultural land where potential for toxicological threat to the species was reported in strategic national documents. To assess the exposure risk on nesting sites in Croatia we have analysed topsoil contaminants from all three *M. rivulata* populations yearly 2023 to 2025. Elevated Cu and Cd were associated with cultivated plots, while Ni and Cr were confirmed as geogenic. Screening for over 300 pesticide residues, PCBs, and PAHs showed concentrations mostly below detection limits. Monitoring baselines were recorded and environmental contaminant exposure risk at nesting sites is low over all three populations of *M. rivulata* in Croatia.

Keywords: turtle nests, soil contamination, heavy metals, agricultural impact, nature conservation

Introduction

Various environmental contaminants can be present in agricultural land due to chemical agrotechnical measures, as persistent residues, chemical metabolites, off-site environmental transport, or traffic (Cai et al., 2022; Carter et al., 2025; Knuth et al., 2024; Vane et al., 2014). Physiological, developmental, and reproductive effects of environmental contaminants have been demonstrated in different turtle species, as they often remain within a limited home range, are long-lived, and can accumulate toxins in their tissues (fat, liver, and shell). Heavy metals persist in the environment and interfere with enzyme functions and organ health (Burger and Gochfeld, 2004). The immunotoxic effect of mercury (Hg) has been shown in *Chelydra serpentina* (Hopkins et al., 2013), while neurological impairment of the "righting response" in hatchlings has been observed for lead (Pb), and nephrotoxicity of cadmium (Cd) and its interference with calcium metabolism have been reported (Dong et al., 2024). Polycyclic aromatic hydrocarbons (PAHs) have been shown to be genotoxic in *Emys orbicularis*, with exposure during the egg stage leading to developmental deformities such as shell anomalies (Merleau et al., 2026). Polychlorinated biphenyls (PCBs) are legacy contaminants with endocrine-disrupting effects that can override temperature in temperature-dependent sex determination (TSD) (Bergeron et al., 1994). Organochlorine pesticides act as anti-androgens, decreasing male fertility (de Solla et al., 1998). Modern herbicides (e.g. atrazine or glyphosate) have been shown in turtles to stress the liver, leading to death during hibernation (Héritier et al., 2017).

Focusing on the trans-shell route of exposure, our aim was to quantify anthropogenic contaminants in topsoil (0–30 cm) at *Mauremys rivulata* nesting sites in Croatia, as first input for contaminant exposure assessment. We also aimed to provide monitoring baselines to support habitat management and restoration strategies.

Material and Methods

Topsoil was sampled at total 18 private agricultural plots with small-scale agricultural production or production for personal use (horse and cow pastures, private gardens, olive orchards, and intensive vineyards) within the Natura 2000 ecological network. Four to five plots were selected for each location per population recorded in Croatia: Konavle population (5), Ston field (4), and Majkovi (two Makovi ponds 5, one Prljevići pond 4). Three plots per population were sampled continuously during the three years (Table 1). Samples were taken at the nesting site areas, within a 1.5 km radius of recorded *M. rivulata* specimens. Soil sampling was conducted three times (2023–2025) for physicochemical analysis and heavy metal quantification, while organic pollutants (PAH, PCB, pesticide residues) were assessed for all plots in 2023.

Composite samples (15–20 subsamples, 0–30 cm layer) were prepared according to ISO 11464. Physicochemical methods included potentiometric pH determination (EN ISO 10390), humus by the dichromate method (Škorić, 1982), total N (ISO 11261), and available P_2O_5 and K_2O by the AL-method (Egner et al, 1960). Heavy metals were extracted with aqua regia and quantified by AAS (ISO 2004; ISO 2006; ISO 2022). Organic pollutants were analysed by Eurofins Croatiakontrola Zagreb protocols (RU-OTV-121, 2024). Pesticide screening (300+ substances) followed EN 15662 protocol (LOQ 0.01 mg kg^{-1}). PCBs and PAHs were analysed by chromatography-mass spectrometry (MSZ EN 17322, 2021; MSZ 21470-84., 2002).

The normality and equal variance tests, Welches one-way ANOVA and post hoc tests examining effect of the population or year on the element quantity were preformed in R 4.5.2 (R Core Team, 2025). Preliminary monitoring baseline per population for a certain contaminant is determined using the 95th percentile of measurements from clean locations as a cut-off for background levels.

Table 1. Nine private agricultural plots in four Natura 2000 areas monitored for heavy metals during 2023–2025 and analysed for soil contaminants (PAH, PCB, and pesticide residues) in 2023

Parcel ID	Parcel size (m^2)	Land use	Location	Natura 2000 area	Population
a1	4200	pasture meadow	Konavle	HR2000946	Konavle
b8	1155	natural meadow			
c9	1777	intensive vinyard			
d2	1303	garden, orchard	Ston	HR2001364	Ston
e3	1977	meadow			
f7	1082	arable land meadow			
g4	425	orchard, garden	Majkovi	HR2000947	Majkovi
h5	3187	old olives	Prljevići	HR2000555	
i6	1108	garden			

Results and Discussion

Physicochemical analyses of the 33 topsoil samples (2023–2025) indicated slightly alkaline to neutral conditions, with pH values ranging from 7.76 to 8.50. Humus content varied from 2.99% in olive orchards to over 5.00% in natural pastures. Plant-available phosphorus was generally low in pastures (2.1 mg 100 g^{-1}) but higher in cultivated vegetable gardens (up to 65.2 mg 100 g^{-1}), while potassium levels ranged from 17.0 to 60.5 mg 100 g^{-1}. These values are consistent with land use and previous findings for the Dubrovnik hinterland (Čoga et al., 2022).

None of the 33 samples exceeded the maximum allowed quantities (MAQ) of mercury, lead or molybdenum in agricultural land according to the Regulation on the protection of agricultural land from pollution (NN 71/19). Arsenic exceeded the MAQ in one sample (31.5 mg kg^{-1}). Zinc and cobalt exceeded the MAQ in two samples each (152–198 and 63.4–66.6 mg kg^{-1},

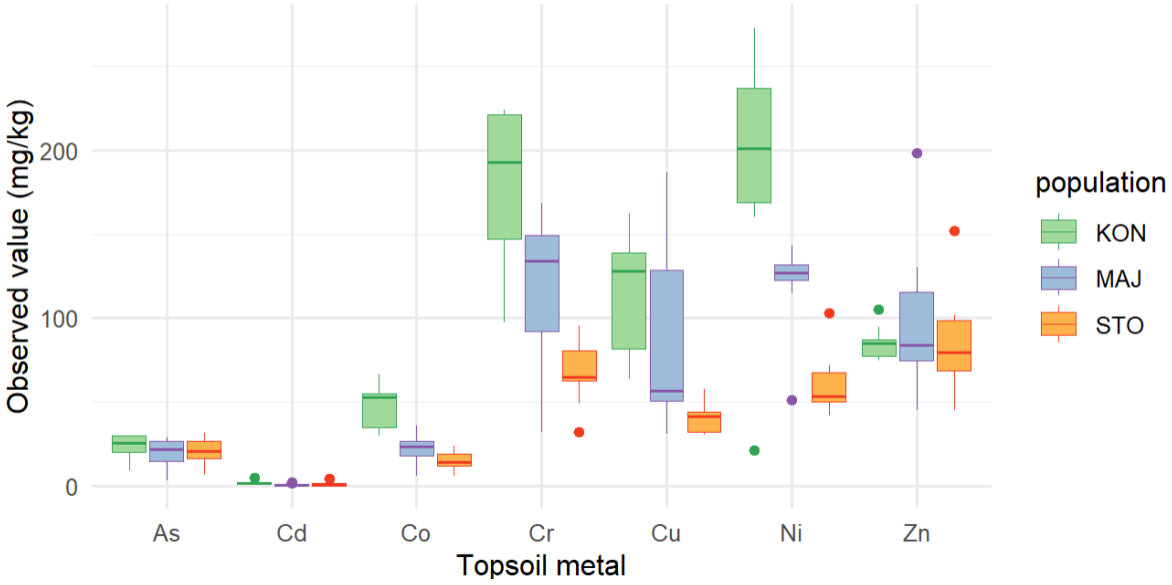
respectively). Cadmium exceeded the MAQ in 15% of samples (2.04–5.19 mg kg^{-1}), copper in 26%, chromium in 52% (190–221 mg kg^{-1}), and nickel in 55% (213–273 mg kg^{-1}) of samples. Except for zinc in one sample (152 mg kg^{-1}), and arsenic in one sample (31.5 mg kg^{-1}) in 2024 none of the metals exceeded MAQ in Ston population nesting sites.

Elevated levels of chromium and nickel in Dubrovnik-Neretva County have previously been recorded and attributed to geogenic sources, specifically the local carbonate and colluvial parent material (Čoga et al., 2022). Only one sample exceeding the nickel MAQ was found at Ston population nesting sites, and one-way ANOVA confirms ($F = 33.607$, $p < 0.001$) that Ston topsoil samples have significantly lower nickel content than those from Majkovi and Konavle (est = -62.1, $p < 0.0001$ and est = -130, $p < 0.001$, respectively). No samples in the Ston area exceeded the chromium MAQ (graph 1).

Other elevated values of heavy metals can be considered of anthropogenic origin, for example, copper found in vineyard parcels or cadmium in active gardens. While cadmium topsoil concentration showed a significant effect of population ($F = 3.64$, $p = 0.038$), the effect was weak and more sampling is required to estimate the significant differences between the populations. Copper (graph 1), however, showed a significant location effect ($F = 6.645$, $p = 0.004$), with Cu topsoil concentrations in Ston lower than in Majkovi and Konavle population nesting sites (est = -49.6, $p = 0.014$ and -72.8, $p < 0.001$, respectively). Additionally, none of the copper concentrations in Ston population samples exceed MAQ. Copper and cadmium bioaccumulate in the kidney and liver of turtles. Copper exposure was found to trigger a cascade of intestinal structural damage, impaired mucosal barrier function, suppressed gene expression, and dysbiosis of the gut microbiota in species of the genus Mauremys (Ali et al., 2025), while cadmium was found to structurally damage the liver and kidney (Dong et al., 2024), with increased mortality, abnormal sexual development, and decreased fertility in *Chrysemys picta* due to endocrine (TSD) disruption in embryos. All tested organochlorine pesticides, specifically Aldrin, Dieldrin, Endosulfan isomers, and Lindane, were below the limit of quantification (<0.01 mg kg-1 dry matter). These results confirm the absence of legacy organochlorine contamination, which is particularly significant for the protection of *M. rivulata* embryos, as these substances are known to interfere with reptilian reproductive success (Sparling et al., 2015).

Extensive screening for organic pollutants demonstrated high environmental safety. All 300+ pesticide residues and seven indicator PCB congeners were below the LOQ of 0.01 mg kg-1. PAHs were below detection in eight samples, while Sample 1 showed a minimal sum of 0.19 mg kg-1, well below the threshold for agricultural soil. In one sample from Konavle, trace amounts of specific PAHs were detected, including phenanthrene (0.04 mg kg-1), fluoranthene (0.05 mg kg-1), pyrene (0.05 mg kg-1), chrysene (0.03 mg kg-1), and benzo[b]fluoranthene (0.02 mg kg-1). The total sum of 19 PAHs reached 0.19 mg kg-1, which is significantly below the Croatian MAQ of 2.0 mg kg-1 for agricultural soils. These traces likely originate from localised biomass burning or atmospheric deposition rather than direct pollution (Vane et al., 2014).

Clean locations were chosen as those with measurements not exceeding anthropogenic MAQ in any of the measured parameters (excluding chromium and nickel) in the measuring year. Of the 33 measurements, 24% were not considered clean: 3 in Konavle, 3 in Majkovi (Prljevići pond), and 2 in Ston. In Ston, zinc and arsenic exceeded MAQ, while all other non-clean cases were due to copper. In the Majkovi population (Prljevići pond), zinc and cadmium exceeded MAQ at the same parcel. Table 2 provides the suggested preliminary monitoring baselines per *M. rivulata* population for heavy metal content monitoring.



Graph 1. Distribution of metal concentrations over the 33 topsoil samples collected at Majkovi (MAJ), Konavle (KON) and Ston (STO) agricultural parcels with *Mauremys rivulata* nesting sites 2023-2025.

Table 2. Preliminary monitoring baselines for heavy metal content monitoring at *Mauremys rivulata* nesting sites in Croatian Natura 2000 areas

population, N		As	Cd	Co	Cr	Cu	Ni	Zn
Konavle topsoil, 6	Min-Max	19.10-30.00	0.68-5.19	30.00-63.40	97.60-224.00	63.50-139.00	169.00-241.00	74.80-94.70
	Median	25.05	1.85	50.95	195.00	85.80	209.00	81.50
	Mean	24.87	2.11	47.52	180.43	97.35	210.83	82.57
	SD	4.71	1.62	12.79	49.41	29.84	26.82	7.84
	Baseline	**30.00**	**4.44**	**61.27**	**224.00**	**136.50**	**240.00**	**92.78**
Majkovi topsoil, 12	Min-Max	3.44-29.00	0.01-1.15	6.17-36.00	32.30-168.00	30.90-180.00	51.00-143.00	45.10-127.00
	Median	21.00	0.36	21.90	139.50	54.45	128.00	82.95
	Mean	19.23	0.44	21.96	119.08	68.44	123.00	86.90
	SD	8.98	0.32	7.68	41.33	40.17	23.91	22.70
	Baseline	**29.00**	**1.07**	**31.82**	**158.65**	**133.14**	**141.35**	**125.35**
Ston, 7	Min-Max	6.55-29.00	0.24-1.74	6.17-23.90	32.30-88.40	30.80-57.50	45.50-103.00	45.10-98.40
	Median	21.00	0.33	14.30	64.90	34.40	53.30	71.00
	Mean	18.53	0.66	15.30	63.47	38.79	62.99	72.93
	SD	8.78	0.63	5.73	18.71	9.83	20.02	16.82
	Baseline	**28.25**	**1.64**	**22.52**	**86.00**	**53.42**	**93.73**	**94.08**

Conclusion

Topsoil at *M. rivulata* nesting sites in Konavle and Majkovi shows elevated nickel and chromium of geogenic origin. Ston topsoil samples present the lowest ecotoxicological risk compared to other locations. Localised increases in copper and cadmium were observed, while the absence of pesticides and PCBs indicates minimal environmental contaminant exposure at the analysed nesting sites of the total Croatian *M. rivulata* population. Baselines for heavy metals per population are recommended for habitat monitoring. Further research should focus on internal doses in biomonitoring, surficial sediments of freshwater lentic habitats, and seasonal variation in PAH and pesticide residues.

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Session 5

Agribusiness and Smart Agriculture

ORIGINAL SCIENTIFIC PAPER

Optimization of the exploitation factors of the vacuum seeder in the sowing of chickpeas

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Summary
The paper presents the calculations of the basic factors of chickpea sowing on the test table when applying a vacuum seeder. Research on the uniformity of sowing was carried out at 3 sowing distances using a sowing plate n=48 and a Z2=16 sprocket on the upper shaft of the seeder. For the sowing of cultivar Katalin (Desi type) chickpea seeds with an absolute mass of 182,625±3,449 g, the required suction hole diameter of 4.25 mm was calculated. In the case of cultivar Twist (Kabuli type) chickpea seeds with an absolute weight of 366.9 g, the required openings on the sowing plate were 5.40 mm. In order for the seeds to adhere to the openings of the sowing plate, it is necessary to provide a negative pressure of 4.25 kPa for Katalin and 6.00 kPa for Twist chickpeas. By simulating sowing, higher QFI indices were achieved by using Twist seeds.

Keywords: chickpeas, seeding uniformity, intra row spacing, vacuum, peripheral speed

Introduction

Daily changes in climatic conditions at the world level directly limit the cultivation of certain agricultural crops. As a result of global warming, it is estimated that not only drought, but also high daily temperatures are the main limitation of chickpea yield. Heat accompanying drought stress is considered a major constraint on agricultural production (Jha et al., 2014). With the increase in temperature on a global level, chickpeas as a culture of the Mediterranean climate have been allowed to grow in the area of the Pannonian basin, as stated by Djalovic et.al., (2024). It is precisely this large lowland area in the southeastern region of Central Europe that becomes an area for growing chickpeas, replacing the cultivation of soybeans. Chickpea (*Cicer arietinum*) belongs to the legume family (*Leguminosae*) and as an annual plant has a deep root (< 1m) and a hairy and branched stem. Chickpeas can be divided into two types based on geographical distribution. The smooth surface Kabuli chickpea is mainly grown in European and African countries surrounding the Mediterranean Sea, while rough surface Desi has a various colors and is grown in Asian and African countries. The basic feature of the pneumatic seeder is based on the rotation of the vertical sowing plate between the vacuum chamber and the seed chamber. Due to the difference in pressure, the seeds adhere to the openings of the sowing plate, and the excess grain remover removes the excess grains so that only one seed remains on each opening. The rotation of the plate brings the grain to the space of atmospheric pressure, and it is separated from the plate and falls due to the action of gravitational force into the furrow opened by the seed feeder. The universality of the seeder is achieved by exchanging plates with different number of holes and diameters, and the seeder can sow a larger number of agricultural crops (Karayel et al., 2022).
The sowing sections are driven by a chain transmission from the driving wheel to the upper shaft and gearbox. The gearbox determines the transmission ratio for driving the lower drive shaft, i.e. the adjustment point for grain spacing within the row. Staggenborg et al., (2004) state

that the correct adjustment of the drill has an important role in the overall production of corn. Lauer (2001) states that between 7 and 10% of plants are lost from sowing to harvest, and according to the authors Banaj et al., (2023) between 8 and 13,000, which can be attributed to poor adjustment of the seeder and incorrect selection of technical sowing factors. Kostić et al. (2011) in their research state the occurrence of "external" and "internal" factors that affect the quality of sowing. Factors in the laboratory include all those that influence the precision of sowing, and are determined by the construction of the sowing device itself, namely the peripheral speed of the sowing plate, working elements of sowing devices such as the excess seed removers, the drive transmission system from the wheels to the sowing device, the method of filling the sowing device, and the method of transporting the seed into the furrow. The speed of movement of the seeder at the time of sowing should be increased to such an extent that the quality of work is not impaired, and the optimal speed will be conditioned by several factors such as: soil preparation, slope of the plot, etc. However, it is mentioned in the literature that variations in the geometry of the seeds can affect the performance, mainly on their suction and retention. Yazgi et al. (2016) state that the diameter of the opening, the peripheral speed and the level of vacuum are the three most important variables that influence the success of precision sowing. The authors state that the peripheral speed of the sowing plate is a variable that limits precise sowing and it should not exceed 0.13 ms^{-1}. Also, the physical properties of the seed play an important role in choosing the appropriate diameter of the opening of the sowing plate and the level of vacuum (the larger the sphericity of the diameter of the seed, the larger the diameter of the opening on the sowing plate is needed, and the smaller the sphericity, the lower the level of vacuum required). Evaluation of the quality of work is determined by indexes as prescribed by ISO standard 7256/1, and it can be additionally evaluated by the precision index (PREC), which is the coefficient of variation of the spacings after omitting the missed and multiple seed drops. An acceptable PREC for seed measurements taken in the field should fall below 29%, which would mean that the standard deviation of spacings within the target region would be ≤ 29% of the theoretical spacing.

Material and Methods

The research of the necessary exploitation factors (speed of the seeder, selection of the sowing plate with regard to the number and diameter of the holes, selection of the peripheral speed of the sowing plate, determination of the position of the excess seed remover, selection of the necessary negative pressure) was carried out under controlled conditions on the test table in the Central Laboratory for Agricultural Techniques and Renewable Energy Sources of the Faculty of Agrobiotechnical Sciences Osijek. In the seeding simulation, in order to gain knowledge about the important factors of seeding, underpressure pneumatic seeders from the company Future Machines d.o.o. were used. Instead of the drive-drive wheel, an electric motor (2 kW) was placed on the testing table as the central drive of the test table with a drive sprocket Z2=16. The second electric motor (4 kW) is installed to drive the fan of the seeder. The number of revolutions of both electric motors according to the needs of the research is controlled by static frequency regulators (*Adjustable Speed Drives*, *Variable Frequency Drives - VFD*). Determining the most favorable position of the seed remover was carried out according to the different distances of the teeth of the excess seed remover in relation to the center of the opening on the sowing plate, whereas other technical factors during measurements are shown in Table 1.

Table 1. Technical factors during measurements (Working speed 1.11 ms^{-1})

Measured value	Vacuum plate n=48 holes*		
Drive wheel to seed plate ratio (i)	0,9286	0,8667	0,7917
Theoretical spacing (cm)	4,375-2C	4,688-1D	5,132-4B
Theoretical set of plants (ha^{-1})	456000	425600	388769
Peripheral speed (ms^{-1})	0,351	0,328	0,299
Seed releasing frequency/s	16,844	15,721	14,361

** Katalin ø 4.0 mm, Twist ø 4.5 mm,* Distance between centers of 2 holes 12,435 mm

Chickpea grains of the Desi type (cultivar Katalin) and Kabuli type (cultivar Twist) were used in the research process. Chickpea cultivar Katalin had an absolute mass of 182.625 ± 3.449 at a grain moisture content of 8.456%, and the average absolute mass of 366.9 ± 4.674 (g) at an average grain moisture content of 8.794% was determined for the cultivar Twist.

Table 2. Average chickpea grains dimensions used in the research

Chickpea type - dimensions	$\bar{x} \pm \sigma$ (mm)	Surface area (mm^2)	Sphericity (%)	Geometric $\bar{x}$ diameter (d_g) (mm)	Roundness (%)
Katalin - Length	8.505 ± 0,461				
Katalin - Width	5.848 ± 0,366	141.742	71.640	6.717	68.760
Katalin - Thickness	6.093 ± 0,423				
Twist - Length	9.567±0.502				
Twist - Width	7.638±0.385	224.371	86.369	8.451	79.830
Twist - Thickness	8.263±0.363				



Figure 1. Chickpea grains of type Desi, cultivar Katalin (left) and type Kabuli, cultivar Twist (right) Source: A. Banaj

Performance evaluation criteria for seed-metering devices is based on the evaluation of seeding uniformity using the methodology specified in the ISO 7256/1 standard using the MULT, QFI and MISS indices, and the second criterion used for seeding uniformity assessment is the Precision index (Ip), calculated by the equation (1):

$$I_p = \frac{S_Q}{D_t} * 100 \quad (1)$$

where: S_Q is the standard deviation within the range of qualified seed spacing, and Dt is the theoretical distance between seeds. This indicator establishes the degree of accuracy in seed deposition.

In order to calculate the required maximum value of the vacuum underpressure in the phase of filling the sowing plate, the equation (2) (Zhao et al. 2016., Kang et al. 2020., Ye et al. (2021).

$$Mmax \frac{80 \cdot K_1 \cdot K_2 . C \cdot G \cdot \left(1 + \frac{v^2}{g \cdot r} + \lambda\right)}{\pi \cdot d^3} \quad (2)$$

Where:

K1 = Suction reliability coefficient, value range 1.8-2.0.; K2 = Coefficient of external conditions, (1.8-2.0).;C = Distance from the seed's center of gravity to the sowing plate (cm),;G = single grain weight (kg).;v = Linear speed of the sowing plate (ms^{-1}); r = sowing plate's radius (m).; g = gravity acceleration, g ≈ 9,81 ms^{-2}.; λ = Comprehensive seed friction coefficient, λ = (6~10) tanθ.; d = diameter of opening of the sowing plate (mm). Determination of the sowing plate opening diameter is determined based on the average geometric diameter of the seeds. The maximum number of holes per sowing plate is determined based on the maximum length of the seed (l), diameter of the sowing plate (dp), and sowing plate's opening diameter (d_k). These parameters are included into equation (3) for evaluation of the maximum number of openings Nk.

$$N_k = (\pi \cdot dp)/(l + dk) \quad (3)$$

Results and Discussion

Results of the air velocity at the sowing plate's openings

Calculated air velocity (ms^{-1}) at the openings of the sowing plate n=48 with vacuum pressure of 4.6 kPa is shown in Table 3.

Table 3. Air velocities at different distances from the center of a hole (d_0= 4,00 mm) at vacuum of 4.60 kPa

Terminal velocity ms^{-1}	Distances from the center of a hole (r_1), mm			
	0	2.000	6.217[1]	12.435[2]
Air velocity (V_{r1}) ms^{-1}	62.838	31.419	3.252	0.813

[1]= distance from the center of the hole to 0.5 distance of the second hole, [2]= Distance between centers of 2 holes

Results of measuring the most favorable position of the excess seed remover

During the sowing simulation, the best results of the sowing quality index (QFI, MISS, MULT) were obtained in the position when the first tooth of the remover was 4.00 mm away from the center of the first hole and the fifth tooth was 2.50 mm away from the Katalin seed. When sowing Twist chickpeas, the best results were obtained at the position when the first tooth of the remover was 4.25 mm away from the center of the first hole and tooth 5 was 3.50 mm away. The obtained positions of the remover were applied during subsequent research in this work.

Results of measurement vacuum pressure requirement

The required maximum negative pressure for the Desi type cultivar Katalin when using the sowing plate n=44 (ø 4.00 mm) at the operating speed of the seeder of 1.11 ms-1 and the peripheral speed of the seed plate of 0.351 ms^{-1} (plant spacing of 4.375 cm) was 4.25 kPa. For

the sowing of Kabuli type cultivar Twist under the same working conditions, using a sowing plate n=44 with a diameter of suction holes ø 4.50 mm, it is necessary to ensure a vacuum of 6.00 kPa. During the simulation of the tested seeder, a maximum vacuum of 4.60 kPa was determined in the housing in the zone of filling the sowing plate, and this was used for other researches of this cultivar.

Cultivar Katalin v= 1.11 ms^{-1}= $Mmax \frac{80 \cdot 1.9 \cdot 1.9 \cdot 0.3 \cdot 0.00179 \cdot \left(1+\frac{0,351^2}{9.81 \cdot 0.095}+4.5\right)}{3,1415 \cdot 0.40^3} = 4.25\ kPa$

Cultivar Twist v= 1.11 ms^{-1}= $Mmax \frac{80 \cdot 1.9 \cdot 1.9 \cdot 0.3 \cdot 0.00360 \cdot \left(1+\frac{0,351^2}{9.81 \cdot 0.095}+4.5\right)}{3,1415 \cdot 0.45^3} = 6.00\ kPa$

Results of calculating the diameter and number of holes on the sowing plate

The diameter of the opening is determined based on the average geometric diameter of the seed, as follows:

$$d_k \text{ Katalin} = (0,64 - 0,66) \cdot d_g = 0,64 \cdot 6.717 = ø\ 4.29 \text{ mm}$$

$$d_k \text{ Twist} = (0,64 - 0,66) \cdot d_g = 0,64 \cdot 8.451 = ø\ 5,40 \text{ mm}$$

When sowing, it is necessary to determine the maximum number of openings per sowing plate in order to be sure that there is enough space for the seeds to be placed on the openings of the sowing plate. Applying equation 3, it is necessary to ensure a maximum number of holes of 47.73 for sowing cultivar Katalin and 43.99 for cultivar Twist.

Results of evaluating the performance of seed metering device

The achieved values of spacing in sowing as well as the values of the sowing index are shown in Table 4.

Table 4. Recorded distances in the sowing simulation of the examined types of chickpeas

Theoretical distance (cm) / chickpea	X ± σ	MULT index	QFI index	MISS index	PREC index
4.375 / Katalin	4.414±1.297	3.049	92.954	3.998	18.904
4.375 / Twist	4.381±1.117	4.048	93.953	1.999	18.927
4.688 / Katalin	4.679±1.357	4.498	92.254	3.248	18.593
4.688 / Twist	4.590±1.052	4.048	95.102	0.850	17.805
5.132 / Katalin	5.166±1.372	2.149	93.803	4.048	16.425
5.132 / Twist	5.093±1.044	2.549	96.802	0.650	16.986

Conclusion

The following conclusions can be drawn from the seeding simulations with a vacuum seeder when sowing chickpeas of the Desi (cultivar Katalin) and Kabuli (cultivar Twist) types at a speed of 1.11 ms^{-1} using a sowing plate n=48;

- for sowing Desi type (cultivar Katalin), the maximum value of the vacuum in the phase of filling the sowing plate is 4.25 kPa, and for larger Kabuli type (cultivar Twist), vacuum is 6.00 kPa.
- The diameter of the opening on the sowing plate is determined based on the average geometric diameter of the seed, for cultivar Katalin is ø 4.29 mm, and for cultivar Twist is ø 5.40 mm.
- Cultivar Twist, which was bigger and with higher roundness, in all sowing intervals achieved better sowing results than cultivar Katalin through the achievement of the required theoretical spacing and the sowing quality index.

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ORIGINAL SCIENTIFIC PAPER

Audit and analysis of tractor repairs in a specialized workshop

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Summary
The paper presents a study on the audit and analysis of agricultural tractor repairs conducted at the company Agrifull, a specialized tractor repair workshop located in Kućanci, covering an area of 200 m². In the first part of the study, an audit was carried out in cooperation with the workshop owner, and the subsequent analysis resulted in an overall very good rating. In the second part, monitoring and analysis of repairs were performed on three CASE IH model PUMA tractors under investigation, where transmission (gearbox) and engine failures were identified. The workshop operates according to a well-organized process, from the reception of a defective tractor to the delivery of a fully functional unit. Furthermore, although the workshop achieved a very good overall evaluation, there is still room for improvement in terms of staff training, the implementation of workshop management software, and the reduction of procurement time for standard spare parts, which would significantly enhance the efficiency and reliability of the specialized workshop.

Keywords: workshop audit, CASE IH, transmission failure of tractor, repair analysis of tractor.

Introduction

With the development of agricultural tractors, repair procedures have become significantly more complex. In order to eliminate complex failures as quickly and cost-effectively as possible while maintaining high repair quality, continuous education and training of workshop personnel are essential. Tractor repair workshops can meet these requirements by systematically monitoring their performance through regular self-assessment, i.e., workshop audits. For this reason, auditing and repair processes are closely interconnected.
In the context of management systems, an audit is defined as a systematic, independent, and documented process for obtaining evidence and evaluating it objectively to determine the extent to which audit criteria are fulfilled. An operational audit encompasses all auditing principles, including the management of the audit program and its implementation, such as defining objectives, scope, and criteria; establishing auditor competence; preparing audit reports and analyses; and presenting audit results (ISO 19011, 2018). A modern approach to complex repairs is reflected in the application of advanced diagnostic methods that surpass manually selected features and increasingly rely on deep learning techniques capable of identifying failure patterns directly from raw signals. Different statistical methods in the analysis of tractor transmission assemblies has demonstrated high accuracy in damage detection, thereby reducing the time required to identify suspicious components and enabling targeted inspection of critical points associated with failure development (Durczak, 2020). In contemporary workshops, failures are often statistically categorized into electrical/electronic and hydraulic systems, regardless of tractor brand. Mechanical systems, such as engines, transmissions, and drive shafts, occur less frequently but are typically associated with more severe consequences in terms of extended downtime and higher repair costs. This distribution reflects the increasing complexity of control systems and the growing reliance on hydraulic systems for the operation of implements and lifting mechanisms (Napiórkowski and Gonera, 2020). Hydraulic failures commonly include clogging of oil filters, pipelines, and related components. Such failures can be diagnosed using acoustic

measurements based on flow and resistance noise analysis. The combination of simple sensors and sound classification techniques enables rapid identification of potentially defective components during failure detection (Gupta et al., 2019). In transmission systems—particularly clutches and transmission assemblies, which represent some of the most costly repairs and are associated with prolonged downtime—the use of specialized software tools, along with vibration measurement and analysis, significantly shortens fault detection time and reduces unnecessary disassembly. Consequently, the repair cycle is optimized and overall downtime is minimized (Da Silva et al., 2019; Hosseinpour-Zarnaq et al., 2022).
The aim of this research is to conduct an audit and analysis of agricultural tractor repairs at the company AGRIFULL, a specialized agricultural tractor repair workshop, and to provide guidelines for further improvement.

Material and Methods

The research on the audit and repair of agricultural tractors was conducted in a specialized agricultural tractor repair workshop owned by the company Agrifull, located in Kućanci, Croatia. The workshop has a total area of 200 m² and performs repairs on tractors of the brands NEW HOLLAND, CASE IH, STEYR, FORD, and FIAT.
The specialized workshop is focused on maintenance and repair services for family farms cultivating between 50 and 200 ha of agricultural land. The owner is actively engaged as a mechanic and employs two qualified mechanics, as well as one administrative staff member responsible for customer communication and service documentation. As there is no authorized service provider for NEW HOLLAND tractors in the area, the strategic objective of the workshop is to obtain authorized service status for NEW HOLLAND in the future. The audit assessment was conducted in cooperation with the workshop owner following the development of a structured questionnaire. The questionnaire consisted of 26 questions that were designed on the basis of a preliminary interview with the owner of the workshop, which were defined by the Department for exploitation, ergonomics and maintenance of agricultural machinery, to which the owner answered individually. Each question was evaluated on a scale from 1 to 5, and the overall workshop rating was calculated based on the aggregated scores. The analysis of agricultural tractor repairs was performed on three CASE IH PUMA tractors over a one-year period. The study focused on significant failures that resulted in prolonged downtime and had a substantial impact on tractor performance and operational reliability.

Results and Discussion

The audit results and analysis of repairs of the studied tractors are presented in the following tables. The repair procedure applied in the investigated workshop consists of a test drive, diagnostic assessment (ranging from simple to complex procedures), measurement of pressure at transmission shift points (gearbox), verification of hydraulic parameters, decision-making regarding disassembly, preparation of a cost estimate, procurement of the required spare parts, installation and reassembly, and final testing. A comparable repair procedure is reported in similar workshops according to authors (Emert et al., 1995; Ćosić, 2022; Durczak and Selech, 2022).
Table 1 indicates a very good audit rating of the workshop (107/120). Despite the high overall score, several deficiencies were identified. These include the absence of systematic employee training, the lack of an equipment register (suggesting insufficient competence development and limited traceability of workshop equipment), and planning practices primarily based on seasonal workload, which represent inadequate monitoring of employee capacity and resource allocation. In addition, prolonged procurement times for standard spare parts (longer then 24 h) (Morosini Frazzon et al., 2014) were identified as a significant limitation, particularly during peak operational periods when rapid fault elimination is essential. Similar observations have been reported by authors (Van Houtum and Kranenburg, 2015; Barač et al., 2023).

Table 1. Audit report of the investigated workshop.

	QUESTIONS	ANSWERS	SCORE
1.	How many workstations does the workshop have?	Four.	5
2.	Number of employees and managers?	Four employees: three workshop mechanics and one administrative employee.	5
3.	Are prescribed work clothing and personal protective equipment provided?	Yes.	5
4.	Is occupation-specific training conducted for employees?	No.	1
5.	What is the average number of repairs and services per year?	Approximately 200 machines.	5
6.	Is an annual maintenance budget planned?	Yes.	5
7.	Is there an equipment register and how is it maintained?	No.	1
8.	Type of maintenance organization (centralized/decentralized/combined)?	Centralized.	5
9.	Maintenance strategy (concept)?	Planned maintenance.	5
10.	Are there daily, weekly, and long-term plans?	Seasonal.	3
11.	Are preventive maintenance plans established and implemented?	Yes.	5
12.	Are diagnostic examination plans established and implemented?	Yes.	5
13.	Is there a work order system and how is it managed?	Yes, analogously.	5
14.	Is a work order issued for all tasks?	Yes.	5
15.	Are the prescribed rules for different work order types followed?	Yes.	5
16.	Are reports on completed work orders submitted?	Yes.	5
17.	Is the execution of work orders monitored and controlled?	Yes.	5
18.	What is the procedure from fault occurrence to repair completion?	Fault reporting, preparation of a preliminary defect report and cost estimate, disassembly and preparation of a detailed offer, ordering of spare parts, installation and final testing.	5
19.	How are spare parts managed?	Sinesis for parts and CNH ordering portal.	5
20.	What is the average procurement time for standard spare parts?	3 to 10 days.	2
21.	What is the total value of spare parts inventory?	148,000 €	-

22.	Is the effectiveness of spare parts management analyzed?	Yes.	5
23.	Is the backlog (outstanding work) monitored?	Yes.	5
24.	Is a warranty provided for repairs? If so, for how long?	Yes, in accordance with the warranty period for spare parts (6 months).	5
25.	Is there a plan to expand the workshop or invest in new equipment?	Yes.	5
26.	What challenges are encountered and where is there room for improvement?	Additional employee training, expansion of workspace capacity, and enhancement of employee motivation.	-
		TOTAL:	107/120

Table 2 presents the identified tractor failures. A reduction in engine power and transmission slippage were observed in all three investigated tractors. In addition, a severe engine failure was confirmed in the third tractor. Similar types of failures and performance deviations have been reported by authors (Ćosić et al., 2023; Hosseinpour-Zarnaq et al., 2022).

Table 2. Overview of repairs performed on the investigated tractors.

Tractors	Repairs
CASE IH PUMA 215	- Gearshift slippage in the D range (Figure 1). - Parts replaced: clutch plate set and complete gearshift seal kit.
CASE IH PUMA	- Transmission slippage in the M range and occurrence of engine–transmission speed mismatch (Figure 1). - Parts replaced: transmission gasket set, Teflon piston seal for the M range clutch pack, clutch plate set, hydraulic filter, O-rings, and sealing compounds.
CASE IH MxM 155	- Loss of engine power and irregular engine operation, accompanied by blue exhaust smoke, oil leakage at the cylinder head gasket, lubricant contamination on the exhaust manifold, a damaged piston crown, and cracked piston rings (Figure 1). - Parts replaced: piston, piston ring set, cylinder head gasket, set of main and connecting rod bearings, valve stem seals, injector, and associated consumable components.



CASE IH PUMA 215



CASE IH PUMA



CASE IH MxM 155

Figure 1. Visual presentation of the repairs performed on the investigated tractors [(CASE IH PUMA 215 - transmission D clutch); (CASE IH PUMA - transmission M clutch); (CASE IH MxM 155 - engine disassembly).

Conclusion

The research conducted has determined the following conclusions:

1) With regard to the audit and repair analysis, the investigated specialized workshop demonstrates a very high level of performance in these segments, characterized by a clearly structured workflow from the receipt of a tractor repair request to final testing and delivery of the tractor to the customer.
2) However, certain shortcomings limiting the workshop's overall excellence were identified, including insufficient employee training, the absence of systematic daily and weekly planning, and prolonged procurement times for standard spare parts.
3) The proposed guidelines for improvement include the implementation of comprehensive operational planning, continuous and structured employee training, reduction of standard spare part procurement time to within 24 hours, and the introduction of workshop management software to enhance control over spare parts procurement and work order management. The adoption of these measures would enable the investigated specialized agricultural tractor repair workshop to shorten the service cycle from tractor intake to delivery and to increase the overall reliability and quality of repairs provided to end users.
4) In future research, the number of tractors studied should be increased in order to use statistical tools, in order to obtain better accuracy of the audit itself and fault findings.

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ORIGINAL SCIENTIFIC PAPER

Estimating the climate change mitigation potential of CAP interventions in Slovenia

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Summary
Agriculture is both a source of greenhouse gas emissions and a sector with mitigation potential. The aim of this paper is to assess the potential contribution of interventions under the Slovenian CAP Strategic Plan 2023-2027 to climate change mitigation in agriculture using a qualitative expert assessment. The highest ratings were assigned to interventions linked to Specific Objective 4, especially agri-environment-climate measures and payments for areas facing natural or other specific constraints. Several biodiversity-related, land-management and investment interventions were also assessed as relevant. The results show that mitigation potential is distributed across several groups of interventions.

Keywords: climate change, mitigation, CAP Strategic Plan, expert assessment, Slovenia

Introduction

Agriculture has a dual role in climate change, as it is both an important source of greenhouse gas (GHG) emissions and a sector with considerable mitigation potential. It can contribute to climate change mitigation through measures such as improved nutrient management, livestock feeding, manure treatment, reduced energy use, and increased carbon sequestration in soils and biomass (Smith, 2011).

Within the European Union, the Common Agricultural Policy (CAP) is one of the main policy frameworks shaping agricultural practices and, indirectly, the environmental and climate performance of the sector (Pe'er et al., 2020). In the 2023-2027 programming period, CAP Strategic Plans provide Member States with greater flexibility in designing interventions, which makes country specific assessments of their likely environmental effects particularly relevant. However, the mitigation potential of individual CAP interventions is not always straightforward to evaluate, since it depends not only on their formal objectives, but also on the mechanisms through which they influence production practices, input use, livestock management, land use, and investment decisions (Santeramo et al., 2020).

As a result, evidence on their expected mitigation relevance at the level of individual interventions remains limited, particularly in the context of national CAP Strategic Plans. In Slovenia, this issue is especially relevant in relation to the amended CAP Strategic Plan 2023-2027 (Ministry of Agriculture, Forestry and Food, 2026).

This paper therefore aims to assess the potential contribution of interventions under the Slovenian CAP Strategic Plan 2023-2027 to climate change mitigation, focusing on their expected effects on methane and nitrous oxide emissions, carbon sequestration, energy use, and renewable energy production. Because no systematic methodological approach has yet been established in Slovenia for the ex ante evaluation of the climate mitigation potential of CAP interventions and related environmental parameters, an expert workshop was conducted to provide a transparent and comparable assessment of these interventions.

Material and Methods

In May 2025, an expert workshop was conducted to systematically assess the potential contribution of individual interventions and operations under the Slovenian CAP Strategic Plan 20232027 to climate change mitigation and their effects on selected climate-related parameters. Expert-based qualitative evaluation has also been applied in previous climate-related agricultural assessments (Travnikar et al., 2025).The workshop brought together 11 experts in agriculture and forestry, from the Agricultural Institute of Slovenia and the Slovenian Forestry Institute, who evaluated the expected effects of individual interventions on methane, nitrous oxide and ammonia emissions, nitrogen and phosphorus balances, energy use, renewable energy production, and nitrate pollution of water.

For the analysis, a matrix was prepared containing all interventions and sub-interventions under the Slovenian CAP Strategic Plan 2023-2027, their specific objectives, and the selected assessment parameters. In total, the expert assessment covered 112 interventions and operations, grouped into four thematic areas: competitiveness and resilience of the agricultural sector; environmental and climate protection; rural development, food quality and health; and knowledge transfer, innovation and digitalisation.

Interventions were identified using the official codes of the CAP Strategic Plan (e.g. INP for income support interventions and IRP for investment-related interventions). The classification into thematic groups followed the structure of the CAP Strategic Plan and was retained as defined in the original policy framework.

The qualitative rating scale was defined for this assessment to ensure a consistent evaluation of expected effects. The potential effectiveness of each intervention was assessed using the following scale: strong negative effect (---), medium negative effect (--), weak negative effect (-), no contribution (0), weak positive effect (+), medium positive effect (++), strong positive effect (+++), and not relevant (NR). The assessment refers to the substantive or technical potential of individual interventions to influence the selected parameters and does not consider the expected scale or actual extent of implementation. Consequently, an intervention may be assessed as having high mitigation potential even if its uptake in practice is limited.

Results and Discussion

The highest ratings were assigned to interventions linked to Specific Objective 4, which addresses climate change mitigation and adaptation and sustainable energy. In particular, agri-environment-climate-measures (AECM) and payments for areas facing natural or other specific constraints (ANC) stood out, as they were evaluated as having the strongest potential to reduce methane, nitrous oxide and ammonia emissions.

Overall, no intervention was assessed as having a strong negative effect on climate mitigation. However, a weak negative effect (-) was identified for some biodiversity-related interventions and for Organic Farming, mainly because these measures may increase methane emissions through changes in feed composition or adapted feeding practices in livestock production. At the same time, they were assessed positively for nitrous oxide emissions and soil carbon stocks, suggesting that their overall environmental effect cannot be judged by a single parameter. Coupled income support for livestock production was also associated with a potential increase in methane emissions. Similarly, some crop-management measures, such as successive crops and intercrops or greening of arable land over winter, may increase energy use and, indirectly, carbon dioxide emissions.

By contrast, almost no interventions were assessed as increasing nitrous oxide emissions. Payments for natural or other constraints which were evaluated as having a weak negative effect from a climate mitigation perspective. Without this support, part of the land could be abandoned, likely reducing methane and nitrous oxide emissions, lowering agricultural energy use, and increasing above-ground carbon stocks through natural vegetation regrowth. However,

this finding should be interpreted with caution, given the intervention's positive effects on landscape maintenance, biodiversity and the socio-economic conditions of rural and remote areas, which is consistent with broader discussions on CAP trade-offs and multifunctionality (Pe'er et al., 2020; Cuadros-Casanova et al., 2023).

Table 1. Interventions of the Slovenian CAP Strategic Plan 2023–2027 with strong (+++) and moderate (++) positive effects on selected climate mitigation dimensions

Methane (CH_4)	
+++	++
Renewable energy investments (**IRP36)**; Additives to reduce ammonia and GHG emissions (**INP08.04)**; Targeted feeding of cattle (**PS.1);** Targeted feeding of sheep and goats **(PS.3)**; Livestock welfare adaptation investment (**IRP29)**	Animal welfare **(IRP28)**
Nitrous oxide (N_2O)	
+++	**++**
Additives to reduce ammonia and GHG emissions **(INP08.04)**; Targeted feeding in fattening pigs **(PS.2)**; Integrated production systems (crop, vegetable, hop, fruit/olive and grape production) (**NV.3** – **NV.7)**; Precision Fertilization and Spraying **(NV.11)**; Grassland and habitat conservation measures (**BK.2** – **BK.6**, **BK.14, BK.15)**	Basic income support for sustainability (**INP01)**; Complementary redistributive income support for sustainability (**INP02)**; Investments in productivity, technological development and digitalisation of agricultural holdings (**IRP02)**; Investment in competitiveness and market orientation of organic farms **(IRP04)**; Coupled income support for protein crops (**INP07)**; Investments in efficient use of nitrogen fertilisers (**IRP17);** Organic farming (**IRP19)**; Fertilisation with low-emission organic fertilisers (**INP08.03)**; Successive crops and intercrops **(INP08.05)**; Greening of arable land over winter (**INP08.06)**; Targeted feeding of cattle (**PS.1)**; Targeted feeding of sheep and goats (**PS.3)**; Water resources **(NV.1)**; Special grassland habitats **(BK.1)**; Livestock welfare adaptation investment **(IRP29)**
Carbon sink	
+++	**++**
Investments in forest rehabilitation and restoration following natural disasters and adverse climatic events **(IRP15)**; Successive crops and intercrops (**INP08.05)**; Greening of arable land over winter (**INP08.06)**; Conservation tillage (**INP08.07**); Water resources **(NV.1)**; Maintenance of Crop Rotation **(NV.2)**; Conservation of wetlands and peatlands **(BK.5)**	Investments in primary wood processing and digitalisation **(IRP08)**; Investments in the establishment and development of forest tree nurseries **(IRP09)**; Integrated production systems (crops, vegetables and hops) (**NV.3** – **NV.5)**; Precision Fertilization and Spraying (**NV.11);** Grassland, habitat and landscape conservation measures **(BK.1** – **BK.4**, **BK.7**, **BK.10, BK.14)**
Energy consumption	
+++	**++**
Investments in productivity, technological development and digitalisation of agricultural holdings (**IRP02)**; Collective investment in market preparation and resilient food value chains **(IRP03)**; Investments in competitiveness and market orientation of organic farms (**IRP04)**; Agro-melioration and land consolidation **(IRP05)**; Agro-melioration and land consolidation **(IRP35)**	Investments in the improvement of forest infrastructure **(IRP06)**; Investments in the purchase of new machinery and equipment for work in the forest (**IRP07)**; Investments in primary wood processing and digitalisation **(IRP08)**; Construction of multi-user irrigation systems **(IRP13)**; Investments in renewable energy sources **(IRP36)**; Conservation tillage **(INP08.07)**
Renewable energy sources	
+++	**++**
Investments in productivity gains and digitalisation of agricultural holdings **(IRP02)**; Investments in competitiveness and market orientation of organic farms **(IRP04)**; Investments in processing and marketing of agricultural products (**IRP35)**; Investments in renewable energy sources (**IRP36)**; Intervention Support for non-agricultural activities, bio-economy and conservation of heritage **(IRP25)**	Investments in the purchase of new machinery and equipment for work in the forest **(IRP07)**; Investments in primary wood processing and digitalisation (**IRP08)**; Investment in adaptation to climate change in permanent crops (**IRP16)**; Investments in the adaptation of agricultural holdings to the implementation of above-standard livestock welfare requirements **(IRP29)**

Source: Authors' own elaboration based on expert assessment; Note: For ease of presentation, the official names of interventions have been shortened in the table.

Table 1 summarises interventions assessed as having a potential strong or moderate positive effect. The examination did not consider the extent of implementation of individual interventions. Knowledge-transfer and innovation-related interventions (IRP31, IRP32 and IRP38) were consistently assessed as having moderate positive effects across all assessed

dimensions. Their contribution is primarily indirect, through support for the uptake of mitigation practices, innovation and advisory capacity.

Methane (CH_4). Interventions evaluated as having potential strong and medium positive effect on methane reduction are associated primarily with measures targeting methane emissions from the digestive tract of livestock. In particular, this includes financial incentives for the use of feed additives that reduce methane formation in enteric fermentation, as well as interventions that contribute to methane mitigation through improved feed quality for ruminants and targeted feeding of cattle and small ruminants, taking into account, among other factors, the results of chemical feed analyses. Furthermore, highly rated interventions also comprise measures aimed at reducing methane emissions from livestock manure storage, either through the introduction of pasture-based animal husbandry or through methane capture by biogas plants.

Nitrous oxide (N_2O). The reduction of nitrous oxide emissions is associated primarily with interventions that improve the efficiency of nitrogen fertiliser use and thereby reduce the need for mineral nitrogen fertilisers. Most notably, this applies to interventions requiring increased greening of arable land and different forms of precision fertilisation. Among the most effective are interventions that prohibit or strongly restrict the use of nitrogen fertilisers, such as Organic Farming and most operations aimed at preserving biodiversity. Moreover, strong positive effects were also identified for incentives supporting the use of urease, nitrification and denitrification inhibitors, as well as for investment interventions contributing to improved nitrogen use in agriculture. Basic Income Support for Sustainability and Complementary Redistributive Income Support for Sustainability were likewise assessed as relevant for nitrous oxide mitigation. On the one hand, these interventions require compliance with the Prescribed Behaviour Requirements; on the other hand, income support reduces the dependence of agricultural holdings on market revenues, which are generally associated with the intensification of agricultural production and processing.

Carbon sink. Interventions assessed as having a strong positive effect (+++) on the carbon sink are implemented mainly within the framework of AECM and ANC payments, and interventions in the field of Environmental and Climate Protection. Particularly important in this respect are investments in the rehabilitation and restoration of forests following natural disasters and adverse climatic events, incentives for conservation tillage, and payments for the conservation of wetlands and bogs. Furthermore, interventions requiring increased greening of arable land, together with measures aimed at preserving grassland habitats, hedgerows and high-density meadow orchards, contribute to increasing organic carbon inputs to the soil and above-ground biomass. In the forestry sector, moreover, alongside investments in forest rehabilitation and restoration, interventions supporting the development of forest arboriculture were also assessed as relevant.

Energy consumption. The analysis showed that interventions including technological development, digitalization and efficient use of natural resources, as well as other investments contributing to the optimization of energy consumption in agriculture, contribute mainly to the reduction of energy consumption.

Renewable energy sources. As expected, the intervention Investments in Renewable Energy Sources was assessed as having a strong positive effect on the production of renewable energy sources (RES), thereby directly contributing to increased use of renewable energy and reduced dependence on fossil fuels. In addition, interventions promoting technological development and digitalisation, including investments in renewable energy production such as micro-biogas plants, were also assessed as having a strong positive effect.

Conclusion

The expert-based assessment showed that the climate change mitigation potential of the Slovenian CAP Strategic Plan 2023-2027 is distributed across several groups of interventions rather than concentrated in a single policy instrument. The highest ratings were assigned primarily to interventions linked to Specific Objective 4, especially AECM and ANC payments, which target key mitigation-related parameters, including methane, nitrous oxide and ammonia emissions.

Several biodiversity-related, land management and investment interventions were likewise assessed as relevant, although in some cases their effects involved important trade-offs between climate mitigation and other environmental or socio-economic objectives. In addition, knowledge-transfer and innovation-related interventions were identified as relevant supporting measures through their contribution to advisory capacity, innovation uptake and the wider adoption of mitigation practices.

The present assessment reflects the substantive or technical potential of interventions and does not account for their actual uptake or implementation intensity. Future research should therefore complement this qualitative assessment with information on the financial allocation, expenditure and implementation of individual interventions. Linking estimated mitigation potential with financial support and actual uptake would provide a more realistic basis for assessing the overall contribution of the Slovenian CAP Strategic Plan 2023-2027 to climate change mitigation in agriculture. This also highlights the need for a more systematic and continuous approach to the assessment of CAP interventions, in order to support more consistent monitoring, evaluation and future design of mitigation oriented measures.

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ORIGINAL SCIENTIFIC PAPER

Spatial Patterns and Hotspots of Agricultural GHG Emissions at the Municipal Level in Slovenia

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Summary
The study quantifies methane (CH_4) emissions in Slovenia using a spatially bottom-up approach at the municipal level. Emissions are estimated based on grazing livestock data and emission factors for enteric fermentation and manure management. To enable spatial comparison, emissions are also expressed as emission intensity per selected unit and contextualised within EU-27 averages. The results reveal pronounced spatial heterogeneity. They improve understanding of the spatial distribution of CH_4 emissions, identify emission hotspots, and may support the identification of priority areas for climate policy implementation.

Keywords: methane emissions, emission hotspots, grazing livestock, spatial distribution

Introduction

Agriculture contributes significantly to non-CO_2 greenhouse gas emissions (GHG), primarily methane (CH_4) and nitrous oxide (N_2O). In agriculture, CH_4 is mainly linked to enteric fermentation and manure management in livestock, whereas N_2O emissions are associated mainly with manure management and agricultural soils, including fertilisation, atmospheric nitrogen deposition, leaching and runoff (Wójcik-Gront, 2020). In Slovenia, agriculture accounted for 10.6% of total national GHG in 2024, while CH_4 represented the largest share of agricultural emissions, with 59.7% originating from enteric fermentation and 11.7% from manure storage. Although N_2O is also part of the agricultural emission profile, this study focuses on CH_4 because of its dominant role in cattle production, which accounted for 68.6% of agricultural emissions (Climate Report, 2026). CH_4 is a short-lived climate pollutant with a perturbation lifetime of 11.8 ± 1.8 years (IPCC, 2021). In livestock production, CH_4 is generated through microbial processes. Emissions from manure storage depend on manure collection, storage duration, manure temperature and CH_4 capture (Beauchemin et al., 2022; Montes et al., 2013).

At the national level, CH_4 emissions from agriculture are linked to both direct and indirect technological, economic and socio-demographic factors that vary by region. Increased demand for livestock and crop products can lead to expanded production areas or higher livestock numbers, resulting in higher emissions (Liu et al., 2024; Hasan et al., 2025). In Slovenia, due to structural change, intensification is evident in the long-term increase in cattle numbers per farm and milk production per dairy cow, although these trends differ significantly between regions (Sadar et al., 2025).

Data on GHG emissions at the national level are reported in the Slovenian National Inventory Report (Mekinda Majaron et al., 2026). Although national GHG inventories provide the basis for emission monitoring, they do not capture within country variation in CH_4 emissions. A regional analysis can therefore provide insights into spatial variation in CH_4 emission intensity. This study aims to analyse spatial patterns and identify CH_4 emission hotspots at the municipal level in Slovenia, focusing on emissions from grazing livestock.

Material and Methods

To assess CH_4 emissions from grazing livestock at the regional level, a bottom-up approach was applied at the level of municipalities (NUTS 5). The analysis was based on the number of grazing livestock by individual categories and their spatial distribution in 2024.

For grazing livestock on regional level, an internal database developed within the national project was used (DSLFS, 2025). Compared to the official statistics of the Statistical Office of the Republic of Slovenia, it covers a broader range of holdings, as official statistics generally include farms above the threshold of 1 livestock unit (LSU). This improved grazing livestock coverage on local level.

The analysis included: three cattle categories (dairy cows, suckler cows, and other cattle), sheep, goats, and horses. These categories were selected based on their key contribution to CH_4 emissions from livestock production. According to data (Climate Report, 2026), grazing livestock accounted for 96.3% of total CH_4 emissions from livestock in 2024 (enteric fermentation and manure management), corresponding to 1,123.6 kt carbon dioxide equivalents (CO_2 eq) (Figure 1).

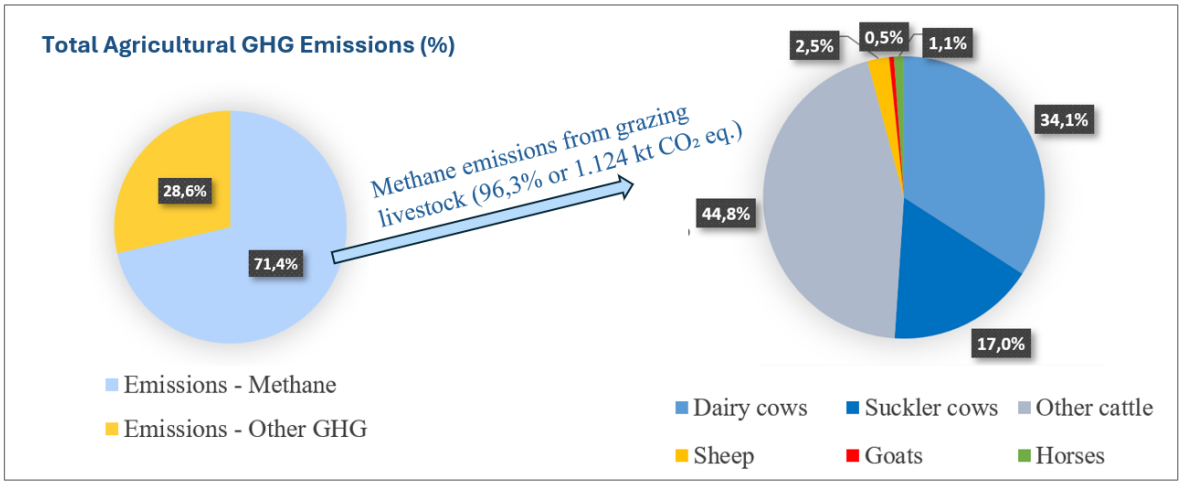

Figure 1: CH_4 emissions in Slovenia from grazing livestock, 2024 (%)

For CH_4 emission calculations, emission factors for 2024 were used (Slovenian National Inventory Report; Mekinda Majaron et al., 2026). Two separate emission factors were applied for each grazing livestock category: (i) CH_4 emissions from enteric fermentation (kg CH_4/head/year) and (ii) CH_4 emissions from manure management (kg CH_4/head/year). For dairy cows, a more detailed approach was applied by adjusting emission factors according to the average milk yield per feeding day at the municipal level. Municipality-specific coefficients linked to milk production productivity improved the accuracy of the estimates.

Based on the bottom-up approach used in this paper, we estimated that grazing livestock in Slovenia generated 1,140.4 kt CO_2 eq of CH_4 emissions in 2024. To convert CH_4 emissions into CO_2 equivalents, we used a global warming potential factor (GWP_{100}) of 28 (IPCC, 2014). The deviation from the nationally reported value was +1.5%, confirming the methodological consistency and reliability of the applied approach.

To improve comparability between municipalities, CH_4 emissions were analysed using two indicators of CH_4 intensity: (i) CH_4 emissions per hectare of utilised agricultural area (ha UAA) and (ii) CH_4 emissions per livestock unit (LSU) of grazing livestock.

To place the Slovenian results in a broader European context, average CH_4 emissions per unit were also calculated for the EU-27. Data on total CH_4 emissions were obtained from the European Environment Agency (EEA, 2026), while data on ha UAA and LSU of grazing livestock were sourced from Eurostat (Eurostat, 2026). Average CH_4 emission intensity in

Slovenia amounted to 2.4 t CO_2 eq per ha of UAA and 3.1 t CO_2 eq per LSU of grazing livestock. In the EU-27, average CH_4 emission intensity amounted to 1.5 t CO_2 eq per ha of UAA and 3.8 t CO_2 eq per LSU of grazing livestock.

Results and Discussion

The following section presents CH_4 emission intensity indicators (per ha of UAA in the Figure 2 left and per LSU in the Figure 2 right; classification legends are provided in Tables 1 and 2) based on a spatially explicit bottom-up analysis at the municipal level.

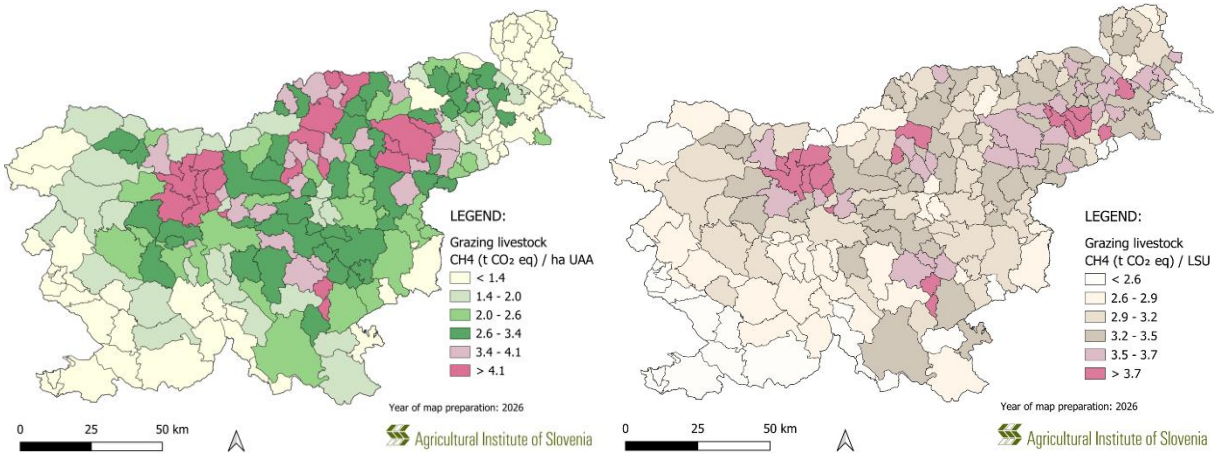

Figure 2: CH_4 emissions (tonnes CO_2 eq) per ha of UAA and per LSU

Spatial analysis of emission intensity per ha of UAA reveals three bigger clusters where emissions per unit significantly exceed both the Slovenian and EU-27 averages. These areas potentially indicate higher spatial pressure and may be suitable as case study regions for analysing CH_4 mitigation strategies in livestock systems. Overall, slightly more than 40% of municipalities fall within low emission intensity classes relative to both the Slovenian and EU-27 averages (Table 1).

Table 1: Grazing Livestock Emission Intensity Classes (per ha of UAA)

Class	Emission range (t CO_2 eq/ha UAA)	Description	Share of municipalities
Very low emission intensity	< 1.4	Below the Slovenian and EU-27 average	26.9%
Low emission intensity	1.4–2.0	Around the EU-27 average	16.5%
Moderate emission intensity	2.0–2.6	Around the Slovenian average and above the EU-27 average	14.6%
Elevated emission intensity	2.6–3.4	Above average (above the Slovenian and EU-27 average)	20.8%
Marginal emission hotspots	3.4–4.1	Noticeably above average (above the Slovenian and EU-27 average)	11.3%
Emission hotspots	> 4.1	Areas with concentrated emission pressures	9.9%

It is noteworthy that a substantial share of municipalities falls within higher emission classes compared to the EU average. However, interpretation of these results requires caution. Higher average CH_4 emissions per ha of UAA in Slovenia compared to the EU-27 are partly driven by the relatively smaller share of UAA in Slovenia (approximately 24% of national territory, compared to almost 40% in the EU-27). In simpler terms, the extent of agricultural land in Slovenia is considerably smaller than the EU-27 average, which increases emission intensity per unit of land.

In future analyses, comparisons should therefore preferably be made with structurally similar countries rather than EU-27 averages, as Slovenia's specific land-use structure leads to systematically higher emissions per unit of agricultural area.

In contrast to emission intensity per ha of UAA, CH_4 emission intensity per LSU reflects livestock system intensity and thus captures the scale of livestock production rather than land-based emission intensity. This indicator reveals a distinctly different spatial pattern. Nearly all municipalities fall into classes where emissions per LSU are below the European average (Table 2). This suggests that, despite the importance of livestock production in Slovenia, the scale and structural intensity of livestock systems remain comparatively lower than in the EU-27. Lower emissions per LSU in Slovenia are related to the particularly smaller average herd size (Slovenia: 9.1 LSU/holding; EU-27: 27.8 LSU/holding).

Table 2: Grazing Livestock Emission Intensity Classes (per LSU of Grazing Livestock)

Class	Emission range (t CO_2 eq/LSU)	Description	Share of municipalities
Very low emission intensity	< 2.6	Significantly below the Slovenian and EU-27 average	11.3%
Low emission intensity	2.6-2.9	Below the Slovenian and EU-27 average	16.5%
Moderate emission intensity	2.9-3.2	Around the Slovenian average and below the EU-27 average	25.5%
Elevated emission intensity	3.2-3.5	Slightly above the Slovenian average and below the EU-27 average	24.5%
Marginal emission hotspots	3.5-3.7	Above the Slovenian average and below the EU-27 average	13.7%
Emission hotspots	> 3.7	Areas with concentrated emission pressures	8.5%

Nevertheless, approximately 9% of municipalities exceed the Slovenian average and approach EU-27 levels. These cases require more detailed analysis, particularly in relation to natural conditions and farm structure. It is relevant to examine whether these areas correspond to more favourable agricultural regions (i.e. flat areas) which allow greater livestock concentration or correspond to areas with limited agricultural potential. However, a more detailed regional-scale analysis would be needed to better understand the underlying drivers of these patterns.

Measures to reduce CH_4 emissions differ between species and livestock categories, so future research should identify hotspots within each species or category to ensure measures are as targeted as possible. As total emissions at the regional level do not reflect actual livestock product output, further research should also examine emission intensity at the product level (meat or milk). Research should also investigate relationships between individual parameters at the regional level, for example, whether an area with low product-level intensity is characterised by high LSU-level intensity (so-called unrealized production potential), as different CH_4 intensity parameters provide insights from distinct perspectives. Finally, since political climate agreements, such as the Paris Agreement, focus on reducing additional global warming, further research should include temporal changes and use metrics that better capture the effects of emissions on temperature.

Conclusions

We identified spatial patterns of emission intensity and highlighted so-called emission hotspot areas. However, these results are not intended as a tool for "finger-pointing" at individual regions, but rather as an analytical basis for understanding structural differences in livestock emissions. In many cases, emission hotspot municipalities are located in areas dominated by less-favoured and permanent grassland regions, which play a key role in maintaining national food security through milk and meat supply and reduce the need for importing these products

(and the associated embodied emissions in imports), as Slovenia achieves a high level of self-sufficiency in these commodities.

The main added value of the results lies in providing spatial evidence on CH_4 emission distribution, improving understanding of regional emission patterns. Such insights can also support the identification of priority areas for climate policy interventions in the livestock sector and inform targeted mitigation strategies at the regional level.

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REVIEW ARTICLE

Exploring technology adoption trends in agri-food supply chains: Applications of the unified theory of acceptance and use of technology

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Summary
Digital technologies are being increasingly adopted in agri-food supply chains to enhance traceability, efficiency, and transparency. The Unified Theory of Acceptance and Use of Technology (UTAUT) is a widely applied framework for understanding technology adoption factors, yet its application in the agri-food context remains fragmented. This paper presents a narrative review of studies applying UTAUT and its extensions in agri-food supply chains. A total of 11 empirical studies published between 2020 and 2026 were analysed, identifying trends in technologies examined, constructs used, and research gaps. Results show that performance expectancy, social influence and facilitating conditions are the most consistent factors of adoption, while context-specific factors such as trust and perceived risk are often incorporated to fit the agri-food context. The findings highlight the need for broader empirical coverage across geographic regions and types of technology.

Keywords: UTAUT, technology adoption, agri-food, blockchain, literature review

Introduction

Digital technologies are increasingly being integrated into agri-food supply chains to address challenges related to food safety, traceability, quality control, and operational efficiency (Vu et al., 2023; Fernando et al., 2025). Existing reviews have examined blockchain and digital technology adoption in food supply chains (Yogarajan et al., 2023; Wong et al., 2024). However, the specific application and contribution of the Unified Theory of Acceptance and Use of Technology (UTAUT) to understanding digital technology adoption in food supply chains requires more attention. Technologies such as blockchain, the Internet of Things (IoT), precision agriculture tools, cold chain systems, and digital platforms are being adopted from production to consumption (Yu et al., 2025). Despite this growing adoption, uptake remains uneven across regions and supply chain segments, influenced by diverse socio-economic, institutional, and behavioural factors (Yogarajan et al., 2023; Yu et al., 2025). Among the theoretical frameworks used to explain technology adoption, UTAUT, developed by Venkatesh et al. (2003), is one of the most widely applied. UTAUT is based on eight prior models, integrated into four constructs: performance expectancy (PE), effort expectancy (EE), social influence (SI), and facilitating conditions (FC), moderated by age, gender, experience, and voluntariness of use. Its extension, UTAUT2 (Venkatesh et al., 2012), introduces additional constructs such as hedonic motivation, price value, and habit, making it more suitable for consumer-oriented and voluntary adoption contexts. Both versions have been applied across a range of sectors (Venkatesh et al., 2003, 2012), with growing interest in the agri-food domain (Shi et al., 2022; Zhang et al., 2024).

Despite this increasing attention, the application of UTAUT in the agri-food sector remains fragmented. While UTAUT is primarily designed to explain individual-level behaviour, it has been widely applied to different actors in agri-food supply chains, particularly farmers and

consumers. However, its application to organisational-level adoption remains more limited and often requires model extensions. Moreover, existing studies tend to focus predominantly on blockchain adoption (Yogarajan et al., 2023; Wong et al., 2024), with fewer focusing on IoT, precision agriculture, or cold chain technologies. Moreover, the geographic distribution of studies is uneven, leaving Central and Eastern European contexts underexplored (Yogarajan et al., 2023; Yu et al., 2025). The aim of this paper is to provide a narrative review of existing studies that have applied the UTAUT model and its extensions within agri-food supply chains, identifying key trends in terms of technologies examined, UTAUT constructs employed, and existing research gaps.

Material and Methods

This study adopts a narrative review approach to explore the application of UTAUT and its extensions in agri-food supply chains. Relevant studies were identified through searches in major scientific databases (Web of Science, Google Scholar), using keyword combinations using operators AND and OR, including "UTAUT", "technology adoption", "agri-food", "supply chain", and "agriculture", from 2020 to 2026. Additional studies were identified by screening recent review articles related to the topic (Yogarajan et al., 2023; Wong et al., 2024; Yu et al., 2025). A total of 11 empirical studies met the inclusion criteria and were selected for analysis. The analysis is descriptive, identifying and comparing the main technology types, UTAUT constructs and key findings regarding technology adoption in the agri-food context.

Results and Discussion

As shown in Table 1, blockchain emerges as the most frequently studied technology in this scope of work, mostly in the context of traceability, quality management and supply chain transparency, which is consistent with previous reviews (Yogarajan et al., 2023; Yu et al., 2025). The remaining studies apply UTAUT to IoT, precision agriculture, cold chain technology, digital crowdfarming and digital platforms for agri-food waste and circular economy initiatives. Based on Table 1, the dominance of Asian studies may partly be explained by the suitability of UTAUT for analysing individual-level adoption decisions, particularly when farming is characterised by a high share of smallholders. However, this should not be overgeneralised, as countries such as China combine small-scale farming with rapidly advancing digitalisation and large-scale agricultural enterprises. In contrast, European agri-food systems are often more institutionalized, policy-driven, with technology adoption influenced by regulatory frameworks, subsidies, and organisational capacities. As a result, European research tends to focus more on actual adoption behaviour, implementation challenges and structural barriers, often applying broader frameworks such as TOE, not relying solely on UTAUT. Also, performance expectancy is the most consistently confirmed predictor of adoption intention, for blockchain and agri-food supply chains (Sharma et al., 2023; Toader et al., 2024; Cricelli et al., 2025; Chen and Solis, 2026). IoT in agriculture (Shi et al., 2022), cold chain technology (Mkunda, 2025), and precision agriculture (Zhang et al., 2024). **Facilitating conditions** are significant mainly in contexts with weak infrastructure or where organisational readiness is decisive (Mkunda, 2025; Toader et al., 2024), although Zhang et al. (2024) find FC predicts actual use rather than intention. **Social influence** is most relevant in smallholder and developing-country settings (Shi et al., 2022; Sharma et al., 2023; Mkunda, 2025; Chen and Solis, 2026; Upadhyay and Kaur, 2026), and affects retailers more than farmers in digital platform contexts (Padthar et al., 2024).

Table 1. Overview of UTAUT-based studies on technology in agri-food supply chains

Authors	Technology	UTAUT version	Country	Actor	Level of analysis	Product/ Sector	Key findings
Shi et al. (2022)	IoT	UTAUT2	Bangladesh	Farmers	Individual	Agri-food	PE, EE, SI, FC, HM, PV all significant; trust, government support and personal innovativeness added as extensions
Pienwisetkaew et al. (2023)	Digital platform	UTAUT2	Thailand	Farmers and retailers	Individual	Fruits and vegetables	SI and PV vary across demographic segments; older and higher-income users more influenced by SI, PV, and trust
Sharma et al. (2023)	Blockchain	UTAUT extended	India	SC stakeholders and managers	Organisational	Agri-food	PE, EE, FC, SI all significant; inter-firm trust and transparency added as key constructs
Li et al. (2024)	IoT	UTAUT + TOE	China	Farmers	Individual	Vegetables	Government support and complexity strongest predictors; individual UTAUT constructs mostly non-significant when combined with TOE
Padthar et al. (2024)	Digital platform	UTAUT2	Thailand	Farmers and retailers	Individual	Fruits and vegetables	SI affects retailers but not farmers; HM more significant for farmers; multigroup differences across SC actors
Toader et al. (2024)	Blockchain	UTAUT extended	EU (8 countries)	Agri-food company representatives	Organisational	Agri-food	PE and EE significant; introduced FSCPP, perceived trust and organisational blockchain readiness as novel constructs
Zhang et al. (2024)	Precision agriculture	UTAUT2	China	Farmers	Individual	Agri-food	PE, EE, and HM significant; SI, and PV not significant, FC significant only for actual use
Cricelli et al. (2025)	Blockchain	UTAUT extended	Italy	Wineries/ SMEs	Organisational	Wine	PE and SI significant; EE and FC not significant; extended UTAUT with market dynamics construct
Mkunda (2025)	Cold chain	UTAUT	Tanzania	Vegetable producers	Individual	Vegetables	PE strongest predictor; SI and FC significant; EE non-significant
Chen and Solis (2026)	Blockchain	UTAUT + DOI	Philippines	SC stakeholders	Individual	Coffee	PE, EE, FC, SI all significant; collective inertia moderates the intention–behaviour gap
Upadhyay and Kaur (2026)	Digital crowdfarming	UTAUT extended	India	Smallholder farmers	Individual	Agri-food	PE, EE, FC, SI all significant; trust positively and perceived risk negatively affect adoption

Effort expectancy shows mixed results, suggesting that farmers may tolerate complexity when the perceived benefits of the technology outweigh usability concerns (Mkunda, 2025). Notably, when UTAUT is combined with TOE, organisational and environmental factors can override the individual UTAUT constructs (Li et al., 2024).

Several studies extend UTAUT with context-specific constructs. **Trust** is frequently added in blockchain studies (Sharma et al., 2023; Toader et al., 2024; Upadhyay and Kaur, 2026), **perceived risk** in crowdfarming adoption (Upadhyay and Kaur, 2026), and domain-specific constructs such as agri-food supply chain partner preparedness (FSCPP), organisational blockchain readiness and market dynamics in European blockchain studies (Toader et al., 2024; Cricelli et al., 2025). Chen and Solis (2026) integrate collective inertia from the diffusion-of-innovation literature as a moderator of the intention–behaviour gap. Studies applying UTAUT2 show that hedonic motivation matters in several settings (Shi et al., 2022; Zhang et al., 2024; Padthar et al., 2024 – for farmers only), while price value is less consistently significant, appearing only in Shi et al. (2022). Demographic segmentation further moderates these effects (Pienwisetkaew et al., 2023).

Despite these insights, important gaps remain. Digital traceability platforms are still understudied through the UTAUT lens, and have recently emerged (Padthar et al., 2024; Yu et al., 2025). Also, most studies were conducted in Asian countries, so the findings cannot be generalized to other geographical regions, such as Central and Eastern Europe (Yogarajan et al., 2023). Lastly, most blockchain technology studies examine adoption intention rather than showing its translation into action in supply chains, which is only beginning to be explored (Chen and Solis, 2026).

Conclusion

This paper reviewed the application of the UTAUT model and its extensions for technology adoption in agri-food supply chains. The analysis of 11 empirical studies covering multiple technologies such as blockchain, IoT, precision agriculture, cold chain, and digital platform technologies provides several inisights.

Performance expectancy and facilitating conditions were the most consistently significant predictors of adoption intention, with other context-specific extensions such as trust, perceived risk, and collective inertia also being added to the model. The results confirm the value of extending UTAUT with constructs specific to the context, in this case agri-food, rather than relying on the basic model alone, since the explanatory power of the models tended to occur.

The review also reveals significant imbalances in the literature. Blockchain dominates the research landscape, while other technologies remain underexpled. In addition, empirical evidence is heavily concentrated in Asian countries, limiting the generalisability of findings to other regions, particularly Europe.

Some limitations should be aknowledge. The narrative approach and the relatively small number of selected studies restrict the scope of the analysis. Furthermore, the heterogeneity of technologies and contexts makes direct comparison challenging.

Future research should apply UTAUT to underexplored technologies, as well as the research should expand the geographic focus that has mostly been Asian countries for now. Exploring more than pure intention to use, and studies comparing adoption drivers across different supply chain actors would provide a more comprehensive understanding of technology adoption trend in agri-food systems.

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ORIGINAL SCIENTIFIC PAPER

Modelling the chemical composition of apple waste using machine learning models

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Summary
In this study data on the chemical composition of apple waste were collected from relevant scientific literature, and predictive machine learning models were developed and analysed to estimate fibre content. The dataset comprised 34 samples with variables. For prediction, an artificial neural network model (ANN), a random forest (RF), and multiple linear regression (MLR) were developed and compared. The ANN model achieved the best results, with a coefficient of determination $R^2 = 0.96$ and a mean absolute percentage error of 9.11%, while multiple linear regression showed the weakest performance ($R^2 = 0.08$), confirming the nonlinear nature of the relationship between the observed variables.

Keywords: apple waste, fibers, machine learning models, artificial neural networks

Introduction

Waste from agricultural production is often a valuable source of nutrients such as fibre, phytochemicals and other nutrients. Large quantities of this waste often end up in landfills, despite containing unused resources. Apple waste is one of the most common industrial by-products and forms of food waste, and contains significant amounts of fibre, bioactive compounds and other nutrients (Ahmed et al, 2022). Numerous scientific studies focus on apples as a fruit containing substances desirable in a healthy human diet. Polyphenolic compounds, as powerful antioxidants, provide most of the health benefits of apples. Jancikova and Dordevic (2020) state that a high proportion of dietary fibre in apple waste is largely retained in the processed product, thus improving its nutritional composition. It is crucial to find safe and effective ways to use apple waste in the food and related industries. As apple waste is mainly generated during industrial juice production, its large-scale valorisation could provide significant economic benefits. For a long time, it has been shown that residues from agricultural production and the food industry can be converted into useful products, as well as renewable energy sources such as biofuel. For this reason, the application potential of apple waste is increasingly being investigated to maximise its utilisation (Lyu et al., 2020). Despite growing research on the valorisation of apple waste, accurate assessment of nutritional composition, especially fibre content, remains challenging due to high variability between different varieties, processing conditions, and drying methods. This creates opportunities for the use of advanced machine learning methods as a complement to conventional prediction and modelling techniques. As a key discipline within artificial intelligence, machine learning enables computer systems to build predictive algorithms capable of translating input variables – such as the known chemical constituents of a given product – into targeted outputs such as fibre concentration, by learning patterns from existing datasets (Davies et al., 2021). The science of nutrition is increasingly generating large-scale, high-dimensional data, which requires more sophisticated analytical approaches. Given the complexity and nonlinearity of such data, machine learning represents a suitable methodology, offering an effective approach to working with complex datasets (Kirk et al., 2022).

Material and Methods

The data for the study were collected from the scientific literature (Ahmed et al, 2022; Fátima et al, 2011; Hosein et al, 2019; Kieliszek et al, 2020; Lyu et al, 2020; Selmi et al, 2025; Younis and Ahmad, 2015; Jancikova and Dordevic, 2020; Younas et al, 2015). The literature search was conducted using predefined keywords and their combinations. For each included study, relevant data were stored in a structured database. In total, data were collected for 34 apple waste samples. After collection, all data underwent a cleaning process to ensure completeness and reliability. Basic descriptive statistical parameters were calculated for all observed variables. To estimate the fibre content in apple waste, three predictive models were developed and compared: artificial neural networks (ANN), random forest models (RFR), and multiple linear regression (MLR). All models were trained on the same data set of 34 observations. The artificial neural network model was developed as a multilayer perceptron (MLP) with two hidden layers, the first containing 10 neurons and the second 5 neurons. The ReLU (Rectified Linear Unit) function was used as the activation function, and the weighting coefficients were optimised using the Adam algorithm. The random forest model was developed as an ensemble of 200 decision trees, with unlimited tree depth, allowing each tree to grow fully. Multiple linear regression was performed using the method of least squares (Ordinary Least Squares, OLS), which estimated regression coefficients that minimise the sum of squared residuals between actual and predicted values. The accuracy of the model was determined by cross-validation assessment (k = 5), in which the data set (n = 34) was divided into five subsets; in each iteration, four were used for training and one for testing. The models were evaluated using the coefficient of determination (R^2), Pearson's correlation (R), MAE, RMSE, and MAPE as the arithmetic mean across all iterations.A summary of the architecture and parameters of all three models is presented in Table 1.

Table 1. Model parameters and architecture settings for ANN, Random Forest, and Multiple Linear Regression models applied to predict Fiber content (%) in apple pomace (n = 34).

Parameter	ANN (MLP)	RF	MLR
General			
Target variable	Fiber (%)	Fiber (%)	Fiber (%)
Input variables (n)	5	5	5
Sample size	34	34	34
Architecture / Structure			
Model type	Multilayer Perceptron	Ensemble of decision trees	Ordinary Least Squares
Hidden layers	2 (10 → 5 neurons)	—	—
Activation function	ReLU	—	—
Optimizer	Adam	—	Analytical (OLS)
Number of trees	—	200	—
Max tree depth	—	None	—
Feature selection / split	—	√n_features	—
Random state (seed)	42	42	—

ANN – Artificial neural network; RF – Random forest; MLR - Multiple Linear Regression

Results and Discussion

Table 2 shows descriptive statistical parameters for all six observed variables (moisture, protein, fat, fiber, ash, and carbohydrates) calculated based on 34 samples collected from the literature.

Table 2. Descriptive statistics of the observed research variables

Variable	N	Mean	Std Dev	Min	Med	Max	CV (%)
Moisture	34	7.35	1.24	3.97	7.35	10.50	16.92
Protein	34	4.43	5.73	0.10	3.08	35.30	129.28
Fat	34	2.97	3.41	0.20	1.93	19.70	114.65
Fiber	34	31.41	17.69	0.63	33.79	62.67	56.34
Ash	34	1.96	0.73	0.50	1.96	5.34	37.20
Carbohydrates	34	44.85	13.13	10.00	44.85	83.80	29.28

Among all variables, fibre content had the highest average value (M = 31.41%), with high data variability (CV = 56.34%), indicating significant differences in fibre composition among the analysed apple residue samples. High coefficients of variation were also recorded for protein (CV = 129.28%) and fat (CV = 114.65%), while moisture showed the lowest variability (CV = 16.92%), as expected given the relatively narrow physiological limits of water content in apple waste.

Figure 1 presents the Pearson correlation matrix for the observed variables of the chemical composition of apple waste.

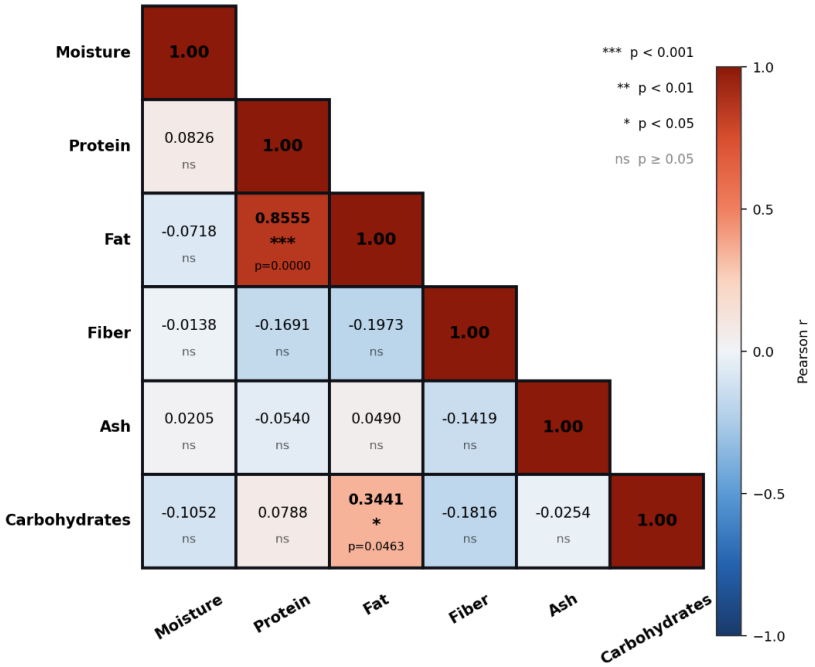

Figure 1. Pearson correlation matrix of chemical composition variables in apple waste.

The most pronounced and statistically highly significant positive correlation was found between protein and fat ($r = 0.8555$; $p < 0.001$), indicating that samples with higher protein content also tend to have higher fat content. A statistically significant, though moderate, positive correlation was also found between fat and carbohydrates ($r = 0.3441$; $p < 0.05$). All other correlations between the observed variables were not statistically significant ($p \geq 0.05$), including all correlations with fibre as the target variable, indicating the relative independence of fibre content from other components of the chemical composition of apple waste.

The performance of all three developed models for estimating fiber content in apple waste is shown in Table 3.

Table 3. Performance of evaluated machine learning models for estimating fiber content

Metric	ANN (MLP 10–5, ReLU)	RF (200 trees)	MLR
Coefficient of Determination (R^2)	0.96	0.81	0.08
Pearson Correlation (R)	0.97	0.93	0.28
RMSE	3.55	7.47	16.70
MAE	2.02	6.11	14.15
MAPE (%)	9.11%	116.98%	286.39%

ANN – Artificial neural network; RF – Random forest; MLR - Multiple Linear Regression

Davies et al. (2021) conducted a study using a KNN machine learning model to predict fibre content in packaged foods, achieving good accuracy (R^2 = 0.84), lower error (MAE ≈ 1.52 g/100 g), and better performance than the existing manual approach. In contrast, Monticeli et al. (2021) used an ANN to predict the thermal degradation of plant fibres, achieving exceptionally high accuracy (R^2 > 0.99) with very low errors (<2%), with the predicted curves showing excellent agreement with experimental data and high model reliability.
The artificial neural network model (ANN MLP 10–5, ReLU) demonstrated the best predictive performance among all evaluated models, with a coefficient of determination R^2 = 0.96 and a Pearson correlation coefficient R = 0.97, as well as a relatively low prediction error (RMSE = 3.55; MAE = 2.02; MAPE = 9.11%). The random forest model (Random Forest, 200 trees) achieved moderately good results (R^2 = 0.81; R = 0.93), but with significantly higher error values (RMSE = 7.47; MAE = 6.11; MAPE = 116.98%), indicating some instability in predictions at more extreme values. Multiple linear regression proved to be the least suitable model for predicting fibre content, with a very low coefficient of determination (R^2 = 0.08) and extremely high errors (RMSE = 16.70; MAE = 14.15; MAPE = 286.39%), suggesting that a linear relationship between input variables and fibre content is insufficient to adequately model this complex dependence.

Conclusion

The aim of this study was to develop and compare predictive machine learning models for estimating the fibre content of apple waste based on chemical composition data collected from the literature. The high variability of the data and the absence of statistically significant correlations between fibre and other variables confirmed the complexity of the predictive problem and the limitations of classical linear methods, as also demonstrated by the poor performance of multiple linear regression (R^2 = 0.08). The artificial neural network model was the most effective approach, with high prediction accuracy (R^2 = 0.96; MAPE = 9.11%), confirming the potential of deep learning for modelling the nutritional composition of food industry by-products.

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PRELIMINARY COMMUNICATION

The impact of branding domestic agricultural products on the preferences of young people in the Republic of Croatia

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Summary
In increasingly competitive and globalized markets, the growing complexity of science and technology has intensified the need for effective product differentiation, particularly through branding in the agricultural sector. This study examines whether branding agricultural products influences the preferences of young consumers in Croatia. A survey was conducted among 50 respondents aged 18-30, and the data were analyzed using SPSS. The results show that young consumers pay attention to brands in agriculture and are willing to pay more for branded products. Social networks play an important role in product visibility. The findings suggest that branding domestic agricultural products improves market positioning and competitiveness.

Keywords: agriculture, branding, marketing, preferences, young people

Introduction

Brand represents a company's promise to customers, not merely a name or marketing message, but a carrier of the core attributes of a product or service (Yakup and Sevil, 2011). It is more than a traditional promotional tool; it encompasses a word, logo, sound, geographical designation, geometric shape, figure, and even emotion (Gherasim, 2014). Branding refers to creating a unique product or corporate identity, guaranteeing a certain level of quality as a symbol of trust, and consistently communicating and delivering these values (Okereafor et al., 2023). Therefore, a brand is of strategic importance for every company (Urbanek, 2015). In a dynamic marketing environment, brand storytelling has become a powerful instrument for attracting and engaging audiences, moving beyond simple promotion to construct compelling narratives that promise meaningful experiences or appealing visions (Yuan, 2024). A brand is also a functional identification of value, a complex of elements, and a promise of uniqueness (Bivainiene, 2011). Branding agricultural products represents a strategic approach through which agricultural enterprises seek to increase product visibility, guarantee quality, and enhance sales. Traditionally perceived as commodities competing primarily on price, agricultural products can, through branding, move beyond price-based competition by developing distinctive identities and adopting premium pricing models (Chaturvedi and Sinha, 2025). Branding also stimulates innovation within agriculture (Meng, 2021) and contributes to better product valorization and higher prices (Uzelac et al., 2022). Key branding approaches include value-added branding through quality differentiation and certification, retail branding in cooperation with organized retail chains, varietal branding based on patented superior crop varieties, packaging as a tool for brand recognition and product protection, and the use of geographical indications (GI) to differentiate and promote products, particularly in export markets (Rajput et al., 2022). Previous research has extensively examined agricultural branding and youth perception. Yang et al. (2015), on a sample of 351 young respondents in China, found that branding significantly increases demand for agricultural products. Sun et al. (2022), analyzing 576 respondents, concluded that consumer

habits change when products are branded. Zheng and Li (2024) reported a 1.6% income increase among farmers who adopted branding. Wei (2023), based on 208 respondents, identified storytelling combined with social media as the most effective strategy for building positive perceptions among young consumers. Zhang and Liu (2023) emphasized that storytelling in agricultural branding should incorporate elements of ecology, authenticity, and tradition. According to Prial (2021), companies selling through storytelling promote not only products but complete narratives. Overall, findings suggest that youth perception of agricultural products improves when branding strategies are applied.

In the context of globalization and increasing market competition, branding of agricultural products in Croatia is gaining importance. Croatia's natural resources, diverse agroecological conditions, and production tradition provide strong potential for developing competitive agricultural brands. Products such as Baranjski kulen, Istarsko maslinovo ulje, Paški sir, and Lički krumpir illustrate how geographical origin and strong identity can enhance market recognition. Branding strategies in Croatian agriculture include value addition through innovative packaging, certification, promotion of local origin, and linking products with regional tradition, with geographical indications playing a crucial role (Rajput, Ghosh and Labanya, 2022). Moreover, digital channels and storytelling approaches are increasingly used to attract younger consumers and create emotional connections (Meng, 2024). The perception of the younger generation in Croatia regarding branded agricultural products therefore represents a relevant research issue addressed in this study.

Material and Methods

The empirical research was conducted on a sample of 50 respondents from Croatia, primarily from Zagreb, Split, Osijek and Rijeka. The main sampling criteria were that the respondents were aged between 18 and 30. A convenience sampling method was used, as participants were selected based on their availability and willingness to participate. Participants were surveyed using a questionnaire. The questionnaire included the following categories of questions: sampling and sociodemographic questions (origin, gender, age), questions related to the purchasing process of agricultural products, and questions related to branding. The questionnaire was distributed online via Google Survey, and the collected data were analyzed using the SPSS statistical software package.

Results and Discussion

At the beginning of this section, it should be noted that all respondents in the sample are from Croatia (100%) and that all of them are between 18 and 30 years of age. Data on age and gender are presented in the following table.

Table 1. Sociodemographic data

Variable	Category		
	Variable type	Frequency	Percent (%)
Gender	Male	24	48.0
	Female	26	52.0
Age	18 – 20	15	30.0
	21 – 25	11	22.0
	26 – 30	24	48.0

Based on the previous table (Table 1), it can be concluded that 24 respondents (48%) were male, while 26 respondents (52%) were female. The following section presents respondents' attitudes regarding the purchase of agricultural products. Respondents were asked to evaluate a

series of statements using a five-point Likert scale, where 5 represents the highest rating and 1 the lowest rating.

Table 2. Descriptive Statistics (1)

Variable	Category		
	N	Mean	Std. Deviation
I believe that it is important to brand agricultural products.	50	4.85	0.0320
I am willing to pay more if it is a branded agricultural product.	50	4.95	0.0223
Branding of agricultural products indicates to me that the product is of high quality.	50	4.55	0.0232
There are not enough branded agricultural products in Croatia.	50	4.00	0.3241
I believe that marketing is very important in agriculture and contributes to higher sales of agricultural products.	50	4.95	0.0021

Based on the results presented in the previous table, it can be concluded that most young people support the branding of agricultural products and perceive branded products as synonymous with quality. Given this perception, it is also noteworthy that most young respondents are willing to pay more for branded products. Similar findings were reported by Suo and Huang (2023), who, in a sample of 444 respondents, concluded that young people are willing to pay more for premium-branded agricultural products. Likewise, Hao et al. (2022), in a study of 601 respondents, found that branding is the most effective motivator for young people to purchase specific agricultural products. The following section presents results related to young people's attitudes toward the branding process.

Table 3. Descriptive Statistics (2)

Variable	Category		
	N	Mean	Std. Deviation
I spend most of my time online, so agricultural products need to be more visible and present on social media.	50	4.95	0.1593
Branding of agricultural products must be accompanied by a specific story (storytelling).	50	4.95	0.13562
Branding of agricultural products should not focus	50	4.20	0.1578

only on the product itself, but also on its packaging.			
Geographical indication is extremely important in the branding of agricultural products.	50	4.10	0.3659
I would rather buy a branded agricultural product than one that is not branded.	50	4.20	0.3263

The results presented in Table 3 provide insights and information that can be useful for agricultural producers planning to brand their products. Social media represents a solid foundation that can be leveraged during the branding process. Previous research by Soulignac et al. (2025) shows similar findings. Branding of agricultural products should also involve storytelling, as noted by Yueh and Jheng (2019). Respondents in this study indicated that product packaging is very important, as is the geographical indication, and that they are more willing to purchase (and pay for) a branded agricultural product compared to an unbranded one. All of this information can serve as a significant support for agricultural producers, primarily in understanding young consumers' perceptions regarding branding, and secondarily in guiding the steps necessary for successful branding of agricultural products.

Conclusion

Branding represents a secure path and effective means for increasing the visibility and sales of agricultural products. This study aimed to explore the perception of young people regarding the branding of agricultural products. Both theoretical and empirical results indicate that young people in Croatia consider branding important and are more likely to purchase branded agricultural products than unbranded ones. The findings also show that social media provides a suitable platform and starting point for building an agricultural product brand, with storytelling, sharing compelling narratives, being one of the most effective implementation methods. The results presented in this study can be applied practically and serve as a foundation for agricultural producers who are planning to bring their products to the market and develop them into recognizable brands. Based on the data presented, it becomes clearer how to engage the younger population in Croatia with branded agricultural products. Despite the valuable insights provided, this study has certain limitations that should be acknowledged. The sample size of 50 respondents is relatively small and does not allow for fully representative or generalizable conclusions. Therefore, the findings should be interpreted with caution. Future research should include a larger and more diverse sample in order to ensure greater reliability and broader applicability of the results.

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ORIGINAL SCIENTIFIC PAPER

Education of youth for successful agribusiness: Managerial and entrepreneurial competencies

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Summary
This paper analyses the role of youth education in sustainable agribusiness, focusing on managerial and entrepreneurial competencies. It assumes that quality of education influences young people's readiness for agribusiness. Based on literature and a survey of students in agribusiness programs, results reveal a gap between formal education and labour market needs, especially in management, marketing, and financial planning skills. The study highlights the need to modernise curricula with practical and interdisciplinary content to enhance employability and sustainable entrepreneurship.

Keywords: entrepreneurship, employability, managerial competencies, sustainable agribusiness, youth education.

Introduction

Education of youth for successful agribusiness represents a key component in the development of managerial and entrepreneurial competencies necessary for effective human resource management, strategic planning, and innovation in the agricultural sector. Today, agribusiness faces numerous challenges, such as climate change, technological transformation, and global competition, which require new generations to possess not only technical knowledge but also business and leadership skills (Agustina and Rosalia, 2025). The literature indicates a significant gap between formal education and labor market needs in agribusiness, particularly in the areas of management, financial planning, and entrepreneurship. Formal academic programs are often focused on theoretical content without sufficient practical components, resulting in a limited ability of young people to confront real business challenges after graduation (Siregar and Nasution, 2026). Empirical research has shown that practice and experiential learning (work-based learning), including field instruction, project-based activities, and collaboration with agribusiness companies, have a positive impact on the development of key youth competencies. Such integration of theory and practice enables students to develop practical skills directly applicable to business operations, including decision-making, resource management, and adaptation to market changes (Kassem et al., 2021). A particularly valuable study on youth engagement in agribusiness across Africa used a systematic literature review and empirical data to identify factors influencing the perception of agribusiness as a career option. The authors found that access to education, mentorship, and specialized training significantly foster the development of agribusiness competencies and entrepreneurial capacities among youth, while socioeconomic barriers (e.g., limited access to capital) remain a substantial challenge (Boye et al., 2024). Additionally, research indicates that specific training programs and support initiatives for young agripreneurs positively affect their business performance.

This includes increased income, productivity, and risk management capacity, especially when training programs are structured to combine theoretical knowledge with practical experience and mentorship (Adeyanju et al., 2021). Furthermore, curriculum development that integrates business skills with agronomy-oriented knowledge results in better employment outcomes and greater readiness among young people to assume active roles in agribusiness within their communities (Cukanovic Karavidic et al., 2018). Such an approach fosters interdisciplinary competencies essential for strategic management, innovation, and sustainable operations in the agricultural sector. Despite these positive findings, the integration of human resource management practices within agribusiness education systems remains a significant challenge, particularly for small enterprises facing limited resources for professional development. This underscores the need for modernization of educational programs in order to strengthen managerial and entrepreneurial competencies among youth through practical, experiential, and contextually relevant learning methods.

Material and Methods

The research is based on an review of relevant scientific literature, the key findings of which were presented in the previous chapter. The literature review served as the theoretical foundation for defining the research problem, operationalizing variables, and formulating research hypotheses. The primary objective of the study was to determine which of the identified managerial competencies has the most statistically significant contribution to the business success of young agripreneurs, as well as whether there is a statistically significant relationship between the level of development of managerial competencies and agribusiness performance. The research also aimed to examine whether managerial competencies were acquired during studies at the Faculty of Agronomy and whether this form of education, in addition to agronomic knowledge, provides sufficient knowledge in management and business administration. Primary data were collected from a sample of young agripreneurs, that is, individuals who completed studies in agronomy and subsequently launched their own agribusiness ventures using the knowledge and competencies acquired through formal education.

The sample consists of 50 young agripreneurs (up to 40 years of age). The main sampling criteria were: the respondent completed a degree in agronomy; the respondent is up to 38 years old; the respondent established their own agribusiness venture. A purposive sampling method was applied. Primary data were collected through a structured survey questionnaire (January - February 2026), which included the following groups of questions: filter questions (confirming fulfillment of sampling criteria), sociodemographic questions (gender, age, duration of business operation), questions related to managerial knowledge and competencies acquired during agronomy studies, and questions concerning the application of managerial and entrepreneurial competencies in practical business operations. Competencies were measured using a five-point Likert scale (1 – strongly disagree; 5 – strongly agree), enabling quantitative analysis of respondents' perceptions regarding the development of specific competencies.

Accordingly, the following research hypotheses were formulated: (H1) The most important managerial competency in agribusiness is strategic planning; (H2) There is a high degree of correlation between managerial competencies and business success in agribusiness; (H3) Faculties of agronomy provide sufficient knowledge in management and entrepreneurship necessary for entrepreneurs to successfully manage their agribusinesses. The data were analyzed using descriptive statistical methods (mean and standard deviation) and inferential statistics. Pearson's correlation coefficient was planned for hypothesis testing, alongside descriptive statistical analysis. The research was conducted in accordance with fundamental ethical principles of scientific work. Participation in the study was voluntary, and respondents were informed prior to completing the questionnaire about the purpose of the research, data

processing procedures, and the anonymity of their responses. Data were collected anonymously, without gathering identifying information that could lead to the identification of respondents or their business entities. Participants were informed that they could withdraw from the study at any time without any consequences.

Results and Discussion

In this section, the research results are presented and interpreted in accordance with the defined research objectives and hypotheses. The analysis begins with the presentation of sociodemographic characteristics of the respondents, followed by descriptive and inferential statistical analysis.

Table 1. Sociodemographic data

Variable	Category		
	Variable type	Frequency	Percent (%)
Gender	Male	32	64.0
	Female	18	36.0
Age	Up to 30 years	14	28.0
	31 – 35	21	42.0
	36 – 38	15	30.0
Duration of business	Up to 3 years	17	34.0
	4 – 7 years	19	38.0
	Over 7 years	14	28.0

The sample consists predominantly of HR managers (96%), which confirms the relevance of the respondents for the subject of the research. The largest number of respondents belongs to the age group 31–35 years (42%), while the majority have more than 10 years of experience in agribusiness (64%), indicating a high level of professional competence within the sample, according to results on the sample.

Table 2. Descriptive statistics of key variables

Variable	Category		
	N	Median	Std. Deviation
Strategic planning competence	50	4.12	0.63
Financial planning competence	50	3.98	0.71
Leadership competence	50	4.05	0.66
Entrepreneurial orientation	50	4.18	0.59
Perceived business success	50	4.09	0.64
Adequacy of faculty education in management	50	3.54	0.82

The results indicate relatively high average values for all analyzed competencies. Entrepreneurial orientation (M = 4.18) and strategic planning (M = 4.12) show the highest mean values, suggesting that respondents perceive themselves as highly competent in these domains. The average perceived business success is also high (M = 4.09), indicating a positive self-assessment of business performance. However, the adequacy of faculty education in management and entrepreneurship records a somewhat lower mean value (M = 3.54), suggesting moderate satisfaction with managerial knowledge acquired during formal education.

Table 3. (H1) Strategic planning has a statistically significant positive impact on business success in agribusiness

Variable	Category	
	Strategic planning vs Agri-business success	
Strategic planning	1	.647
Sig. (2-tailed)		.000
N	50	50
Business success	.647	1
Sig. (2-tailed)	.000	
N	50	50

A strong positive correlation was identified between strategic planning competence and perceived business success ($r = .647$, $p < .001$). The correlation is statistically significant at the 0.01 level, thus supporting hypothesis H1. The findings indicate that higher levels of strategic planning competence are associated with higher levels of business success among young agripreneurs.

Table 4. (H2) There is a high degree of correlation between managerial competencies and Agri-business success

Variable	Category	
	Overall managerial competencies vs Agri-business success	
Managerial competencies	1	.712
Sig. (2-tailed)		.000
N	50	50
Agri-business success	.712	1
Sig. (2-tailed)	.000	
N	50	50

The results show a strong positive correlation between overall managerial competencies and business success ($r = .712$, $p < .001$). Since the p-value is lower than 0.01, hypothesis H2 is accepted. This indicates that the development of managerial and entrepreneurial competencies significantly contributes to the success of agribusiness ventures. Descriptive results indicate a moderate mean value ($M = 3.54$), suggesting partial agreement among respondents that agronomy faculties provide sufficient managerial and entrepreneurial knowledge. Although respondents recognize the importance of education, the findings imply that additional practical managerial training may be necessary to fully prepare graduates for entrepreneurial challenges. Therefore, H3 is only partially supported. The results of this study confirm a strong and statistically significant relationship between managerial competencies—particularly strategic planning—and perceived business success among young agripreneurs. These findings are consistent with prior research emphasizing the critical role of managerial capabilities in small and medium-sized enterprises (SMEs). Specifically, the strong correlation identified between strategic planning and business success ($r = .647$, $p < .001$) aligns with the findings of Wiklund and Shepherd (2005), who demonstrated that entrepreneurial orientation and strategic decision-making positively influence small firm performance. Their study highlights that proactive strategic behavior and opportunity-oriented thinking significantly enhance competitive advantage, which supports our empirical evidence from the agribusiness sector. Furthermore, the strong association between overall managerial competencies and business success ($r = .712$, $p < .001$) corresponds with the theoretical framework developed by Man, Lau, and Chan (2002), who conceptualized entrepreneurial competencies as key determinants of SME performance. Their model emphasizes strategic, conceptual, and opportunity competencies as central drivers

of business growth dimensions that are directly reflected in the constructs measured in this research. In the agribusiness context specifically, our findings are also consistent with the analysis of Vesala and Pyysiäinen (2008), who argue that modern farmers increasingly operate as entrepreneurs whose managerial and strategic skills significantly influence farm performance. This supports our conclusion that agronomic expertise alone is insufficient without complementary managerial capabilities. Regarding the moderate evaluation of faculty-provided managerial education (M = 3.54), our findings resonate with the broader literature on entrepreneurial education. Fayolle and Gailly (2015) demonstrate that while entrepreneurship education positively affects entrepreneurial intentions and competencies, its effectiveness depends heavily on pedagogical design and practical integration. The moderate perception observed in our study may therefore indicate the need for stronger experiential learning components and closer university–industry collaboration in agronomy programs.

Conclusion

The findings of this study confirm that managerial and entrepreneurial competencies play a decisive role in achieving business success in agribusiness among young entrepreneurs. Strategic planning, financial management, leadership skills, and entrepreneurial orientation show strong positive associations with perceived business performance. Although respondents generally assess their competencies at a high level, the moderate evaluation of faculty-provided managerial knowledge indicates the need for stronger integration of practical entrepreneurship and management education within agronomy programs. Strengthening these competencies through formal education and continuous professional development represents a key prerequisite for enhancing the competitiveness and long-term viability of youth-led agribusiness ventures.

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ORIGINAL SCIENTIFIC PAPER

Electricity Production Cost from the Anaerobic Digestion of Swine Manure

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Summary
This study estimates electricity production costs from the anaerobic digestion of swine manure. With a 10-year life and 8% interest rate, costs ranged from $0.15 to $1.52 per kWh. Farm size, manure storage system, and digester type impact cost. Economies of scale and existing infrastructure significantly impact costs. Covering an existing anaerobic lagoon at a 30,000 finish pig space facility results in $0.24 per kWh, while constructing a new tank digester and covered secondary storage for a 5,000-head deep-pit facility increases the cost to $0.51 per kWh. Production costs exceed current wholesale electricity cost of $0.05 per kWh in the US, or $0.095 per kWh in the EU.

Keywords: electricity, cost, swine, manure, biogas

Introduction

The anaerobic digestion of swine manure presents a pathway for renewable electricity generation, yet its economic feasibility remains heavily dependent on farm scale and existing manure storage infrastructure. In addition to generating renewable energy, the anaerobic digestion of swine manure reduces manure odor, improves manure plant nutrient availability, and provides for the collection and destruction of GHG emissions from manure storage systems. Biogas produced from the anaerobic digestion of swine finishing manure can be combusted in reciprocating engine generators to produce electricity. However, the capital-intensive nature of these systems makes their economic feasibility highly variable.

Previous baseline analyses, such as the 2007 USDA Technical Note No. 1, estimated the cost to produce electricity from swine manure digesters to be between $0.07 and $0.11 per kWh (Beddoes et al., 2007). When adjusting these cost estimates using the Producer Price Index for new industrial building construction cost between 2007 and 2025, by applying a 1.99 multiplier (U.S. Bureau of Labor Statistics, 2026) to reach the 2025 equivalent, the baseline electricity production cost estimate rises to $0.14 - $0.22 per kWh. It is important to note that the USDA Technical Note No. 1 cost estimates do not include the labor costs required to operate the anaerobic digester and electrical production generator systems. Data from international commercial digester operations further confirms these estimates of biogas electricity production cost. An analysis of eight operational 2.0 MW manure-based anaerobic digesters in Croatia reveals a 2023 electricity production cost of approximately $0.243 per kWh. While these European systems rely on co-digesting manure with other substrates, the increasing purchase cost of materials like corn silage has been the primary driver in raising their overall production costs in recent years (Bošnjaković, 2023).

Within the United States, standard wholesale commercial electricity rates are currently average $0.05 per kWh (EIA, 2025), and average $0.095 per kWh averaged across the European Union (IEA, 2026). This creates a significant deficit between the cost of manure biogas based electricity and the wholesale market value that a producer would be paid for electricty sold to

the grid in the absence of any renewable energy subsides. Consequently, the financial viability of integrating digesters on swine farms is dependent on supplementary revenue streams, such as the payment through the U.S. Environmental Protection Agency's Renewable Identification Numbers (RIN) program for the production of a biogas as renewable fuel, and state-level Low Carbon Fuel Standards (LCFS) in the United States, or via the continuation of adequate Feed-In-Tarriffs in the European Union.

To better define the current economic landscape and establish the minimum feasible production costs, updated cost estimations are required that account for modern construction expenses, diverse facility setups, and labor. This paper reports on the evaluation of six different swine finish farm facility configurations to provide a comprehensive range of estimated electricity production costs. By modeling scenarios across nine different farm sizes ranging from 1,250 to 50,000 finish pig spaces, and comparing existing lagoon-based storage retrofits against deep-pit barns that require the construction of new, external (out-of-barn) manure storage systems.

Material and Methods

A spreadsheet model to estimate electricity production costs was developed based on six different swine facility configurations to establish a range of projected electricity production costs for swine finisher farms. For configurations that include existing lagoon-based manure storage, systems considered in the analysis included 1) covering the existing lagoon and combust the biogas to produce electricity, 2) adding a tank digester between the barn and the uncovered existing lagoon and combust biogas to produce electricity, and 3) adding a tank digester between the barn and the existing lagoon and then cover the existing lagoon and combusting the biogas from both the tank digester and the covered lagoon for electricity production. Three additional options were considered for deep-pit finishing pig facilities where a new, out of barn storage must be constructed; 4) build a new tank digester, 5) add a tank digester before the new uncovered manure storage tank, and 6) add a tank digester and build a new covered tank manure storage. Manure and methane production was estimated as follows. Pigs were assumed to generate 1,658 liters of manure per finish pig space per year, which was averaged to 4.54 liters of manure per pig space per day (not adjusted for actual pig occupancy), and assumed to excrete 0.314 kg $VSday^{-1}$ (ASABE, 2005). The methane potential was set at 0.35 m^3 CH_4/kg of volatile solids (VS) (Moody, 2011). This equates to 40 m^3 CH_4 per pig space per year. The conversion efficiency of potential to actual methane production was set at 47% for covered manure storages (this is the average efficiency of all lagoon-based projects listed in the AgStar database (AgSTAR, 2023). Digester efficiency for heated digesters was set at 75% of the theoretical methane potential. When covered manure storage followed a lagoon, the lagoon methane production was estimated to be 46% of the methane potential added. Table 1 provides a summary of methane production per pig for covered lagoons, tank digesters, covered lagoon following a tank digester and tank digester followed by a covered lagoon combined. Tank digesters were defined as mesophillic Continuously Stirred Tank Reactors (CSTR), and lagoon covers were defined as impermeable covers. The lagoon/manure storage impermeable cover was estimated based on the manure volume and a three-meter deep manure storage lagoon. The cover price in US dollars per square meter was estimated using Equation 1, $CC\$ = 1852 * Area^{-0.53}$ with *Area* entered as square meters. This leads to a declining price as the manure storage surface area increases. The lowest price possible was set to \$14.05 per m^2 to match the largest storage covered in the dataset. The digester cost in US dollars was estimated using Equation 2, $Digester\$ = 9900 * Volume^{0.59}$ with *Volume* entered as cubic meters.

Table 1. Estimated methane potential and methane levels that could be achieved with different digester and covered and uncovered lagoon configurations. Electricity production was based on a generator achieving a 40% conversion efficiency.

	Methane Potential	Covered Lagoon	Tank Digester	Covered Lagoon Following Tank Digester	Digester & Covered Lagoon
Methane (m^3 CH_4/pig space-yr)	40	18	30	5	35
MMBtu*/pig space-yr	1.4	0.7	1.1	0.2	1.2
kWhr/pig space-yr	168	77	126	19	145

*MMBtu –million British thermal units

The digester volume was set based on a 30-day hydraulic retention time, with 85% of the volume assumed to be the working volume. When new manure storage needed to be constructed, it was estimated to cost $0.04 a liter, and a year-long retention time was planned. This represents the price of a concrete or glass-lined steel tank. An unlined earthen manure storage would be cheaper ($0.01 per liter), but more difficult to permit in many locations, so we did not consider this option in this analysis. The generator cost was estimated at $1,000 per kW of capacity. Total kWhr for each system was used to determine the kW size required for the digester assuming a 50% runtime for the generator over the course of the year. For covered lagoon systems, gas production is seasonal, to have sufficient capacity generation during the highest month of gas production a 3x factor was used to size the generator. For systems with a digester and covered storage, no correction was made as the majority of gas comes from the digester, but we note that generator operation would be slightly more than estimated during peaking periods when the lagoon was generating biogas. Operating and maintenance costs were assumed to be 10% of capital costs plus a $100,000 a year for salary and benefits for a trained employee to manage the facility. No gas cleaning was assumed prior to combustion. Capital cost was assumed to be borrowed at an 8% interest rate. Each scenario was evaluated for finish pig farm sizes of (1250, 2500, 5000, 7500, 10000, 20000, 30000, 40000, and 50000) and for an estimated design life of 10 years. Table 2 shows estimated costs per kWhr for systems that originally used lagoon manure management and for systems that originally used deep pit manure management.

Table 2. Cost per kWhr for systems for existing lagoon and deep pit systems.

Pig Spaces	**Existing Lagoon ($)**	**Digester ($)**	**Digester +Cover ($)**	**Deep Pit +Lagoon ($)**	**Digester ($)**	**Digester +Cover ($)**
1,250	1.27	1.03	0.96	1.52	1.16	1.07
2,500	0.74	0.63	0.59	0.99	0.77	0.71
5,000	0.47	0.41	0.39	0.72	0.55	0.51
7,500	0.37	0.33	0.31	0.63	0.47	0.44
10,000	0.33	0.28	0.27	0.58	0.43	0.40
20,000	0.26	0.21	0.20	0.51	0.36	0.33
30,000	0.24	0.18	0.18	0.49	0.33	0.31
40,000	0.23	0.16	0.16	0.48	0.31	0.29
50,000	0.22	0.15	0.15	0.47	0.30	0.28

Results and Discussion

The levelized cost of energy (LCOE) to produce electricity via a reciprocating engine generator using biogas from swine finishing manure ranges from $0.15 to $1.52 per kWh. This broad distribution is heavily dependent on farm size, existing manure infrastructure, and the specific digester technology implemented. Economies of scale play a distinct role in project feasibility. For instance, a 30,000 pig space facility that simply adds an impermeable cover to an existing anaerobic lagoon can generate electricity at an estimated $0.24 per kWh. In contrast, a smaller 5,000 head deep-pit facility that must construct both a new tank digester and a new covered secondary storage tank faces a significantly higher production cost of $0.51 per kWh. Electricity production costs from swine manure based anaerobic digester currently exceed the rates that farmers could expect to be paid when selling electrical power to utilities. Wholesale commercial electricity rates that would be paid for electricity purchased by utilities from farmers are much lower however, averaging $0.05 per kWhr in the United States and $0.095 per kWhr in the European Union. The cost to produce renewable electricity from swine manure anaerobic digestion systems falls within the range of current residential and business electricity rates in some cases. Residential electricity rates currently average $0.16 per kWh in the United States (Woo et al., 2024), and $0.33 per kWh for residential customers and $0.22 per kWh for business customers, averaged across the European Union (Eurostat, 2025). It should be noted that electricity prices vary significantly across different EU member states, with countries like Germany reporting the highest residential prices of around $0.44 per kWh and countries like Hungary reporting the lowest residential prices around $0.12 per kWh (Eurostat, 2025). The model's outputs align closely with both historical inflation-adjusted data and modern international commercial operations. Adjusting 2007 USDA Tech Note 1 estimates for construction inflation results in a 2025 baseline cost range of $0.14 to $0.22 per kWh, excluding necessary labor costs. Our calculated minimums align well with these historical adjustments. Furthermore, data from operational 2.0 MW manure-based digesters in Croatia show a current production cost of $0.243 per kWh inclusive of labor and co-digestion feedstock purchases. The Croatian data also reveals the vulnerability of co-digestion economics; electricity production costs at these plants rose from $0.173 per kWh in 2021 to $0.243 per kWh in 2023 primarily due to the increasing purchase price of corn silage. While corn silage generated 77% of the total electricity for these specific European plants, swine manure remained the most economically efficient substrate.

Conclusions

Electricity production costs from generators fueled with biogas from the anaerobic digestion of swine manure ranged from $0.15 to $1.52 per kWh across a range of farm sizes, manure storage systems, and digester types. For example, adding a cover to an existing anaerobic lagoon at a 30,000 finish pig space facility results in a cost of $0.24 per kWh, while constructing a new tank digester and covered secondary storage for a 5,000-head deep-pit finish pig facility increases the cost to $0.51 per kWh. Swine manure based electricity production costs are significantly higher than current wholesale electricity cost in either the United States, or the European Union. As such these projects are not economically viable through electricity sales alone. The future financial feasibility of electricity production from manure digestion relies fundamentally on renewable energy subsidies, specifically pathways like the Renewable Identification Number (RIN) programs and Low Carbon Fuel Standards (LCFS) in the United States, or the continuation of Feed-In-Tariffs in European Union Member States.

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ORIGINAL SCIENTIFIC PAPER

The impact of label design on the branding of Dalmatian extra virgin olive oil: An empirical study of consumer perceptions

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Summary
Regional origin is important in the marketing of geographically identified food products. Using an online survey of 274 consumers who evaluated hypothetical olive oil labels, this study examines how label design affects perceptions of origin, perceived quality, and purchase intention for Dalmatian extra virgin olive oil. Regression results indicate that perceived origin and perceived quality significantly predict purchase intention. Label elements emphasising geographical origin lead to stronger perceptions of authenticity and greater consumer trust.

Keywords: branding, label design, origin, geographical indication, olive oil

Introduction

In markets for geographically identified food products, regional origin has become an important basis for product differentiation. Consumers frequently associate regional origin with authenticity, traditional production, and higher product quality. These perceptions are particularly strong in the olive oil sector, where production practices, terroir characteristics, and regional reputation significantly influence consumer evaluations. Dalmatian extra virgin olive oil represents a product whose value is closely associated with geographical identity. The region's Mediterranean climate, long-standing cultivation traditions, and historical connection with olive growing contribute to its reputation as a premium product. At the same time, the expansion of global olive oil markets and rising international competition make it more difficult for producers to communicate these distinctive attributes. In this environment, packaging and label design play an important role in marketing communication. Product labels provide mandatory information while simultaneously acting as visual cues that shape consumer expectations before purchase. Through elements such as typography, colour, imagery, and references to regional heritage, labels communicate signals of origin, craftsmanship, and quality. Despite growing interest in packaging design, empirical research examining how specific label elements influence consumer perceptions in the olive oil sector is still scarce. Although previous studies have examined country-of-origin effects and broader aspects of brand equity, the visual mechanisms through which packaging communicates regional origin have received less attention. The present study examines how label design elements influence perceptions of origin, perceived quality, and purchase intention for Dalmatian extra virgin olive oil. Drawing on perspectives from brand equity theory (Aaker, 1991; Keller, 2013), signalling theory in consumer markets (Spence, 2002), research on geographical indications and origin-based products (Barham and Sylvander, 2011; Belletti et al., 2015), and studies of visual cues in packaging design (Spence, 2002), the research aims to provide a clearer understanding of how visual signals shape consumer decision-making in regional food markets. Brand equity represents the value that a brand adds to a product through consumer perceptions, associations, and loyalty (Aaker, 1991). Keller (2013) conceptualizes brand equity as consumer responses

arising from brand knowledge that influence purchasing behaviour. In food marketing, brand equity is shaped by both intrinsic and extrinsic product cues. Intrinsic attributes include sensory characteristics such as taste and aroma, while extrinsic cues include packaging, labelling, brand reputation, and price. Because consumers typically cannot evaluate intrinsic qualities before purchase, extrinsic cues often play a crucial role in shaping expectations and purchase decisions. For regionally associated products, origin-related cues may constitute an important component of brand equity, generating associations related to provenance, craftsmanship, and traditional production. Geographical indications (GI) link products to specific territories and production traditions, serving as mechanisms for protecting regional heritage and product reputation. From a consumer perspective, GI labels function as signals of origin and quality assurance, often increasing trust and willingness to pay premium prices. In the olive oil sector, origin signals frequently appear through visual and textual elements on product labels, including certification logos, references to protected designations of origin, regional symbols, and imagery associated with local landscapes or cultural heritage. In food marketing, packaging serves both protective and communication functions. Visual elements such as colour, typography, imagery, and layout contribute to brand meaning and influence consumer perceptions of quality. Research in consumer psychology suggests that visual design elements can shape expectations before direct product experience. Colours may evoke associations with naturalness or freshness, while typographic styles can communicate tradition or craftsmanship. In olive oil packaging, imagery related to Mediterranean landscapes, olive groves, and traditional agricultural practices often reinforces perceptions of heritage and geographical authenticity. Origin has become an increasingly important element in contemporary food marketing, as consumers often seek products perceived as authentic, locally rooted, and linked to traditional production methods. Consumers form perceptions of product origin through visual cues, production narratives, and branding strategies. For regional food products, packaging plays a central role in communicating these messages. Labels emphasizing geographical origin, heritage, and traditional practices can strengthen perceptions of authenticity and positively influence consumer attitudes and purchase intentions.

Material and Methods

This study employed a between-subject experimental survey design using a 2 × 2 factorial structure manipulating visual attractiveness and origin cue salience, to ensure experimental control and transparency. Respondents were randomly assigned to one label condition. Data were collected online (n = 327; valid n = 274). All constructs were measured using multi-item scales adapted from prior research. Responses were recorded on a five-point Likert scale (1 = strongly disagree; 5 = strongly agree): Visual Attractiveness (3 items; Orth and Malkewitz, 2008); Perceived Origin (4 items; Beverland, 2005; Napoli et al., 2016); Perceived Quality (3 items; Zeithaml, 1988); Perceived Authenticity (3 items); Purchase Intention (3 items; Dodds et al., 1991). Data analysis (SPSS v26) included reliability testing, correlation analysis, regression, and ANOVA. Only perceived origin and perceived quality were retained as predictors to ensure model parsimony, while other variables were treated as antecedents. The analytical procedure followed four steps; reliability and validity assessment; Cronbach's alpha, composite reliability (CR), and average variance extracted (AVE) were calculated. Pearson correlation coefficients were used for exploratory analysis. Multicollinearity was assessed using variance inflation factors (VIF) and tolerance values. Multiple regression analysis Purchase intention was regressed on perceived origin and perceived quality. Standard regression assumptions (normality, homoscedasticity, independence of errors) were tested. A one-way ANOVA tested differences in purchase intention across age groups. Tukey HSD post hoc tests and effect sizes (η^2) were reported. The labels varied along two key dimensions: Visual attractiveness (low vs. high); Salience of geographical origin cues (low vs. high). This resulted

in the following label conditions: Label 1: Low attractiveness / Low origin salience; Label 2: High attractiveness / Low origin salience; Label 3: Low attractiveness / High origin salience; Label 4: High attractiveness / High origin salience. Each respondent evaluated one randomly assigned label, ensuring independence of observations.
The label variations were created by systematically modifying four core visual components commonly used in olive oil packaging: 1. Colour Scheme- Low attractiveness: muted, low-contrast colours (e.g., grey, pale green), High attractiveness: saturated, high-contrast colours (e.g., dark green, gold accents); 2. Typography- Low attractiveness: standard sans-serif fonts with minimal stylistic differentiation, High attractiveness: stylized serif fonts evoking tradition and craftsmanship; 3. Imagery- Low origin salience: abstract or generic graphic elements, High origin salience: images of olive groves, Dalmatian landscapes, and traditional harvesting scenes; 4. Origin Cues and Symbols- Low origin salience: minimal or absent reference to geographical origin, High origin salience: explicit references such as: "Dalmatia" or "Dalmatian olive oil" geographical indication (GI) symbols traditional motifs associated with the region.

Results and Discussion

Perceived quality was measured using three items evaluating the respondent's expectation of product quality based on label information (Zeithaml, 1988). Purchase intention is measured using three items assessing the likelihood of purchasing the product if available in a retail setting (Dodds et al., 1991).

Table 1. Demographic characteristics of the sample

Characteristic	Distribution (%)
Female	52
Male	48
Age 18–34	32
Age 35–54	41
Age 55+	27

The sample included respondents with diverse educational and income backgrounds, reflecting the structure of typical urban food-consumer segments. Data analysis proceeded in three sequential steps. Cronbach's alpha coefficients were calculated to assess the internal reliability of the measurement scales. All constructs demonstrated acceptable reliability levels exceeding the commonly recommended threshold of 0.70. Pearson correlation analysis was used to examine the relationships between the main constructs.
While the correlation matrix includes visual attractiveness and perceived authenticity alongside perceived origin and perceived quality, these variables were not all included in the regression model. Visual attractiveness and perceived authenticity showed moderate to strong correlations with perceived origin ($r = 0.42$ and $r = 0.61$, respectively) and perceived quality ($r = 0.55$ and $r = 0.52$, respectively), indicating that their effects on purchase intention may be mediated through these core perceptual constructs. This pattern supports the conceptual distinction between design-related cues (visual attractiveness) and interpretative perceptions (authenticity, origin, quality).

Table 2. Correlations between variables

Variable	Visual attractiveness	Geographical origin	Perceived quality	Perceived authenticity	Purchase intention
Visual attractiveness	1				
Geographical origin	0.42	1			
Perceived quality	0.55	0.4	1		
Perceived authenticity	0.38	0.61	0.52	1	
Purchase intention	0.46	0.49	0.63	0.67	1

Visual attractiveness primarily functions as an initial stimulus influencing higher-order perceptions, while perceived authenticity overlaps conceptually with perceived origin. A multiple regression analysis was conducted to examine the effects of perceived origin and perceived quality on purchase intention. The overall model was statistically significant, $F(2, 271) = 185.40$, $p < 0.001$, explaining 58% of the variance in purchase intention ($R^2 = 0.58$; adjusted $R^2 = 0.57$). Both predictors were statistically significant. Perceived origin had a stronger effect ($\beta = 0.48$, SE = 0.05, $t = 9.60$, $p < 0.001$, 95% CI [0.38, 0.58]), followed by perceived quality ($\beta = 0.36$, SE = 0.06, $t = 6.00$, $p < 0.001$, 95% CI [0.24, 0.48]). Multicollinearity diagnostics indicated no concerns, with variance inflation factor (VIF) values of 1.42 for both predictors. The Durbin–Watson statistic (1.89) suggested no autocorrelation in residuals. These results confirm that both perceived origin and perceived quality are robust and independent predictors of purchase intention. A one-way ANOVA was conducted to examine differences in purchase intention across age groups. The results revealed a statistically significant effect of age, $F(2, 271) = 6.87$, $p = 0.001$, with a small-to-moderate effect size ($\eta^2 = 0.048$). Post hoc comparisons using Tukey HSD indicated that respondents aged 55 and above reported significantly higher purchase intention than those aged 18–34 ($p = 0.002$) and moderately higher than those aged 35–54 ($p = 0.048$). No statistically significant difference was found between the two younger groups. Manipulation checks confirmed that labels designed with high visual attractiveness and strong origin cues were rated significantly higher on perceived attractiveness and perceived origin ($p < 0.001$), supporting the validity of the experimental design.

Table 3. Multiple regression results predicting purchase intention

Predictor variable	β (Stand.)	SE	t-value	p-value	95% CI (Lower, Upper)	VIF
Geographical origin	0.48	0.05	9.60	<0.001	(0.38, 0.58)	1.42
Perceived quality	0.36	0.06	6.00	<0.001	(0.24, 0.48)	1.42

The exclusion of visual attractiveness and perceived authenticity from the regression model reflects their role as upstream explanatory variables, whose influence is largely captured through perceived origin and perceived quality. This approach ensures a theoretically consistent and parsimonious model while avoiding redundancy among closely related constructs.

Table 4. Model statistics

Statistics	Value
R^2	0.58
Adjusted R^2	0.57
F-statistic	185.40
Degrees of freedom	(2, 271)
p-value	<0.001
Durbin–Watson	1.89

Consumers interpret origin-related visual and textual cues on packaging as signals of product credibility and quality. These findings are consistent with prior research emphasizing the importance of territorial identity and origin in the marketing of premium food products. In the case of olive oil, visual and textual cues displayed on product labels can reinforce the perceived connection between the product and its place of origin, thereby strengthening consumer trust.

Table 5. Reliability and convergent validity of constructs

Construct	Cronbach Alpha	Composite reliability	AVE
Visual attractiveness	0.84	0.87	0.63
Geographical origin	0.81	0.85	0.60
Perceived quality	0.86	0.89	0.67
Perceived authenticity	0.88	0.91	0.71
Purchase intention	0.90	0.92	0.75

The study also highlights the role of packaging design as a mediator between origin signaling and consumer perception. The product label therefore functions not only as packaging but also as a communication tool through which producers signal origin, tradition, and product quality.

Conclusion

Label design significantly influences consumer perceptions, with perceived origin and quality as key drivers of purchase intention. Origin-related cues enhance authenticity and trust, supporting origin-based branding strategies. Limitations include use of hypothetical stimuli, cross-sectional design, and lack of mediation testing. Future research should apply structural equation modelling and broader samples.

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REVIEW ARTICLE

Apple Waste Recovery Potential: A Review of Available Databases

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Summary
This research evaluates the availability and completeness of apple waste data across databases and scientific literature, focusing on waste type, origin, and disposal methods. Several databases, including projects and 27 scientific papers, were reviewed, making 38 sources in total. The results indicate that apple cultivation and processing generate large amounts of waste, including both pruning residues and pomace, with pomace being the most frequently reported. Although the data are fragmented and incomplete, they show strong potential for apple waste valorization through bioproducts, bioenergy, and other circular applications.

Keywords: apple residues, apple pomace, circular bioeconomy, valorization, databases

Introduction

Apple production is one of the most widespread fruit production systems globally, generating substantial quantities of biomass along the value chain (Selmi et al., 2025). Alongside primary products, significant amounts of waste are produced at different stages, including pruning residues from orchards and pomace from juice and cider processing (Zaky et al., 2024; Sharma et al., 2025). These by-products represent both an environmental challenge and an opportunity for resource recovery within circular economy frameworks (Piwowarek et al., 2022; Tasić et al., 2022; Tulej and Głowacki 2022).

In recent years, increasing attention has been given to the valorization of agri-food waste streams, particularly in the context of climate change mitigation, bioeconomy development, and sustainable resource management (Piwowarek et al., 2022; Ampese et al., 2025). Apple waste is rich in organic matter, fiber, and bioactive compounds, making it suitable for a wide range of applications, including bioenergy production, extraction of high-value compounds, animal feed, and soil amendments (Lyu et al., 2020; Gołębiewska et al., 2022; Fraterrigo Garofalo et al., 2025). Despite growing interest, data on apple waste remain fragmented, non-uniform, and incomplete across scientific publications and various databases. These sources differ in scope, methodology, and level of detail, often lacking harmonized reporting on key parameters such as waste origin and type and disposal pathways. As a result, comparing results and assessing the true recovery potential of apple waste is challenging.

In this review, the current generation and utilization of apple waste were studied over the last 10 years of publications, aiming to evaluate the availability and completeness of apple waste data across selected databases and scientific literature, with a focus on waste type, origin, and disposal methods. By identifying existing gaps and inconsistencies, the study seeks to provide a clearer basis for assessing apple waste recovery potential and to support the development of more standardized and comprehensive reporting approaches.

Material and Methods

A systematic review approach was applied to assess the availability and quality of data on apple waste. In total, 38 data sources were analyzed, collected from several databases: EIP-AGRI, Eurostat, FAOSTAT, Zenodo, and scientific literature indexed in major bibliographic databases (Web of Science) (Table 1). The search was conducted using combinations of keywords, with inclusion criteria covering studies and reports that provided information on at least one of the following parameters: (i) origin of raw material (EU, non-EU or global), (ii) waste origin (primary production, processing, or other), (iii) waste type (pomace, pruning residues, or other), and (iv) disposal or utilization method. Data on apple varieties and the amount of waste were uneven and vaguely defined, so they were not considered. Only explicitly stated values were recorded, without estimation. After data extraction, information was organized into a structured dataset according to the defined parameters.

Table 1. Reviewed databases

Databases	EIP-AGRI projects	EUROSTAT	FAOSTAT	ZENODO	WoS	Other
Source quantity	4	2	1	2	27	2
Total	38 data sources					

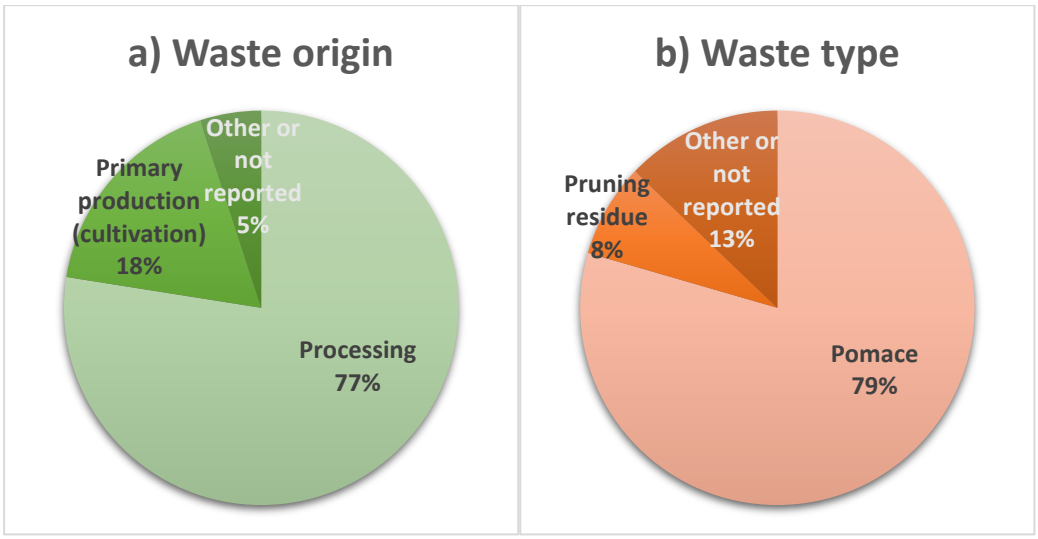
Results and Discussion

The analysis shows that apple waste data are unevenly distributed across regions, with 39% referring to the EU, 33% to non-EU countries, and 28% presenting global data (Table 2). Regarding waste origin, most studies distinguish between primary production and processing stages, with processing-related waste more frequently reported (Graph 1 (a)). In terms of waste type, as shown in Graph 1 (b), apple pomace is the dominant category across the reviewed sources, while pruning residues and other waste types are considerably less represented. This imbalance reflects both the industrial relevance of processing waste and the lack of systematic reporting for field-based residues. Across the reviewed sources, it is frequently estimated that approximately 25–35% of processed apples end up as pomace, confirming its significance as the main waste stream (Petrović et al., 2021; Putra et al., 2023).

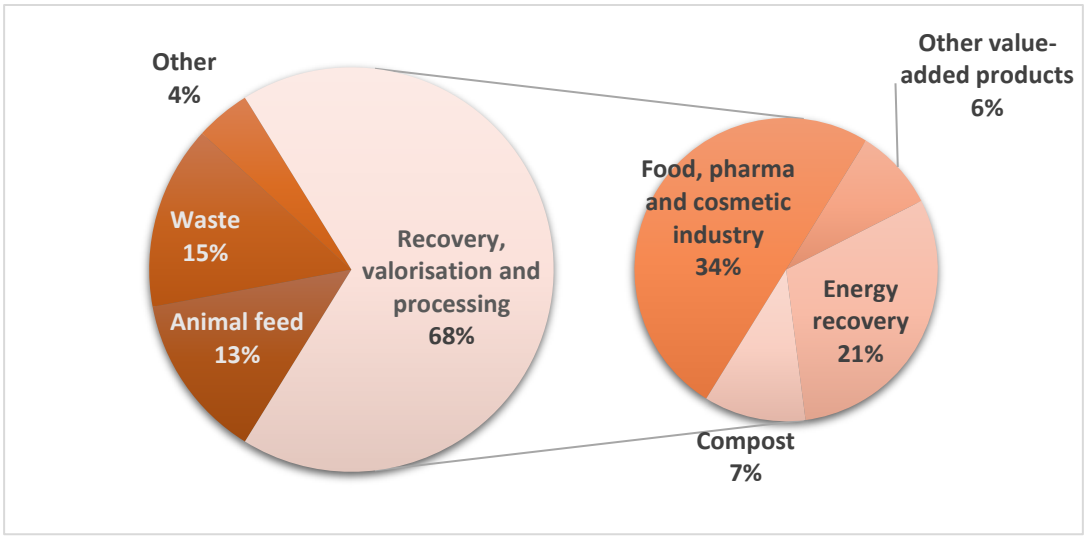
As shown in Graph 2, disposal methods vary widely among the analyzed sources, with a clear emphasis on recovery and valorization pathways. The most commonly reported approaches include utilization in the food, pharmaceutical, and cosmetic industries, including creation of value-added products and the extraction of high-value compounds. Energy recovery methods, particularly anaerobic digestion for biogas production, are also frequently mentioned. In addition, composting, direct soil application, landfilling, and incineration appear less frequently. However, disposal methods are often described in general or combined terms, and in some cases are not specified at all, which limits comparability between studies. Overall, the results highlight both the strong potential for apple waste utilization and the need for more consistent and detailed reporting.

Table 2. Origin of raw material

Location	EU	Non-EU	Global (EU + non-EU)
Number of locations	14	13	11



Graph 1. Categorization of apple waste according to (a) Origin and (b) Type



Graph 2. Disposal methods of apple waste

Conclusion

The results show that apple waste is utilized through various disposal methods, with increasing emphasis on recovery and valorization in different industries, as well as the production of value-added products and energy. Apple pomace is the most frequently reported waste type. The lack of standardized reporting limits data comparability; therefore, improved data harmonization is essential to support more efficient utilization of apple waste.

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ORIGINAL SCIENTIFIC PAPER

Development of an AI-based system for detection of olive fruits and yield estimation

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Summary
This paper describes the development of an intelligent system that uses unmanned aerial vehicles for collecting images in olive groves. These images are further being analysed by deep neural networks in order to detect olive fruits and their characteristics. Next step is estimation of olive fruit maturity and the expected yield on an individual tree as well as in the entire olive grove. During year 2025, more than 800 images were collected from July to November with focus on three chosen olive cultivars ('Oblica', 'Istarska bjelica' and 'Leccino'). After first phase of olive images annotation and training of an initial model for olive detection, obtained results show encouraging precision and recall values.

Keywords: deep neural networks, unmanned aerial vehicles, olive fruits detection, yield estimation

Introduction

The application of artificial intelligence and machine learning in agriculture is increasingly intensive due to its ability to understand, learn and react to different situations (based on learning) in order to increase the efficiency and quality of production. As it is a very broad field of possible research and applications, within the framework of research that will be presented here, the focus will be on the introduction of new technologies and artificial intelligence in olive growing.

Olive (*Olea europaea* L.) is one of the most widespread plants and plantations in the world and olive oil is a basic ingredient in Mediterranean diet, popular all over the world. The consumption of olives (oil olives and table olives) in the world is constantly increasing and is estimated around 6.2 million tons in 2026 (IOC, 2026). For olive growers, estimating the maturity of the olive fruit determines the optimal timing for harvesting. Also, the crop assessment is necessary for planning and coordinating harvest operations, enabling efficient management of resources and logistics. Accurate yield estimation is essential for decision-making by farmers, policymakers and farms, enabling them to make informed decisions regarding production and resource allocation.

The use of drones for data collection in olive growing enables high spatial resolution, the time of recording images is determined by the user, and data can be collected even in cloudy weather. Various camera types are available (RGB, multispectral, hyperspectral, thermal), so these data are of significantly higher quality than those collected by satellite (Matese et al., 2015, Marques et al., 2024). Collected images can be used after applying different computer vision algorithms for different types of applications, such as assessing fruit maturity (Benalia et al., 2017), assessing crops (Nevavuori et al., 2019), plant diseases (Liu et al., 2021), etc.

As in many other areas, deep learning has played an increasingly important role in the field of image processing in agriculture in recent years (Kamilaris and Prenafeta-Boldú, 2018). The RGB camera is emerging as the first choice for fruit detection in the field due to its low cost and simple implementation, and the main features for fruit detection are color, texture and shape (Gongal et al., 2015). Changes in lighting, camera position, distance, and fruit occlusion significantly affect the performance of classical methods compared to methods that use deep learning. Compared to classical methods, the approach using deep learning requires larger computing resources and larger databases of labeled images for learning. In a review of research focused on fruit detection and crop estimation (Koirala et al., 2019), it can be noted that approaches using transfer learning dominate. A modified Inception-ResNet architecture was used to estimate tomato fruit number (Rahnemoonfar and Sheppard, 2017). The model was trained and verified on synthetically generated images, the test results on natural images of objects showed an average accuracy of 91%.

There are several possible approaches for yield estimation (Koriala et al., 2019 and Meghraoui et al., 2024). One is to count all the fruits by recording the plant from multiple angles. This approach is time-consuming with the additional problem of multiple detection of the same fruit. The second approach is based on the use of one shot and the introduction of a correction factor for fruits that are obscured. It is easiest to use only one correction factor for each orchard (depending on the canopy structure), but it should be determined again every season. Improvement of this approach is possible by correcting factors for each tree individually. Fruit and canopy area, number of visible fruit in the images were used as input parameters in the artificial neural network, and the assessment results were compared with the real results of apple tree yields (Rozman et al., 2012). In the paper (Cheng et al., 2017), features for crop estimation were collected using classical color segmentation and passed to a neural network for crop prediction. Research related to the detection of olives, assessment of ripeness and yield are also numerous, but they are dominantly based on the analysis of images of collected olive fruits and a controlled recording environment (Ponce et al., 2019). The first research and paper representing the approach of automatic detection of olives on the tree uses mathematical morphology to detect fruit illumination reflection patterns in image preprocessing and a convolutional neural network for fruit identification (Aquino et al., 2020).

In the folloving sections, we present proposed approach and current status of our research that is focused on development of a complete solution for estimation of fruit maturity and yield cased on unmanned aerial vehicle (UAV)-collected images and application of deep neural networks (DNN).

Material and Methods

The collection of images from the field to form a image database that will be used in developing image processing algorithms and proposing an optimal architecture started in July, 2025. Before the actual collection of images using an unmanned aerial vehicle (for fruit detection, assessment of ripeness and yield), characteristics of the image database were defined (resolution, distance of the camera from the object, cultivars of olives that will be monitored, time of year when the fruits will be photographed, time of day and weather conditions). Initial recordings were made for several different distances of the UAV from the canopy in order to verify the success of existing algorithms and models in fruit detection. In this way, the maximum recommended distance of the available UAV from the canopy is determined for the future surveys.

Annotating collected images is time-consuming process since expected average number of objects (olives) in an image is larger than 500. It should be noted that, due to the need to collect images of all important periods of the olive's annual cycle, it takes at least one year to form the initial database.

Evaluation of the maturity index and classification of the detected fruits and the proposal of an algorithm for estimating the total expected yield of an olive tree implies the availability of a sufficient number of test images taken at different times of the plant's annual cycle. To evaluate the maturity index, it is necessary to form special subsets of the detected fruits depending on the olive cultivars. In addition, for the evaluation of the results, a comparison with the so-called ground-truth data is needed. Such data is collected by measuring the actual yield in the olive grove (and for each tree separately) and chemical analysis of fruits/samples taken from the tree at different time intervals.
Estimate of yield will be possible for each tree and the entire olive grove and will be based on the correlation between the visible surface of the crown in the picture, the number (and size) of the detected olive fruits and the actual data obtained in two annual cycles for individual trees or the entire olive grove.

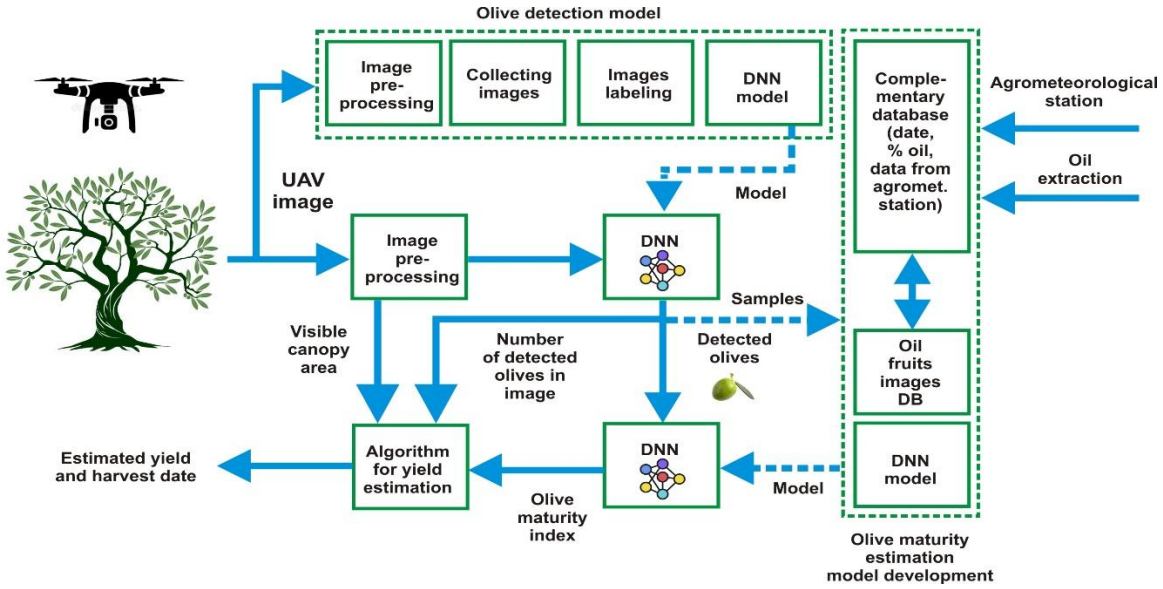

Figure1. Initial system concept.

For the experimental olive grove, olive grove of the family farm Bobanović in Polača near Benkovac is chosen. The location of the plantation is 44°00'27.3"N and 15°29'22.7"E and the altitude of the locality is 65 m. The olive grove was planted in 2007 on an area of 12 ha. Planting distance is 6 meters between rows and 5 meters tree to tree. The olive grove contains several different cultivars, of which the cultivars 'Oblica', 'Leccino' and 'Istarska bjelica' were selected for detailed analysis and tests. All agrotechnical and elaiotechnical measures are implemented in the plantation. Fruit sampling for chemical analysis are carried out on three trees of each cultivar in three repetitions (3 cultivars * 3 trees = 9 samples per one inspection, 10 inspections per year). It should be noted that this procedure will be carried out during two annual cycles in order to achieve the greatest possible reliability and reduce the impact of extraordinary weather conditions in a particular year. Chemical analysis is carried out in an attested laboratory. As for programming tools, open source software (PyTorch, Python) is chosen, MATLAB software package is used for other tests and analysis and CVAT for images annotation.
As data collection is limited to time periods within the annual olive cycle, monitoring and recording is planned for at least two years. In this way, the dependence on weather conditions will be reduced and the reliability of the collected data will be increased. Comparison of the obtained results with the data obtained by chemical analysis of fruits from samples collected during all stages of fruit growth will enable the improvement of algorithms for evaluating fruit maturity in relation to existing research in literature. Proposed architecture is based on DNN,

considering that deep neural network approaches have shown superiority over classical methods in recent years. Complete intelligent system is presented in Figure 1.

Results and Discussion

Although only the first year of the research has passed, some results have already been obtained. 817 images of olive trees were collected and, at the moment of writing this, 265 images with 105224 olive fruits are annotated (Table 1). Several state-of-the-art DNN architectures and their performance on two previously available databases (Musić et al., 2025) were compared. Obtained results (mAP50 ranging from 68% to 95% depending on the used architecture) demonstrated the applicability of the DNN-based approaches. The most important outcome of the comparison is the conclusion that database size and quality (diversity) on DNN's final performance is crucial while the choice of a neural network architecture is less critical for the success of detection. Therefore, we used pre-trained YOLOv11 architecture for further training of the initial detection model. Original images were tiled into 640×640 pixel images (70% for training, 20% for validation and 10% for test). Current results are: mAP_{50}: 0.80351, $mAP_{50\text{-}95}$: 0.5337, Precision: 0.82131, Recall: 0.71493.

Table 1. Deep neural net (state on 15.03.2026.)

Olive cultivar	Number of images	Number of annotated olives
'Istarska bjelica'	73	66218
'Leccino'	29	14951
'Oblica'	123	13925
'Ascolana tenera'	28	3790
'Pendolino'	8	4124
'Frantoio'	1	594
Others	3	1621
Total	**265**	**105224**

Conclusion

After comparing available image databases, the obtained results indicate (and confirm) the importance of forming an image database for training that has good generalization, which implies variability of learning examples. Therefore, our goal will be continuing development of a new, large image database with images of three olive cultivars ('Oblica', 'Istarska bjelica' and 'Leccino') that will enable improvement of detection metrics values. Special care will be given to the labeling process and its influence on neural network performance and ultimately, olive yield estimation. Additional preprocessing of images such as exclusion of very small objects (mainly olives in the background and other trees) is expected to improve overall detection results. Exclusion of blurred images from the training dataset should additionally improve detection rate. Information obtained by multispectral camera as the additional (fourth) channel as the input to the neural network is expected to improve precision and recall of the detection as well. Although two season weather data is most likely not enough for a reliable assessment and inclusion of this information in predicting fruit maturity, an attempt will be made to use this information to improve the assessment of the moment of optimal ripening so that visual information about the appearance of the fruit is not the only information used. In any case, it is expected that a quality framework will be set up and an intelligent system for detecting and assessing fruit maturity will be established, which will be able to be developed even after the formal completion of the project since additional funding for continued research (after 2027) has already been secured.

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ORIGINAL SCIENTIFIC PAPER

Consumer perceptions of influencer marketing in the agri-food sector: Attitudes, trust, and purchasing behaviour

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Summary
This paper investigates the role of influencers in the digital marketing of agri-food products based on an online survey of 105 respondents conducted in January 2025. Non-parametric statistical tests (Spearman's rank-order correlation and Kruskal-Wallis test) were applied given that normality assumptions were not met. The results indicate that while respondents generally view social media as a useful information channel and show moderate trust in influencer recommendations, actual influencer-driven purchasing behaviour remains limited. Trust and prior purchasing behaviour were identified as the strongest differentiating factors in attitudes toward influencer marketing, highlighting a gap between positive consumer attitudes and actual purchasing action in the agri-food sector.

Keywords: influencers, digital marketing, agri-food products, consumer behaviour, purchase intention

Introduction

As the world has become increasingly digitalised, digital technologies and media have been adopted in marketing, expanding the traditional concept into what is now known as digital marketing. Digital marketing can be defined as the achievement of marketing objectives through the use of the internet and other digital and information-communication technologies in business operations, which increases the overall efficiency and effectiveness of marketing (Mahmutović, 2021). According to Radeljak et al. (2019), it represents the process of promoting products and services via digital media in order to reach a targeted audience, with the goal of building and maintaining mutual trust between a company and its customers, carried out across various digital channels including the internet, television, and mobile devices. One of the most prominent forms of digital marketing today is influencer marketing, a contemporary concept that emerged from the development of innovative digital marketing strategies. It relies on individuals who hold a degree of social influence to reach and affect the end consumer, primarily through social media, using a two-step communication flow that creates a unique relationship between a brand, an influencer, and the consumer, ultimately improving brand positioning and driving sales (Kostić Stanković et al., 2020). Influencers can be classified according to the type of content they produce, including fashion, travel, food, fitness, and lifestyle, among others. According to Lim et al. (2017), consumers are more inclined to purchase products recommended by someone they trust, whether known personally or through digital platforms, which is why followers often choose to buy products promoted by influencers they follow. The key elements underpinning the effectiveness of influencer marketing are therefore the building of trust and the maintenance of credibility in the eyes of the audience. Despite a growing body of research on influencer marketing, empirical studies focused on the agri-food sector remain limited, with most research concentrated on fashion, beauty, or general consumer goods. In light of this, this paper aims to investigate the role of influencers in the

digital marketing of agri-food products and to identify the factors shaping the relationship between influencer exposure and consumer purchasing behaviour.

Material and Methods

Primary data were collected using the survey method, with an online questionnaire used as the research instrument. The study on the role of influencers in the digital marketing of agri-food products was conducted in January 2025 using the Google Forms online survey software. The questionnaire as a whole explored the role of influencers in the purchasing of agri-food products. The complete questionnaire contained a total of 35 open- and closed-ended questions divided into several groups, with questions relating to the sociodemographic characteristics of the respondents added at the end of the survey. The 17 Likert-scale statements measuring attitudes towards influencer marketing were developed specifically for this study, based on existing literature on influencer marketing and consumer behaviour in the food sector.

To reduce the risk of sample bias, a post-hoc quality control procedure was applied, whereby responses submitted in under five minutes were removed on the grounds that they likely indicated inadequate engagement with the survey items (Vecchio et al. 2020). Responses exhibiting straight-lining behaviour, that is, identical answers given across all Likert-scale items were also reviewed, and those occurring alongside unusually short completion times were likewise discarded (Jelić Milković et al., 2026). The resulting analytical sample of 105 respondents satisfied all quality criteria, thereby ensuring the reliability of the data.

All participants were made aware that their involvement was entirely voluntary, and were guaranteed that their responses would remain confidential and anonymous. They were clearly informed of their right to withdraw from the study at any point without any personal data being retained. The research was conducted in accordance with the ethical standards for online survey research set out by the European Society for Opinion and Market Research (ESOMAR, 2016).

The data collected through the survey were analysed using IBM SPSS Statistics software (V26). Descriptive statistics were first computed for all variables, including frequencies, percentages, means, medians, and standard deviations, in order to provide an overview of the sample characteristics and the distribution of responses across all items. Prior to selecting the appropriate inferential tests, the normality of the distribution was assessed for all 17 Likert-scale items. using the Shapiro-Wilk test, which is considered more suitable for samples of this size (n=105) than the Kolmogorov-Smirnov test. Since the results of the Shapiro-Wilk test indicated a statistically significant departure from normality for all items ($p < 0.001$), non-parametric statistical tests were employed for all subsequent analyses. Internal consistency of the 17 Likert-scale items was assessed using Cronbach's alpha coefficient ($\alpha = 0.954$), indicating excellent scale reliability. Specifically, Spearman's rank-order correlation coefficient was used to examine relationships between ordinal variables, while the Kruskal-Wallis test was applied where group comparisons were required. This approach ensured that the assumptions underlying each statistical procedure were met and that the findings of the study are both valid and reliable.

Results and Discussion

The sample comprised 79.0% female respondents, 20.0% male respondents, and 1.0% who did not wish to answer the question. Of the respondents, 47.6% were aged 55 or older, 41.7% were aged between 25 and 44, and 10.5% were between 18 and 24 years old. The majority (80.0%) were from urban areas, and 79.0% were employed. Regarding education, 57.1% had higher education (university or professional degree, master's, or doctorate), while 42.9% had secondary or lower education (primary or lower secondary school). Most respondents lived in households with three to four members (61.0%). The income distribution is relatively balanced

between €1,501 and €3,500, with a noticeable dip only in the lowest income group. The sample leans slightly towards middle-income households.

In the survey, respondents were also asked how often they use social networks. Of these, 93.3% stated every day, 3.8% a few times a week, and 2.9% a few times a month. The results show that the majority of respondents use Instagram (74.3%), followed by Facebook with 70 responses (66.7%). YouTube is used as a social network by 61 respondents (58.1%), while TikTok is used by 40 respondents (38.1%). Fewer than 12% of respondents use LinkedIn (11.4%), followed by X (6.7%), other platforms without specifying which ones (5.7%), while Pinterest and digital newspapers were each mentioned by one respondent (1.0%). A total of 66.7% stated that they follow influencers on social networks, while 33.3% do not. Respondents most commonly follow influencers dealing with food and culinary topics (54.5%), followed by fitness and health (51.1%), travel (50%), fashion (48.9%), beauty (44.3%), lifestyle (31.8%), and technology (8.0%). Fewer than 5.0% of respondents follow influencers covering home decor, current news, and similar topics (1.0%).

Out of 105 respondents, just over half (56.2%) reported never having purchased an agri-food product based on an influencer's recommendation, while 36.2% said they do so occasionally, and a small minority purchase such products often or very often (2.9% and 4.8%, respectively), suggesting that influencer-driven purchasing behaviour remains limited in this sample. Despite this, attitudes toward social media as a source of food and agri-food information were notably more positive: 22.9% rated it very useful and 44.8% rated it useful, meaning nearly 68% of respondents view social media as a source of information as at least useful, with only 7.6% considering it of little value. Trust in influencer recommendations was more cautious 17.1% reported trusting them, 59.0% partially trusted them, and approximately 21.0% expressed little trust, while only 2.9% expressed no trust whatsoever. Taken together, these results are interesting, showing that respondents are generally open to social media as an information channel and display moderate levels of trust in influencers. However, this does not readily translate into actual purchasing behaviour, indicating a gap between perceived usefulness and consumer action.

Spearman's rank-order correlation analysis revealed that all 17 Likert-scale items were significantly and positively correlated with one another ($p < 0.01$), indicating a coherent and internally consistent pattern of responses. Correlation coefficients ranged from weak-to-moderate ($r = 0.198$, between perceiving social media as an important food information source and viewing influencers as sincere) to very strong ($r = 0.859$, between willingness to try and willingness to purchase agri-food products after influencer endorsement). Notably strong correlations were also found between frequency of recommendations and confidence in product quality ($r = 0.771$), and between willingness to pay more and perceived credibility ($r = 0.773$), highlighting trust and frequency of exposure as central connecting factors. Overall, the pattern of correlations suggests that respondents who are more receptive to influencer marketing in one domain whether in terms of trust, perceived authenticity, or behavioural intention tend to be more receptive across all other measured dimensions as well. These findings are consistent with Qin et al. (2024), who demonstrated that influencer marketing platform intervention positively affects both purchase intention and purchasing behaviour in the food sector, and that consumers who trust influencer recommendations are more likely to convert that trust into actual purchasing behaviour.

Table 1. Kruskal-Wallis test p-values for attitudes toward influencer marketing by social media use, purchasing behaviour, perceived usefulness, and trust in influencer recommendations

	Social media use	Purchase behaviour	Perceived usefulness	Trust
Influencer marketing plays a key role in my food purchasing decisions.	0.175	0.001**	0.040*	0.013*
Social media is an important source of information about food.	0.303	0.101	0.052	0.150
Influencers help me discover new agri-food products.	0.018*	0.003**	0.000**	0.003**
I am more likely to purchase agri-food products promoted by influencers I follow.	0.110	0.003**	0.013*	0.008**
Positive reviews from influencers significantly influence my decision to purchase agri-food products.	0.097	0.000**	0.008**	0.006**
I am more inclined to try agri-food products when I see an influencer using them.	0.224	0.001**	0.044*	0.003**
I am more inclined to purchase agri-food products after seeing them endorsed by influencers on social media.	0.274	0.000**	0.006**	0.001**
Influencer recommendations make me more confident in the quality of agri-food products.	0.136	0.002**	0.043*	0.001**
The authenticity of an influencer affects my intention to purchase the agri-food products they promote.	0.171	0.002**	0.023*	0.005**
The frequency of influencer recommendations affects my decision to purchase agri-food products.	0.192	0.001**	0.067	0.008**
I feel that influencer recommendations are more credible than other types of recommendations.	0.530	0.001**	0.120	0.001**
I believe that influencers on social media give honest opinions about agri-food products.	0.057	0.003**	0.000**	0.000**
I believe that influencers on social media genuinely use the products they promote.	0.057	0.004**	0.005**	0.001**
I perceive influencers on social media as sincere when they share both positive and negative aspects of a product.	0.033*	0.016*	0.002**	0.002**
I consider influencer content on social media about agri-food products to be informative and useful.	0.689	0.003**	0.000**	0.002**
I trust influencers who disclose their sponsorships and brand affiliations.	0.548	0.003**	0.001**	0.020*
I am willing to pay more for agri-food products recommended by influencers, even if they are more expensive.	0.589	0.000**	0.276	0.007**

Four Kruskal-Wallis tests were conducted to examine whether respondents' attitudes toward influencer marketing differed significantly according to four grouping variables: frequency of social media use, prior purchasing behaviour based on influencer recommendations, perceived usefulness of social media for food information, and trust in influencer recommendations (Table

1). Frequency of social media use produced the weakest differentiation only two of the seventeen items reached statistical significance: the belief that influencers help discover new agri-food products (H=10.113, p=0.018) and the perception of influencers as sincere when sharing both positive and negative aspects of products (H=8.706, p=0.033). This is unsurprising given that 93.3% of respondents use social media every day, leaving very little group variation to detect. Prior purchasing behaviour showed statistically significant differences across groups for sixteen out of seventeen statements, the sole exception being the perception of social media as an important source of food information (H=6.219, p=0.101). The rank means consistently show the same pattern: respondents who purchase agri-food products based on influencer recommendations very often or often have considerably higher mean ranks across all attitude items than those who never purchase, indicating that actual purchasing behaviour is closely tied to more positive and trusting attitudes toward influencer marketing. Perceived usefulness of social media for food information also yielded significant differences for the majority of items (13 out of 17), with respondents who rated social media as very useful consistently showing higher mean ranks on items related to purchase intention, trust, and credibility than those who found it of little use. Non-significant items included the frequency of influencer recommendations affecting purchase decisions, perceived credibility compared to other recommendations, and willingness to pay more. Trust in influencer recommendations also produced significant differences on sixteen of the seventeen items, with those who fully trust influencer recommendations showing markedly higher mean ranks particularly on items related to sincerity, authenticity, and purchase intention. Notably, the perception of social media as an important information source (p=0.150) did not differ significantly across trust groups.

These findings are consistent with previous research indicating that credibility and content quality play a central role in shaping consumer purchasing intentions in the food sector (Kilumile et al., 2026). Similarly, Ma and Aung (2022), who demonstrated that trust and professionality are central determinants of purchase intentions for agri-food products, further supporting the conclusion that credibility-related attitudes are the primary factors through which influencer marketing affects consumer behaviour.

Conclusion

The results reveal that despite widespread daily social media use and positive attitudes toward social media as an information channel, the majority of respondents have never purchased an agri-food product based on an influencer's recommendation, pointing to a clear gap between perceived usefulness and actual purchasing behaviour. Trust and credibility emerged as the main factors linking attitudes towards influencer marketing with purchase intentions. Respondents who trust influencer recommendations consistently showed more positive and receptive attitudes across all measured dimensions, which aligns with existing research in the food sector. The findings underline the need for agri-food producers and marketing professionals to prioritise partnerships with credible, food-specialist influencers who disclose brand affiliations transparently. To bridge the attitude–behaviour gap, influencer campaigns should go beyond raising awareness and include direct purchase motivators such as discount codes or product sampling, which reduce the gap between positive consumer attitudes and actual purchasing decisions.

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ORIGINAL SCIENTIFIC PAPER

Women who carry rural communities: Experiences of LAG and FLAG leaders

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Summary
The paper examines the professional challenges and enabling factors faced by women leaders of Local Action Groups (LAGs) and Fisheries Local Action Groups (FLAGs) in Croatia. A focus group with eight leaders reveals persistent gender stereotypes, administrative and structural constraints, and difficulties balancing work and family life. Despite these challenges, women show strong motivation, expertise, and resilience, making significant contributions to rural development. The findings highlight the need to strengthen institutional support and gender-sensitive rural policies.

Keywords: women leaders, rural development, LAG, FLAG, gender roles

Introduction

The role of women in rural areas is increasingly recognised as essential for the sustainable development of local communities. In the European, and therefore Croatian, context, Local Action Groups (LAGs) and Fisheries Local Action Groups (FLAGs) are recognised as instruments of rural development based on the bottom-up approach, in which local actors actively participate in planning and implementing development projects.

Although this approach creates opportunities for women, those in leadership positions in LAGs and FLAGs continue to face gender stereotypes, limited access to resources, and challenges related to balancing private and professional expectations. Their presence in leadership often results from personal persistence, community support, and external development initiatives, yet opportunities remain constrained by local social norms and limited institutional support (Bock et al., 2023; Shortall and Marangudakis, 2022).

Gender inequalities in rural communities are reflected in various aspects, from access to resources and finances to underrepresentation in strategic decision-making and the political sphere. Shortall and Marangudakis (2022) emphasise that agriculture in Europe is still perceived as a "male domain." Even when women assume professional roles, they remain restricted to "softer" sectors such as education, culture, and tourism. These patterns are evident in Croatia as well, where women's leadership in LAGs is often undervalued or questioned due to social stereotypes (Grass Ceiling Project – EC Horizon Europe, 2025; Idžanović, 2024).

Research further indicates complex barriers affecting the personal and professional affirmation of women. Although they constitute a significant portion of the EU's rural population, women are economically and politically underrepresented, facing limited access to education, ownership, the labour market, and decision-making structures (Bock et al., 2023; Franić and Kovačićek, 2019). While European gender equality policies recognise the problem and propose measures such as care services, lifelong learning, micro-funds, and thematic subprogrammes, implementation remains uneven, and effects are insufficiently monitored through gender-disaggregated data (European Parliament, 2017).

Existing research in Croatia shows that women, despite numerous challenges, are important drivers of development, particularly in communities with systematic support, strong local networks, and access to financial and educational resources (Idžanović, 2024). However, many questions remain: What are the key obstacles in their work? Which factors support them? How do the leaders themselves perceive their position and role in the local community?
The aim of this paper is to identify the obstacles faced by women in leadership positions in Croatian LAGs and FLAGs, and to determine the enabling factors that support their professional lives and career development.

Material and Methods
To achieve the research objectives, a focus group method (Skoko and Benković, 2009) was used as a qualitative research approach involving group discussion on a specific topic. This method encourages in-depth discussion and reveals the reasons why participants hold positive or negative views on an issue. The focus group was conducted in June 2025 via Microsoft Teams and included eight female leaders from LAGs and FLAGs. Participants were selected based on their leadership positions, and anonymity was ensured. Data were processed through thematic coding, categorising participants' statements according to the research questions. After initial open coding, key themes were identified as the basis for interpreting the results.
The focus group questions were divided into four thematic groups:

1. balancing private and professional life,
2. gender stereotypes,
3. structural and social changes in rural areas,
4. women as drivers of future change.

Results and Discussion
A total of eight women participated. According to 2025 data, Croatia has 56 registered LAGs and 14 FLAGs. Of the FLAGs, 12 (85%) were led by women; of the LAGs, 42 (75%) had women leaders (Table 1).

Table 1. Number of LAGs and FLAGs in Croatia and the share of women leaders (2025)

Type of action group	Total number	No. of women leaders	Share of women leaders (%)
LAG	56	42	75.0
FLAG	14	12	85.0

BALANCING PRIVATE AND PROFESSIONAL LIFE
All participants emphasised that professional duties often extend into evenings and weekends, particularly due to deadlines and the administrative workload associated with EU projects. Work frequently encroaches on the private sphere, where household and family responsibilities await, contributing to feelings of overload. Limited availability of childcare services, especially in smaller communities, further complicates work-life balance. Despite this, women report strong motivation, commitment to their work, and a sense of contributing to their communities.

"The municipality I come from did not have a kindergarten, so it was difficult for me to organise everything, especially because my husband is a professional fisherman and does not have regular working hours." (Participant 1)
"The lack of available places in nurseries and kindergartens, the lack of full-day childcare… that is one of the biggest problems." (Participant 2)
"A lack of time for both one and the other, constant balancing of private and professional life." (Participant 3)

"A lack of time to achieve all the planned goals. The distance from larger centres makes it even more difficult." (Participant 4)
"It is very difficult to balance both precisely because there are no kindergartens that provide full-day service." (Participant 6)
"Working from home is only possible if I just need to reply to emails, because I cannot concentrate on work while the children are at home with me." (Participant 7)

GENDER STEREOTYPES AND PERCEPTIONS OF FEMALE LEADERSHIP

Most participants encounter double standards: assertiveness is viewed positively in men but often judged negatively in women. Some leaders feel they must involve a man for their decisions to be taken seriously. These stereotypes diminish over time, but the initial stages often require additional self-proving.

"Sometimes I need to have a man beside me to confirm what I am saying." (Participant 2)
"If you are a man, you are immediately in a better position; you are perceived differently, you have more authority, and you are taken more seriously." (Participant 4)
"I feel that if I were male, I would be more successful." (Participant 3)
"You know, somehow in life men have always had it a bit easier, and they have always been taken more seriously." (Participant 6)

STRUCTURAL AND SOCIAL BARRIERS IN RURAL AREAS

Participants expressed frustration with administrative burdens, frequent regulatory changes, unclear EU rules, and limited professional development opportunities in smaller communities. The lack of services and slow information flow negatively affect the functioning of LAGs and FLAGs.

"Distrust in institutions arises from generally weak and slow responses to users' inquiries, extensive administration, uncertainty caused by relatively frequent changes in legal regulations, and uncertainty resulting from unclear and contradictory interpretations of EU calls and eligible investments." (Participant 2)
"A barrier is also the small community and the lack of opportunities for professional advancement, development, and job offers." (Participant 4)
"Knowledge and skills contribute to developing self-confidence, authority, and the strength to make and implement even difficult decisions." (Participant 5)
"In our area, the perception still somewhat prevails that a woman should stay at home and take care of the children. Women are still fighting their battles." (Participant 6)

WOMEN AS DRIVERS OF FUTURE CHANGE

Participants believe women will play an increasingly important role in rural development over the next 10-20 years, highlighting the significance of education, digital literacy, care services, and professional networks.

Some expressed concerns about depopulation and the loss of local identity, while others noted the growth of women's entrepreneurship.

"I definitely believe that women in rural areas will, in 10 to 20 years, have an even stronger role in the development of their communities. I see them as educated, digitally literate, and active members of the local economy." (Participant 3)
"I hope that women will secure their place under the sun even more. I believe we are moving in that direction, and through our calls we further encourage women in agriculture, but primarily to work." (Participant 6)

"In such a community, a woman is, alongside a man, an equal pillar of society, complementing and supporting one another." (Participant 2)

The analysis of the conducted focus group indicates that women in leadership positions are often the initiators and principal drivers of projects with significant social and cultural impact; however, their roles within decision-making structures are not always equally recognised. This pattern is supported by European research, such as the report from the Grass Ceiling project – EC Horizon Europe (2025), which highlights that women frequently take on roles in so-called "softer" sectors, namely tourism, culture, and education, while infrastructural and capital-intensive projects remain predominantly under male control.

This division is not merely a matter of personal preference or competence; rather, it is often the result of deeply embedded social norms and expectations. Bock et al. (2023) emphasise that gender roles defined at the community level shape opportunities for access to resources and projects, thereby perpetuating inequalities over the long term. The focus group revealed that, although women successfully manage the responsibilities associated with project leadership, their visibility and influence in strategic decision-making processes remain limited. Moreover, the double standards applied when evaluating the competencies and behaviour of (F)LAG leaders, described by participants through concrete examples from practice, align with Shortall and Marangudakis (2022), who argue that gender stereotypes in rural communities remain deeply entrenched regardless of formal equal-opportunity policies.

An additional valuable insight is provided by Idžanović (2024), who, in her analysis of LAG Moslavina, notes that although women initiate and implement projects important to the local community, they often must invest extra effort to gain an equivalent level of trust from local decision-makers. These expectations affect not only the daily perception of female leaders but also their access to funding opportunities, the types of projects entrusted to them, and their broader role in shaping local development strategies. For example, the more challenging inclusion of women in infrastructural projects, which carry significant political capital, may have consequences not only for their professional advancement but also for the formulation of development priorities within rural communities.

To enhance the professional status of women in rural areas, and drawing on the experiences reported in the focus group, it is necessary to promote a gender-balanced distribution of project types, introduce clear and concrete criteria, and establish a systematic allocation of leadership responsibilities among team members based on predefined rules. Administrative burdens should be reduced through the standardisation of forms, the establishment of a single point of contact for clarifying regulations, and the definition of clear deadlines for responses from competent authorities. Furthermore, the institutionalisation of mentorship, along with the development of national-level networks of female leaders to facilitate the exchange of experiences and the monitoring of the work of more experienced colleagues, could help increase the visibility of women.

It would also be advisable to integrate care services for dependent family members into development policies, as the inclusion of nursery programs, kindergartens, extended school programs, and organized transportation within local development strategies is a prerequisite for the equal participation of women in leadership roles. Ultimately, it is desirable to continuously enhance the visibility and recognition of women's achievements, which in addition to further motivating women in rural areas, may also influence the transformation of societal perceptions of female leadership within local communities.

Conclusion

To further implement the proposed recommendations, the findings suggest several concrete policy- and practice-oriented measures. First, administrative burden on LAG and FLAG

management should be reduced by introducing standardised application and reporting templates, establishing a single institutional contact point for clarification of regulations and procedures, and defining binding response deadlines for relevant authorities.
Second, a more gender-balanced allocation of project types should be actively promoted by managing authorities, particularly by encouraging and supporting the involvement of women leaders in infrastructural and capital-intensive projects that carry greater strategic and political significance. This requires transparent project assignment criteria and the systematic distribution of leadership responsibilities within LAG and FLAG teams.
Third, the professional position of women leaders could be strengthened through the institutionalisation of mentorship schemes and the development of national-level networks of female LAG and FLAG managers. Such networks should facilitate regular exchange of experience, peer learning, and the monitoring and support of less experienced leaders.
Finally, rural development policies should explicitly integrate care-related services and mobility solutions, including access to nurseries, kindergartens, extended school programmes, and organised transport, as these services are a prerequisite for the equal participation of women in leadership roles. The systematic recognition and public visibility of women's achievements in rural development should be further enhanced, as this may contribute both to the motivation of women leaders and to the long-term transformation of perceptions of female leadership in rural communities.

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ORIGINAL SCIENTIFIC PAPER

Latest outlook on beef meat production in the Republic of Croatia

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Summary
The Croatian beef sector has undergone significant structural transformation since EU accession in 2013. Despite a relatively stable total cattle population, the number of dairy cows declined sharply by 57.8%, signaling a gradual structural break in beef production. Domestic beef and veal production has fallen by 21.4% since 2013, while import dependency has grown substantially, with imports increasing from 10.2 to 38.4 thousand tonnes over the same period. Self-sufficiency in beef and veal meat declined from 76% at EU accession to 59% in 2024. A baseline simulation using the AGMEMOD partial equilibrium model simulates a continued contraction of the sector through 2030, with production expected to fall by a further 11.6%, imports to rise by 6.0%, and self-sufficiency to deteriorate to 51%. These findings point to persistent structural vulnerabilities and underscore the need for targeted policy interventions to support long-term sector resilience.

Keywords: Croatian beef sector, AGMEMOD, baseline outlook

Introduction

The accession of Croatia to the European Union on 1 July 2013 fundamentally reshaped its agricultural landscape. Integration into the EU Single Market exposed domestic producers to intensified competitive pressures, while the adoption of Common Agricultural Policy (CAP) instruments necessitated substantial structural adjustments across the entire agri-food sector (Erjavec et al., 2006; Kranjac et al., 2020). These effects proved particularly pronounced in the livestock sector, where family-scale farms with limited economies of scale struggled to maintain competitiveness (Csaki and Jambor, 2013; Swinnen and Vranken, 2009).

Within Croatian livestock production, the beef and dairy sectors hold central importance, together accounting for a combined share of total agricultural output that fell from 19.8% in 2013 to 15.8% in 2024, while self-sufficiency in beef and veal meat declined from roughly 75% to 60% over the same period (MP, 2014, 2025). These figures reflect broader structural challenges that have persisted and intensified throughout the period of EU membership.

Changes in the composition of the Croatian cattle herd between 2013 and 2024 are presented in Table 1. Although total cattle numbers have remained relatively stable at around 420-450 thousand heads, significant structural shifts are evident within the herd. Most notably, the dairy cow population declined sharply by 57.8%, falling from 168.0 to 70.9 thousand heads.

As a result of the declining dairy herd, domestic beef and veal production has also trended downward, falling from 47.3 thousand tonnes in 2013 to 37.2 thousand tonnes in 2024, a decline of 21.4%. Simultaneously, imports have risen sharply from 10.2 to 38.4 thousand tonnes over the same period. Despite moderate growth in exports from 3.4 to 9.4 thousand tonnes, the beef sector has experienced a significant deterioration in self-sufficiency, which declined from 76% at EU accession to 59% in 2024. These trends reflect the structural imbalances between domestic supply capacity and growing domestic demand, increasingly bridged through import flows.

Table 1. Changes in the Croatian cattle herd (2013-2024)

Category (1000 Heads)	2013	2015	2017	2019	2021	2023	2024
Young cattle under 1 year	144.762	147.990	155.868	151.584	156.331	144.760	150.028
- for slaughter	19.267	19.964	20.263	19.179	19.737	18.596	19.366
- female	51.689	47.067	57.081	62.449	65.248	56.215	57.891
- male	73.806	80.959	78.524	69.956	71.346	69.949	72.771
Cattle between 1 and 2 years	92.178	88.167	99.923	106.689	106.663	115.311	114.150
- heifers (breeding)	37.771	38.101	40.319	49.824	47.535	54.216	43.560
- heifers for slaughter	7.450	7.296	7.115	8.688	8.734	9.429	9.339
- male	46.957	42.770	52.489	48.177	50.394	51.666	61.251
Cattle over 2 years	205.492	203.935	194.966	161.966	164.593	155.133	158.352
- dairy cows	168.025	151.502	139.443	130.025	102.333	71.423	70.884
- other cows (beef)	13.460	18.999	21.550	11.867	39.807	59.927	59.150
- other (bulls, etc.)	24.007	33.434	33.973	20.074	22.453	23.783	28.318
Total Cattle	**442.432**	**440.092**	**450.757**	**420.239**	**427.587**	**415.204**	**422.530**

Source: Authors' calculation based on Croatian Bureau of Statistics (CBS) database 2013-2024.

Given these observed trends, the aim of this paper is to project the medium-term trajectory of key indicators within the Croatian beef and veal sector through to 2030, using a partial equilibrium model-based approach. The central hypothesis is that, under current policy conditions and prevailing market trends, the sector will continue its structural contraction without any meaningful recovery in production or self-sufficiency by the end of the decade.

Material and Methods

The medium-term outlook for the Croatian beef and veal sector was generated using the AGMEMOD partial equilibrium (PE) model, a well-established modelling framework applied within the EU agricultural economics research community (Salamon et al., 2017; Chantreuil et al., 2012). Partial equilibrium models are grounded in neoclassical economic principles and are designed to simulate supply, demand, trade, and price interactions within specific commodity markets. Market equilibrium in each period is established through the following balance condition:

$$Production_t + Import_t + Beginning\ stocks_t = Consumption_t + Export_t + Ending\ stocks_t$$

The AGMEMOD framework employs a bottom-up, commodity-specific modelling approach, in which individual national models are developed according to a standardised EU-level template and subsequently aggregated into a comprehensive EU-wide model (Hanrahan, 2001). The Croatian national model captures the interrelationships between crop and livestock sub-models, integrating crop-livestock linkages through feed use, land allocation, and cross-price elasticities. The core dataset consists of production-consumption balances for beef and veal, incorporating annual data on beginning stocks, production, imports, domestic consumption, exports, and ending stocks. These data are sourced from the Croatian Bureau of Statistics (CBS) and Eurostat. Within the model, these variables are treated as endogenous. Exogenous inputs include macroeconomic indicators such as GDP per capita, population, and inflation, as well as CAP policy variables including coupled support payments and decoupled direct payments.

Econometric behavioral equations were estimated for key supply and demand variables, including the breeding herd, slaughter numbers, slaughter weights, meat production, and per capita consumption. Equations were validated using standard statistical diagnostics, including the t-test, F-test, coefficient of determination (R^2), White test for heteroscedasticity, and

Durbin–Watson test for autocorrelation (Bartova and M'barek, 2008). Additional expert validation was carried out at both international and national levels to ensure plausibility and consistency with observed market dynamics. The baseline simulation assumes a continuation of current CAP instruments and measures (2023-2027) throughout the simulation period, along with stable climatic conditions and the absence of major external market shocks. Domestic demand is assumed to follow its current trend without structural disruption.

Results and Discussion

The AGMEMOD baseline simulation for the Croatian beef and veal sector projects a continued structural contraction through 2030 (Table 2). The total cattle population is expected to follow a steady downward trajectory, declining from 422.5 thousand heads in 2024 to 404.5 thousand heads by 2030, a reduction of 4.3%. Beef and veal meat production is projected to fall by 11.6%, reaching 32.9 thousand tonnes by 2030, continuing the downward trend observed since EU accession. Domestic use is simulated to rise modestly by 3.0%, reaching 64.8 thousand tonnes, with this growing gap between supply and demand addressed almost entirely through increased imports.

Table 2. Baseline outlook simulation of the Croatian beef and veal sector (2013-2030)

Year	Live animals (1000 heads)	Production (1000 t)	Domestic use (1000 t)	Imports (1000 t)	Exports (1000 t)	Net exports (1000 t)	Self-sufficiency (%)
2013	442.4	47.3	55.8	10.2	3.4	-6.9	76%
2014	440.6	44.4	56.8	14.5	4.2	-10.3	71%
2015	440.1	42.3	57.5	17.4	4.3	-13.1	66%
2016	444.6	44.4	61.1	20.0	6.5	-13.4	62%
2017	450.8	42.2	61.3	22.0	5.9	-16.2	58%
2018	414.1	43.8	62.6	22.6	7.6	-15.0	65%
2019	420.2	45.4	63.3	24.7	7.7	-17.1	66%
2020	422.9	43.4	62.7	22.5	6.2	-16.3	66%
2021	427.6	43.2	62.7	26.8	6.7	-20.2	66%
2022	421.8	41.2	63.0	31.3	7.1	-24.2	65%
2023	415.2	38.4	62.1	35.4	7.4	-28.1	62%
2024	422.5	37.2	62.9	38.4	9.4	-29.0	59%
2025*	418.8	36.5	62.9	38.7	9.1	-29.6	58%
2026*	415.5	36.1	63.0	39.0	9.2	-29.9	57%
2027*	410.6	35.3	63.2	39.3	8.7	-30.2	56%
2028*	407.9	34.6	63.5	39.6	8.4	-31.0	54%
2029*	406.3	33.8	64.1	40.0	8.1	-32.1	52%
2030*	404.5	32.9	64.8	40.7	7.8	-33.5	51%
Change (%) 2030/2024	**-4.3%**	**-11.6%**	**3.0%**	**6.0%**	**-17.0%**	**15.5%**	**-13.6%**

*Data from 2025 onwards represent baseline simulation results. Source: AGMEMOD v8.0 modelling results.

Imports are projected to increase by 6.0%, rising from 38.4 to 40.7 thousand tonnes by 2030, while exports are expected to decline sharply by 17.0%, falling from 9.4 to 7.8 thousand tonnes. Net exports are consequently projected to deteriorate by 15.5%, reaching -33.5 thousand tonnes. As a result of these dynamics, the self-sufficiency rate is projected to decline from 59% in 2024 to 51% by 2030, representing a reduction of 13.6%. This means that by the end of the decade, Croatia will be able to satisfy barely half of its domestic beef and veal demand from national

production. The simulated decline in Croatian beef sector performance is consistent with broader patterns documented across Central and Eastern European Countries (CEECs) following EU accession. Multiple studies indicate that market liberalisation and CAP adoption accelerated structural adjustment in CEEC livestock sectors, often resulting in declining herd sizes, farm exits, and a growing divergence between crop and livestock production (Csaki and Jambor, 2013; Némethová and Hudáková, 2019). Persistent structural constraints, including insufficient farm consolidation, limited capital investment, and slow modernisation of production facilities, have further eroded the competitiveness of livestock sectors across the region (Jambor and Gorton, 2025). The declining self-sufficiency projected through 2030 raises important considerations for national food security. Growing import dependence may increase exposure to supply chain disruptions during periods of geopolitical tension or market volatility, concerns that have gained renewed relevance in the aftermath of the COVID-19 pandemic and recent geopolitical conflicts (Puma et al., 2015; Clapp, 2022). At the same time, recent CAP reforms have progressively shifted support away from production-coupled payments toward decoupled and environmentally oriented instruments, which may inadvertently accelerate structural adjustment in structurally vulnerable livestock sectors such as Croatian beef production (Scown et al., 2020; Matthews, 2018). In the Croatian context, where beef production is dominated by relatively small farms with limited capacity for capital investment, the baseline projections suggest that the current policy mix is unlikely to reverse the ongoing contraction of the sector by 2030. Reversing these trends would require targeted measures that simultaneously address structural modernisation, productivity improvement, and the sustainability objectives embedded within the evolving CAP framework.

Conclusion

The Croatian beef and veal sector has undergone significant structural transformation since EU accession, characterised by a recomposition of the cattle herd, a sustained decline in domestic production, and growing import dependency. The baseline simulation conducted using the AGMEMOD partial equilibrium model projects a continuation of these trends through 2030, with production expected to fall by 11.6%, self-sufficiency to deteriorate to 51%, and import reliance to increase further. These findings indicate that, under current policy and market conditions, the structural challenges facing Croatian beef production are likely to persist without significant policy intervention.

Future prospects for the sector depend on the development of coherent policy approaches that balance environmental and sustainability objectives with the economic viability of livestock production. Strengthening investment support for farm modernisation, promoting structural consolidation, and maintaining targeted coupled support for beef production within the CAP framework represent key policy levers for improving the sector's long-term resilience and competitiveness.

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ORIGINAL SCIENTIFIC PAPER

Human resource management challenges in agribusiness under digitalisation and AI

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Summary
The paper investigates human resource management challenges in agribusiness enterprises within the context of digitalisation and artificial intelligence (AI). Digital transformation and AI reshape recruitment, work planning, and employee development, while generating new managerial challenges. The study combines literature analysis and survey data from managers and employees. Findings based on survey responses and literature analysis indicate that respondents perceive AI as a tool with potential to improve specific HR functions such as recruitment efficiency, training processes, and workforce planning; however, they simultaneously report insufficient digital skills and a lack of trained personnel for its effective implementation. Strategic HR planning and continuous education are essential for sustainable agribusiness development.

Keywords: agribusiness, human resource management, digitalisation, artificial intelligence, management.

Introduction

Digital transformation represents one of the most important and significant changes in the modern business environment, but its importance is particularly pronounced in the agribusiness sector. Agribusiness encompasses primary agricultural production, processing, distribution, and food supply chains, and faces increasing pressures from global competition, climate change, market instability, and demands for sustainable production. Given the above, it can be concluded that this sector, like all others, must implement certain changes, adapt to new trends, and embrace the benefits of artificial intelligence and digitalization in all its spheres, including human resource management. According to the Food and Agriculture Organization of the United Nations (2019), digital technologies can significantly enhance productivity, optimize resource utilization, and increase efficiency in the agribusiness sector. The specificity of agribusiness is reflected in the seasonality of work, high dependence on natural factors, dispersed production units, and the traditional structure of the workforce. These characteristics create additional challenges in human resource management. The lack of qualified personnel, migration from rural areas, aging workforce, and low levels of digital competencies represent serious limitations for the modernization of the sector. According to the World Bank (2021), the digital divide in rural areas and limited access to education further slow down the integration of modern technologies into agricultural systems. The introduction of artificial intelligence in agribusiness involves the application of yield data analysis systems, predictive models for crop management, automated production monitoring systems, and optimization of logistics and distribution. However, the success of implementing these solutions largely depends on the human factor. Research indicates that technological investments without the parallel development of employee competencies often do not yield the expected results (Stone et al., 2015). In agribusiness, where an operational mode of work is traditionally present, the transition

to digital management requires a change in work practices and continuous employee education. Literature on e-HRM emphasizes the importance of integrating information systems into workforce management processes (Marler and Fisher, 2013), but in the agribusiness context, this integration implies additional challenges such as limited infrastructure, lower technological equipment, and insufficient institutional support. According to Bondarouk and Brewster (2016), the digital transformation of HR functions must be aligned with broader organizational and sectoral strategies in order to achieve agro-sustainability, which is a key priority. Beyond operational challenges, the application of AI in agribusiness raises questions regarding the ethical use of data, information security, and transparency of algorithmic decisions (Raisch and Krakowski, 2021). Agribusiness enterprises often possess large amounts of data on production, market, and workforce, but do not have sufficiently developed mechanisms for their strategic use. Contemporary research indicates that agribusiness organizations that successfully integrate digital technologies with human capital development achieve greater resilience to market shocks and climate risks (Brynjolfsson and McAfee, 2014). Nevertheless, empirical literature specifically analyzing the challenges of human resource management in agribusiness in the context of AI and digitalization is still limited. This research gap highlights the need for a more detailed examination of the organizational, competency-related, and strategic challenges faced by agribusiness enterprises in the process of digital transformation. In practical terms, digital transformation in agribusiness includes precision agriculture, digital farm management systems, and AI-based decision-support tools that improve productivity and efficiency. Precision agriculture uses satellite imagery, IoT sensors, and GIS technologies to monitor soil conditions, crop health, and weather patterns in real time (Wolfert et al., 2017). Large agribusiness companies also implement farm management information systems (FMIS) that integrate production, financial, and workforce data to support better planning and resource allocation. In addition, AI-driven technologies such as autonomous machinery and machine learning systems, exemplified by companies like John Deere, optimize planting and harvesting processes (Lowenberg-DeBoer et al., 2020). Overall, these technologies enhance operational performance but also require significant workforce adaptation, continuous training, and development of digital competencies within organizations.

Material and Methods

In the research process, both secondary and primary data were used. The secondary data were presented in the previous chapter and relate to relevant theoretical and empirical findings in the fields of agribusiness, digital transformation, and human resource management. Primary data were collected from a sample of 25 medium-sized agribusiness companies in Croatia. The main sampling criteria were that the companies had more than 50 employees (classified as medium-sized according to European Union criteria) and that they applied artificial intelligence in their business processes to some extent (especially in human resource management). The sample was purposive. The survey questionnaire was completed by HR managers in 24 cases, while in one case the questionnaire was completed by the company director (specific case). The research was conducted using an electronic questionnaire distributed via e-mail. The aim of the research was to examine the relationship between the level of artificial intelligence application and the efficiency of human resource management, as well as its contribution to the improvement of recruitment and selection processes in agribusiness enterprises. The questionnaire consisted of three thematic sections: Sociodemographic data (gender, age, work experience in agribusiness, position in the company, company size); Level of artificial intelligence application in HRM – questions related to the frequency and scope of AI tool usage in workforce planning, recruitment, selection, performance evaluation, and administrative HR activities; Perception of HR efficiency and process improvement – questions focused on assessing: the degree of efficiency in human resource management after AI implementation, the speed and quality of

recruitment and selection processes, reduction of hiring costs, objectivity and transparency of HR decision-making, and the overall contribution of AI to strategic HR management. Most questions were closed-ended with a five-point Likert scale (1 – strongly disagree to 5 – strongly agree), allowing quantification of respondents' attitudes and statistical testing of hypotheses. The Likert scale was used to measure respondents' level of agreement with statements related to AI usage and HR performance, where 1 indicated "strongly disagree" and 5 indicated "strongly agree". Higher scores indicated a higher perceived level of AI implementation and greater perceived efficiency of HR processes. The scale allowed the transformation of qualitative perceptions into quantitative data suitable for statistical analysis. The independent variable in the study was the level of artificial intelligence application in HRM processes, while the dependent variables were: perception of HR efficiency and improvement of recruitment and selection processes.

The main research hypotheses were: (H1) There is a statistically significant positive relationship between the level of artificial intelligence application and the efficiency of human resource management in agribusiness enterprises and (H2) A higher level of artificial intelligence application contributes to the improvement of recruitment and selection processes in the agribusiness sector. Data were analyzed using descriptive statistics and Pearson correlation analysis to determine statistically significant relationships between variables in accordance with the stated hypotheses. To ensure the ethical conduct of the research, respondents were guaranteed data anonymity, participation was voluntary, and informed consent was obtained. The data were used exclusively for scientific research purposes.

Results and Discussion

In this section, the research results will be presented. To begin with, the sociodemographic data related to the sample on which the research was conducted will be shown. The sample consists of 25 respondents, with a slightly higher proportion of males (56.0%) compared to females (44.0%). In terms of age distribution, the largest group falls within the 41–50 range (44.0%), followed by those aged 30–40 (32.0%), while respondents over 50 years old make up 24.0% of the sample.

Table 1. Descriptive statistics

Variable	Category		
	N	Median	Std. Deviation
Level of AI application in HRM	25	3.84	0.62
HRM efficiency (perceived)	25	3.92	0.58
Improvement of recruitment & selection process	25	4.08	0.55
Years in agribusiness	5 - 10	9	36.0
	11 - 20	10	40.0
	Over 21	6	24.0

Regarding professional roles, the vast majority of participants are HR managers (96.0%), with only a small proportion holding director positions (4.0%). In terms of experience in agribusiness, respondents are relatively evenly distributed, with 40.0% having 11–20 years of experience, 36.0% having 5–10 years, and 24.0% having more than 21 years of experience.

The average score for the level of AI application in HRM (M = 3.84, SD = 0.62) indicates a relatively high perceived level of implementation on a five-point Likert scale. The results for

HRM efficiency (M = 3.92, SD = 0.58) and improvement in recruitment and selection processes (M = 4.08, SD = 0.55) reflect respondents' perceptions rather than objective performance indicators, meaning that improvements are based on subjective assessments of HR managers and directors. Overall, higher mean values suggest a positive perceived impact of AI on HR-related processes in agribusiness enterprises.

Table 2. (H1) There is a statistically significant positive correlation between the level of application of artificial intelligence and the efficiency of human resource management in agribusiness companies

Variable	Category	
	AI level vs HRM efficiency in agribusiness	
AI Level	1	.621
Sig. (2-tailed)		.001
N	25	25
HRM efficiency in agribusiness	.621	1
Sig. (2-tailed)	.001	
N	25	25

A strong positive correlation was found between the level of AI application and the perception of HRM efficiency ($r = .621$, $p = .001$). The result is statistically significant at the $p < 0.01$ level, thus supporting H1. A higher level of AI application is associated with a higher perception of human resource management efficiency in agribusiness.

Table 3. (H2) A greater level of application of artificial intelligence contributes to improving the recruitment and selection process in the agribusiness sector.

Variable	Category	
	AI level vs Recruitment and selection improvement	
AI Level	1	.558
Sig. (2-tailed)		.004
N	25	25
Recruitment and Selection improvement	.558	1
Sig. (2-tailed)	.004	
N	25	25

The results show a moderately strong positive correlation between the level of AI application and the improvement of recruitment and selection processes ($r = .558$, $p = .004$). Since the p-value is < 0.01, hypothesis H2 is accepted. This indicates that a greater integration of AI tools contributes to more efficient and higher-quality hiring processes in the agribusiness sector. The findings of this study demonstrate statistically significant positive relationships between the level of artificial intelligence application and the perception of HRM efficiency, as well as the improvement of recruitment and selection processes in agribusiness enterprises. Based on these results, it can be concluded that AI clearly has the potential to transform human resource management in agribusiness companies. In an integrative review, Qorri et al. (2024) highlighted that technology is increasingly used in agriculture and agribusiness to save labor and enable more efficient resource planning, including digital and automation tools that positively impact employee performance and organizational productivity. Furthermore, Saini (2025) indicates that AI particularly contributes to the improvement of recruitment processes, the speed of candidate selection, and the objectivity of decisions, which is especially relevant for hypothesis

H2 in this study. Additionally, Agustina and Rosalia (2025) emphasize in their analysis that high-quality human resource management in agribusiness - including HR strategy development, professional training, and human capital planning - significantly enhances organizational efficiency and business sustainability. Their analysis supports the finding that technologies such as artificial intelligence further amplify these effects, especially when used to support managerial functions. However, most of the literature still highlights the challenges of AI implementation, including the need to develop digital competencies among employees and managers in agribusiness, as well as issues related to the ethical use of algorithmic solutions. This represents an important guideline for future research and the implementation of HRM practices.

Conclusion

The study examined the role of artificial intelligence in human resource management within agribusiness enterprises, with a focus on its perceived impact on HR efficiency and recruitment and selection processes. The findings indicate that organizations with higher levels of AI adoption report more positive perceptions of HRM efficiency, particularly in terms of faster recruitment processes, improved decision-making support, and reduced administrative workload. However, when compared to the current state of digital maturity in agribusiness, the results suggest that AI implementation remains at a moderate level, indicating that the sector is still in an early stage of digital transformation. In relation to existing industry practices, agribusiness organizations are increasingly adopting basic digital tools, while more advanced AI-driven HR solutions are still limited to a smaller number of firms. This gap highlights a disparity between technological potential and actual implementation capacity, primarily due to limited digital competencies and insufficient organizational readiness. Therefore, the findings emphasize that technological investment alone is not sufficient without parallel development of human capital and organizational capabilities. From a practical perspective, the results suggest that agribusiness enterprises should focus on developing structured digital HR strategies that integrate AI tools with workforce development programs. This includes investing in continuous employee training, improving digital literacy among HR staff, and ensuring alignment between technological adoption and organizational objectives. Furthermore, management should adopt a phased implementation approach to AI integration in HRM, allowing gradual adaptation and reducing resistance to change. Finally, the study highlights that sustainable benefits from AI in HRM can only be achieved through a balanced approach that combines technological advancement, strategic human resource management, and ethical considerations in data use and decision-making processes.

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ORIGINAL SCIENTIFIC PAPER

Effects of using e-learning platforms in the education of agricultural producers in Croatia

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Summary
The increasing complexity of science and technology heightens the need for continuous education in agronomy, especially in the era of digitalisation and artificial intelligence. This study examines the effects of e-platforms on educating agricultural producers in the Republic of Croatia. A sample of 50 farmers was surveyed, and data were processed using SPSS. The results indicate that e-platforms significantly enhance competences, knowledge, and daily work practices, while increasing awareness of innovations and business opportunities. Participants also reported income growth after training. Key limitations include high course costs and limited availability of content in Croatian.

Keywords: agriculture, e-learning, effects, platforms, producers

Introduction

The need for continuous education is evident in agriculture, especially regarding digitalisation, technological innovation, and the use of artificial intelligence. Modern farmers must continually update their knowledge to enhance productivity, competitiveness, and sustainability. The traditional ex-cathedra approach is increasingly supplemented by digital learning models that offer flexibility, accessibility, and adaptation to individual needs (Abdulkareem and Afolabi, 2021; Dahiya et al., 2012; Dissanayeke and Wickramasuriya, 2010). Globally, agriculture faces challenges such as food security, climate change, market volatility, and technological transformation (Leary and Berge, 2005). Digital platforms and mobile applications provide access to real-time information, optimise production processes, and support informed decision-making (Palit et al., 2024). Research confirms that e-learning has become a key tool in modernising the agricultural sector (McFadden et al., 2023).

E-learning refers to distance education delivered via the internet, enabling interaction between learners and content (Raab and Abdon, 2020). In agriculture, it is particularly valuable in rural areas where access to formal educational institutions is limited (Valsamidis et al., 2011). This model increases access to knowledge on sustainable practices, resource management, and market trends (Shaik et al., 2022). Its main advantages include lower costs, temporal and spatial flexibility, and the possibility of selecting individualised content (Agarwal and Kumar, 2013). E-learning is increasingly delivered through mobile applications (Sanga et al., 2016), while artificial intelligence further improves learning and decision-making processes (Elbehri and Chestnov, 2021).

In Croatia, several digital platforms are available to farmers. The national ePoljoprivreda portal, developed by the Ministry of Agriculture, Forestry and Fisheries of the Republic of Croatia, enables electronic management of administrative procedures and provides access to educational materials. Advisory and educational support is also offered by the Croatian Agency for Agriculture and Food (HAPIH). The Agroklub portal features professional articles, advice, and digital tools, while Agrivi provides farm management solutions and education in smart

agriculture. Internationally, farmers can use the FAO eLearning Platform, as well as commercial platforms such as Coursera, FutureLearn, and edX, which offer courses in sustainable agriculture, agricultural economics, precision farming, and smart agriculture.
The literature confirms that digital platforms are a key tool in transforming the agricultural sector by improving producers' knowledge, competencies, and economic efficiency. In addition to enhancing technical skills, e-learning helps farmers develop better decision-making abilities, adopt innovative practices, and respond more effectively to market changes. These platforms support continuous professional development, enabling farmers to stay updated with new research, trends, and regulations. They also foster collaboration and knowledge exchange among peers, strengthening professional networks. For rural farmers in particular, e-platforms overcome geographic and logistical barriers, making training accessible that would otherwise be out of reach. The flexibility of self-paced learning allows individuals to balance education with ongoing farm operations. Furthermore, the integration of multimedia content, such as videos and interactive modules, increases engagement and information retention. Through these digital tools, farmers can experiment with new techniques and apply learned concepts immediately, resulting in measurable improvements in productivity and sustainability. Finally, the combination of local and international e-learning resources broadens perspectives and exposes farmers to global best practices, further enhancing their capacity to innovate and remain competitive.

Material and Methods

The empirical research was conducted on a sample of 50 respondents. The main sampling criteria were that participants were from Croatia and used some form of e-platform for agricultural education. The participants completed a structured questionnaire. The questionnaire included the following categories: sampling and socio-demographic questions (origin, gender, age), questions on experience with e-learning platforms for agricultural education, and questions on the effects of using e-platforms in agriculture. The questionnaire was distributed online via Google Survey, and the collected data were processed using the SPSS statistical software package.

Results and Discussion

At the beginning of this section, it should be noted that all respondents in the sample are from Croatia (100%) and all use some form of e-platform for agricultural education. Of the total respondents, 27 (54%) use platforms in Croatian, while the remaining 23 (46%) use e-platforms in English. Data on age and gender are presented in the following table.

Table 1. Sociodemographic data

Variable	Category		
	Variable type	Frequency	Percent (%)
Gender	Male	42	84.0
	Female	6	12.0
Age	18 – 25	2	4.0
	26 – 35	5	10.0
	36 – 45	18	36.0
	46 – 55	19	38.0
	56 – 65	6	12.0

Based on Table 1, it can be concluded that 42 respondents (84%) were male and 6 respondents (12%) were female. The following section presents participants' views on their experiences with e-learning platforms. Respondents rated statements on a Likert scale from 1 to 5, where 5 is the highest rating and 1 the lowest.

Table 2. Statements (1)

Variable	Category		
	N	Mean	Std. Deviation
E-platforms are an excellent way to acquire new knowledge.	50	4.85	1.2568
E-platforms are excellent because people from rural areas have easier access to knowledge.	50	4.95	1.1152
Education through e-platforms is expensive.	50	3.52	0.2312
Foreign e-platforms are better than domestic ones.	50	3.20	0.2532
Learning through e-platforms was useless to me.	50	2.20	1.2563

Based on the results presented in Table 2, most respondents consider e-platforms an excellent way to acquire new knowledge in agriculture and find them particularly useful for people living in rural areas, as they allow learning from home via phone or laptop. However, nearly 50% of respondents believe this form of education is expensive and that foreign platforms are superior to domestic ones. Respondents do not agree that e-platforms are useless, indicating they have effectively applied the knowledge gained through them. Similar results were reported in studies by Tjernström et al. (2021) and Aubert et al. (2012).

Table 3. Statements (2)

Variable	Category		
	N	Mean	Std. Deviation
The knowledge and skills I acquired through education using e-platforms, I have successfully implemented in my daily activities.	50	4.95	0.12569
Thanks to the knowledge gained through e-platforms, I have increased my personal income.	50	4.20	0.23569
Thanks to the knowledge gained through e-platforms, I have reduced my expenses.	50	4.15	0.34589
Thanks to e-platforms, I have acquired knowledge that I successfully share with other farmers.	50	4.85	1.36952

The results presented in Table 3 represent the experiences of farmers with education through e-platforms. Owing to the knowledge and skills gained via e-platforms, farmers improved their business operations, increased their income, and reduced their expenses. Notably, after acquiring new knowledge, farmers often shared it with colleagues and other farmers, thereby disseminating valuable information that can enhance the operations of others. Similar results were reported in the studies by Greig et al. (2024), Morepje et al. (2024), and Narayanamurthy et al. (2025).

Conclusion

The new era brings new rules. It is certain that new forms of learning and education exist, and they should be recognised and utilised. This applies to all fields, including agriculture. Agricultural education improves business operations for farmers, influencing both cost

reduction and income growth. These forms of education also enhance information sharing among farmers.

Learning through e-platforms has had a largely positive effect on farmers, increasing learning opportunities for those in rural areas and allowing them to participate in education from home. It is also important to note that the Government of the Republic of Croatia has developed numerous learning platforms for farmers, while domestic farmers also willingly use English-language platforms to further enhance their knowledge.

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ORIGINAL SCIENTIFIC PAPER

Consumer awareness, attitudes and behavioural intentions towards agroforestry in Croatia

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Summary
This study examines awareness, attitudes and behavioural intentions of consumers towards agroforestry in Croatia using survey data (N = 185). The sample was predominantly female (56.2%), with a mean age of 37.6 years, and mainly consisted of urban, medium-income respondents with secondary and higher education. Results indicate low prior knowledge despite generally positive attitudes, particularly regarding environmental benefits, while behavioural intentions remain moderate. Cluster analysis identified two consumer segments (Engaged and Passive consumers). The results highlight the need for targeted strategies to increase awareness of agroforestry market development.

Keywords: agroforestry, consumer attitudes, cluster analysis, marketing

Introduction

Agroforestry is a land-use system that integrates woody perennials (trees and shrubs) with agricultural crops and/or livestock on the same land unit (FAO, 2015). Numerous studies have highlighted its role in climate change mitigation and adaptation, biodiversity conservation and the improvement of soil and water quality (Nair, 2012; Abebaw et al., 2025). Agroforestry is increasingly recognised as a strategy for developing resilient and environmentally sustainable agricultural systems, while also contributing to income diversification and the stability of farm production (Aguilar and Cai, 2010). In addition, previous research suggests that consumers tend to perceive agroforestry products positively, particularly when associated with traditional and environmentally friendly production systems (Bondesan et al., 2016; Cole et al., 2024). Despite the recognised environmental and economic benefits, the adoption of agroforestry systems remains limited in many regions, particularly in Southeast Europe (Szerb et al., 2020; Cole et al., 2024). Existing research has predominantly focused on ecological aspects, while socio-economic dimensions, especially consumer awareness and attitudes, remain comparatively underexplored (Szerb et al., 2020). Previous studies indicate that consumer perceptions play a critical role in the market success of sustainable products (Aguilar and Cai, 2010; Bondesan et al., 2016; Cole et al., 2024). However, empirical evidence on consumer behaviour in the context of agroforestry is still limited. This gap highlights the need for further research examining consumer attitudes and behavioural intentions towards agroforestry products. Understanding consumer perceptions is crucial, as the market success of agroforestry products largely depends on their acceptance by consumers. Therefore, the aim of this study is to examine consumer awareness, attitudes, and behavioural intentions towards agroforestry, to assess perceptions of its economic, environmental, and social benefits, and to identify consumer segments.

Material and Methods

The study was conducted using a structured questionnaire designed to examine consumer awareness, attitudes, and behavioural intentions towards agroforestry. The questionnaire included socio-demographic characteristics and an initial assessment of respondents' prior

knowledge and familiarity with agroforestry. Subsequently, a definition of agroforestry was provided, including examples of agroforestry products (e.g. fruits, nuts, honey, and animal products from silvopastoral systems), which served as a reference point for respondents when evaluating purchase-related statements. A set of statements aimed at assessing attitudes towards environmental, economic, and social benefits was then presented. These statements were measured using a five-point Likert scale (1 = strongly disagree; 5 = strongly agree). The questionnaire was based on previous research addressing consumer perceptions of agroforestry and sustainable agricultural products (Bondesan et al., 2016; Szerb et al., 2020; Cole et al., 2024).

Survey was distributed via social media platforms (primarily WhatsApp). Sample consists of more digitally active, urban, and higher-educated individuals. Therefore, the findings should be considered in light of the sample characteristics.

Furthermore, descriptive statistical methods were applied, including frequencies and percentages for categorical variables and mean values for Likert-scale items. Exploratory factor analysis (EFA) was conducted to identify underlying dimensions of consumer attitudes and behavioural intentions. The suitability of the data for factor analysis was confirmed by a Kaiser–Meyer–Olkin (KMO) measure of 0.913, indicating valid sampling adequacy, and a statistically significant Bartlett's test of sphericity ($\chi^2 = 1530.033$, $df = 66$, $p < 0.001$), confirming that the correlation matrix was appropriate for factor analysis. Additionally, the anti-image correlation matrix showed that all Measures of Sampling Adequacy (MSA) exceeded the recommended threshold of 0.70, further supporting the adequacy of the data. Factor scores obtained from EFA were used as input variables for cluster analysis.

Prior to clustering, six cases with unusually large distances from other observations were identified and excluded based on hierarchical clustering (between-group linkage), as they could disproportionately influence the clustering solution. The optimal number of clusters was determined using the elbow criterion, and a two-cluster solution was selected. Cluster analysis was performed using a hierarchical approach based on Ward's method and squared Euclidean distance.

Chi-square (χ^2) tests were used to examine the association between socio-demographic variables and cluster membership. Furthermore, independent samples t-tests were employed to evaluate differences in mean responses between identified consumer segments across attitude and behavioural intention items. Homogeneity of variances was assessed using Levene's test, and where this assumption was violated, results based on unequal variances were reported. Statistical significance was set at $p < 0.05$.

Results and Discussion

The sample consisted of 185 respondents. In terms of gender, females were more represented than males (56.2% vs. 42.7%), while a small proportion of respondents (1.1%) preferred not to disclose their gender. The average age of respondents was 37.6 years. Regarding education level, the largest proportion of respondents had completed secondary education (39.5%), followed by those with graduate (24.3%) and undergraduate (20.5%) degrees. A notable share of respondents held postgraduate qualifications (15.1%), while only a negligible proportion had primary education (0.5%). In terms of place of residence, respondents were predominantly from urban areas (64.3%), while a smaller proportion lived in rural areas (35.7%). In terms of household income, most respondents reported medium income levels (61.1%), followed by higher income categories (26.5%). A relatively small share of respondents reported low (7.0%) or high income (4.9%), while very low income was almost negligible (0.5%).

More than half of respondents (63.2%) have heard of agroforestry, yet their self-assessed knowledge was generally low, with the majority indicating weak or only moderate familiarity with the concept. This suggests that positive attitudes may not be based on well-established

knowledge. This pattern is consistent with previous findings showing limited consumer understanding of agroforestry despite exposure (Szerb et al., 2020; Cole et al., 2024).
Prior to cluster analysis, six respondents were excluded from the analysis based on hierarchical clustering (distance measures) to avoid distortion of the clustering solution. The final sample used for segmentation consisted of 179 respondents. Two respondent segments were identified (Table 1). Cluster 1 (Engaged consumers; N = 134) exhibited consistently higher levels of agreement, particularly in behavioural intentions, whereas Cluster 2 (Passive consumers; N = 45) showed lower agreement levels. The most pronounced differences between clusters were observed in intention-related variables, while general attitudes remained relatively similar. This aligns with previous research indicating that differences in pro-environmental behaviour are more strongly reflected in behavioural intentions than in general attitudes (Szerb et al., 2020).

Table 1. Differences in mean agreement scores between consumer segments

Statement	Overall	Cluster 1 (Engaged consumers)	Cluster 2 (Passive consumers)	Difference	p-value
Agroforestry has a positive impact on the environment.	4.11	4.28	3.98	0.30	0.012
Agroforestry contributes to sustainable agriculture.	4.11	4.26	4.02	0.24	0.039
Agroforestry is an important system for the future of food production.	3.98	4.16	3.80	0.36	0.011
Agroforestry products are more environmentally friendly.	3.90	4.08	3.64	0.44	0.002
Agroforestry contributes to society.	4.03	4.17	3.96	0.21	0.102
Agroforestry is economically beneficial for farmers.	3.66	3.84	3.44	0.39	0.007
Agroforestry is applicable in Croatia.	3.92	4.06	3.84	0.22	0.117
I would support the development of agroforestry in Croatia.	4.28	4.44	3.98	0.46	<0.001
I would like to learn more about agroforestry.	3.93	4.16	3.47	0.69	<0.001
I would buy agroforestry products if I saw them in a store.	3.96	4.30	3.27	1.03	<0.001
I would choose agroforestry products over conventional products.	3.84	4.21	2.84	1.37	<0.001
A quality label 'Agroforestry product' would encourage me to purchase such products.	3.73	4.16	2.62	1.54	<0.001

Note: Values represent mean scores. Likert scale ranged from 1 (strongly disagree) to 5 (strongly agree).

Chi-square analysis showed that gender significantly influences segment membership (χ^2 = 6.236, p = 0.013). Women were more represented in the engaged consumer segment. Age showed marginal significance ($p \approx 0.05$), with younger respondents more likely to belong to the passive consumer segment.
Knowledge of agroforestry significantly influenced cluster membership ($p < 0.05$), as respondents with higher self-reported knowledge were more likely to belong to the engaged segment, which is consistent with evidence that knowledge positively affects behavioural intentions in sustainable consumption (Stampa et al., 2020). No significant differences were

found between segments for place of residence, income, prior awareness of agroforestry or education level.

The results indicate positive attitudes of respondents towards agroforestry (Table 1). The highest levels of agreement were observed for statements related to environmental and sustainability benefits. Strong agreement was also found for the statement 'I would support the development of agroforestry in Croatia'.

While the economic aspect of agroforestry is perceived positively, it is evaluated less favourably compared to its environmental and social benefits. Purchase-related intentions are also moderately positive and respondents showed a moderate intention to choose agroforestry products (as defined in the questionnaire, e.g. fruits, nuts, honey and animal products) over conventional alternatives.

However, the influence of quality labels appears somewhat weaker. No statistically significant differences between clusters were found for perceived social benefits or the applicability of agroforestry in Croatia.

Despite generally positive attitudes, a clear gap between attitudes and behavioural intentions was observed, as attitudes were relatively homogeneous while significant differences emerged in purchase-related intentions. This supports established attitude to behaviour gap in sustainable food consumption, where favourable perceptions do not necessarily translate into actual behaviour (Otter and Langenberg, 2020; Cole et al., 2024). The identification of engaged and passive consumer segments is consistent with previous research (Szerb et al., 2020), while the significant role of knowledge suggests that better-informed consumers are more likely to exhibit stronger behavioural intentions (Stampa et al., 2020).

Overall, the findings indicate that agroforestry products remain in a relatively latent market phase, characterised by positive perceptions but limited behavioural engagement (Szerb et al., 2020).

Conclusion

Consumers exhibit generally positive attitudes towards agroforestry, particularly regarding environmental benefits, despite relatively low levels of knowledge. However, behavioural intentions towards purchasing agroforestry food products remain moderate, revealing a gap between favourable perceptions and actual purchasing behaviour.

The results further demonstrate that consumer segmentation is primarily driven by differences in behavioural intentions rather than general attitudes. This suggests that positive perceptions alone are insufficient to ensure market adoption, as they do not automatically translate into purchasing decisions.

These findings highlight the need for targeted communication and marketing strategies focused on increasing consumer awareness, strengthening perceived value, and facilitating the translation of positive attitudes into actual purchasing behaviour. In addition, the results provide relevant insights for policymakers by emphasising the importance of education, labelling, and market development measures in supporting the wider adoption of agroforestry systems.

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ORIGINAL SCIENTIFIC PAPER

Perceptions of slow tourism among Croatian tourists

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Summary
Slow tourism is the shift in focus from quantity to the quality of experiences, generally fewer and more conscious, while on holiday. This study examined the knowledge, travel behaviour, and attitudes of Croatian tourists toward slow tourism. It was conducted through an online survey of 264 respondents conducted in June 2024. Results show that awareness of slow tourism is very low, only 12% of respondents knew what it was. Current travel behaviour is largely misaligned with slow tourism principles, marked by short stays, frequent trips and heavy reliance on private cars. Despite this, attitudes toward slow tourism are strongly positive and 86% of respondents expressed interest in slow travel for the future. The main barriers identified were lack of free time, cost and insufficient information. These findings suggest that the gap between slow tourism values and actual behaviour in Croatia is driven primarily by lack of awareness and structural constraints, not by a rejection of slow tourism ideals.

Keywords: slow tourism, tourist behaviour, awareness, attitudes, Croatia

Introduction

Tourism is one of the most economically significant sectors globally, yet its dominant form, mass tourism, has brought growing concerns about environmental degradation, overcrowding and loss of destination quality. Croatia illustrates these pressures clearly. Its tourism is heavily concentrated along the Adriatic coast and is marked by strong seasonality, with most visitors arriving in summer. This has led to uncontrolled capacity growth, infrastructure strain and overtourism in the most visited destinations. Croatia continues to attract mostly medium and lower purchasing power visitors, arriving largely out of inertia rather than because of a genuinely improved tourism offer (Dujmović and Vitasović, 2024).

Slow tourism has emerged as a relevant alternative. It is generally understood as a conscious approach to travel that emphasises quality of experience over quantity of destinations visited. It focuses on staying longer in one place, engaging with local culture and communities, using lower-carbon transport, and supporting local food and services (Dickinson and Lumsdon, 2010). The concept originated from the Slow Food movement founded by Carlo Petrini in Italy in 1986. It later extended through the Cittaslow network into tourism (Serdane et al., 2020; Lowry and Lee, 2016). The absence of a single agreed definition is a gap well documented in the literature (Mavrić et al., 2021). Despite this, the core of slow tourism is widely understood to be an attitudinal phenomenon driven by a slow mindset rather than a fixed set of behaviours (Oh et al., 2014). Klarin et al. (2023) have proposed a conceptual framework that consolidates this research and calls for more empirical studies in diverse geographic contexts.

Research on slow tourism motivations has identified six recurring drivers: relaxation, self-reflection, escape, novelty-seeking, engagement and discovery (Oh et al., 2014). Survey-based studies in Central Europe have found that tourists hold positive attitudes toward slow tourism elements, particularly local food, culture and destination discovery. Sustainability related dimensions attract much less support (Ernszt and Marton, 2021).

No prior empirical study has examined how Croatian tourists understand, practise or relate to slow tourism. This paper addresses that gap. The research objectives are: (1) to assess how well

the travel behaviour of Croatian tourists aligns with slow tourism principles; (2) to determine the level of awareness Croatian tourists have about slow tourism; and (3) to explore Croatian tourists' attitudes toward slow tourism and their intentions for future slow travel.

Material and methods

The research was based on primary data collected through an online survey examining three areas: tourist travel behaviour, knowledge and awareness of slow tourism, and attitudes toward slow tourism and future travel intentions. The survey consisted of 28 questions in five sections, using multiple-choice questions, open-ended questions and 5-point Likert scales (1 = strongly disagree, 5 = strongly agree). The questionnaire design was informed by the slow tourism motivation and travel mode scales developed by Oh et al. (2014).

The survey was created using the 1KA online tool and distributed in June 2024 via social media (Instagram, Facebook, WhatsApp), using a combination of random sampling and the snowball method. Of 357 respondents who opened the survey, 264 completed it fully (completion rate 74%). Data were analysed using descriptive statistics in MS Excel.

The sample was predominantly female (86%), with an average age of 35 years (SD = 11.58, range 18–74). Most respondents were employed (67%), held a university degree or higher (40%), lived in urban areas (64%) and reported a good financial situation (76%). The gender imbalance and snowball sampling are limitations that should be considered when interpreting results.

Results and discussion

Travel behaviour and alignment with slow tourism principles

The travel frequency of respondents is high: 32% travel three to five times per year and 18% more than five times per year. Stays are short, with 58% staying five days or fewer and 87% staying seven days or fewer (average 5.9 days, SD = 4.19). This pattern of frequent but brief trips directly contradicts slow tourism's emphasis on fewer, longer and more immersive travel (Oh et al., 2014).

Table 1. Length of stay at destination

Length of stay	Respondents (%)	Cumulative (%)
1–3 days	21	21
4–5 days	37	58
6–7 days	29	87
8–10 days	8	95
More than 10 days	5	100

Transport is dominated by private cars (49%) and aeroplanes (27%), together accounting for 76% of choices. Only 5% of respondents use trains, despite low-carbon transport being considered a core dimension of slow tourism (Dickinson and Lumsdon, 2010). Serdane et al. (2020) found the same pattern in Latvia and attributed it to infrastructure limitations rather than environmental indifference. The same explanation could apply here, given Croatia's limited rail connections to inland and rural areas.

When rating destination choice factors on a 5-point scale, respondents ranked safety highest (mean 4.2), followed by accessibility and cleanliness (both 4.0). Slow tourism-relevant attributes scored lower: kindness of the local population (3.6), local cuisine (3.5) and untouched nature (3.5). Nightlife and entertainment ranked last (2.6). This places Croatian tourists in an

interesting middle ground, they are not seeking hedonistic mass tourism, but they are not yet actively prioritising the cultural and community dimensions that define slow tourism.
The primary travel motives were rest and relaxation (26%), escape from everyday life (19%) and getting to know the culture (16%) - closely matching the motivational framework of Oh et al. (2014). They identified relaxation and escape as the two strongest universal slow tourism drivers. The strong showing of cultural curiosity in third place is noteworthy.

Awareness and knowledge of slow tourism
Awareness of slow tourism among Croatian tourists is very low. As shown in Table 2, only 12% of respondents knew what slow tourism was, 39% had heard of it but could not explain it, and 49% had never heard of it at all.

Table 2. Self-reported knowledge of slow tourism

Response	n	%
Yes, I know what slow tourism is	31	12
I am not sure I can explain it accurately	103	39
No, I do not know what slow tourism is	130	49

This is consistent with findings from comparable markets. Serdane et al. (2020) found in Latvia that even tourists engaging in slow tourism practices had limited awareness of the concept as a named phenomenon. Ernszt and Marton (2021) reported the same in Hungary. More broadly, the literature suggests that slow tourism demand exists latently in many markets but has not been translated into named consumer awareness (Mavrić et al., 2021; Klarin et al., 2023). Croatia fits this pattern well. The desire is there, the label is not.
Among the 31 respondents who were aware of slow tourism, the most common associations were food and culture (7 mentions each), relaxation (6), nature (5) and hiking or cycling (5). Crucially, 83% of aware respondents had already practised slow travel, and all 29 who answered the recommendation question said they would recommend it. This suggests that awareness, once reached, converts quickly into practice and advocacy. The main source of knowledge about slow tourism was electronic media (56%), followed by family and friends (11%) and school or college (11%). Only 4% learned about it through travel agencies.
Among those who had previously travelled slowly, the primary motives were getting to know local cultural heritage (31%), local food and drink (24%) and connecting with local people (23%). Environmental protection was cited by only 19%. This mirrors the Hungarian findings of Ernszt and Marton (2021), where the local and cultural dimensions scored highest and sustainability ranked last. The pattern suggests that cultural and experiential appeal and not environmental concern is the realistic entry point for slow tourism promotion in this region.

Attitudes toward slow tourism and future travel intentions
Attitude scores were collected from all 264 respondents. Those unfamiliar with slow tourism were first given a brief description. As shown in Table 3, all benefit-related statements scored above 4.0, with consistently low standard deviations, indicating strong and broad agreement across the sample.

Table 3. Attitudes of Croatian tourists toward slow tourism (1 = strongly disagree, 5 = strongly agree)

Statement	Mean	SD
Slow tourism contributes to preservation of tradition and local culture	4.3	0.77
Slow tourism has a positive impact on rural and urban development	4.2	0.73
Slow tourism contributes to development of cultural and gastronomic offer	4.1	0.74
Slow tourism encourages preservation of cultural resources	4.1	0.77
Slow tourism improves the living standards of local people	4.1	0.78
Slow tourism has more positive than negative impacts overall	4.0	0.82
Slow tourism encourages preservation of natural resources	3.9	0.87
Slow tourism requires more time than other forms of tourism	3.8	0.99
Slow tourism is characterised by higher prices than other forms	3.1	0.90

The lowest-rated statement concerned higher prices (mean 3.1), with respondents neither agreeing nor disagreeing. Since cost is often assumed to be a primary deterrent to slow tourism adoption, this finding is important. It suggests that education and communication are more effective tools than price reduction for encouraging behaviour change.

Regarding future intentions, 86% expressed interest or strong interest in slow travel. The most commonly cited barriers were lack of free time (37%), more affordable prices (28%), better information (18%) and better transport connections (16%). These results align with Serdane et al. (2020), who found that Latvian tourists attributed their limited slow tourism engagement to structural constraints rather than a lack of interest.

Looking at specific future travel preferences, respondents most strongly agreed with wanting mental rest (mean 4.6) and escaping everyday routine (mean 4.6). They also wanted to travel at their own pace (mean 4.1), while doing as many things as possible under time pressure scored lowest (mean 3.4). These are precisely the motivational drivers of slow travel identified by Oh et al. (2014).

The overall picture is a gap between values and behaviour that is not rooted in a conflict of values. Tourists value culture, rest, authenticity and self-directed travel. They hold strongly positive views about slow tourism's benefits for communities and destinations. Yet they travel frequently and briefly, rely on cars and planes, and are almost entirely unaware of slow tourism as a concept. This gap is structural, caused by lack of knowledge, inadequate transport infrastructure and an absence of slow tourism products in the Croatian market (Dujmović and Vitasović, 2024).

For practitioners, the most immediate implication is product design. Slow tourism offers should be built around realistic Croatian holiday durations of five to seven days. Agencies should become active promoters of slow tourism. For policymakers, investment in rail and public transport to inland and rural Croatia would directly address the structural transport barrier and open new slow tourism territories. Digital awareness campaigns are the most cost-effective channel, given that 56% of currently aware tourists learned about slow tourism through electronic media.

Conclusion

Three main conclusions emerge from this study. First, the travel behaviour of Croatian tourists is not aligned with slow tourism principles: frequent short trips, reliance on private cars and aeroplanes, and destination choices driven by safety and accessibility rather than cultural depth. This reflects the broader structure of Croatian tourism, still dominated by coastal, seasonal, high-volume travel.

Second, slow tourism awareness is very low (12%). Yet among those who are aware, 83% have already practised it and 100% would recommend it. This makes closing the information gap the single highest-leverage intervention available for growing slow tourism demand in Croatia. This finding is consistent with Central and Eastern European evidence.

Third, attitudes are strongly positive and future intentions are high. The emotional needs Croatians describe for their next trip are exactly what slow tourism delivers.

This study provides the first survey-based empirical evidence on slow tourism from the perspective of Croatian tourists and establishes a baseline for future research. Priorities for follow-up include more representative samples, a gender-balanced design, and longitudinal tracking of whether stated intentions translate into behaviour. A key open question is why 83% of respondents practised slow tourism without ever knowing the term - qualitative research into that group's motivations and barriers would considerably deepen understanding of latent slow tourism demand in Croatia.

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ORIGINAL SCIENTIFIC PAPER

Comparative analysis of the dairy sector: Slovenia and Croatia

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Summary
The development of milk production in Slovenia and Croatia has been very similar for decades. This is not surprising given the politics and economic development of the former common state. The development of milk production remained comparable in the first two decades after independence, but in recent years there have been significant differences in production between the countries. In order to understand the developments on the milk market in both countries, we have conducted a brief comparative analysis of both sectors. We have sought explanations for the differences in key production indicators. The slower pace of structural change in Slovenia compared to Croatia appears likely to be the result of a combination of factors, including potentially more favourable socio-economic and infrastructural conditions, such as diverse employment opportunities in rural areas, better access to local markets, or specific national agricultural support policies that alleviate economic pressures on smaller farms. Further research is needed to quantify the relative impact of these factors on the observed deviation.

Keywords: dairy sector, Slovenia, Croatia, comparative analysis

Introduction

Milk production had a great self-sufficiency and social importance on smaller farms for decades. In the former common state, market milk production was based on larger agricultural enterprises (in Slovenia also in cooperatives), while the private sector was fragmented and limited in production. After the collapse of the former state, significant structural changes began to occur in the field of agriculture, both in Slovenia and in Croatia, as also indicated by agricultural census data (SURS, DZS). At the same time, specialization of agricultural holdings also took place with considerable intensity in both countries (Zeleno poročilo, 2025, MPŠR, 2025). While the two sectors were similar at the end of the former state and for some time after independence, significant differences have emerged in the last decade. Thus, the purpose of this paper is to review trends in milk production in Slovenia and Croatia over the last two decades and to demonstrate the key causes of the changes that occurred and to explain them.

Material and Methods

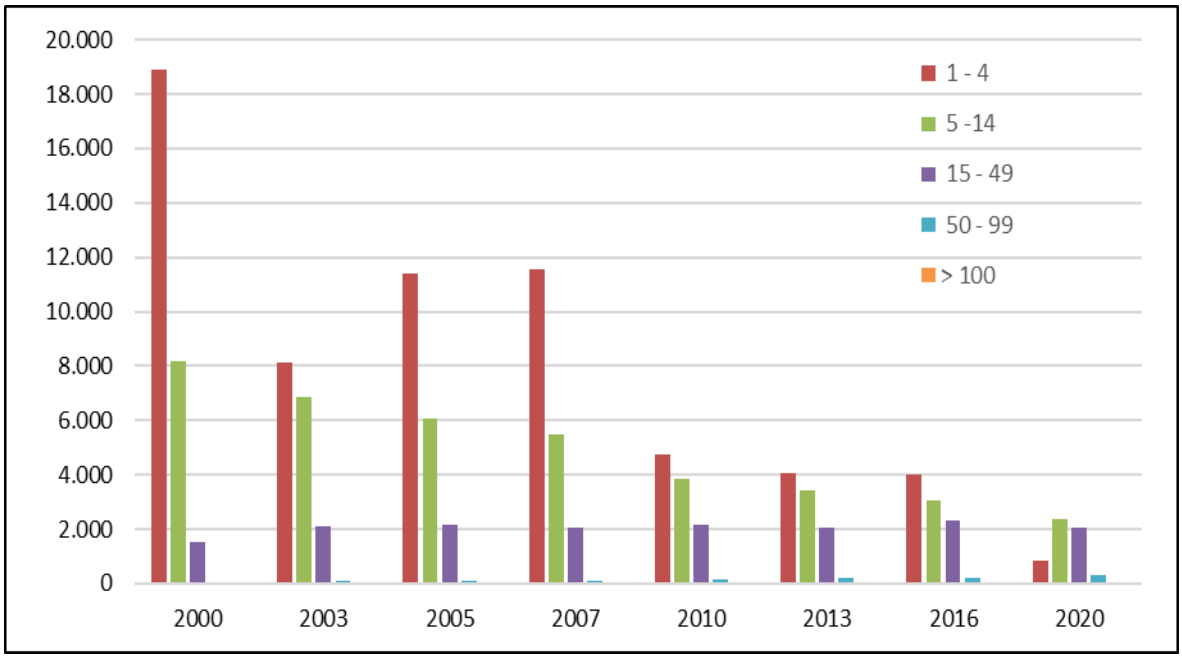
This comparative analysis uses available official statistics and reports from national statistical offices, agricultural ministries, and specialized agencies for the period 2000-2024. Data for primary production included dairy farm structure (number of holdings by size categories), dairy cow numbers, annual milk production levels, and average milk yield. Foreign trade analysis is focused on aggregated import and export volumes of milk and dairy products, and self-sufficiency rates. Trends were identified through descriptive statistical analysis, focusing on year-on-year changes and multi-year averages, to highlight convergences and divergences between the two countries.

Results and Discussion

In Slovenia, milk production is the most important agricultural sector (Zeleno poročilo, 2025). It contributes around 15% to the total value of agricultural production in recent years, and 34% to the value of livestock farming.

Due to its natural conditions, the structure of agricultural production in Croatia is quite different from that in Slovenia. Due to the large share of arable land, the production of cereals and oilseeds has the largest share in the value structure of agricultural production (DZS, 2026), while milk production, according to the Economic Accounts of Agriculture, represented just under 6% of the total value of agricultural production, and within livestock production, just under 14% of the value. As recently as 2010, milk production represented around 12% of the value of agricultural production, and around 31% of the value of livestock production. The significant decrease in the share in fifteen years indicates structural changes and contraction of the sector, which is also confirmed by other statistical data.

Studies concerning the farm structure in Slovenia indicate that structural adjustments within the dairy sector have been the most pronounced across all livestock sectors over the past decade, particularly since 2007. There has been a significant reduction in the number of holdings specializing in dairy farming; by 2020, approximately 8% of all farms were involved in milk production. The most substantial decrease in dairy operations occurred in the size categories with fewer than 14 cows.



Graph 1. Dairy farm structure; Slovenia, 2000-2020
Source: SURS

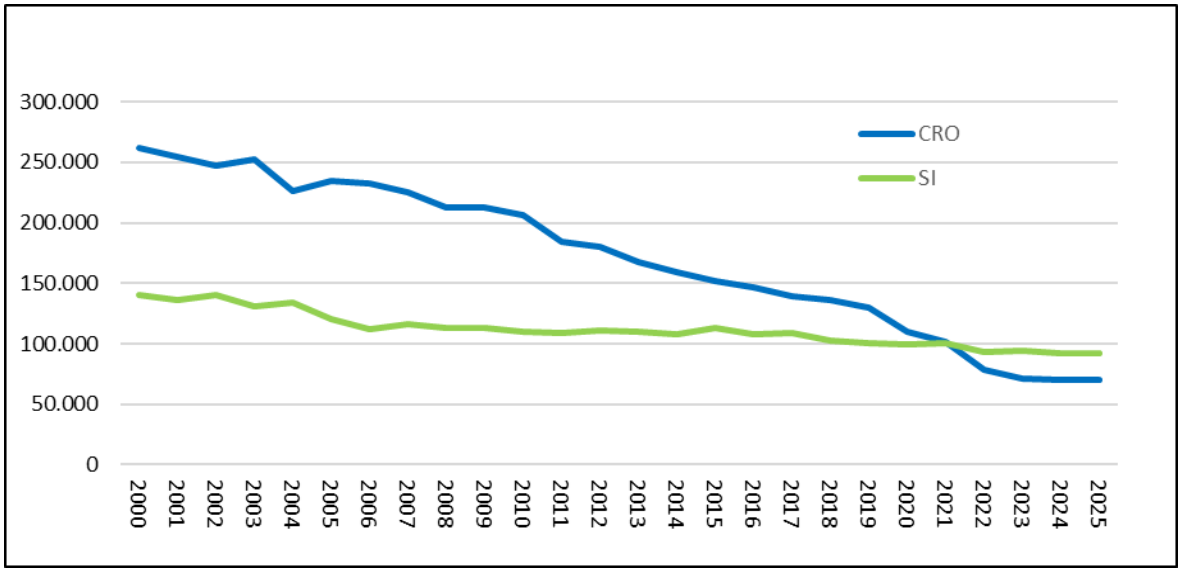
Structural changes have also been occurring in Croatia, becoming most evident over the last decade. As shown in Table 1, the number of dairy herds with up to 10 animals has decreased rapidly, while the decline in the 11–30 cow category was significantly less intense. This indicates the income viability threshold for smaller herds; however, these are not specialized operations and they supplement their income with other revenue sources.

Table 1. Dairy farm structure; Croatia, 2020-2024

No. of dairy cows	2020	2021	2022	2023	No. of dairy cows	2024
<6	14.777	142.41	11.542	10.553	<6	9.535
6-10	2.626	2.402	2.019	1.911	6-10	1.853
11-30	2.486	2.436	2.239	2.198	11-30	2.102
31-100	636	635	638	626	31-100	649
101-250	71	77	69	66	101-1.000	96
>250	33	32	30	32	>1.000	5

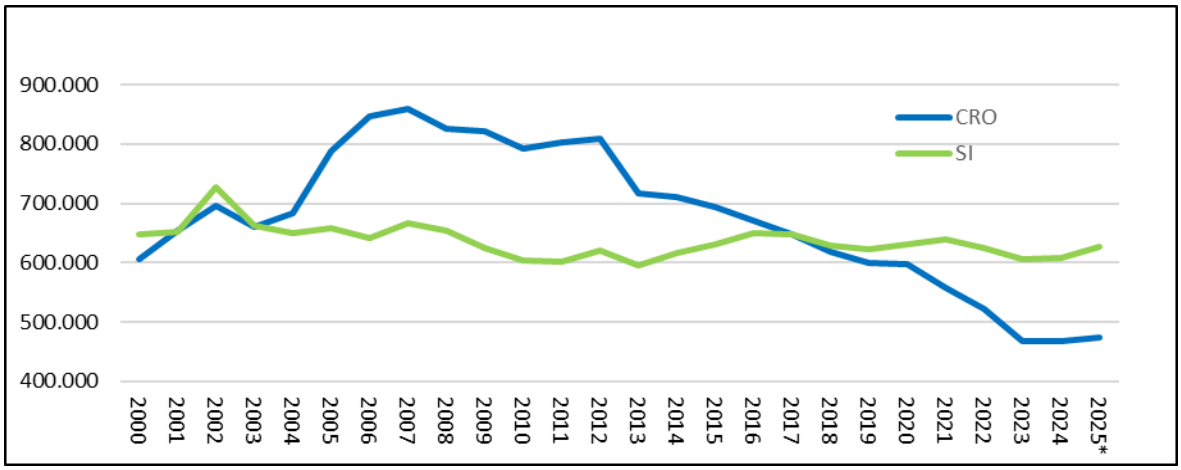
Source: MPŠR, HAPIH

The trend of decreasing the number of dairy farms in Slovenia continued after 2020 (CPZ Govedo, 2026), and the number of dairy cows also decreased. Thus, in 2015, there were over 110 thousand dairy cows in Slovenia, while in recent years there have been barely over 90 thousand (SURS). On the contrary, average milk yield increased. From around 5.500 kg/cow, it increased by 1.300 kg/cow in a decade (SURS), and an even greater increase in milk yield is shown by data from cows in milk yield control (CPZ Govedo, 2026).
The reasons are not only in better management of dairy cow nutrition, selection and changes in breed structure, but also in the fact that dairy cows, which were characterized by lower milk yield, are no longer in breeding (Zeleno poročilo, 2022).



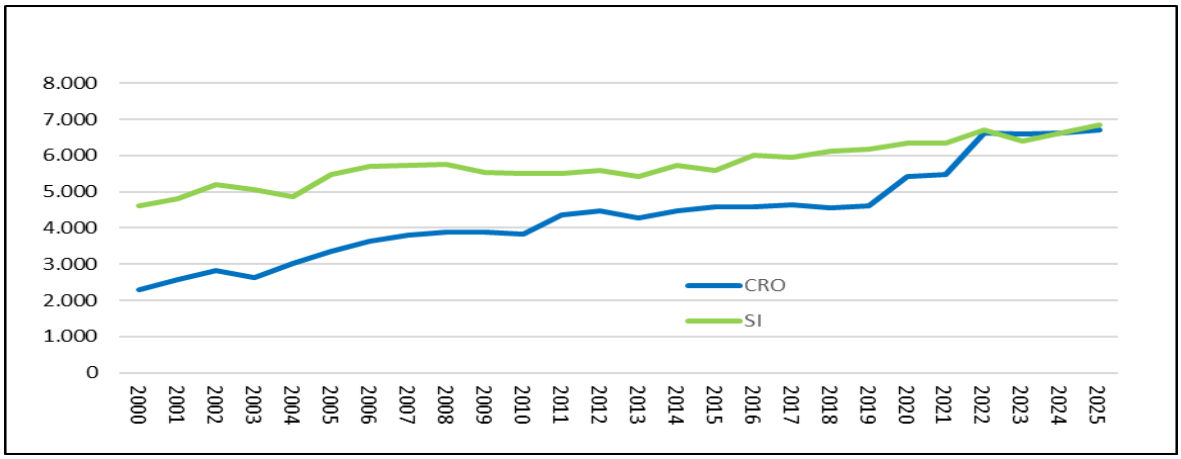
Graph 2. Number of dairy cows; Croatia*, Slovenia, 2000-2025
* estimation for period 2021-2025
Source: SURS (SI), DZS (CRO)

The number of dairy cows in Croatia has more than halved since 2000, when there were 262,200. In 2020, there were only 109,800, which is around 10 thousand more than in Slovenia, and in 2021 the number of dairy cows was similar to that in Slovenia. A significant drop in the heifer population occurred in 2022, when there were only around 79 thousand dairy cows in breeding, and in 2025 there were only around 71 thousand.



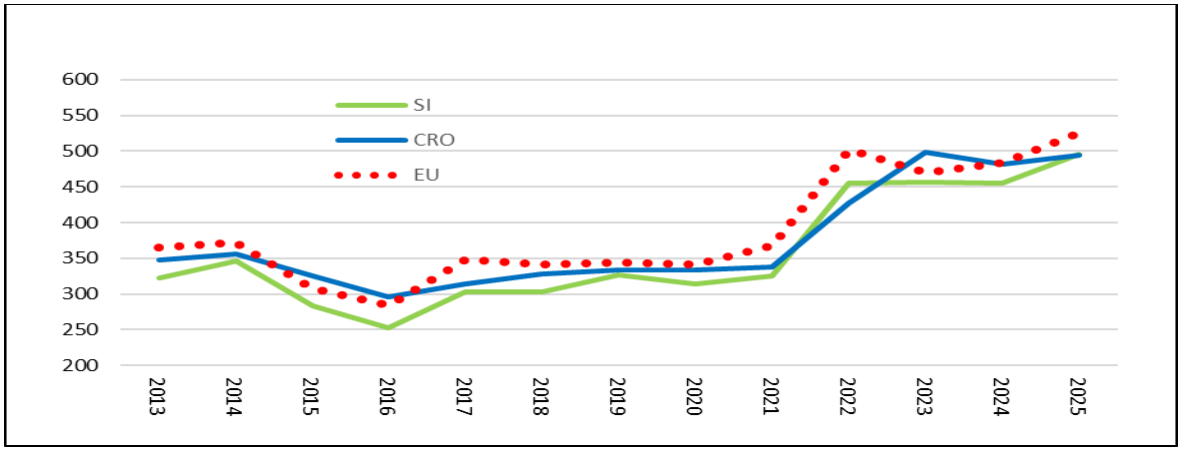
Graph 3. Milk production; Croatia, Slovenia, 2000-2025 (ton)
* estimation for year 2025
Source: SURS (SI), DZS (CRO)

In line with the decrease in the number of dairy cows, significant changes in Croatia are also reflected in the amount of produced milk. In 2000, it amounted to 605 thousand tons, and just under 25 years later, it was 468 thousand tons (in 2024), or 23% less. Since the decrease in the total amount of produced milk is less intense than the decrease in the number of dairy cows, it means that the average milk yield has been increasing rapidly. The calculation for year 2000 shows an average milk yield of 2.300 kg/dairy cow, while in 2024 it is almost 3 times more. Similar to Slovenia, during the initial period of recording dairy cows, there were numerous cows from subsistence farms, where milk production was at a level typical of suckler cows. Also Croatia, the increase in average milk yield clearly confirms the intensive structural changes. Smaller farms have abandoned milk production for various reasons. In addition to economic reasons, this is certainly also a consequence of generational leaps. In Croatia, the contraction of the so-called small family farms is more noticeable than in Slovenia.



Graph 4. Average milk yield; Croatia, Slovenia, 2000-2025 (kg/dairy cow)
* estimation for period 2021-2025
Source: SURS (SI), DZS (CRO)

Prices of raw milk in Slovenia and Croatia are flexibly adapting to the conditions on the European dairy market, but prices are significantly influenced by regional markets, especially in Italy. Milk prices in Croatia have been consistently higher than in Slovenia, but in both countries they are below the European average.



Graph 5. Price of raw milk - delivered; Croatia, Slovenia, EU, 2013-2025 (EUR/ton)
Source: Market Information System, EC

Slovenia is a net exporter of milk and dairy products. The foreign trade balance has averaged around 150 thousand tons over the past decade. Despite occasional fluctuations, the volume of foreign trade is increasing and, according to the latest available data, amounted to 711 thousand tons in 2024.

Table 2. Foreign trade with milk and dairy products, self-sufficiency; Slovenia, Croatia

SLOVENIA	**2020**	**2021**	**2022**	**2023**	**2024**
Export (000 tons)	400.115	418.944	395.517	405.775	424.624
Import (000 tons)	244.289	248.192	238.046	263.114	285.897
Self-sufficiency (%)	133,2	135,5	133,9	130,7	129,0
CROATIA	**2020**	**2021**	**2022**	**2023**	**2024**
Export (000 tons)	61.045	56.751	62.175	58.867	65.390
Import (000 tons)	262.403	276.382	298.156	311.788	325.103
Self-sufficiency (%)*	60	55	48	40-42	43-45

* estimation
Source: SURS (SI), DZS (CRO)

In contrast, Croatia is a net importer of milk and dairy products. The trade deficit in 2020 amounted to just over 200 thousand tons, and in the period 2021-2024 it increased due to increasing imports of milk and dairy products, which coincides with a noticeable decline in domestic milk production. Exports of milk and dairy products ranged between 60 and 66 thousand tons in 2020-2024.

Conclusion

Changes in the dairy sector in Slovenia and Croatia have taken place at different rates. The economics of milk production are certainly a reason for the abandonment of production on small farms. In Slovenia, the process has been slower, which can probably be attributed to socio-economic and infrastructural factors, such as employment opportunities outside agricultural activities, short distances to market routes, etc. In Croatia, by contrast, milk production is increasingly concentrated and based mainly on large agricultural enterprises. The observed structural changes, in particular the decrease in milk production on smaller farms, are likely the result of a complex interaction of economic pressures, the political environment and socio-demographic factors.

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ORIGINAL SCIENTIFIC PAPER

Economic sustainability of family farms - Empirical analysis

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Summary
Modern agriculture in the Republic of Croatia is undergoing structural changes affecting the competitiveness of family farms. The purpose and goal of this paper is to examine how these changes shape the perception of economic sustainability of family farms. The results show that farm size is not a decisive factor, as no significant correlation was found with sustainability, while a statistically significant moderate correlation was identified between involvement in agribusiness chains and economic sustainability ($r = 0.328$; $p = 0.004$). This indicates that market integration plays a key role in income stability. Strengthening cooperation and agribusiness partnerships is essential for future agricultural policy and rural income stability.

Keywords: economic sustainability, agricultural holdings, agribusiness, structural changes

Introduction

Agriculture plays a significant role in the Croatian economy. The agricultural sector in Croatia has been undergoing significant structural changes in recent years. Structural changes refer to the reduction in the number of farms as well as the increase in their average size. Such a process results in a higher level of specialization and increasing involvement in agribusiness value chains.

The supply value chain in agribusiness represents a network of interconnected actors and activities (from input suppliers, producers, processors, distributors to end consumers), they are involved through the flows of materials, information and finance with the aim of delivering agricultural products to the end user (Dza, 2024). Furthermore, it is a complete system, which includes inputs (seeds, fertilizers, equipment), production, processing, logistics and distribution, market and consumers.

As they state Grgić et al. (2019), the low level of association of farmers in producer organizations makes it difficult for them to access agribusiness chains, which makes family farms more vulnerable to market price fluctuations and income instability.

Family farms continue to face challenges of economic sustainability, unstable incomes, as well as dependence on state aid. Small farms face lower levels of competitiveness and are vulnerable to turbulent market changes. Furthermore, additional aggravating circumstances are rising costs related to energy, raw materials and labour costs. Greater involvement in agribusiness systems (buyers, manufacturing industry) can contribute to higher revenues, lower costs and better market position, and thus greater competitiveness.

According to Zrakić Sušac and Grgić (2020), it is crucial for the long-term survival of Croatian agriculture to achieve economic sustainability, especially for small family farms that form the backbone of the rural area.

It is important to look at how farmers themselves perceive the impact of structural changes on the economic sustainability of their farms. The modern concept of farm management implies the application and understanding of basic managerial functions in business. Young farmers are becoming key carriers of innovation, and with investment in technology and digitalization, good foundations are being created for efficient business and long-term sustainability.

Although Croatia owned significant natural resources, transition processes and accession to the European Union have imposed strict market criteria on family farms that often threaten their economic survival (Franić and Kumrić, 2012). On the other hand, family farms have been given the opportunity to use grants from the Rural Development Program and thus improve their business. However, despite the availability of funds from the Rural Development Programme, many small farms still face the problem of low productivity and difficult access to supply chains (Baćanac, 2021).

According to available data, there were about 233,000 farms in Croatia in 2010, and by 2020 this number had decreased to approximately 162,000, representing a decline of nearly 30%. However, according to the Agency for Payments in Agriculture, Fisheries and Rural Development, the number of registered agricultural holdings in the Register of Farmers amounted to 170,837, indicating certain differences between statistical and administrative data sources. Despite these discrepancies, both sources confirm a clear trend of structural consolidation, characterised by a declining number of farms alongside an increase in their average size (Eurostat, 2024; Croatian Bureau of Statistics, 2023; APPRRR, 2024).

Considering these trends and challenges, there is a need for a more detailed empirical study of the relationship between the structural characteristics of economies and their economic sustainability. The aim and purpose of this paper is to examine the perception of the impact of structural changes on the economic sustainability of family farms, i.e. to examine the correlation between family farm size, involvement in agribusiness and the level of economic sustainability in the context of the observed decrease in the number of farms and the process of their consolidation in the last decade.

Overview of the structure of agricultural holdings in the Republic of Croatia

To better understand the topic, it is important to have an insight into the official data on the structure of agriculture in Croatia. Official data state that Croatian agriculture is characterized by fragmentation of estates and the dominance of family farms.

Table 1. Structure of agricultural holdings in the Republic of Croatia by used area

Category (ha)	Number of holdings	Percentage (%)
< 2	52.410	31,5
2 – 5	48.120	28,9
5 – 10	25.330	15,2
10 – 20	16.480	9,9
>20	24.160	14,5
TOTAL:	**166.500**	**100**

Source: Croatian Bureau of Statistics, 2023.

Table 1 shows why the size of holdings is not decisive as the observed variable in this study (because most farms belong to the category of small, under 5 ha), so their sustainability cannot be based solely on economies of scale.

Similar observations are made by the authors Mikuš et al. (2020) and say that small producers in the Republic of Croatia face sales problems regardless of physical capacity.

The importance of knowing that the competitiveness of the economy in modern conditions also depends on the quality of management, and not exclusively on the area, is also stated by Gugić et al. (2021).

Since the official data according to the Croatian Bureau of Statistics (2023) results from Table 1. They show that more than 60% of farms in Croatia are less than 5 ha, the results of this research have practical value. They indicate that the sustainability of Croatian agriculture should be based on strengthening the market positions of small producers through joint market

appearances. A similar perspective is reflected in European agricultural policy (Eurostat, 2022). Integration into agribusiness chains, i.e. vertical connection with buyers and the manufacturing industry, proves to be a significant predictor of economic sustainability. It is observed that there are still many farms that face problems of low technological equipment and difficult access to capital (Ministry of Agriculture, 2022).

Material and Methods

The analysis and research are based on primary data collected through an online survey questionnaire created in a Google Form. The survey was available in the period from February 1st to 28th 2026. and was intended for owners and members of farms in the Republic of Croatia. The research was conducted on a sample of 76 farms. Participation was anonymous and voluntary. The questionnaire contained 10 questions that included the general characteristics of the economy (size, years of business), perception of structural changes, economic sustainability and the role of agribusiness. The results of the research provide insight into perceptions and trends among respondents. For the purpose of analysing the correlation between the size of the holding and economic viability, the key questions from the questionnaire were used, which refer to: (1) the size of the farm expressed through the total utilised agricultural area (in hectares), (2) the assessment of the economic viability of the holding measured by the Likert scale from 1 to 5 and (3) the perception of the impact of involvement in agribusiness chains on income stability, also measured by the Likert scale. These variables were used in further statistical analysis to examine their interconnection.

Most of the claims were rated using a Likert scale of 1 to 5. Statistical processing was carried out in the JASSP program, and descriptive statistics and spearmen correlation were used to examine the correlation between the size of the farm, the importance of linking in agribusiness chains and perceived economic sustainability. The Spearmen correlation was chosen because the data was also collected using the Likert scale, which means that they are ordinal in nature and do not have to follow a normal distribution. Since this test does not depend on strict mathematical assumptions, it allows for a more reliable analysis of the correlation between attitudes and actual indicators on the holding and allows for a more precise determination of the correlation of attitudes between the ranked variables. As part of the research, the following hypothesis was put forward:

H1: There is a correlation between farm size, involvement in agribusiness chains and economic viability.

To test the hypothesis, Spearman's correlation coefficient was used, given the ordinal nature of the data. The level of statistical significance is set at $p < 0.05$.

The farm size variable was measured through categories of total utilised agricultural area (expressed in hectares), while economic viability was assessed using a Likert scale from 1 to 5, while involvement in agribusiness chains was measured using claims assessed using the Likert scale, related to cooperation with buyers and market integration.

Results and Discussion

In Table 2, the results of Spearmen's coefficient (p) between farm size, involvement in agribusiness chains and perceived economic sustainability are presented. Looking at the variables, the analysis shows that there is no significant correlation between farm size (ha) and inclusion in agribusiness chains ($r = 0.097$; $p = 0.406$), as well as between farm size and economic viability ($r = 0.042$; $p = 0.717$). Consequently, the results suggest that the physical size of the holdings in this sample alone does not play a key role in how integrated the economy is in market chains or how respondents assess their long-term viability.

The key finding of the research is a statistically significant correlation of moderate intensity between involvement in agribusiness chains and economic viability ($r = 0.328$; $p = 0.004$). Since

the p-value is less than 0.01, it can be confirmed with a high level of reliability that the farms that are more involved in purchases and processing (agribusiness chains) are also those that see their economic future as safer and more sustainable.

Table 2. Spearman's correlations

Variable		1. Farm size (ha)	2. Involvement in agribusiness chains	3. Economic sustainability
1. Farm size (ha)	Spearman's rho	—		
	p-value	—		
2. Involvement in agribusiness chains	Spearman's rho	0.097	—	
	p-value	0.406	—	
3. Economic sustainability	Spearman's rho	0.042	0.328**	—
	p-value	0.717	0.004	—

* p < .05, **p < .01, ***p < .001

Consequently, the hypothesis (H1) has been partially confirmed. The correlation between involvement in agribusiness and economic viability was confirmed (the result is 0.328 with p = 0.004), which is statistically significant and important, however, the correlation between farm size and other variables was not confirmed (because the values of p = 0.406 and 0.717, which is higher than the limit of 0.05), which assumes a positive correlation between farm size, involvement in agribusiness chains and economic viability. This finding indicates that integration into market chains has a greater impact on the perception of long-term sustainability than the physical size of the property itself.

Conclusion

The conducted research provided insight into the connection between the key structural characteristics of farms and their long-term economic sustainability. Based on the statistical analysis and interpretation of the results obtained, the following conclusions can be drawn: that the size of the farm is not a decisive factor of sustainability, then that the key role of agribusiness chains is that it indicates that integration into market flows provides farmers with a greater sense of security and more stable incomes. Following the results obtained, the initial hypothesis of the research was partially confirmed. Although the size of the economy did not play a role, the importance of market coupling proved to be a key factor. The contribution of the paper is manifested in pointing out the importance of vertical connection within the agri-food sector. A strict conclusion is imposed that future agrarian policies should put more emphasis on encouraging associations and strengthening cooperation between primary producers and the processing sector. This ensures income stability regardless of the fragmentation of agricultural land and strengthens the long-term sustainability of domestic farms.

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PRELIMINARY COMMUNICATION

Internal control systems: The case of Naše Klasje d.o.o.

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Summary
An internal control system ensures reliable financial reporting, asset protection, fraud prevention, and compliance with legislation. The framework was developed by the Committee of Sponsoring Organizations of the Treadway Commission, and the 2013 Integrated Framework became an international standard. The aim of this paper is to present the practical application of internal control within the Croatian food industry using the example of Naše Klasje d.o.o. which uses ERP (SAP Business One) and EDI systems, automation and the standardisation of postings increase transparency and reporting accuracy, with further potential improvements through the formalisation of internal control strategies.

Keywords: COSO, corporate governance, financial reporting reliability, accounting information systems, financial transparency

Introduction

The modern business environment is characterised by constant change, global competition, and complex business processes. In this context, the internal control system ensures reliable financial reporting, safeguards assets, prevents fraud, and maintains compliance with laws and regulations. The Committee of Sponsoring Organisations of the Treadway Commission (COSO) defined a key framework for internal control processes in its 2013 Integrated Framework document, which is widely recognised as an international standard in this field. According to COSO, internal control is a process conducted by the board of directors, management, and other employees, designed to provide reasonable assurance regarding the achievement of objectives in three key areas: operational effectiveness and efficiency, reliability of financial reporting, and compliance with applicable laws and regulations (COSO, 2013).

Organisations do not implement internal control as a one-time effort; rather, it is a continuous process comprising daily activities and tasks. The purpose is not for internal control to represent the ultimate objective; rather, it serves as a means of achieving the broader range of the organisation's strategic and operational aims. The human factor represents a key component.

Research by Yuliyanto et al. (2023), conducted at PT ABC President Indonesia, demonstrated that a coordinated approach through the five components of the COSO framework contributes to the accuracy of financial reporting, risk reduction, and greater business transparency. Ong'ang and Nyamita (2014) examined the impact of internal control systems on the financial performance of small and medium-sized enterprises. Their findings confirm that implementing internal controls has a positive, significant effect on financial performance, despite challenges related to a lack of technical personnel and financial resources, as well as insufficient knowledge of such systems among business owners. Akinyomi (2009), focusing on the banking sector, concludes that effective internal control systems play a crucial role in preventing and detecting fraud. Wu and Zeng (2022) highlight the importance of balancing investment in research and development with other investments, and of carefully managing internal control systems to maximise enterprise value. Alawaqleh (2021) investigated the impact of internal

control on employee performance in small and medium-sized enterprises in Jordan, emphasising the important mediating role of the Accounting Information System (AIS).
Collectively, these studies confirm that effective internal control systems, tailored to the specific characteristics of individual sectors and organisations, represent a key factor in improving financial performance, preventing fraud, managing risks effectively, and enhancing employee performance. They are an indispensable component of modern corporate governance (Grubišić, 2025).
The aim of this paper is to present the practical application of internal control within the Croatian food industry using the example of Naše Klasje d.o.o.

Material and Methods

The 2013 COSO internal control model consists of five interrelated components, based on 17 principles. This framework is applicable to organisations regardless of their size, activity, or legal status. The first component is the control environment. It sets principles of integrity and ethics, ensuring the reliability and effectiveness of the control system. It includes developing a culture of integrity, safeguarding supervisory independence, defining organisational structures, managing human resources, and providing ongoing employee training.
The second component is risk assessment. The organisation must focus on understanding potential threats at all levels of its operations. This allows it to identify and evaluate factors that could negatively affect its effectiveness and stability.
The third component consists of control activities. These include a series of specific measures implemented to mitigate previously identified risks and maintain business stability. Their function is to ensure the implementation of management's strategic and operational decisions through clearly defined procedures.
Such actions contribute to achieving set objectives. Information and communication represent the fourth key component of the internal control model. Their significance lies in ensuring timely access to relevant, accurate, and high-quality data necessary for informed decision-making. The final component concerns monitoring and supervision, aimed at ensuring the proper and effective functioning of all system elements. Monitoring may be continuous and integrated into daily activities or conducted periodically. Its key purpose is to regularly evaluate the presence and functionality of all control elements.
Naše Klasje d.o.o. is a Croatian company headquartered in Zagreb, specialising in the production of food products, particularly pasta and cereal-based products. The company was established as a greenfield investment in 2001. It operates in the manufacturing sector with the aim of creating a modern production facility that combines high-quality raw materials, advanced technology, and high hygiene standards (Direktorij, 2022).
We conducted a semi-structured interview with the head of the company financial management department in February 2025. The goal of the interview was to determine the approaches, challenges, and strategies for implementing internal control systems, as well as the impact of internal controls on the company's operational efficiency.
In addition, the objective was to examine how the financial management department uses the ERP system (SAP Business One) and an EDI system, since these tools help establish and implement internal controls to ensure data accuracy, maintain legal compliance, and safeguard company assets.

Results and Discussion

One of the key control elements in the financial module of the ERP system concerns predefined posting schemes established by the accounting department and integrated into SAP Business One. According to the respondent from Naše Klasje d.o.o., a predefined posting scheme exists for each type of business transaction. This approach enables a high degree of standardisation

and consistency in financial reporting. When entering a business document, the system automatically generates a journal entry in the background in accordance with predefined rules. Users do not have access to or the ability to modify automatically generated journal entries, significantly reducing the risk of errors, manipulation, and subjective interpretation of accounting rules. Such system design represents an example of good internal control practice, as it ensures that all business event records comply with internal accounting policies and legal regulations. Despite the prominent level of automation, the system does not automatically detect content-related errors. For example, even if an incorrect amount is stated on an incoming document, the system will still execute the posting according to the predefined scheme, without warning, validation, or transaction blocking. This highlights the system's limitations in terms of substantive control, as SAP Business One, like most ERP solutions, assumes the accuracy of input data. This also emphasises the need for supplementary control mechanisms, such as subsequent reviews, validation rules, internal control reports, and software solutions to detect exceptions and inconsistencies in postings.

Electronic Data Interchange (EDI) plays a crucial role in enhancing the accuracy and reliability of business processes. The use of the EDI system eliminates the need for manual data entry, as communication takes place directly between the information systems of business partners. This elevated level of automation significantly reduces the risk of operational errors, particularly those arising from incorrect transcription or subjective interpretation of documents.

The EDI system is based on a contractual arrangement with the EDI service provider, and it ensures data security and integrity. Formalised mechanisms and procedures protect data from unauthorised access and modification, representing a key segment of information control within the company. The EDI system integrates with SAP Business One, enabling automated, continuous data exchange with business partners. The accuracy of financial data is further ensured through systematic and regular reconciliation of open items with partners. In practice, statements of account are exchanged to provide a transparent overview of mutual receivables and liabilities. In the event of discrepancies, customer or supplier ledger accounts are exchanged to identify and clarify differences. When discrepancies result from invoicing inaccuracies or settlement procedures, additional supporting documents, such as certified delivery notes or returns, are used to confirm the correctness of the postings.

When discrepancies are identified, the company applies a systematic and proactive approach. A detailed analysis is conducted to determine the source and stage of the error, followed by manual data corrections where necessary.

Within SAP Business One, fixed asset management is supported through defined asset classes linked to accounts and depreciation periods. This enables systematic recording, monitoring, and depreciation calculation, contributing to transparency and accuracy in financial reporting. The company also conducts regular annual stocktakes and detailed inventories of fixed assets, which serve as key control measures to ensure alignment between accounting records and physical assets, thereby safeguarding assets against loss or irregularities. The SAP Business One ERP system for the Croatian market has been adapted to current legislation through local implementation. The system allows the definition of internal policies by parameterising user settings, ensuring compliance with relevant legal norms and transparency in business record-keeping. Specifically, the system has been configured in accordance with tax legislation amendments effective from 1 January 2024, which allow for a reduction in tax liability for uncollected invoices within 6 months, provided that all necessary measures have been taken in accordance with the principle of prudent business conduct. This demonstrates the ERP system's capacity to support the timely implementation of legal changes and lawful financial management. However, the absence of formalised internal control strategies indicates room for further development and implementation of additional procedures for systematic monitoring, evaluation, and continuous improvement of the system.

The interview highlighted that although the company does not face significant difficulties in ensuring the accuracy of financial data, there are currently no formalized strategies in place aimed at continuously improving the precision and reliability of information systems. Nevertheless, the use of ERP and EDI technologies is recognized within the company as a key factor in optimizing financial reporting and control processes, with particular emphasis on the timely correction of posting errors, alignment of business documentation with relevant legal and regulatory requirements, strict oversight of accounting policies, and ensuring the credibility and transparency of financial statements.

The research confirms previous findings that consistent and systematic implementation of internal controls contributes to the accuracy of financial reporting, reduces the risk of fraud and irregularities, and maximizes company value (Yuliyanto et al., 2023; Ong'ang and Nyamita, 2014; Akinyomi, 2009; Wu and Zeng, 2022). The importance of the role of Accounting Information Systems in internal control systems was also confirmed (Alawaqleh, 2021).

Conclusion

The internal control system plays a crucial role in ensuring the efficiency, transparency, and legality of business operations. The COSO model provides comprehensive guidance for understanding and implementing internal controls, emphasising the integration of the control environment, risk assessment, control activities, information and communication, and monitoring. These components function as a unified system contributing to business stability and long-term sustainability.

Naše Klasje d.o.o. utilises the SAP Business One ERP system and an EDI system as mechanisms of internal control. Process automation, standardised postings, and the elimination of manual data entry reduce the risk of errors, enhance financial reporting accuracy, and improve regulatory compliance. Nevertheless, opportunities for improvement remain, particularly in implementing formalised strategies to continuously enhance internal controls and introduce additional error-prevention mechanisms. The systematic application of internal controls based on the COSO model, supported by modern technological solutions, is an indispensable tool in contemporary corporate governance. It is recommended that companies such as Naše Klasje d.o.o. continue to develop their control processes through formalised strategies and ongoing monitoring of effectiveness in order to ensure long-term sustainability, reduce risks, and further strengthen stakeholder confidence in business operations.

This study is limited by its single-case design and reliance on qualitative data. Future research could include multi-company analyses to provide broader insight into the implementation and effectiveness of internal control mechanisms in the food industry and other sectors.

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PRELIMINARY COMMUNICATION

Competitiveness assessment of typical dairy farms in Slovenia

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Summary
The paper presents an analysis of typical dairy farms in Slovenia. The study examines costs, revenues, and other indicators, and develops a farm competitiveness factor. Thirty-four dairy farms with varying production capacities and milk production intensities were analysed. For econometric modelling, a farm competitiveness factor was defined to represent the current level of adaptability in managing agricultural production costs. By determining the level of sensitivity or the ability of farms to adapt to variable costs, the level of flexibility was established, reflecting the competitive performance of farms in Slovenia. The model also considered the theoretical structure of the milk price within the income of agricultural holdings. The estimated level of flexibility ranged between 1 and 2; the higher the level of competitiveness, the greater the scope for adjusting variable costs, and vice versa. The results are divided into two groups of agricultural holdings, with scores between 1–1.5 and 1.51–2.0. The first group included 22 agricultural holdings, and the second group included 12. The findings provide insights intoa deeper reflection on the ability of agricultural holdings in Slovenia to adapt to milk production, their dependence on changes in the price of milk, and provides general insight into the relationship between the size of agricultural holdings and the level of flexibility.

Keywords: Typical dairy farms, econometric modelling, competitiveness, Slovenia

Introduction

Milk production in Slovenia is one of the leading branches of agriculture, accounting for approximately 17% of the country's total agricultural production (Petkovšek, 2026). However, in recent years, it has faced increasing challenges. The sector is primarily exposed to the volatility of raw milk purchase prices and rising production costs, which affect the income stability and competitiveness of agricultural holdings (European Parliament, 2024; European Parliament, 2023). Given the volatility and instability within the agricultural milk production sector, it is important to understand the concept of agricultural holding competitiveness, which in this paper is defined as the ability of agricultural holdings to adjust production costs in response to certain unfavourable changes in income.
Therefore, we have defined the following three hypotheses, which form the basis of the research idea.
H1: We assume that the competitiveness of agricultural holdings engaged in milk production is influenced by their ability to respond quickly to changes in production costs.
H2: We assume that the competitiveness of agricultural holdings engaged in milk production is influenced by milk prices on the market.
H3: We assume that as production intensity increases, the possibilities for adjusting production costs decrease, while dependence on milk purchase prices increases.

Material and Methods

In this research, we analysed data from the publication Catalogue of Typical Farms 2022, which was produced in cooperation between the Biotechnical Faculty of the University of Ljubljana, the Agricultural Institute of Slovenia, and the Agricultural and Forestry Institute Murska Sobota. The Catalogue of Typical Farms (TKMG) presents farm models that cover the most important sectors of Slovenian agriculture. Typical farms are simplified models of real farms, so the results should be interpreted with appropriate caution. For each sector, farms are presented whose main source of income is the selected production sector. This approach shows the operation of the farm as a whole, not just individual activities carried out on the farm (TKMG, 2022). We analysed 34 farms from the Catalogue of Typical Farms. We summarised data on herd size (number of dairy cows), production intensity (kg of milk), variable costs, revenues, gross value added, own labour force, and budget supplements. The integrated models approach was identified (according to Siniscalchi 2023, as a suitable methodology for this analysis. A model can be classified as integrated in a number of ways. Substantively integrated models encompass a number of different activities within the same analytical framework.Using econometric modelling in IBM SPSS Statistics 20, we tested a integrated model to analyse the impact of selected economic factors on the competitiveness of dairy farms. Integrated models are used to study research problems from an interdisciplinary perspective.

The first model [1] included four independent variables. The dependent variable was the competitiveness factor, and the independent variables were variable costs, production volume (the amount of products produced by the farm in a given period), gross value added, and own labour.

In the second (2+"1") model [2], we added the level of flexibility and the impact of the milk price on the farm's income position. The competitiveness factor represents the ratio of variable production costs to revenue volume. The level of flexibility represents the (elasticity) change in the competitiveness factor relative to the farm's production volume. After reviewing the results, we assessed, using econometric, statistical, and economic criteria, that the most suitable models were logarithmic functions. Model specification can be expressed as:

$$\text{Model 1: } Lny = -3.611 + 0.380\ lnx1 + 0.009\ lnx2 - 0.383\ lnx3 - 0.395\ lnx4 + ut \quad [1]$$

$$\text{Model 2+"1": } Lny = -1.405 + 0.769\ lnx1 - 0.622\ lnx2 - 0.138\ lnx3 - 0.147\ lnx4 - 0.633\ lnx6 + ut \quad [2]$$

It is important to understand that the methodological approach to analysing the research question is not based on a separate comparison of two econometric models developed in parallel, but rather on their mutual complementarity.

Results and Discussion

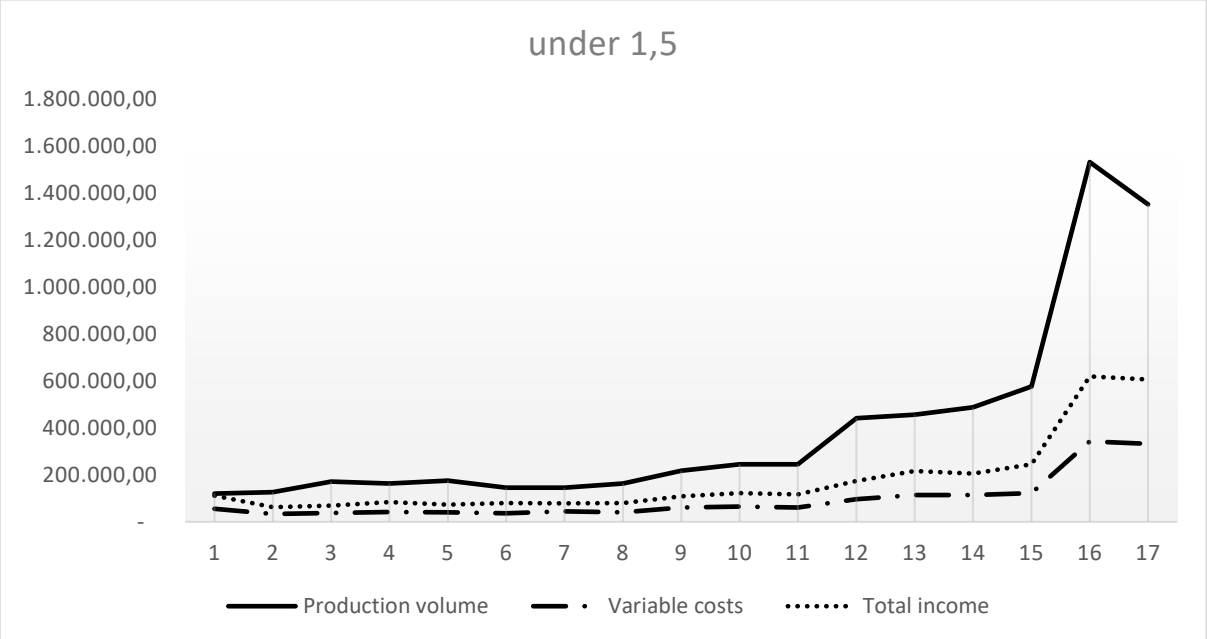
This section presents the results and estimates of regression models 1 and 2, as well as the calculation of the ability of agricultural holdings with milk production to respond quickly to changes in production costs.

In regression model 1, the competitiveness factor is defined as the dependent variable, while the independent variables are variable costs, production volume, gross value added, own labour force, and budget allowance. In model 2, the level of flexibility and the impact of milk price on the farm's income position were added as independent variables. Based on economic and econometric criteria, the most appropriate logarithmic functional form of the model was tested using IBM SPSS Statistics 20. The estimated coefficients of determination ($R^2 = 0.976$ and $R^2 = 0.995$) indicate an extremely high level of explanatory power for the model. The high R^2 values indicate strong explanatory power; however, given the small sample size and potential

multicollinearity, results should be interpreted with a small restraint. The high correlation is understandable given the dependence between the volume of agricultural production and the increase or decrease in costs, revenues, and other economic parameters. The t-test value in model 1 (20.670) indicates a statistically significant positive impact of variable costs on the competitiveness factor, while the value in model 2 (−9.785) indicates a statistically significant negative impact of variable costs on the competitiveness factor. These high values confirm a significant relationship between the variables under consideration and that the estimated impact is not due to chance. Significant values of $p < 0.001$further confirm the significance of the model. The independent variables statistically affect the dependent variable and thus significantly contribute to the explanatory power of the model. The Durbin-Watson criterion values (1.851 and 1.956) indicate that there is no serious autocorrelation in the model. This means that the model errors are not systematically correlated, which is an important condition for the validity of the parameter estimates.

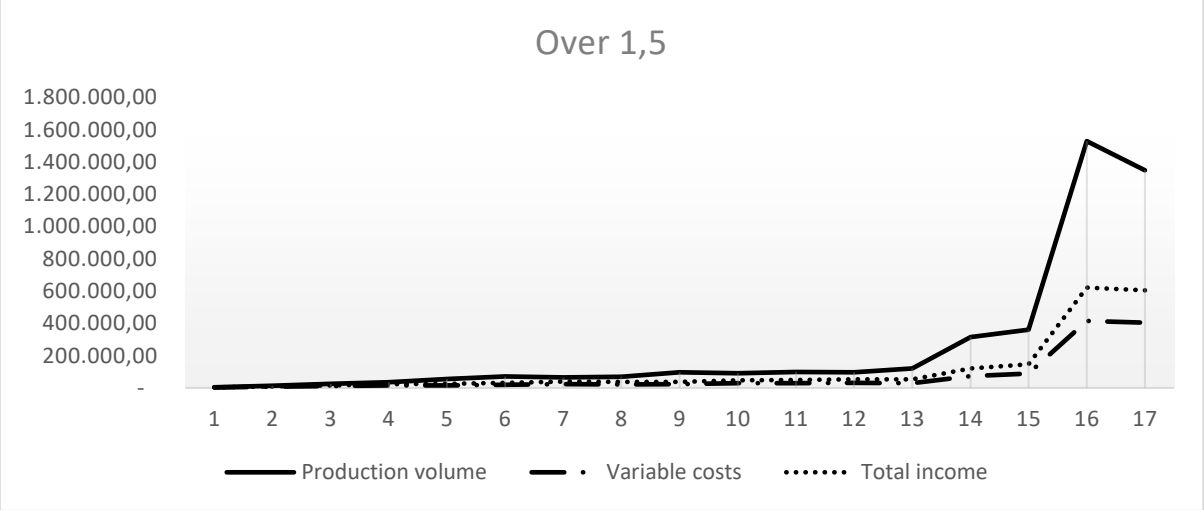
The estimated elasticity in model 1 indicates that a 1% increase in variable production costs leads to a 0.38% increase in the competitiveness factor of the agricultural holding. In model 2, the elasticity shows that a 1% increase in the impact of the milk price on the income position of the agricultural holding results in a 0.633% decrease in the competitiveness factor. The ability to respond quickly to changes in production costs for agricultural holdings engaged in milk production is calculated as the competitiveness factor divided by the level of flexibility. The values obtained for these agricultural holdings range between 1 and 2. We divided the group distribution into those with greater potential (values above 1.5) and those with lesser potential (values up to 1.5).

Smaller-scale farms demonstrate a better ability to respond (value above 1.5) in terms of adjusting production costs to the production capacity of dairy farms. The calculated average income for this group of agricultural holdings is estimated at 112,672€, with an average milk production volume of 258,705.9kg per lactation period. The ability to respond, interpreted as greater flexibility in cost adjustment to production volume, decreases as production volume increases (value below 1.5).



Graph 1. Characteristics of agricultural holdings group with a response value to changes in production costs under 1.5.

The average estimated income for this group of agricultural holdings is 179,183.6€, with an average milk production volume of 397,205.9kg per lactation period. The frequency distribution of agricultural holdings was even between the groups (17 below and 17 above the value of 1.5). Graphs 1 and 2 illustrate a more accurate alignment of cost and income curves with the production volume curve for values above 1.5 compared to those below this value. A smaller spatial gap thus represents greater flexibility in the adjustment of agricultural holdings in the adaptation of agribusiness.



Graph 2. Characteristics of agricultural holdings group with a response value to changes in production costs over 1.5.

The graphical representation of both charts shows the difference in the area of the plot deviation between the production volume and variable cost curves, which is larger in graph 1.

Conclusion

The conclusion of the research outlines the complex logic of the analysis, which assumes greater competitiveness of the agricultural sector if the share of variable costs in the structure of agricultural production increases. From the perspective of production stability and the stabilisation of conditions, such a generalised approach to arguing for the competitiveness of the agricultural sector is difficult to accept. However, it allows smaller agricultural holdings to control production costs and provides a detailed explanation of the limited competitiveness of intensive agricultural production. This analysis also adds value by demonstrating that as production intensity increases, the ability to adjust production costs decreases, while dependence on purchase prices increases. The conclusion ends with an analysis of the hypotheses presented.

H1: We assume that the competitiveness of dairy farms is influenced by their ability to respond quickly to changes in production costs.

Hypothesis 1 is confirmed. The results show that a 1% increase in variable production costs leads to a 0.38% increase in the competitiveness factor of an agricultural holding. This suggests that agricultural holdings which adapt efficiently and promptly, based on their ability to respond to changes in costs (for example, by optimising production, adopting new technology, or improving resource management), demonstrate a higher level of competitiveness. The result confirms the assumption that flexibility in cost management is an important factor in the competitiveness of agricultural holdings engaged in milk production.

H2: We assume that the competitiveness of dairy farms is influenced by milk prices on the market.
Hypothesis 2 can be confirmed. The results show that a 1% increase in the impact of milk prices on the income position of the dairy farm results in a 0.633% decrease in the competitiveness factor. This means that the income position of dairy farms in Slovenia is moderately dependent on fluctuations in milk purchase prices on the market. Farms that are more exposed to price fluctuations and have fewer opportunities to diversify their income or optimise their production are more vulnerable and less competitive.
H3: We assume that as production intensity increases, the possibilities for adjusting production costs decrease, while dependence on milk purchase prices increases.
Smaller farms have a better ability to respond to changes in production costs (farms with an average income of €112,672 and an average volume of 258,705.9 kg of milk per lactation period).
The ability to respond to changes in production costs decreases as production volume increases (this includes farms with an average income of €179,183.6 and an average volume of 397,205.9 kg of milk per lactation period).
The general summary of the research explains the greater dependence on milk purchase prices and the reduced flexibility in optimising production costs in extensive production, while in intensive production it encourages the search for solutions and opportunities for faster adaptation to the optimisation of production costs in the dairy sector. This is possible primarily on certain, mostly larger, typical agricultural farms that regularly employ external labour (according to TKMG, 2022). In any case, such an approach to optimising costs at the labour level offers an opportunity to improve the model by seeking solutions to upgrade the methodology, taking opportunity costs into account.

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REVIEW ARTICLE

Income stabilisation or structural adjustment? CAP and farm size dynamics in Croatia

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Summary
The paper examines whether the Common Agricultural Policy (CAP) induced structural adjustment or primarily stabilised income in Croatian family farms. Using official statistics and FADN data, changes in farm number, average size and income composition during 2014–2020 are analysed. Results indicate continuous farm exit and limited land consolidation, while direct payments account for a dominant share of income. CAP functioned mainly as an income stabilisation mechanism, with gradual and selective structural adjustment.

Keywords: CAP, family farms, structural adjustment, farm income, Croatia

Introduction

The Common Agricultural Policy (CAP) has historically been justified as a stabilising instrument in a sector characterised by production risk, price volatility and structural rigidities (Tracy, 1996). However, direct payments are primarily designed to stabilise farm income rather than directly stimulate production or structural change, although their structural effects remain heterogeneous across the EU (Lillemets et al., 2022). EU-wide evidence confirms a persistent decline in farm numbers alongside gradual land concentration over the past decade (Lowder et al., 2021; Eurostat). Nevertheless, structural adjustment trajectories differ significantly between older member states and the newer EU member states of Central and Eastern Europe. In agricultural systems characterised by fragmented land distribution and small-scale dominance, adjustment processes tend to be slower and strongly path-dependent, reflecting institutional legacies and historical ownership structures (OECD, 2025). Empirical evidence from several post-socialist CEEC member states further demonstrates that dual farm structures and inherited organisational patterns significantly condition post-accession restructuring dynamics (Swinnen, Van Herck and Vranken, 2009). Croatia's accession to the EU similarly induced notable shifts in its agricultural markets, with crop production expanding relative to livestock sectors (Kranjac et al., 2020). These findings suggest that the impact of CAP instruments cannot be understood independently of initial ownership structures and farm size distribution.

Among CEEC countries, the Czech Republic represents a particularly illustrative case of a post-socialist agricultural system with a pronounced dual structure and significantly larger average farm size. Its post-accession consolidation trajectory provides a useful comparative benchmark for assessing the Croatian experience within the same CAP framework.

Croatian agriculture is numerically dominated by family farms operating under structural limitations related to small average size, limited capital accumulation and demographic ageing. Under these conditions, the role of CAP income support requires closer examination. CAP income support influences farm structure through two main channels. In this paper, structural adjustment is understood narrowly as changes in farm size, while income stabilisation refers to the role of direct payments in buffering income variability. Income buffering may reduce exit pressure and prolong the survival of marginal farms, while liquidity enhancement may enable

investment and productivity-driven restructuring. The structural trajectory depends on which channel dominates under given institutional and ownership conditions.
This paper therefore examines whether the 2014-2020 CAP period in Croatia resulted primarily in income stabilisation or in measurable structural transformation of family farms. It is hypothesised that during this period the stabilisation effect of direct payments dominated their investment effect, leading to gradual farm exit and limited structural consolidation.

Material and Methods

The analysis is based on secondary data from the Croatian Bureau of Statistics (CBS), the Farm Structure Survey (FSS), Eurostat and Farm Accountancy Data Network (FADN). The period 2014-2020 represents the first full CAP programming cycle in which Croatia participated as a Member State, allowing assessment of policy effects under relatively stable institutional conditions. Farm number trends were derived from CBS annual statistics, while structural changes in utilised agricultural area were assessed using FSS 2013 and Agricultural Census 2020 data. Economic indicators are based on FADN three-year averages (2018-2020) to reduce annual volatility. The share of direct payments (primarily decoupled Pillar I income support) in farm income was used as a proxy for income stabilisation. Although this does not allow observation of individual farm behaviour, it is sufficient to identify broader structural trends and patterns of income dependence. FADN data cover commercially oriented holdings and provide harmonised income indicators at EU level, although they do not capture the smallest subsistence units. The approach is descriptive and comparative, focusing on sector-level tendencies rather than micro-level behavioural modelling.

Results and Discussion

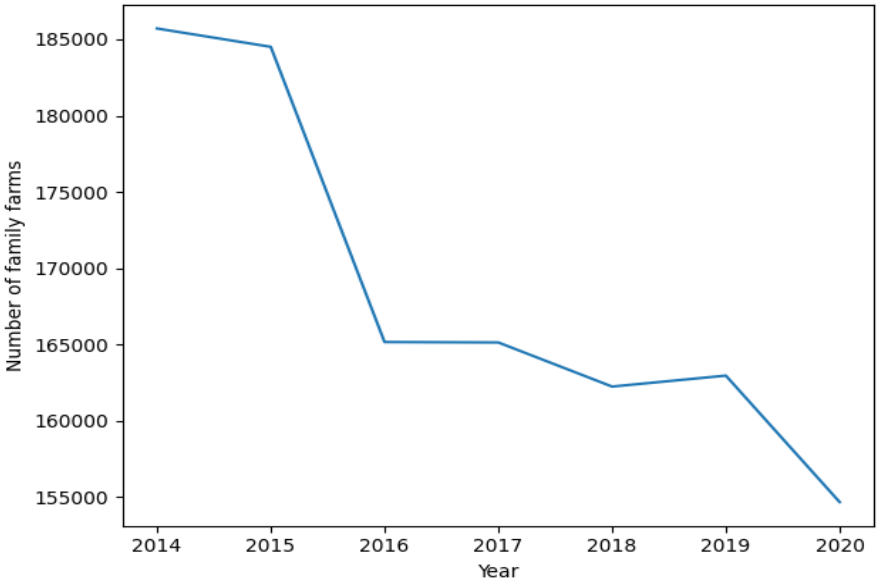
Between 2014 and 2020, the number of family farms in Croatia declined steadily.

Table 1. Number of family farms in Croatia (2014-2020)

Year	Number of family farms	Annual change (%)
2014	185,713	-
2015	184,511	-0.6 %
2016	165,167	-10.5 %
2017	165,139	-0.0 %
2018	162,248	-1.8 %
2019	162,966	+0.4 %
2020	154,679	-5.1 %

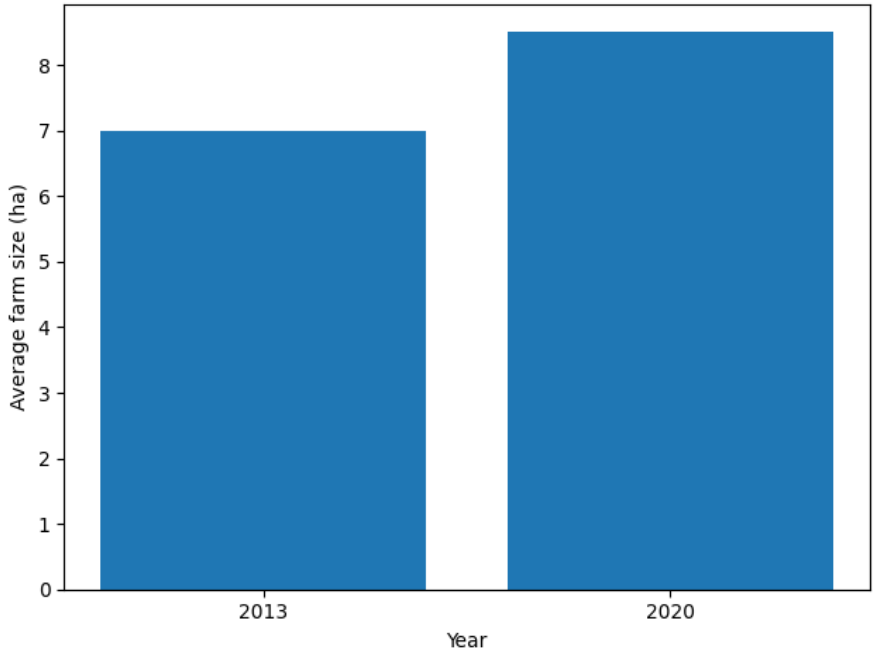
Source: CBS data; author's calculation.

Part of the observed decline reflects administrative adjustments. Therefore, the reported decline should be interpreted as a combination of structural exit and statistical reclassification.
The cumulative decline of approximately 17% indicates a sustained reduction in the number of farms rather than a short-term fluctuation. The decline is particularly pronounced after 2015, indicating continued adjustment. However, the absence of any observable stabilisation phase suggests that CAP income support did not reverse exit dynamics, even if it may have moderated their intensity.



Graph 1. Trend in number of family farms in Croatia (2014-2020).
Source: Croatian Bureau of Statistics (Državni zavod za statistiku), Agricultural Statistics and Farm Register data.

Average farm size increased from approximately 7.0 ha in 2013 to 8.5 ha in 2020.



Graph 2. Average farm size in Croatia (2013 and 2020, ha).
Source: Croatian Bureau of Statistics (CBS), Farm Structure Survey 2013; Agricultural Census 2020.

The observed increase remains modest relative to consolidation magnitudes documented in several older member states, reinforcing the interpretation of gradual rather than transformative structural adjustment (Eurostat). Czechia, by contrast, has consistently reported one of the highest average farm sizes in the EU, exceeding 120 ha per holding in recent structural surveys. In Croatia, however, income transfers cushioned adjustment pressures, while structural change remained limited. In other words, identical policy instruments operated on structurally unequal

starting points. The data suggest statistical consolidation resulting primarily from the exit of smaller units rather than dynamic land restructuring.
FADN results for small farms (low economic size classes, typically below €8,000 standard output) reveal limited economic scale and substantial dependence on direct payments.

Table 2. Economic indicators of small family farms (average 2018–2020)

Indicator	Value
Standard output (€)	6,000
Farm net income (€)	2,363
Share of direct payments in income (%)	77.6
Labour input (hours ha^{-1})	369

Source: Farm Accountancy Data Network (FADN), standard results.

Direct payments account for more than three quarters of income in small holdings. This level of dependence is markedly higher than the EU average, where direct payments typically account for a significantly lower share of farm income (European Commission, 2022; Joint Research Centre, 2023), implying that a substantial portion of farm income in Croatia remains directly tied to policy transfers rather than market-based returns. Such income dependence constrains reinvestment capacity and weakens the link between support and structural upgrading, while its capacity to induce productivity-enhancing restructuring appears limited.
Croatian-specific research shows that participation in rural development measures is uneven and conditioned by demographic and regional factors (Pečurlić et al., 2025), further constraining uniform structural upgrading. In the Croatian context, income transfers appear to have cushioned adjustment pressures, while observable indicators of consolidation and scaling remained limited. The evidence does not suggest a break with existing structural patterns; rather, adjustment seems to have unfolded gradually, primarily through the exit of smaller units. In addition, coupled support schemes continue to play a significant role in sustaining production in sectors such as dairy and beef (Kranjac et al., 2021). This suggests that changes in such payments may affect domestic production levels, although the primary objective of direct payments remains income stabilisation rather than production expansion. Under these conditions, structural weaknesses may be reinforced rather than alleviated.
The findings suggest that structural change materialised predominantly through gradual farm exit, while capital deepening and scale enlargement remained limited.

Conclusion

The results demonstrate a persistent decline in the number of Croatian family farms between 2014 and 2020, accompanied by only modest growth in average farm size. Structural adjustment materialised primarily through gradual exit of smaller units rather than through dynamic consolidation or capital deepening. Income composition data reveal substantial dependence on direct payments, particularly among small farms.
The evidence suggests a clear conclusion: CAP in Croatia operated primarily as an income stabilisation mechanism. This reflects the sector's inherited structural configuration - fragmented land distribution, demographic constraints, and the predominance of small family holdings - which fundamentally framed the scope of adjustment. Consistent with findings for several CEEC countries (Swinnen, Van Herck and Vranken, 2009), post-accession restructuring was shaped less by policy design alone and more by historical ownership patterns.
The Croatian case reflects a broader tension within Central and Eastern European agricultural systems. Income-based CAP instruments can strengthen short-term resilience, yet their transformative potential remains limited where fragmentation and small-scale dominance

persist. This suggests that income support alone is unlikely to drive substantial structural change.

Future policy design needs to more explicitly link income stabilisation with investment incentives, structural consolidation and generational renewal. Whether future CAP reforms will alter this balance remains uncertain, as their effectiveness will ultimately depend less on policy design alone and more on the structural conditions within which they operate.

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REVIEW ARTICLE

Migration turnaround driven by structural demographic changes

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Summary
The Republic of Croatia is facing a persistent demographic crisis characterized by population decline, negative natural increase, and population ageing. The aim of this paper is to analyse demographic trends and migration dynamics in the period 2020–2025, with particular emphasis on the role of immigrant labour and the integration of foreign workers. In 2024, 8.7% of the labour force consisted of workers from third countries, most commonly employed in tourism, construction, industry, and agriculture. The research is based on secondary statistical data, international reports, and a descriptive analysis of the labour market and migration policy.
The results confirm that immigration alleviates labour shortages in the short term, but does not address the structural causes of the demographic crisis. Key challenges include population ageing, the emigration of highly educated individuals, and the need for more effective integration of foreign workers. The synergy of population, migration, and development policies is crucial for the long-term social and economic stability of the Republic of Croatia.

Keywords: migration, demographic trends, labour force, Croatian returnees

Introduction

The Republic of Croatia is experiencing a profound demographic crisis. This is the result of the long-term influence of various destabilising factors of demographic development which, through their interaction over recent decades, have produced significant demographic depopulation, as well as the absence of a clear, long-term and comprehensive strategy for demographic revitalisation (Živić, 2024). More than a decade ago, Wertheimer-Baletić et al. (2014) warned of substantial demographic changes reflected in a rapid population decline and an increasingly pronounced negative natural increase.
The aim of this paper is to analyse demographic trends, the importance of immigrant labour, and the return of Croatian citizens, that is, to define migration dynamics in the Republic of Croatia in the period 2020-2025, with particular emphasis on the integration of foreign workers.
According to the OECD, the Republic of Croatia is among the countries with the fastest population decline within the OECD group, with a decrease of approximately 8% between 2014 and 2024, alongside projections of further decline by 2060.
This trend is largely the result of low fertility rates and high levels of emigration, particularly among young and highly skilled workers (www.oecd.org). During the same period, migration flows have also shifted. After a decade of negative net migration, the Republic of Croatia recorded a positive net migration balance with in 2022, 2023, and 2024, when the number of immigrants significantly exceeded the number of emigrants (www.dzs.hr). However, migration flows are not homogeneous.
The structure of countries of origin, reasons for migration (employment, family reunification, protection), and labour market demands in specific sectors such as tourism, construction, industry, and seasonal agricultural work have all evolved. Notably, only 7.6% of employed persons in Croatia occasionally worked from home in 2024, whereas this trend is increasing across European Union countries, with an average of 13.3% of employees (EU-27) working

remotely at least occasionally, placing the Republic of Croatia below the European average (https://eures.europa.eu/).

Materials and Methods

For the purposes of the analysis, the following sources were used: secondary statistical data from the Croatian Bureau of Statistics on population migration (2020-2024); international reports and publications of the OECD on migration and the labour market in the Republic of Croatia (2024-2025); media and analytical sources on the labour force and foreign workers in key sectors; data from the Ministry of Demography and Immigration; as well as other documents related to immigration policy and legislation of the Republic of Croatia and the European Union.

The methods include descriptive statistical analysis, comparisons across employment sectors, and the presentation of relevant legal and social issues through an analytical narrative.

Results and Discussion

In 2024, more than 1.7 million people were active in the labour market in the Republic of Croatia. The employment rate stood at 65.8%, showing a slight increase (EU-27 average: 70.4%), while the unemployment rate was 6.2% (EU-27 average: 6.1%). In the same year, 89.5% of the labour force consisted of Croatian nationals, 1.9% of citizens from other EU Member States, and 8.7% from third countries (EU-27 average: 10.5% from third countries). Global aging, driven by lower fertility and higher life expectancy, is both a major demographic challenge and a historic human achievement.

Demographic ageing alters the age structure of the population, leading to an increase in both the number and share of older persons, which poses significant challenges for the economy and society as a whole, particularly when the working-age population is simultaneously declining (Čipin, 2024). Table 1 presents an analysis of demographic trends in the Republic of Croatia for the period 2020–2024.

Table 1. Demographic trends in the Republic of Croatia (2020.-2024.)

Year	Population	Absolute Change	Change Index	Average Annual Rate of Relative Change (%)	Natural Increase (per 1,000 inhabitants)	Vital Index (live births per 100 deaths)
2020.	3.914	-	-	-	-5,4	62,9
2021.	3.879	-0,035	99,11	-0,89	-6,8	58,2
2022.	3.856	-0,023	99,41	-0,59	-6,0	59,5
2023.	3.860	+0,004	100,10	+0,10	-5,0	62,7
2024.	3.866	+0,006	100,16	+0,16	-4,9	62,8

Source: Author's calculation based on data www.dzs.hr

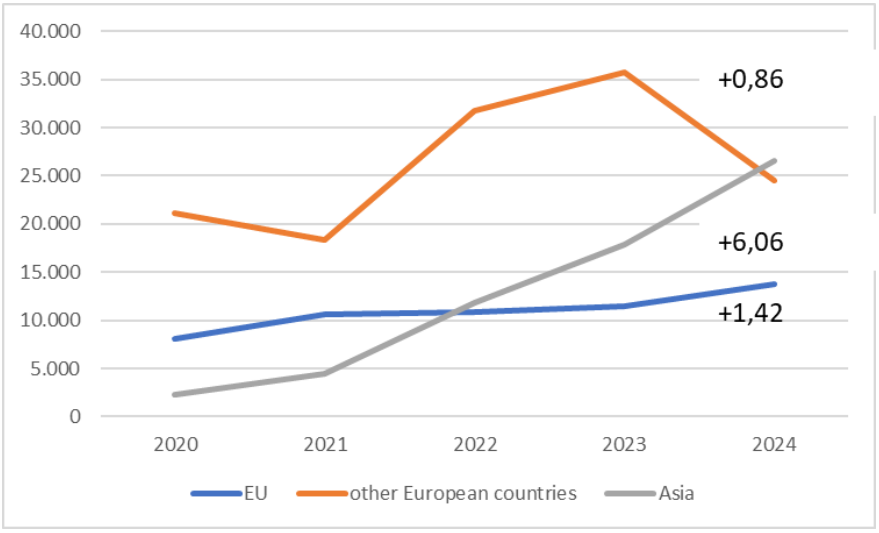
The change population index from 2020 to 2024 is 1.05, while the total absolute change in the number of inhabitants amounts to -0.068. The average annual rate of change (2020–2024) is -0.31. All of the above indicates a long-term negative demographic trend. Such developments are most often the result of negative natural increase and emigration, which inevitably lead to population ageing, a reduction in the labour force, and increased pressure on the pension and healthcare systems.

Table 2. Migration balance in the Republic of Croatia (2015.-2024.)

	Immigrants	Emigrants	Migration balance
2015.	11.706	29.651	-17.945
2016.	13.985	36.436	-22.451
2017.	15.553	47.352	-31.799
2018.	26.029	39.515	-13.486
2019.	37.726	40.148	-2.422
2020.	33.414	34.046	-632
2021.	35.912	40.424	-4.512
2022.	57.972	46.287	+11.685
2023.	69.396	39.218	+30.178
2024.	70.391	38.997	+31.394

Source: www.dzs.hr

The positive migration balance has been continuously increasing (Table 2). An increase in the number of immigrants is also expected in 2025, which would indicate a continuation of the immigration trend. However, significant emigration rates are observed among the young and highly educated population. Many individuals seek higher wages and better career development opportunities abroad, which affects industries such as IT, engineering, and healthcare. Although job seekers find more employment opportunities locally, these positions are usually of lower quality compared to those available abroad. For employers, retaining skilled workers is crucial. As the labour force in the Republic of Croatia decreases, an increasing number of people arrive from neighbouring countries and beyond to fill positions in sectors such as construction, healthcare, tourism, and agriculture. This means that job seekers face greater competition for certain positions in these areas. Employing foreign workers helps employers mitigate the local shortage of qualified personnel without the need to improve working conditions or raise wages. However, bringing in workers from third countries involves a lengthy process of obtaining work permits, as well as providing support to help these workers adapt to the new environment. On the other hand, EU citizens have the same rights as Croatian nationals to work in the Republic of Croatia, with free access to the Croatian labour market. This equal treatment simplifies the hiring process for employers and supports the free movement of workers within the European Union. According to the analysed data, there is an upward trend in the immigrant population from all observed regions between 2020 and 2024. The most significant growth is among immigrants from Asia (annual growth rate +6.06%), primarily from Nepal, India, and the Philippines, representing a recent development in Croatia's migration structure (Graph 1). In 2025, 170,723 residence and work permits were issued to foreign nationals (www.dzs.hr). According to the most recent data and in accordance with the Aliens Act (for the period 1 January to 28 February 2026), a total of 28,437 residence and work permits have been issued. The largest numbers of permits were granted to nationals of the following countries: Philippines (5,648), Bosnia and Herzegovina (4,858), Nepal (4,626), Serbia (2,938), India (2,097), Uzbekistan (1,567), North Macedonia (1,526), Kosovo (898), Egypt (605), and Turkey (575) (www.mup.gov.hr). Policies to attract foreign workers, together with engagement with the Croatian diaspora, can help mitigate the effects of population ageing and address skill shortages. Guiding the selection of foreign workers based on skills forecasts, while simultaneously improving integration services, can help attract the necessary qualifications. Building a diaspora network, supporting job search, and reintegration initiatives can also encourage the return of Croatians from abroad (Banić et al., 2024).



Graph 1. Immigrant population in the Republic of Croatia in the period 2020-2024.
Source: author's calculation based on data www.dzs.hr

Returnees and skilled foreign workers bring new knowledge, experience, and international contacts that stimulate economic growth. Their skills and business networks facilitate the creation of new enterprises, attract investment, and enhance the competitiveness of the Croatian economy in the international market. There are labour shortages in agriculture, particularly in seasonal production, as well as a lack of skilled workers in the manufacturing and service tourism sectors. European Union member states show an annual growth rate of arrivals to the Republic of Croatia of +1.42%. According to data from the Croatian Bureau of Statistics, the most significant arrivals (immigration) come from Germany and Slovenia. Other European countries (annual growth rate +0.86%) show more moderate but stable growth in arrivals, with some fluctuations in individual countries. In this group, the most prominent immigrants are from Bosnia and Herzegovina, although they show a slight downward trend, while Serbia and Ukraine exhibit positive trends. Based on estimates from Croatian diplomatic missions and consular offices, Croatian Catholic missions, as well as censuses in countries where Croatian emigrants and their descendants reside, it is estimated that around 3,200,000 Croatian emigrants and their descendants live outside Croatia and worldwide (https://mdu.gov.hr/). According to estimates from various countries, approximately 1,200,000 Croatians and their descendants live in the United States (37.05%), followed by Germany with around 500,000 Croatians (15.6%) and Argentina with about 300,000 Croatians (9.37%). Croatian emigrants are also significantly represented in Australia, Chile, Canada, Peru, and in European countries such as Austria (around 90,000), Italy (around 60,000), Switzerland (around 80,000), and Sweden (around 40,000), among others. Demographic developments in the Republic of Croatia indicate a critical phase in which immigration temporarily offsets depopulation losses but does not resolve the underlying causes of demographic decline. Without an integrated, long-term, and empirically based strategy that combines population policy, migration management, labour market regulation, and regional development, Croatia will continue to face the cumulative effects of ageing and a shrinking labour base. Sustainable demographic recovery requires simultaneous action in three areas: promoting fertility and family policies, actively and selectively managing immigration with strong integration mechanisms, and creating a supportive socio-economic environment to retain and attract the return of the domestic population (Nejašmić, 2014; Šterc and Komušanac, 2012). Only the synergy of these measures can reverse current trends and ensure the country's long-term demographic and economic stability.

Conclusion

Demographic trends in the Republic of Croatia from 2020 to 2025 confirm the continuation of deep structural imbalances, characterized by negative natural increase, population ageing, and a shrinking working-age population. Despite a positive migration balance since 2022, the overall population decline and the average annual rate of decrease (-0.31%) indicate a long-term depopulation trend. Projections suggest further reductions in the labour force and an increasing share of older adults, placing growing pressure on pension, healthcare, and social welfare systems, potentially slowing economic growth. The rise in immigration, particularly of workers from third countries, addresses labour market needs in tourism, construction, industry, and agriculture. However, migration is largely employment-driven and often temporary, without systematic integration policies and it cannot ensure long-term demographic stability. Key challenges include recognition of qualifications, language adaptation, social inclusion, and retention of domestic, especially young and highly educated, workers. In conclusion, demographic revitalization requires a comprehensive, coordinated approach. Priorities include stronger family and housing policies to support fertility, extending working life and increasing participation of women and older adults in the labour market, active and selective immigration management with effective integration mechanisms, and competitive working conditions to encourage the return of emigrants. Population ageing need not be a constraint - it can drive institutional modernization, innovative social services, and a more sustainable economic model. The synergy of population, migration, and development policies is essential for long-term social and economic stability.

Acknowledgement

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REVIEW ARTICLE

Optimizing the use of pesticides in an apple orchard

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Summary
The project proposal envisages a two-year study in an apple orchard to investigate the influence of technical and technological factors of pesticide application on the main properties of research: Residues of active pesticide substances in the apples, occurrence and spread of pests, fruit quality, and deposition and drift of the spray. The technical and technological factors of the application are the spraying rate, the spray concentration and the nozzle type with its sub-factors.The spraying rate is determined according to the TRV method (tree row volume) and a 50% reduction, while the concentration is determined according to the recommended (FIS registration – Croatian Fitosanitary Information System) and its 20 and 40% reduction. According to the technological map for the protection of an apple orchard (17 pesticide active substances), two types of nozzles are used for spraying: Standard TR and Air-Injector ITR. The residues of the active substances are determined using the liquid chromatography with mass spectrometer (LC-MS/MS) method in an accredited laboratory, according to QuEChERS protocol, while the quality of the fruit and the occurrence and development of the pests are determined in the Faculty of agrobiotechnical sciences central laboratory. The deposit and spray drift are determined using the spectrophotometric method defined by the ISO 22866:2005 standard.

Keywords: sprayer, an apple orchard, pesticide, residues, nozzles, drift, deposit

Introduction

This manuscript is presenting a scientific project funded by the EU funds 581 – Recovery and Resilience Mechanism through the NextGenerationEU platform under the national NPOO program. Therefore, the text represents a two-year scientific project starting on 1 October 2025 in an apple orchard. It explains the reasons for applying the mentioned project, the main research objectives, the research methodology and the expected research results with the application projection.

Intensive and modern agricultural production aims to achieve high apple yields per hectare and to meet all trade standards for classification and standardisation, enabling large producers to access the major EU market. Such fruit often has reduced physiological qualities and contains high levels of pesticide residues. This results from the stringent requirements for first-class apples, which do not permit any scab or spotting caused by plant diseases. Consequently, farmers and agrochemical companies have adopted apple protection protocols involving more than 30 pesticide applications (Tadić, V. et al., 2025). Based on the above examples, we conclude that these production technologies, the apples are loaded with residues of pesticide active substances that exceed the permitted MRLs (maximum residue levels) - non-compliance with phytosanitary quality (Elsavier, 2016, Ahmadi, et al., 2024). Maximum residues of active pesticide substances in apples for consumption and processing are regulated for each substance in precisely defined amounts and can be found in the MRLs EU pesticide database (European Commission database of pesticide MRLs). The MRL control system in the Republic of Croatia, for both domestic production and imports, processes about 500 samples annually. For the

system to be more efficient, this number should be significantly higher (565 samples in 2023; FIS system, 2023).

Various studies have investigated pesticide residues in apples across different regions. Lozowicka, B. and Kaczynski, P. (2011) analysed 220 apple samples from northeastern Poland and found that 56% contained pesticide residues with 95% of cases below the MRLs. The same authors continued similar research (Lozowicka, B. et al., 2025) and analysed 1,223 apple samples, detecting residues in 64.2% of samples and identifying a total of 57 active substances. The fungicide captan and the insecticide acetamiprid were most frequently detected, with concentrations ranging from 0.14 to 5.80 ppm, captan being present at higher levels. Estonian scientists are investigating pesticide residues in potatoes and apples from 18 school canteens (Kutman, A. et al., 2025). No pesticides were found in organic apples and potatoes, or in conventional potatoes, while traces were detected in 94.4% of conventional apple samples, all of which were below EU limits. The most common pesticide was captan, found in 83% of samples, and small amounts of banned pesticides (carbendazim and spirodiclofen) were detected in three samples.

Once applied, pesticides remain in varying proportions in different plant parts, with 15–24.7% remaining on leaves, 0.37–0.58% on fruits, 46.3–60.9% reaching the soil, and 22.5–37.3% being dispersed during application as drift (Wu, Y. et al., 2022). Degradation begins immediately after application, caused by various factors: biodegradation (9.6–64.8%), dilution due to fruit growth (29.8–75.8%), and leaching by precipitation (11.3–47.6%). Research by Hwang, J. I. et al. (2025) shows that different active substances degrade at different rates: tebufenozide 0.2–0.4 days (the fastest), captan 8.6–9.9 days, lufenuron 11.3–16.1 days, and teflubenzuron 24.6–29.3 days. Similar results are reported by Sadlo, S. et al. (2023), who stated that captan degraded the fastest (9–13 days), followed by fluopyram (13 days) and tebuconazole (14 days), while carbendazim had the slowest degradation (24–32 days). Carbendazim requires 152 days after application for the residue in apple fruit to fall below 0.01 ppm, and insecticides applied before 15 July do not leave any residues. Study by Odabas, E. et al. (2024) found pesticides in 82% of samples, while 18% contained no measurable residues. In 9% of samples, concentrations exceeded the maximum permitted levels (MRL), specifically for dimethoate, tebuconazole, captan, and chlorpyrifos (banned in the EU). Tong, J. et al. published similar findings, detecting pesticide residues in 76% of samples, with 14% exceeding the MRL. Multiple residues (two or more pesticides) were found in 58% of positive samples.

In the Republic of Croatia, there is no comprehensive multidisciplinary scientific research on the above-mentioned topic. Empirical results will be obtained by applying current technological maps of plant protection in an experimental design, and, as one of the research outcomes, residues of active substances in fruits will be determined.

Working Hypotheses

The research is designed as a field experiment in an apple orchard, where the influence of technical and technological factors on pesticide application is examined. The technical factors in the study are the nozzle flow rate (Tadić et al., 2014) defined by the ISO standard, and the nozzle type, defined by the spectrum of generated droplets. The technological factors are the spraying rate (Tadić, 2023), determined by the TRV method and its 50% reduction, and the spray concentration: 100% concentration as registered for the pesticide, and its 20% and 40% reductions. The factors to be investigated based on these technical and technological application parameters are: pesticide residues in apple fruits, physiological and pomological quality of apples, the occurrence and progression of pests on leaves and fruit, and the determination of spray deposits in the canopy and drift.

The colour of the nozzles, that is, the ISO number, is adjusted according to the first factor of the trial – the spraying norm – while the nozzle type is the third factor of the study. If the nozzle

has the same ISO number but a different type, this primarily affects the spectrum of droplets produced by the nozzle (Tadić et al., 2024).
Pesticide residues are determined in accredited laboratories, that use standardised certificates and two techniques, LC-MS/MS and GC-MS/MS, in accordance with HRN EN ISO/IEC 17025:2017 (ISO/IEC 17025:2017; EN ISO/IEC 17025:2017), following the QuEChERS protocol (Mladenova et al., 2009; Isharat et al., 2025).
The occurrence and progression of pests during the growing season on fruits and leaves will be used for a standardised evaluation of infection intensity under research and field conditions - EPPO standards, where 0 indicates the absence of symptoms and 5 indicates very pronounced symptoms with more than 75% of the leaf area affected. The observation of the morphological, pomological and physiological characteristics of the fruit will be conducted in the central laboratory of the Faculty of Agrobiotechnical Sciences Osijek.
The last two properties investigated were technical, indicating how much spray reached the canopy and how much spray resulted in drift on the ground and in the air. These two measurements are conducted using a spectrophotometric method with a water-soluble dye of known wavelength, following the ISO 22866:2005 standard (Petrović, 2018; Petrović et al., 2019a; 2019b). The technological map for apple protection (Table 1) includes 18 active substances, and their application is carried out in accordance with all rules and measures specified in the product registration.

Table 1 Technological map of pesticide application

Pesticide	Dosage / Concentration	Type of pesticide^{+}	Time of application*	MRLs mg kg^{-1} (ppm)	No.of applic.	PHI (days)
Pyriproxyfen	40 ml/100 l 0,04 %	Ics	Ae	0,05	1	30
Deltamethrin	50 ml/100 l 0,05 %	Ics	Al/Jne/Jym	0,20	3	7
Tau-fluvalinate	60 ml/100 l 0,06 %	Ics	Ae/Jnm	0,30	2	30
Abamectin	60 ml/100 l 0,06 %	IAcs	Jne/Jye	0,03	2	3
Paraffin oil + Cu (hydroxide)	2 l/100 l 2,00 %	FIAc	Winter spraying	5,00	1	SSTA
Acetamiprid	30 g /100 l 0,03 %	Isy	Ml/Jnl	0,07	2	14
Pirimicarb	60 g/ 100 l 0,06 %	Isy	Mye	0,50	1	14
Ciram	330 g/ 100 l 0,33 %	Fc	Ml/Ae	0,10	2	SSTA
Sulfur	600 g /100 l 0,6 %	Fc	Me/Al /Mym Jnm/Jnl	-	3	14
Captan	180 g /100 l 0,18 %	Fc	Ml/Ae/ Jne/Jnl/Jyl	10,0	7	21
Cu (oxychloride)	200 g /100 l 0,20 %	FBc	Winter spraying	5,00	2	SSTA
Boscalid	80 g /100 l 0,08 %	Fc	Mye/Jnl/Jym	2,00	3	7
Pyraclostrobin	80 g /100 l 0,08 %	Fsy	Mye/Jnl/Jym	0,50	3	14
Azoxystrobin	200 ml/100 l 0,20 %	Fsy	Al/Mym	0,01	2	21
Dodin	125 ml/100 l 0,12 %	Fsy	Ml/Am	0,90	2	60

Pyrimethanil	200 ml/100 l 0,20 %	Fsy	Al/Jnm/Jye	15,00	3	52
Difenconazole	15 ml/100 l 0,01 %	Fsy	Ml/Am/Mye/Jne	0,80	4	35
Kresoxim-methyl	20 g /100 l 0,02 %	Fsy	Mye/Jnl	0,20	2	35

[+]I – Insectcide; A - acarcide; F – fungicide; B – bactericide; c – concact; s – stomach; sy - systemic
*M – March; A – April; My – May; Jn – June; Jy – July; e – early; m – middle; l - late
PHI – pre-harvest inerval; SSTA – safe secured by time of application

Expected results and discussion

Several working hypotheses can be derived from the research objectives:
(1) The current technological map of apple orchard protection with the recommended concentrations and spraying rates controls the development and spread of pests well, but with active pesticide substance residues above the permitted MRL levels. The literature rarely specifies how many times active substances were used during the growing season – most research focuses on food monitoring – or how often pesticides were applied to the orchard, although this is a key factor on which residues largely depend. As already mentioned, it is realistic to expect that, with current protection maps, pesticide residues will exceed the MRLs, which is the main hypothesis of this study. Research by Pogacean et al. (2013) shows that eight applications were made during the growing season, and measurement results indicated that immediately after treatment, initial residue concentrations ranged from approximately 0.1 to 1.4 ppm, depending on the pesticide. Within the first few days, a significant decrease (about 30–50%) occurred due to precipitation leaching, photodegradation, and volatilisation. Further decreases followed first-order kinetics, with calculated degradation half-lives generally between 3 and 7 days, meaning the amount of pesticide was halved within about a week. Until harvest, concentrations decreased continuously and in most samples were below the maximum permissible levels (MRL) or close to the detection limit, with no visible accumulation despite repeated applications during the season. If the input of pesticides, according to the technological map of apple protection in Table 1, is significantly higher than in previous research, it is realistic to expect that the residues will also be significantly higher and that there will be over MRLs.
(2) Pest control with a 20% reduction in concentration and the same or lower spray rates will achieve the same biological effect as the recommended treatments with an efficiency of 70-80% - verified by fruit quality, residues of active substances and pest control indices.
(3) Reducing the spraying rate and the recommended concentration of sprays will also control the pests satisfactorily during the growing season, with the obligation to improve the quality of spraying (different nozzle types and technical settings), but with active substance residues below the MRL levels. This is supported by the fact that smaller droplets achieve greater coverage of the treated surface, therefore the pesticide effect is better, and it is possible to reduce the recommended dose (Tadić, 2024).
(4) Fruit quality is better in treatments where lower amounts of pesticides are sprayed (either spraying rates or concentrations).
(5) Technical factors in spray application are important for spray deposition and drift. Better deposition is achieved with small droplets and TR type nozzles, while drift reduction is achieved with larger droplets and ITR nozzles (Tadić et al., 2014; 2024; Petrović et al., 2018; 2019).

Conclusion

The project will focus on determining the relationship between the main parameters of plant protection and application efficiency, the level of pesticide residues in fruit, crop quality, pest control success and occurrence, as well as the processes of spray deposition and drift. Special emphasis is placed on the use of technologies that apply reduced quantities and concentrations

of active pesticide substances (low-volume approach), in accordance with the FAO guidelines from 2022. The main objectives of this project proposal concern the planned field research under real-time conditions (Real Time Research): a) the impact of agricultural machinery (ISO number and type of nozzle), and b) the impact of crop protection technologies on pesticide residues in fruits of permanent crops; pest occurrence and development during the growing season; fruit characteristics (morphological, pomological, and physiological); and spray deposition and drift.

The plant protection process is highly complex, as even a single factor can significantly affect the success of pesticide application and the amount of active substance residues. Key elements determining effectiveness include the type and characteristics of the sprayer, nozzle type, spray concentration and volume, waiting period, frequency of treatment during the growing season, physicochemical properties of the preparation, crop type, weather conditions at the time of application and throughout the growing season, biological characteristics of the pest, and the method of spray deposition and drift.

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REVIEW ARTICLE

Regulatory asymmetry of fiscalization in cashless operations of agricultural entities

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Summary
This paper analyzes fiscalization in cashless B2C transactions in Croatia, focusing on agricultural entities. Results show that cashless transactions, despite high traceability, are treated as cash transactions, although they carry lower fiscal risk. Comparative analysis of Slovenia, Slovakia, and the Czech Republic shows risk-based approaches focused on cash transactions. Findings indicate a mismatch between fiscal risk and regulatory intensity, resulting in disproportionate administrative burden.

Keywords: fiscalization, regulatory asymmetry, cashless operations, agricultural entities, administrative burden

Introduction

Digital transformation significantly affects business operations across all sectors, including agriculture. The development of information technologies, electronic banking, and digital platforms has led to the increasing use of cashless payment methods in business transactions (OECD, 2021; European Commission, 2022). In such an environment, regulatory systems are expected to follow technological changes and ensure proportionality between the level of fiscal control and the actual fiscal risk. The fiscalization system (i.e., a system of recording and transmitting transaction data to the tax authority in real time) in the Republic of Croatia is regulated by the Law on Fiscalization in Cash Transactions (Narodne novine, 2012a). According to the legal definition, fiscalization represents a set of measures aimed at monitoring transactions realized in cash, where the concept of cash transactions also includes payments made by cards and other similar payment methods, except payments made to a transaction account (Narodne novine, 2012a, Art. 2). Taxpayers subject to fiscalization are required to issue invoices for delivered goods and services (Narodne novine, 2012a, Art. 3) and to include all prescribed invoice elements regardless of the payment method (Narodne novine, 2012a, Art. 9). Within the system, two main models are distinguished – F1 and F2. The F1 model applies to transactions with final consumers (B2C) and includes real-time fiscalization, while the F2 model applies to transactions between business entities (B2B) and does not involve such a level of fiscal control. This regulatory approach is based on the formal status of the customer rather than the payment method, which in practice leads to the inclusion of cashless transactions in the fiscalization system. Cashless transactions are characterized by a high level of traceability, as they are conducted through financial institutions and leave a digital record, significantly reducing the potential for tax evasion (Slemrod, 2019; Alm, 2012). Despite this, in the Croatian system they are subject to the same regulatory treatment as cash transactions, indicating the existence of regulatory asymmetry between the level of fiscal risk and the intensity of regulatory requirements. In contrast, certain European Union Member States, such as Slovenia, Slovakia, and the Czech Republic, apply fiscal control systems primarily focused on cash transactions and sectors with higher fiscal risk, aiming to achieve a better alignment between regulatory requirements and actual risk levels. Although the fiscalization system applies to a wide range

of taxpayers, this paper places particular emphasis on agricultural entities as a specific segment of the economy. Their operations are characterized by seasonality, limited organizational capacity, and an increasing reliance on cashless payments, which makes the impact of regulatory requirements particularly pronounced. In such conditions, additional administrative obligations may significantly affect business efficiency and sustainability. Accordingly, the aim of this paper is to analyze the application of fiscalization in cashless B2C transactions and to identify the existence of regulatory asymmetry between the level of fiscal risk and the intensity of regulatory requirements, with a particular focus on implications for agricultural entities.

Materials and Methods

This study employs a qualitative research approach based on the analysis of the regulatory framework and a comparative analysis of fiscal systems. The primary source of data is the legislative framework of the Republic of Croatia, with a particular focus on the Law on Fiscalization in Cash Transactions (Narodne novine, 2012a), while secondary sources include relevant scientific literature and official documents of international institutions. Content analysis is applied to identify key elements of the regulatory framework, including the definition of cash transactions (Narodne novine, 2012a, Art. 2), the obligations of taxpayers subject to fiscalization (Narodne novine, 2012a, Art. 3), and the requirements related to invoice issuance regardless of the payment method (Narodne novine, 2012a, Art. 9). Based on these elements, an analytical interpretation of the relationship between payment methods and regulatory requirements is conducted. The comparative analysis includes selected European Union Member States (Slovenia, Slovakia, and the Czech Republic), focusing on their systems of transaction recording and fiscal control. The comparison is based on available official sources and legislation, with the aim of identifying differences in regulatory approaches and levels of fiscal control. The concept of regulatory asymmetry is used as the analytical framework to assess the alignment between the level of fiscal risk and the intensity of regulatory requirements. Particular emphasis is placed on cashless transactions, which, due to their high level of traceability, represent a lower fiscal risk but are subject to the same regulatory requirements as cash transactions within the Croatian system. A limitation of the study arises from the fact that the analysis is based on the regulatory framework and secondary sources, without the application of quantitative methods. Nevertheless, this approach provides insight into the key characteristics of the regulatory system and their implications for business operations, particularly in the context of agricultural entities. The analysis is conducted through a comparison of key elements of fiscal systems (scope of application, transaction recording mechanisms, and regulatory focus), whereby differences in the treatment of cashless transactions between B2C and B2B segments are identified.

Results and Discussion

The results of the conducted analysis clearly indicate the existence of regulatory asymmetry in the application of the fiscalization system in the Republic of Croatia, particularly in the segment of cashless transactions. It is found that the fiscal treatment of transactions is not primarily based on the level of fiscal risk, but rather on the formal classification of the customer (B2C or B2B), which leads to different regulatory requirements for transactions that are, in essence, equally transparent and traceable. According to Article 2 of the Law on Fiscalization in Cash Transactions (Narodne novine, 2012a), the concept of cash transactions also includes payments made by cards and other similar payment methods, thereby including cashless B2C transactions within the fiscalization system. In the context of cashless operations, where transactions are conducted through financial institutions and leave a clear digital trail, such an approach results in additional administrative burden for certain groups of taxpayers, particularly in the B2C

segment. At the same time, B2B transactions, although equally recorded within the financial system, are not subject to the same level of fiscal control.

To further contextualize these findings, a comparative overview of selected European fiscal systems is presented. The analysis includes Slovenia, Slovakia, and the Czech Republic, which apply different approaches to transaction monitoring and fiscal control (Financial Administration of the Republic of Slovenia, 2015; Financial Administration of the Slovak Republic, 2019; Ministry of Finance of the Czech Republic, 2016). The analyzed systems are primarily focused on cash transactions and risk-based control mechanisms, reflecting the need to combat tax evasion in higher-risk sectors. Such a comparison provides a clearer understanding of the specific features of the Croatian model in relation to other European practices. Nevertheless, certain regulatory justifications for the existing system may exist, including the need to combat tax fraud, ensure a unified control system, and address limitations of existing technical solutions. However, in the context of cashless transactions, where the level of traceability is already high, such justifications are not sufficiently supported in relation to the actual level of fiscal risk.

Table 1. Comparative overview of fiscal systems

Country	System	Scope	Payment logic	Regulatory focus
Croatia	Fiscalization (F1/F2)	B2C and B2B	Not payment-dependent	Customer status
Slovenia	Fiscal cash registers	B2C (cash transactions)	Cash-focused	Transaction risk
Slovakia	eKasa (VRP)	Sales recording	Broad recording	Control of transactions
Czech Republic	EET	Sales recording	Cash-focused	Risk sectors

As shown in Table 1, most of the analyzed systems are primarily focused on cash transactions and apply fiscal control mechanisms based on transaction risk assessment. In contrast, the Croatian fiscalization system is not exclusively linked to the method of payment, but rather based on the formal classification of the customer. Such an approach results in a specific regulatory framework that deviates from prevailing European practices.

Table 2. Comparison of F1 and F2 Fiscalization in Cashless Operations

Criteria	F1 (B2C)	F2 (B2B)
Payment method	Transaction account	Transaction account
Cash flow	None	None
Fiscalization requirement	Yes	No
Fiscal cash register	Mandatory	Not required
Administrative burden	High	Lower
Financial effects	More pronounced	Limited

The presented comparison clearly shows that differences in fiscal treatment do not arise from the method of payment, but from the formal classification of the customer. In both analyzed cases, transactions are conducted through a transaction account and are fully recorded within the financial system, implying a high level of transparency and traceability. Despite this, B2C transactions are subject to real-time fiscalization, including the use of fiscal cash registers and additional technical requirements, while B2B transactions are not subject to the same regulatory framework. This confirms the existence of regulatory asymmetry between the level of fiscal risk and the intensity of regulatory requirements. Such an approach results in an increased administrative burden for taxpayers in the B2C segment, without clear justification in terms of reducing tax risk. In the context of agricultural entities, which often operate with limited resources and an increasing reliance on cashless payments, this asymmetry may have a particularly pronounced negative impact on business efficiency and sustainability. The findings indicate the need to reconsider the existing regulatory approach and to adapt the fiscalization

system to contemporary forms of digital business, with a stronger alignment between the level of fiscal control and the actual fiscal risk. The costs of compliance with the fiscalization system include the acquisition of fiscal equipment, digital certificates, intermediary service fees for data transmission, and increased accounting costs due to additional administrative requirements. In addition to financial costs, the system also generates a significant time burden associated with administrative and digital obligations, which is particularly pronounced for small agricultural holdings without employees. Despite recent legislative amendments, the fundamental logic of the fiscalization system remains unchanged, as the obligation to fiscalize transactions is still based on the formal classification of the customer rather than the method of payment, meaning that the identified regulatory asymmetry has not been eliminated.

Conclusion

The conducted analysis confirms that the application of the F1 fiscalization model in cashless B2C transactions represents a regulatorily unjustified and disproportionate approach to fiscal control. It is established that such transactions are carried out through financial institutions and are inherently transparent and traceable, thereby significantly reducing fiscal risk by the very nature of the payment method. Despite this, the F1 model imposes real-time fiscalization requirements, including the use of fiscal cash registers and additional technical obligations, resulting in increased administrative and financial burdens for taxpayers. At the same time, comparable cashless B2B transactions (F2 model) are not subject to the same regulatory regime, clearly indicating inconsistency and the existence of regulatory asymmetry. The comparative analysis of European systems further confirms that most countries focus fiscal control on cash transactions and higher-risk sectors, while cashless transactions are not subject to equally intensive forms of control. This approach enables a better alignment between the level of fiscal control and the actual fiscal risk.

The findings are particularly relevant for agricultural entities, which operate with limited resources and an increasing reliance on cashless payments, making them more vulnerable to the negative effects of additional regulatory requirements on efficiency and competitiveness. In conclusion, the results clearly indicate the need to redefine the existing fiscalization model in the Republic of Croatia, particularly in relation to cashless transactions. Introducing a regulatory approach based on the actual assessment of fiscal risk, rather than on the formal classification of the customer, could contribute to reducing administrative burdens while maintaining the effectiveness of tax control. Although the study does not include quantitative measurement of effects, the findings are grounded in a normative and comparative analysis of the relevant regulatory framework.

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PRELIMINARY COMMUNICATION

Analysis of employer perceptions of agricultural students' readiness for the labor market

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Summary
This paper analyzes employer perceptions of agricultural students' readiness for the labor market. The research was conducted on a sample of 30 employers in Croatia using a structured questionnaire. Employers evaluated the importance of selected competencies and their level of development among students. The results indicate that technical knowledge is generally well developed, while practical, generic, and organizational competencies remain less developed. The largest gaps were identified in communication, problem-solving, adaptability, and organizational skills. The findings confirm a mismatch between educational outcomes and labor market expectations and highlight the need for stronger practical training and better alignment of curricula with sectoral demands.

Keywords: agriculture, competencies, employability, labor market, students

Introduction

Graduate employability in agriculture is increasingly shaped by structural changes within the sector, which challenge the traditional relationship between education and work. While disciplinary knowledge remains a necessary foundation, it is no longer sufficient to ensure effective integration into the labor market. Employers increasingly expect graduates to demonstrate not only technical competence, but also the ability to apply knowledge in practical situations, communicate effectively, and adapt to changing work environments.

Employability can be understood as a multidimensional concept that extends beyond formal qualifications. It reflects the capacity of graduates to translate acquired knowledge into functional performance within specific organizational settings. The notion of an employability gap is particularly relevant, referring to the discrepancy between competencies expected by employers and those demonstrated by graduates (Noel and Qenani, 2013). In agriculture, this gap is most often manifested through the imbalance between theoretical preparation and its practical application.

Empirical studies consistently indicate that graduates are generally well prepared in terms of theoretical knowledge, but less equipped with practical experience and transferable skills. Graham (2001) emphasized that employers tend to value applied competencies more strongly than purely academic knowledge. Similarly, Van Der Zanden and Reinert (2009) highlighted the importance of communication and problem-solving skills, particularly in early career stages. More recent research confirms that these discrepancies persist (Ghimire et al., 2022).

Formal education should provide certain knowledge, skills and competences that correspond to social, personal and cultural needs in the labor market. The success of the real sector is reflected in the conditionality and mutual functioning of the education system and the labor market (Sudarić et al., 2022; Sudarić et al., 2024.). These findings are not limited to international research, but are also evident in studies conducted within the Croatian context. Research conducted within the Croatian context provides additional insight into the relationship between higher education and employability in agriculture. Studies focusing on student perceptions

indicate that graduates often recognize discrepancies between acquired knowledge and labor market expectations, particularly in relation to practical competencies and employment opportunities (Lončarić et al., 2015). Furthermore, research on agricultural study programs highlights the need for stronger alignment between educational content and labor market demands, especially in the context of employability of agronomy graduates (Zrakić and Grgić, 2015). In addition, structural analyses of the labor market point to limitations in employment opportunities for agronomy graduates, emphasizing the importance of adapting educational outcomes to sectoral needs (Grgić et al., 2017).

At the same time, the agricultural sector is undergoing gradual but significant transformation. The increasing integration of digital technologies, data-driven decision- making, and sustainability requirements is reshaping competence demands. Seti et al. (2025) interpret these changes within the framework of the fourth industrial revolution, where hybrid competence profiles become increasingly relevant.

European policy frameworks further emphasize the importance of green and digital competencies (European Commission, 2022). In the Croatian context, these challenges are intensified by structural characteristics of the agricultural sector and labor shortages. Recent research also highlights the need for stronger alignment between educational and labor market expectations (Parrella et al., 2024). The aim of this study is to examine employer perceptions of agricultural students' readiness for the labor market and to identify gaps between required and developed competencies.

Material and Methods

The empirical research was conducted in 2025 on a sample of 30 employers operating within the agricultural sector in the Republic of Croatia. The sample included respondents from different subsectors, such as crop production, livestock farming, horticulture, and agribusiness. The intention was not to achieve statistical representativeness, but to capture indicative patterns across diverse organizational contexts. Data were collected using a structured online questionnaire administered to employers in the agricultural sector. The questionnaire included evaluation of the importance of selected competencies and assessment of their level of development among students. A five-point Likert scale was used (1 = lowest level, 5 = highest level). Competencies were grouped into five categories: technical, practical, generic, digital, and organizational. This classification was applied for analytical purposes, acknowledging that in practice these competencies often overlap.

Data analysis was conducted using descriptive statistical methods, including mean values. In addition, a gap analysis was applied to identify differences between expected and actual levels of competencies. Given the exploratory nature of the study and the relatively small sample size, the analysis focused on descriptive indicators rather than inferential statistical methods. Although the sample size is limited, the objective of the study was not statistical generalization but the identification of prevailing patterns in employer perceptions. The consistency of responses across different competency categories suggests that the results reflect broader tendencies rather than isolated opinions.

Results and Discussion

The assessment of graduate employability is increasingly understood as a function of the alignment between educational outcomes and labor market expectations, particularly in sectors characterized by structural change (Ghimire et al., 2022; Sudarić et al., 2024). Results indicate a consistent discrepancy between the perceived importance of competencies and their level of development among students. Employers rated all competencies as highly important, with mean values above 4.0, suggesting that modern agricultural work requires a broad and integrated competence profile.

Table 1. Importance and level of competencies (N=30)

Competency	Importance	Level	Gap
Technical	4.6	4.1	-0.5
Practical	4.5	3.3	-1.2
Generic	4.4	3.2	-1.2
Digital	4.2	3.4	-0.8
Organizational	4.3	3.1	-1.2

Source: author's calculation

In order to provide a clearer interpretation of the results, it is necessary to further specify the structure of the analyzed competencies, as defined within the questionnaire. Technical competencies included knowledge related to production technologies, plant protection, soil management, and understanding of core agricultural processes. Practical competencies referred to the ability to apply agrotechnical measures, perform operational tasks in real production conditions, and adapt theoretical knowledge to specific field situations. Generic competencies encompassed communication skills, teamwork, problem-solving, and critical thinking. Digital competencies included the use of precision agriculture tools, basic data handling, and digital technologies applied in agricultural production. Organizational competencies referred to planning, coordination of tasks, time management, and understanding of production economics. The smallest gap is observed in technical competencies, indicating a solid theoretical foundation. However, larger gaps in practical and generic competencies suggest limited readiness for real work environments. These findings are consistent with previous research (Armoogum and Ramasawmy, 2016; Ghimire et al., 2022) and are also supported by findings from Croatian studies, which indicate that students often face difficulties in translating acquired knowledge into practical competencies (Lončarić et al., 2015; Zrakić and Grgić, 2015). These findings also reflect broader structural challenges in the employment of agronomy graduates identified in Croatian research (Grgić et al., 2017). Organizational competencies show the lowest level of development, while digital competencies show moderate gaps. This reflects ongoing changes in agriculture and increasing demand for broader skill sets (Seti et al., 2025). Overall, students are perceived as moderately prepared, but not fully ready for independent work. This aligns with EU policy directions emphasizing the importance of green and digital competencies (European Commission, 2022).

Conclusion

The study confirms the existence of a gap between educational outcomes and labor market expectations in the agricultural sector. While technical knowledge is well developed, practical, generic, and organizational competencies remain insufficient. Improving graduate employability requires a shift toward competence-oriented education, with greater emphasis on experiential learning and real-world application of knowledge. Strengthening cooperation between universities and employers could further support alignment with labor market needs. The results should be interpreted as indicative rather than representative due to the limited sample size. However, their consistency and alignment with existing research suggest broader relevance. These findings support recent research emphasizing the need to integrate employability skills into agricultural education and continuously adapt curricula to sectoral demands.

This study has several limitations that should be acknowledged. The sample size is relatively small and not statistically representative, which limits the generalizability of the findings. In addition, the results are based on employer perceptions, which may introduce subjective bias. Future research should include larger and more diverse samples, as well as combine employer and student perspectives. Further studies could also apply inferential statistical methods to deepen the analysis of competency gaps.

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REVIEW ARTICLE

Autonomous Harvesting and Picking Robots in Agriculture: An Overview of Technologies and Applications

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Summary

This paper delves into the application of robots in agriculture for harvesting and picking purposes. Harvesting and picking robots are machines designed to automate the process of harvesting and picking agricultural produce. The paper explores the history of automation in agriculture, the essential components for robot operation, including the platform, sensors, cameras, computers, and manipulators. It also delves into the principles of robot operation and provides examples of robots employed for harvesting and picking, such as Harvest CROO Robotics, Sweeper, Metomotion, Tevel Robots and Tortuga AgTech. The paper concludes that while utilizing robots in agriculture leads to time and resource savings, the initial investment costs are substantial.

Key words: robots, automation, sensors, cameras, manipulators, picking, harvesting

Introduction

The development of technology, particularly with regard to functional capabilities and cost-effective integration, is advancing at an accelerated rate. Robotics enables human operators to circumvent hazardous tasks, while contemporary robotic systems have the potential to foster economic growth, enhance quality of life, and improve operational efficiency (Christensen et al., 2021). Droukas et al. (2023) emphasize that the advancement of robotic technologies and their implementation in agricultural practices has become an increasingly prominent subject of scholarly interest and consideration, with a marked increase in research endeavors observed over recent decades. This phenomenon aligns with an emerging trend in agriculture, commonly designated as precision agriculture or low-carbon-footprint agriculture. Precision agriculture is founded upon data-driven decision-making processes in crop cultivation, wherein management decisions are underpinned by accurately acquired information derived from satellite remote sensing, aerial or terrestrial surface mapping, soil chemical analyses, the integration of GPS-enabled agricultural machinery, and related technological modalities (Zimmer et al., 2016). Harvesting robots constitute automated agricultural machinery employed to facilitate the mechanized collection and reaping of diverse agricultural commodities, including fruits, vegetables, cereals, cotton, and analogous produce. The integration of automation technologies significantly mitigates agricultural labor shortages while concurrently enhancing harvest quality, operational efficiency, picking accuracy, and processing throughput. In the context of global demographic expansion and rising population demands, the advancement of both the quantity and quality of food production systems has become imperative. Furthermore, the implementation of automated solutions in agricultural operations exerts a positive influence on environmental sustainability, promotes resource conservation, and contributes to the reduction of associated operational costs.

Beyond harvesting and reaping applications, robotic systems are increasingly deployed across multiple agricultural domains. These include autonomous platforms for site-specific fertilizer

application, robotic weeding systems for selective weed eradication, specialized harvesters for vegetable crops, citrus-picking robots, mushroom-harvesting systems, and automated fruit sorting and grading machinery. Such diversification underscores the expanding role of robotics in optimizing precision agriculture practices and supporting sustainable food production frameworks. Agricultural robots represent complex integrated systems that employ a synergistic combination of heterogeneous technologies to autonomously execute field-based operational tasks (Droukas et al., 2023). These robotic platforms utilize multimodal sensor arrays and computer vision systems to facilitate navigation, environmental perception, and the identification of mature produce. Furthermore, robotic manipulators—commonly referred to as articulated arms—are employed to perform delicate harvesting operations, ensuring precise fruit detachment while minimizing mechanical damage and preserving product integrity. Popović (1996) defined a sensor as a transducer device that converts a measurable physical quantity into an electrical signal, characterized by compact dimensions, superior technical specifications, and integrated signal processing capabilities. Furthermore, the author emphasizes that sensors enable robotic systems to perceive and interpret their operational environment by detecting and discriminating among diverse contextual elements—such as soil properties, vegetation characteristics, meteorological conditions, and physical obstacles. These sensory inputs facilitate the acquisition of environmental data, which subsequently undergoes computational processing to inform motion planning algorithms, thereby generating navigational trajectories and orchestrating sequential operational directives for autonomous robotic execution.

Computing system

The onboard computing unit processes sensor-derived data to generate navigational trajectories and orchestrate the execution of robotic operational tasks. Through dedicated software frameworks, the robotic system is programmed with functional modules governing locomotion, navigation, manipulation, and inter-device communication protocols. The Global Positioning System (GPS) enables high-precision geospatial positioning via satellite-transmitted radio frequency signals for pseudorange measurement, facilitating terrestrial georeferencing and route planning (Jurišić and Plaščak, 2009). Fundamentally, the objective of navigation systems is to enable efficient trajectory planning from an origin point (A) to a destination point (B) via the most optimal path. Modern GPS receivers are capable of storing hundreds of geospatial coordinates, commonly designated as *waypoints*, which serve as reference markers for autonomous navigation sequences. Furthermore, according to Link 5. indicate that the Real-Time Kinematic (RTK) positioning method is employed to enhance the accuracy of Global Navigation Satellite Systems (GNSS). The RTK architecture (Figure 1 left) operates through a differential correction framework comprising a fixed base station situated at a precisely known geodetic reference point and a mobile rover receiver positioned at an unknown location. The RTK system functions by first enabling the base station to compute its nominal position, subsequently calculating systematic measurement errors—such as atmospheric delays, satellite clock drift, and orbital inaccuracies—and transmitting these correction data in real time to the mobile receiver such as harvesting with EtaRob (Figure 1. right). This differential processing significantly reduces positional uncertainty, enabling centimeter-level localization accuracy essential for precision agricultural applications and autonomous robotic navigation.

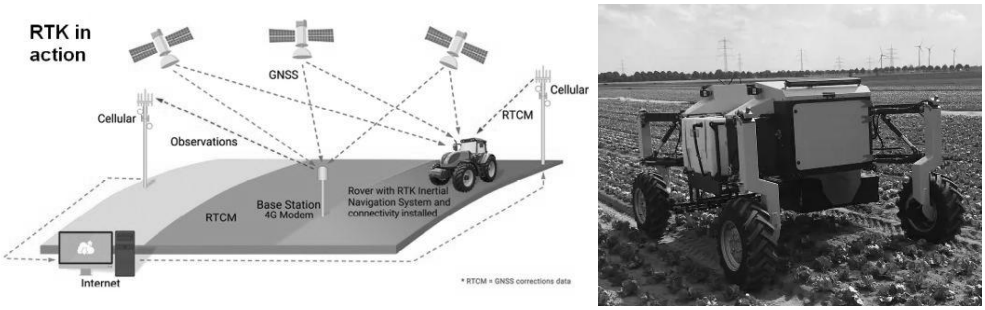

Figure 1. Schematic Representation of the Real-Time Kinematic (RTK) Positioning System (Source: Link 1.) (left), EtaRob in harvesting with RTK navigation (Source: Link. 4.)

Implementations of various agricultural harvesting and reaping robots

The Harvest CROO Robotics platform represents an autonomous combine harvester specifically engineered for the selective harvesting of strawberries (*Fragaria × ananassa*). The system is capable of independent navigation along predefined trajectories through strawberry cultivation plots. The robotic architecture comprises modular subsystems, including dedicated computer vision units, multimodal environmental sensors, and a specialized end-effector manipulator designed for the delicate detachment and collection of ripe strawberries. The integrated vehicle platform operates autonomously along crop rows within agricultural parcels, following optimized path-planning algorithms. A Light Detection and Ranging (LiDAR) system is embedded within the vehicle chassis, generating high-resolution three-dimensional point clouds of the terrain and canopy structure. This spatial data facilitates robust (Fig 2.) row-following capabilities, obstacle avoidance, and precise intra-row navigation, thereby enhancing operational reliability and minimizing crop damage during harvesting operations (Harvest CROO Robotics LLC, 2022). The SWEEPER system (Fig 3.) constitutes an autonomous robotic platform designed for the selective harvesting of sweet pepper fruits (*Capsicum annuum*). The SWEEPER technology optimizes horticultural production systems by streamlining harvesting operations through the implementation of automated robotic solutions, thereby reducing labor dependency and enhancing operational efficiency. The visual perception subsystem employed by SWEEPER integrates a hybrid imaging architecture combining a Red-Green-Blue (RGB) color camera with a Time-of-Flight (ToF) depth-sensing camera. Both modalities utilize a shared optical assembly and sensor chip, thereby generating synchronized RGB-D (color-depth) imagery that facilitates simultaneous colorimetric analysis and three-dimensional spatial reconstruction. This custom-designed camera prototype was specifically engineered for the SWEEPER platform by the Swedish corporation Fotonic. To mitigate the adverse effects of variable ambient illumination conditions, the robotic system is equipped with purpose-built, high-intensity LED lighting modules powered by rechargeable battery systems. These illumination units ensure consistent and uniform lighting conditions for image acquisition, thereby maintaining imaging quality during both diurnal and nocturnal operations. With appropriate maintenance protocols, regular cleaning procedures, and daily battery recharging cycles, the system is designed to sustain operational functionality for up to 20 hours per day. The maturity assessment of detected peppers is performed through a computational analysis of two discriminative features for each fruit: (1) colorimetric characterization, quantified as the mean hue value within the detected fruit region; and (2) morphological shape descriptors, expressed as the circularity ratio (perimeter²/area). Following successful harvesting, the robotic manipulator deposits the collected fruits onto a conveyor belt system for subsequent transportation and storage (Wageningen University and Research, 2019).



Figure 2. Harvest CROO Autonomous Strawberry Picker
(Source: Growing produce, 2018)

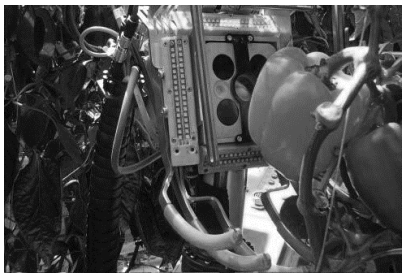

Figure 3. Actuation and Grasping Mechanism of the SWEEPER Autonomous Harvester
(Source: Wageningen University & Reasearch, 2018)

Tevel Aerobotics Technologies (2020) reports that the autonomous aerial robotic system developed by Tevel (Figure 4 left) is operated through sophisticated guidance algorithms and advanced flight control mechanisms that ensure operational precision, enhanced maneuverability, and continuous real-time data acquisition pertaining to the harvested fruit. According to Link 2. the system architecture comprises a central ground-based platform that serves as both the command hub and primary power distribution unit. This stationary base station is interconnected with a swarm of up to six unmanned aerial vehicles (UAVs), each equipped with a specialized end-effector mechanism designed for the selective harvesting of designated fruit crops. The tethered configuration enables sustained operational endurance through continuous power supply from the central platform, while simultaneously facilitating bidirectional data transmission and coordinated swarm intelligence protocols for optimized harvesting efficiency. MetoMotion (Israel Innovation Authority and Trendlines Group) develops Greenhouse Robotic Worker platform (Fig 4 right), designed for automated harvesting of greenhouse crops and analytical data processing to optimize yield. The system employs AI for 3D perception, advanced path-planning algorithms, and adaptive motion control enabling precise navigation among plants. During tomato harvesting, the robot assesses fruit ripeness based on user-defined color criteria using integrated sensors and cameras, while two automated arms allow simultaneous picking from both sides of the row.



Figure 4. Robots Tevel (left), (Source: Tevel Aerobotics Technologies, 2020.) and MetoMotion (Right), (Source: MetoMotion)

Tortuga AgTech is an automated robot (Fig 5.) designed for harvesting strawberries and grapes. AgTech states that the robot is primarily designed for harvesting but can also collect weather data, treat plants with UV-C light, and perform pruning. According to Tortuga AgTech (2024), the robot utilizes a combination of sensors, cameras, and artificial intelligence (AI) to autonomously assess fruit ripeness, along with a robotic arm for harvesting.



Figure 5. Tortuga AgTech robot line (left) and picking (right) (Source: Link 3.)

Robotic manipulators are essential components for executing precision agricultural tasks, particularly selective harvesting. The articulated manipulator arm enables the dexterous handling of target objects, including crop produce, agricultural implements, and agrochemical applicators. Furthermore, the integration of task-specific end-effectors onto the manipulator significantly enhances both operational accuracy and process quality during field interventions.

Table 1. Type of manipulators

Tip	Description	Advantages	Limitations	Applications
Serial (open-chain)	Joints connected in an open kinematic chain; each joint supports the load of subsequent links	Large workspace, high dexterity, simpler kinematics	Cumulative positioning error, lower stiffness, higher inertia	Industrial assembly, welding, harvesting
Parallel (closed-chain)	Multiple kinematic chains connecting the base and end-effector	High stiffness, precision, fast response, load capacity	Limited workspace, complex kinematics, singularities	Flight simulators, precision machining, surgery
Hybrid (parallel–serial)	Combination of serial and parallel substructures	Balance between workspace and stiffness, modularity	More complex control and calibration	Large-component machining, mobile robotics

Conclusion

The transition from manual and mechanized harvesting to robotic systems represents a critical trajectory in modern agriculture. Robotic harvesters enhance operational efficiency, product consistency, and picking precision while offering long-term economic viability. Ongoing technological advancements have streamlined harvesting workflows, increased throughput, and mitigated labor shortages across high-demand agricultural tasks. Key improvements—including faster cycle times, robust fruit maturity detection, extended battery endurance, and optimized energy management—collectively drive the global integration of automation in precision agriculture. Despite persistent challenges related to acquisition costs, scalability, and infrastructure, continued refinement of autonomous harvesting platforms holds strong potential to advance agricultural sustainability, productivity, and resilience amid evolving demographic and environmental pressures.

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Session 6

Youth and Education in Life Sciences

ORIGINAL SCIENTIFIC PAPER

Bibliometric Overview of Scientific Publications on Drought Stress in Maize (*Zea mays* L.)

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Summary
This study presents a bibliometric analysis of scientific papers on drought stress in maize indexed in the Web of Science Core Collection. A total of 8,160 papers published between 1968 and 2025 were analyzed. The results showed a clear increase in the number of publications over time. In the early years, only a small number of papers were published, but research activity started to grow after 2000 and increased strongly after 2010. The highest number of papers was recorded in 2025. Publication output increased markedly over time. Of the total 8,160 papers, only 11 were published in 1968–1978, compared with 4,415 papers in 2019–2025, representing an approximately 400-fold increase. The highest annual output was recorded in 2025, with 768 papers. The journal analysis showed that Frontiers in Plant Science published the most papers, followed by Plants, Agricultural Water Management, and Agronomy. The impact factors of the leading journals ranged from 1.9 to 6.5, indicating that research on maize drought stress is published in journals with moderate to high scientific impact. At the institutional level, CGIAR was the most productive organization, followed by the United States Department of Agriculture and the Chinese Academy of Sciences. Many Chinese institutions were among the leading contributors. The country analysis showed that China had the highest number of published papers, followed by the United States, India, and Pakistan. At the continental level, Asia was the leading region, followed by Europe and North America.

Keywords: maize, drought stress, bibliometric analysis, climate change, Web of Science, scientific publications

Introduction

Drought is one of the most important abiotic stress factors limiting agricultural production worldwide. Climate change, which increases the frequency and intensity of drought events, further exacerbates water scarcity, particularly in vulnerable agroecosystems. Among major field crops, maize (*Zea mays L.*) plays a crucial role due to its high economic value and widespread use in human nutrition, animal feed, and industry. However, maize is highly sensitive to drought stress, especially during critical growth stages, which can result in significant yield losses (Cruz-González et al., 2025).

Consequently, research on drought stress in maize has become a key focus in agronomy, plant physiology, and molecular biology (Poudel, 2023; Kipkulei et al., 2025). Over the past decades, a substantial increase in the number of scientific publications on this topic has been observed, reflecting the growing interest of the scientific community in understanding drought tolerance mechanisms and developing more resilient crop varieties.

Bibliometric analysis is a valuable tool for systematically assessing the development of a scientific field, enabling the identification of major research trends, leading institutions, countries, and journals, as well as the evaluation of publication dynamics (Singh and Shau, 2019; Wu et al., 2023). Therefore, the aim of this study was to analyze global research trends related to drought stress in maize using data from the Web of Science Core Collection, with a

particular focus on publication trends, institutional and geographical contributions, and key scientific sources.

Material and Methods

The data used in this study were obtained from the Web of Science Core Collection database. The literature search was conducted using a topic-based query with the following keywords: "*maize" or "corn", and "drought" and "stress"*. The search covered the period from 1968 to 2025, and all records meeting the defined criteria were included in the analysis. The analysis included the annual number of publications, the most productive institutions, leading journals, and the distribution of publications by country and continent.

Standard bibliometric approaches commonly used in the analysis of scientific production were applied in this study. The methodology was based on previously published bibliometric studies investigating research trends in agriculture, climate change, and plant abiotic stress. In particular, the guidelines proposed by Donthu Naveen et al. (2021) were followed, as well as the methodological framework implemented in the bibliometrix tool developed by Aria Massimo and Cuccurullo Corrado (2017).

Similar bibliometric studies have been conducted in the field of drought stress, climate change, and plant physiology, enabling comparison of findings and supporting the validity of the applied methodology.

Results and Discussion

A total of 8,160 papers published in the Web of Science Core Collection database during the period from 1968 to 2025 were analyzed (Figure 1). The number of published papers on drought stress shows a clear long-term upward trend. In the earlier period, publication output was very low, with a gradual increase becoming evident after 2000 and a much more pronounced rise after 2010.

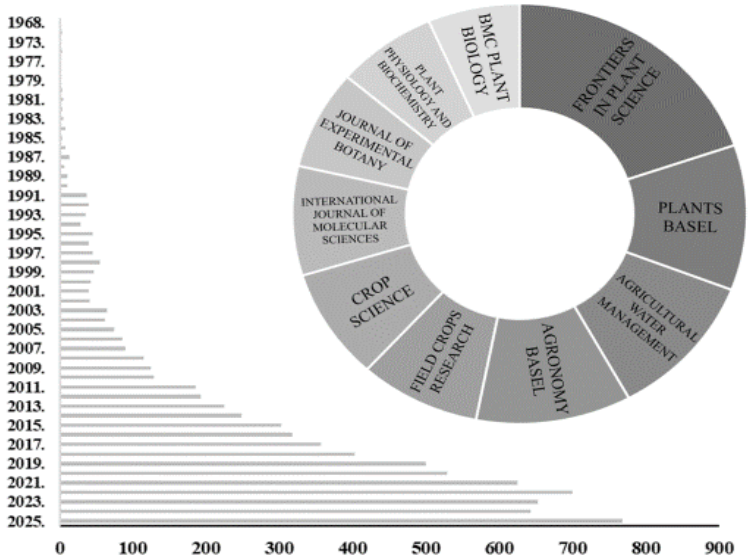

Figure 1. Annual publication output and leading journals in maize drought stress research indexed in the Web of Science Core Collection (1968–2025), and the most productive journals during the period 1968–2025

The highest number of papers was recorded in 2025 (768), while high publication activity was also observed in 2022 (700), 2023 (652), and 2024 (642). The lower number of papers in 2025 (191) is likely due to the fact that the year is not yet complete. In total, 8,160 papers were analyzed, confirming that drought stress has become a highly relevant and increasingly

studied research topic.Of the total 8,160 papers, only 11 papers were published in 1968–1978, followed by 57 in 1979–1988, 345 in 1989–1998, 658 in 1999–2008, 2,483 in 2009–2018, and 4,415 in 2019–2025. This indicates a clear long-term growth trend: publication output increased about 5.2-fold from 1968–1978 to 1979–1988, about 6.1-fold from 1979–1988 to 1989–1998, about 1.9-fold from 1989–1998 to 1999–2008, about 3.8-fold from 1999–2008 to 2009–2018, and about 1.8-fold from 2009–2018 to 2019–2025. The highest publication output was recorded in the most recent complete period, 2019–2025, while 2026 was not included in the interval comparison because it represents an incomplete publication year with 191 papers recorded so far.

Table 1. Top 20 institutional contributions to drought stress in maize research based on publication output Web of Science Core Collection

University / Institution	**Number of published papers**
Consultative Group on International Agricultural Research (CGIAR)	420
United States Department of Agriculture USDA	334
Chinese Academy of Sciences	309
Chinese Academy of Agricultural Sciences	301
China Agricultural University	294
Egyptian Knowledge Bank EKB	268
Northwest A F University China	262
International Maize Wheat Improvement Center CIMMYT	226
University of Agriculture Faisalabad	216
Indian Council of Agricultural Research ICAR	194
French National Research Institute for Agriculture, Food and Environment (INRAE)	160
Henan Agricultural University	149
Institute of Crop Sciences CAAS	147
Islamic Azad University	124
King Saud University	116
State University System of Florida	114
University of Florida	107
Sichuan Agricultural University	104
Huazhong Agricultural University	99
Consejo Superior de Investigaciones Científicas CSIC	96

The journal analysis (Figure 1) showed that Frontiers in Plant Science published the highest number of papers on drought stress (349), indicating its prominent role as a major outlet for research in this field. It was followed by Plants (198), Agricultural Water Management (197), and Agronomy (196), all of which also contributed substantially to the dissemination of drought stress research. Other important sources included Field Crops Research, Crop Science, International Journal of Molecular Sciences, Journal of Experimental Botany, Plant Physiology and Biochemistry, and BMC Plant Biology. The distribution of publications across these journals suggests that drought stress in maize is a highly interdisciplinary topic, spanning plant science, agronomy, crop production, plant physiology, molecular biology, and water management. The impact factors (IF) of the analyzed journals ranged from 0.2 to 24.1 according to the 2024 Journal Citation Reports. Most of the leading journals publishing research on drought stress in maize were classified in the Q1 category within their respective subject areas, including Agricultural Water Management (IF 6.5), Field Crops Research (IF 6.4), Frontiers in Plant Science (IF 4.8), Journal of Experimental Botany (IF 5.7), Plant Physiology and Biochemistry (IF 5.7), and Plant Physiology (IF 6.9). Several additional journals belonged to Q2–Q4 categories, indicating that research on maize drought stress is disseminated across journals with varying levels of scientific visibility and specialization.

The institutional analysis (Table 1) showed that CGIAR was the most productive organization, with 420 published papers, followed by the United States Department of Agriculture (USDA) with 334 papers and the Chinese Academy of Sciences with 309 papers in the period fro 1968 to 2025. A strong presence of Chinese institutions was evident, as several of them ranked among the leading contributors, including the Chinese Academy of Agricultural Sciences, China Agricultural University, Northwest A&F University, Henan Agricultural University, the Institute of Crop Sciences CAAS, Sichuan Agricultural University, and Huazhong Agricultural University. International and national agricultural research organizations, such as CIMMYT, ICAR, INRAE, and CSIC, also made substantial contributions, highlighting the global scientific interest in drought stress research. Overall, the results indicate that research output in this field is driven predominantly by major agricultural research centers, national research institutions, and specialized universities.

The country-level analysis (Figure 2) revealed a strong geographical concentration of drought stress research output. China was the leading contributor with 2,675 published papers, followed by the United States (1,556), while India (657) and Pakistan (589) also showed substantial research activity. Other major contributors included Germany, Iran, Brazil, Mexico, Egypt, and Australia, indicating that drought stress is a globally relevant topic, particularly in countries where water limitation represents a major constraint to agricultural production.

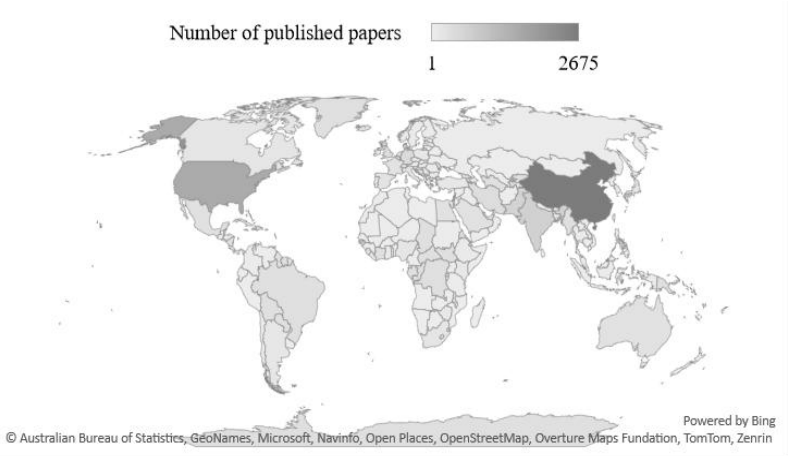

Figure 2. Distribution of published papers on drought stress ima maize by country (from 1968 to 2025)

At the continental level, Asia clearly dominated publication output, driven primarily by China, India, Pakistan, Iran, Saudi Arabia, Japan, and several other Asian countries. Europe also showed strong representation, with important contributions from Germany, France, England, Spain, Italy, Poland, and other European countries. North America was represented mainly by the United States, Mexico, and Canada, while South America contributed through Brazil, Argentina, Chile, Colombia, Peru, Uruguay, and others. Africa demonstrated notable research activity as well, particularly through Egypt, Nigeria, South Africa, Kenya, Ethiopia, Ghana, and several additional countries, reflecting the high relevance of drought-related issues across the continent. Oceania, although represented by fewer countries, showed a meaningful contribution mainly through Australia and New Zealand.

Generally, the results indicate that drought stress research is characterized by broad international participation, but with a particularly strong concentration in Asia, followed by Europe and North America. This distribution highlights the global scientific importance of drought stress, especially in regions where climate variability, water scarcity, and agricultural sustainability are major concerns.

Conclusion

Agricultural production is increasingly challenged by climate change, particularly by the rising frequency and severity of drought events. The strong growth in scientific output on maize drought stress—8,160 papers published between 1968 and 2025—highlights the global importance of this issue. Leading contributors particularly China, the United States of America, represent regions and institutions where drought poses major threats to crop productivity. Generally, the findings confirm that drought is one of the most critical constraints to maize production and underscore the need for continued research and development of more resilient crop varieties.

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SCIENTIFIC REVIEW PAPER

The Role of Social Media and Apps in Second-Language Learning

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Summary
In this paper, the role of mobile applications and social media in second-language (L2) acquisition will be discussed. These are powerful tools with advantages and disadvantages for second-language learning. Some of the advantages mentioned are flexibility and accessibility. The major weakness of social media and apps in second-language learning is that the focus is given to the vocabulary and grammar rules, but the production of speaking and writing skills is ignored. Social media and mobile apps do not have the ability to receive personalized feedback. The other major concern is the overuse of informal language. In addition, the overuse of Anglicism may hinder the achievement of a high level of culturally appropriate and accurate vocabulary use. Although social media and mobile apps can provide beneficial learning experiences, because of their limitations, a more integrated approach to second-language learning is necessary.

Keywords: social media, mobile apps, second- language acquisition, motivation, Anglicism

Introduction

Social media and mobile applications influence the learning of a second language (L2) greatly. Unlike traditional learning in classrooms, this way of learning offers many new opportunities. Learners can now communicate with native speakers in different environments.
There are also many challenges this type of learning brings; one of them is the informality of language. As Thorne (2010) explains, learners are exposed to colloquial language, abbreviations and nonstandard forms. Such forms affect the development of formal language, which is needed for professional and academic communication. Learners become unaware of the mistakes they make. The increase of Anglicism is another common challenge. According to Croatian Encyclopedia (n.d.), an anglicism is a word taken from English and incorporated into another language as a loanword, adapted to fit the linguistic system of that language.
This paper examines both advantages and challenges of social media and mobile apps in second-language learning. It will address the interaction with native speakers and learner motivation, but also issues such as informal language use, which refers to the use of language associated with an informal context, suitable for everyday work and family communication and a simple, ordinary style of expression. The paper aims to provide a balanced view of the role of social media and apps in language learning.

Authentic Language Exposure

Authentic language exposure is one of the main benefits of social media in second-language learning. Bahrani et al. (2014) define it as "language used in real-life situations outside formal context", and explain that platforms such as Facebook, X, Instagram, and TikTok provide more natural language use, while in classrooms, grammar and vocabulary are often taught via simplified forms made for specific learning.
Using social media and apps, learners get to experience real language without leaving their country. Taguchi (2023) believes that the latter helps with faster language acquisition, and learners can acquire speech patterns, cultural nuances, and nonstandard expressions.

Thorne (2010) emphasises the importance of social-media posts and videos for a better understanding of idioms, humour, and cultural references. Due to the exposure to informal language such as slang or regional-language variations, cultural competence is also developed (Dembe, 2024). Platforms such as Youtube expose learners to real-life situation videos, which improve language flexibility in students who thus learn how language functions in real sitautions (Godwin-Jones, 2021).

Fostering Interaction with Native Speakers

Social media also plays a significant role in facilitating interaction with native speakers. Traditional methods often fail to provide opportunities for interacting with native speakers, especially for languages that are not commonly spoken. Social media, however, remove these barriers and enable real-time communication with native speakers from different parts of the world. This can be done by joining different groups to interact with native speakers, and receive feedback on performance (Hamadeh, 2020).

HelloTalk and Duolingo are examples of social media platforms that facilitate communication with native speakers through text, voice, or video communication. These platforms provide opportunities for learners to practice language in a relaxed environment and receive feedback in real-time (Schroeder, 2021). According to research, communication with native speakers enhances the development of the target language (Othman, 2020).

This also enhances listening skills, as learners are exposed to different accents and speaking styles (Godwin-Jones, 2021).

Social media also facilitate the development of sociolinguistic competence, as learners are exposed to different cultures, which enhances the development of pragmatic competence (Alamri, 2018).

Motivation

Motivation is an essential aspect of second-language learning, and social media is a useful tool that increases motivation through interesting learning activities. Motivation is a critical aspect of language learning, as discussed by Ushioda (2016). Social media is a useful tool that keeps learners motivated by constantly changing learning activities and opportunities for interaction. It also provides a learner with a feeling of association with the language and its speakers. Learners follow native speakers, join different groups, and engage in different activities. This provides them with a feeling of how language is used in real-life situations, which increases their motivation for learning (Reinhard, 2019). Barrot (2023) also discussed the significance of active participation and interaction with others who have similar learning goals.

Social media provides a learner with a feeling of belonging. Learners comment, share, and engage with others, which reduces the feeling of loneliness. It also provides different formats for learning, which increases the learner's interest. Formats such as videos or memes make learning a personal experience for the learner.

Exposure to interesting learning activities increases a learner's motivation (Godwin-Jones, 2021). Learners also engage in different activities through commenting and sharing, which increases the learner's engagement and provides them with a strong association with learning.

Accessibility and Flexibility in Mobile Application

Mobile apps provide a very accessible and flexible learning experience for language learners. This is very beneficial for language learning since learners can access learning materials anywhere and anytime, which is very convenient for scheduling learning activities within a busy day.

This provides learners with the opportunity to make the most of every moment, even when they are doing day-to-day activities like traveling to work. This is beneficial for language learning since learners will be constantly exposed to learning activities (Godwin-Jones, 2021).
Mobile apps also provide a flexible and personalized learning experience that is beneficial for language learners since they will be motivated to continue learning. (Loewen, 2019). Mobile apps also provide learners with opportunities for learning even when they are doing day-to-day activities (Haque, 2023).

Gamification

Gamification is an integral part of mobile language learning apps. Gamification is defined by Deterding et al. (2011) as ″the use of game elements and mechanics to increase user engagement″. Gamification makes learning interactive and motivating for language learners.
Language learning apps like Duolingo, Babbel, and Lingvist apply gamification by using reward systems to encourage learners to learn. Gamification motivates language learners by helping them reach their personal learning goals (Godwin-Jones, 2021). Gamification also makes learning interactive by using elements like competition and leaderboards (Teske, 2017). Learners are given the opportunity to practice interactive language learning, and repeat and revise what they have learned. Learner persistence is increased via apps due to their sense of accomplishment (Sailer & Homner, 2019). Gamification makes language learning interactive by helping learners learn and practice different language skills like vocabulary and grammar (Signori et al., 2018).

Limitations

However, there are also limitations in the use of mobile apps for learning. For instance, some apps only provide learning in terms of vocabulary and grammar, but not much is done regarding meaningful communication. Apps also provide activities that help learners improve their receptive skills, but not much is done regarding productive skills such as speaking and writing (Reinders & Stockwell, 2017). Most apps provide feedback which is usually not detailed enough and may cause learners to make the same mistakes over and over again without understanding them.
Another limitation is that most apps provide learners with too much gamification, which may lead to surface learning (Deterding et al., 2011). Such learning only focuses on learning for the sake of winning, not for understanding. Another limitation is that not all learners will have access to mobile learning, as it is dependent on devices, internet, and software updates (Godwin-Jones, 2021). Most apps also provide learners with passive learning, i.e. learners only do activities without really being involved (Nasution, 2022).

The Overuse of Informal Language

Another criticism leveled against social media in the context of language acquisition is the use of informal language. According to Nuri (2023), informal language includes the use of colloquial or non-standard language in day-to-day online communication. Although this type of language is used in real-life situations, a learner must be able to differentiate between informal and formal situations.
Exposure to informal language can result in a learner adopting a wrong way of using the language. Informal language includes the use of abbreviations, slangs, and simple sentence structures (Thorne, 2010). X or Instagram promote the use of short and creative words, which may be spelled in a non-standard way.
This can result in a learner using informal language in a wrong context. In addition, fast communication can result in a learner paying little attention to grammar (Godwin-Jones, 2021).

The Proliferation of Anglicisms

Another phenomenon related to the use of social media and apps is the spread of Anglicisms. Anglicisms refer to the use of English words and structures in other languages, especially in digital communication (Ashrafova, 2023).

However, this may affect the learner negatively. They may not develop their vocabulary adequately by using English words instead of native words (Živić, 2024). Social media may use words like "like," "hashtag," or "selfie," which may become part of their daily vocabulary (Álvarez Valencia & Fernández Benavides, 2018).

Moreover, the overuse of Anglicisms may cause the learner to have a shallow understanding of the foreign language and a lack of cultural awareness (Kultanova & Dusengaliyeva, 2020).

Besides, the learner may learn and adopt the wrong grammatical structures without even recognizing them (Thorne, 2010).

Conclusion

Mobile applications and social media have revolutionized second-language learning. They provide the learner with flexibility and access to second languages at any time. Second-language learning has become part of the daily routine.

Second-language learning through mobile applications provides the learner with opportunities to communicate with native speakers. The learner gets exposed to real second language use, different accents, and cultural settings.

However, there are some limitations to second-language learning through mobile applications. First, most mobile applications are designed for receptive skills rather than productive skills. Second, mobile applications lack adequate feedback for second-language learners. Third, second-language learning through mobile applications involves too much gamification, leading to surface learning rather than deeper learning. Moreover, this approach involves a lot of informal language use, which may negatively influence the development of formal second-language skills.

This way of learning should therefore be used in conjunction with other forms of second-language learning.

Second-language learning through mobile applications should therefore be developed further to provide detailed feedback for second-language learners. Second-language learning through mobile applications should also be developed further to support productive second-language skills. It is also important to develop second-language learning through mobile apps in such a manner that emphasis should be put on formal second-language skills.

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