IMPACT OF FOLIAR NANOCALCIUM FERTILIZATION ON YIELD AND WINE CHARACTERISTICS OF 'VRANAC' WINE GRAPES

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This study investigates the impact of foliar application of calcium nanofertilizer (Fitosmart®) on the yield and quality of *Vitis vinifera* L. (cv. 'Vranac') grapes. The experiment was conducted during the 2023 growing season at a commercial vineyard in Montenegro, where vines were treated with a 0.5% calcium nanofertilizer solution at three critical phenological stages: pre-bloom, post-bloom, and veraison. The results showed a significant increase in grapevine yield, primarily driven by an enhanced number of clusters per vine, while cluster weight exhibited only a minor increase. Chemical analysis revealed delayed sugar accumulation in treated grapes, which impacted must and wine composition by slightly reducing total soluble solids and final alcohol content. Treated wines demonstrated increased levels of total polyphenols and anthocyanins, factors that contribute to the sensory quality of red wines, including aroma authenticity, harmony, and overall impression. These findings suggest that the foliar application of nanocalcium fertilizers can enhance both grape yield and wine quality, making it a promising, sustainable alternative to conventional mineral fertilizers. However, further research is needed to explore its broader applicability across diverse climatic conditions and grapevine varieties.

Keywords: Vranac, calcite nanofertilizer, wine, anthocyanins, polyphenols

Introduction

Foliar fertilization plays a critical role in viticulture, allowing grapevines to efficiently absorb essential nutrients through buds, green berries, and leaves. Although soil fertilization is preferred due to its low cost, foliar fertilization has been regularly used as an important agrotechnical measure to avoid deficiencies and to improve quality. Numerous authors [1-3] reported that foliar application of nutrients improves grape yield and quality, resistance to diseases [4], and tolerance to other stress factors [5]. Absorbed by plant surfaces via stomata, the cuticle and cuticular irregularities, trichomes, veins, and other modified epidermal structures, such as lenticels [6], it enables rapid uptake of macro and micronutrients. This is especially important when it comes to calcium, the constitutive element that faces challenges in absorption and immobility in xylem and floem [7, 8]. Calcium is an indispensable mineral nutrient for fruit tree growth and development [9-12], building cell walls and membrane structure [13, 14]. Furthermore, calcium affects and supports plant resistance to diseases [15] and the sugar accumulation of photosynthesis in plants [16].

Grape is a calcium-loving plant, with much higher demand for calcium than other fruit species [17]. Foliar application mainly accumulates calcium in the grape berry skin, delaying softening and fungal diseases [18], improving yield [19], overall firmness [20, 21], and storage life [21-23]. That is why Ca fertilization is standard practice, especially in table grape production. Although most studies have focused on table grape production, in recent years, there have been numerous studies regarding foliar calcium application and its positive effect on wine grapes' growth and berry quality. Calcium affects yield [24, 25], total phenolic and anthocyanin berry content [26], delaying berry maturation and increasing fruit firmness and flavonol content [27], which makes it one of the important limiting factors for high-quality wine production [7].

Previous studies on grapes have been based on the use of various calcium supplements, such as carbonate [25], nitrate [15, 17, 19, 28], chloride [18, 27, 29], chelate [24, 30], sugar alcohol [7] and other forms, provided through foliar application. Although the application of na-

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notechnology in agriculture has advanced, with numerous positive effects on plants (31-35), including grapevines [36, 37], the usage of nanofertilizers in agriculture is still underexplored. Fitosmart® is a natural and ecological calcium fertilizer derived from nanotechnology, containing 93.80% $CaCO_3$, suitable for both conventional and organic production. It is a micronized calcite obtained from finely ground limestone sediment, activated by a tribomechanical process that creates particles with micrometer dimensions, large specific surface area, and greater reactivity. Following foliar application [38], calcite nanoparticles can easily penetrate through the stomata into the leaves [39], where they split into CO_2 and CaO [40], being immediately available for the plant as the driving force of photosynthesis.

'Vranac' is an autochthonous grape variety from Montenegro, within ecological-geographical group *Proles pontica* (*Convarietas pontica*). It is the national brand, one of the most important grapevine varieties for Montenegro red wines production, occupying about 70% of total production from autochthonous varieties [41], the most recognizable and the best product of the "13 jul Plantaže" [42]. Outside of Montenegro, it is planted in the Republic of Macedonia, Serbia, and Croatia (Dalmatia). 'Vranac' grapes are considered to have a strong polyphenol potential [43], while wines are of premium quality, with dark red ruby colour, full body, fruity taste, and pleasant astringency with aging potential in oak barrels [44].

Wine contains numerous compounds such as polyphenols, organic acids, carbohydrates, aroma compounds (alcohols, esters), metals, etc. Phenolic compounds are considered to be the main factors responsible for the quality of grapes and wines. They are biologically active compounds, thanks to their effects in the prevention of various diseases caused by oxidative stress, and an integral part of health and medical research [45]. Acting with antioxidant, cardioprotective, anticancer, anti-inflammatory, and antimicrobial effect [46, 47], they bring benefits against cardiovascular diseases, tumors, and inflammation [48-51]. Phenolic compounds significantly determine wine quality [52], affecting color, sensory characteristics such as mouthfeel, astringency and bitterness [53], oxidation reactions, and changes in wine during aging. Phenolic compounds in grapes originate mainly from seeds and skins [54], however the content of polyphenols in wine depends on numerous factors such as grape variety, vineyard location, cultivation system, climate and soil type, vine cultivation method, harvest time, production process, and aging [55]. Wine phenolics extracted from grape skins and seeds during fermentation, interact with insoluble cell wall material from the grapes [56], and undergo a range of chemical transformations during maturation [57].

Anthocyanins are red, blue, or purple pigments [47] localized in the skin of the berry [58, 59] of red and, blue-skinned grape varieties. These pigments are responsible for the red color of wines, contributing little to the taste of wine, playing an important role in tannin retention during aging. The concentration of anthocyanins in wine varies depending on: variety, maturity, seasonal conditions, and viticultural practices [58]. According to [60] cv. 'Vranac' is characterized by a high total anthocyanin content.

The main objective of this study was to evaluate the effect of natural nanocalcium fertilizer on the grape yield, and the content of polyphenols and anthocyanins in the wine of cv. 'Vranac'. Another objective was to explore the potential of nanocalcium fertilizers as a sustainable alternative to mineral fertilizers, aiming to enhance grape quality and wine performance while supporting environmental sustainability.

Material and methods

Field management and experimental design

The trial was carried out during the 2023 growing season in the commercial vineyard of the "13 jul Plantaže" in the Ćemovsko field that belongs to the sub-region of Podgorica. The study was conducted in a 7-year-old vineyard planted at the Bunar-4 micro-locality, with local 'Vranac' variety (clone 3), grafted onto Paulsen 1103 rootstock, trained to a modified double Guyot training system, with planting distance 2.6m x 1.2 m. Winter pruning was carried out, leaving 14 buds per vine. Removing the leaves from clusters was applied in the middle of May, for regulating yield components and improving grape quality. All standard agro-technical operations with drip irrigation were applied.

At harvest, cluster number as well as the total yield was recorded on 15 tagged vines per treatment. The components of the generative potential, such as the number of clusters, the cluster weight, as well as the yield of grape per vine (kg), were determined by counting and weighing the clusters harvested from each vine. The yield per ha (t) was calculated by multiplying the number of vines per ha (3.205) by the yield per vine. In the laboratory, the following variables of 6 kg representatively collected clusters for each treatment were estimated: cluster weight, number of berries per cluster, berry percentage in cluster, berry length and width, and weight of 100 berries. Berry, cluster, and yield data were presented as average.

During the three weeks of the ripening period, sugar in grapes was measured by refractometry every 5-6 days in order to monitor the dynamics of sugar accumulation. The total soluble solids (TSS) of grape juice were determined by refractometry and pH values achieved by pH meter and titratable acidity (TA) with 0.1 N NaOH and bromothymol blue as an indicator (expressed as g/L of tartaric acid equivalents). Determination of total (titratable) acidity is quantified by titration with a standard 0.1 M sodium hydroxyde solution of a sample to the point of pH 7.0, determined with electrode Unitrode Pt 1000, with automatic titrator 785 DMP Titrino Metrohm. 10 mL of the sample was pipetted in a 100 mL beaker, and 40 mL of boiled distilled water was added. The beaker with

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the sample was placed on the automatic sampler of the titrator, the titration was performed, and the calculated TA in g/L of tartaric acid was given.

At the moment of optimal technological maturation (21.3-23.6 °Brix), grapes were harvested manually and transported to the experimental cellar. Microvinification was carried out to evaluate the influence of the nanocalcium treatment on wine composition and quality. For both treatments, approximately 60 kg of grapes were stored overnight in a cool chamber (4 °C) and the following day warmed to room temperature before being slightly crushed. During grape crashing, 5 g/100 kg potassium metabisulfite (Agroterm KFT, Hungary), was added and in fermenting tank 3 g/hL of enzyme Fintage FCE, Lamothe-Abiet, Bordeaux, France and 150 g/hL of oak chips MT+ (Nadalié OAK ADD INS, LUDON-MÉDOC France). To adjust total acidity and pH to the appropriate values tartaric acid (2 g/L, Caviro Extra spa - Villorba, Italy) was used. All musts were immediately inoculated with selected *Saccharomyces cerevisiae* yeast (25 g/hL, Lalvin EC 1118, Lallemand Inc., Montreal, Canada) and Go-ferm protect (30 g/hL), yeast nutrient. Two days after inoculation lactic acid bacteria (E'CO-Malic, 0.5 g/hL, Lallemand Inc., Montreal, Canada) was added in order to perform malolactic fermentation. Nutrients (Fermaid E, 25 g/hL, Lallemand Inc., Montreal, Canada) for yeast were added when 1/3 of alcoholic fermentation had passed. Fermentations were conducted in small inox tanks at 25 °C for up to 9 days.

Basic chemical parameters of grape must and wine were determined according to the European Union Commission regulation (EC) 1234/07 and Commission regulation (EC) 606/09. Analysis of wine chemical parameters was done after alcohol fermentation. These analyses included determination of: alcohol content (AlC), pH, total acids (ToA), volatile acids (VA), malic acid (MA) and lactic acid (LA) content, reducing sugars (RS), total anthocyanins (TAN) and total polyphenols (TP) content. The total polyphenols [61], total anthocyanins [62] of the wine were determined by UV-vis spectrophotometry. Spectrophotometric measurements of absorbance at 420, 520, and 620 nm were made using a 1 mm quartz cuvette.

The sensory evaluation of experimental wines was carried out using the 100-point scale OIV system, according to the OIV 332A/2009 resolution, which is a widely accepted sensory method in Montenegro. Six panelists, trained and with long-term experience in wine sensory analysis, applied the descriptive analysis with the following score ranking for visual category: limpidity (clarity), aspect other than limpidity (maximum score ranking up to 15 points); nose category: positive intensity, genuineness, quality (maximum score ranking up to 30 points); taste category: positive intensity, genuineness, harmonious persistence, quality (maximum score ranking up to 44 points); and harmony category: overall judgement (maximum score ranking up to 11 points).

The standard error for the measured parameters was calculated. The data were statistically analyzed using analysis of variance (ANOVA). The significance of differences between the mean values of treatments was performed using the Tukey's test. Data analysis was performed using the statistical software package STATISTICA, version 8 (StatSoft, Inc., Tulsa, OK, USA).

Results and discussion

Effect on yield

According to [63], the structure and the number of clusters per vine are important yield components, under the strong influence of mineral nutrition. In our research, calcium treatment improved grapevine yield (Table 1) mainly by increasing the number of clusters per vine (by 1.6 cluster) rather than by increasing cluster mass. Comparing cluster weight of treated and control group, we determined the differences in only 15.6 g per cluster in favor of the treated group. Consequently, the improvement of these two yield components contributed to the higher yield per vine of the treated group by 0.8 kg, effecting the enlargement of yield per ha by 2.58 t in comparison with the control group.

Table 1. Effect of the treatment on cluster characteristics and grape yield

Treatment	Cluster number/ vine	Cluster weight (g)	Yield (kg)/vine	Yield (t)/ha
Fitosmart 0.5%	13.6 ± 1.37	397.0 ± 54.00	5.38 ± 1.28	17.25 ± 4.11
Control	12.0 ± 0.58	381.4 ± 37.81	4.58 ± 0.36	14.67 ± 1.12
p value	>0.01	>0.01	>0.01	>0.01

Table 2 shows the cluster structure and berry characteristics of the treated and control group. In our research, cluster fertility was affected by nanocalcium treatment with an average difference of 11.8 berries per cluster in favor of the treated group. Cluster structure expressed by berry percentage in cluster was with small differences among treated and control group (97.3% and 96.9%, respectively). Berry size is an important factor that affects the quality of the grapes. According to [64], small berry size improves wine quality due to the higher concentration of aromatic and phenolic compounds in the grape skin. This is in correlation with [65], who stated that larger berries can contribute to the dilution of the polyphenol content in the grapes. Our results show that the values of berry length and width of the treatment (17.89 mm and 16.04 mm, respectively) were similar to those in the con-

trol group (17.86 mm and 15.88 mm, respectively), indicating that calcium doesn't affect the berry size in wine grapes. The previous investigations [66,28] showed that Ca treatment decreases the weight of 100 berries, which is in contrast to our results where similar values were detected among the treated and control groups (420.5 g and 403.6 g, respectively).

Table 2. Effect of the nanocalcium treatment on berry characteristics

Treatment	No of berries per cluster	Berry % in cluster	Berry length (mm)	Berry diameter (mm)	Weight of 100 berries (g)
Fitosmart 0.5%	148.6 ± 35.07	97.3 ± 0.31	17.89 ± 1.42	16.04 ± 1.27	420.5 ± 121.69
Control	136.8 ± 39.78	96.9 ± 0.50	17.86 ± 0.35	15.88 ± 0.36	403.6 ± 103.56
p value	>0.01	>0.01	>0.01	>0.01	>0.01

Effect on grape and wine quality

The primary requirement for the production of high-quality wine is grape quality. According to [67], fertilization may affect earlier grape ripening, bigger berries, and intensive colour. Table 3 shows grape must sugar levels in three three-week measurement period. Our results show different dynamics of sugar accumulation in the treated and control group. In the initial period, sugar content growth in the treated group was slower, but in the second half, it accelerated and became equal to the sugar content in the control group. Our observations are in accordance with [28], who stated that calcium treatment may be associated with a delay in berry maturation.

According to [30], the quality of grapes is determined primarily by the acidity and sugar content of the juice of the berries. Our results (Table 4) show that the delay in berry maturation, which is crucial for balancing acids and sugars, consequently had an impact on the oenological parameters of the must. The lower values in the treated group in terms of TSS (ºBrix) imply that the ripening of grapes and the accumulation of sugar in treatment was moved to a slightly later period. Also, the higher yield in the treated group, and the competitive relation among clusters should be taken into account.

The acid content impacts the taste, color, and microbial stability of the must. The acid concentration in must and resultant wine is important to structural/textural balance. Titratable acidity (TA) in grapes normally ranges between 5.0 and 16.0 g/L as tartaric acid, but in warm climates such as the Basin of Skadar Lake region in Montenegro, the grape juice acidity is usually low [65]. Given that TA is influenced by multiple factors—including grape variety, vineyard management practices, and berry ripeness, maintaining optimal acidity levels is essential for preserving the sensory characteristics of the wine. The results indicating similar TA and pH values between treated and control groups suggest that the nanocalcium treatment did not significantly alter the overall acid composition of the must.

Table 3. Sugar accumulation in grapes during ripening

Date of measurement during 2023.	Fitosmart 0.5% °Brix	Control °Brix
23.08.	16.7	16.7
29.08.	18.2	19.1
04.09.	19.4	22.0
11.09.	22.3	22.1
13.09.	23.0	23.0

Table 4. Effect of the nanocalcium treatment on must chemical parameters

Treatment	TSS (°Brix)	pH	TA (g/L)
Fitosmart 0.5%	23.81±0.17	3.86±0,07	4.00±017
Control	24.06±0.18	3.84±0.13	3.95±0.26
p value	>0.01	>0.01	>0.01

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Lower sugar content in the must of the treated wines resulted in a slightly but significantly lower AIC (by 0.28% v/v) in comparison with wine from control vines (Table 5a). Moderate alcohol content in the must is desirable in warm climate zones where high temperatures often cause excessively high alcohol levels and reduced acidity in wines, potentially leading to so-called flat wines, lacking a pronounced refreshing character. Although the treated wines had higher ToA (4.0 g/L) compared to the control wines (3.95 g/L), our results showed that the LA content and VA in the control wines (2.0 g/L and 0.35 g/L respectively) were higher than in treated wines (1.5 g/L and 0.25 g/L respectively). The reduced content of volatile acids in treated wines may indicate a more stable alcoholic fermentation process. Some chemical parameters, such as the pH value and MA content (Table 5a and 5b), were very similar among the investigated groups.

Also, results in Table 5b showed that with nanocalcium application treated wines achieved significantly higher content of RS, TAN and TP (2.4 g/L, 963.37 mg/L and 2.52 mg/L respectively) compared with control wines (2.3 g/L, 926.62 mg/L and 2.33 mg/L respectively), which points to the fact that calcium affects the quality parameters of the wine. The higher residual sugar content in treated wines also contributed to a more rounded and harmonious taste, while the increased total phenol and tannin content in treated wines suggests that nanocalcium positively influenced the content of extractive compounds from skin and the seeds, thereby improving the structure and aging potential of the wine.

Over the last few decades, agriculture is the sector that has been hard hit by climate changes, due to high temperatures, water deficit, rainfall, and radiation, with consequently reduced plant productivity and final product quality. All these changes have had an extreme impact on viticulture. Warmer weather conditions lead to an imbalance between phenological stage and ripeness because of advancement in plant growth periods, with significantly earlier veraison and harvest dates [68]. Under higher temperatures, berry ripening occurs earlier in summer, leading to incomplete phenolic maturity [69]. Additionally, the reduction in anthocyanin content, colour and aroma expression, as well as the appearance of polyphenols with unpleasant sensory properties [70], could lead to “flabby” wines [71]. Additional consequences are high sugar concentration, an increase in alcohol content, and reduction in acidity of wines in many regions, leading to the production of organoleptically disrupted wines. Our results showed that the positive effect of Ca treatment may be essential for wine production in warmer climate regions. Hence, vine growers and winemakers need to implement novel techniques and adapt viticulture to the effects of climate.

Table 5a. Effect of the nanocalcium treatment on wine chemical parameters

Treatment	AIC (% v/v)	pH	ToA (g/L)	VA (g/L)
Fitosmart 0.5%	14.27±0.06	3.86±0.07	4.00±0.17	0.25±0.03
Control	14.55±0.03	3.84±0.12	3.95±0.26	0.35±0.01
p value	**	>0.01	>0.01	**

Table 5b. Effect of the nanocalcium treatment on wine chemical parameters

Treatment	MA (g/L)	LA (g/L)	RS (g/L)	TAN (mg/L)	TP (mg/L)
Fitosmart 0.5%	0.20±0.03	1.50±0.06	2.40±0.02	963.37±22.04	2.52±0.01
Control	0.20±0.02	2.00±0.11	2.30±0,03	926.62±2.66	2.33±0.02
p value	>0.01	**	**	**	**

Effect on sensory evaluation

In wines, sensory evaluation plays a very important role since it analyzes important attributes in wines such as color, clarity, odor, taste, and astringency, among others. Achieving a red wine with a good organoleptic balance is of great interest. The sensory characteristics of wine depend not only on the variety, location and environment of the vineyard and the winemaking process, but also on viticultural practices [72]. Additionally, sensory characteristics can be modified by the presence of polyphenolic compounds and their low solubility [73].

According to the results shown in Table 6, the enrichment in TAN and TP of wine produced from treated grapes slightly increased visual aspects, aroma authenticity, aroma quality, taste harmonious persistence, and overall impression. That resulted in a higher total score for wine from nanocalcium treatment (93.2) in comparison to the control (92.2). However, further investigation would be needed to confirm the practical significance of these improvements.

Table 6. Effect of the nanocalcium treatment on sensory characteristics of wine

Sensory characteristics	Fitosmart 0.5%	Control
Limpidity (clarity)	5.0	5.0
Aspect other than limpidity	9.6	9.2
Nose positive intensity	7.4	7.6
Nose genuineness	5.8	5.6
Nose quality	15.2	14.8
Taste positive intensity	7.2	7.4
Taste genuineness	5.6	5.6
Harmonious persistence	7.4	7.2
Taste quality	19.6	19.6
Overall impression	10.4	10.2
Total	**93.2**	**92.2**

Conclusion

The application of nanocalcium fertilizer demonstrated a positive impact on both grape yield and wine quality of the cv. 'Vranac'. The increase in yield was mainly due to a higher number of grape clusters per vine, although berry size and cluster weight were minimally affected. The treated vines exhibited a delayed sugar accumulation, which also influenced certain wine parameters, including total polyphenol and anthocyanin content, enhancing the wine's sensory profile. These findings highlight the potential of calcium nanofertilizers, such as Fitosmart®, to serve as a sustainable alternative to conventional fertilization, aligning with environmentally-friendly viticulture practices. Despite these promising results, the study emphasizes the need for further research across diverse growing conditions and grape varieties to establish broader efficacy and optimize application protocols. This research offers valuable insights for viticulturists and enologists and provides a basis for developing advanced, sustainable practices aimed at improving grape quality and wine production in a climate-conscious manner.

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Izvod

UTICAJ FOLIJARNE NANOKALCIJUMSKE FERTILIZACIJE NA PRINOS I KARAKTERISTIKE VINA SORTE 'VRANAC'

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Ova studija istražuje uticaj folijarne primene nanođubriva sa kalcijumom (Fitosmart®) na prinos i kvalitet grožđa vrste *Vitis vinifera* L. (sorta 'Vranac'). Eksperiment je sproveden tokom vegetacione sezone 2023. godine u komercijalnom vinogradu u Crnoj Gori, gde su čokoti tretirani rastvorom nanođubriva sa 0,5% kalcijuma u tri ključne fenološke faze: pre cvetanja, nakon cvetanja i u fazi šarka. Rezultati su pokazali značajno povećanje prinosa po čokotu, pre svega zahvaljujući većem broju grozdova po čokotu, dok je masa grozda pokazala samo blago povećanje. Hemijska analiza ukazuje na usporenu akumulaciju šećera u tretiranom grožđu, što je uticalo na sastav šire i vina, sa blagim smanjenjem ukupnih rastvorljivih suvih materija i konačnog sadržaja alkohola. Tretirana vina su pokazala povećan sadržaj ukupnih polifenola i antocijanina, komponenti koje doprinose senzornom kvalitetu crvenih vina, uključujući autentičnost arome, sklad i ukupan utisak. Ovi nalazi sugerišu da folijarna primena nanođubriva sa kalcijumom može poboljšati i prinos grožđa i kvalitet vina, predstavljajući obećavajuću i održivu alternativu konvencionalnim mineralnim đubrivima. Ipak, potrebna su dodatna istraživanja kako bi se ispitala njegova šira primenljivost u različitim klimatskim uslovima i kod različitih sorti vinove loze.

Ključne reči: vranac, nanođubrivo sa kalcitom, vino, antocijanini, polifenoli

CRediT authorship contribution statement:
Ivana Bakić: conceptualization, methodology, writing-original draft, writing - review & editing.
Aleksandar Radović: formal analysis, data curation.
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Sanja Radonjić: conceptualization, validation, writing-original draft. Arif Atak: methodology, visualisation, supervision.
Milena Peruničić: investigation, validation.
Slavica Čolić: writing-original draft, writing-review & editing, supervision.
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