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Influence of nano fertilization on the chemical composition and bioactive components in grapevine

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ABSTRACT

Nanofertilizers represent a major advancement in agriculture, offering enhanced nutrient transport and regulation, resulting in improved plant growth and crop yield. Essential nutrients such as nitrogen, phosphorus, potassium, and micronutrients are crucial for healthy plant development, and the application of nanofertilizers has demonstrated improved soil properties, better water retention, and increased plant resilience to stressors. This study investigated the effects of a newly developed organic nano NPK+Zn fertilizer on the nutrition of the 'Cardinal' grape variety, focusing on its influence on the chemical composition of grape leaves and the bioactive compound content in grape fruit. The experiment included four variants: 1. Control (untreated); 2. NPK mineral fertilizer (soil); 3. Nano NPK + Zn (3 g l^{-1} foliar); and 4. Nano NPK + Zn (5 g l^{-1} foliar). Treatments were applied four times during the vegetation period: 15 days before and after flowering, during grain growth, and at veraison. Results showed that Nano NPK fertilization led to a higher ($p<0.05$) concentration of key nutrients and bioactive compounds in both leaves and grape bunches compared to the control. Notably, the 3 g l^{-1} concentration of the Nano NPK + Zn foliar treatment yielded the highest ($p<0.05$) content in the examined parameters. While the findings indicate the promising potential of Nano NPK fertilization to enhance grapevine nutrition and fruit quality, further research is necessary to assess long-term effects on soil health, grapevine longevity, and the sustainability of agricultural practices. This approach may offer a more sustainable alternative to conventional fertilization methods.

Keywords: *nanofertilizers, smart plant nutrition, sustainable agricultural production, antioxidant potential.*

INTRODUCTION

The growing global population has led to an increased demand for food, necessitating the extensive use of fertilizers. However, due to resource limitations and the low efficiency of conventional fertilizers, farmers are experiencing significantly rising costs. Nanotechnology offers a promising solution by enabling the production of fertilizers with specific chemical compositions that enhance nutrient use efficiency (Nisar et al., 2019).

Over the past few decades, both chemical and organic fertilizers have been developed to ensure the production of safe and affordable food. In this context, new alternative technologies have emerged, focusing on increased efficiency and reduced toxicity. These include genetically modified plant varieties and seeds, as well as the recent innovation of nanofertilizers (NFs) (Silva et al., 2018). Sustainable agriculture is vital for addressing global challenges such as food security, soil degradation, and climate change. Traditional agricultural practices often struggle with declining soil health, inefficient nutrient use, and environmental pollution caused by excessive fertilizer application. Nanotechnology, particularly the development of NFs, presents a viable solution for improving nutrient use efficiency (NUE) and mitigating nutrient deficiencies in crops (Shebl et al., 2019). Nanofertilizers contain essential minerals and nutrients, including nitrogen (N), phosphorus (P), potassium (K), iron (Fe), and manganese (Mn), which are either independently bonded or combined with nanoscale adsorbents (Nongbet et al., 2022).

Grapevine (*Vitis vinifera* L.) is one of the most economically significant drought-resistant crops cultivated worldwide (Gambetta et al., 2020). The mineral content of grapes and wine is influenced by multiple factors, such as soil composition, viticulture techniques, and environmental conditions (Daccak et al., 2022). Viticulture and winemaking play essential roles in agriculture, closely tied to the traditions, culture, and lifestyle of specific regions. Though these practices evolve at a slower pace than other agricultural activities, they continue to advance with the incorporation of modern scientific and technological innovations. Research on grapes and grape-derived products has been extensive, mainly due to their rich composition of biologically active secondary metabolites. By employing advanced agronomic methods, particularly in nutrition management, it is possible to enhance vine growth significantly. This can be achieved by applying the correct doses and types of fertilizers at optimal times, ultimately improving grape quality (Stojanova et al., 2024a).

Fertilization is a crucial agricultural practice in modern viticulture for ensuring consistent, high-quality, and profitable grape production (Stojanova et al., 2023). Factors influencing grape yield and quality include grapevine biological properties, climatic conditions, soil quality, and nutrient management (Stojanova et al., 2024b). Proper fertilization enhances root development and nutrient uptake efficiency, directly impacting grape yield and composition.

Nanotechnology offers an innovative approach to slow-release fertilizers, which enable controlled nutrient release into the soil. This ensures a steady or, when necessary, uniform nutrient supply over an extended period, improving agronomic efficiency while reducing fertilizer application rates. Consequently, this prevents pollution and eutrophication of water resources.

The use of nanofertilizers allows for a gradual and controlled nutrient release, resulting in increased fertilizer efficiency, reduced volatilization and leaching, and minimized environmental risks. These fertilizers have the potential to boost crop productivity by enhancing seed germination, seedling development, photosynthesis, nitrogen metabolism, and the synthesis of proteins and carbohydrates, while also increasing stress tolerance. Furthermore, their application in smaller quantities reduces transportation costs and simplifies fertilizer usage (Solanki et al., 2016; Zulfiqara et al., 2019).

This research aimed to evaluate the effects of a newly developed nano NPK+Zn fertilizer on the nutrition of the ‘Cardinal’ grape variety by assessing its influence on the chemical composition of the leaves and bioactive compound content in the grapes fruit.

MATERIALS AND METHODS

Experimental Design

The research was conducted in a vineyard located in the Skopje vineyard region of North Macedonia, using the ‘Cardinal’ grapevine variety. The vineyard was at full bloom during the study. The grapevines were planted with a spacing of 2.8 x 1.0 m, totaling 3571 vines per hectare, and trained on a trellis system with a stem height of 70 cm. For the basic autumn tillage, the vineyard received NPK 20-20-20 fertilizer at a rate of 450 kg ha^{-1} (variant 2). Fertilization was carried out in furrows, which were dug to a depth of 40 cm and placed 60 cm away from the vines. The field experiment utilized a drip irrigation system, and throughout the growing season, all necessary agricultural and ampelotechnical practices were applied.

The experiment included the following variations:

1. Control (untreated);
2. NPK (20-20-20) Mineral fertiliznig (soil);
3. Nano NPK (20-20-20) + Nano Zn (3 g l^{-1} foliar);
4. Nano NPK (20-20-20) + Nano Zn (5 g l^{-1} foliar).

Each variant involved 10 grapevines, totalling 120 vines across the entire experiment. Four foliar treatments were applied throughout the growing season: 15 days before flowering, 15 days after flowering, during the berry growth phase, and at the phase of veraison. The fertilizers were applied using a hand sprayer by spraying the leaves.

Grapevine Cultivar Used

The ‘Cardinal’ grape variety originates from California and was developed through the crossbreeding of Flame Tokay and Ribier. It is cultivated in nearly all parts of the world, with significant presence in Spain, Italy, Romania, and Mexico. This variety thrives best in regions where the maturation period is free from excessive rainfall and where winter temperatures do not pose a risk of severe frost (Popovic et al., 2020).

The grapes are large, weighing between 250 and 550 grams, with an elongated shape and an amber-yellow hue. The bunches can be either loosely packed or moderately dense. In North Macedonia, ‘Cardinal’ is predominantly grown in warm vineyard areas. As a late-ripening variety, it reaches maturity between late September and early October. However, if the flowering phase coincides with rainy and cold weather, the grape clusters tend to develop a reddish coloration.

Nanofertilizer Production in Laboratory Conditions

Organic material rich in nitrogen, phosphorus, and potassium (NPK) is first fermented. After fermentation, the compost is dried at temperatures between 40-50°C and then ground into a fine powder. The resulting NPK organic fertilizer is then converted into a nano-fertilizer with a particle size ranging from 1 to 100 nm using the green synthesis method. To enhance the mixture, 0.5% zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$) is added, and the mixture is stirred at 400 rpm for an additional 30 minutes. The pH of the solution is adjusted to fall between 6 and 7. Next, the mixture is centrifuged at 10,000 rpm for 15 minutes at room temperature. This process is repeated 2 to 3 times, with distilled water being added each time. The gel-like mixture is then dried under vacuum, and the nanoparticles are collected for further use.

Harvesting

The harvest took place when the grapes reached full maturity, with the grapes being collected separately by variety and repetition.

Soil Analysis

Soil fertility was assessed by taking soil samples before the experiment was set up at depths of 0–30 cm, 30–60 cm, and 60–80 cm (Stojanova, 2019). In the laboratory, the soil samples were air-dried and prepared for agrochemical analysis, where the following parameters were measured: pH value – measured potentiometrically using a pH meter (Stojanova, 2017); Easily available nitrogen content – determined using the Tjurin and Kononova method (Stojanova, 2017); Easily available phosphorus content – determined by the AL method and spectrophotometric reading (Stojanova, 2017); Easily available potassium content – determined by the AL method and spectrophotometric reading (Stojanova, 2017); Humus content – determined using the permanganate method of Kotzman (Stojanova, 2017); Carbonate content – determined with a Schaibler Calcimeter (Stojanova, 2017).

Determination of Chemical Composition of Grapevine Leaves

Following the harvest, during the period of maximum stability of both macro and microelements, leaf samples were collected to analyze their chemical composition. The lower leaves and the first cluster of leaves from all sides of the grapevine were selected for the analysis. In the laboratory, the leaf samples were first washed with a 0.05% HCl solution, then rinsed with distilled water, and subsequently dried at room temperature. A total of 20 leaves were sampled separately from each variant and replication for the study (Stojanova, 2017).

The following parameters were analyzed: Nitrogen (N) content – determined using the Kjeldahl method (Stojanova, 2017); Phosphorus (P_2O_5) content – determined using atomic emission spectroscopy with inductively coupled plasma (ICP - AEC) (Stojanova, 2017); Potassium (K_2O) content – determined by incinerating the material with concentrated H_2SO_4 and using a flame photometer (Stojanova, 2017); Calcium (Ca) and Zinc (Zn – determined using atomic emission spectroscopy with inductively coupled plasma (ICP - AEC) (Stojanova, 2017).

Determination of Biologically Active Compounds in Grapevine Fruit

The total carbohydrate content in the grapes was determined using a modified method by Stojanova et al. (2022) in microplates. Absorbance was measured with a spectrophotometer at a wavelength of 490 nm. The total carbohydrate content was calculated based on the calibration curve (absorbance versus concentration) of a standard D-glucose solution.

The total acidity was determined by O.I.V. (2001) standard methods.

The total phenol content was measured using the method of Stojanova et al. (2021), adapted for microplates. This method is based on the reaction between phenols and Folin–Ciolcateu reagent, which forms a colored complex. The phenol content was calculated using the calibration curve (absorbance as a function of concentration) of a standard gallic acid solution.

The flavonoid content was determined using a modified method by Stojanova et al. (2021), also adapted for microplates. This method relies on the ability of flavonoids and flavoglycosides to form complexes with metal ions, with the Al-complex being particularly significant. The method is based on the formation of a colored complex, with a maximum absorption at 430 nm. The flavonoid content was calculated using the calibration curve (absorbance as a function of concentration) of a standard quercetin solution.

Statistical analysis

The data were analyzed using SPSS 20 software. To determine statistically significant differences, an ANOVA test with post hoc Tukey's test ($p<0.05$) was performed.

RESULTS AND DISCUSSION

Soil Fertility and Composition

Soil, as an ecological factor, directly or indirectly influences the life functions of the grapevine. Ideal soil for grape cultivation is loose, deep, slightly acidic to slightly alkaline, and easily warmed. It should offer good aeration, adequate water and nutrient supply, a lighter texture, and high microbiological activity. Additionally, the presence of various macro and microelements plays a crucial role in determining the soil's suitability for growing grapes (Stojanova, 2023).

The soil, as indicated in Table 1, shows moderate fertility for nitrogen, phosphorus, and potassium. The nitrogen level of 6.30 mg/100g places the soil in a moderately fertile category, which is crucial for the proper growth of grapevines. The phosphorus content of 14.83 mg/100 g suggests a medium fertility level, which is adequate for promoting root development and overall vine health. The potassium content of 23.53 mg/100 g is classified as good, which is vital for regulating various physiological processes, including water retention and enzyme activation.

However, the humus content is low (1.43%), indicating that organic matter may need to be supplemented to improve soil fertility, microbial activity, and nutrient retention. The slightly carbonated nature of the soil (2.23% calcium carbonate) does not significantly affect its suitability for grapevine growth. Furthermore, the neutral pH of the soil enhances nutrient availability and is favorable for grapevine development.

Table 1. Agrochemical analysis of soil

Cultivar	Depth (cm)	pH		Available (mg/100 g soil)			Humus (%)	$CaCO_3$ (%)
		H_2O	KCl	N	P_2O_5	K_2O		
‘Cardinal’	0–30	7.55	7.25	7.10	13.80	25.00	1.40	2.50
	30–60	7.40	7.20	6.50	17.50	23.20	1.50	2.30
	60–80	7.42	7.22	5.30	13.20	22.40	1.38	1.90
	Average 0-80	7.46	7.22	6.30	14.83	23.53	1.43	2.23

Effect of Nano NPK Fertilization on the Chemical Composition of Grapevine Leaves

Macronutrients are essential elements that play a critical role in grapevine growth, grape yield, and wine quality. Currently, plant tissue and soil sampling, followed by chemical analysis, provide valuable insights into whether a nutrient is deficient, adequate, excessive, or present in toxic concentrations (Debnath et al., 2021). Among these nutrients, nitrogen (N) and potassium (K) are particularly mobile, allowing them to be rapidly translocated from older leaves to younger leaves or fruiting bodies when the demand exceeds the supply from the soil. This movement can lead to chlorosis and necrosis in the plant (Jegadeeswari et al., 2020).

Zinc, a micronutrient, is primarily absorbed as a divalent cation (Zn^{2+}), but in soils with higher pH, it may also appear as a monovalent cation ($Zn(OH)^+$). Additionally, it can be absorbed in the form of Zn-chelates. The absorption process is active, and zinc can also inhibit the uptake of iron, copper, and manganese ions. Zinc is predominantly found in chloroplasts, cell walls, young leaves, and roots (Stojanova et al., 2024).

As an essential component of various plant enzymes, zinc accelerates metabolic activities. Without these enzymes, plant growth and development would cease, and the production of carbohydrates, proteins, and chlorophyll would be severely reduced in zinc-deficient plants. The application of zinc oxide nanofertilizers to the soil enhances the availability of zinc to plants, thus improving crop yields. Moreover, zinc oxide nanofertilizers have been shown to reduce zinc leaching from the soil, minimizing the risk of environmental contamination (Yadav et al., 2023).

According to Table 2, the foliar application of Nano NPK (20-20-20) combined with nano zinc (Zn) had a substantial effect on increasing the nutrient content in the grapevine leaves, particularly for nitrogen (2.40%), phosphorus (1.92%), potassium (1.69%), and zinc (19.50 $mg \cdot kg^{-1}$), compared to both control variant and variant treated with conventional mineral NPK fertilizer. The highest nutrient concentrations were observed in variant 3 (Nano NPK + Zn 3 g l^{-1}), and these differences were statistically significant ($p<0.05$). This suggests that the Nano NPK formulation, particularly with the zinc addition, facilitates better nutrient uptake and translocation within the grapevines compared to traditional mineral fertilization. These findings align with the general consensus that nano-formulations enhance the bioavailability of nutrients due to their improved surface area and mobility in the plant system.

Table 2. Chemical composition of grapevine leaves (% of dry matter)

Variant	n	N	P_2O_5	K_2O	CaO	Zn ($mg \cdot kg^{-1}$)
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
1	20	2.12 ± 0.01^a	1.53 ± 0.06^a	1.02 ± 0.13^a	2.12 ± 0.10^a	13.10 ± 0.19^a
2	20	2.21 ± 0.07^b	1.67 ± 0.12^b	1.27 ± 0.09^b	2.29 ± 0.05^b	17.25 ± 0.11^b
3	20	2.40 ± 0.03^c	1.92 ± 0.10^c	1.69 ± 0.17^c	2.38 ± 0.03^c	19.50 ± 0.08^c
4	20	2.35 ± 0.11^d	1.83 ± 0.02^d	1.55 ± 0.09^d	2.40 ± 0.16^d	15.20 ± 0.12^d

$^{a, b, c}$ – values for the same parameter of the different variants marked with different letters have statistically significant differences ($p < 0.05$), ANOVA, *post hoc* Tukey's test.

The aim of the research conducted by Mohammed and Ahmed (2023) was to study the effectiveness of nano NPK fertilization on the vegetative growth, mineral content, and response of grape cultivars to nano-fertilizers, as well as to determine the optimal method for applying nano-fertilizers to grape seedlings. Three levels of nano-NPK (0, 2, and 4 g) were applied to the soil, either alone or in combination with foliar application of nano-NPK, which was sprayed three times at concentrations of 0, 1, and 2 g l^{-1}. These treatments significantly promoted all vegetative growth traits, including the height and diameter of the main stem, the number of leaves, chlorophyll concentration, and leaf area.

The investigation also aimed to improve the yield and quality of the 'Thompson Seedless' grape cultivar by using both bulk and nano-formulations of zinc, iron, and manganese (zinc 6.11%, iron 6.06%, and manganese 6.05%). The highest zinc content (38.2 ppm) was found in the variant treated with 2000 ppm of Nano Zn + Fe + Mn (Mohamed, 2020).

In the study by Aljubori et al. (2024), the use of NPK fertilizer (20:20:20) and chelated Nano Zinc significantly improved the number of leaves per vine in 'Taifi' and 'Kamali' grape cultivars. The maximum number of leaves per vine (2640.20) was observed with the application of 200 $mg \cdot l^{-1}$ zinc, compared to the lowest number (2290.8) in the control treatment.

Determination of Biologically Active Compounds in Grapevine Fruit

Polyphenols, the most studied compounds in grapes, are plant secondary metabolites with over 8,000 variations. These compounds, which include anthocyanins, flavan-3-ols, flavonols, phenolic acids, and stilbenes, have antioxidant, anti-inflammatory, anti-cancer, and protective effects on the brain and heart. They also influence the taste and complexity of wine and juice. Grape polyphenol concentration varies by grape variety, climate, environment, and production process. The highest polyphenol content is found in the seed, followed by the skin, which contains tannins and other phenols (Hornedo-Ortega et al., 2020; Stojanova, 2024a).

Table 3. Chemical composition of grapevine fruit

Variant	n	TPC (mg GAE/100 g)	TFC (mg CAE/100 g)	Total carbohydrates (g/100 g)	Total acidity (g/L)
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
1	10	98.15 ± 0.03[a]	59.27 ± 0.10[a]	16.95 ± 0.09[a]	7.21 ± 0.11[a]
2	10	130.84 ± 0.11[b]	65.17 ± 0.16[b]	18.41 ± 0.02[b]	10.38 ± 0.10[b]
3	10	156.32 ± 0.08[c]	68.02 ± 0.07[d]	21.07 ± 0.13[c]	15.69 ± 0.15[c]
4	10	148.11 ± 0.01[d]	69.89 ± 0.02[c]	19.64 ± 0.16[d]	14.06 ± 0.12[d]

[a, b, c] – values for the same parameter of the different variant marked with different letters have statistically significant difference ($p < 0.05$), ANOVA, *post hoc* Tukey's test.

The data from Table 3 reveals that Nano NPK fertilization also positively influenced the grape fruit's biologically active compounds, such as phenols, carbohydrates, total acidity, and flavonoids. Variant 3 (Nano NPK + Zn 3 g l^{-1}) exhibited significantly higher ($p < 0.05$) concentrations of total phenols (156.32 mg GAE/100g), total carbohydrates (21.07 g/100 g), and total acidity (15.69 g l^{-1}) compared to the control and other variants. Additionally, the highest flavonoid content (69.89 mg CAE/100g) was recorded in this variant, suggesting that Nano NPK fertilization promotes the synthesis of these valuable compounds, which contribute to the health benefits and sensory properties of the grape fruit. This result supports the notion that nano-fertilization can enhance the nutritional value and quality of grape fruit by stimulating the production of secondary metabolites.

The study by Hamza et al. (2019) demonstrated that all Nano Potassium fertilizer treatments resulted in increased nitrogen and potassium concentrations in the petioles of 'Flame Seedless' grapevines and enhanced various vegetative growth traits, including shoot length, shoot diameter, and leaf area. Furthermore, a significant increase in yield per vine was observed when compared to the control treatment. The treatment combining 50% mineral fertilizer with 250 ppm of Nano K produced the highest values for soluble solids concentration (SSC), SSC/acid ratio, total anthocyanins, and total sugars, while also yielding the lowest acidity values across both study seasons.

In the research conducted by Arji et al. (2022), the highest values for the number of berries per bunch, number of bunches per vine, bunch weight, bunch length, yield per vine, and yield per hectare were recorded in 'Yaqouti' grapes treated with a combination of chelated nanofertilizers and chemical fertilizers.

Lopez et al. (2019) reported that the total concentration of polyphenolic compounds was consistently higher in seed extracts compared to skin samples, with the highest concentration of polyphenols reaching 1,545 g GAE/100 g of sample in the seed extract of the 'Winemaker 2' variety. Similarly, Flancy (2000) also found that seed polyphenol concentrations surpassed those in the peel, with tannins being the predominant phenolic compounds.

Kennedy et al. (2000) highlighted that phenols in seeds play a crucial role in dormancy regulation and seed viability. These phenolic compounds enhance seed permeability to water, facilitating germination. Additionally, they noted that hydrolyzable tannins are present in smaller quantities compared to the more abundant extractable and condensed tannins.

Further research by Maryem (2020) examined the phenolic content of grape skins, revealing total phenol levels of 168.55 mg/100 g GAE, flavonoid content of 61.10 mg/100 g, and an IC50 of 1,065.19 ppm. This study utilized the standard curve equation to quantify total phenols and flavonoids, finding that phenolic compounds were present in greater amounts than flavonoids, as flavonoids represent a subclass of phenolic compounds.

The improved nutrient status and elevated levels of biologically active compounds in the grape fruit from the Nano NPK + Zn treatments suggest that these nano-formulations may offer a more efficient and sustainable alternative to traditional mineral fertilization. Nanofertilizers have been shown to enhance nutrient uptake by plants due to their small particle size, which increases their surface area and reactivity. Additionally, the inclusion of zinc, an essential micronutrient, likely played a role in stimulating the synthesis of secondary metabolites, further improving the quality of the grapes.

CONCLUSION

The findings of this experiment underscore the potential benefits of using Nano NPK fertilization on grapevine nutrition and the enhancement of biologically active components in the grape fruit. The higher concentrations of essential nutrients such as nitrogen, phosphorus, potassium, and zinc in the leaves treated with Nano NPK + Zn demonstrate the efficacy of nano-sized fertilizers in increasing nutrient availability and uptake. This is particularly significant for grapevines, which require a balanced nutrient supply to ensure optimal growth, development, and fruit quality. The positive effects of Nano NPK fertilization are particularly evident in the increased levels of total phenols, flavonoids, and carbohydrates in the grape fruit. These compounds are known for their antioxidant properties and contribute to the health benefits of grape consumption. The significant increase in total acidity in variant 3 further indicates that the treatment has an impact on the fruit's taste profile, potentially enhancing its flavor complexity. However, it is important to note that while Nano NPK fertilization showed positive effects in this study, further research is needed to explore the long-term impacts of such fertilization practices on soil health, grapevine longevity, and the overall sustainability of agricultural systems. Future studies should also investigate optimal application methods, concentrations, and combinations of nano-formulations for different grapevine varieties and growing conditions.

In conclusion, Nano NPK fertilization holds great promise for improving grapevine nutrition, enhancing fruit quality, and potentially reducing the environmental impact of conventional fertilization methods. By promoting both the nutritional and sensory properties of the fruit, it may contribute to higher quality grape production and, subsequently, better wine or fresh fruit outcomes.

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