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Research Article

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ZnO nanoparticles improve bioactive compounds, enzymatic activity and zinc concentration in grapevine

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Abstract

The low availability of micronutrients in the soil leads to a deficit of these micronutrients in crops, causing malnutrition in the population. Approximately 3,000 million people in the world have health problems caused by inadequate Zn intake. Agronomic biofortification is a way to produce crops rich in micronutrients and mitigate malnutrition problems. Nano biofortification with zinc oxide nanoparticles (NPs-ZnO) is a good strategy to mitigate and increase the nutritional content of the edible part of the plant. The aim was to determine the effect of foliar spraying with NPs-ZnO on yield and biosynthesis of enzymatic and non-enzymatic antioxidant compounds and their bioaccumulation. In this study, the effect of foliar fertilization with NPs-ZnO: 0, 25, 50, 75, and 100 mg L^{-1}, on yield, the content of bioactive compounds and their bioaccumulation in grapevine berries was evaluated. The distribution of the treatments was under a completely randomized design, each treatment consisted of 10 plants, each representing one experimental unit. The treatments were applied by foliar sprays at fruit formation, in veraison and 15 days before harvest. Foliar spraying with NPs-ZnO positively modifies yield, the content of bioactive compounds, and their bioaccumulation. Doses of 50-75 mg L^{-1} of NPs-ZnO increased crop yield, and oenological parameters. In addition, all doses evaluated modified enzymatic and non-enzymatic antioxidants, and improving Zn concentration in grapevine berries. Foliar spraying of NPs-ZnO is an alternative to improve grape quality and yield, in addition to enriching its nutritional and antioxidant content.

Keywords: agronomic biofortification; bioactive compounds; yield; *Vitis vinifera* L.

Introduction

Zinc (Zn) is an essential micronutrient for organisms, in humans its deficiency affects 17.3% of the world population (Ramalho *et al.*, 2022), the health problems associated with this insufficiency are neurological

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disorders, autoimmune conditions, age-related degenerative diseases, Wilson's disease (disorder that causes copper to accumulate in your liver, brain and other vital organs), cardiovascular problems, and diabetes, among others (Kumar *et al*., 2022). In plants, Zn is essential for genetic regulation, photosynthesis, enzyme activator, auxins, membrane integrity, and protein production (Umair *et al*., 2020), its application to crops via edaphic and/or foliar is mainly aimed at reducing the deficiency of this element, due to its low availability especially in calcareous soils and alkaline pH, which causes a low content of this element in crops (Preciado *et al*., 2021). Agronomic biofortification is a strategy to try to supply this lack of trace elements in the population (De Groote *et al*., 2021), using this technique, in addition to increasing the mineral content and bioactive compounds in the edible part of the plant, the nutritional quality of food is substantially improved (Martínez-Ballesta *et al*., 2018). On the other hand, the growing demand for food of plant origin, forces to increase the production and quality of them through innovative technologies that are more sustainable in modern agriculture (Rai-Kalal & Jajoo, 2021); in this sense the use of nanomaterials represents an innovation in the agricultural sector since nanomaterials can be used as biostimulants of plant growth and as biactivators of the antioxidant activity (enzymatic and non-enzymatic) of plants (Saleem *et al*., 2022). Zinc oxide nanoparticles (NPs-ZnO), have been successfully evaluated in agricultural production for their antifungal, antibacterial, and nutritional properties (Rossi *et al*., 2019); they have shown an increase in yield, biomass, root, germination, and chlorophyll concentration (Servin *et al*., 2015); they also protect plants from biotic and abiotic stress by activating non-enzymatic (proline, phenolic compounds) and enzymatic (CAT, POD, SOD) antioxidants to alleviate stress (Hong *et al*., 2021). However, excessive zinc in plants can lead to nutrient imbalances, toxicity, and root damage, resulting in poor growth and overall plant health issues. (Rico *et al*., 2015). Therefore, it is of utmost importance to determine the appropriate doses of NPs-ZnO that stimulate crop yield and quality. On the other hand, grapevine (*Vitis vinifera* L.), is one of the most important perennial crops in the world (Tello *et al*., 2019) and is considered a functional food due to its high content of bioactive compounds, which are related to disease prevention (Huber *et al*., 2021; Krasteva *et al*., 2023; Kaushik *et al*., 2023), and it is also a crop very sensitive to Zn deficiencies (García-López *et al*., 2019). For the above-described NPs-ZnO can be an alternative to increasing the yield and quality of grapevine berries. Based on the above, the present study aims to determine the effect of foliar spraying with NPs-ZnO on yield and biosynthesis of enzymatic and non-enzymatic antioxidant compounds and their bioaccumulation.

Materials and Methods

Study area and experimental conditions

The experiment was carried out in a commercial orchard located in Monterrey, Durango, Mexico, at coordinates 25°29'20''N, 103°37'37'W. The climate of the study area is dry; average temperature is 21 °C and annual precipitation is 253 mm (García, 2004). The soil was subjected to a characterization analysis, whose results are shown in Table 1.

Planting material

Eight-year-old 'Cabernet Sauvignon' grapevine plants were used. The planting system was 1 m between plants and 3 m between rows, with a plant density of 3,333 plants ha^{-1}. The study lot received the same agronomic management applied to the entire vineyard by the producer, regarding irrigation, fertilization (80, 20 units of N and P_2O_5 ha^{-1}), phytosanitary control, and pruning.

Zinc oxide nanoparticles

The NPs ZnO was obtained by wet chemistry methodology in the form of wurtzite crystals with an average size of 50 nm with no contaminants, a purity level of 99.7%, and a density of 5.61 g cm^{-3}. The material was provided from the Mexican company Investigación y Desarrollo de Nanomateriales S.A. de C.V. and was characterized in a previous study by Ponce-Garcia *et al.* (2019).

Table 1. Soil characteristics where the experiment was carried out

Soil characteristic	Value
Sand (%)	81
Silt (%)	14
Clay (%)	5
Bulk Density (g cm-^{3})	1.67
pH	8.37
Water Holding Capacity (%)	25.2
Electrical Conductivity (dS m-^{1})	1.28
Organic Matter Content (mg kg-^{1})	1.18
Total Nitrogen (mg kg-^{1})	32.8
Available Phosphorus (mg kg-^{1})	24.4
Removable Potassium (mg kg-^{1})	90.43

Treatments and experimental design

Four doses of NPs-ZnO were used: 25, 50, 75, 100 mg L^{-1}, and a control treatment (0 mg L^{-1}), In all formulations, 0.1% urea was added as the carrier ion and a non-toxic commercial surfactant (INEX-A®, 0.02% v:v). The pH of the solutions was adjusted to 6.5 to facilitate foliar uptake in formulations with metallic nutrients. The distribution of the treatments was under a completely randomized design, each treatment consisted of 10 plants, each representing one experimental unit (EU). The treatments were foliar applied three times during the crop cycle (fruit formation, veraison, and 15 days before harvest).

Yield components

Harvesting was carried out on the same day for all treatments when the total soluble solids (TSS) of randomly harvested berries remained constant for a few days. Yield per plant and average bunch weight were recorded for each plant using a hanging scale

Oenological parameters

Total soluble solids (TSS) and titratable acidity (TA) were measured in the juice of 50 berries per treatment and replicated. TSS were measured with a 0-32% manual refractometer (Sper ScientificR 30001, SperScientific LTD, Scottsdale AZ, USA) at 20 °C and expressed in °Brix. TA was determined following AOAC methodology (AOAC, 1990), using NaOH (0.1 N) and phenolphthalein (1%) as indicators; results were expressed as a percentage of tartaric acid per 100 g. The pH was measured with an electronic pH meter (Thermo Scientific, USA), and finally, the maturity index was calculated with the TSS/TA ratio. The probable degrees of alcohol PGA) were calculated using the formula: PGA = (0.675*°Brix)-2.0839.

Non-enzymatic antioxidants

For the preparation of extracts were taken two grams of fresh pulp were mixed in 10 mL of ethanol (80%) in a test tube, which was placed on a rotary shaker (ATR Inc., USA) for 6 h at 5 °C and 20 rpm. Subsequently, the tubes were centrifuged at 3,000 rpm for 5 min and the supernatant was removed for analytical testing.

Phenolic content

Total phenolic content was determined by the Folin-Ciocalteau method (Garcia-Nava, 2009). Samples were quantified in an ultraviolet (UV)-Vis spectrophotometer at 760 nm (Metash, UV-6000). The standard was prepared with gallic acid. The results were expressed in mg GAE 100 g^{-1} fresh weight (FW).

Flavonoid content

Total flavonoids were determined by colorimetry (Garcia-Nava, 2009). Samples were quantified in a UV-Vis spectrophotometer at 510 nm (Metash, UV-6000). The standard was prepared with quercetin dissolved in absolute ethanol. Results were expressed as mg QE 100 g^{-1} FW.

Antioxidant capacity

Total antioxidant capacity was measured by the in vitro $DPPH^+$ method (Brand-Williams *et al.*, 1995). Samples were quantified in a UV-Vis spectrophotometer at 517 nm (Metash, UV-6000). The standard was prepared with Trolox (0.1-1.0 mM, $r^2 = 0.998$). Results were expressed as μM Trolox equivalent 100 g^{-1} FW.

Vitamin C content

Vitamin C was determined according to Hernandez-Hernandez *et al.* (2019). 10 g of fresh fruit were ground with 10 mL of 2% hydrochloric acid, then with the help of a funnel and filter paper, the sample was filtered and the extract obtained was made up to 100 mL with deionized water. Then, 2,6 dichlorophenolindophenol (1×10^{-3} N) was used to titrate 10 mL of the dilute. To determine the titration, the reddish color should persist for a few seconds. If it disappears when the sample is shaken, it means that there is still vitamin C without oxidation. Once we obtain the reddish color, we stop adding dye and calculate the volume spent. The result is reported as mg 100 g^{-1} FW.

Anthocyanins

The total anthocyanin content was determined according to the methodology indicated by Lira *et al.* (2017). One gram of grape sample was placed into a 25 mL Erlenmeyer flask, followed by the addition of a 10 mL anthocyanin extraction solution composed of HCl:methanol:water (0.02:8:1.8; v/v/v). The flask was placed in a sonicator (Branson, 1,800: 1.8 L/0.5 GAL) at 50 °C for 1 h. The extract obtained was centrifuged. The extract obtained was centrifuged. The extract obtained was centrifuged at 3,000 rpm for 10 min at 4 °C and the supernatant was collected and stored in an amber vial at 4 °C until use. Finally, absorbance was measured at a wavelength of 525 nm in a spectrophotometer (Metash, UV-6000) and the concentration of total anthocyanins in the grape sample was reported as μg cyanidin 3-glucoside equivalent g^{-1} grape sample.

Enzymatic antioxidant

The determination of catalase activity (CAT) (EC 1.11.1.6) (the mM equivalent of H_2O_2 consumed per milliliter per minute), was carried out according to David *et al.* (2008). An enzyme extract was prepared, and 0.5 g of grape pulp was weighed, to which 5 mL of a potassium and sodium phosphate solution with the following characteristics was added: 100 mM, pH 7.0 at 4 °C and 50 mg of polyvinylpyrrolidone (PVP) was added. After this procedure, it was mixed in a mortar. The mixture was centrifuged at 11,000 g for 11 min at 4 °C and the supernatant was taken to determine the catalase activity, then mixed with 3 mL of sodium phosphate buffer at 300 μM, pH 6.8, 1 mL of H_2O_2 at 100 μM, and 1 mL of the enzyme (from the enzyme extract) as recommended diluted at a ratio of 1:20. The measured reaction time was 1 min at 240 nm. Peroxidase activity (PXA) (EC 1.11.1.7) (the mM equivalent of H_2O_2 consumed per milliliter per minute) was determined using a modification of the method of Nickel and Cunnnigham (1969), where absorbance was measured at 420 nm in a spectrophotometer (Metash, UV-6000), the reaction mixture contained 20 mL H_2O, 2 mL enzyme extract plus 1 mL guaiacol and 1 mL H_2O_2. Then, the reaction time was taken as 10 min.

Protein determination

The protein content in each extract was determined through the method developed by Bradford (1976) using bovine serum albumin (BSA) as the standard.

Zinc content analysis

The Zn content was determined by triacid digestion (Guillén-Enríquez *et al.*, 2022). One gram of each sample was weighed on an analytical balance (HR-120), with an accuracy of 0.0001 g. The sample was then placed in a 250 mL beaker with boiling beads, and 25 mL of triazide mixture (1 L of HNO_3, 100 mL of HCl, 25 mL of H_2SO_4) was added. Following this, the sample was placed in a digester grill in a fume hood for one hour. At the end, the resulting samples were filtered into 50 mL volumetric flasks (stock solution), gauged, and stirred with triple distilled water. Finally, samples were poured into 50 mL tubes to centrifuge them. The concentrations of Zn were performed by Coupled Plasma Atomic Emission Spectroscopy, ICP-OES (Agilent Technologies 700 Series ICP-OES, California, USA), and the results were expressed in mg kg^{-1} dry weight (DW).

Statistical analysis

Data were analysed by one-way ANOVA by the GLM method of SAS statistical package version 9.1 (SAS Institute, 2009). The Tukey simultaneous test was used for comparing statistical means ($P \leq 0.05$).

Results and Discussion

Yield components

Foliar spraying of NPs presents advantages when applying Zn in nanometric form, thanks to its large surface area intensive surface charge, and high resonance of its particles, which includes high absorption efficiency, as well as rapid uptake and movement within the vascular tissues of the plant (Abou El-Nasr *et al.*, 2021). In the present study, foliar spraying with 75 mg L^{-1} of NPs-ZnO promoted a yield increase of 14.89%, relative to that obtained in untreated plants (Figure 1). This effect is attributed to the fact that Zn is a nutrient strongly related to plant growth and yield since it is necessary for the synthesis of nucleic acids and proteins (Toor *et al.*, 2020), carbohydrates, and the translocation of photosynthates to the reproductive parts (Bana *et al.*, 2021). Also, higher Zn availability contributes to higher carbohydrate accumulation and a favorable effect on plant yield (Saha *et al.*, 2023). Although, higher doses of NPs-ZnO >2000 mg L^{-1} may cause plant toxicity on grass and buckwheat (Liu and Lal, 2015; Alvarez *et al.*, 2017) and imply an environmental risk (Gomez *et al.* 2021).

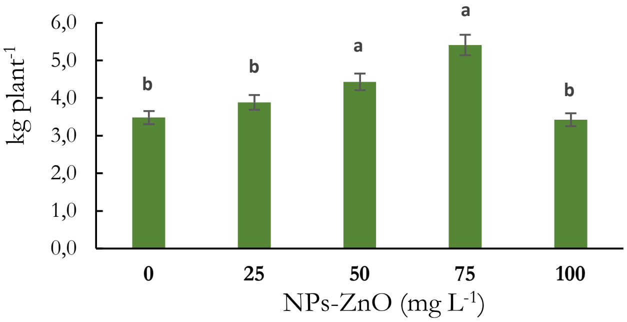

Figure 1. Effect of foliar NPs-ZnO spraying on yield of grapevine plants
Different letters indicate a difference between treatments (Tukey test $P \leq 0.05$). n = 10 ± standard error.

Oenological parameters

pH, TSS, TA, and TA/TSS ratio are used as parameters to define the optimum harvest time (Ramírez-Gottfried *et al.*, 2023). Foliar spraying of NPs-ZnO caused significant differences in the oenological parameters evaluated. The observed variations in grape quality parameters, such as pH, TSS, TA/TSS ratio, and PGA, stem from the differential impact of dosage levels on grape physiology and chemistry. The medium dose appears to create an environment that optimizes the balance between acidity and sugar content, resulting in grapes well-suited for winemaking with a potentially higher PGA during fermentation. Conversely, the high dose disrupts this balance, leading to unfavourable changes in grape chemistry and a subsequent decrease in the TA/TSS ratio and PGA, signalling diminished suitability for high-quality wine or alcohol production. On the other hand, any dose of NPs-ZnO decreases TA (Table 2). Zn plays an important role in many biochemical pathways (Song *et al.*, 2015), such as regulating tryptophan synthesis and directly influencing fruit quality (Garcia-Lopez *et al.*, 2019), increasing TSS content and decreasing TA in grapevine berries (Abou El-Nasr *et al.*, 2021; Daccak *et al.*, 2022;). High TSS and low TA lead to a low TA/TSS ratio attributable to the fact that organic acids are used as substrate for sugar synthesis during berry ripening (Sariñana-Navarrete *et al.*, 2021). Sugar content plays an important role in consumer acceptability, mainly due to its interaction with organic acid and phenol synthesis, sensory properties, alcohol concentration in winemaking, and aroma compounds (Walker *et al.*, 2021). Higher doses likely cause some type of stress or toxicity since, depending on the form and dose, Zn can act as an antioxidant or as a toxic agent (Estrada-Domínguez *et al.*, 2020).

Table 2. Effect of foliar spraying of NPs-ZnO on oenological parameters in grapevine berries

NPs ZnO mg L^{-1}	pH	TSS °Brix	TA %	TA/TSS Ratio	PGA %
0	3.91±0.11a*	18.33±1.24b	8.00±0.81a	2.32±0.29b	10.29±0.31b
25	4.03±0.06a	20.33±0.94a	5.66±0.47b	3.61±0.34a	11.64±0.84a
50	4.07±0.07a	20.33±0.47a	5.66±0.43b	3.62±0.43a	11.64±0.63a
75	3.80±0.28ba	18.66±0.47b	6.33±0.47b	2.96±0.18ba	10.51±0.31b
100	3.44±0.18b	17.66±0.45b	6.33±0.45b	2.81±0.26b	9.84±0.31b

*Data are shown as the mean ± standard deviation (SD, n = 25). Different letters indicate a difference between treatments (Tukey test $P \leq 0.05$). n = 10 ± standard error.

Non-enzymatic antioxidants

Foliar spraying with NPs-ZnO significantly increased non-enzymatic antioxidants (Figure 2). With 75 mg L^{-1}, the Vitamin C and anthocyanin content increased by 69.53 and 21.56% compared to the control treatment. With 100 mg L^{-1} phenols, flavonoids and antioxidant capacity increased by 18.42%, 68.91%, and 38.07%, respectively, compared to untreated berries. These results are in agreement with previous studies reporting increases in non-enzymatic antioxidant content by Zn use (Hong *et al.*, 2021; Saleem *et al.*, 2022. Plant response to Zn depends on the concentration and chemical species used and can range from increased development and productivity to stress or toxicity (Khan *et al.*, 2023). In this study, it was observed signs of stress, notably in the form of reduced crop yield and changes in oenological variables, as detailed in Table 1 and 2. This suggests that the observed stress may have been linked to the higher concentrations of zinc applied. Additionally, the increase in non-enzymatic antioxidants, as noted in our results, may be attributed to the plant's natural response mechanism aimed at protecting itself from potential oxidative damage, as suggested by the research of Sutuliené *et al.* (2023). For the above mentioned, the increased levels of Vitamin C and antioxidant capacity can be attributed to the positive influence of zinc, yet the complex nature of the impact of zinc on plants, encompassing both beneficial and stress-inducing effects, necessitates careful consideration in agricultural practices.

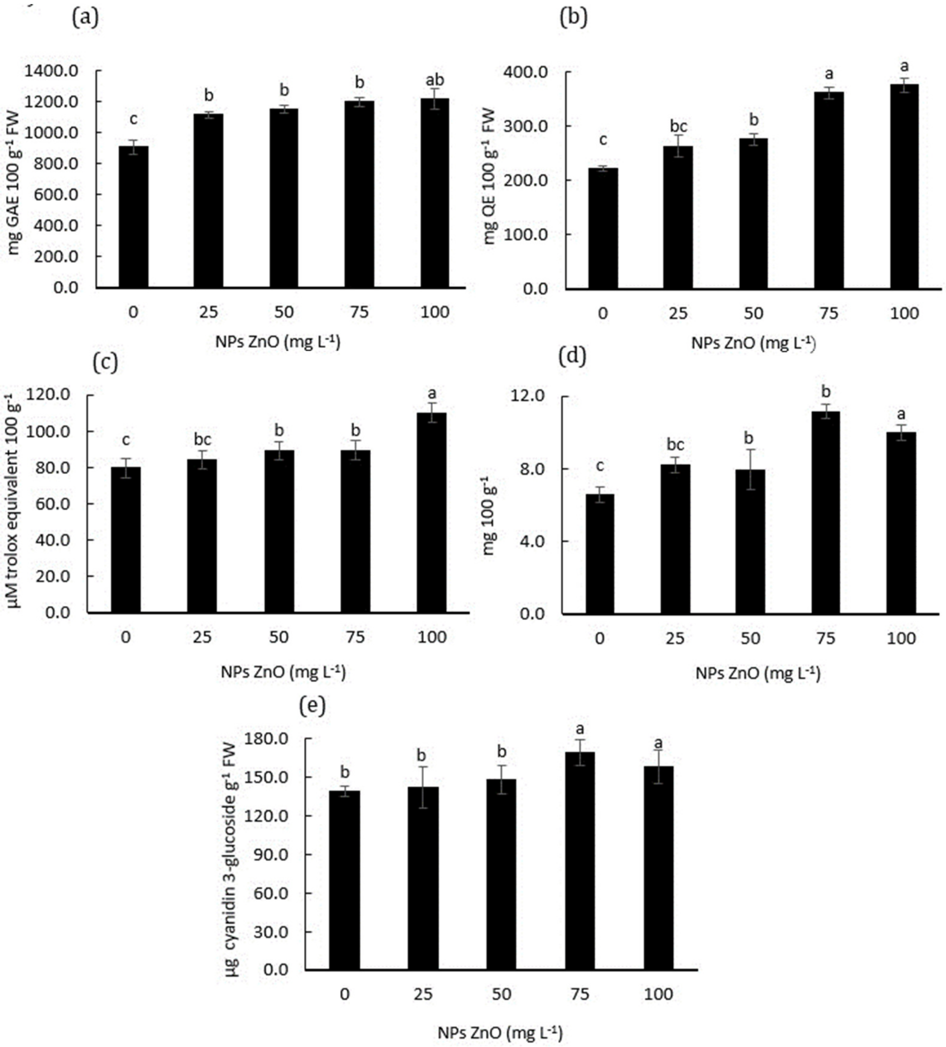

Figure 2. Effect of foliar NPs-ZnO spraying on phenolic compounds (a), flavonoids (b), antioxidant capacity (c), vitamin C (d) and anthocyanins (e)

Different letters indicate a difference between treatments (Tukey test $P \leq 0.05$). n = 10 ± standard error.

Enzymatic activity

Regarding enzymes and proteins evaluated in grapevine after foliar application of NPs-ZnO (Table 3). It was identified that the concentration of 50 mg L^{-1} induced an increase in catalase activity compared to the control. At a concentration of 75 mg L^{-1}, peroxidase activity was increased compared to the control. Whereas, when using a concentration of 25 mg L^{-1}, the protein content increased compared to the control. The increase in enzyme activity is because Zn acts as a cofactor for the activity of many enzymes, such as superoxide dismutase and glutathione peroxidase (Faizan *et al*., 2021); these enzymes increase the plant's tolerance to oxidative stress (Sariñana-Navarrete *et al*., 2023); however, it has been shown that high doses of Zn can induce stress and inhibit enzyme activity (Cakmak, 2000). The rise in protein content could be attributed to the plant responding to the applied stressor by attempting to bolster its defense mechanisms (Etesami *et al*., 2021). Proteins play vital roles in a multitude of cellular processes, including pathways associated with stress responses (Kaur *et al.,* 2021). Therefore, the elevated protein content may indicate an adaptive reaction to the presence of zinc, potentially aimed at mitigating the stress induced by the application.

Table 3. Effect of foliar spraying of NPs-ZnO on enzymatic antioxidants in grapevine berries

NPs ZnO mg L^{-1}	Catalase activity mM min^{-1}	Peroxidase activity mM min^{-1}	Protein mg mL^{-1}
0	0.01333±0.004b*	0.1999±0.031a	0.0191±0.001b
25	0.0266±0.012ba	0.3389±0.045a	0.0472±0.007a
50	0.0366±0.009a	0.3618±0.138a	0.0376±0.016a
75	0.0300±0.004ba	0.5147±0.336a	0.0421±0.009a
100	0.0300±0.005ba	0.2252±0.017a	0.0338±0.001a

*Data are shown as the mean ± standard deviation (SD, n = 25). Different letters indicate a difference between treatments (Tukey test $P \leq 0.05$). n = 10 ± standard error.

Zinc content in berries

Although Zn deficiency in the population is associated with excessive consumption of processed foods and cereals rich in phytates (Meneghelli *et al.*, 2021). Zn content in plants depends on the content and availability of Zn in the soil or on soil or foliar biofortification. Our results indicate that Zn content in grape berries was influenced by the dose used. Higher doses of Zn application promoted increases in Zn content concentration in treated berries (Figure 3). Studies in what and rice gain have shown that biofortification with NPs-ZnO is a practical way to increase the content of this element in the edible part of the plant (Dhaliwal *et al.*, 2021). All sources of Zn applied contribute to a significant increase in the Zn content in the edible part of the plant (Du *et al.*, 2019); however, caution should be exercised in the dose used since Zn being a heavy element can cause toxicity (Obrador *et al.*, 2021), in this sense, it is crucial to underline that the use of NPs-ZnO in high concentrations could raise concerns in terms of environmental impact (Tong *et al.*, 2022). The inherent risk of excessive accumulation of these particles in the environment could lead to adverse effects on the surrounding ecosystems (Lai *et al.*, 2020). It is therefore essential to advocate for accurate and responsible management of NPs-ZnO, considering both their potential benefit and the potential environmental challenges that could arise.

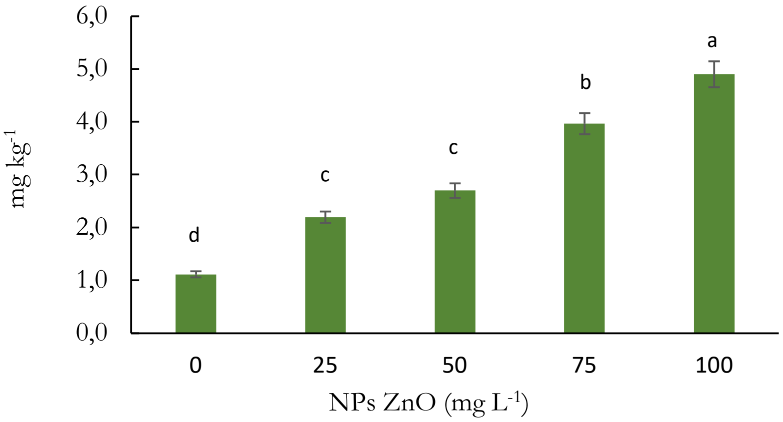

Figure 3. Effect of foliar NPs-ZnO spraying on zinc bioaccumulation in grapevine berries
Different letters indicate a difference between treatments (Tukey test $P \leq 0.05$). n = 10 ± standard error.

Discussion

The study demonstrated that foliar spraying with NPs-ZnO at a concentration of 75 mg L^{-1} led to a significant increase in yield, which is consistent with previous research emphasizing the role of zinc in promoting plant growth (Abou El-Nasr *et al.*, 2021). Zinc is known to be a crucial nutrient for plants, influencing processes like nucleic acid and protein synthesis, carbohydrate translocation, and photosynthate distribution (Toor *et al.*, 2020). These findings support the hypothesis that zinc, when delivered in nanometric form, can enhance plant growth, yield and bioactive compounds. The study also found that different doses of NPs-ZnO had varying effects on oenological parameters, including pH, TSS, TA, and TA/TSS ratio. This indicates that zinc application can influence the quality of grapes, with moderate dosages promoting a better balance between acidity and sugar content, making the grapes more suitable for winemaking. In contrast, high doses disrupted this balance, resulting in less suitable grapes for high-quality wine production. These results align with the concept that nutrient availability, such as zinc, plays a crucial role in shaping grape quality for winemaking. The study revealed a significant increase in non-enzymatic antioxidants, including Vitamin C, anthocyanins, phenols, flavonoids, and antioxidant capacity. These findings correspond with previous studies that have shown increased non-enzymatic antioxidant content in response to zinc application (Hong *et al.*, 2021; Saleem *et al.*, 2022). However, it's essential to recognize that these increases could be the result of the natural plant defense mechanism against potential oxidative stress induced by higher concentrations of zinc. The study observed changes in enzyme activities and protein content, with the concentration of 50 mg L^{-1} increasing catalase activity and 75 mg L^{-1} enhancing peroxidase activity. This can be attributed to zinc's role as a cofactor for various enzymes that boost the tolerance of the plant to oxidative stress. However, it is worth noting that higher doses of zinc can inhibit enzyme activity (Cakmak, 2000). The increased protein content may be linked to the plant's adaptive response to the stressor, as proteins are integral in stress response pathways (Kaur *et al.*, 2021). The study found that the zinc content in grape berries was influenced by the dose used, with higher doses promoting increased zinc concentration. This finding aligns with the concept of biofortification, wherein zinc content in edible plant parts can be increased through controlled application of zinc (Dhaliwal *et al.*, 2021). However, it is crucial to recognize the potential environmental impact and toxicity associated with excessive zinc accumulation in plants and the environment. It is important to acknowledge that this study identified signs of stress and potential toxicity at higher zinc concentrations, which might have implications for both plant health and the environment. Further research is needed to determine the optimal dosages of NPs-ZnO for different crops and soil conditions. Additionally, studies on the long-term effects of zinc application, including its impact on soil health and environmental sustainability, are essential.

Conclusions

Foliar application of NPs-ZnO increased yield, enological parameters, enzymatic and non-enzymatic antioxidants in grapevine berries at doses of 50-75 mg L^{-1} of NPs-ZnO. In addition, all doses evaluated (25-100 mg L^{-1} of NPs-ZnO) modified enzymatic and non-enzymatic antioxidants, as well as improved Zn concentration. Foliar spraying of NPs-ZnO is an alternative to improve grape quality and yield.

Authors' Contributions

Conceptualization: RRGE, PPR; Methodology: ESCh, MFH; Validation: SYMG; Formal analysis: MFH, Investigation: RRGE; Data curation: BEP; Funding acquisition: PPR; Project administration: BEP. Writing: ESCh; Review and editing PPR. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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