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INFLUENCE OF ZINC OXIDE NANOPARTICLES ON BIOACTIVE COMPOUNDS IN MELON FRUITS (*Cucumis melo* L.)

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ABSTRACT

Foliar application of metal nanoparticles represents a promising strategy for enhancing both yield and nutritional quality in horticultural crops. This study focused on the foliar application of zinc oxide nanoparticles (ZnO NPs) across a range of concentrations (0, 150, 200, 250, 300, and 350 mg L^{-1}) and assessed their impact on the yield and nutraceutical profile of melon fruits. Our findings revealed specific effects at different concentration levels: lower concentrations (150 mg L^{-1}) improved yield and biophysical quality; intermediate concentrations (250 mg L^{-1}) enhanced enzymatic and non-enzymatic antioxidants; while higher concentrations (300–350 mg L^{-1}) resulted in reduced yield and enzymatic activity, accompanied by elevated Zn concentration in melon pulp. The utilization of ZnO NPs presents a viable avenue for enhancing both the biophysical quality and antioxidant content of melon fruits. However, it is crucial to note that their effects are dose-dependent, capable of inducing either beneficial or inhibitory outcomes. Consequently, further comprehensive investigations are warranted to elucidate optimal application dosages and maximize the potential benefits of ZnO NPs in horticultural production.

Keywords: *Cucumis melo* L, antioxidants, nano-biofortification.

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INTRODUCTION

The increasing micronutrient deficiency in plant foods, especially zinc (Zn), poses malnutrition challenges, with approximately one-third of the world's population at risk of zinc deficiency, particularly among children under five years old, who require higher zinc intake for proper growth and development (Praharaj et al., 2021). In fact, zinc deficiency in humans is associated with various diseases, including immunological and neurological disorders (Gupta et al., 2020). In plants, zinc is an essential micronutrient as it regulates growth through protein synthesis and enzyme activation, controls gene expression, influences hormone functions, supports photosynthesis, aids in carbohydrate metabolism, and enhances disease resistance, all of which are crucial for proper physiological development (Umair et al., 2020). Zn deficiency in plants manifests itself through symptoms such as a smaller leaf area on young leaves, chlorotic leaves, and general stunted growth (Balafrej et al., 2020). The limited availability of Zn in calcareous soils with alkaline pH, combined with the use of micronutrient-deficient fertilizers, contributes to low zinc content in plants (Preciado et al., 2021). Nano-biofortification, involving the application of nanomaterials to crops, is an agronomic approach aimed at mitigating zinc deficiency, increasing zinc content in edible parts, and enhancing human dietary intake. While it can improve crop productivity, quality, and soil remediation, it also poses risks such as toxicity and nano-contamination, affecting both the environment and human health (Silva et al., 2021; Khan et al., 2021).

Zinc oxide nanoparticles (ZnO NPs) are notable among metallic NPs due to their beneficial impact on crop production, including antifungal and antibacterial properties, improved germination, stress mitigation, enzyme modulation, and gene regulation (González-Merino et al., 2021). However, determining the optimal dose for ZnO NPs remains a challenge, as their effects vary depending on factors such crop type, nanoform, duration of exposure, and interactions of nanoelements (Guillén-Enríquez et al., 2022). Therefore, it is crucial to determine the correct doses that promote both yield and quality in various crops (Vishwakarma et al., 2023).

Melon (*Cucumis melo* L.), belonging to the Cucurbitaceae family, is known for its rich nutritional profile, containing proteins, lipids, vitamin C, beta carotenes, antioxidants, bioactive polyphenols, and other phytochemical compounds crucial for disease prevention (Rivera-Gutiérrez et al., 2021; Manchali et al., 2021; Guo et al., 2023). Given its economic importance and continued growth in production, melon is an ideal candidate for Zn biofortification (Kubo et al., 2021; Davidson et al., 2023). Consequently, this study aims to evaluate the impact of foliar spraying with ZnO NPs on melon crops, evaluating its effects on yield, commercial quality, nutraceutical properties, and fruit bioaccumulation.

MATERIALS AND METHODS

Location

The study was carried in Ejido Concordia, San Pedro de las Colonias, Coahuila, Mexico (25° 48′31′′ north latitude and 103°5′56.4′′ longitude, with an altitude of 1,016 m). The area experiences a semi-warm climate, characterized by average annual temperatures of 20 to 22°C and annual rainfall ranging from 125 to 400 mm, with hot summers and mild winters.

Vegetation and growing conditions

The melon hybrid Cv Crusier (Harris Moran®) was used. Land preparation was typical for this region, which included fallow, cross-cropping, and leveling. Double-row borders were constructed forming beds at a distance of 4 m between borders and a plant spacing of 30 cm for a density of 16,665 plants per hectare. Fertilization was according to Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias recommendations, consisting of 120-60-00 (N-P_2O_5-K_2O); applying all the phosphorus and half of the nitrogen at planting and the rest of the nitrogen at flowering. The fertilizers used were $NH_4H_2PO_4$ and NH_4SO_4. Irrigation was provided by gravity. Before sowing, one irrigation of 30 cm was applied to ensure adequate soil moisture. During the crop cycle, six auxiliary irrigations of 10 cm each were applied at intervals of approximately 10–15 days, resulting in a total of 90 cm of water applied.

Treatments and experimental design

The ZnO nanoparticles were provided by Compañía Mexicana Investigación y Desarrollo de Nanomateriales S.A. de C.V. The nanoparticles have an average size of less than 50 nm, a purity of 99.7%, and a density of 5.61 g cm^3. The procedure for preparing the treatments involved the use of a ZnO NPs stock solution at a concentration of 1,000 mg L^{-1}. From this stock, five doses (150, 200, 250, 300, and 350 mg L^{-1}) were prepared by serial dilution in distilled water, with each dose mixed in a one-liter flask before application. A randomized block design was employed, comprising six treatments and six

replicates, resulting in a total of 36 experimental units. Each experimental unit measured 4 meters in length by 10 m in width, totaling an area of 40 m^2. The application of treatments was carried out directly on the plant using a 20 L manual sprayer. Each concentration of ZnO NPs was dissolved in 10 L of distilled water. Three applications were made, the first one 20 days after planting, and the following two applications were made every 20 days.

Fruit weight and yield

The fruits of all treatments were harvested at commercial maturity (well-formed mesh and when the peduncle was easily detached). All harvested fruits were weighed using a digital scale (Torrey®, Mexico) with a maximum capacity of 5 kg. Yield was estimated per hectare considering the total weight of fruit in each experimental unit. The polar and equatorial diameter was measured using a digital vernier (Truper®, Mexico) and the result was reported in cm.

Soluble solids and firmness

The determination of total soluble solids (TSS) and firmness was carried out on one fruit per replicate. TSS (°Brix) was measured with a manual refractometer with a measuring range from 0 to 32% (Atago® Master 2311). Firmness was measured using a penetrometer model FH20000 (Extech®, USA) with an 8 mm measuring head. The method involved peeling the fruit, placing it on a sturdy, level surface, creating four punctures per fruit, averaging the readings, and presenting the findings as the maximum compression force in Newtons (N).

Preparation of extracts for non-enzymatic antioxidants

One melon was randomly chosen from each treatment and replication for the assessment of non-enzymatic antioxidants. Each fruit was sampled by extracting two grams of fresh pulp and combining it with 10 mL of 80% ethanol in a plastic tube sealed with a screw cap. The tube was then placed in a rotary shaker (ATR Inc, USA) and shaken for 6 hours at 5 °C and 20 rpm. Subsequently, the tubes were centrifuged at 3000 rpm for 5 min and the supernatant was removed for analytical tests.

Total phenolic content

The total phenolic content was determined using a modified version of the Folin-Ciocalteau method, as described by Guillén-Enríquez et al. (2023). A volume of 30 µL of the sample was combined with 270 µL of distilled water in a test tube. Subsequently, 1.5 mL of Folin-Ciocalteau reagent (Sigma-Aldrich) diluted at a ratio of 1:15 was added, and the mixture was agitated briefly using a vortex mixer for 10 seconds. After waiting for 5 min, 1.2 mL of sodium carbonate solution (7.5% w/v) was introduced into the test tube and vortexed for an additional 10 seconds. The resulting solution was then incubated in a water bath set to 45°C for 15 min. Finally, the solution was allowed to cool to room temperature before further analysis. The absorbance of the solution was measured at 765 nm using a HACH 4000 spectrophotometer. The phenolic content was assessed by employing a standard curve with gallic acid (from Sigma) as the reference standard, and the outcomes were expressed as milligrams of gallic acid (GA) equivalent per gram of fresh weight (mg equiv GA g^{-1} FW).

Antioxidant capacity

The assessment of antioxidant capacity was conducted through the in vitro DPPH+ method, with a modification based on the method of Brand-Williams (1995). To determine the antioxidant capacity, 50 µL of the sample was mixed with 950 µL of $DPPH^+$ solution, and after a 3-minute reaction period, the absorbance of the mixture was measured at 515 nm. A standard curve was created using Trolox (Sigma-Aldrich), and the results were presented as equivalent antioxidant capacity in micromoles (µM) of Trolox per gram of fresh weight (µM equiv Trolox g^{-1} FW).

Vitamin C

The Vitamin C content was analyzed according to Hernandez-Hernandez et al. (2019) by taking 10 grams of fresh fruit, which were then ground with 10 mL of 2% hydrochloric acid. After filtering the mixture with a funnel and filter paper, an extract composed of 100 mL of deionized water was obtained. Subsequently, a titration was conducted using 2,6 dichlorophenolindophenol (1×10^{-3} N) and 10 mL of the diluted extract. The titration endpoint was determined by the continuous presence of a reddish color for a brief period. Upon reaching the reddish color, the addition of the dye was stopped, and the volume used for titration was recorded. The final result is expressed as mg per 100 grams of fresh weight (FW).

Enzymatic activity

The method described by David et al. (2008) was used to measure catalase activity (EC 1.11.1.6), quantifying the millimolar equivalent of H_2O_2 consumed per milliliter per minute. To prepare the enzyme extract, 0.5 grams of melon pulp were homogenized with 5 mL of a cold potassium and sodium phosphate buffer (100 mM, pH 7.0),

containing 50 mg of polyvinylpyrrolidone (PVP) to prevent phenolic compound interference. The mixture was homogenized in a mortar, kept on ice to preserve enzymatic activity, and centrifuged at 11,000 g for 11 min at 4 °C. The resulting supernatant was used for catalase activity assessment by combining it with 3 mL of sodium phosphate buffer at 300 µM and pH 6.8, along with 1 mL each of H_2O_2 at 100 µM and the enzyme extract (diluted at a 1:20 ratio). The reaction was observed for one minute at a wavelength of 240 nm.

Peroxidase activity (EC 1.11.1.7), represented as the millimolar equivalent of H_2O_2 consumed per milliliter per minute, was measured using a modified version of the approach outlined by Nickel and Cunningham (1969). The absorbance was measured at 420 nm, and the reaction mixture comprised 20 mL of water, 2 mL of enzyme extract, 1 mL of guaiacol, and 1 mL of H_2O_2. The reaction was allowed to continue for 10 minutes before taking readings.

Total protein

For the determination of total protein, 3 g of fresh sample were homogenized in a mortar placed on ice, adding 0.1 g of PVP and 3 mL of a sodium-potassium solution (100 mM, pH 7 and 0.1 mM EDTA) as extraction buffer, then centrifuged at 1,200 rpm at 5 °C for 5 min. The supernatant was utilized to quantify total protein content following the Bradford method (1976), with bovine serum albumin serving as the reference standard. The results were expressed as mg g^{-1} FW.

Zn content

The zinc concentration in melon pulp was determined in accordance with AOAC (1990) guidelines using atomic absorption spectrophotometry with an air-acetylene flame (VARIAN-SPECTR AA 3110, Palo Alto, CA, USA). The results were expressed as µg kg^{-1} dry weight (DW).

Data analyses

The data obtained underwent Bartlett's test to assess variance homogeneity, and normality was examined using the Kolmogorov-Smirnov and Shapiro-Wilk W tests. Following these tests, an analysis of variance (ANOVA) was conducted, identifying differences between treatments where applicable; Tukey's test was also used ($p \leq 0.05$).

RESULTS AND DISCUSSION

Yield and biophysical quality

The yield, weight, and size of melon fruits were significantly influenced by the applied ZnO NP doses. The highest yield (24.11 ton ha^{-1}) and fruit weight (1.88 kg) were observed at 150 mg L^{-1}, while higher doses of 300 and 350 mg L^{-1} resulted in a decline in yield to 20.81 and 17.59 ton ha^{-1}, respectively. These effects were statistically significant ($p \leq 0.05$) as shown in Table 1. Fruits exhibited greater weight and size at the 150 mg L^{-1} dose. These findings are attributed to zinc's involvement in enzymatic reactions, which are essential for auxin biosynthesis, particularly its role as a cofactor for enzymes in the tryptophan-dependent pathway, which enhances indole acetic acid production. In turn, this promotes cell elongation and division, protein synthesis, and efficient photosynthate translocation to developing fruits (Saboor et al., 2021). Zinc serves as a precursor to tryptophan, essential for indole acetic acid biosynthesis, which stimulates cell division and elongation, thus enhancing crop yields (Faizan et al., 2020). However, elevated concentrations of zinc in ionic form may compete

Table 1. Yield, fruit weight, total soluble solids, and firmness of melon fruit subjected to different doses of ZnO NPs.

NPs ZnO mg L^{-1}	Yield ton ha^{-1}	Fruit weight kg	Diameter Polar cm	Diameter Equatorial cm	Firmness N	Total Soluble Solids ºBrix
Control	19.92 a*	1.52b	15.10b	13.94b	15.52a	11.61c
150	24.11a	1.88a	16.24a	15.24a	16.88a	14.39a
200	20.99a	1.54b	14.89b	13.61b	15.74a	13.39ab
250	20.99a	1.55b	14.79b	13.89b	16.9a	13.09b
300	20.81a	1.58b	15.05b	14.06b	15.96a	13.13b
350	17.59b	1.59b	15.05b	13.82b	13.60b	11.43c

*Means with different letters in the same column are statistically different (Tukey $p \leq 0.05$).

with other cations for identical binding sites (Sturikova et al., 2018), leading to physiological disruptions and growth inhibition (Barrera-Medina et al., 2014), along with increased reactive oxygen species (ROS) production, causing lipid peroxidation (Lin et al., 2012).

From a commercial perspective, sweet and firm melon fruits are essential as they enhance consumer acceptance. The results obtained from foliar spraying at 150 mg L^{-1} show a significant increase in firmness and TSS in melon fruits. However, doses above this level (200-350 mg L^{-1}) result in a gradual decline in these parameters, with the most pronounced negative effects observed at 350 mg L^{-1} (Table 1). This dose-dependent response highlights the importance of optimizing ZnO NP application to avoid adverse effects associated with higher concentrations. The higher TSS concentration is likely due to zinc's crucial role in enzyme activity related to photosynthesis and carbohydrate metabolism, as well as in sugar transport to fruit demand points (Marschner, 2012). The decrease in TSS is attributed to a lower photosynthetic rate and reduced photosynthate production, leading to lesser sugar accumulation in the fruit (Al-Zahrani et al., 2021). Additionally, the increase in fruit firmness induced by ZnO NPs could be attributed to zinc's involvement in protein and carbohydrate transfer and synthesis, as well as the maintenance of cell membrane structural stability (García-Lopez et al., 2019). However, high doses of ZnO NPs result in decreased fruit firmness due to accelerated ethylene biosynthesis, leading to faster ripening and softening of melon flesh (Khan et al., 2021).

Non-enzymatic antioxidants

Foliar spraying with ZnO NPs has a notable impact on the bioactive compound content in melon pulp (Table 2). The bioactive compound content showed a dose-dependent response, with the highest concentration observed at 250 mg L^{-1}, which enhanced antioxidant capacity significantly. However, doses above this threshold (300-350 mg L^{-1}) caused a decline, probably due to oxidative stress induced by excess ZnO NPs García-López et al., 2018). However, higher doses lead to decreases in these phytochemical compounds due to stress induced by the NPs. The application of ZnO NPs generates reactive oxygen species, altering the response of enzymatic and non-enzymatic antioxidants to induced stress (Ahmed et al., 2018). These reactive oxygen species play a dual role; at low concentrations, they act as signalers, triggering a moderate stress response in plants and activating the biosynthesis of bioactive compounds. However, at high concentrations, they disrupt cellular homeostasis, damaging cellular structures, proteins, DNA, and lipids (Kumar et al., 2016).

Enzymatic activity

The application of ZnO NPs via foliar spray positively impacted proteins (Table 3). The highest protein concentration was achieved with the dose of 350 mg mL^{-1}. Previous studies have indicated that the application of ZnO NPs increases protein biosynthesis (Rai-Kalal et al., 2021; Wang et al., 2019). This increase in protein content may be because Zn is involved in various cellular metabolic processes, including the activation of key enzymes involved in protein synthesis (Fiazan et al., 2021). Additionally, zinc has been observed to function as a cofactor for key regulatory proteins in gene expression associated with protein biosynthesis, which could contribute to the enhancement induced by ZnO NPs (Pejam et al., 2021; Thounaojam et al., 2021).

The activity of enzymatic antioxidants, such as catalase (CAT) and peroxidase (POD), in detoxifying oxygen-free radicals induced by ZnO NPs is emphasized (Priyanka et al., 2021). Interestingly, the highest catalase activity was observed at 350 mg L^{-1}, despite a lower zinc concentration in the melon fruit at this dosage.

Table 2. Effect of foliar spraying of NPs ZnO on non-enzymatic antioxidants in melon fruits.

NPs ZnO mg L^{-1}	Flavonoids mg QE 100 g^{-1} FW	Total phenolic mg AG 100 g^{-1}FW	Antioxidant Capacity mg equiv Trolox 100 g^{-1} FW	Vitamin C mg 100 g^{-1} FW
Control	76.36d*	126.29e	67.06b	35.50e
150	89.20c	173.07d	88.43a	53.90c
200	85.69cd	181.20c	91.16a	59.50b
250	125.39a	224.23a	87.00a	65.90a
300	103.59b	191.73b	88.57a	52.40c
350	114.59ab	188.29b	89.66a	44.70d

*Means with different letters in the same column are statistically different (Tukey $p \leq 0.05$).

Table 3. Effect of foliar spraying of NPs ZnO on enzymatic antioxidants and Zn content in melon fruits.

NPs ZnO mg L^{-1}	Proteins mg g^{-1}	Catalase mM/min	Peroxidase mM/min	Zn *u*g kg^{-1}
Control	0.030c*	1.06c	414.4b	21.92b
150	0.034bc	1.41b	1415.0a	31.76a
200	0.050abc	1.54b	1480.3a	30.30ab
250	0.057ab	1.71b	1333.0a	34.33a
300	0.068a	1.63b	585.57b	31.57a
350	0.069a	2.35a	439.7b	32.40a

*Means with different letters in the same column are statistically different (Tukey $p \leq 0.05$).

This suggests that the enzymatic response to oxidative stress is not directly proportional to zinc accumulation in the pulp but rather reflects the plant's attempt to mitigate reactive oxygen species (ROS) generated at higher ZnO NP concentrations. Conversely, at 250 mg L^{-1}, where zinc accumulation was higher, catalase activity was comparatively lower, indicating a more balanced redox state and reduced ROS production under moderate stress conditions. These antioxidants play a crucial role in neutralizing free radicals, maintaining redox balance, and alleviating oxidative stress caused by ZnO NPs exposure. However, high doses of ZnO NPs lead to the inhibition of these enzymatic antioxidants (Table 3). Similar findings were reported by Farghaly et al. (2020), indicating increased CAT activity but decreased POD activity at high doses. This suggests a decrease in Km and Vmax of POD due to non-competitive inhibition by ZnO NPs. The stress from ZnO NPs probably results in elevated ROS production, surpassing the compensatory capacity of antioxidants. This complexity in enzymatic responses to high ZnO NP doses highlights differential regulation of CAT and POD, potentially due to specific non-competitive inhibition by ZnO NPs, contributing to subtoxic oxidative stress levels in the biological system.

Zinc concentration

The concentration of Zn in melon pulp varied according to the treatments applied, with higher doses resulting in increased Zn concentration (Table 3). The observed zinc concentrations in melon pulp at higher ZnO NP doses (300-350 mg L^{-1}) raise concerns about potential health risks for consumers. While these levels remain within the tolerable limits established by regulatory agencies, further studies are recommended to evaluate the bioavailability and potential toxicity of zinc from nanoparticle-enriched fruits. Nano-biofortification using ZnO has the potential to help fulfill the daily dietary Zn requirements, which average 11 mg for men and 8 mg for women (Chasapis et al., 2020). However, caution is necessary when dealing with metal nanoparticles, as elevated levels of ZnO NPs can accumulate in the soil, potentially disrupting microbial communities, reducing soil fertility, and leading to environmental issues in surrounding ecosystems (Fortis-Hernandez et al., 2022; Khan et al., 2021). Notably, no visible symptoms of phytotoxicity, such as necrosis or reduced photosynthetic activity in leaves, were observed. Hence, achieving a balance between the beneficial effects of ZnO NPs on crops and their potential environmental impact is crucial.

CONCLUSION

The response of the crop is closely linked to the concentration of ZnO NPs used. Lower concentrations of ZnO NPs (150 mg L^{-1}) significantly improve yield and biophysical quality, while intermediate concentrations (250 mg L^{-1}) enhance enzymatic and non-enzymatic antioxidant activity. Conversely, higher concentrations (300-350 mg L^{-1}) inhibit antioxidant activity and result in excessive zinc accumulation in the pulp. These findings highlight the importance of optimizing ZnO NPs dosages to balance crop productivity and nutritional quality. High doses of ZnO NPs are not recommended due to their potential to induce oxidative stress, which negatively impacts yield and nutritional quality. Optimizing ZnO NPs application is crucial for enhancing crop productivity and achieving sustainable agricultural practices.

Author contributions

The authors declare active participation in the bibliographic review by Alain Buendía-García and Melisa C. Hermosillo-Alba; in the

development of the methodology: Daniel Ruiz-Juarez, Oscar Sariñana-Aldaco; in the discussion of the results: Reyna R. Guillén-Enríquez and Pablo Preciado-Rangel; in review and approval of the final version of the article: Pablo Preciado-Rangel and Leticia Alfaro-Hernandez.

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