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Article

Copper nanobiofortification in watermelon

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Abstract

Nanomaterials such as copper oxide nanoparticles (CuO NPs) are of utmost importance due to their applications in very diverse aspects, such as in agriculture, in which they allow the increase in the organoleptic characteristics of the edible part of the plant. The present work evaluated different concentrations of CuO NPs to show their effect on yield, fruit quality, bioactive quality, and Cu concentration in watermelon fruits. Six treatments of CuO NPs (0, 50, 100, 150, 200, 250 mg L^{-1}) were evaluated. The results showed that the foliar application of CuO NPs does not affect yield; nevertheless, at high concentrations, fruit quality, bioactive quality, and Cu content increase, obtaining better results with the 250 mg L^{-1} treatment. Therefore, nanobiofortification with CuO NPs resulted in better-quality watermelon fruits due to the accumulation of bioactive compounds.

Keywords:

Citrullus lanatus, bioactive compounds, nanoparticles.

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Introduction

Nanotechnology is considered an important technology in a large number of application areas, such as agronomy, industry, medicine, and chemistry, among others (Urquilla, 2019). Nanoparticles (NPs) can be synthesized from different materials and natural elements, including plants, bacteria and organic waste (Gómez-Garzón, 2018), which can be used to increase crop productivity (Cumplido-Nájera *et al*., 2019).

Copper oxide nanoparticles (CuO NPs), when synthesized by natural elements, exert a certain organic coating that activates abiotic stress in the plant (Ananda *et al*., 2018). That is why biofortification is used in crops, as it is a strategy to increase the nutritional content of micronutrients in the fruits of the plants (Schiavon *et al*., 2020).

In addition, copper, being an essential micronutrient in plant mineral nutrition, participates in oxidation-reduction reactions and is part of cuproenzymes that participate in fundamental processes for plants, such as electron transport in photosynthesis and respiration, nitrogen, and carbon metabolism (León Morales, 2012; Lopez-Lima *et al*., 2021).

On the other hand, watermelon is a cucurbit that is produced in most parts of the world, and its consumption is important for humans due to its high contents of beta-carotene, lycopene, antioxidants, vitamins (B, C and E), and minerals (phosphorus, magnesium, calcium, and iron) (Cavalcante *et al*., 2019). In addition, watermelon is one of the most important crops in Mexico due to the large production it exports internationally (Sandoval *et al*., 2019). In the year alone, Mexico ranked 12th as a world producer (SIAP, 2021).

That is why one of the strategies to increase the content of Cu in food is through biofortification, which consists of potentiating the bioactivity and content of Cu in the edible parts of plants (Hernández-Hernández *et al*., 2019). This research aimed to evaluate different concentrations of CuO NPs in watermelon crops, which showed their effect on yield, bioactive quality, and Cu concentration in watermelon fruits.

Materials and methods

Study area

The experiment was carried out under field conditions in Ejido 13 de Marzo, Municipality of Gómez Palacio, Durango, Mexico, which is located at coordinates 25° 29' 0" north latitude and 103° 44' 25" west longitude and 1 110 masl.

Copper oxide nanoparticles

The nanoparticles used in the experiment were donated by the Applied Chemistry Research Center (CIQA), for its acronym in Spanish in Saltillo, Coahuila, Mexico. Nanoparticles of hemispherical morphology, black-brown with a dark appearance (powder), with a size of 95 nm, having a purity of 99.8%, and synthesized by green synthesis.

Application of nanoparticles

The treatments evaluated consisted of the foliar application of copper nanoparticles using the following concentrations: (0, 50, 100, 150, 200, 250 mg L^{-1}) (Rivera-Gutiérrez *et al*., 2021). The method used to prepare the different doses was a CuO-NPs stock solution. Subsequently, the five doses of nanoparticles were prepared in a one-liter volumetric flask, each of the nanoparticle concentrations was poured separately and completed with distilled water.

The finished solutions were then transferred to handheld sprayers with a capacity of 1 000 ml. Each treatment was applied to five plants, representing an experimental unit (EU). Spraying was carried out every fifteen days after transplanting (DAT) until the end of the crop cycle (90 days), with a total

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of eight applications. These applications were carried out in the morning, specifically between 7:00 and 9:00 am, using a manual sprayer. The experimental design was completely randomized.

Plant material and crop conditions

The vegetative material used was red watermelon (*Citrullus lanatus*, hybrid Syngenta®). Direct sowing was carried out on March 1, 2021. Double-row borders were built, forming a micro plot (seed bed) at a distance of 4 m, both between lines and between plants. Sowing was done with a dibble stick, depositing 3 to 4 seeds m^{-1} per hole at a depth of 10 cm. In the irrigation area, the borders were drawn with widths of 1.5 m, with an irrigation channel bottom of 30 cm. The population density was 3 200 plants ha^{-1}, sowing density: 1.5 kg ha^{-1}, distance between rows of 2 m and distance between plants of 1 m.

The preparation of agricultural soil consisted of fallowing at a depth of 30 cm, with double harrowing, adding leveling to the agricultural soil, building seed beds and borders for conduction in the retention of irrigation water. The fertilization dose used was 160-80-00 (N-P_2O_5-K_2O), in which the phosphorus was completely incorporated with half of N at the time of starting the sowing and the rest of the nitrogen when the plant began flowering. The fertilizers used were $NH_4H_2PO_4$ and $(NH_4)_2SO_4$. Irrigation was provided with water from the Francisco Zarco dam and a deep well. A pre-sowing irrigation of 30 cm was applied, and during the crop's vegetative cycle, eight supplemental irrigation sheets of 15 cm were provided.

Variables evaluated

Fruit yield and quality

The fruits were harvested 96 days after transplantation once they reached commercial maturity (when the basal spot turns from white to creamy and with low sounds to percussion). Fruit weight (FrW) was quantified in all harvested fruits, which were weighed on a digital scale (Truper®) with a capacity of 20 kg. Total soluble solids (TSS) were measured with a handheld refractometer (Atago Master 53M).

Bioactive compounds

Sample processing

Two grams of watermelon fruit were amalgamated with 10 ml of 80% ethanol. The mixture was kept in stirring for a day using a 'Stuart' stirrer. The tubes were then centrifuged at 120 x g for the same time. The supernatant, referred to as ethanolic extract (EE), was separated for subsequent analytical procedures.

Phenolic content

Total phenolic content was quantified using the Folin-Ciocalteau method (Sariñana-Navarrete *et al*., 2021). Samples were quantified with an ultraviolet (UV)-Vis spectrophotometer at 760 nm (GENESYS 10S UV-Vis), and results were expressed as mg GAE 100 g^{-1} fresh weight (FW).

Flavonoid content

The colorimetry method quantified the flavonoid content (Sariñana-Navarrete *et al*., 2021). Samples were quantified with an ultraviolet (UV)-Vis spectrophotometer at 510 nm (GENESYS 10S UV-Vis), and results were expressed as mg QE 100 g^{-1} FW.

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Antioxidant capacity

Total antioxidant capacity was measured using the method of DPPH+ (Brand-Williams *et al*., 1995). Samples were quantified with an ultraviolet (UV)-Vis spectrophotometer at 517 nm (GENESYS 10S UV-Vis), and results are presented in the form of µM Trolox equivalents 100 g^{-1} FW.

Vitamin C

It was determined by titration according to the method described by Hernández-Hernández *et al*. (2019). The absorbance of the samples was then measured at 515 nm using a GENESYS 10S UV-Vis spectrophotometer. Results are expressed as mg of vitamin C per 100 g^{-1} FW.

Lycopene

It was performed by the method reported by Gómez-Romero *et al*. (2007) with some modifications. Absorbances were measured three times using a UV-Vis spectrophotometer (UV-Vis, GENESYS 10S), wavelength range at 340-1 000 nm, and a spectral bandwidth 5 nm at 503 nm. Absolute hexane was used as a blank. Lycopene amounts in tissues were calculated using the following formula: lycopene (mg/kg)= (x/y) × A503 × 3.12. Where: x= amount of hexane (ml); y= the weight of the sample; A= absorbance at 503 nm; and 3.12= extinction coefficient. Results were expressed in mg kg^{-1}.

Enzyme activity

Superoxide dismutase (SOD) (U ml^{-1}. Where: U is defined as the amount of enzyme needed to exhibit 50% superoxide radical dismutation) was determined using the Cayman SOD 706002® kit (Hernández-Hernández *et al*., 2019).

Catalase (CAT) (U TP^{-1}. Where: U is equal to the mM equivalent of H_2O_2 consumed per milliliter per minute) was analyzed by the method of Luna *et al*. (2019) and performed in two steps [at time 0 (T0) and at time 1 (T1)]. Absorbance was measured in a UV-Vis spectrophotometer (Thermo Fisher Scientific, model G10S, Waltham, MA, USA) at 270 nm.

Glutathione peroxidase (GPX) [U per gram of total protein (U TP^{-1}). Where: U is equal to the mM equivalent of reduced glutathione (GSH) per milliliter per minute] was evaluated using the method of Cosat Da and Shaiama (2016). Absorbance was measured using a UV-Vis spectrophotometer (Mettler toledo, model UV7) at 412 nm.

Copper content

The copper content in watermelon was determined by atomic absorption spectrophotometry. The watermelon pulp was dried in the oven at 70 °C on brown paper and then macerated in a mortar. Cu was quantified in the atomic absorption (AA) spectrophotometer; the results were expressed in µg kg^{-1} dry weight (AOAC, 1990).

Analysis of statistical data

Data were evaluated by one-way analysis of variance and mean comparison with Tukey's test ($p \leq 0.05$) using the Statistica software (version 10.0; StatSoft, Tulsa, OK, USA).

Results and discussion

Yield

Foliar application of CuO NPs did not affect yield (Figure 1); however, the highest fruit weight was achieved in those treated with the dose of 250 mg L^{-1}. Watermelons treated with CuO NPs obtained a higher yield than the control treatment. Copper is among the eight essential micronutrients needed

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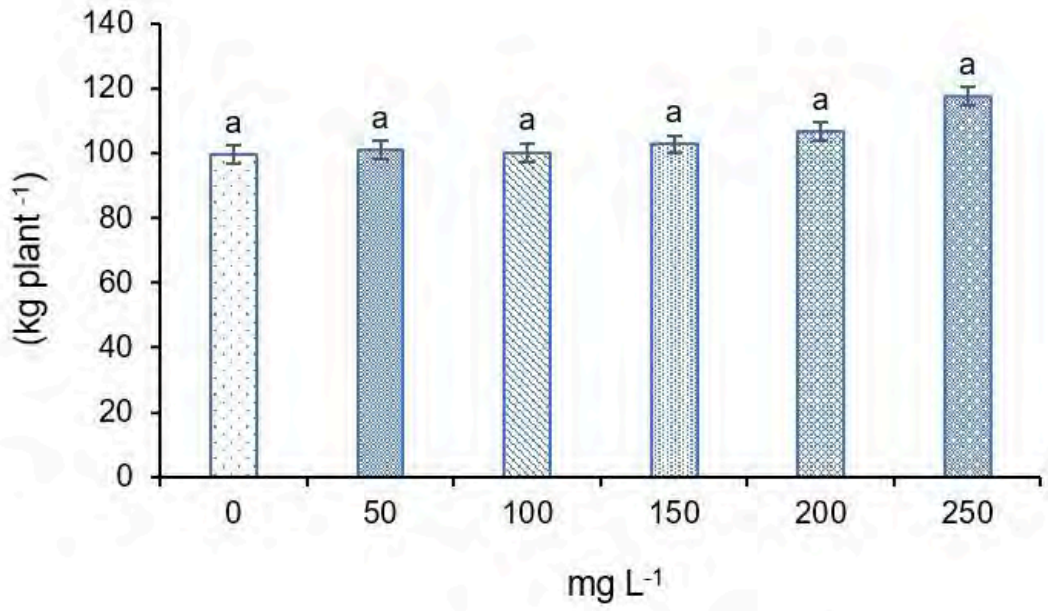
for plant growth (Nazir *et al*., 2019) and is associated with numerous physiological and biochemical processes (García-Bueno and Marín, 2021).

Figure 1. Watermelon yield due to the effect of the different concentrations of CuO NPs applied via foliar. Different letters indicate a significant difference ($p \leq 0.05$) according to Tukey's test.



In addition, it is a structural component of numerous regulatory proteins and plays key roles by participating in mitochondrial respiration, cell wall metabolism, photosynthetic electron transport, oxidative stress responses, protein synthesis, hormone signaling, and ethylene detection (Li *et al*., 2019; Nazir *et al*., 2019). Likewise, it can be incorporated into electron transporter proteins (Pilon *et al*., 2006; Zhang and Liu, 2020) and positively affect crop growth and yield (Cota-Ruiz *et al*., 2022).

The latter is probably due to the biochemical effect of the positive regulation of oxidative stress caused by Cu. On the other hand, high concentrations of CuO NPs can cause cellular redox homeostasis to be altered in the plant, resulting in an excessive accumulation of reactive oxygen species (ROS) that damage biomolecules (lipids, proteins and DNA) and inhibit crop growth and yield (Zhao *et al*., 2022).

Fruit quality

The application of copper oxide nanoparticles significantly affected the quality of the watermelon fruit, mainly total soluble solids (TSS) by 58%; on the other hand, in titratable acids and ripeness index, they did not show a significant difference (Table 1).

Table 1. Effect of the application of CuO NPs on the quality of watermelon fruits.

Treatment (mg L^{-1})	Total soluble solids (°Brix)	Firmness (Newton)	Titratable acidity (% AcC)	Ripeness index
0	7.666 ab*	19.62 bc	0.477 a	18.559 a
50	7 b	25.42 a	0.574 a	12.534 a
100	8 ab	21.12 b	0.3878 a	20.691 a

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Treatment (mg L^{-1})	Total soluble solids (°Brix)	Firmness (Newton)	Titratable acidity (% AcC)	Ripeness index
150	8.333 ab	18.05 bc	0.3716 a	17.443 a
200	8.666 a	17.03 c	0.5934 a	14.598 a
250	8 ab	16.62 c	0.3258 a	24.872 a

*= different letters indicate significant differences ($p \leq 0.05$) according to Tukey's test.

As for the increase in TSS in fruits treated with CuO NPs, it is probably because Cu promotes the increase in the concentration of soluble sugars (sucrose, glucose, and fructose) that participate as osmoprotectants during osmotic adjustment; it also causes the activation of cuproenzymes, antioxidants such as superoxide dismutase (SOD) and ascorbate peroxidase (APX), and the non-enzymatic antioxidant system in which phenolic compounds are found, in order to keep reactive oxygen species (ROS), such as O2 and H2O2, at minimum levels (Shabbir *et al*., 2020a) and this, in turn, has a preponderant effect on the accumulation of soluble solids in fruits (Mir *et al*., 2021).

Likewise, watermelon fruits vary not only in size and shape but also in the accumulation of various metabolites, the most obvious of which include the horticulture-important metabolites of external and internal pigmentation, the volatiles responsible for the fruit's smell, and the carbohydrates and organic acids responsible for sweetness and acidity (Naz *et al*., 2020).

In addition, fruit hormones, such as ethylene and abscisic acid, and pulp composition (cellulose, hemicellulose and pectin) determine fruit ripeness, shelf life, and chemical compositions that influence product quality, such as fruit firmness, pulp chewiness and gumminess, and ripeness (Anees *et al*., 2021). This is consistent with what was pointed out by Deng *et al*. (2022), who mention that foliar applications of Cu improve soluble solids and fruit quality.

Bioactive quality

The foliar application of copper oxide nanoparticles showed significant differences for total phenols, flavonoids, and antioxidant capacity (Figure 2), in watermelon fruits, the treatment of 250 mg L^{-1} of CuO NPs was the one that obtained the highest concentration, exceeded the control by 15.76% in total phenols, by 12.96% in flavonoids, by 14.79% in antioxidant capacity; in contrast, in vitamin C and lycopene, there was no significant difference between treatments.

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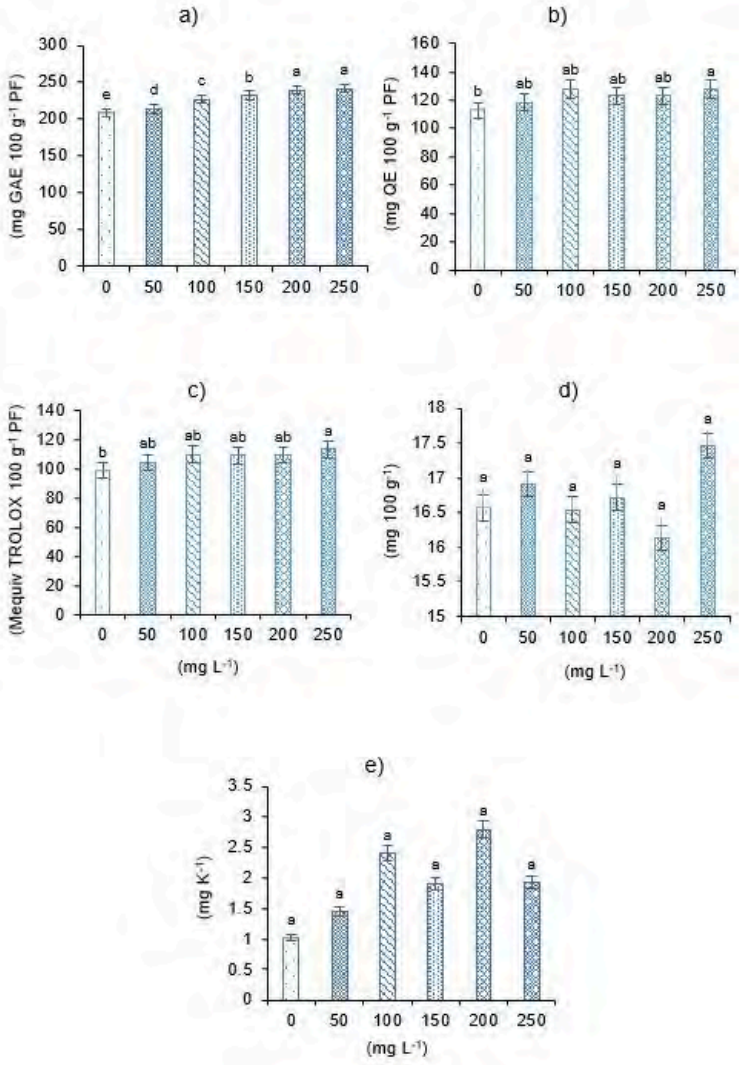
Figure 2. Effect of the concentration of foliarly applied CuO NPs on the content of total phenols (a); total flavonoids (b); antioxidant capacity (c); vitamin c (d) and lycopene (e) in watermelon fruits. The average values in columns with different letters differ statistically ($p \leq 0.05$).



CuO NPs have potent antioxidant functions, thereby improving the bioactive quality of the edible part of the crop (Lasso-Robledo *et al*., 2022) and reducing the production of ROS, such as O_2 and H_2O_2 (Kusiak *et al*., 2023). CuO NPs in adequate concentrations improve nutraceutical quality and accumulation of bioactive compounds (Kusiak *et al*., 2023). A concentration of 250 mg L^{-1} of CuO NPs in cucurbits has a positive effect on the elimination of free radicals, antioxidant capacity, and phenolic compound content (Fortis *et al*., 2022).

The lycopene content in watermelon fruit increased significantly with the dose of 250 mg L^{-1}, compared to the control treatment and higher doses of CuO NPs. Watermelon is characterized by several bioactive compounds showing different chemical structures, such as carotenoids, lutein, phenolics and citrulline. In addition to this, when the watermelon fruit turns from green to red, the antioxidant system of carotenoids and xanthophylls, responsible for the different colors of the watermelon pulp, is activated.

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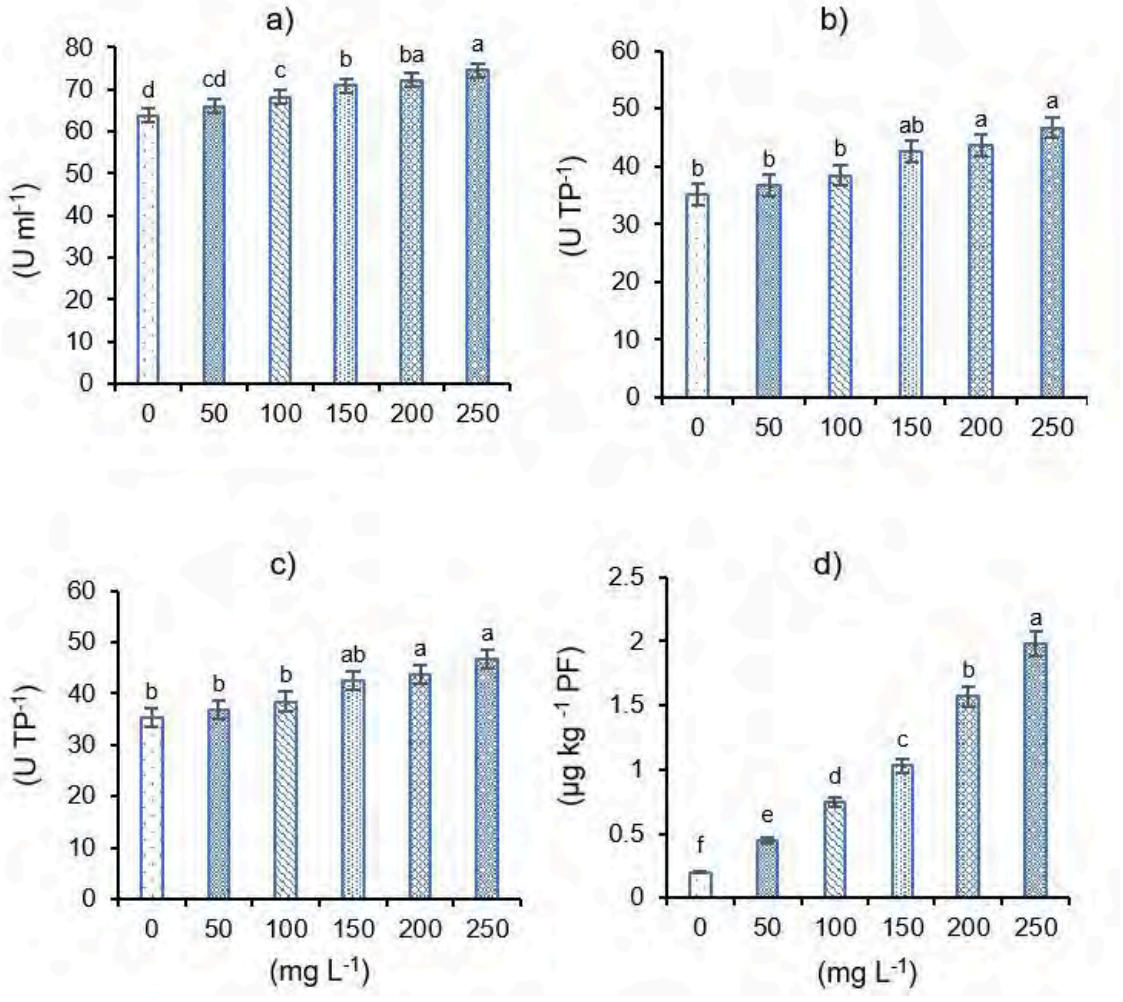
Lycopene is the main carotenoid present in red- and pink-fleshed watermelons (Kang *et al*., 2010). Watermelon represents an important source of carotenoids, being around 15 mg kg in the red pulp (Zamuz *et al*., 2021). As the fruit turns red, ascorbate and glutathione decrease as the fruit matures.

This decrease is due to the presence of carotenoids (lycopene and β-carotene), activated mainly in the fruit ripeness stage. In addition, the good amount of total polyphenols, vitamin C, and citrulline, and the excellent contribution of lycopene, which represented more than 40% compared to raw tomatoes, give a measure of the importance of this fruit compared to other crops considering the beneficial effects of these compounds on human health.

CuO NPs significantly modified the enzymatic activity of superoxide dismutase (SOD), catalase (CAT), and glutathione in watermelon fruits (Figure 3). The use of NPs at the 250 mg L^{-1} dose increased enzyme activity by 16.87, 32.67, and 15.26% over the non-nanoparticle treatment. Copper interacts in plant cell metabolism as catalytic protein centers by regulating enzyme activities (Saleem *et al*., 2020).

Figure 3. Effect of the concentration of foliarly applied CuO NPs on the contents of SOD (a); CAT (b); glutathione (c); Cu content in watermelon fruits (d). The average values in columns with different letters differ statistically ($p \leq 0.05$).



The enzymatic antioxidant system comprising superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POX), and non-enzymatic

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antioxidants, such as vitamins, flavonoids, stilbenes, and carotenoids, extinguish excess ROS, thus providing a shield against oxidative stress (Sachdev *et al*., 2021).

Therefore, CuO NPs could improve the enzymatic antioxidant defense mechanism of watermelon fruits. In addition, CuO NPs are also considered to be an important activator of the PAL enzyme in plants, which are the main precursor of phenylpropanoid synthesis in plants that act as inhibitors of singlet oxygen formation, free radical scavengers, and reducing agents against abiotic and biotic stress (Gaucin *et al*., 2022).

Copper content in watermelon pulp

The addition of 250 mg L^{-1} increased the Cu content in watermelon fruits; there is a positive correlation between copper in fruits and the applied dose (r^2= 0.99) (Figure 3d). The Cu content in the crop depends on the age of the plant, the plant species, the chemical form of the element that was applied, its concentration, and the method of application (Santás-Miguel *et al*., 2023).

Previous studies show that biofortification significantly increases the amount of essential elements in the edible part of plants (Buturi *et al*., 2021), which, in crops biofortified with Cu, can increase by up to 20% over untreated crops (Fortis *et al*., 2022b). Cu in plants is metabolized along with zinc within plant tissues, transforming into cuproenzymes (Rietra *et al*., 2017), accelerating the transport, accumulation, volatilization, and tolerance of Cu in the plant (Guardiola-Márquez *et al*., 2022).

Conclusions

The foliar application of CuO NPs improves the bioactive quality, antioxidant capacity, and concentration of Cu in watermelon fruits, in addition to increasing the activity of enzymes (GPX, CAT and SOD), without negative effects on yield. Therefore, the application of CuO NPs results in watermelon fruits of better quality for the human diet due to the accumulation of bioactive compounds.

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