Revista Mexicana de Ciencias Agrícolas
REMEXCA
DOI: https://doi.org/10.29312/remexca.v15i8.3851
Article

Biofortification with copper nanoparticles improves yield and bioactive compounds in melon fruits

Alain Buendía-García[1]
Pablo Preciado-Rangel[2]
José R. Paredes-Jácome[1]
Reyna R. Guillén-Enríquez[2]
Selene Y. Márquez-Guerrero[2]
Ricardo I. Ramírez-Gottfried[1,§]

1 Unidad Laguna-Universidad Autónoma Agraria Antonio Narro. Periférico Raúl López Sánchez y Carretera Santa Fe s/n, Torreón, Coahuila, México. CP. 27010. (alain75191@hotmail.com; gottfried-ricardo@hotmail.com; rafaelparedes93@hotmail.com).

2 Tecnológico Nacional de México-Instituto Tecnológico de Torreón. Carretera Torreón-San Pedro km 7.5, Ejido Ana, Torreón, Coahuila, México. CP. 27170. (reguillen@outlook.com; selenne.marquez@hotmail.com).

Autor para correspondencia: gottfried-ricardo@hotmail.com

Abstract

The use of nanotechnology allows greater sustainability in agricultural systems by reducing the environmental impact of agrochemical use. Among the main nanoproducts, metal nanoparticles (NPs) have been used to improve yield and modulate bioactive compounds in crops. The present study was conducted during the spring-summer cycle of 2022 with the aim of evaluating the foliar spray of five increasing doses of copper nanoparticles (CuO NPs): 150, 200, 250, 300, and 350 mg L^{-1} and a control treatment in melon crops. During harvest, yield, nutraceutical quality, enzymatic activity, and its bioaccumulation in melon fruits were determined. The foliar application of CuO NPs induced an increase in yield and biosynthesis of bioactive compounds, as well as their bioaccumulation in the pulp; nevertheless, high doses cause the opposite effect due to their accumulation. The responses of melon crops to CuO NPs depend on the dose used as they can induce beneficial or negative effects; therefore, further research is needed.

Keywords:

Cucumis melo L., antioxidants, nanobiofortification.

DOI: https://doi.org/10.29312/remexca.v15i8.3851

Introduction

Melon (*Cucumis melo* L.) is a fruit with high nutritional properties that belongs to the cucurbit family; it is characterized by containing a large amount of proteins, lipids, vitamin C, beta-carotene, antioxidants, and bioactive polyphenols (Mosquera-Vivas *et al*., 2019; Rivera-Gutiérrez *et al*., 2021); it also contains other phytochemicals that are also important for disease prevention (Manchali *et al*., 2021; Guo *et al*., 2023).

Approximately 27 million tons are produced globally (Kubo *et al*., 2021; Davidson *et al*., 2023). In Mexico, the area cultivated with melons reached 19 104 ha year^{-1}, with a production of 591 574 t (SIAP, 2021). On the other hand, due to the type of diet of the current population, the deficiency of trace elements, such as iron, copper, iodine, selenium, and zinc, is common, which represents a global health problem (Gío-Trujillo *et al*., 2022).

Cu is an essential microelement; however, the deficiency or excess of Cu can affect human health (Raha *et al*., 2020). Cu deficiency leads to serious disorders, such as anemia and neutropenia (Wahab *et al*., 2020), while in excess, it causes liver disorders and diseases such as Alzheimer's, as well as nervous breakdown (Taylor *et al*., 2020).

In plants, Cu is involved in oxidation-reduction reactions, cellular and molecular processes, such as chlorophyll synthesis, photosynthesis, respiration, and protein and carbohydrate metabolism (Gaytan-Aleman *et al*., 2021). Nonetheless, sandy soils with low organic matter content and high pH values prevail in many areas; in these soils, the deficiency of Cu is usually accentuated (Shabbir *et al*., 2020). Therefore, it is important to adopt proper crop management practices to prevent its deficiency, both in the plant and in humans.

An alternative to increase the content of Cu in the edible part of crops and improve human nutrition is crop biofortification since the nutritional content in the edible parts of plants is improved and, in this way, it is possible to satisfy the requirements of this trace element because not all the population can acquire commercial mineral supplements (Dhaliwal *et al*., 2022). Nanotechnology is a technological innovation that increases sustainability in agricultural systems by reducing the environmental impact of agrochemical use (Gutiérrez-Ruelas *et al*., 2021).

Among the main nanoproducts, metal nanoparticles (NPs) have been used to improve yield and modulate bioactive compounds in crops (Kalisz *et al*., 2021). Among the metallic NPs, CuO NPs stand out considerably due to their optical, catalytic, mechanical, and electrical properties (Amer and Awwad, 2021). It has been shown that when Cu is applied in *Arabidopsis thaliana*, *Capsicum annuum*, *Solanum lycopersicum* L, it has a stimulating function, increasing the accumulation of bioactive compounds, firmness, and quality of the fruits (López-Vargas *et al*., 2018).

This work aimed to evaluate the effect of foliar spraying of CuO NPs in melon crops and to determine its effects on yield and commercial and nutraceutical quality, as well as its bioaccumulation in fruits.

Materials and methods

Location

This research work was conducted in the spring-summer cycle of 2022 under field conditions in the ejido Concordia, Municipality of San Pedro de las Colonias, Coahuila, Mexico, which is located at 25° 48' 31" north latitude and 103° 5' 56.4" west longitude. This area has a semi-warm climate with average annual temperatures of 20 to 22 °C and an average annual rainfall of 125 to 400 mm.

Plant material and cultural work

The melon hybrid cv. Crusier (Harris Moran®) was used as plant material. Direct seeding was carried out on March 20, 2022. Double-row ridges were constructed, forming beds at a distance of 4 m between ridges and a separation between plants of 30 cm for a density of 16 665 plants ha^{-1}. Fertilization was in accordance with INIFAP recommendations, consisting of: 120-60-00 (N-P_2O_5-

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K_2O), applying all the phosphorus and half of the nitrogen at the time of sowing and the rest of the nitrogen at the time of flowering.

The fertilizers used were $NH_4H_2PO_4$ and NH_4SO_4. Irrigation was provided by gravity. In pre-sowing, irrigation was applied with a 30 cm sheet; subsequently, six supplemental irrigations were applied with sheets of 15 cm each; in total a sheet of 120 cm was applied during the crop cycle.

Treatments and experimental design

The nanoparticles used were donated by the Applied Chemistry Research Center of the city of Saltillo, Coahuila. The CuO NPs were obtained by green synthesis and their characterization is reported by Ortega-Ortiz *et al*. (2022). The method to develop the treatments consisted of using a stock solution of CuO NPs. From it, five different doses were prepared in one-liter flasks; the applied concentrations were poured in each of the flasks, which consisted of five increasing doses of CuO NPs: 150, 200, 250, 300 and 350 mg L^{-1}.

Only distilled water was used in the control treatment. The design used was randomized blocks with six treatments and six replications for a total of 36 experimental units. Each experimental unit was 4 m long by 10 m wide for a total of 40 m^2. The treatments were directly applied on the plant by using a 20 L manual sprayer. Each concentration of CuO NPs was dissolved in 10 L of distilled water. Three applications were made; the first 20 days after sowing and the following two applications were made every 20 days.

Variables evaluated

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 on a digital scale (Torrey®, Mexico) with a capacity of 5 kg. The yield was estimated per hectare considering the total weight of the fruits in each experimental unit. The polar and equatorial diameters were measured using a digital vernier (Truper®, Mexico), reporting the result in cm.

Soluble solids and firmness

TSS and firmness were determined in a fruit per replication; soluble solids (°Brix) were measured using a manual refractometer with a measurement range of 0 to 32% (Atago® Master 2311). The firmness was measured using a FH20000 penetrometer (Extech®, USA) with an 8 mm measuring head; the procedure consisted of removing the peel from the fruit, then it was placed on a rigid and flat surface, four penetrations were made per fruit, they were averaged and the results are expressed in maximum compressive force in Newton units.

Preparation of extracts for non-enzymatic antioxidants

From each treatment and repetition, a melon was randomly selected for the quantification of non-enzymatic antioxidants; subsequently, two grams of fresh pulp were taken from each fruit and mixed in 10 ml of 80% ethanol in a plastic tube with a screw cap, which was placed in a rotary stirrer (ATR Inc., USA) for 6 h at 5 °C and 20 rpm. The tubes were then centrifuged at 3 000 rpm for 5 min and the supernatant was removed for analytical testing.

Total phenolic content

Total phenolic content was measured by a modification of the Folin-Ciocalteau method (Esparza *et al*., 2006). Thirty microliters of sample were mixed with 270 µl of distilled water in a test tube and 1.5 ml of diluted (1:15) Folin-Ciocalteau reagent (Sigma-Aldrich) was added, stirring in vortex for 10 s. After 5 min, 1.2 ml of sodium carbonate (7.5% w/v) was added and it was stirred for 10 s.

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The solution was placed in a double boiler at 45 °C for 15 min and then left to cool to room temperature. The absorbance of the solution was read at 765 nm in a HACH 4000 spectrophotometer. Phenolic content was calculated using a standard curve with gallic acid (Sigma) as standard and results were reported in mg gallic acid (GA) equivalents per g of fresh basis sample (mg equiv GA g^{-1} FB).

Antioxidant capacity

Antioxidant capacity was evaluated according to the *in vitro* method $DPPH^+$, using a modification of the method published by Brand-Williams (1995). To determine the antioxidant capacity, 50 µl of sample and 950 µl of $DPPH^+$ solution was mixed, and after 3 min of reaction, the absorbance of the mixture was read at 515 nm. A standard curve was prepared with Trolox (Aldrich) and the results were reported as equivalent antioxidant capacity in µM Trolox equivalents per g fresh basis (µM equiv Trolox g^{-1} FB).

Vitamin C content

The vitamin C content was determined according to what was reported by Hernández-Hernández *et al*. (2019). Ten grams of fresh fruit were taken and ground with 10 ml of 2% hydrochloric acid. Later, a funnel and filter paper were used. The sample was filtered and the extracted obtained was made up to 100 ml with deionized water. Next, 2,6-dichlorophenolindophenol (1×10^{-3} N) was used to perform a titration with 10 ml of the dilute. To determine titration, the reddish color should persist for a few seconds. Once the reddish color was obtained, the coloring stopped being added and it was calculated with the volume spent. The result is expressed in mg 100 g^{-1} FB.

Cu content in pulp

The concentration of copper in melon pulp was determined according to the AOAC (1990) by atomic absorption spectrophotometry with air-acetylene flame (Varian-Spectr AA 3110, Palo Alto, CA, USA); the results were expressed in µg kg^{-1} dry weight (DW).

Statistical analysis

The data of the variables were analyzed with an analysis of variance and the comparison of means by Tukey's test ($p \leq 0.05$) using the statistical package of the Statistical Analysis System Institute (SAS) version 9.4.

Results and discussion

Yield and commercial quality

The yield and weight of melon fruits were affected by the different doses of CuO NPs sprayed since these variables decreased as the concentration of applied NPs increased (Table 1). The weight of the fruits treated with the highest dose of CuO NPs showed a 11% decrease compared to the control. The weight of melon fruits fluctuates from 0.5 to 4 kg (Espinoza-Arellano *et al*., 2023). The results found in the present study are within this range.

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

CuO NPs (mg L^{-1})	Yield (t ha^{-1})	Fruit weight (kg)	Total soluble solids (°Brix)	Firmness (N)
Control	19.77 ±1.4c	1.81 ±0.3 a*	12.77 ±0.79 ab	17.08 ±1.4 ab
150	23.78 ±1.7 a	1.63 ±0.2 ab	13.49 ±0.54a	17.83 ±2.0ab

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CuO NPs (mg L^{-1})	Yield (t ha^{-1})	Fruit weight (kg)	Total soluble solids (°Brix)	Firmness (N)
200	24.09 ±1.09 a	1.62 ±0.4 b	12.52 ±0.31ab	19.64 ±1.6 a
250	23.15 ±1.4 ab	1.62 ±0.73ab	12.62 ±0.17 ab	16.34 ±1.1 b
300	19.92 ±1.3 abc	1.55 ±0.2 b	12.69 ±0.19 ab	18.41 ± 2.2b
350	17.58 ±1.5 c	1.53 ±0.1 b	10.66 ±0.67 c	12.24 ±1.4 c

[*] = means with different letters in the same column are statistically different (Tukey $p \leq 0.05$); n= 6 ± standard deviation.

On the other hand, the yields obtained by plants treated with 150 and 200 mg L^{-1} exceeded those obtained by the control treatment by 16 and 18%. It has been reported that applications of CuO NPs can increase crop yields (Rajput *et al*., 2018); however, high doses of this metal cause an alteration of DNA repair kinetics (Shabbir *et al*., 2020), negative effects on morphology, physiology, and biochemistry (Da-Costa *et al*., 2016), as well as a phytotoxicity to crop (AlQuraidi, *et al*., 2019).

From a commercial point of view, sweet (minimum 10 °Brix) and firm fruits are required since, in this way, the acceptance of melon fruits by the consumer is improved. The results obtained indicate that the use of low doses of CuO NPs increases firmness and TSS and high doses cause a decrease in these parameters (Table 1).

Similar results were found by López-Vargas *et al*. (2018) as they reported that high doses of CuO NPs decrease TSS and fruit firmness. Da-Costa and Sharma (2016) explain that the decrease in TSS is due to the decrease in the content of photosynthetic pigments in the leaves, bringing with it a lower production of photosynthates and, consequently, less accumulation of sugars in the fruits.

On the other hand, the increase in fruit firmness caused by CuO NPs could be due to the lignification of the cell wall (López-Vargas *et al*., 2018), an effect that is related to the increase in the activity of the phenylalanine ammonia lyase enzyme since phenylalanine is a precursor of lignin synthesis (Wang *et al*., 2013). Nevertheless, high doses of CuO NPs drastically decrease fruit firmness (Hong *et al*., 2016).

The diversity of responses to NPs depends on the dose used since they can induce positive or negative responses or cause no effect; this behavior is called hormesis and has been reported when NPs are applied as biostimulants in crops (Juárez-Maldonado *et al*., 2019), which is why more research should be carried out on the dose, species, and vegetative stage of the crops.

Bioactive compounds in fruits

The production of foods with a high content of bioactive compounds is desirable because these compounds can help prevent chronic degenerative diseases and promote the physiological functions of the body. The results obtained show that foliar spraying with CuO NPs affected the content of bioactive compounds in melon pulp (Table 2). The foliar application of 150 mg L^{-1} increased the bioactive compounds: 28, 10, and 43% (flavonoids, phenols, and antioxidant capacity), compared to the fruits of the untreated plants. Authors such as Juárez-Maldonado *et al*. (2018) mention that Cu NPs have a beneficial effect on the accumulation of bioactive compounds and increases the antioxidant capacity in *M. oleifera*.

Table 2. Effect of the foliar spraying of CuO NPs on bioactive compounds in melon fruits.

CuO NPs (mg L^{-1})	Flavonoids (mg QE 100 g^{-1} FB)	Phenols (mg GA 100 g^{-1} FB)	Antioxidant capacity (mg equiv Trolox 100 g^{-1} FB)	Vitamin C (mg 100 g^{-1} FB)
Control	73.32 ±1.7 c[*]	184.87 ±1.8 c	53.38 ±1 c	40.48 ±4.1 b
150	101.97 ±1.9 a	206.51 ±2.8 a	93.76 ±6 a	54.3 ±1.6 a
200	85.77 ±1.5 b	187.26 ±1.4 bc	71.67 ± 4b	42.82 ±2.0 b
250	77.82 ±3.7 bc	192.29 ±6 bc	63.65 ±2.9 b	32.98 ±0.57 c
300	77.21 ±1.2bc	188.28 ±8.4 bc	64.38 ±0.51 b	31.2 ±1.0c
350	71.68 ± 4c	165.84 ±6.1 d	52.07 ±15.1c	20.6 ±1.9d

[*] = means with different letters in the same column are statistically different (Tukey $p \leq 0.05$); n= 6 ± standard deviation.

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Nonetheless, the highest dose caused a drastic decrease, of 29, 19 and 44%, compared to the best treatment (150 mg L^{-1}). The application of CuO NPs produces reactive oxygen species, which modify the response of enzymatic and non-enzymatic antioxidants in response to induced stress (Ma *et al*., 2010; Ahmed *et al*., 2018). Reactive oxygen species play a dual role since, at low concentrations, they act as signalers, generating a moderate stress response in plants, thus activating the biosynthesis of bioactive compounds, while at high concentrations, cell homeostasis is interrupted, damaging cellular structures, proteins, DNA and lipids (Kumar *et al*., 2016).

Regarding vitamin C, an increase of 25% was found with the foliar application of 150 mg L^{-1}, compared to the control. López-Vargas *et al*. (2018) report similar results with the application of 50-250 mg L^{-1}; however, a higher dose (500 mg L^{-1}) reduces its content. Vitamin C is a cofactor used for the redox reaction of many enzymes. For example, ascorbic acid plays a vital role in plant cells, especially in the ascorbic acid-glutathione cycle, which is responsible for electron donation.

Through this cycle, ascorbate peroxidase, an enzyme, uses two molecules of ascorbic acid to transform H_2O_2, a potentially harmful substance, into water and monodehydroascorbate (MDA) (Hernández-Hernández *et al*., 2019). Therefore, an increase in copper availability could potentially increase the enzymatic activity of dehydroascorbate dehydrogenase. As for copper oxide nanoparticles, these could allow the delivery of copper to the plant to be more efficient and regulated, limiting the toxicity of copper when applied in excessive amounts (Juárez-Maldonado *et al*., 2018).

Cu content in pulp

Cu is one of the essential trace elements for humans; it is absorbed through the diet in the small intestine and is quickly integrated into the circulation, associated with long proteins, which play an important role in the function and maintenance of the immune system (Raha *et al*., 2020). The results obtained show that the copper content in the pulp of melon fruits increased as the doses applied increased (Figure 1).

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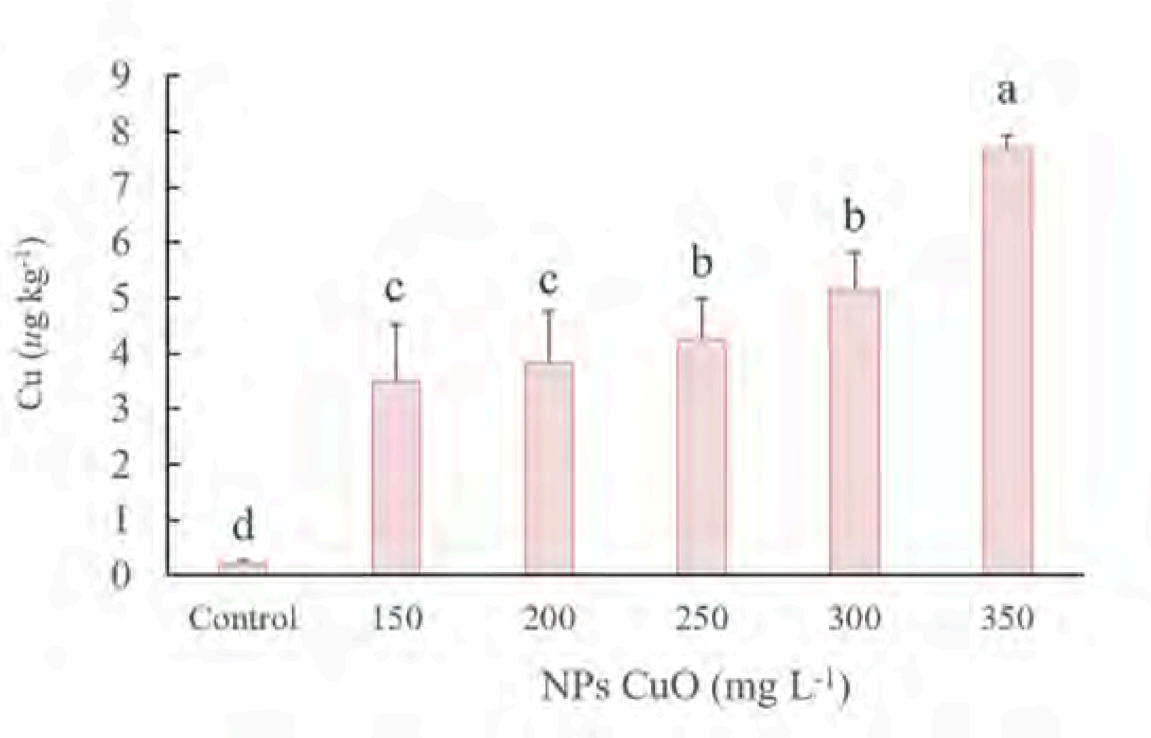
Figure 1. Effect of foliar spraying of CuO NPs on its accumulation in melon pulp. Means with different letters in the same column are statistically different (Tukey *p*≤ 0.05); n= 6 ± standard deviation.



CuO NPs can penetrate the interior of the plant, move through the phloem, and travel to other organs (Pérez-de-Luque, 2017). The requirements of Cu in the diet of the population fluctuate between 1 and 3 mg day^{-1} to avoid any deficiency, with an average recommended dose of 1 200 µg day^{-1} (Al-Hakkani, 2020), so under the conditions of this study, there is no risk in consumption and it could be an effective alternative to enrich melon fruits with Cu (Al-Hakkani, 2020).

Conclusions

Foliar spraying of 150 mg L^{-1} of CuO NPs improves yield and induces an accumulation of bioactive compounds and Cu in the pulp of melon fruits. High doses of CuO NPs decrease yield and phytochemical compound synthesis due to copper accumulation in fruits. The application of high doses of CuO NPs is not recommended as it causes an overproduction of reactive oxygen species, causing cellular stress, affecting nutraceutical yield and quality.

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Biofortification with copper nanoparticles improves yield and bioactive compounds in melon fruits

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 September 2024
Date accepted: 01 November 2024
Publication date: 11 January 2025
Publication date: Nov-Dec 2024
Volume: 15
Issue: 8
Electronic Location Identifier: e3851
DOI: 10.29312/remexca.v15i8.3851

Categories

Subject: Articles

Keywords:

Keywords:

Cucumis melo L.

antioxidants

nanobiofortification

Counts

Figures: 1

Tables: 2

Equations: 0

References: 36

Pages: 0