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Research Article

Biofortification with copper nanoparticles (Nps Cu) and its effect on the physical and nutraceutical quality of hydroponic melon fruits

Manuel FORTIS-HERNÁNDEZ[1], Jaime ORTIZ-LOPEZ[2], Pablo PRECIADO-RANGEL[1], Radames TREJO-VALENCIA[3], Erika LAGUNES-FORTIZ[4], Alfonso ANDRADE-SIFUENTES[5]* Edgar O. RUEDA-PUENTE[6]*

[1]*National Technological Institute of Mexico-Campus Instituto Tecnologico de Torreon (TecNm-ITT), Antigua Carretera Torreon-San Pedro km 7.5, Torreon, Coahuila C.P. 27170, Mexico; fortismanuel@hotmail.com; ppreciador@yahoo.com*
[2]*Master of Science in Soils National Technological Institute of Mexico-Campus Instituto Tecnologico de Torreon (TecNm-ITT), Antigua Carretera Torreon-San Pedro km 7.5, Torreon, Coahuila C.P. 27170, Mexico; alfred_jaime@hotmail.com*
[3]*National Technological Institute of Mexico-Campus Instituto Tecnologico de Minatitlan (TecNm-ITM), Blvd. Institutos Tecnologicos S/N, Col. Buena Vista Norte, Minatitlan, Veracruz 96848, Mexico; radamestv@hotmail.com*
[4]*Autonomous University of Chapingo, Phytotechnics Department, Carretera Mexico-Texcoco km 38.5 Chapingo, Texcoco 56230, State of Mexico, Mexico. erika_lagunes@hotmail.com*
[5]*Doctorate in Sciences in Water and Soils National Technological Institute of Mexico-Campus Instituto Tecnologico de Torreon (TecNm-ITT), Antigua Carretera Torreon-San Pedro km 7.5, Torreon, Coahuila C.P. 27170, Mexico; ing.andrade_85@hotmail.com (*corresponding author)*
[6]*University of Sonora, Department of Agriculture and Livestock, Boulevard Luis Encinas y Rosales s/n, Colonia Centro, Hermosillo, Sonora C.P. 83000, Mexico; erueda04@santana.uson.mx (*corresponding author)*

Abstract

Currently the use of nanoparticles is having an impact on agricultural production. There is evidence that copper nanoparticles have a strong impact on the growth and development of different crops. Biofortification specifically with (NPs Cu) improves the nutritional quality of food and its consumption has a positive influence on the health of humanity. The objective of this study consisted in evaluating the foliar application of copper nanoparticles (NPs Cu), on the weight of the fruit, nutraceutical quality and concentration of copper in melon fruit pulp. The treatments consisted of five doses of Cu NPs: 0, 1.8, 3.6, 5.4, 7.2 and 9.0 mg L^{-1} sprinkled foliarly. The variables evaluated were fruit weight, polar and equatorial diameter, firmness, total soluble solids, bioactive compounds and copper content in melon pulp. The results obtained indicated that the foliar application of NPs Cu, improved the physical and nutraceutical quality and the concentration of Cu in melon fruits. The highest weight and the best diameters of the fruit were obtained with the highest concentrations of NPs Cu (7.2 and 9.0 mg L^{-1}). The concentration of 3.6 mg L^{-1} Cu NPs presented the highest antioxidant capacity with a value of 117,713 mg equiv. Trolox * 100 mg $^{-1}$ PF, and higher content of phenols with 243.68 mg ac. gallic / 100 g FP, exceeding the concentration of 1.8 mg L $^{-1}$ by 39% and the control treatment by 48%. The 3.6 and 5.4 mg L^{-1} treatments obtained the highest amount of flavonoids with values of 149.903 and 148.29 mg QE / 100 g $^{-1}$ FP, respectively. Regarding the copper concentration in the melon fruit pulp, the 9.0 mg L^{-1} treatment presented the highest concentration with a value of 5.39 mg kg^{-1} PS; The results show that, statistically, there is a correlation between the copper nanoparticles and the

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phytochemical variables in melon fruits. It is concluded that the use of Cu NPs can be an alternative to enrich melon fruits, and could help to solve the copper deficiency in the diet of the population.

Keywords: antioxidants; *Cucumis melo* L; nanoparticles

Introduction

The melon (*Cucumis melo* L.) is one of the vegetables that is cultivated in many regions of the world; In 2019, 1,039,691 hectares were reported, with China, Iran and Turkey being the main producing countries (FAOSTAT, 2020). In Mexico in the same 2019, about 20 thousand hectares were reported, especially in places that have hot climates and little precipitation (SIAP, 2019).

Nanoparticles have been used as nanofertilizers and nanopesticides, for nutrient management, genetic improvement, treatment of plant diseases and growth promotion. When applied in small amounts, they are capable of promoting plant growth since they can be considered biostimulants (Juárez *et al.*, 2018) or nanofertilizers. They have been reported to induce oxidative stress in plants (Chandra *et al.*, 2020) and have an influence on photosynthetic and antioxidant activity (Juárez *et al.*, 2016).

Through nanotechnology, crops are being biofortified, a promising technique which consists of applying nanoparticles (NPs) of any element to crops, with positive results (Elemike *et al.*, 2019). It has been possible to biofortify edible plant tissues in different crops such as tomato, potato, pepper, lettuce, rice, jalapeño pepper, melon, etc. (Wang *et al.*, 2019; Rivera-Gutiérrez *et al.*, 2021) with easily assimilated mineral elements that are commonly lacking in the human diet such as iron (Fe), zinc (Zn), copper (Cu), calcium (Ca), magnesium (Mg), iodine (I) and selenium (Se), and increase their concentration, without generating a possible toxicity (White *et al.*, 2009). However, there are few studies on the effect of these metallic nanomaterials in melon cultivation, being that this crop provides an appreciable amount of vitamins and minerals (300 g), in addition to 36.7 mg of vitamin C, which corresponds to 76% of the recommended daily intake. It provides a large amount of antioxidants, which play an important role in human health, as they help prevent cancer, cardiovascular diseases and other chronic diseases (Lester *et al.*, 1997).

In relation to copper, studies indicate that it is an essential metal for the normal growth or development of plants, being an essential cofactor for many metalloproteins and various enzymes, which participates in various physiological and cellular processes such as oxidation and reduction reactions involving the enzyme oxidase (Rajput *et al.*, 2018). Cu NPs can function as reducers or oxidants in biochemical reactions within the cell, which is why they can catalyze the production of reactive oxygen species (ROS) and induce oxidative stress (Somasundaran *et al.*, 2010). As it is a transition metal, it catalyzes the formation of H_2O_2, an increase in the concentration of this substance results in an increase in peroxidase activity. For humans, it participates in the growth and strengthening of bones, in the transport and assimilation of iron in the intestine, the production of melanin, the regeneration of body tissues, the stability of the immune system and the health of blood vessels. In the organs where it is mostly found is the liver, brain, kidneys and heart. The previously indicated, guides us to orient our efforts to generate knowledge in the so-called "functional foods" that contain a wide variety of bioactive compounds that are beneficial for human health (Maietti *et al.*, 2012).

Based on the above, the objective of this research was to identify and determine the most efficient concentration of copper nanoparticles (NPs Cu) that affect the biofortification, physical and nutraceutical quality of melon fruits produced in hydroponic gutters under conditions of shade cloth.

Materials and Methods

The study was carried out in a shadow house of the Technological Institute of Torreon (ITT), during the spring-summer agricultural cycle of 2020. The ITT is located in the municipality of Torreon Coahuila, Mexico at parallel 26° 30′ 15″ N - 103° 22′ 07″ W. The shade mesh is a 2 mm thick galvanized steel support structure with 1.25″ and 1.5″ square profiles and anti-insect mesh (crystal colour) with threads 25 x 25-inch, 720-gauge, UV-treated polyethylene with diffuse light and 30% shade.

Plant material and growing conditions

Melon seeds (*Cucumis melo* L.) of the Cruiser Harris Moran® hybrid were used, which were germinated in 200-cavity polyethylene trays and later transplanted into black polypropylene gutters of 5.0 x 0.30 x 0.30 m long, wide and high, a capacity of 0.45 m^3 which contained a substrate based on river sand and perlite (80:20 vol/vol). The substrate was washed and sterilized with a 5% sulfuric acid (H_2SO_4) solution, allowing it to stand for 24 hours to later be washed with water (Preciado *et al.*, 2021). Once the gutters were prepared, the seedlings were placed 30 cm apart (3 plants per linear meter) in single rows.

The treatments consisted of the foliar application of concentrations 1.8, 3.6, 5.4, 7.2 and 9.0 mg L^{-1} of copper nanoparticles (NPs Cu) plus a control treatment based on distilled water. The size of the nanoparticles was from 20 to 60 nm, with a purity of 97%, white in color and with spherical and polygonal structural shapes. They were synthesized by the method of Ramírez *et al.* (2019) at the Center for Research in Applied Chemistry (CIQA), Saltillo, Coah., Mexico. Four foliar applications of NPs Cu were made, the first at 10 days after transplantation (dat), when the crop showed foliage expansion and subsequently every 15 days, using cylindrical atomizers with an output of 0.14 mL and for a better adherence, a non-ionic acidifier - adherent (AF-OPTIMUS®) was added at a dose of 1 - 2 ml·L of spray water. The foliar applications were made in the morning and without the presence of wind. A completely experimental design was used, considering five treatments and five repetitions.

The water needs of the melon were covered by applying the water through an automated irrigation system using a 8000 gauge strip with emitters every 15 cm, from the T-tape® brand. A pressure gauge was used to monitor the constant pressure of 15 lb. To define when to apply the irrigation, moisture sensors of the substrate brand Soonhua model 1434700 (Three-way meter®) were used, which measured the volumetric content of water in the substrate; depending of phenological stage of cultivation irrigations were with a certain concentration (35, 50, 70 and 100%) of the nutrient solution Steiner (Precious *et al.*, 2021) at a pH of 6.5 and an electrical conductivity (EC) of 2 dS m^{-1}.

The environmental conditions of temperature (T), relative humidity (HR), photosynthetically active radiation (PAR: me·$M^{-2} s^{-1}$) and the mean annual insolation (Ima·h·day) in the shade house are shown in Table 1.

Table 1. Environmental conditions (T, HR, photosynthetically active radiation (PAR: me·$m^{-2} s^{-1}$) and mean annual insolation (Ima h·day) present in the different phenological stages of the melon crop grown in shade house

Phenologic stage	T (°C)	H.R. (%)	PAR (me· $m^{-2} s^{-1}$)	Ima (h·day)
Seedling / transplant (April)	39 ± 3	<46	550.33	14.20
Pre-flowering (May)	38 ± 2	<50	555.22	14.10
Flowering and fruiting (June)	37 ± 3	<39	510.32	13.30
Harvest (July)	36 ± 5	<42	477.43	13.40

Variables measured with the Uni-T UT333 and BIKING LX1330B (Hygrometer, Luxometer) equipment.

Crop management

The cultivation was conducted to a single stem, tutored with polypropylene agricultural raffia, which was attached to the base of the plant and vertically to the structure of the shade mesh, in a similar way the fruits were placed in plastic mesh bags and they were tied to the structure to prevent their detachment. A hive of worker bees (*Apis mellifera*) was used for pollination.

Green aphids (*Aphis gucipi)* and whiteflies (*Bemisia tabaci)* pests were controlled by applying potassium soap, organic repellants based on Neem extracts and bioinsecticides (Pirecris®). The first fruits were harvested at 90 dat. when they reached their commercial maturity, presenting a well-formed network and when the peduncle was easily detached from the main branch.

Variables evaluated

Average fruit weight, size (polar, equatorial diameter), soluble solids and firmness

For this, a digital scale (Adir®, USA) with a capacity of 5,000 g was used. The results were expressed in kilograms and to determine the effect that the treatments induced on the biophysical quality of the fruits, the size of the fruit was evaluated through the polar diameter (DP) and equatorial diameter (DE) using a digital Vernier caliper (Steren Brand); the soluble solids content (determined in °Brix) measured with a Master-T refractometer (Atago®, Tokyo, Japan); fruit firmness (FF) with a penetrometer using an 8 mm plunger (FHT200, Extech Instruments®, USA) The exocarp (shell) was removed and placed on a flat surface, four penetrations were made per fruit Azam *et al.* (2015) and the results were averaged by registering as maximum compressive force (N).

Nutraceutical quality: phenolic content, flavonoids, antioxidant capacity, vitamin C and copper accumulation in the fruit

For the determination of bioactive phytochemicals, three randomly selected melon fruits from each treatment were analyzed, each one extracting 150 g of pulp. Then, they were crushed manually (in a mortar) and samples of 5 g of fresh pulp were obtained which were placed in 10 mL of methanol in plastic tubes with screw cap and then placed on a rotary shaker (ATR Inc.) during 6 h at 20 rpm and 5 °C. The tubes with the mixture were centrifuged at 3000 rpm for 10 min and the supernatant was extracted for analysis (Andrade *et al.*, 2020).

The total phenolic content was determined by a modification of the Folin-Ciocalteau method (Singleton *et al.*, 1999); 50 μL of ethanolic extract were used, diluted in 3 mL of Milli-Q water (MQ, Damstadt, DE), subsequently 250 μl of the Folin-Ciocalteau reagent (1 N) was added, stirred and allowed to react for 3 min. Subsequently 750 μL of Na_2CO_3 (20%) and 950 μL of MQ water were added. The solution was allowed to stand for 2 h, and the samples were quantified in an ultraviolet (UV) -Vis spectrophotometer at 760 nm. The standard was prepared with gallic acid. The results were expressed in mg GAE / 100 g^{-1} fresh weight.

Total flavonoids were determined by colorimetry (Moretti *et al.*, 2013); 250 μL of ethanolic extract were taken, mixed with 1.25 mL of MQ water and 75 μL of NaNO2 (5%). After 5 min of standing, 150 μL of $AlCl_3$ (aluminium) 1 ethyl 3 methylimidazolium (Sigma Aldrich, St. Louis, MO, USA) were added. Subsequently, 500 μL of NaOH (1 M) and 275 μL were added of MQ water, they were shaken vigorously and the samples were quantified in a UV-Vis spectrophotometer at 510 nm. The standard was prepared with quercetin dissolved in absolute ethanol (Y = 0.0122x-0.0067; r^2 = 0.965). The results were expressed in mg QE / 100 g^{-1} fresh weight.

The total antioxidant capacity was measured by 2,2-diphenyl-^{1}-picryl-hydrazyl hydrate *in vitro* ($DPPH^+$) (Brand *et al.,* 1995). A DPPH + solution (Sigma-Aldrich, St. Louis, MO, USA) was prepared in ethanol at a concentration of 0.025 mg mL^{-1}. 50 μL of ethanolic extract were mixed with 1950 μL of DPPH + solution; after 30 min the samples were quantified in a UV-Vis spectrophotometer at 517 nm. The results were expressed in μM equivalent in Trolox / 100 g-^{1} fresh weight.

The content of vitamin C in the fruit was determined by the titration method (Bona *et al.*, 2016), when 10 g fruit samples were taken, crushed together with 10 mL of 2% hydrochloric acid, filtered and made up to 100 mL with distilled water. in an Erlenmeyer flask; then, 10 ml of the dilute was titrated with 2,6 dichlorophenolindophenol (1 x 10^{-3} N).

For the accumulation of copper in the fruit, the dried melon samples were ground in a porcelain mortar and digested with nitric and perchloric acid (3: 1) using a plate and heating to 100 °C. The solution was filtered and boiled to 100 mL of working solution with deionized water. The copper concentration in melon fruits was determined by atomic absorption spectrophotometry (AOAC, 2005). The results were expressed in µg kg^{-1} of weight of nuts.

Statistic analysis

The data of the variables were analyzed by analysis of variance and mean comparison test using the Tukey test ($P \leq 0.05$) with the statistical package SAS (Statistical Analysis System Institute) version 9.4 Pearson's correlation coefficients were established between the phytochemical variables and copper content in melon fruits.

Results and Discussion

Average fruit weight, size (polar, equatorial diameter), soluble solids and firmness

The average weight of the melon fruit was positively affected by the foliar application of NPs Cu, since as the concentration of NPs applied increased, there was also an increase in the weight of the fruit (Table 2). The highest weight of the fruit was obtained with 9 mg L^{-1} Cu, exceeding the control treatment by 41%. In the present experiment, the increase in fruit weight can be explained by the effect of NPs Cu by stimulating a greater production of indoleacetic acid (IAA) (Dimkpa *et al.*, 2012). Due to the fact that with the application of copper nanoparticles, the stress of both the plants and the soil microorganisms is reduced, thus the biological activity is accelerated, which causes an increase in the production of indoleacetic acid, being reflected in the growth of the plant and its fruits (Tucuch-Haas *et al.*, 2015), which results in greater elongation and cell division. Saharan *et al.* (2015) report an increase in biomass in tomato treated with Cu NPs. For the variables polar and equatorial diameter, the largest size was obtained with the T5 of 7.2 mg L^{-1}, with values of 15.28 and 12. 67 cm, respectively; however, this treatment was statistically similar to the polar diameter T6 (9 mg L^{-1}), and T4 (5.4 mg L^{-1}). Garcia *et al.* (2019) report significant values for cantaloupe melon of 15.19 and 14.69 cm, for polar and equatorial diameter, respectively. These quality parameters are desirable since they are related to post-harvest handling and the quality of the fruit in the sense that the fruits do not suffer damage when transported to the market and reach the consumer with acceptable characteristics, which are: Well-formed fruits, almost spherical and uniform in appearance, smooth peduncle scar, absence of scars, sunburn or surface defects. Firm and with its sugar content of no less than 9% soluble solids in its pulp (Martínez-González *et al.*, 2017).

Regarding the variable firmness, there were significant statistical differences between the concentrations of Cu NPs applied. However, it could be observed that the T6 (9 mg L^{-1} Cu NPs) exceeded 29% to the control treatment and for the variable of total soluble solids; the higher values were obtained with high concentrations (5.4, 7.2 and 9.0 mg L^{-1} Cu NPs) which are statistically equal (Table 2). Similar results were reported by López *et al.* (2018), who with higher doses of copper nanoparticles in tomato obtained greater firmness. In this regard, Juárez *et al.* (2016), reports that the application of NPsCu + chitosan increased the firmness of tomato fruits by 9%.

Regarding the TSS in fruit, these were increased by 25% by the highest dose of Cu NPs compared to the fruit of the control treatment (Table 2). It is worth mentioning that the SST values obtained are within the ranges reported for this crop in the Lagunera region (6.5 to 11 °Brix). Values of 9 °Brix are optimal for the optimal internal quality of the melon and according to international standards, melons with sugar content of 12 - 14 °Brix are suitable for the international market (Bower *et al.*, 2002).

Table 2. Comparison of means of fruit weight, polar and equatorial diameter, firmness and total soluble solids of melon fruits with foliar applications of copper nanoparticles (NPsCu)

Treatments (mg L-1 Cu NPs)	Fruit weight (kg)	Diameter *Equatorial polar* (cm)		Firmness (Newton)	Total soluble solids (°Brix)
T1 = Control	0.86±0.10 c*	11.50±2.86 c	10.33±5.11 c	10.14±0.26 b	8.93±0.06 d
T2 = 1.8	1.24±0.64 ab	12.28±2.30 bc	11.58±1.55 b	11.28±0.34 ab	9.46±0.15 c
T3 = 3.6	1.17±0.02 b	12.76±2.02 b	11.58±3.38 b	11.32±0.17 ab	10.36±0.51 b
T4 = 5.4	1.18±0.07 b	14.52±2.15 a	12.53±3.43 ab	12.05±0.85 a	11.70±0.10 a
T5 = 7.2	1.40±0.05 ab	15.28±6.03 a	12.67±4.28 a	12.04±0.58 a	11.80±0.10 a
T6 = 9.0	1.46±0.15 a	14.43±2.22 a	11.76±2.23 a	12.51±0.20 a	11.90±0.00 a
DMS	0.257	8.726	9.700	1.255	0.298
CV	7.665	2.417	3.410	3.961	1.017
R^2	0.863	0.963	0.873	0.808	0.994

DMS = Minimum significant difference; CV = Coefficient of variation; R^2 = Coefficient of determination

* Values with different letters within each column are statistically different according to Tukey's test ($P \leq 0.05$).

Nutraceutical quality: phenolic content, flavonoids, antioxidant capacity, Vitamin C and accumulation of copper in the fruit

In the case of the nutraceutical quality of melon fruits, the analysis of variance showed significant differences ($P \leq 0.05$) for the antioxidant capacity. This means that the applications of NPsCu had an effect on this component, being the concentration of 3.6 mg L^{-1} where the highest antioxidant capacity was achieved with a value of 117.713 mg equiv. Trolox * 100 mg $^{-1}$ FP. This concentration exceeded the control treatment by 50% and the T2 (1.8) and T5 (9.0) treatments by 20 and 17%, since they presented the lowest values with 58.1, 93.79 and 98.56 mg equiv. Trolox * 100 mg $^{-1}$ FP, respectively (Figure 1a). These results may be associated with the foliar application of NPs having a greater potential for transport, greater bioavailability and absorption that allows them to interact with intracellular structures that stimulate the formation of ROS (García-López *et al.*, 2019).

For phenolic compounds, the treatment that obtained the highest content was the concentration of 3.6 mg L^{-1} NPsCu (243.68 mg gallic ac. / 100 g FP) exceeding by 39% the lowest applied concentration of 1.8 mg L^{-1} and in 51.29% to the control treatment. In Figure 1b, a clear tendency of increasing in the values of the phenological compounds is appreciated when increasing the concentrations of the NPs Cu. This happened when going from the concentration of 3.6 mg L^{-1} to 5.4 and so on. T6 (9.0 mg L^{-1}), decreased the amount of phenological compounds by 24.50% with respect to T3 (3.6 mg L^{-1}).

In the case of flavonoids, the treatments of 3.6 and 5.4 mg L^{-1} NPsCu were those that obtained the highest amount with values of 149.903 and 148.29 mg QE / 100 g $^{-1}$ PF, respectively, exceeding 28.2% to T1 (Control) and by 14.7% at T2 of 1.8 mg L^{-1} (Figure 1c). The amount of flavonoids present in melon depends on the genotype, ripening of the fruit and the date of harvest. They have various functions, it acts as an excellent antioxidant agent, it has antimicrobial compounds, UV protectors, insect protectors, etc.

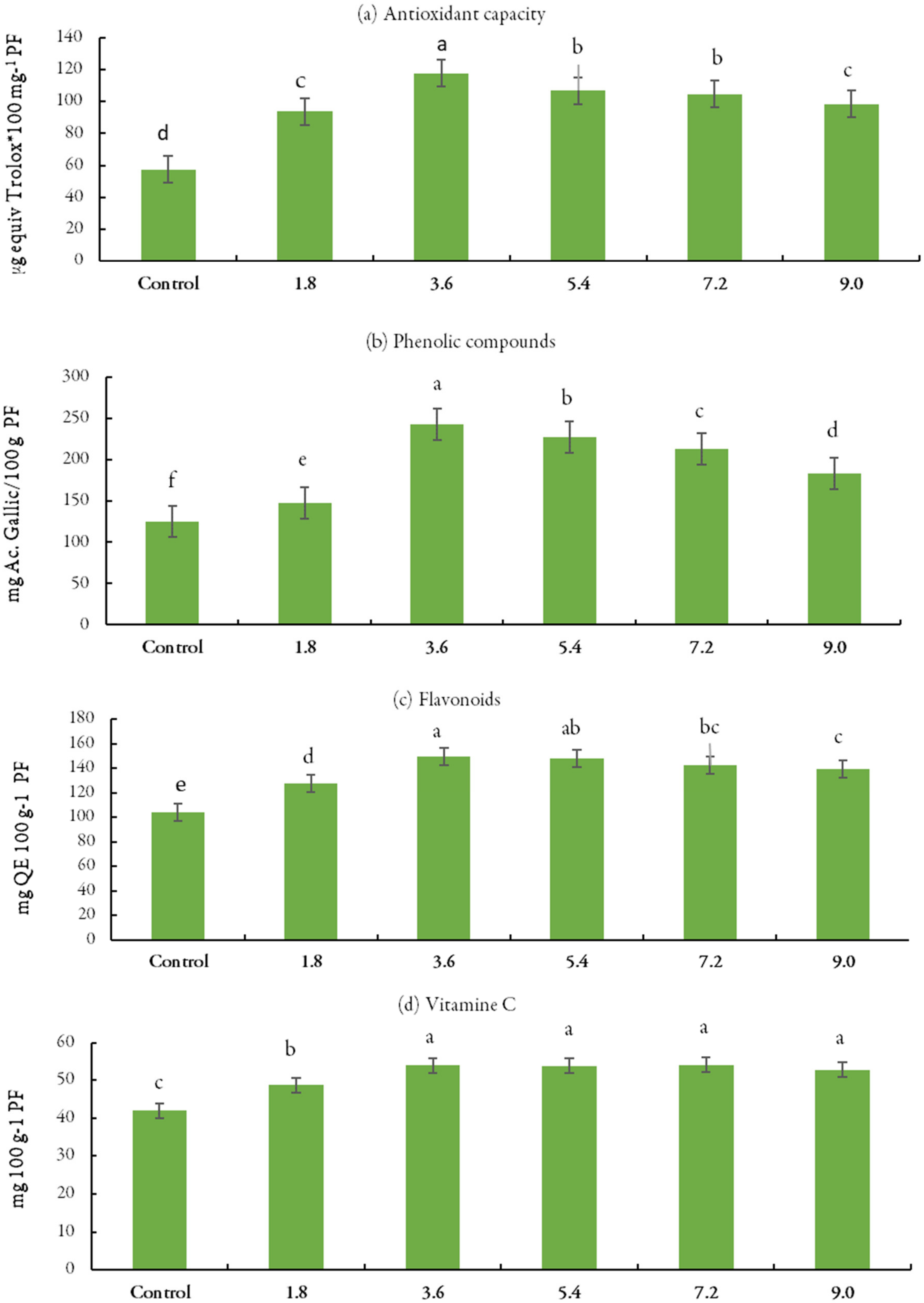

Figure 1. Effect of the foliar application of copper nanoparticles (NPs Cu) on the antioxidant capacity, phenological compounds, flavonoids and vitamin C, in cantaloupe melon fruits produced in a hydroponic system

Different letters mean statistical differences according to the honest Tukey significant difference test ($P \leq 0.05$).

The analysis of variance for vitamin C showed significant differences ($P \leq 0.05$), which means that the applied NPsCu concentrations affected its behavior. The T3 (3.6 mg L^{-1}), T4 (5.4 mg L^{-1}), T5 (7.2 mg L^{-1}) and T6 (9 mg L^{-1}) presented the highest values (53.90, 53.71, 53.99 and 52.70 mg 100 g^{-1} FP), respectively. These treatments were statistically equal to each other and exceeded T2 by 9%, which contained the lowest concentration of NPs CU (1.8 mg L^{-1}) and by 22% to the control treatment (Figure 1d). Rivera-Gutiérrez *et al.* (2021), report vitamin C contents of 9-60 mg /100 g of melon pulp, results that agree with those obtained in the present study. Similarly, Fundo *et al.* (2018), report vitamin C values of 88 mg / 100 g for Cantaloupe melon pulp. The values obtained in our work were 38% below the highest value reported by Fundo *et al.* (2018). The possible explanation is possible that it is due to the low concentrations of applied NPs Cu. Studies carried out in tomato cultivation by López-Mora *et al.* (2018) show that applications of 250 mg L^{-1} NPs of Cu significantly increased the concentration of vitamin C in its fruits, which increased its nutraceutical quality. Similarly, Pérez-Labrada *et al.* (2019) found that the foliar application of Cu NP increased the content of biocomposites including phenols, β-carotene and vitamin C also in tomato fruits. On the other hand, Cumplido *et al.* (2019) when applying NPs Cu + potassium silicate in high and low concentrations, they did not obtain significant differences in this variable. While Hernández *et al.* (2018) mention that the use of Cu NPs in combination with chitosan tends to improve the growth and activation of bioactive compounds in plants. Improving the amount of vitamin C in melon fruits is very important, since vitamin C is an antioxidant, whose effects reduce human diseases such as atherosclerosis and cancer, which occur due to oxidative damage to tissues (Padayatty *et al.*, 2003). In general, diets rich in fruits and vegetables biofortified with NPs Cu, could reduce the risk of this type of disease.

The analysis of variance for the copper concentration in the pulp of melon fruits (Figure 2), showed significant differences ($P \leq 0.05$) due to the effect of the copper NPs. Being the treatment of 9.0 mg L^{-1} (T6), the one that presented the highest concentration of copper with a value of 5.39 mg kg^{-1} PS, while the treatment of 1.8 mg L^{-1}, presented the lowest concentration (3.14 mg kg^{-1}), 41.7% lower (Figure 2).

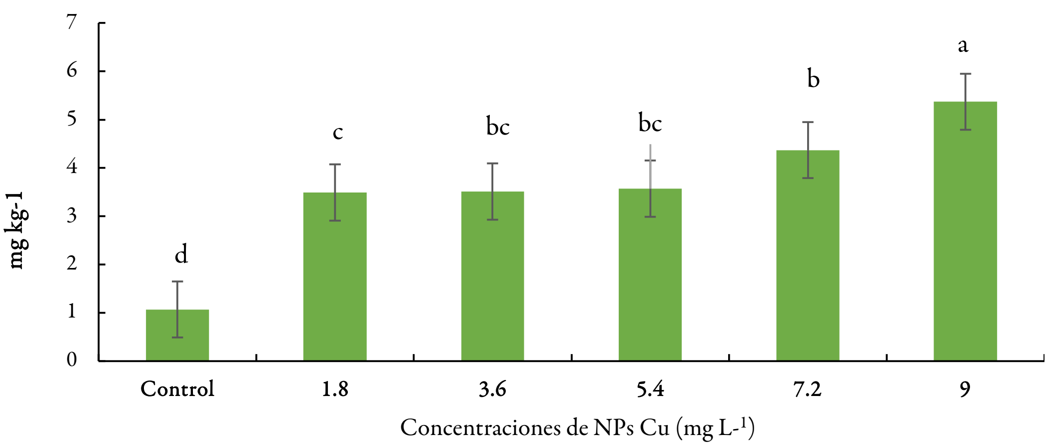

Figure 2. Copper content in melon fruit pulp due to the foliar application of NPsCu in different concentrations

Bars with different letters are statistically different according to Tukey's test ($P \leq 0.05$).

It is important to note that a positive trend is observed between the increase in the concentration of the applied nanoparticles and the increase in the concentration of copper in the melon pulp. This is relevant as this suggests that its value can still be increased. Robledo-Torres *et al.* (2005), report copper concentrations of 0.22 mg / 100 g for melon fruits. On the other hand, López-Vargas *et al.* (2018), point out that copper is essential for building strong tissue and maintaining blood volume in human beings, in addition to producing energy in their cells. Studies indicate that values of 0.04 milligrams of copper can be found for every 100 grams of melon pulp, indicating that it would be 2% of the recommended daily intake of copper, in this study these values would be exceeded.

Table 3. Pearson's correlation coefficients of the phytochemical variables and copper content

	Flavonoids	Phenolic compounds	Antioxidant capacity	Vitamin C	Copper
Flavonoids	1.0000<.0001	0.9309<.0001	0.96669<.0001	0.94791<.0001	0.726810 <.0006
Phenolic compounds	0.9300<.0001	1.0000 <.0001	0.8936<.0001	0.8772<.0001	0.50620<.0.032
Antioxidant capacity	0.9666<.0001	0.8936<.0001	1.0000<.0001	0.9249<.0001	0.7067<.0010
Vitamine C	0.947<.0001	0.877<.0001	0.924<.0001	1.000<.0001	0.777<.0001
Copper	0.726<.0.0006	0.506<.0.0321	0.706<.0.0010	0.777<.0.0001	1.000<.0001

N = 18; Prob> lrl assuming H0: Rho = 0.

According to the correlation coefficient, it can be observed (Table 3) that the concentrations of copper nanoparticles (NPsCu) applied in the present study influence on the phytochemical variables of melon fruits. These results may be associated with the foliar application of NPsCu having a greater potential for transport, greater bioavailability and absorption that allows them to interact with intracellular structures that stimulate the formation of ROS (Ghosh *et al.*, 2016; García-López *et al.*, 2019). Once the NPs have penetrated the plant tissue, they present a slow and gradual availability of the active principle at the cellular level, which results in a greater accumulation of the $^{++}$ ion and, therefore, the generation of oxidation stress (Gaschler *et al.*, 2017; Mosavat *et al.*, 2019). The high concentration of metallic NPs in plant tissues can influence the production of reactive oxygen species (ROS) and lipid peroxidation (MDA) (Gohari *et al.*, 2020; Huang *et al.*, 2011; Kalal *et al.*, 2021). ROS reduce atmospheric oxygen molecules such as superoxide radical O2 •, hydrogen peroxide H_2O_2, singlet oxygen O_2 and hydroxyl radical OH •, which are highly reactive and can cause oxidative stress in organisms (Mittler *et al.*, 2017) and can affect proteins, lipids, carbohydrates, and DNA. NPs can also alter photosynthetic efficiency, photochemical fluorescence and plant performance, due to their interactions with photosystems I and II, since studies have shown that chlorophylls transfer energy to NPs (Olejnik *et al.*, 2013; Rico *et al.*, 2015).

Conclusions

Due to the research, the use of copper nanoparticles could be an effective way to enrich melon fruits, and could help solve the copper deficiency in the diet of the population. In addition, a correlation between copper nanoparticles was statistically demonstrated. and the phytochemical variables in melon fruits.

Authors' Contributions

Investigation, M.F.H. and P.P.R.; Formal analysis, M.F.H. and E.L.F.; Investigation, J.O.L. and M.F.H.; Visualization M.F.H. and R.T.V. and E.O.R.P. and E.L.F.; Writing—original draft, M.F.H. and A.A.S. and E.O.R.P.; Writing—review and editing, A.A.S. and E.O.R.P.

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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