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Foliar biofortification with copper nanoparticles and its effect on phytochemical quality and enzymatic activity in lettuce

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ABSTRACT

The objective of this study was to evaluate the foliar application of copper oxide nanoparticles (NPs-CuO) on the phytochemical quality, yield, catalase and peroxidase activity of the hydroponic lettuce crop. Five concentrations (5, 10, 15, 20 and 25 mg/L) of NPs-CuO and a control (0 mg/L) were compared. The experiment was established in a completely randomized design with five repetitions. Data were analyzed with analysis of variance and mean comparisons using Tukey's test (0.05). The results indicate that, under the hydroponic conditions, the lettuce plants showed a greater number of leaves, leaf width and the peroxidase enzyme activity was recorded with the concentration of 15 mg/L NPs-CuO. The concentration of 10 mg/L of NPs-CuO yielded higher contents of total phenols, total flavonoids and antioxidant capacity, being 46.63, 34.21 and 28.23% higher compared to the control treatment, respectively. Regarding the concentration of Cu in the lettuce leaf, the 20 mg/L treatment showed the highest concentration with a value of 9.93±2.40 µg/L DW. The use of copper nanoparticles could be an effective alternative to mitigate copper deficiency in the diet of the human population.

Keywords: *Lactuca sativa*, antioxidants, total phenols, NPs-CuO, peroxidase.

RESUMO

Biofortificação foliar com nanopartículas de cobre e seu efeito na qualidade fitoquímica e atividade enzimática em alface

O objetivo deste estudo foi avaliar a aplicação foliar de nanopartículas de óxido de cobre (NPs-CuO) na qualidade fitoquímica, produtividade, atividade de catalase e peroxidase da cultura hidropônica de alface. Foram comparadas cinco concentrações (5, 10, 15, 20 e 25 mg/L) de NPs-CuO e um controle (0 mg/L). O experimento foi instalado em delineamento inteiramente casualizado com cinco repetições. Os dados foram analisados com análise de variância e comparações de médias pelo teste de Tukey (0,05). Os resultados indicam que, nas condições hidropônicas, as plantas de alface apresentaram maior número de folhas, largura de folhas e a atividade da enzima peroxidase foi registrada com a concentração de 15 mg/L de NPs-CuO. A concentração de 10 mg/L de NPs-CuO proporcionou maiores teores de fenóis totais, flavonóides totais e capacidade antioxidante, sendo 46,63, 34,21 e 28,23% maiores em relação ao tratamento controle, respectivamente. Em relação à concentração de Cu na folha de alface, o tratamento 20 mg/L apresentou a maior concentração com valor de 9,93±2,40 µg/L de MS. O uso de nanopartículas de cobre poderia ser uma alternativa eficaz para mitigar a deficiência de cobre na dieta da população humana.

Palavras-chave: *Lactuca sativa*, antioxidantes, fenóis totais, NPs-CuO, peroxidase.

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By the year 2050, it is estimated that the world production must increase by 70%. Consequently, there is a need to improve agricultural production systems in the short term (Xiong *et al*., 2021). New technologies or alternatives are required to protect plants from stress caused by biotic or abiotic factors and improve the efficiency of the use of agrochemicals to achieve food security safely and sustainably (Zhao *et al*., 2020). An emerging and promising alternative is agronanotechnology which involves the supply of nanoproducts of different metal oxides in the agricultural field, causing beneficial effects on growth parameters, crop productivity development and reducing the negative impact on the environment (Priyanka *et al*., 2019). According to the wide range of nanoproducts, nanoparticles are used in agriculture, biomedicine, pharmaceuticals, transportation, biosensors, consumer products, catalysts, and industrial products (Xiong *et al*., 2021). Nanopesticides and nanofertilizers could reduce the ecological footprint caused by agricultural activities, they can increase crop production while improving plant traits against various environmental stresses, such as pathogens (Xiong *et al*., 2021). Its use in the remeasurement and conservation of soil and water could replenish these depleted resources to improve agricultural production (Bakshi & Kumar, 2021). In addition, to improve crop performance, resistance to pathogens and bioactive compounds that help prevent chronic and degenerative diseases are enhanced (Fortis-Hernández *et al*., 2022). With the application of nanoparticles in agricultural crops, eating disorders caused by the deficit in the intake of micronutrients contained in food could be reduced (Elemike *et al*., 2019).

In particular, copper oxide nanoparticles (NPs-CuO) have great potential due to their broad-spectrum antimicrobial activity, and their small size facilitates their uptake by plants. Cu is an essential trace element for humans and animals. In plants it plays an important role in different physiological processes, such as photosynthetic and respiratory electron transport chains, nitrogen fixation, antioxidant activity, protein metabolism, structural and catalytic component of proteins and enzymes (Zhao *et al*., 2020). Biofortification of plants by applying nanoparticles via the foliar route and/or in the nutrient solution increases essential minerals such as copper, zinc, calcium, magnesium and iodine in the human diet (Jampílek & Králová, 2019). Copper deficiency in humans causes disorders such as anemia and neutropenia, while excess causes liver disorders and diseases such as Alzheimer's and nervous breakdown (Gaucin-Delgado *et al*., 2022). There is evidence that the application of NPs-CuO via the foliar route or in the growth medium modifies the behavior of growth, development and productivity of plants, such as black mustard (*Brassica nigra*) (Zafar *et al*., 2020), tomato (*Solanum lycopersicum*) (Hernández-Hernández *et al*., 2018), lettuce (Liu *et al*., 2016), garlic (*Allium sativum*) (Mottaleb *et al*., 2021), among other plant species of commercial interest. In particular, in several studies it has been shown that the use of NPs-CuO has managed to favor the enzymatic and non-enzymatic antioxidants of lettuce leaves (Ahmad *et al*., 2021). Foliar spraying of NPs-CuO increases the phenolic and flavonoid contents in plants subjected to saline stress; these increases in bioactives could be a strategy to mitigate damage from saline stress. High doses of NPs-CuO on plants produce stress and/or phytotoxicity, generating reactive oxygen species (ROS), increasing the content of antioxidant enzymes and non-antioxidant enzymes: glutathione, vitamin C, ascorbate peroxidase, superoxidase dismutase and catalase, among others (Xiong *et al*., 2021). However, research is frequently carried out with different plant species to determine the optimal dose of NPs-CuO without causing phytotoxicity.

On the other hand, lettuce (*Lactuca sativa*) is one of the main vegetables grown and consumed worldwide; in 2019 its production reached 29,134,653 tons (SIAP, 2020). In 2019, the main producer of this vegetable was China with 16,310,175 tons, Mexico ranked ninth with a production of 516,000 tons (SIAP, 2020). Lettuce is a healthy vegetable due to its high content of fiber, carotenoids, phenolic compounds, antioxidants, vitamins A, C and minerals, it is low in calories, fat, sodium, minerals and fiber, as well as bioactive compounds such as folates, beta-carotene, lutein and phenols that are organoleptic and nutraceutical properties that benefit human health. Therefore, the objective of this study was to evaluate the foliar application of copper oxide nanoparticles (NPs-CuO) on the quality and yield of the hydroponic lettuce crop.

MATERIAL AND METHODS

Place of study

This research was carried out in the autumn-winter 2022 agricultural cycle in a shade mesh of the Technological Institute of Torreon, in the city of Torreon, Coahuila, Mexico (24°30'27"N, 102°00'104'40"W, 1120 m altitude).

Genetic material and growth conditions

The plant material used was lettuce (*Lactuca sativa*) cv. orejona (Rancho los Molinos®), which was planted in phenolic foam trays (agricultural foam) with 100 cavities, applying irrigation every 24 h. The transplant was carried out 23 days after sowing (DAS), when the seedlings presented an average of 6 to 7 true leaves in a Nutrient Film Technique (NFT) hydroponic system, with the following features; 4-inch diameter hydraulic PVC pipes 6 m long, with a 10% slope and 25 cm spacing between each hole. For the nutrient solution (NS) circulation system, a ½ horsepower centrifugal pump (BOAP-1/2A2, Truper) was used. After transplantation, the electrical conductivity of the SN was dosed according to the development stages of the culture. From the transplant to the seventh day after the transplant (DAT) an electrical conductivity of 0.8 dS/m was applied, the conductivity increased 1.2, 1.6 and 2 dS/m, at 8, 19, 29 DAT, respectively. During the 67 days of the crop cycle, the minimum and maximum temperature inside the shade mesh fluctuated between 17.7 and 31.6°C, respectively, while the minimum and maximum relative humidity ranged between 30 and 70%. The universal nutrient solution was prepared from calcium nitrate [$Ca(NO_3)_2$], potassium nitrate (KNO_3), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), potassium sulfate (K_2SO_4), phosphate monopotassium (KH_2PO_4), plus micronutrients (Librel Mix-AL®). The electrical conductivity of the SN was 2 dS/m and the pH was 6.0.

Treatments and experimental design

The copper oxide nanoparticles (NPs-CuO) used belong to the nanoparticle collection of the Applied Chemistry Research Center (CIQA), Saltillo, Coahuila, Mexico. The size of the nanoparticles was 30 nm, spherical morphology, with a purity of 98%, and they were obtained by chemical synthesis.

The experimental design used was completely randomized, with six treatments and five repetitions, each plant was considered as an experimental unit. Copper nanoparticles can act as stimulants or elicitors in small doses (Juárez-Maldonado *et al*., 2019), in this sense they were evaluated at the following concentrations: 0, 5, 10, 15, 20 and 25 mg/L. By the stock solution method, the different concentrations were prepared separately. The concentrations of NPs-CuO were deposited individually in volumetric flasks with a capacity of one liter, immediately after volumetric with distilled water. The solutions were applied foliarly during the first hours of the morning with manual sprayers with a capacity of 100 mL. The first application of the treatments was at seven DAT, later they were applied every 15 DAT until 67 DAT (Gaucin-Delgado *et al*., 2022).

Variables evaluated

The lettuce plants were harvested at 67 DAT, the variables evaluated at harvest time were: leaf height, number of leaves, crown diameter, leaf width and length, fresh root weight, chlorophyll, compound phenolics, total flavonoids, antioxidant capacity, enzymatic activity (peroxidase, catalase) and copper concentration. To determine the fresh weight (crop yield, g/plant), the lettuces were harvested and weighed on an analytical balance.

Phytochemical quality

For the determination of total phenols, flavonoids and antioxidant capacity, 2 g of a lyophilized lettuce leaf sample were mixed with 10 mL of ethanol in plastic tubes with screw caps. A "Stuart" type stirrer was used to keep the mixture agitated for 24 h. Subsequently, the tubes were centrifuged at 3000 rpm for 5 min and the supernatants (ethanolic extracts) were used for their respective analyzes. The phenolic content was determined using a modification of the Folin-Ciocalteu considering 30 μL of extract were mixed with 270 μL of distilled water in a test tube, then 1.5 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, St Louis MO, USA) diluted (1:15) was added and vortexed for 10 s. After 5 min, 1.2 mL of sodium carbonate (7.5% w/v) was added and stirred for 10 s. The solution was placed in a water bath for 15 min and then allowed to cool to room temperature. The absorption of the solution was read at 765 nm in a UV spectrophotometer (Genesys 10). To calculate the phenolic content, a standard curve was made using gallic acid as a standard, the results were recorded in mg of gallic acid equivalent per 100 g based on fresh weight (mg gallic ac./100 g FW); analyzes were performed in triplicate.

The flavonoid content consisted in use 250 μL aliquot of the supernatant of the ethanolic extract and then adding 1.25 mL of distilled water and 75 μL of 5% $Na^{+}NO^{-}_{2}$, shaking the mixture in a vortex and allowing it to react for 5 min. Then, 150 μL 10% $AlCl*H_20$ was added, shaking the mixture in a vortex, allowing it to react for 6 min. Subsequently, 500 μL 1 M NaOH and 275 μL water were added, vortexing. The absorption was read in a UV spectrophotometer (Genesys 10) at a wavelength of 510 nm. To quantify the concentration, the standard curve (y= 0.0122x-0.0067; R2= 0.9653) prepared with quercetin was performed. The results were expressed in mg quercetin equivalents (QE) per 100 g based on fresh weight (mg QE/100 g FW). Analyzes were performed in triplicate.

The antioxidant capacity was determined *in vitro* using reagent 2,2-diphenyl-1-picrylhydrazyl DPPH taking account a solution of DPPH (Aldrich, St. Louis, Missouri, USA) in ethanol and adjusting the absorbance of the solution to 1.100 ± 0.010 at a wavelength of 515 nm. The antioxidant capacity was determined by mixing 50 μL of sample and 1950 μL of DPPH+ solution, and after 30 min of reaction, the absorbance of the mixture was read at 517 nm in a UV spectrophotometer (Genesys 10). Readings were taken in triplicate and ethanol was used as blank. The standard curve was prepared with trolox (Aldrich, St. Louis, Missouri, USA) and the results reported as equivalent antioxidant capacity in μM trolox equivalent per 100 g on a fresh weight basis (mg trolox equiv./100 g FW).

The chlorophyll content was carried out by the techniques of Nagata & Yamashita (1992). 10 mg of lyophilized tissue was weighed, added 2 mL of hexane:acetone (3:2 v:v) and vortexed. An aliquot was taken and the absorbance was measured at 645 and 663 nm in a UV-VIS spectrophotometer model UV-6300PC, subsequently the following equations were applied.

Chl a = 0.999 x Abs 663 - 0.0989 x Abs 645

Chl b = -0.328 x Abs 663 + 1.77 x Abs 645

For the peroxidase enzyme, the method described by Pascual *et al*. (1983) we used guaiacol and hydrogen peroxide as substrates. The assay conditions were 1.0 mL 0.01 M Tris (HCl), pH 7.0, 0.75 mL 100 mM guaiacol, 0.02 mL 30 mM H_20_2, 0.1 mL enzyme extract. Absorbance was measured in a Jenway® 7305 UV-visible spectrophotometer at 470 nm, in 1 cm light path cuvettes every 30 and 60 s at room temperature. It was reported as U/g of FW, where U = unit of enzyme activity and one unit equals the decomposition of 1 umol/min H_2O_2.

For catalase, enzyme was evaluated by the method described by Luck, cited by Blackman & Hardham (2008), using 3 mL of 10 mM Tris-HCl (pH 8.5) and 0.1 mL of 0.88% hydrogen peroxide in 100 mM Tris-HCl were placed in cells. The reaction is initiated by adding 0.1 mL of crude extract and the change in absorbance at 240 nm was observed and the enzyme activity was reported as U/g of FW. The assays were performed at room temperature.

The determination of Cu was carried out at the National Center for Disciplinary Research in Relation, Water, Soil, Plant, Atmosphere (CENID-RASPA), located in the city of Gomez Palacio, Durango, Mexico. The determination was by atomic absorption spectrophotometer (AOAC, 1990), the results were expressed in μg/kg dry weight (DW).

Statistical analysis

The data obtained from all the variables described above were subjected to the Shapiro Willk and Bartlett tests to test the normality and homogeneity of variance, respectively. Analysis of variance was performed to determine the differences between the treatments and Tukey's multiple test (P≤0.05) for the comparison of means

± standard deviation. The analyzes were carried out with the statistical software Statistical Analysis System 9.0.

RESULTS AND DISCUSSION

Morphological characteristics

The foliar application of NPs-CuO in the concentrations used did not show significant differences in crown diameter, lettuce leaf length and plant height (p>0.05). The concentration of 15 mg/L of NPs-CuO showed higher averages in crown size (43.90±3.04 cm) and leaf length (26.33±0.57 cm), being higher by 8.13 and 8.22% compared to the control treatment, respectively. (Table 1). These results are consistent with those reported for lettuce cv. Parris Island that were subjected to foliar NPs-CuO doses that fluctuated from 0.5 to 6 mg/L without significant difference in crown diameter and leaf length, finding that the concentration of 4 mg/L NPs-CuO (39.1±1.94 cm), the crown diameter increased by 11.72% in relation to the control treatment (0 mg/L of NPs-CuO) and the concentration 1 mg/L NPs-CuO with a value of 32.8±4.29 cm, a 34.43% increase in leaf length in relation to the control treatment (Gaucin-Delgado *et al*., 2022).

Table 1. Comparison of means for agronomic variables in lettuce plants (*Lactuca sativa*) produced with foliar application of NPs-CuO. Torreon, Mexico, Technological Institute of Torreon, 2022.

NPs-CuO (mg/L)	NH	AP	DC	LH	AH	Yield (g)
	(cm)					
Control	41.60±1.51c†	25.60±2.70	40.60±3.78	24.33±0.57	15.66±1.52b	808.33±3.51
5	43.60±1.94bc	30.40±3.28	42.20±1.82	25.33±1.52	17.66±1.15ab	810.66±2.08
10	46.00±2.54abc	29.40±2.60	43.50±2.17	24.66±1.15	17.33±0.57ab	811.66±3.51
15	50.80±1.92a	29.40±1.08	43.90±3.04	26.33±0.57	19.66±0.57a	814.00±2.00
20	48.80±4.81ab	30.00±1.58	42.60±1.67	25.66±1.52	17.33±1.15ab	811.33±3.51
25	47.60±2.88ab	28.90±3.24	42.30±2.10	26.00±1.00	17.00±1.00ab	811.33±3.51
LSD	5.5195	4.9936	4.9808	3.1001	2.8909	8.5023
Significance	*	NS	NS	NS	*	NS

†Values with the same letters within each column are statistically similar (Tukey, p≤0.05); NS= not significant; *= significant, NH= number of leaves, AP= plant height, DC= crown diameter, LH= leaf length, AH= leaf width, LSD=Less significant difference data are shown as mean ± standard deviation (n= 30).

The plants developed in the 5 mg/L treatment of NPs-CuO showed a higher average plant height (30.40±3.28 cm), registering an increase of 18.75%, compared to the control treatment (25.60±2.70 cm) (Table 1). Similar results were reported by Zafar *et al*. (2020) in the cultivation of black mustard (*Brassica nigra*) with applications of NPs-CuO at doses of 12.5, 25 and 50 mg/L that increased plant height in relation to the control. These last authors suggest that the increases in plant growth could be due to the nutritional behavior of the dissociated ions of the nanoparticles, which regulate the synthesis of phytohormones. Increases in the accumulation of metals such as Zn and Cu have a negative effect on biomass and plant growth, so it is important to evaluate concentrations of NPs-CuO without generating phytotoxicity in plants.

The increase in the growth of plant species due to the effect of NPs-CuO is due to the induction of activity in chloroplasts, synthesis of antioxidant enzymes and nitrate reductase, depending on the shape of the NPs, concentration, form of application, stage of the application and the plant species (Nekrasova *et al*., 2011). Another possible explanation is that Cu is an essential nutrient for plants and in an adequate quantity it is essential for the normal development and growth of plants. It plays crucial roles in photosynthetic and respiratory electron transport chains, in ethylene synthesis, cell wall metabolism, protection against oxidative stress, and biogenesis of the molybdenum cofactor (Yruela *et al*., 1996). For example, Hernández-Hernández *et al*. (2018) report that chitosan and NPs-Cu hydrogels improve tomato crop growth, in addition, they stimulated the expression of jasmonic acid and superoxide dismutase genes under saline stress, which suggests that they can mitigate abiotic stress (salinity) at through the regulation of oxidation and ionic stress. Therefore, low doses (<50 mg/L) of NPs-CuO stimulate the growth and development variables of the lettuce crop in hydroponic systems (Liu *et al*., 2016). NPs-Cu exhibit antimicrobial properties due to their small size and ability to release ions. They can be used as a broad-spectrum antimicrobial agent to protect agricultural crops against pests and diseases due to their multifaceted action against various microorganisms. The small size of NPs-Cu facilitates their absorption by plants, improving their biological efficacy, thus avoiding danger due to excessive use and runoff (Bakshi & Kumar, 2021).

Yield

The foliar application of NPs-CuO in the concentrations used presented highly significant differences in the number of leaves and leaf width (p≤0.01). The treatment with the dose of 15 mg/L reported the highest number of leaves with an average of 50.80±1.92, being 22.1% higher than the control treatment (Table 1). The number of leaves registered in the treatments with the concentrations of NPs-CuO was higher than the average of 32 leaves in lettuce plants cv. Vanda developed with foliar application of 20 mg/L NPs-Cu

(Kohatsu *et al*., 2021). Foliar applications at low concentrations ranging from 10 to 20 mg/L NPs-CuO promote an increase in the number of leaves and improve the resistance of the *Allium cepa* cv. Giza 20 (Mottaleb *et al*., 2021). High doses of NPs-CuO (100 and 1000 mg/L) via foliar application in lettuce cv. Ramosa Hort decrease the number and growth of leaves, due to a high accumulation of Cu on the leaf surface, causing phytotoxicity and altering the nutritional status of plants, especially in nutrients such as Mn, K and Ca (Xiong *et al*., 2021).

In the present study, the 15 mg/L dose registered the greatest leaf width with an average of 19.66±0.57 cm, resulting in 25.5% higher than the control treatment (Table 1). Similarly, Zafar *et al*. (2020) reported that NPs-CuO applications at concentrations of 50 mg/L in black mustard crops increase leaf width. The applications of NPs-Cu (0.02, 0.2, 2 and 10 mg applied to the substrate) increase the number of leaves, fresh weight of the stem and fresh weight of the root, number of fruits in tomato plants, so it could be used as stimulants of growth, because NPs-Cu improve photosynthesis by increasing the electron transport chain and phosphorylation during the light reaction, in the same way, increasing enzymatic activity in the dark phase and participating in carbon and nitrogen metabolism (Hernández-Hernández *et al*., 2018).

With respect to yield, the statistical model indicated that this variable did not present a significant difference due to the effect of foliar application of NPs-CuO in hydroponic lettuce ($p>0.05$), however, yield was not affected by NPs-phytotoxicity CuO in plants (Table 1). The doses used in this work do not exceed the doses of NPs-Cu to generate phytotoxicity in lettuce crops, however, the toxicity thresholds vary between species, therefore the doses applied in this study are within the optimal ranges. Therefore, the hypothesis that the application of NPs-Cu at low doses favors the development and growth of crops without causing phytotoxicity is strengthened. It has been reported that doses of 1000 mg NPs-Cu are toxic in zucchini (*Cucumis pepo*) crops, causing a 77% reduction in root length, causing a 90% reduction in plant biomass in relation to the control plants (Stampoulis *et al*., 2009). Similarly, the application of 500 mg/L of NPs-CuO in the zucchini culture showed a 2 to 5-fold higher Cu uptake, suggesting a complete deregulation of Cu uptake (Pagano *et al*., 2017). In addition to the above, high doses of NPs-CuO reduce the growth and productivity of agricultural crops, because they increase the production of free radicals causing oxidative stress and low doses increase yields since they prevent oxidative stress, improving the capacity of plant species to resist cellular oxidation caused by reactive oxygen species under stress conditions (Gaucin-Delgado *et al*., 2022).

Similarly, it has been shown that both silver nanoparticles (NPs-Ag) and NPs-Cu have antifungal activity with respect to pathogens that cause damping off, the benefits of these nanoparticles are sufficient to be useful as alternatives for chemical prevention or control and thus improve the growth and yields of agricultural crops, therefore contributing to reduce the impact on the environment (Aleksandrowicz-Trzci´nska *et al*., 2018). It is documented that hydrogels integrated in NPs-CuO improve the uptake of P, Mn, Zn and Mg and increase the levels of organic acids compared to the control of diseased plants with *Fusarium oxysporum* f. sp. lactucae. Increasing these organic acids (salicylic acid) and decreasing levels of jasmonic acid and abscisic acid with the addition of different forms of Cu can improve resistance to fungal diseases (Shang *et al*., 2021).

Phytochemical quality

Lettuce is the most important vegetable in the group of leafy vegetables and its main use is as a fresh vegetable in salads. Lettuce is low in calories, fat, sodium, minerals, and fiber, as well as bioactive compounds such as folates, beta carotene, lutein and phenols. In our results, the foliar application of NPs-CuO in the concentrations used showed highly significant differences in the content of total phenols and total flavonoids ($p\leq0.01$) (Figure 1), behavior that coincides with what was established by Surjit *et al*. (2018), who with foliar applications of NPs-CuO of 250 mg/L increased the concentration of phenolic compounds by 5.43%, likewise, 50 mg/L NPs-CuO increased by 36.14% the concentration of total flavonoids in fruits of tomato, compared to the control. In the same way, it has been reported that the foliar spraying of NPs-CuO increases the contents of phenols and flavonoids in leaves and fruits of tomato crops subjected to saline stress. These increases are favorable since the production of these bioactives could be a feasible strategy to mitigate the damage caused by saline stress (Surjit *et al*., 2018).

Phenolic compounds, ascorbic acid, glutathione, non-protein amino acids and a-tocopherols are important up-regulated low molecular weight non-enzymatic antioxidants that can serve as free radical scavengers to protect plants from oxidative damage (Surjit *et al*., 2018). Cu generates Reactive Oxygen Species (ROS) in cells through the Fenton reaction. ROS sequestering enzymes and antioxidant molecules are a plant defense mechanism to ROS stress. It is possible that the biosynthesis of these metabolites is activated in response to Cu-induced ROS stress (Zhao *et al*., 2020). Polyphenols (phenolic compounds and flavonoids) have received attention from researchers and consumers due to their potential medicinal properties. Experimental results both *in vitro* and *in vivo* have provided information to support the possible role of polyphenols in the human diet in preventing and curing various degenerative diseases, in particular cancer, cardiovascular diseases, and neurodegenerative diseases (Surjit *et al*., 2018).

In addition to the above, nanoparticle-containing essential elements are widely used in agricultural systems due to their specific physicochemical properties, including their high catalytic capacities, ability to modify electron exchange, and high surface area/volume ratio. They can be very effective than classical fertilizers (Najafi *et al*., 2020). Among the physiological processes improved by NPs-Cu nanoparticles in plants are: photosynthetic rate, stability of the thylakoid membrane, stomatal conductance, stress mitigation, antioxidant system (enzymatic and non-enzymatic), gene regulation for growth, nutrient assimilation, (enzymatic and non-enzymatic homeostasis, stress tolerance antioxidant production) (Ahmad *et al*., 2021).

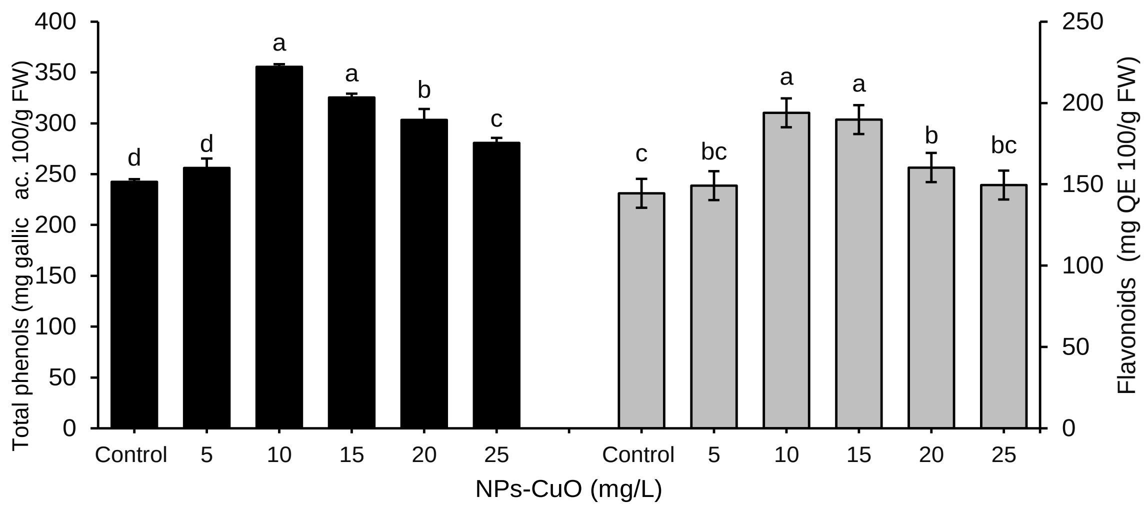

Figure 1. Effect of foliar application of NPs-CuO on the content of phenols (left) and flavonoids (right) in lettuce plants produced in an NFT system. All data values represent means ± standard deviation (n= 30). Bars with different letters are statistically different according to the Tukey test ($p \leq 0.05$). Torreon, Mexico, Technological Institute of Torreon, 2022.

In Figure 2, it can be seen that the peroxidase enzyme varied due to the effect of foliar application of NPs-CuO ($p \leq 0.01$). The concentration of 15 mg/L of NPs-CuO showed higher averages in the peroxidase content (1.08±0.14 µm/L). These results confirm that the production of enzymatic antioxidants is significantly affected in plants treated with NPs-CuO. These results agree with those obtained by Gaucin-Delgado *et al*. (2022) who observed that in lettuce plants developed with foliar application of NPs-CuO the glutathione peroxidase enzyme increased by 125%, compared to the control. Similarly, it has been reported that foliar application of NPs-Cu in soybean seedlings (*Glycine max*) with concentrations ranging from 100, 200, 400 and 500 mg/L increased the level of hydrogen peroxide and the activity of the peroxidase enzyme. Peroxidases represent a family of isozymes that actively participate in the oxidation of ROS. Plants possess very efficient enzymes (superoxide dismutase, catalase, glutathione reductase, monodehydroascorbate reductase, glutathione peroxidase, glutathione-S-transferase) which are antioxidant defense systems that work together to stop uncontrolled oxidation cascades and protect plant cells from oxidative damage by elimination of ROS.

In the catalase enzyme, there was no statistical difference ($p>0.05$) due to the effect of the evaluated treatments. However, with 15 mg/L NPs-CuO the highest concentration of catalase was recorded (1.02±0.03 µg/mL) (Figure 2). Similar results were found in evaluating NPs-Cu in tomato (10, 50 and 250 mg/L) (Hernández-Hernández *et al*., 2018) and lettuce (5, 10, 20 mg/L (Hong *et al*., 2015) in which no significant differences were found in the concentrations of the catalase enzyme. Cu acts as a protein catalytic center in plant cell metabolism and is capable of regulating the activities of the enzymes superoxide dismutase, peroxidase, catalase, phenylalanine ammonia lyase, and ascorbate peroxidase, which play an important role as ROS scavengers and form the first line of defense against oxidative stress (Hernández-Hernández *et al*., 2018). This allows us to assume that the foliar application of NPs-CuO promotes the increase of antioxidants, which is desirable since higher levels of antioxidants improve the resistance of plant species to stress caused by biotic and abiotic factors.

In the case of antioxidant capacity, a highly significant difference was shown due to the effect of NPs-CuO in lettuce ($p \leq 0.01$). The concentrations of 10 and 15 mg/L NPs-CuO were statistically equal, in addition, they registered the highest values of antioxidant capacity with values of

106.44±2.25 and 101.07±1.14 mg equiv. Trolox/100 g FW, resulting in 28.23 and 21.76% superior to the control treatment (82.01 mg equiv. Trolox/100 g FW), respectively (Figure 2). Applications of 3.6, 10 and 25 mg/L NPs-CuO significantly increase the antioxidant capacity in melon (*Cucumis melo*) fruits and tomato and moringa (*Moringa oleifera*) leaves, respectively. This could be due to the fact that NPs-CuO generate a low level of stress in plants, which results in an increase in ROS, which in turn activates the antioxidant defense mechanism (enzymatic and non-enzymatic antioxidants) that result in the production of a series of enzymatic antioxidant compounds (Hernández-Hernández *et al*., 2018; Fortis-Hernández *et al*., 2022). The results of this work and those described above suggest that NPs-CuO could be a viable alternative to obtain leaves and/or fruits with higher contents of antioxidant compounds and greater antioxidant capacity in plant tissues, which is extremely important to contribute in disease prevention thus contributing to public health.

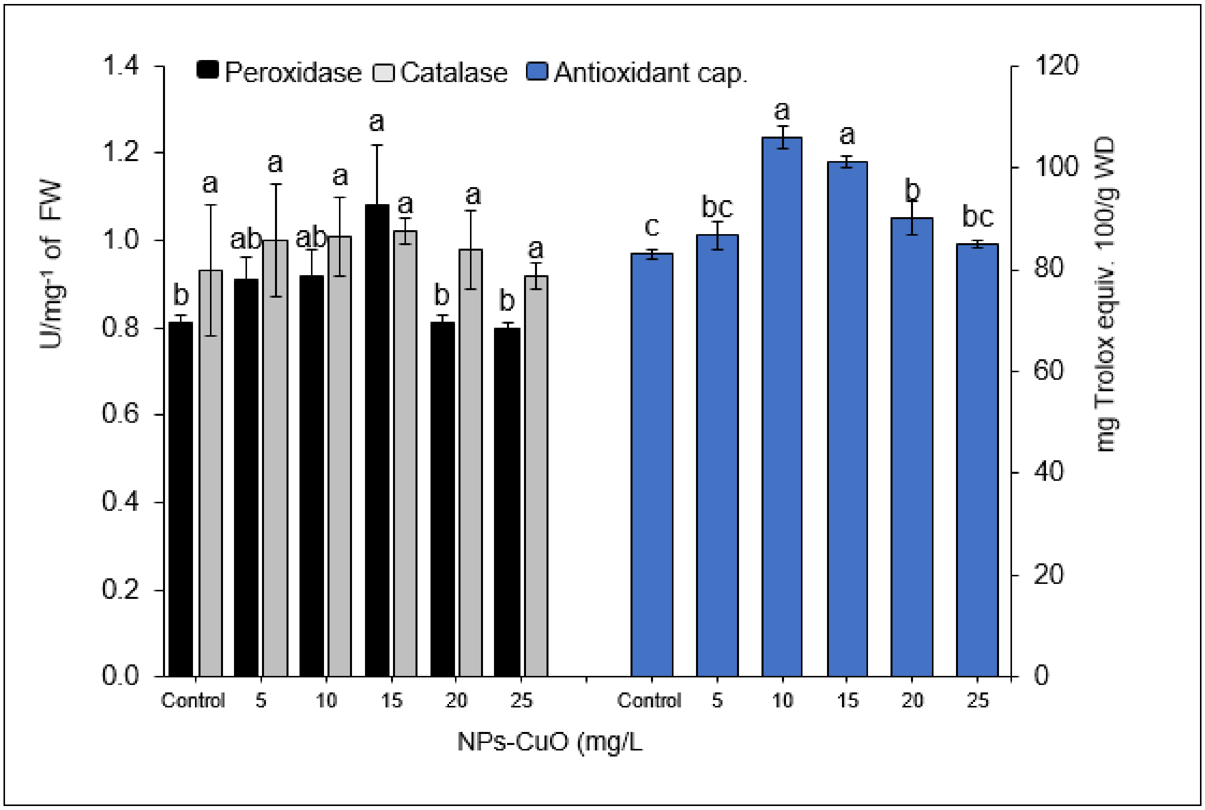

Figure 2. Effect of foliar application of NPs-CuO on peroxidase and catalase content (left) and antioxidant capacity (right) in lettuce plants produced in an NFT system. All data values represent means ± standard deviation (n= 30). Bars with different letters are statistically different according to the Tukey test (p≤0.05). Torreon, Mexico, Technological Institute of Torreon, 2022.

The chlorophyll content of the lettuce leaf also varied significantly due to the effect of the NPs-CuO (p≤0.01). The treatments with 10 and 15 mg/L NPs-CuO were statistically equal, in addition, showing increases in the concentration of chlorophyll with values of 9.94±0.10 and 9.88±0.79 mg/100 g, resulting in 39.41 and 38.38% higher than the control treatment (7.14 mg/100 g), respectively (Figure 3). The improvement in photosynthetic efficiency has been associated with greater accumulation of biomass. Tamez *et al*. (2019) in sugarcane (*Saccharum officinarum*) using increasing doses of NPs-Cu (20, 40 and 60 mg/L), found that the chlorophyll content increased significantly when applying 20 mg/L. NPs-CuO increase the content of photosynthetic pigments in lettuce and cucumber leaves, therefore, an increase in photosynthesis is reflected. Chlorophyll increases could be due to the protection provided by metallic NPs, which may be caused by antioxidant agents in the chloroplast membrane. This response depends on the doses used since NPs-CuO can induce negative responses to certain doses and plant species, while in others they can induce the opposite effect or have no effect (Gaucin-Delgado *et al*., 2022). As described, the application of NPs-CuO decreases total chlorophyll in duckweed (*Lemma* spp.) and aquatic weeds (*Landoltia punctata*) (Nekrasova *et al*., 2011; Shi *et al*., 2011). This behavior, called hormesis (what does not kill, strengthens), has been reported when metallic nanoparticles are applied as biostimulants in crops. The favorable effects of NPs-CuO on lettuce can be attributed to its modification in the photosynthesis system of plants with a higher rate of transpiration and higher stomatal density conductance (Kranjc & Drobne, 2019).

The results indicate that the foliar application of NPs-CuO did not significantly affect the Cu content in lettuce leaves produced in the NFT system (Figure 3). Obtaining the highest Cu content in lettuce treated with 20 mg/L, with an average of 9.93 µg/kg DW, surpassing the rest of the treatments. These results can be attributed to the fact that the Cu concentrations used in this research were low, therefore no variation was found in the Cu content in the leaf. The increase of Cu in the leaf is important since it is part of the plastocyanin in the chloroplasts, which helps the transfer of electrons in the lumen of the thylakoids, which is vital to mediate photosynthesis in plants (Zafar *et al*., 2020). However, the results of the present study differ from Xiong *et al*. (2021), who reported that the foliar uptake, biotransformation and effects of NPs-CuO in *Lactuca sativa* var. Ramosa, where the copper content in the leaves and root of the plant increased with the foliar application of concentrations 100 and 1000 mg/L, which can be toxic for human consumption. Wang *et al*. (2019) have reported that concentrations ranging from 200 to 400 mg/L respectively increase the accumulation of Cu in the roots, which can be toxic when finding remains in shoots and leaves, a high concentration of Cu can cause a type of stress in the plant and affect its physiological processes. The results obtained in this experiment suggest that low doses <15 mg/L of NPs-CuO allow improving the growth walls and quality of lettuce leaves, increasing the concentration of Cu in the lettuce leaf without causing phytotoxicity to the plant. The increase of Cu in the leaf could be a viable alternative to increase the nutraceutical quality of the edible part of the lettuce, thus contributing of Cu in the human diet. The application of NPs-CuO in concentrations lower than 15 mg/L improved all biochemical and physiological traits of lettuce plants, such as growth, enzymatic, and antioxidant parameters. The use of copper nanoparticles could be an effective alternative to enrich lettuce leaves and, therefore, mitigate copper deficiency in the diet of the human population.

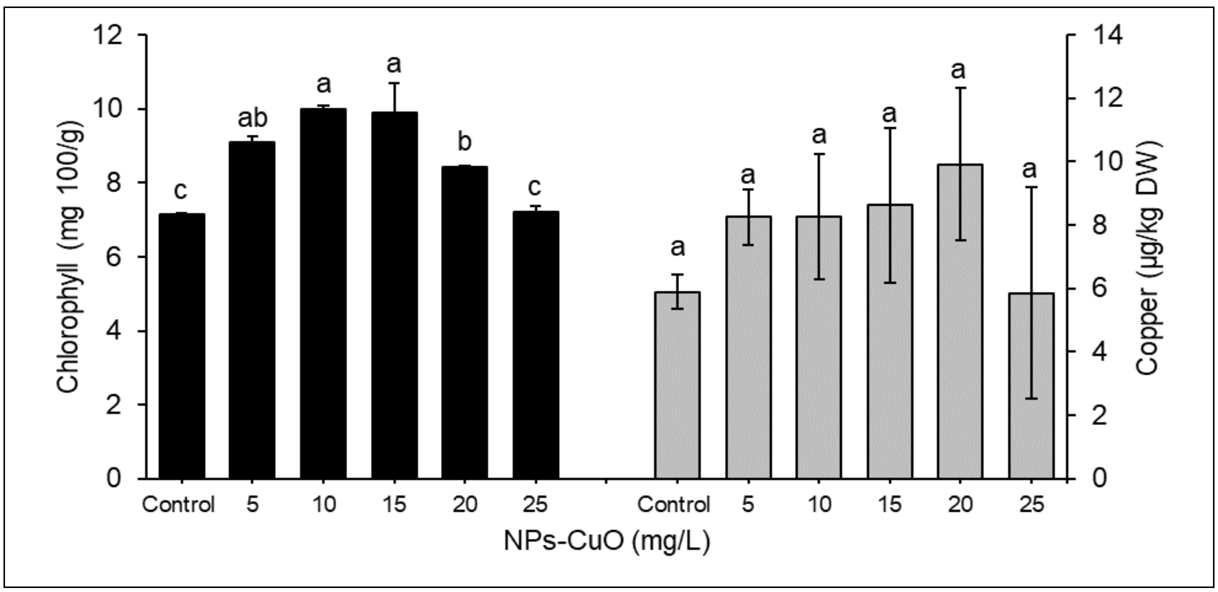

Figure 3. Effect of foliar application of NPs-CuO on chlorophyll (left) and copper (right) content in lettuce plants produced in an NFT system. All data values represent means ± standard deviation (n= 30). Bars with different letters are statistically different according to the Tukey test (p≤0.05). Torreon, Mexico, Technological Institute of Torreon, 2022.

REFERENCES

AHMAD, ASS; HASHMI, JM; PALMA, YFJ; CORPAS. 2021. Influence of metallic, metallic oxide, and organic nanoparticles on plant physiology. *Chemosphere*: 133329.

ALEKSANDROWICZ-TRZCI´NSKA, MA; SZANIAWSKI, J; OLCHOWIK, YS; DROZDOWSKI. 2018. Effects of copper and silver nanoparticles on growth of selected species of pathogenic and wood-decay fungi *in vitro*. *The Forestry Chronicle* 93: 109-118.

AOAC. 1990. Official methods of analysis of the association of official analytical chemists. p1023, Arlington, Virginia, USA.

BAKSHI, M; KUMAR, A. 2021. Copper-based nanoparticles in the soil-plant environment: Assessing their applications, interactions, fate and toxicity. *Chemosphere* 281: 130940.

BLACKMAN, LM; HARDHAM, AR. 2008. Regulation of catalase activity and gene expression during *Phytophthora nicotianae* development and infection of tobacco. *Molecular Plant Pathology* 9:495-510. doi: 10.1111/j.1364-3703.2008.00478.x.

ELEMIKE, E; UZOH, I; ONWUDIWE, D; BABALOLA, O. 2019. The role of nanotechnology in the fortification of plant nutrients and improvement of crop production. *Applied Sciences* 9.

FORTIS-HERNÁNDEZ, M; ORTIZ-LOPEZ, J; PRECIADO-RANGEL, P; TREJO-VALENCIA, R. LAGUNES-FORTIZ, E; ANDRADE-SIFUENTES, A; RUEDA-PUENTE, EO. 2022. Biofortification with copper nanoparticles (Nps Cu) and its effect on the physical and nutraceutical quality of hydroponic melon fruits. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 50.

GAUCIN-DELGADO, JM; ORTIZ-CAMPOS, A; HERNANDEZ-MONTIEL, L; FORTIS-HERNÁNDEZ, GM; REYES-PEREZ, JJ; GONZALES-FUENTES, JA; PRECIADO-RANGEL, P. 2022. CuO-NPs improve biosynthesis of bioactive compounds in lettuce. *Plants* 11(7).

HERNÁNDEZ-HERNÁNDEZ, H; JUÁREZ-MALDONADO, A; BENAVIDES-MENDOZA, A; ORTEGA-ORTIZ, H; CADENAS-PLIEGO, G; SÁNCHEZ-ASPEYTIA, D; GONZÁLEZ-MORALES, S. 2018. Chitosan-PVA and copper nanoparticles improve growth and overexpress the SOD and JA genes in tomato plants under salt stress. *Agronomy* 8(9).

HONG, J; CYREN, M; ZHAO, L; ADEYEMI, S; KELLER, A; PERALTA, V; GARDEA, J. 2015. Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). *Environment Science* 177-185. 10.1039/C4EM00551A

JAMPÍLEK, J; KRÁLOVÁ, K. 2019. Beneficial effect of metal and metalloid-based nanoparticles on crop production. In: PANPATTE, GD; JHALA, YK (eds). *Nanotecnhology for agriculture*. Advances for sustainable agriculure. Springer Nature Singapore

JUÁREZ-MALDONADO, AH; ORTEGA-ORTÍZ, A; MORALES-DÍAZ, B; GONZÁLEZ-MORALES, S; MORELOS-MORENO, A; CABRERA-DE LA FUENTE, M; SANDOVAL-RANGEL, A; CADENAS-PLIEGO, G; BENAVIDES-MENDOZA, A. 2019. Nanoparticles and nanomaterials as plant biostimulants. *International Journal of Molecular Science* 20: 62 doi:10.3390/ijms20010162.

KOHATSU, MY; LANGE, VM; PELEGRINO, T; PIERETTI, JC; TORTELLA, G; RUBILAR, O; BATISTA, BL; SEABRA, AB; JESUS, TAD. 2021. Foliar spraying of biogenic CuO nanoparticles protects the defence system and photosynthetic pigments of lettuce (*Lactuca sativa*). *Journal of Cleaner Production* 324.

KRANJC, E; DROBNE, D. 2019. Nanomaterials in plants: A review of hazard and applications in the agri-food sector. *Nanomaterials* 9: 1094. https://doi.org/10.3390/nano9081094

LIU, R; ZHANG, H; LAL, YR. 2016. Effects of stabilized nanoparticles of copper, zinc, manganese, and iron oxides in low concentrations on lettuce (*Lactuca sativa*) seed germination: nanotoxicants or nanonutrients? *Water, Air, & Soil Pollution* 227(1).

MOTTALEB, SA; HASSAN, AZA; EL-BAHBOHY, R; MAHMOUD, AWM. 2021. Are copper nanoparticles toxic to all plants? A case study on onion (*Allium cepa* L.). *Agronomy* 11(5).

NAGATA, M; YAMASHITA, I. 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruits *J. Jpm. Soc. Food Sci. Technol.* 39: 925-928.

NAJAFI, F; GAMALELDIN, F; ROBIN, C; EFTYCHIOS, P; LATHAM, E; CUNNINGHAM, P; CHURCHLAND, K. 2020. Excitatory and inhibitory subnetworks are equally selective during decision-making and emerge simultaneously during learning. *Neuron* 105: 165-179. https://doi.org/10.1016/j.neuron.2019.09.045

NEKRASOVA, GF; USHAKOVA, OS; ERMAKOV, AE; UIMIN, MA; BYZOV, IV. 2011. Effects of copper (II) ions and copper oxide nanoparticles on *Elodea densa* Planch. *Russian Journal of Ecology* 42: 458-463.

ONSA, GH; SAARI, N; SELAMAT, J; BAKAR, J. 2004. Purification and characterization of membrane-bound peroxidases from metroxylon sagu. *Food Chemistry* 85: 365-376. https://doi.org/10.1016/J.FOODCHEM.2003.07.013.

PAGANO, L; PASQUALI, F; MAJUMDAR, S; TORRE-ROCHE, R; ZUVERZA-MENA, N; VILLANI, M; ZAPPETTINI, A; MARRA, RE; ISCH, SM; MARMIROLI, M; MAESTRI, E; DHANKHER, OP; WHITE, JC; MARMIROLI, N. 2017. Exposure of *Cucurbita pepo* to binary combinations of engineered nanomaterials: physiological and molecular response. *Environmental Science: Nano* 4: 1579-1590.

PASCUAL, MR; PEREDA, CM; PÉREZ, R. 1983 citado por MÁRQUEZ, M. 1997 em: Purificación de peroxidasas de rábano picante *Armoracia rusticana*. Universidad de la Habana. Cuba.

PRIYANKA, N; GEETHA, N; GHORBANPOUR, M; VENKATACHALAM, P. 2019. Role of engineered zinc and copper oxide nanoparticles in promoting plant growth and yield: Present status and future prospects. *Advances in Phytonanotechnology*. p.183-201.

SHANG, HC; LI, JMA; ZHAO, C; ELMER, W; WHITE, JC; XING, B. 2021. Copper oxide nanoparticle-embedded hydrogels enhance nutrient supply and growth of lettuce (*Lactuca sativa*) infected with Fusarium oxysporum f. sp. lactucae. *Environ. Sci. Technol*. 55: 13432-13442.

SHI, J; AAMIR, D; ABID, M; KENNEDY, M; KRASSIMIRA, R; HRISTOVA, W; SILK, YK. 2011. To duckweeds (*Landoltia punctata*), nanoparticulate copper oxide is more inhibitory than the soluble copper in the bulk solution. *Environmental Pollution* 159: 1277-1282. https://doi.org/10.1016/j.envpol.2011.01.028.

SIAP. 2020. *Servicio de información agroalimentaria y pesquera.* Anuario Estadístico de Producción Agrícola. http://www.siap.gob.mx/ Accessed July 12, 2022.

STAMPOULIS, D; SINHA, SK; WHITE, JC. 2009. Assay-dependent phytotoxicity of nanoparticles to plants. *Eviron Sci Technol* 43: 9473-9479.

SURJIT, SO; CHANDRA, PN; LAISHRAM, L; TEWARI, M; DHOUNDIYAL, R; JOSHI, K; PANDEY, YCS. 2018. Effect of CuO nanoparticles on polyphenols content and antioxidant activity in Ashwagandha (*Withania somnifera* L. Dunal). *Journal of Pharmacognosy and Phytochemistry* 7: 3433-3439.

TAMEZ, C; MORELIUS, E; HERNANDEZ, V; PERALTA, V; GARDEA, J. 2019. Biochemical and physiological effects of copper compounds/nanoparticles on sugarcane (*Saccharum officinarum*). *Science of the Total Environment* 649: 554-562.

WANG, Y; LIN, Y; XU, Y; YIN, Y; GUO, H; DU. W. 2019. Divergence in response of lettuce (var. ramosa Hort.) to copper oxide nanoparticles/microparticles as potential agricultural fertilizer. *Environmental Pollutants and Bioavailability* 31: 80-84.

XIONG, T; ZHANG, T; XIAN, Y; KANG, Z; ZHANG, S; DUMAT, C; SHAHID, M; LI, S. 2021. Foliar uptake, biotransformation, and impact of CuO nanoparticles in *Lactuca sativa* L. var. ramosa *Hort*. *Environ Geochem Health* 43: 423-439.

YRUELA, I; PUEYO, J; ALONSO, P; PICOREL, R. 1996. Photoinhibition of photosystem II from higher plants: effect of copper inhibition. *Journal of Biological Chemistry* 271: 27408-27415.

ZAFAR, H; AZIZ, T; KHAN, B; MANNAN, A; REHMAN, RU; ZIA, M. 2020. CuO and ZnO nanoparticle application in synthetic soil modulates morphology, nutritional contents, and metal analysis of *Brassica nigra*. *ACS Omega* 5: 13566-13577.

ZHAO, L; LU, L; WANG, A; ZHANG, H; HUANG, M; WU, H; XING, B; WANG, Z; JI, YR. 2020. Nano-biotechnology in agriculture: Use of nanomaterials to promote plant growth and stress tolerance. *J Agric Food Chem* 68: 1935-1947.

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