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Research Article

Copper oxide nanoparticles biosynthetized improve germination and bioactive compounds in wheat sprouts

Hortensia ORTEGA-ORTIZ[1], Jazmín M. GAUCIN-DELGADO[2*], Pablo PRECIADO-RANGEL[2*], Manuel FORTIS-HERNANDEZ[2], Luis G. HERNANDEZ-MONTIEL[3], Efraín DE La CRUZ-LAZARO[4], Liliana LARA-CAPISTRAN[5]

[1]*Applied Chemistry Research Center, Department of Advanced Materials, Enrique Reyna H. No. 140, San José of the Cerritos, 25294, Saltillo, Coahuila, Mexico; hortensia.ortega@ciqa.edu.mx*
[2]*National Technological Institute of Mexico/ Torreon Technological Institute, Carretera Torreón-San Pedro km 7.5, 27170, Torreón, Coahuila, México; jazmontse@hotmail.com (*corresponding author); ppreciador@yahoo.com.mx (*corresponding author); fortismanuel@hotmail.com*
[3]*Biological Research Center of the Northwest, Av. Politecnico Nacional 195, Col. Playa Palo Santa Rita, 23090, La Paz, Baja California Sur, Mexico; lhernandez@cibnor.mx*
[4]*Academic Division of Agricultural Sciences-Juarez Autonomous University of Tabasco, Km. 25, Villahermosa-Teapa Highway, Ra. The Huasteca, 2nd. Section. Center, Tabasco 86280, Mexico; efrain.delacruz@ujat.mx*
[5]*Veracruzana University-Xalapa Campus, Gonzalo Aguirre Beltrán University Circuit s/n, university area, Xalapa, Veracruz, Mexico; llaracapistran@gmail.com*

Abstract

Metal nanoparticles have many positive effects in improving crop production and productivity and allow for increased germination and rapid crop establishment under field conditions. The metallic nanoparticles applied in this study were copper oxide nanoparticles (CuONPs) biosynthesized using orange peel (*Citrus X sinensis*) as a reducing agent to avoid or reduce toxicity in wheat seeds and sprouts. This study determined the effect of CuONPs on germination, radicle and plumule length, as well as the production of phytochemical compounds in wheat sprouts. The seeds were treated with suspensions of CuONPs at the following concentrations: 0, 0.5, 1, 2, 4 and 6 mg mL^{-1}. The results indicate that the use of low doses of CuONPs (0.5 mg mL^{-1}), improved germination, vigor, plumule and radicle length, in addition to increasing the biosynthesis of phytochemical compounds in wheat shoots. A high concentration of CuONPs (6 mg mL^{-1}) causes inhibitory effects due to Cu accumulation and phytotoxicity in plant tissue. The use of CuONPs for green synthesis is a viable alternative to obtain beneficial effects in germination and seedling development, as well as greater secondary metabolite production.

Keywords: antioxidants; biosynthesized; CuONPs; *Triticum aestivum* L.

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Introduction

Nanotechnology is revolutionizing agriculture by producing agriproducts, such as nanopesticides, nanoherbicides and nanofertilizers that reduce environmental impact and increase crop yield (Kumar and Pandey, 2017; Younes *et al.*, 2020). Among the main nanoproducts, metallic nanoparticles (NPs) have been the most used in producing nanofertilizers (Kolesnikov *et al.*, 2019).

Metallic NPs increase seed germination in diverse plant species (Joshi *et al.*, 2020) since they induce a greater synthesis of antioxidant compounds, such as enzymatic superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPx) and non-enzymatic phenols, carotenoids, glutathione, and vitamins. These compounds increase greater bioactivity (germination and content of phytochemical compounds) in seeds of *Sinapis alba* (Rajput *et al.*, 2018), *Lactuca sativa* L *(Pelegrino et al., 2020), Medicago sativa* (Hong *et al.*, 2015), *Eleusine coracana Gaertn* (Sathiyabama and Manikandan, 2018), *Ipomea batatas* (Bonilla-Bird *et al.*, 2020), among others.

Antioxidant compounds are responsible for neutralizing and transforming toxic free radicals in non-harmful substances to maintain cellular homeostasis (Al-Hakkani, 2020). However, high doses of NPs of Ag, Ni, Zn, Ce, Ti, among other, have cytotoxic and genotoxic effects, decreasing plant germination and growth (Antisari *et al.*, 2018).

Among the metallic NPs, those that stand out are copper (CuONPs) because they show antimicrobial and antiviral effects (Bridley *et al.*, 2020). Their application in agriculture has demonstrated to have positive effects in plants because of their stability (Mali *et al.*, 2019) and low concentrations used (Hernández-Hernández *et al.*, 2019), besides showing they improve content of bioactive compounds (Lopez-Lima *et al.*, 2020).

Moreover, wheat (*Triticum aestivum* L.) is one of the most important cereals at world level since it occupies a predominant role in total cereal production and global food security (Faraji *et al.*, 2018). To assure an adequate productivity of this crop, high quality seed is required for an adequate germination since this stage is one of the most sensitive to adverse conditions and has an influence directly on crop yield (Victorava and Feoktistova, 2018). Therefore, the objective of this study is to assess the effect of CuONPs on wheat germination, growth, and phytochemical compounds.

Materials and Methods

Study site and experimental conditions

This study was performed in a biotechnology laboratory located in Institute Technologic, Torreon, Mexico at 24°30'27" N latitude, 102°104'40" W longitude at 1120 m.a.s.l.

Synthesis of copper oxide nanoparticles (CuONPs)

The CuONPs synthesis was performed following the procedure described by Markova *et al.,* (2010) and Taghavi *et al.* (2018), using copper salt $CuSO_4 \bullet 5H_20$ at 100 mM (>99.8%, Fermont®), aqueous solution and orange (*Citrus* × *sinensis*) peel as a reducing agent in a ratio of 4:1 (v/w). The mixture was maintained stirring at 250 rpm at 25°C for 24 h in a 4 L glass reactor on a heating blanket (EVELSA®). The activation of the chemical reaction was by colour change from emerald-green to dark green. Subsequently, the extract was vacuum filtered with Whatman #1 and #40, and heating was applied at 100 °C until solid CuONPs were obtained. Once the CuONPs synthesis was performed, the product was characterized by dynamic light scattering (DLS), X-Rays (XRD), Fourier-transform infrared (FTIR) spectroscopy by attenuated total reflection (ATR) and ultraviolet-visible (UV-vis) spectroscopy (synthesis and characterization were replicated five times).

Characterizing copper oxide nanoparticles by dynamic light scattering (DLS)

The sample was prepared dispersing 1 mg CuONPs in 5 mL of deionized H_2O (Items S.A de C.V., MX) adding one drop of Tween® 80 (Merck, S.A de C.V, MX) at 1%. This technique was used to characterize nanoparticle size and distribution inferior to 1 nm with the particle size analyzer (Microtrac®, Nanotrac Wave II Q).

Characterizing copper oxide nanoparticles by fourier-transform infrared by ATR

This study measured spectra in the region 4000-500 cm^{-1} on an infrared spectrometer 550 FTIR (Nicolet®) using KBr powder. A spectrum was taken as the average of 160 scans to increase the signal of the relationship noise and 4 cm^{-1} spectral resolution and band frequency precision at 0.01 cm^{-1}. Peak absorption intensities in this study were determined using the reference method (Vijilvani *et al.*, 2019). The varieties in frequencies and band areas were determined with precision from the corrected spectrum from the original base line up to the corresponding control and treated samples. To detect absorption band intensities at the corresponding functional group concentration (Izumi *et al.*, 2015), all spectral manipulation was performed with the software 8.0 for Windows (OMNIC Specta).

Characterizing copper oxide nanoparticles by x-ray diffraction (XRD)

The x-ray diffraction analysis was performed in a diffractometer (SIEMENS® D-500) using CuKα (25 mA, 35 kV) radiation, operating at a 40 kV and 40 mA at room temperature in a range of 2θ from 10° to 80° and a speed of 0.02 °/s. This technique was used to identify the crystalline stage and approximate size of the biosynthesized CuONPs. The calculus to obtain average particle size was performed with the software Maud following Halder-Wagner's (Santos Filho *et al.*, 2019) method.

Characterizing copper oxide nanoparticles by ultraviolet-visible spectroscopy

Absorbance of CuONPs solution was recorded using an UV-vis spectrophotometer (Shimadzu®, UV-2401PC) in a wavelength range of 190 nm at 500 nm. The sample was prepared by dispersing 1 mg CuONPs in 5 mL of deionized H_2O (Items S.A de C.V., MX), adding one drop of Tween® 80 (Merck®) at 1%. This technique was used to identify transition metal ions in the solution.

Treatments

Copper oxide nanoparticles were dispersed in 50 mL sterile deionized H_2O suspension; 0.5 mL of Agrex® (Agroenzimas S.A de C.V., MX) and Tween® 80 (Merck®) at 1% were added to the CuONPs solution with a concentration of 100 mg and stirred for 30 min in a shaker vortex (Fisher Scientific®, Vortex Genie 2). Subsequently, 950 mL of sterile deionized H_2O were added, complementing the solution with 0.5 mL Agrex® (Agroenzimas S.A de C.V., MX) and 0.5 mL of Tween at 1% to a 1 L volume and stopped shaking at 12 h to avoid adding the dispersed particles (Chung *et al.*, 2019). The CuONPs resulting suspension was diluted in distilled water and adjusted to concentrations 0, 0.5, 1, 2, 4, and 6 mg mL^{-1} (Li *et al.*, 2019).

Plant material

Wheat (*Triticum aestivum* L.) seeds -donated by the Cereal Program of Universidad Autónoma Agraria Antonio Narro were used with 96% germination, disinfected with ethanol solution at 75% for 5 min and washed four times with sterile distilled water (Li *et al.*, 2019).

Germination and growth parameter assay

Seeds were placed in six batches with 10 seeds each (n = 60). The treatments were applied only once to the seed by inhibition using 50 mL of each CuONPs concentration. Seeds were placed within an artificial growth chamber (Labnet International Inc.®, 211DS) at a temperature of 25 °C ± 2 °C, respectively, with 60% relative humidity for 8 h in darkness (Salas-Pérez *et al.*, 2016).

Once the inhibition period was completed, 10 seeds were deposited on Whatman #1 (Productos quimicos del sur S.A. de C.V., MX) filter paper wet with 5 mL deionized water inside a Petri dish; then seeds were placed carefully with the embryo located downward. Subsequently, the Petri plates were sealed and placed in an artificial growth incubator (Labnet International Inc.®, 211DS) with a day/night 12-h cycle at 25 ± 2 °C, respectively, with 60% relative humidity measured with (BOSCH Digital Multi-Scanner GMS120G). The treatment distribution consisted of a completely randomized design with six replicates per treatment, considering a Petri plate as an experimental unit. Seed germination was quantified daily according to the International Seed Testing Association (ISTA, 1999), and growth parameters (germination percentage (G), germination Index (GI), seed vigor (V), plumule (PL), radicle (RL), fresh weight and dry weight were recorded for seven days. Seed germination was considered when sprout length reached half the seed length (2 mm long) (Faraji *et al.*, 2018).

Parameters tested during bioassay development

The following parameters were calculated: Germination percentage (G). For determining germination percentage, total count of normal seedlings was considered, and the result was expressed in percentage at seven days after germination.

$$G = \frac{N° \; germinated \; seeds}{Total \; n° \; of \; seeds} \times 100 \qquad (1)$$

Germination Index (GI). GI represents the product of relative seed germination by the radicle relative growth. It constitutes an interaction indicator of the factors that promote or inhibit germination, as well as the respective factors that favour or hinder radicle growth. This index expresses both germinated seed and growth percentages reached by the radicle during the bioassay. The germination index was calculated with seed relative germination (SRG = # of germinated seeds in the extract × 100) and relative radicle growth (RRG = radicle elongation in the extract × 100).

$$GI \; (\%) = (\text{SRG} \times \text{RRG})/100 \qquad (2)$$

Seed vigor (V). The biological potential that favors rapid an uniform establishment under conditions is seed vigour, including unfavorable ones in field (Taghavi *et al.*, 2018), that determine the activity and performance in growth since it allows identifying the differences between germination and emergence in the field, mainly when field conditions can cause stress. At day 4 after sowing, the first count, at 5 days the second count, at 6 days the third count and at seven days the fourth was performed for seedling (that have well root and plumule) data with a total development of 2.0 cm measured individually, with a digital vernier (Truper, CALDI-6MP; 14388), to determine seed vigour expressed in percentage.

$$V \; (\%) = \frac{Normal \; seedlings}{Total \; n° \; of \; seeds} \times 100 \qquad (3)$$

Plumule (PL) and radicle (RL) length. Plumule length was measured from the root-hypocotyl junction up to the cotyledon base, whereas radicle length was measured from the hypocotyl base to the radicle at its apex. The values were expressed in centimetres.

Fresh weight. Fresh weight was recorded in sprouting mg^{-1}. The sprouts of each experimental unit were placed in a watch glass recording sample weight in an analytical balance (Mettler Toledo, ADN® HR-200).

Dry weight. Dry weight was expressed in sprouting mg^{-1}. Sprouts were placed in perforated and labelled brown paper bags. After that, they were placed in a drying stove (Novatech S.A de C.V, Ohasus® 547A) at a temperature of 72 °C for 24 h. At the end of each period, sample weight was recorded in an analytical balance (Mettler Toledo, ADN® HR-200).

Extract preparation for phytochemical compounds

To obtain the extracts, 2 g of fresh sprouts were mixed in 10 mL ethanol at 80% with constant centrifugation at 0.31 RCF (relative centrifugal force) for 24 h. Subsequently, extracts were centrifuged at 13.61 RCF for 5 min, and the supernatant was extracted for its subsequent analysis.

Phytochemical compounds

Total phenolic content was determined using a modification of Folin-Ciocalteau method (Singleton *et al.*, 1999); 50 µL of ethanolic extract were taken, diluted in 3 mL of Milli-Q (MQ, Damstadt, DE) water; 250 µL of Folin-Ciocalteau reagent (1N) was added, stirred, and left in reaction 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 UV-Vis spectrophotometer at 760 nm. The standard was prepared with gallic acid, and the results were expressed in mg GAE 100 g^{-1} fresh weight.

Total flavonoids were determined by colorimetry (Hidalgo *et al.*, 2019); 250 µL of ethanolic extract were taken, mixed with 1.25 mL of MQ water and 75 µL of $NaNO_2$ (5%). After a 5-min rest, 150 µL $AlCl_3$ (aluminum chloride-1-Ethyl-3-methylimidazolium chloride, Sigma-Aldrich, St. Louis, MO, U.S.A.) were added. Subsequently, 500 µL of NaOH (1M) and 275 µL of MQ water were added and vigorously stirred; samples were quantified in an UV-Vis spectrophotometer at 510 nm. The standard was prepared with quercetin dissolved in absolute ethanol ($y = 0.0122x-0.0067$; $r^2 = 0.965$), and the results were expressed in mg QE 100 g^{-1} fresh weight.

Total antioxidant capacity was measured by in-vitro 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH+) method (Li *et al.,* 2019). A DPPH+ solution (Sigma-Aldrich, St. Louis) was prepared in ethanol at 0.025 mg mL^{-1} concentration; 50 µL of ethanolic extract were mixed with 1950 µL of DPPH+ solution; after 30 min the samples were quantified in an UV-Vis spectrophotometer at 517 nm. The results were expressed in µM equivalent in Trolox 100 g^{-1} fresh weight.

Copper

Cu content in wheat sprouts was determined by atomic absorption spectrophotometry (Cartaya *et al.*, 2017). Sprouts were dried in a stove at 70 °C in brown paper and subsequently macerated in a mortar. Cu was quantified in a hydride generation atomic absorption (AA) spectrophotometer (Lab Wrench Varian Spectr AA®, model 220Fast). The results were determined in µg kg^{-1} dry weight.

Statistical analyses

The results obtained were analysed by analysis of variance (ANOVA) and comparison of means with Tukey's test ($p \leq 0.05$) using the statistical package SAS (Statistical Analysis System Institute) version 9.4. Normality of data expressed as a percentage (germination percentage (G), germination Index (GI), seed vigor (V), was verified with Kolmogorov-Smirnov Test and were transformed applying arcsine and square root transformation before analysis of variance.

Results and Discussion

Green synthesis of copper oxide nanoparticles (CuONPs)

Nanoparticles were obtained in a size of >95 nm by DLS (Figure 1). The size of the particles depends on the biological entity and biochemical processing, as the effect of environmental conditions such as temperature, affect the size, shape, and morphology of the biosynthesized nanoparticles (Fardood *et al.*, 2019). The nanoparticles obtained showed a cone shape with a length from 80-120 nm and thickness from 15-20 nm, specific characteristics of CuONPs (Ghidan *et al.*, 2016). The nanoparticles measured by XRD indicated the specific formation of CuONPs with diffraction angles of 43° and 56°, assigned to the plane orientation 111 and

200 of CuO, respectively (Figure 2), indicating its crystalline nature (Wei *et al.*, 2016). Therefore, the powder is characterized as CuONPs according to the international data base of the Joint Committee on Diffraction Standards for Dust (JCDPS 9-43) and the Joint Committee on Standards for Diffraction of Dust (No. 089-2838) (Trang *et al.,* 2018; Van den Bruinhorst *et al.*, 2016).

Figure 1. Micrographics obtained by histogram of the size distribution of the dynamic light scattering (DLS) The particle size was determined by dynamic light scattering (DSL) as the relative percentage of the grains of each of the different size fractions represented in a sample and the data was analyzed in the Origin program to generate this type of technical graphs. that allows to analyze them and generate advanced and personalized graphs for investigations.

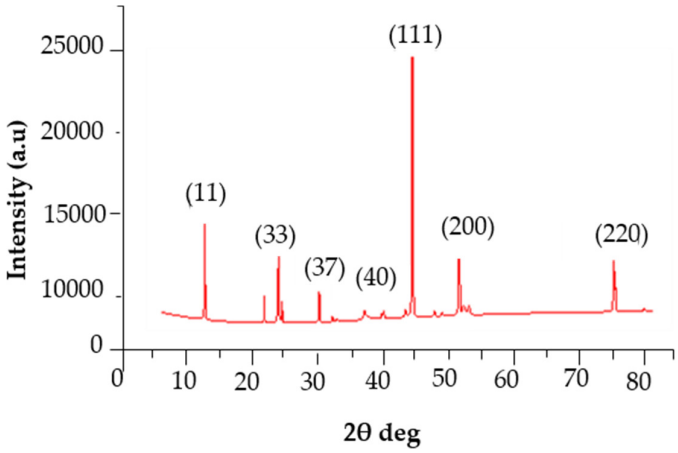
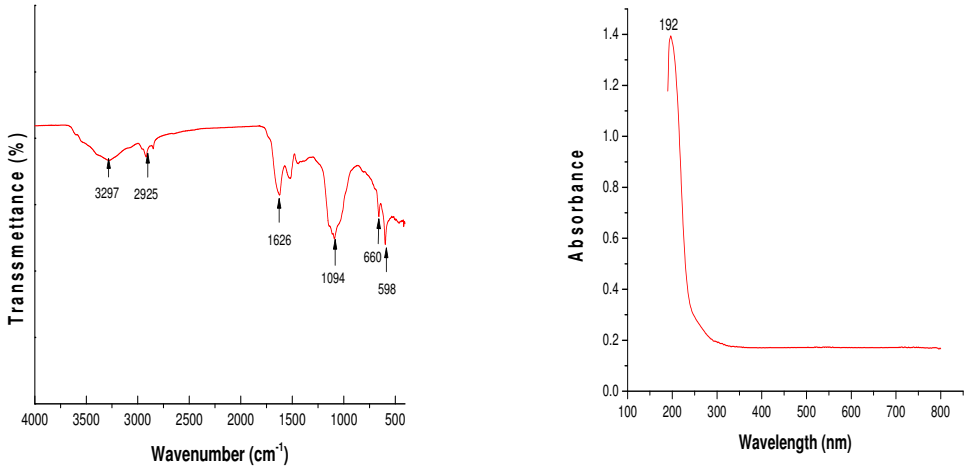


Figure 2. XRD sample patterns of CuONPs powder obtained by green synthesized with orange peel extract and $CuSO_4 \cdot 5\ H_2O$ to 100 mM for 24 h and treated subsequently at 100 °C

The functional groups of the responsible components of the ion bio-reduction and Cu ion stabilization were identified by FTIR spectrometer (Figure 3a) (Tadjarodi *et al.*, 2015). The absorption peaks that appear at 1626 and 3297 cm^{-1} could be water molecule flexing and stretching and the superficial hydroxyl groups in the CuONPs adsorbent surface (Nazar *et al.*, 2018; Siddiqi *et al.*, 2020). Absorption of infrared bands close to 589-660 cm^{-1} show CuONPs characteristic vibration, confirming the formation of the expected CuO starting from green synthesis (Bhagat *et al.*, 2020). Different authors have performed CuO synthesis studies and

obtained characteristic links by FT-IR found from 400-700 nm (Siddiqi *et al.*, 2020; Chaudhary *et al.*, 2019; Qamar *et al.*, 2020), which agree with CuO monoclinic phase (Taghavi *et al.*, 2018). Possibly the carbonyl group of amino acid waste has a strong linkage capacity with metal, which suggests the formation of an organic cover on CuONPs to avoid agglomeration and provide medium stability (Chaudhary *et al.*, 2019).

Figure 3b indicate the absorption spectra of UV-vis/ with the presence of the purified and dispersed CuONPs in deionized water, observing an absorption band close to 192 nm. This result confirms that reported (Le Van *et al.*, 2016) by the maximum appearance of absorbance at 200 nm for CuONPs, assigned to transition between central electron bands of Cu metals and CuO nanocrystals (Oli *et al.*, 2018).



(a) (b)

Figure 3. Spectra of (a) Fourier-Transform Infrared (FTIR) and (b) ultraviolet-visible (UV-vis) of CuONPs obtained by green synthesized with orange peel extract and $CuSO_4$*5 H_2O to 100 mM for 24 h and treated subsequently at 100 °C

Effect of copper oxide nanoparticles on germination of wheat seed

Germination and fresh weight are some of the main properties implied in seed quality (Prieto-Méndez *et al.*, 2019). The highest dose of CuONPs affected the variables related with germination negatively (Table 1), due to cyto- and genotoxicity of CuONPs (Bonciu *et al.*, 2018; Quiterio-Gutiérrez *et al.*, 2019) and an alteration of DNA repairing kinetics (Escobedo-Paredes *et al.*, 2020).

Table 1. Effect of copper oxide nanoparticle (CuONPs) doses on germination percentage (G), germination index (GI), vigor (V), plumule length (PL), radicle length (RL), fresh weight (FW) and dry weight (DW) in wheat sprouts

CuONPs	G	GI	V	PL	RL	FW	DW
mg mL^{-1}	(%)			(cm)		(mg)	
0.0	90.0 a ± 1.02*	42.0 ab ± 0.25	63.9 ab ± 11.02	7.1 b ± 0.27	6.5 c ± 0.31	4891 a ± 0.04	325 a ± 0.04
0.5	90.0 a ± 0.98	43.2 a ± 0.07	81.4 a ± 9.25	9.1 a ± 0.057	7.2 a ± 1.17	4974 a ± 0.07	354a ± 0.07
1.0	66.6 b ± 1.21	39.9 abc ± 0.09	64.1 ab ± 21.32	7.7 ab ± 0.047	6.8 b ± 0.51	4924 a ± 0.09	349 a ± 0.09
2.0	54.9 c ± 2.41	27.8 bc ± 0.04	61.3 ab ± 11.25	7.1 b ± 0.78	5.5 c ± 0.75	3564 b ± 0.06	302 b ± 0.06

4.0	45.1 d ± 1.04	26.7 c ± 0.05	55.1 b ± 13.44	6.0 b ± 0.065	5.2 d ± 0.85	3216 bc ± 0.07	295 bc ± 0.07
6.0	27.5 e ± 2.12	16.4 d ± 0.08	36.4 c ± 10.74	3.7 c ± 0.074	4.3 e ± 0.074	3051 c ± 0.05	115 c ± 0.05

*Data are show as the mean ± standard deviation (SD, n = 50). Values with same letters in each column are equal according to Tukey's test ($p \leq 0.05$).

The abiotic stress caused by NPs reduced germination growth (Heikal *et al.*, 2020). In contrast, with low doses of CuONPs, the variables related with germination improved (Chaudhary *et al.*, 2019; Shabbir *et al.*, 2020) when they showed a stimulating effect in shoot and radicle growth (Figure 4) as indicated by (García-Gómez and Fernández, 2019).

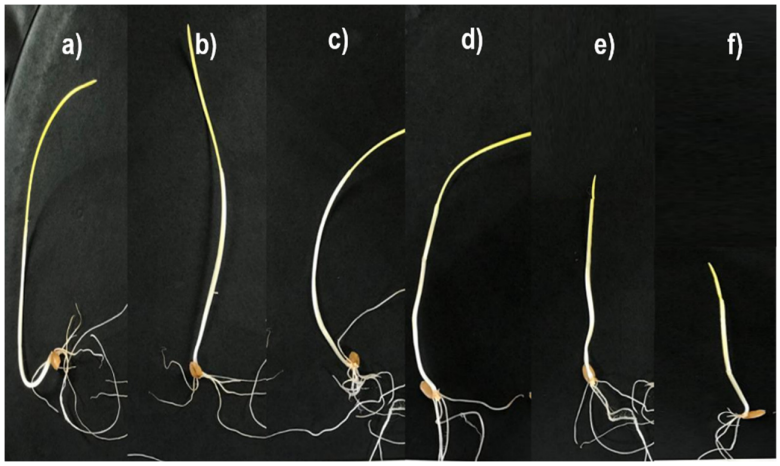


Figure 4. Germination and emergence of wheat seeds treated with copper oxide nanoparticles (CuONPs): (a) 0, (b) 0.5, (c) 1, (d) 2, (e) 4 and (f) 6 mg mL^{-1}

Metal NPs improve water and micronutrient imbibition, accelerate mobilization of reserves during the first stages of the germination process (Costa Da and Sharma, 2016) and increase germination vigour (Quiterio-Gutiérrez *et al.*, 2019)[51] because of a greater activation of copper enzymes (Javed *et al.*, 2017) and a fast adenosine triphosphate (ATP) production by the copper dependent enzyme cytochrome C oxidase (Ramzan *et al.*, 2021). This process takes place when molecular oxygen (O_2) reduction is catalyzed to water (H_2O), generating an electric gradient used by the mitochondria (Kumar *et al.*, 2020; Mehdizadeh *et al.*, 2020). Where Cu enters the cell through a high affinity Cu (I) uptake system that includes CTR/COPT family proteins (Pham *et al.*, 2022). After entry, cytosolic chaperone proteins (of which ATX1 is a prototype) are responsible for subsequent Cu transfer to key metabolic Cu proteins (Shin *et al.*, 2012). More recently, GSH is implicated as a Cu ion carrier between the importers and metallochaperones (Miras *et al.*, 2008). Then, it activates the development of different efficient cellular mechanisms to cope with Cu excess (Paredes *et al.*, 2020; Rajput *et al.*, 2020), immobilizing it in the cytosol to maintain the metal at a low concentration (Xiong *et al.*, 2017) with vacuole and Cu chelation sequestration with metallothionein (MT) and phytochelatin (PC) (Singh *et al.*, 2018). CuONPs in the lowest concentration increased germination, a very important parameter because this stage is the most sensitive one to adverse conditions and influences directly in crop yield (Rastogi *et al.*, 2017). The germination parameter improvement by metallic NPs agrees with other works (Kolesnikov *et al.*, 2019; Mohan *et al.*, 2016), but a response pattern to the effects caused by CuONPs in seeds and sprouts is difficult to establish since it depends on species, concentration, type of NPs (Bhuvaneshwari *et al.*, 2018; Shabbir *et al.,* 2020), and application method (Xu *et al.*, 2020).

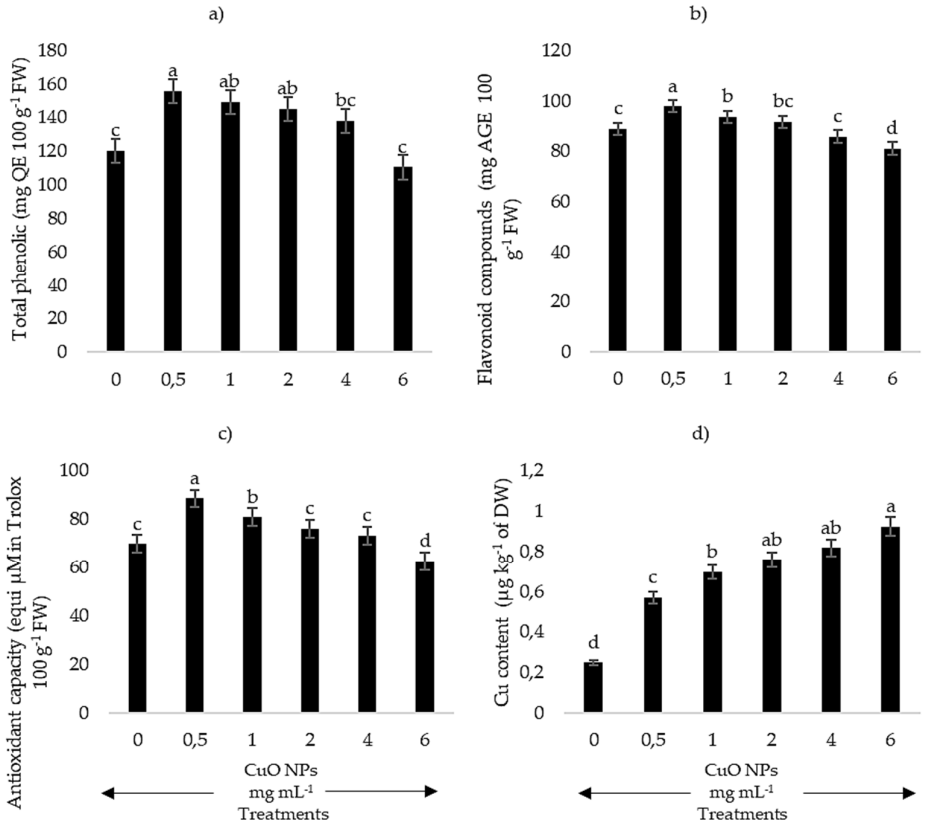


Figure 5. Effect of CuONPs concentration in content of (a) total phenolic and (b) total flavonoid compounds; (c) antioxidant capacity; and (d) Cu content in wheat sprouts
Data are show as the mean ± standard deviation (SD, n = 50).
Average values in the columns with different letters differ statistically among them (Tukey's test $p \leq 0.05$).

Phytochemical compounds

The high CuONPs dose decreased total phenols and flavonoids and the antioxidant capacity of germinated wheat seeds (Figure 5 a-c). CuONPs in high doses cause oxidative stress increasing biosynthesis of these phytochemicals (Ruttkay-Nedecky *et al.,* 2017) due to the high reactive oxygen species (ROS) production through the redox cycle (Mortezaee *et al.*, 2019). This process generates damages in the antioxidant defense system causing a rupture of the normal cellular function besides physiological and morphological damage in different macromolecules, which help promote irreversible damage of lipids, nucleic acids, and cellular proteins (Rao *et al.*, 2018).

Because free radicals tend to cause chain reactions (one radical generates another one, and so on), an unbalance emerges due to excess ROS production and a reduction of antioxidant defense mechanisms (Carvajal *et al.*, 2017). Whereas the use of 0.05 mg mL^{-1} of CuONPs improves secondary metabolite biosynthesis in sprouts because of the stimulation exerted by antioxidant cellular biochemistry (Tocmo *et al.*, 2020; Xu *et al.*, 2020). This stimulation can be explained because copper can accept and donate stable electrons within the atom in its orbit, which avoids decoupling to other molecule, thus the importance of this mechanism for oxide-reduction reactions. Then, the interaction of protector actions against ROS is possible and performed by regulating several enzymes (as superoxide dismutase [SOD], catalase [CAT], ascorbate-glutathione [AG] and glutathione peroxidase (GPx) and non-enzymatic compounds (phenolic, flavonoids, vitamin C, and glutathione) as first line of defense against oxidative stress (Rai *et al.*, 2018; Singh *et al.*, 2018). Likely, CuONPs

function bioactive molecules and dynamic systems that may provide an optimum environment to transport nutrients connectively guiding growth and cellular proliferation, as well as secondary metabolite production due to highly reactive and efficient NPs in their actions (López-Vargas *et al.*, 2018). By participating as copper enzyme of the several enzymatic systems involved in formation and conversion of amino acids as in detoxication of superoxide radicals, they protect the cellular and subcellular system from the cytotoxic ROS effects (León-Silva *et al.*, 2018). Thus, the parameters related with the antioxidant system of sprouts improve, which agrees with that reported by other authors (Fidelis *et al.,* 2019; Prieto-Méndez *et al.*, 2019).

Concentration of copper in wheat sprouts

Copper content in wheat sprouts increased as applied doses increased (Figure 5d). CuONPs distribution in plant species are contrasting because they depend on vegetal species, nanoparticle synthesis (concentration, size, shape) (Kolesnikov *et al.*, 2019) and application routes (seeds, leaves, substrate, among others) (Hernández-Hernández *et al.*, 2019; Kumar *et al.*, 2020).

Cu absorption and translocation within sprouts is determined by activating copper enzymes that oversee unfolding the proteins contained in storage places (Kranjc and Drobne, 2019), which in turn are digested in more simple substances to translocate to growth points by the cellular division of the embryo axis (Sun-Kou *et al.*, 2014). The greatest absorption of this element could have been due to copper, which is captured better by the passive apoplast membrane of the so-called transport proteins (Aguirre-Bolaños *et al.*, 2017; Ruddaraju *et al.*, 2020), since they are incorporated to metabolic pathways, such as plant copper enzymes altering the mitogen-activated protein kinase (El Shafey, 2020). Thus, these proteins participate in signaling the transduction induced by heavy metals (Hu and Gao, 2010) and protein phosphorylation, which are important in the enzymatic activity center (Parsai and Kumar, 2019) and a greater effect in antioxidant activity (Rajput *et al.*, 2020). Similar results have been reported, finding increase in Cu content because of adding CuONPs. Cu accumulation in sprouts could complement daily intake, which fluctuates from 440 to 900 µg (Lugo, 2016).

Conclusions

This study showed that orange peel can be used efficiently for $CuSO_4{\cdot}H_2O$ reduction in CuONPs and the characterization by XRD which confirms copper present in the form of oxide with a particle size of 95 nm. Likewise, CuONPs in low concentration (0.5 mg mL^{-1}) stimulates germination, vigour, and plumule and root development, as well as non-enzymatic antioxidant compound synthesis. Moreover, high doses of CuONPs (6 mg mL^{-1}) caused an inhibitory effect due to phytotoxicity by high Cu accumulation in plant tissues. The use of CuO NPs for green synthesis is a viable alternative to obtain beneficial effects in germination and seedling development, as well as greater secondary metabolite production. Finally, future research should investigate the effects of the CuONPs in other varieties of wheat and confirmed their influence on the growth and productivity of the plants in field.

Authors' Contributions

Conceptualization: JMGD, PPR; Methodology: JMGD, HOO; Validation: LLC, LGHM; Formal analysis: MFH, EdeCL; Investigation: JMGD, LLC; Data curation: PPR, LGHM; Funding acquisition: PPR, HOO; Project administration: PPR. Writing: JMGD, PPR; Review and editing HOO, PPR. 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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