

REGULAR ARTICLE

# Cu Nanoparticles in chitosan-PVA hydrogels as promoters of growth, productivity and fruit quality in tomato

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## ABSTRACT

The encapsulation of copper nanoparticles (Cu NPs) in chitosan hydrogels could improve the yield and quality of fruit of horticultural crops due to the physicochemical properties of the NPs. The objective of this research was to evaluate different concentrations of Cu NPs in Chitosan-polyvinyl alcohol (Cs-PVA) hydrogels and their effects on the growth, productivity and fruit quality in tomato. The treatments were applied to the substrate as follows: 0.02, 0.2, 2 and 10 mg of Cu NPs in Cs-PVA hydrogel, Cs-PVA hydrogel alone and a control. The Cu NPs had significant effects on growth, productivity and fruit quality. They increased the numbers of leaves and clusters, fresh biomass of roots, and dry biomass of stem-leaves and roots of the plants. They also increased the soluble solid content, titratable acidity, lycopene content and total antioxidant capacity in the fruits. The concentration with the best effect on the growth and yield of tomato plants was 10 mg Cu NPs, which increased the stem diameter, dry biomass of stem-leaves (13%) and roots (30%) and the yield (17%), whereas the concentration of 0.02 mg Cu NPs increased the lycopene content (37%) and the total antioxidant capacity of the fruit (10%). The Cu NPs in Cs-PVA hydrogels helped to increase the yield and nutraceutical properties of the tomato fruits.

**Keywords:** Antioxidants; Chitosan; Cu NPs; *Solanum Lycopersicum*; Yield

## INTRODUCTION

Nanomaterials are attracting attention from the scientific community due to their outstanding activity in relation to bulk materials (Naddeo et al., 2015). This heightened activity can be attributed to the high ratio surface-volume area and unique physico-chemical, mechanical and electronic properties of nanoparticles (NPs) (Somasundaran et al., 2010). Advances in nanotechnology have enabled its application to agriculture and the food industry, and particularly in agriculture are becoming popular (Ruttikay-Nedecky et al., 2017). However, its use is still limited due mainly to the lack of information about the toxicity and environmental fate of nanomaterials (Narayanan et al., 2012; Ruttikay-Nedecky et al., 2017), and also the little interest of the application of nanotechnology in plant sciences compared to nanomedicine and nanopharmacology (Wang

et al., 2016). Nanotechnology could play a key role in increasing global food production. Currently, numerous products and patents based on nanomaterials are being developed with the aim of improving the efficiency and sustainability of farming practices (Servin et al., 2015).

The main nanomaterials being studied are carbon-based (fullerol or carbon nanotubes), metals and metal oxides (Peralta-Video et al., 2011). Nanomaterials based on metals and metal oxides that are being evaluated in plants include the ZnO NPs (Landa et al., 2012; Zhao et al., 2013; Zhao et al., 2014), TiO<sub>2</sub> NPs (Landa et al., 2012; Hanif et al., 2015), CeO<sub>2</sub> NPs (Zhao et al., 2013; Zhao et al., 2014; Rico et al., 2014; Hong et al., 2015), Fe<sub>3</sub>O<sub>4</sub> NPs (Trujillo-Reyes et al., 2014), CuO NPs (Adhikari et al., 2012; Hong et al., 2015) and Cu NPs (Lee et al., 2008; Pradhan et al., 2015; Saharan et al., 2015). Some of the ways these nanomaterials are

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being applied to the plants are through a nutrient solution or soil, leaf or in-vitro means (Liu and Lal, 2015; Servin *et al.*, 2015; Anjum *et al.*, 2015). Moreover, the application of nanoparticles in different crop plants has been evaluated, and the effects of this vary greatly with plant species and other factors as dose and type of NPs (Rizwan *et al.*, 2017).

The accepted mode of action of these nanomaterials is cellular penetration, although the exact mechanisms of uptake are not fully known (Zhang *et al.*, 2015; Servin *et al.*, 2015). NPs can be absorbed by plants through the roots or the leaves. They must first penetrate the epidermis and endodermis of the root and then enter the xylem vessel, and they are then transported to the aerial parts. In the leaves, they can penetrate through the stoma, entering the vascular system of the leaves, and then be transported to other parts of the plant through the phloem (Zhang *et al.*, 2015; Servin *et al.*, 2015; Shi *et al.*, 2014; Le *et al.*, 2014). Moreover, the nanoparticles can cross cell walls by several ways: endocytosis, pore formation, carrier proteins, or through plasmodesmata (Pérez-de-Luque, 2017). Even if there are the ion channels, they have size around 1 nm, thus nanoparticles are unlikely to cross the cell wall effectively (Pérez-de-Luque, 2017). Once the NPs are absorbed by the plants, they cause stress and consequently the generation of reactive oxygen species (ROS), activating the plant antioxidant defense system (Rico *et al.*, 2015). This defense system of plants combines the generation of enzymatic and non-enzymatic antioxidant compounds (Rizwan *et al.*, 2017). The changes of enzymatic antioxidants have been demonstrated by Juárez-Maldonado *et al.* (2016) in tomato plants treated with Cu NPs + chitosan, where the catalase activity was more than five times higher than the control. Also Pinedo-Guerrero *et al.* (2017) reported that application of Cu NPs + chitosan generated more concentration of capsaicin in jalapeño pepper, exceeding the control by 51%.

Cu NPs are of particular importance in plants because they enhanced photosynthetic activity by modulating fluorescence emission, photophosphorylation, electron transport chain, and carbon assimilatory pathway under controlled laboratory conditions, as revealed from biochemical and biophysical studies on treated isolated mung bean chloroplast (Pradhan *et al.*, 2015). Recent research studies report that the application of Cu NPs at low concentrations ( $0.05$  to  $1.0$  mg L<sup>-1</sup>) in the soil or by seed imbibition increases the seedling growth, chlorophyll and carotenoid content (Shah and Belozeroova, 2009; Pradhan *et al.*, 2015). However, the application of higher concentrations ( $200$ - $1000$  mg L<sup>-1</sup>) in the nutrient solution reduces growth and biomass accumulation in seedlings (Lee *et al.*, 2008; Kim *et al.*, 2012; Musante and White, 2012; Wang *et al.*, 2012). One of the main problems in using

NPs is their insolubility in water (Lee *et al.*, 2008). Due to this problem, natural polymers such as chitosan (Cs) are being used for the encapsulation and controlled release of NPs metal due to its characteristics of biocompatibility, biodegradability, non-toxicity and adsorption ability (Kashyap *et al.*, 2015). Cs is a biodegradable natural compound, the deacetylated derivative of chitin, obtained from the exoskeletons of crustaceans such as crabs and shrimp. Its industrial and medicinal value derives from its polycationic nature (Bautista-Baños *et al.*, 2006). It is a polysaccharide with great crosslinking ability thanks to the presence of the amino groups (-NH<sub>2</sub>), which explain the unique properties of the Cs because of its behavior cationic in acid solutions and its affinity to metal ions, as well as their antimicrobial properties (Ravi-Kumar, 2000). It is also considered a chelating agent suitable for trapping heavy metals (Shukla *et al.*, 2013). On the other hand, Cs is also used for the synthesis of hydrogels in combination with polyvinyl alcohol (PVA), using glutaraldehyde as a crosslinking polymer (Tripathi *et al.*, 2009; Wang *et al.*, 2004). PVA is soluble in water and acts as an emulsifier and adhesive synthetic polymer (Kanatt *et al.*, 2012) with the ability to release drugs in a controlled way. The main applications and features of Cs in agriculture are founded on its ability to induce a series of defense/stress responses in plants, including the production of H<sub>2</sub>O<sub>2</sub> (Malerba and Cerana, 2015) and nitric oxide; stimulate growth; protect against low temperatures and release nutrients into the soil (Rinaudo, 2006). When it is used as a coating on fruits, it extends their postharvest life (Badawy and Rabea, 2011).

It has recently been shown that Cu NPs coated with Cs are less toxic to seedlings than free Cu NPs or copper sulfate (Aruna *et al.*, 2015; Saharan *et al.*, 2015). Accordingly, this study focused on the application of Cu NPs absorbed in Cs-PVA hydrogels directly to the substrate, evaluating their effect on growth, productivity and fruit quality.

## MATERIALS AND METHODS

### Synthesis of hydrogels of chitosan-polyvinyl alcohol (PVA-Cs) and absorption of Cu NPs

Hydrogels of Cs-PVA were synthesized in the pilot plant of the Research Center for Applied Chemistry (RCAC) according to the following methodology: first, 250 mL of 2% Chitosan from Marine Chemical,  $M_v = 200,000$  g/mol, and 250 mL of 4% polyvinyl alcohol (PVA) from Aldrich,  $M_w = 30,000$ - $50,000$ , in water were mixed for two hours at 60 °C and 300 rpm to form a hydrogel at a 1:2 ratio (CS: PVA). Then, 2.27 mL of crosslinker (50% glutaraldehyde) was added at 450 rpm and 25 °C for 5 min, and 100 mL of 6% NaOH was added at 300 rpm and 25 °C for one hour. The Cs-PVA hydrogels were

immediately washed and purified with distilled water and ethanol, then finally dried and weighed. The Cu NPs used in this work were supplied from SkySpring Nanomaterials, Inc. (Houston, TX, USA) with a reported average size of 25 nm, with a chemical purity of 99.8% and spherical morphology. One hundred milligrams of Cu NPs was dispersed in a solution of Tween 1% by ultrasound for 5 minutes (50 watts and 70% frequency), and dilutions were prepared to obtain concentrations of 10, 2, 0.2 and 0.02 mg, which were subsequently absorbed in 1 gram of Cs-PVA hydrogel and dried at 60 °C.

### Experimental growth of tomato plants in greenhouse

In April 2015, established tomato plants (*Solanum lycopersicum* L.) of the hybrid var. "Cayman", ball type and undetermined, were grown in a greenhouse in the Department of Horticulture of the Agrarian Autonomous University Antonio Narro, under a multi-tunnel with polyethylene cover. The average temperature was 22.4 °C, the average photosynthetic active radiation was 677.15  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and the average relative humidity was 62%. The planting density was three plants per square meter. A mixture of peat moss and perlite (50:50, v/v) was used as the growth substrate, placed in black polyethylene bags of 12 L capacity. Also, a system of targeted irrigation was used. For the treatments, prior to transplantation, 1 g of Cs-PVA hydrogel in the low, medium and high parts of the pot for better dispersion of the Cu NPs in the substrate and root area of the plant. Steiner nutritive solution (Steiner, 1961) with the following micro-nutrients was also used: Fe-EDTA = 3.75 ppm; Mn-EDTA = 1.85 ppm; B = 0.35 ppm; Zn-EDTA = 0.30 ppm; Cu-EDTA = 0.15 ppm; and Mo = 0.10 ppm. The nutritive solution was supplied in different concentrations to provide nutrients to the plants: for the first two weeks after transplantation, it was applied at 25%; 50% for the third and fourth weeks; 75% for the fifth week and the rest of the crop cycle at 100%. The treatments used were as follows: four concentrations (0.02, 0.2, 2 and 10 mg) of Cu NPs absorbed in Cs-PVA hydrogels, an absolute control and one treatment with hydrogel Cs-PVA (2% Chitosan and 4% PVA) to evaluate the effect of the Cs alone.

### Variable growth and yield of tomato

To evaluate the growth and production of tomato plants, the following procedures were performed: 60 days after transplantation (dat) the apex of all plants were cut, and 110 dat agronomic parameters was measured. Plant height (cm) was measured using a tape; the stem diameter (mm) was measured with a digital vernier; the number of leaves, the number of clusters with flowers and fruits tied, and the number of fruits per plant were counted; and the average weight of fruits (g), the fresh weights of stem-leaves and roots (g), and the yield of fruit per plant (g) were determined. The dry weights of the roots, stems

and leaves were obtained after drying in a Drying Oven model DHG9240A for 72 hours at a constant temperature of 80 °C.

### Variable quality of tomato fruits

Fruit quality variables were evaluated as follows: at 90 days after transplant, fruits were randomly selected after the second harvest. It was verified that they had no physical damage and were uniform and in maturity stage 6 (red light), according to the visual color criteria used by the United States Department of Agriculture (USDA).

Harvested tomato fruits were stored for 15 days at a constant temperature of 10 °C and relative humidity of 80% to observe the influence of the Cu NPs on the post-harvest quality. On the first day of harvest, 9 fruits per treatment were weighed to calculate the percentage of weight loss after 15 days of storage. The pH, titrable acidity and soluble solids of 3 fruits per treatment were also measured on the first day of the harvest and after 8 and 15 days of storage. To determine the percentage of weight loss, the fruits were weighed on an OHAUS brand digital balance. The potential of hydrogen (pH) was determined using a digital potentiometer (HANNA®), the soluble solids (°Brix) using a digital Refract meter PR-101ATAGO PALETTE and the percentage of titrable acidity according to the methodology of the AOAC (2000), expressed as a percentage of citric acid.

The lycopene content and total antioxidant capacity of six fruits per treatment from the second harvest were also measured. The lycopene content was determined according to the methodology of Fish et al. (2002). To 3 g of fresh fruit pericarp, 3 mL of buffer phosphate (pH 7) were added; this sample was ground in a mortar, and then 2 mL of the sample and 4 mL of a 3:2 hexane: acetone mixture were centrifuged for 10 min at 3000 rpm. Finally, the absorbance at 503 nm of the resulting supernatant was determined, corresponding to the  $\mu\text{g g}^{-1}$  of lycopene.

The total antioxidant capacity of the fruit was determined using a commercial kit (Antioxidant Assay Kit-Cayman Chemical). First, to 100 mg of lyophilized fruit sample, 2 mL phosphate buffer (pH 7.2) was added and homogenized by vortex for 30 s and ultrasonication for 5 min. The mixture was then centrifuged for 10 min at 12000 g and 4 °C. To 10  $\mu\text{L}$  of the supernatant, 10  $\mu\text{L}$  of metmyoglobin and 150  $\mu\text{L}$  of chromogen were added. To start the antioxidant activity reaction, 40  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$  was added, and the mixture was incubated for 5 minutes at room temperature. The absorbance at 405 nm was measured in a microplate reader for ELISA (LEX-808 IU model). The final value was expressed as millimoles of Trolox Equivalent Antioxidant per gram dry weight.

## Statistical analysis

The crop was established using an experimental Latin square design (6x6), with six treatments and 18 experimental units per treatment for the agronomic variables. For the variables of percentage weight loss, pH, total soluble solids and titratable acidity, a completely randomized design with nine replicates per treatment was used. For the lycopene content and total antioxidant capacity, a completely randomized design with six replications per treatment was used. Statistical analysis of each of the variables was performed using the statistical program R CRAN for the analysis of variance and Fisher LSD mean test ( $p \leq 0.05$ ).

## RESULTS

### Effect of Cu NPs Cs-PVA hydrogels in the growth and yield of tomato

The application of Cu NPs Cs-PVA hydrogels in the substrate had significant effects on the growth variables and yield of the tomato plants ( $p \leq 0.05$ ). Table 1 shows that treatment with 10 mg of Cu NPs resulted in significant differences compared with the control, increasing the stem diameter, fresh root weight (25%) and the number of floral clusters (3%) per plant, whereas treatment with 2 mg Cu NPs increased the number of leaves per plant by 5% compared to the control ( $p \leq 0.05$ ). For the plant height, fresh weight of stem and leaves, number of fruits and average weight of fruits per plant, no significant differences were observed.

Fig. 1 shows that treatment with 0.02 mg of Cu NPs increased the dry weight of the stem-leaves by approximately 20%, and treatment with 10 mg of Cu NPs increased the dry weight of the roots by 30%.

Fig. 2 shows that treatment with 10 mg Cu NPs Cs-PVA hydrogels resulted in the highest fruit yield compared with the control, an increase of 17% per plant.

### Effect of Cu NPs Cs-PVA hydrogels on tomato fruit quality

Table 2 shows significant differences between the treatments for pH, titratable acidity and soluble solids content on the first day of harvest ( $p \leq 0.05$ ). Treatments containing 2.0 and 0.2 mg of Cu NPs increased the titratable acidity 23 and 19% respectively, and decreased the pH by approximately 1% on the first day of harvest with respect to the control. None of the Cu NPs treatments exceeded the control for soluble solids. After 8 days of storage, treatment with 0.02 mg Cu NPs increased the soluble solids content by 11% relative to the control, and no significant differences were found for titratable acidity and pH. After 15 days of storage, treatment with 2.0 mg Cu NPs increased the soluble solids by 14%, and the treatments with 10, 2.0 and 0.2 mg Cu NPs increased the titratable acidity by 7% with respect to the control. No significant differences were observed for pH, and no significant differences in the percentage of weight loss of fruits were found between treatments after 15 days of storage.

The Cu NPs in Cs-PVA hydrogels showed significant differences in the fruit lycopene content and total antioxidant

**Table 1: Effect of Cu NPs in Cs-PVA hydrogels on growth, development and productivity of tomato**

Treatment	Height (cm)	SD (mm)	NL	NC	FWS (g)	FWR (g)	NF	WF (g)
10	166.4 <sup>a</sup>	22.6 <sup>a</sup>	19.8 <sup>abc</sup>	6.2 <sup>a</sup>	2399.4 <sup>a</sup>	98.9 <sup>a</sup>	27.3 <sup>a</sup>	257.9 <sup>a</sup>
2	168.7 <sup>a</sup>	21.6 <sup>bc</sup>	20.4 <sup>a</sup>	6.0 <sup>b</sup>	2207.9 <sup>a</sup>	95.8 <sup>ab</sup>	26.4 <sup>a</sup>	249.9 <sup>a</sup>
0.2	166.8 <sup>a</sup>	22.1 <sup>abc</sup>	19.6 <sup>bc</sup>	6.0 <sup>b</sup>	2204.2 <sup>a</sup>	77.1 <sup>c</sup>	26.2 <sup>a</sup>	254.2 <sup>a</sup>
0.02	169.5 <sup>a</sup>	21.4 <sup>c</sup>	20.3 <sup>ab</sup>	6.0 <sup>b</sup>	2323.7 <sup>a</sup>	78.5 <sup>bc</sup>	27.1 <sup>a</sup>	256.1 <sup>a</sup>
Cs-PVA	167.8 <sup>a</sup>	21.7 <sup>abc</sup>	19.8 <sup>abc</sup>	6.0 <sup>b</sup>	2308.9 <sup>a</sup>	86.6 <sup>abc</sup>	26.6 <sup>a</sup>	256.4 <sup>a</sup>
0	167.2 <sup>a</sup>	22.3 <sup>ab</sup>	19.4 <sup>c</sup>	6.0 <sup>b</sup>	2192.2 <sup>a</sup>	79.0 <sup>bc</sup>	26.6 <sup>a</sup>	239.4 <sup>a</sup>

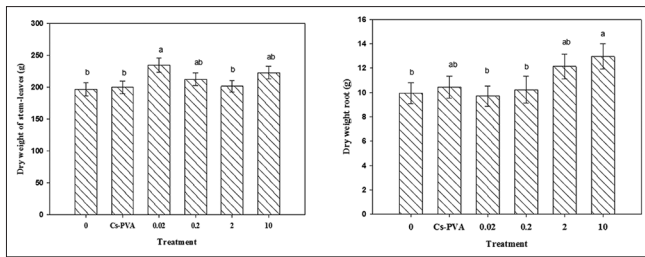
Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, 0= Control, Cs-PVA= Cs-PVA hydrogels only, SD= stem diameter, NL= number of leaf, NC= number of clusters, FWS= fresh weight of shoot, FWR= Fresh weight of root, NF= number of fruits, WF= average fruit weight, means with the same letter in the same column are not different according to Fisher LSD ( $p \leq 0.05$ )

**Table 2: Effect of Cu NPs in Cs-PVA hydrogels on tomato fruit quality**

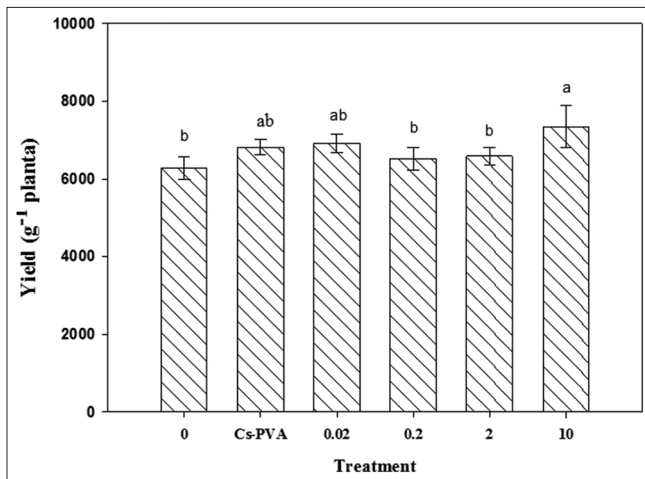
Treatment	WL (%)	pH 1	pH 2	pH 3	SS1 (°Brix)	SS2 (°Brix)	SS3 (°Brix)	TA1 (%)	TA2 (%)	TA3 (%)
Cs-PVA	1.69 <sup>a</sup>	4.27 <sup>a</sup>	4.27 <sup>a</sup>	4.29 <sup>a</sup>	4.18 <sup>ab</sup>	3.60 <sup>b</sup>	4.26 <sup>ab</sup>	1.64 <sup>b</sup>	1.48 <sup>a</sup>	1.64 <sup>b</sup>
2	1.66 <sup>a</sup>	4.17 <sup>b</sup>	4.27 <sup>a</sup>	4.32 <sup>a</sup>	4.07 <sup>ab</sup>	3.75 <sup>b</sup>	4.33 <sup>a</sup>	2.28 <sup>a</sup>	1.51 <sup>a</sup>	1.85 <sup>a</sup>
10	1.42 <sup>a</sup>	4.23 <sup>ab</sup>	4.21 <sup>a</sup>	4.34 <sup>a</sup>	3.98 <sup>b</sup>	3.61 <sup>b</sup>	3.95 <sup>cd</sup>	1.84 <sup>b</sup>	1.62 <sup>a</sup>	1.85 <sup>a</sup>
0.2	1.39 <sup>a</sup>	4.18 <sup>b</sup>	4.21 <sup>a</sup>	4.31 <sup>a</sup>	4.03 <sup>ab</sup>	3.81 <sup>ab</sup>	4.10 <sup>bc</sup>	2.20 <sup>a</sup>	1.50 <sup>a</sup>	1.85 <sup>a</sup>
0	1.37 <sup>a</sup>	4.22 <sup>ab</sup>	4.23 <sup>a</sup>	4.34 <sup>a</sup>	4.22 <sup>a</sup>	3.61 <sup>b</sup>	3.81 <sup>d</sup>	1.85 <sup>b</sup>	1.40 <sup>a</sup>	1.73 <sup>ab</sup>
0.02	1.36 <sup>a</sup>	4.25 <sup>ab</sup>	4.26 <sup>a</sup>	4.30 <sup>a</sup>	4.11 <sup>ab</sup>	4.02 <sup>a</sup>	3.91 <sup>cd</sup>	1.70 <sup>b</sup>	1.60 <sup>a</sup>	1.48 <sup>c</sup>

Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, Cs-PVA=Cs-PVA hydrogels only, 0=Control, WL=weight loss, pH 1=potential hydrogen first day of harvest, pH 2=potential hydrogen storage eight days, pH 3=potential hydrogen storage fifteen days, SS1=soluble solids first day of harvest, SS2=soluble solids storage eight days, SS3=soluble solids storage fifteen days, TA1=titratable acidity First Day of Harvest, TA2=titratable acidity storage eight days, TA3=titratable acidity storage fifteen days. Means with the same letter in the same column are not different according to Fisher LSD ( $p \leq 0.05$ )

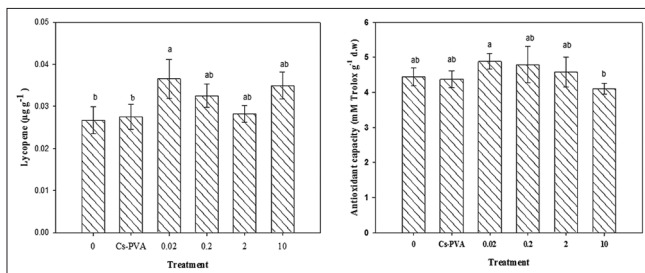




**Fig 1.** Dry biomass production of shoot (stem-leaves), and root of the tomato plants treated with different concentrations of Cu NPs in Cs-PVA hydrogels. Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, Cs-PVA= Cs-PVA hydrogels only, 0= Control. Means with the same letter are not different according to Fisher LSD ( $p \leq 0.05$ ).



**Fig 2.** Effect of Cu NPs in Cs-PVA hydrogels yield tomato. Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, Cs-PVA= Cs-PVA hydrogels only, 0= Control. Means with the same letter are not different according to Fisher LSD ( $p \leq 0.05$ ).



**Fig 3.** Lycopene content and total antioxidant capacity of tomato fruits at different concentrations of Cu NPs in Cs-PVA hydrogels. Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, Cs-PVA= Cs-PVA hydrogels only, 0= Control, d.w= dry weight. Means with the same letter are not different according to Fisher LSD ( $p \leq 0.05$ ).

capacity with respect to the control ( $p \leq 0.05$ ). The 0.02 and 10 mg of Cu NPs in Cs-PVA hydrogel treatments showed higher lycopene content in the fruit, namely, 0.03653 and 0.03495  $\mu\text{g g}^{-1}$  fresh weight, respectively, and the control value was 0.0267  $\mu\text{g g}^{-1}$  fresh weight. These values represent an increase of approximately 37 and 31%, respectively, for the treatments with Cu NPs in comparison with control.

In regards to the total antioxidant capacity of the fruit, the treatment showing the highest amount of antioxidants was 0.02 mg of Cu NPs, which produced an increase of approximately 10% in comparison with the control, whereas treatment with 10 mg of Cu NPs reduced the antioxidants approximately 8% compared to the control (Fig. 3).

## DISCUSSION

The results of this study showed that concentrations of 0.02 to 10 mg Cu NPs in Cs-PVA hydrogels applied to the substrate are not toxic to tomato plants. This nontoxicity could be due to the protection and controlled release of the Cu NPs provided by the Cs-PVA hydrogels during the growth and development of the tomato plants, as mentioned by Kashyap et al. (2015). In addition, the application of high concentrations (200-1000  $\text{mg L}^{-1}$ ) Cu NPs in the nutrient solution is known to be toxic (Lee et al., 2008; Kim et al., 2012; Musante and White, 2012; Wang et al., 2012), in contrast to this study, in which low concentrations were applied to the substrate. It has also been shown that low concentrations (0.05-1  $\text{mg L}^{-1}$ ) of Cu NPs encapsulated in polyethylene glycol (PEG-200) are less toxic than copper sulfate (Pradhan et al., 2015), and that low concentrations of Cu NPs coated with Cs (0.01 to 1  $\text{mg L}^{-1}$ ) are less toxic than free Cu NPs and copper sulfate (Aruna et al., 2015; Saharan et al., 2015). This study used Cu NPs encapsulated in Cs-PVA hydrogels, which could help to reduce the toxicity.

This study also showed that Cu NPs in Cs-PVA hydrogels improved the growth of tomato plants, increasing the diameter of the stem, number of leaves and dry biomass. The diameter of the stem is a very important plant growth parameter to evaluate because it is related to photosynthetic accumulation and transport, as well as to crop performance (Liptay et al., 1981). The literature reports that carbon accumulation in the shoots (stem-leaves) of in *Cyamopsis tetragonoloba* and *Pennisetum glaucum* can be increased by the application of ZnO NPs to the leaves at a concentration of 100  $\mu\text{g mL}^{-1}$  (Burman et al., 2013). It is also known that Cu NPs can play a critical role in photosynthesis, improving the electron transport chain and phosphorylation during the light reaction, as well as improving enzyme activity in the dark phase and participating in the metabolism of carbon and nitrogen (Pradhan et al., 2015). Similarly, Servin et al. (2015) mentioned in their review of literature that different metallic nanoparticles can increase photosynthesis, and the chlorophyll and carotenoid contents in plants.

Some authors, such as Saharan et al. (2015), have shown that the application of 5 ml of Cu NPs coated with Cs at concentrations of 0.08, 0.10 and 0.12% to tomato seeds

placed in Petri dishes with filter paper increase the fresh and dry weight of the tomato seedlings compared with  $\text{CuSO}_4$  and the control. It has also been shown that the imbibition of seeds of *Vigna mungo* in Cu NPs encapsulated in polyethylene glycol (PEG-200) at concentrations of 0.05 and 0.1  $\text{mg L}^{-1}$  for 4-6 hours can increase seedling dry weight compared to  $\text{CuSO}_4$  and the control (Pradhan et al., 2015). On the other hand, Hanif et al. (2015) reported that the application of  $\text{TiO}_2$  NPs (25 to 100  $\text{mg kg}^{-1}$ ) to the soil significantly increases the dry weight of shoots and roots in *Lactuca sativa* with respect to the control. This result shows that the application of Cu NPs coated in Cs have a positive effect on plant growth.

In regard to the reproductive stage, the Cu NPs in Cs-PVA hydrogels increased the number of floral clusters and the yield of fruit per plant. Thus, Cu NPs can efficiently activate the reproductive system of plants and increase fruit production. It is likely that the tomato fruit yield increase resulted from the ability of the Cu NPs to cause a greater accumulation of photosynthates in the supply-demand organs. Alternatively, it could be because the Cu NPs activate genes related to the growth and development of plants, as is the case for ZnO NPs, which regulate the expression of genes related to cell organization and biogenesis, while  $\text{TiO}_2$  NPs are mainly involved in the response of genes to biotic and abiotic stresses in *Arabidopsis thaliana* (Landa et al., 2012). Among the few works that have studied the effect of metal NPs on plant productivity, Wang et al. (2012) reported that the application of  $\text{CeO}_2$  NPs in solution at a concentration of 10  $\text{mg L}^{-1}$  increased the yield of tomato by 10% and suggested that the effect was probably due to the plants transferring more energy to the growth of the fruit. Hong et al. (2015) showed that concentrations of 50, 100 and 200  $\text{mg L}^{-1}$  of  $\text{CeO}_2$  and CuO NPs applied foliarly did not affect the yield of cucumber. However, it has been shown that  $\text{CeO}_2$  NPs applied to the substrate at high concentrations (800  $\text{mg kg}^{-1}$ ) decrease cucumber performance by up to 31.6% (Zhao et al., 2013). This result suggests that the applied amount of NPs directly affects crop growth and can have positive effects at low concentrations and negative effects at high concentrations.

The fruit quality results show that the Cu NPs can increase the content of soluble solids and the percentage of titrable acidity. It is very likely that the increase in soluble solids was due to a greater accumulation of photoassimilates in fruit to form fructose and glucose, as reported in Mustafa et al. (2014). It is also known that the increase in the percentage of titrable acidity is due to better metabolism of organic acids (citric acid) in the fruit (Valero and Serrano, 2010), which helps to improve the proportions of fructose and sucrose (Lobit et al., 2003). On the other hand, some authors report that the content of soluble solids and titratable acidity in fruit increase in plants under abiotic stress conditions

(Yamada et al., 2015; Al-Harbi et al., 2016). Cu NPs induce oxidative stress and can improve the levels of sugar and citric acid in tomato fruits. Similar studies have shown that  $\text{CeO}_2$  NPs applied to the substrate at concentrations of 400 and 800  $\text{mg kg}^{-1}$  do not alter the amounts of reducing sugars (glucose and fructose) in the fruits of cucumber, but the amount of non-reducing sugars (sucrose) is reduced at 400  $\text{mg kg}^{-1}$  and increased at 800  $\text{mg kg}^{-1}$  (Zhao et al., 2014). On the other hand, Dar et al. (2015) mention that copper has a positive and significant correlation with the soluble solids content and total sugars in pear fruits, which may explain the positive effect on tomato fruits observed in this study. In this way, Cu NPs could offer an alternative method to improve fruit condition in post-harvest.

This research also demonstrated that Cu NPs in Cs-PVA hydrogels can increase the lycopene content and total antioxidant capacity in tomato fruits. It has previously been reported that NPs produce oxidative stress in plants, activating the antioxidant defense system to fight against the reactive oxygen species (ROS) (Rico et al., 2015). Juárez-Maldonado et al. (2016) have demonstrated that the catalase activity was more than five times higher than the control in tomato plants treated with Cu NPs + chitosan. Also Pinedo-Guerrero et al. (2017) reported that application of Cu NPs + chitosan increased 51% more the concentration of capsaicin in jalapeño pepper. Corral-Díaz et al. (2014) report that  $\text{CeO}_2$  NPs applied to the soil at a concentration of 250  $\text{mg kg}^{-1}$  resulted in the highest values of total antioxidants in the tuber, and leaves of *Raphanus sativus* L. Kim et al. (2012) showed that suspensions of CuO and ZnO NPs at concentrations of 10 to 1000  $\text{mg L}^{-1}$  increased the activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and peroxidase in the roots of *Cucumis sativus*. Similarly, Trujillo-Reyes et al. (2014) showed that CuO NPs applied in the nutrient solution at a concentration of 10  $\text{mg L}^{-1}$  increased the activity of CAT in the roots and leaves of lettuce. On the other hand, Pradhan et al. (2015) showed that Cu NPs encapsulated in PEG (0.05-1.0  $\text{mg L}^{-1}$ ) did not alter the activity of the enzymes POD, SOD, CAT and GR in the roots and leaves of *Vigna radiata*. Barrios et al. (2015) also reported that  $\text{CeO}_2$  NPs coated with citric acid applied to the soil at concentrations of 62.5 to 500  $\text{mg kg}^{-1}$  increased the activity of the enzyme CAT in tomato leaves. This result suggests that the application of Cu NPs can induce the formation of antioxidant compounds in the fruits, as shown in the results obtained here, and thus might be a strategy to improve the quality of nutraceuticals.

## CONCLUSION

None of the concentrations of Cu NPs in Cs-PVA hydrogels evaluated in tomato plants in this study had

toxic effects. The concentration that produced the greatest increase in the growth and yield of tomato plants was 10 mg of Cu NPs, which increased the stem diameter, the fresh weight and dry weight of the root, and the number of flower clusters and yield. The Cu NPs also increased the content of soluble solids and titratable acidity in the fruit during the first 15 days after harvest, although none of the concentrations showed a clear difference. The highest estimated increase in lycopene content and total antioxidant capacity in the fruit was produced by the treatment containing 0.02 mg Cu NPs. The application of Chitosan-PVA-coated Cu NPs can be used as a tool to increase the nutraceutical properties of tomato fruits as well as the yield of this crop; however, further studies are needed to assess the toxicological profiles of Cu NPs in Cs-PVA hydrogels in other crops before commercial use.

### Author contributions

A.J.M.: Design, formulation and supervision of experiment, and review of manuscript. H.H.H.: Collection of data, did field experiments, and writing of manuscript. A.B.M.: Design and formulation of experiment, and review of manuscript. H.O.O.: Synthesis of nanoparticles. A.D.H.F.: Analysis of tomato fruit quality.

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