ISSN: 2637-7721

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Journal of Plant Biology and Crop Research

Open Access | Research Article

Zinc Oxide Nanoparticles and Their Effects in Crops Growth: Physical, Biochemical and Morphological Point of Views

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Received: Jun 21, 2022
Accepted: Jul 13, 2022
Published Online: Jul 15, 2022
Journal: Journal of Plant Biology and Crop Research
Publisher: MedDocs Publishers LLC
Online edition: http://meddocsonline.org/

Keywords: Ascorbate Peroxidase; Bean; Chlorophyll; Corn; ZnO Nanoparticles.

Abstract

Objective: Metal essential-based nanoparticles have many potential applications, such as improving crop yield. The present study deals with the effects of ZnO nanoparticles on germination and plant growth, chlorophyll content, antioxidative enzyme activity, and root morphological effects in corn (*Zea mays*) and bean (*Phaseolus vulgaris*) to probe their application as an alternative to improve crop yield.

Methods: The seeds were germinated under hydroponic conditions for 15 days, using different concentrations of ZnO nanoparticles (0, 1, 5, 10, 50, 10, and 500 mg/L).

Results and Discussion: The results indicated that each species responds differently to the presence of ZnO nanoparticles in the hydroponic medium, since bean seeds presented a higher percentage of germination than corn seeds, with 5 mg/L being the most favorable concentration reaching 25% and 18% of germination respectively over the control group. Regarding seedling development, evidenced by root and stem elongation, the differences between species were again evident, considering that, in beans, the best response was obtained at 10 mg/L, while in corn at 100 mg/L. In the case of biomass production, concentrations of 100 and 500 mg/L generated a decrease in biomass production in bean seedlings in contrast to corn seedlings, which presented the highest values at these concentrations. The results obtained in the enzymatic activity of Ascorbate Peroxidase did not allow establishing a pattern depending on the species or the concentration of ZnO nanoparticle used. Finally, significant affectations were observed inside the root tissue of both cultures, such as destruction of vascular bundles in beans and lengthening of root cells in corn at high concentrations.

Cite this article: Leura-Vicencio AK, López-Reyes AY, Rosas-Castor JM, Aragón-Piña A, Lara-Martínez EM, et al. Zinc Oxide Nanoparticles and Their Effects in Crops Growth: Physical, Biochemical and Morphological Point of Views. J Plant Biol Crop Res. 2022; 5(2): 1066.

Introduction

Nanoparticles are atomic or molecular aggregates with sizes between 1 and 100 nm, which depending on their nature can be classified as a metallic base, metallic oxides, carbon base, dendrimers, quantum dots and nanocomposites. Agriculture is one of the areas where nanoparticles have an important application, mainly the metallic oxide of Cu, Fe, and Zn, due to the importance of these elements in the development of agricultural crops. [25,30,49]. Zn is considered one of the main nutrients since it plays an important role as an enzyme cofactor in the metabolism of carbohydrates and auxins, proteins and chlorophyll synthesis, as well as it protects cell organelles from the reactive oxygen species and membranes integrity, regulation and gene expression, and others [20]. In natural soils, total Zn concentrations are in a range from 10 to 300 mg/kg. However, it does not represent soil capacity as Zn supplies to plants [1,31]. Worldwide, 30% of agricultural soils are Zn deficient, causing that water and other nutrients necessary to energy production and development of metabolic routes could be wasted by crops, being the crops of bean, maize, citrus, grape, onion, rice and sorghum the most affected due to their sensitivity to the deficiency of this element [4,5,31,41]. In order to counteract Zn deficiency in agricultural soils, huge amounts of fertilizers have been scattered, resulting in an increase of cost production and causing several environmental impacts as soil mineral imbalance and loss of soil structure and fertility [17].

According to World Bank (2019), 107.605 kg/h of fertilizers were employed in 2002, whereas in 2016,140.553 kg/h represented an increase of 131% in 14 years. Therefore, the use of nanoparticles as nano-fertilizers can represent an alternative to currently used fertilizers, which the advantages are: (1) increasing nutrients bioavailability through rapid dissolution and thus improving penetration through membranes; (2) spreading lower doses, leading to lower dose-dependent toxicity; (3) controlling nutrient release; (4) easing target distribution and (5) reducing environmental damage [7,21,24,39].

Studies carried out in corn showed that a concentration of 1000 µg/mL of ZnSO4 nanoparticles can be considered phytotoxic, because it can induce severe damage to the structure of the primary root, an inhibition of up to 40% in the root development and an increase in the number of metaxylems [36]. In cabbage, cauliflower, and tomato vegetable crops, ZnO nanoparticles in concentrations between 1.5 µM y 9.0 µM (0.12 y 0.73 mg/L) enhanced germination, seedling growth, pigments, sugar and protein contents along with increased activities of antioxidant enzymes in all the three test crops [45]. Soybean seedlings exposed to ZnO nanoparticles at 50, 100 and 500 mg/kg showed an interference with the accumulation of macro and micronutrients as Fe, Mo, Cu, K and Mg, compromising crop development [35]. Other study developed with wheat indicated that 50 mg/L of ZnO nanoparticles have a positive effect on seed germination, number of roots, plant biomass, and overall growth of roots, shoots and leaves [6]. In radishes, concentrations of 100 and 1000 mg/L of ZnO nanoparticles were determined to induce deleterious effects on seeds, affected seed germination as reduction in root and shoot length and fresh weight [43].

These studies show that the effect of nanoparticles dependsmainly on plant species, phenologic stage and other factors as nanoparticles concentration, physico-chemical properties, etc. [34,51,56], also tending that the main parameters that are evaluated to determine the effect of nanoparticles on different crops are: (1) seed germination; (2) phenotypic changes as root/shoot length and biomass production; (3) chlorophyll content; (4) reactive production of oxygen species; (5) antioxidant enzymatic activity; (6) gene expression and; (7) nutrient content, which together reveal the health and quality of crops. If the seed germination, plant growth, nutrient and chlorophyll contents increase in crops treated with nanoparticles based on essential elements with respect to crops untreated with nanofertilizers, then it is considered a positive effect and nanoparticles could be used as a strategy to improve crop yields and possible substitutes to the actual commercial fertilizers. On the other hand, if reactive production of oxygen species and antioxidant enzymatic activity increase, it means phytotoxicityto crops [6,18,29,40,50].

Hydroponic systems are increasingly used for various purposes worldwide, such as evaluating nanoparticles effects on crops, mainly due to their direct interactions between seeds or seedlings and nanoparticles, without soil interferences considering that soil is a complex matrix composed by minerals, organic matter, and microorganism (Duran *et al*., 2018; [41]. However, hydroponic systems have some limitations as rapid phytopathogen spread due to high nutrients concentrations which can be controlled by physical, chemical and biological methods (Lee and Lee, 2015). One of the most employed chemical method to reduce phytopathogen activity and spread is the use of sodium hypochlorite in many different concentrations and exposure time for seeds sterilization, due to its effects on breaking seed dormancy, seed germination and specially control in pathogens in many species [3,6,12,28].

Beans and corn represent two of the most important crops worldwide, from the economic, social and nutritional aspects, and are mainly affected by Zn deficiency in agricultural soils. Thus, this study evaluates the physical, biochemical and morphological effects of different concentrations ZnO nanoparticles under hydroponic conditions in order to evaluate their application to improve crop yield and as an alternative to traditional fertilizers.

Materials and methods

Chemicals

All reactives, zinc oxide (ZnO) nanopowder with a nominal primary particle size of 100 nm, (+)-Sodium L-ascorbate ($C_6H_7NaO_6$) ≥ 98%, Potassium phosphate monobasic (KH_2PO_4) ≥ 98%, Hydroxylamine hydrochloride ($NH_2OH·HCl$) ≥ 98%, Poly (vinylpolypyrrolidone) (C_6H_9NO) ~110 µm particlesize, Acetone (CH_3COCH_3) ≥ 99.5% and EDTA ($C_{10}H_{16}N_2O_8$) ≥ 99%, were obtained from Sigma-Aldrich®. NaClO was acquired as commercial bleach Clorox®.

Seeds treatment

Corn (*Zea mays*) and beans (*Phaseolus vulgaris*) seeds were acquired in a local market and sterilized by immersion in a NaClO 10% v/v solution with constant agitation during 10 min. Later, seeds were washed with deionized water until NaClO elimination.

Seeds germination assay

Corn and bean seeds previously sterilized, were germinated in glass flasks with a thin cotton bed saturated with a ZnO-NPs solution (0, 1, 5, 10, 50, 100 and 500 mg/L), containing 1.31 g/L of a commercial N, P and K fertilizer (Triple 17 Royal Garden´s®), to avoid additional stress by absence of another essential nutrient. Concentrations of N, P and K were calculated considering elemental Hoagland formulation. The assay was developed during 3 days with a photoperiod 12/12 (dark/light) and 25°C±2°C, adding 30mL of respective ZnO-NPs solution to each flask every day. Germinated seeds in each treatment were counted after exposure incubation.

Root/Shoot elongation and fresh biomass measurements

Finished the exposure time (15 days), seedlings of corn and bean were extracted from ZnO-NPs solutions, and cotton waste associated to roots was removed. Root and shoot elongations of each seedling was measured, and fresh biomass was determined by weight in aSartorius® analytical balance.

Chlorophyll assay

Corn and bean leaves were collected from seedlings grown in the presence of ZnO-NPs and were stored at 4ºC under dark conditions until analysis. From each ZnO-NPs treatment (0, 5, 10, 50, 100 and 500 mg/L), 50.0±0.5 mg of fresh leave were weighed in a Sartorius® analytical balance and macerated with 1 mL 80% v/v acetone in a mortar and transferred to a flask container with another 9 mL 80% v/v acetone and stored at 4ºC/ 60 min. Finally, extracts absorbance was measured at 646 and 663 nm in a722N Grating UV-Vis spectrophotometer®. Total chlorophyll content was calculated according to the method established by Lichtenthaler and Wellburn (1983) and applied by Gonzaález-Rodríguez et al. (2020).

$$c_b\left(\frac{\mu g}{ml\ plant\ extract}\right) = 12.21A_{663} - 2.81A_{646} \text{ Eq. 1.}$$

$$c_b\left(\frac{\mu g}{ml\ plant\ extract}\right) = 20.13A_{646} - 5.03A_{663} \text{ Eq. 2}$$

$$c_T\left(\frac{\mu g}{ml\ plant\ extract}\right) = C_a + C_b \text{ Eq. 3}$$

Where, C_a represents chlorophyll a, C_b is chlorophyll b, and C_T, total chlorophyll content.

Ascorbate Peroxidase Assay (APX)

Root extracts were obtained according to Ogunkunle *et al.* (2018) and Panfili *et al.* (2019). 0.50 ± 0.05 g of fresh root were homogenized in a relation 1:10 w/v in 5 mL potassium phosphate buffer 100 mM (pH 6), containing 2 mM EDTA, 2% w/v PVP, 4 Mm Dithiothreitol. Extraction was developed at 4°C on ice bath, and it was stored at 4°C until analysis.

For APX activity determination 100 µL of extract were homogenized with 4 µL 0.5 mM ascorbate, 10 µL 0.2 mM hydrogen peroxide and 886 µL 50 mM phosphate buffer (pH 7.4). Absorbance was measured to 290 nm each 15 s/2 min. Extinction coefficient employed (Ɛ) was 2.8 mM^{-1} cm^{-1}

Equations 2 and 3 were employed to obtain enzymatic activity in corn and bean root seedlings.

$$\Delta A\ min^{-} = \frac{Abs_f - Abs_i}{time\ reaction\ (min)} \quad \text{Eq. 2}$$

$$\frac{\mu mol}{mL\ min} = \frac{\frac{\Delta Abs}{min} * \mathrm{V}}{\in * \mathrm{V}} \quad \text{Eq. 3}$$

Where V denotes reaction volume in mL

3.7 Morphological Analysis

Roots samples were sectioned longitudinally and coated with gold in a fine coat ion sputter (JFC-100, JEOL) to examine them by Scanning Electron Microscope (JSM-6610LV, JEOL).

Statistical analysis

Each assay contained 3 replicates. Data was analyzed using software Minitab 18. Means of the data were analyzed using one-way ANOVA (p<0.05) and Fisher´s test.

Results and discussion

Seed germination

The germination capacity of a seed is based on whether or not it germinated, which is considered a qualitative attribute in the germination process and can be converted to a quantitative attribute, generally in percentage (%), and is defined as germination of a seed sample, the percentage of seeds that complete the process under experimental conditions. Seed germination represents a fundamental parameter to evaluate the possible phytotoxicity effects of ZnO nanoparticles on crops [55]. Germination is the first step in growth crops and Zn is an essential micronutrient used to protein synthesis, function membrane, cell elongation, enzymatic cofactor, and others [6,22,37,47,48].

Results presented in Figure 1 showed an increase of germination in beans and corn seeds, as an effect of ZnO nanoparticles into hydroponic medium respect to control group (0 mg/L), being higher effective in bean seeds (Figure 1A) in regard to corn seeds (Figure 1B), due that the highest germination of bean seeds set a value of 88% (25% over control group), and 53% in corn (18% over control group), both at 5 mg/L ZnO nanoparticles.

In seeds of cabbage, cauliflower and tomato, exposed to 0.12, 0.37 and 0.73 mg/L of ZnO nanoparticles by [45], was observed that cabbage germination decreased significantly at 0.12 mg/L; whereas in cauliflower at 0.12 and 0.73 mg/L of ZnO nanoparticles, seed germination was decreased. On the other hand, 0.37 mg/L increased germination regarding to control group. Finally, tomato seed germination was increased over control group at 0.73 mg/L. [8] observed that concentrations of 250 and 500 mg/kg of ZnO nanoparticles do not affect the germination process of alfalfa seeds in soil. However, concentrations of 750 mg/kg increase seed germination by 23% compared to their control group. [57] reported no inhibition of germination in corn seeds in a range of 10 to 1000 mg/L of ZnO nanoparticles. [16] observed no significant inhibition on germination index of wheat seeds treated with 10 mg/L to 1000 mg/L of ZnO nanoparticles. Same effect was observed by [44] at 15 mg/L to 500 mg/L.

Takahashi [46] determined that Zn transport during germination is regulated at the molecular level, due to Zn-induced protein binds to the α-amylase gene. The α-amylase is an involved gibberellin expression responsible for interrupting the dormancy of the seeds and making them germinate. In addition to this, they determined an upregulation of the gene encoding metallothionein involved in metal translocation, which is an important process during germination as well as starch degradation triggered by plant hormones and induction of proteins involved in respiration and photosynthesis. Therefore, the differences in the germination index between corn and beans may be due mainly to the quantity and type of expressed proteins that contribute to the germination process. On the other hand, the differences in the germination index between the concentrations used may be the result of a decrease in the absorption of the nutrients present in the medium such as N, P and K, at higher concentrations of ZnO nanoparticles, according to a study done by Peralta-Videa [35].

Root/Shoot elongation

In addition to the importance of Zn in germination, this micronutrient is important in the regulation of plant growth, as is the development of roots and aerial structures such as shoots and leaves as well as other parameters associated with plant growth. The results (Figure 2) were obtained through an analysis of comparison of means between the control groups (t-student, $P < 0.05$). They show that root development in corn seedlings was more accelerated compared to development root in beans with values of 4.9 ± 2.2 cm and 2.8 ± 1.1 cm respectively. For this comparison, only the control group was considered, since it represents a condition of natural development, without affectation in the presence of ZnO nanoparticles. In the specific case of the results obtained in root development in bean seedlings at different concentrations of ZnO nanoparticles (Figure 2A) it was observed that the concentrations of 1, 5 and 10 mg/L of ZnO nanoparticles induced an increase in root development compared to the control group ($P < 0.05$), reaching lengths of 5.6 ± 1.2, 3.5 ± 1.2 and 6.4 ± 1.0 cm respectively, while the control group recorded an average length of 2.8 ± 1.0 cm.

On the other hand, the concentrations of 50 and 500 mg/L of ZnO nanoparticles significantly decreased the development of root tissue ($P < 0.05$), registering 2.1 ± 0.8 and 1.8 ± 0.5 cm for each, not so 100 mg/L ($P > 0.05$).

In Figure 2B, corresponding to the root elongation in corn seedlings, it was observed that concentrations of 1, 10, 100 and 500 mg/L of ZnO nanoparticles favored root development significantly in relation to the control group (0 mg/L ZnO nanoparticles), with values of 8.0 ± 2.6, 9.4 ± 1.9, 10.3 ± 1.6 and 8.4 ± 1.6 cm respectively, while 5 and 50 mg/L significantly reduced root development.

In relation to shoot elongation, the results in bean seedlings (Figure 3A) indicated that only the concentrations of 1 and 10 mg/L of ZnO nanoparticles presented a positive effect on stem development with values of 27.2 ± 5.5 and 26.2 ± 4.3 cm, which was significant ($P < 0.05$) compared to the control group (17.7 ± 9.7 cm). On the other hand, the concentrations of 100 and 500 mg/L of ZnO nanoparticles affected the development of the stem, since values of 14.5 ± 3.3 and 5.7 ± 2.8 cm were observed. In corn seedlings (Figure 3B) only 10 mg/L of ZnO nanoparticles induced a higher stem growth, registering an elongation of 26.2 ± 4.3 cm, being significant ($P < 0.05$) in contrast to 17.7 ± 9.7 cm of the control group. Finally, the highest concentrations of 100 and 500 mg/L of ZnO nanoparticles, as in the case of beans, affected stem development with values lower than those reported by the control group ($P < 0.05$).

Rao and Shekhawat [38] observed that after 96 h of exposure to concentrations 500, 1000 and 1500 mg/L of ZnO nanoparticles, a decline in root length was induced in *Brassica juncea* seedlings. Yoon [54] indicated a 10 and 89% decrease in soybean root elongation exposed to 50 and 500 mg/kg of ZnO nanoparticles, coupled with a decrease in volume and root area, compared to the control group (0 mg/kg ZnO nanoparticle), while stem development was only affected at 500 mg/kg ZnO nanoparticles with a decrease of up to 76%. Bandyopadhyay [8] determined a 67% increase in soybean root elongation after a 250 mg/kg ZnO nanoparticle treatment. A study developed by Zhang [57] on corn seeds did not show any effects on root elongation at 10 mg/L and 100 mg/L of ZnO nanoparticles, whereas 1000 mg/L significantly inhibited root elongation. For wheat seedlings, Du [16] reported a root length reduction from 43% to 77% respect to control group in a range between 50 mg/L and 1000 mg/L of ZnO nanoparticles, and a shoot length inhibition from 13% to 18% between 100 mg/L and 1000 mg/L. In pearl millet seeds treated with 200 mg/L and 250 mg/L of ZnO nanoparticles was significantly improved the plant height around 35% over control group [30].

Considering then the root and shoot elongation data obtained in this study for bean and corn seedlings as well as the information reported by various authors on different plant species, it can be stated that the development of root and aerial tissue as shoot and leaves represent a primary indicator of nanoparticle toxicity, and such depends on the plant species since each one reacts differently to the presence of ZnO nanoparticles, with some species being more tolerant to concentrations above 500 mg/L, the maximum used in this studio.

In this regard, the results obtained indicated that, unlike corn, the bean turned out to be mostly sensitive to the concentrations of nanoparticles present in the hydroponic medium as far as root elongation is concerned. Considering there was an exposure to a concentration greater than 10 mg/L of ZnO nanoparticles, the growth of root tissue decreased, while concentrations of up to 500 mg/L favored the development of corn. However, not only is the root development important for a healthy growth of crops, but also the formation of other plant structures such as the stem, the leaves and, above all, the fruits. These processes are highly dependent on the signaling processes between the root and the stem. In this regard, Zn plays an important role in the transcription factor ZAT6, related to the growth and control of plant structures [14].

As mentioned above, stem development is also an indicative of the positive or negative effect for the implementation of ZnO nanoparticles as an alternative to the use of conventional fertilizers. Despite the fact that, in corn seedlings, concentrations of up to 500 mg/L of ZnO nanoparticles favored root development, in both cases 10 mg/L turned out to be the concentration with the best results in stem development, since possibly higher concentrations than this exert phytotoxic damage on internal organelles, an alteration in the synthesis of proteins, hormones, among others, which may compromise the development of the stem and, therefore, of agricultural production.

Fresh biomass production

The results of fresh biomass in beans (Figure 4A) indicated that biomass production was reduced at concentrations of 1, 100 and 500 mg/L which, in the case of 1 mg/L, contrasts with the results obtained of root and stem elongation, since at this concentration there was a significant increase in these parameters compared to the control group. The above could be due to a possible decrease in the diameters of the root hairs and of the stem itself, affecting the biomass, this effect has been reported by [54]. On the other hand, da [11] presented information regarding the dissolved fraction of Zn from ZnO nanoparticles due to the effect of root exudates, in which it was observed that at a concentration of 100 mg/L of ZnO nanoparticles in the medium there was a recovery of the 30% Zn, whereas at 1000 mg/L the recovery percentage only reached 2%. This effect was also mentioned by other authors [23]. Considering this information, it could be thought that at this low concentration of 1 mg/L of ZnO nanoparticles, the dissolution of Zn from the nanoparticles was favored, accelerating its uptake and inducing affectations in the development of the plant, resulting in a decrease in biomass.

In the case of 100 and 500 mg/L, phytotoxic concentrations of ZnO nanoparticles could be considered, because they exerted negative effects on biomass production compared to the control group (P <0.05), with values of 0.9 ± 0.3 and 0.8 ± 0.2 g respectively, and that is related to the affectations in root and stem development presented above.

Regarding the results obtained in corn seedlings (Figure 4B), and contrary to what was observed in beans, concentrations of 100 and 500 mg/L of ZnO nanoparticle favored biomass production significantly compared to the control group (P <0.05), having values of 1.3 ± 0.2 and 1.4 ± 0.2 g respectively.

Rao and Shekhawat [38] reported a decline in the fresh weight of *Brassica juncea* seedlings compared to the control group, when exposed to 200, 500, 1000 and 1500 mg/L. Wang [52] observed a decrease of 20% in the fresh biomass of *Arabidopsis* plants after an exposure of 200 mg/L and 80% for those treated with 300 mg/L. The same effect was observed by [13] by exposing Indian mustard seedlings to 200, 500, 100 and 1500 mg/L of ZnO NP. In addition to this information, it has been reported that Zn interferes with the uptake of K in soybean seedlings, which is an important element for the fixation of N_2 [35], recalling that the hydroponic medium of development in addition to ZnO NP, was made up of N, P and K. Therefore, this effect could possibly be most evident in bean seedlings, as it is a crop sensitive to nutrient deficiencies. In addition to this, there are two important differences between crops, and that is that in the case of corn, this represented a monocotyledon and C4 plant, whereas bean corresponds to a dicotyledon and C3 plant and therefore, their Zn requirements for metabolic processes differs. In addition to the differences in the nutritional requirements of the different species, and according to the literature, the expression of genes related to the transport of Zn such as ZIP, NRAMP, YSL and HMA is determined according to the type of plant species [11].

Chlorophyllassay

Chlorophyll is the major sensitive photosynthetic pigment in plants, and it was employed in this study in order to evidence positive effects or affectations in seedlings due to ZnO nanoparticles.

The analysis of the chlorophyll content in beans (Figure 5A) indicated that there was no significant difference (P> 0.05) between the total chlorophyll content of the control group (830.3 ± 169.6 μg/g) and the content of seedling chlorophyll developed in the presence of different concentrations of ZnO NP (0-500 mg/L), with values that were found in a range between 546.8 ± 62.8 μg/g and 764.8 ± 199.9 μg/g.

In the case of corn (Figure 6A), it was observed that a concentration of 10 mg/L of ZnO NP favored the production of chlorophyll reaching a value of 1802.6 ± 147.6 μg/g, which was significant with respect to the control group (P <0.05), which presented a value of 1490.0 ± 172.7 μg/g. On the other hand, concentrations of 100 and 500 mg/L of ZnO NP significantly decreased (P <0.05) the chlorophyll content, registering values of 1222.1 ± 179.4 μg/g and 1136.2 ± 129.8 μg/g, respectively.

Rao and Shekhawat [38] observed a decrease in the chlorophyll content in *Brassica juncea* leaves from a concentration of 1000 mg/L of ZnO NP. Wang [52] reported a decrease in the chlorophyll content in *Arabidopsis* leaves at 100, 200 and 300 mg/L, being more evident at 300 mg/L in approximately 50% compared to the control group. Chen [10] evaluated the phytotoxicity of ZnO nanoparticles in rice and determined that a decrease of chlorophyll contents was associated to a significant reduction in the expression level of CHLD, a chlorophyll synthesis gene. CHLD, encoded D subunits for Mg-chelatase, necessary to insertion of Mg into protoporphyrin during chlorophyll synthesis and, probably, accumulated Zn competed with Mg in this enzymatic centers, diminishing chlorophyll production [10,42]. In addition, it has been described that an excess of Zn can induce the loss of total chlorophyll, causing a disorganization of chloroplasts and a reduction in the number of thylakoids in grana [38]. According to [4,9], Zn is the main constituent of carbonic anhydrase, an enzyme that catalyzes the reversible hydration of CO_2 and its activity is directly related to photosynthesis rate and CO_2 fixation. In C3 plants as bean, there is no direct relationship between carbonic anhydrase activity and photosynthetic process. However, in C4 plants as corn, a high carbonic anhydrase activity is required in chloroplasts to shift the equilibrium to HCO_3^-, limiting photosynthesis.

Ascorbate peroxidase assay

Plants exposed to stress conditions as the presence of ZnO nanoparticlesin the middle of development, could suffer an imbalance process of reactive oxygen species production in their metabolism, generally considered as phytotoxicity. To protect from oxidative stress, plants could activate antioxidative enzymes as ascorbate peroxidase (APX) to catalyze the conversion of H_2O_2 to H_2O, enzyme that was evaluated in this study. The study was not carried out on seedlings of both crops at a concentration of 1 mg/L of ZnO NP because there was not enough fresh biomass.

Results presented in Figure 6 showed different patterns of root APX activity between bean and corn. In bean roots (Figure 6A), all ZnO nanoparticles treatments decreased significantly (P<0.05), the APX activity respect to control group 13.1±1.97 µmol/mL/min. Nevertheless, the lowest activities were determined at 100 mg/L and 500 mg/L with 3.19±0.22 µmol/mL/min and 3.95±0.72 µmol/mL/min respectively.

In corn (Figure 6B) a significative increase of activity at 5 mg/L of ZnO nanoparticles respect to control group (P<0.05) was presented, reaching 25.43±3.23 µmol/mL/min. Significative lowest activities (P<0.05), were determined at 10 mg/L and 50 mg/L with values of 5.71±0.4 µmol/mL/min and 3.43±0.4 µmol/mL/min respectively.

Rao and Shekhawat [38] reported a decrease of APX activity in roots of *Brassica juncea* growth in presence of 200, 100 and 150 mg/L of ZnO nanoparticles. Dogaroglu and Kôleli [15] determined that in roots of *Hordeum vulgare* L. exposed to 5, 10, 20, 40, and 80 mg/kg de ZnO NP there was a significant decrease in APX activity at 80 mg/kg ZnO compared to the control group. In contrast, an increase of APX in red bean was shown at 40, 60 and 80 µg/mL, indicating an increase of reactive oxygen species (Jahan *et al*., 2018). [2] in tomato roots exposed to 300, 600 and 1000 mg/L of ZnO nanoparticles, reported that the enzyme activity of APX at 600 mg/kg 1.5-fold was reduced by 1.5-fold and at 1000 mg/kg by 6-fold compared to control group.

The activity of antioxidant enzymes such as APX has been reported to increase due to the application of nanoparticles. However, the change in enzymatic activities depends on a series of factors such as germination processes, phenological stage, plant species, mechanism of uptake of nanoparticles, concentration, just to mention a few.

On the other hand, there are different reactive oxygen species (ROS) generated during a state of stress, as a consequence the activity of each enzyme will depend on the presence of its substrate. In addition, the APX enzyme is not the only way to respond to oxidative stress, since enzymes such as catalase (CAT) and superoxide dismutase (SOD) are capable, like APX, of switching hydrogen peroxide. In water, therefore, the increase or decrease in activities reported in this study, could be due to the effect of the activation of other enzymes that contribute to decrease oxidative stress. That is why it is important to evaluate more than one antioxidant agent or a product generated during stress in subsequent studies.

Morphological analysis

The effect of ZnO nanoparticles on corn and bean roots were examined by SEM. In bean roots developed at 5 mg/L and 500 mg/L, floem/xylem vessels were observed as ringed tubes (Figure 7), and as in corn roots, an increase of ZnO nanoparticles concentration in growth medium, favored alterations and loss of structures. In control group floem/xylem vessels were not localized.

In corn roots (Figure 8), it was observed that an increase of ZnO nanoparticles concentration in growth medium induced an elongation of vegetal structures until their total compaction to the center of roots and loss of natural structure, which indicated that in spite of not having significative effects on germination index, root elongation, shoot elongation and fresh biomass at 500 mg/L respect to control group, plants were severely damaged internally and, possibly, time exposure was not sufficient to observe negative effects over the other physiological parameters evaluated.

Lin and Xin (2008) observed the root of *Lolium perenne*, epidermal and cortical cells highly vacuolated or collapsed in presence of ZnO nanoparticles. Pokhrel and Dubey [36] reported an elongation in corn cells at a concentration of 100 mg/L of ZnO nanoparticles. This effect was reported as "tunneling-like effect". Regarding vascular bundles, such as those observed in beans, Pokhrel and Dubey [36] indicated that they can increase in number when there is a stress condition, mainly water or in the presence of metallic elements and their respective salts. Rao [38] indicated that *Brassica juncea* seedlings can capture ZnO nanoparticles, establishing the hypothesis that nanoparticles first adhere to root tissue and through the vascular system reach aerial tissues such as stems and leaves. Furthermore, they indicated that the inhibition of root growth leads to a decrease in the absorption of mineral nutrients and water, which in turn affects the vascular bundles as mentioned by Pokhrel and Dubey [36].

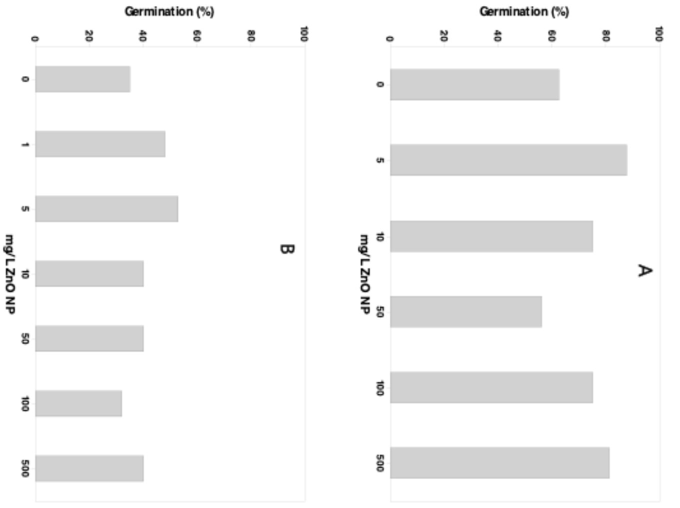

Figure 1: Germinated seeds (%) in **(A)** Bean and **(B)** Corn seeds exposed to 0 (control), 1, 5, 10, 50, 100 and 500 mg/L ZnO nanoparticles.

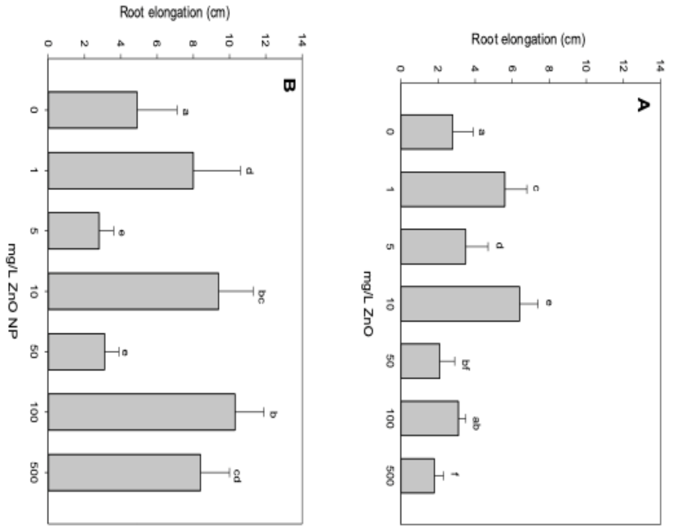

Figure 2: Root elongation in **(A)** Bean and **(B)** Corn seedlings in presence of 0 (control), 1, 5, 10, 50, 100 and 500 mg/L ZnO nanoparticles. Mean ± SD values followed by same letters within each column are not significantly different at α 0.05 (One-way ANOVA and Fisher´s test).

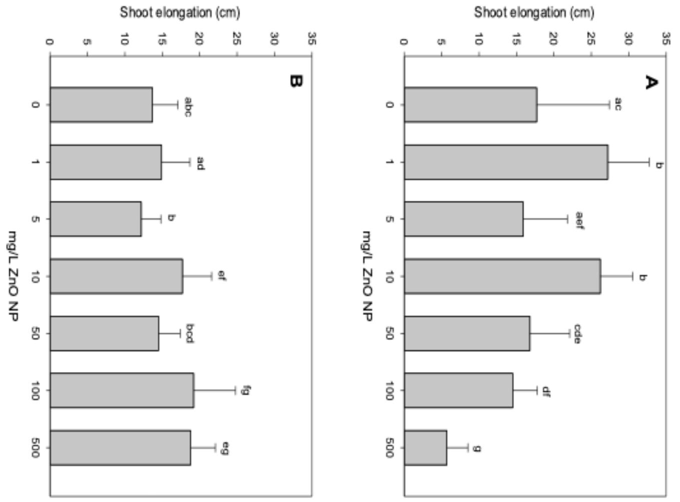

Figure 3: Shoot elongation in **(A)** Bean and **(B)** Corn seedlings in presence of 0 (control), 1, 5, 10, 50, 100 and 500 mg/L ZnO nanoparticles. Mean ± SD values followed by same letters within each column are not significantly different at α 0.05 (One-way ANOVA and Fisher´s test).

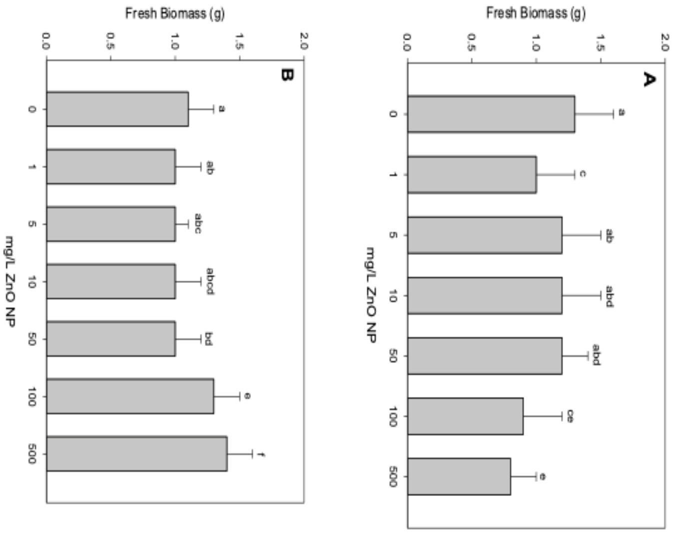

Figure 4: Fresh biomass production in **(A)** Bean and **(B)** Corn seedlings in presence of 0 (control), 1, 5, 10, 50, 100 and 500 mg/L ZnO nanoparticles. Mean ± SD values followed by same letters within each column are not significantly different at α 0.05 (One-way ANOVA and Fisher´s test).

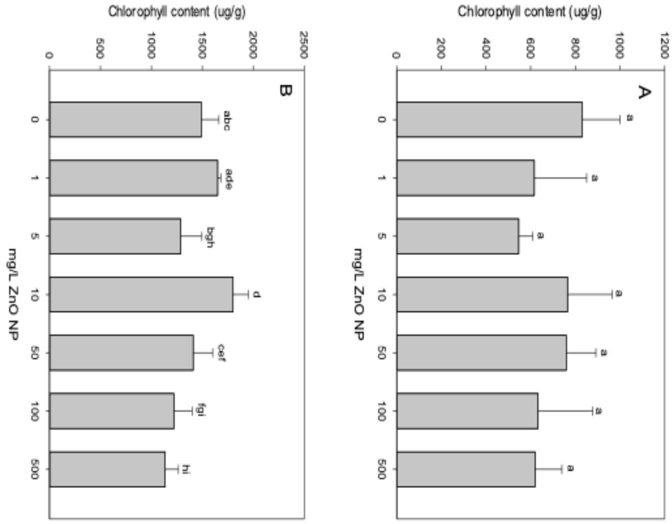

Figure 5: Total chlorophyll content in **(A)** Bean and **(B)** Corn seedlings in presence of 0 (control), 1, 5, 10, 50, 100 and 500 mgL-ZnO nanoparticles. Mean ± SD values followed by same letters within each column are not significantly different at α 0.05 (One-way ANOVA and Fisher´s test).

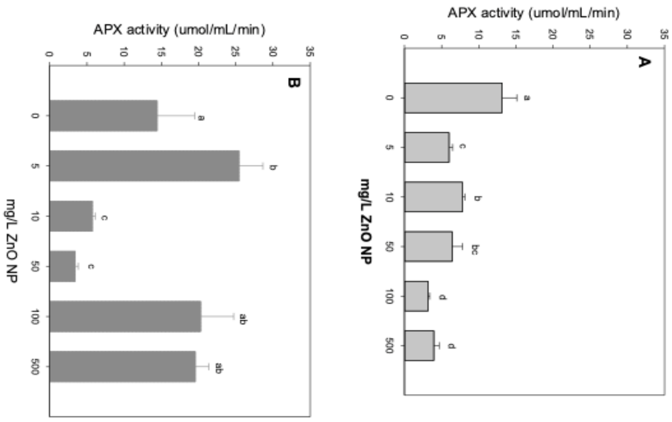

Figure 6: APX activity in **(A)** Bean and **(B)** Corn seedlings in presence of 0 (control), 5, 10, 50, 100 and 500 mg/L ZnO nanoparticles. Mean±SD values followed by same letters within each column are not significantly different at α 0.05 (One-way ANOVA and Fisher´s test).

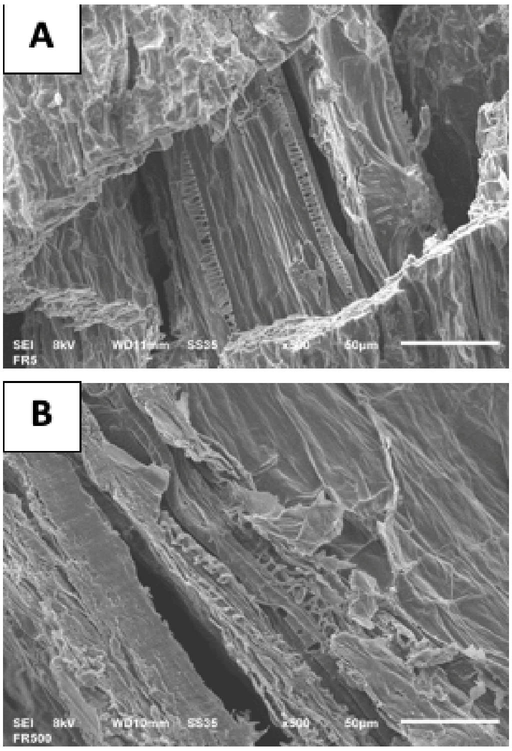

Figure 7: Scanning electron microscope images of bean root growth at **(A)** 5 and; **(B)** 500 mg/L ZnO nanoparticles.

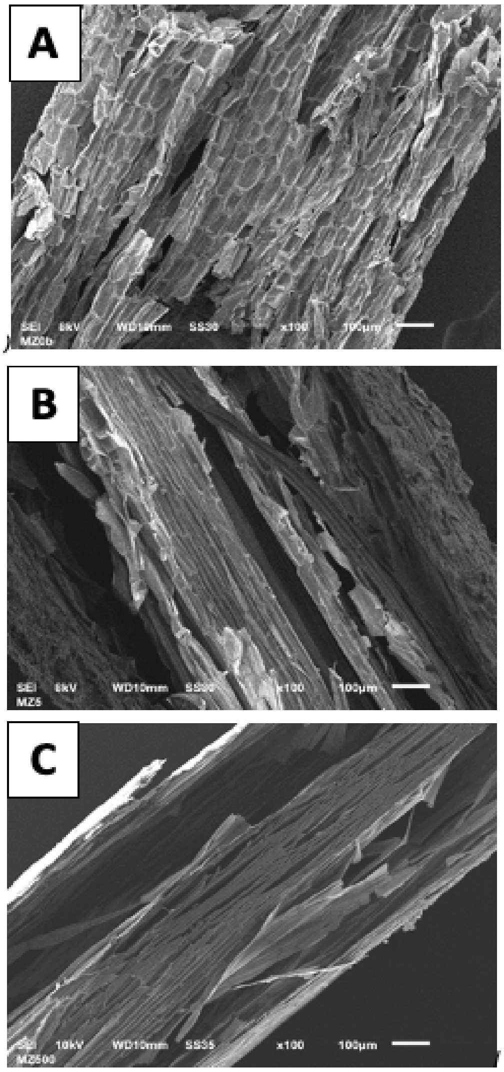

Figure 8: Scanning electron microscope images of corn root growth at **(A)** 0; **(B)** 5 and; **(C)** 500 mg/L ZnO nanoparticles.

Conclusions

The application of nanoparticles based on essential nutrients such as Zn in agricultural crops can represent an important alternative that contributes to the improvement of agricultural yields as well as reducing the use of traditional fertilizers whose formulations may contain elements that are toxic to human health and the environment. However, as observed in this study, each plant species responds differently to the presence of ZnO nanoparticles, either in germination, biomass production or elongation of plant tissues such as root and shoot, some being more sensitive (beans) or more tolerant (corn) at the same nanoparticle concentration used.

It is vitally important to carry out studies that contribute to the understanding of the mechanisms of the uptake of ZnO nanoparticles by different species; since this determines the effect internally generated in plant tissues, the foregoing, considering that the main damage in bean root is the destruction of the vascular bundles was observed, while, in corn, a "tunneling" effect was evidenced. On the other hand, the study showed that in order to obtain a better analysis regarding the oxidative stress that can possibly be induced by ZnO nanoparticles, it is necessary to evaluate more than one antioxidant enzyme or to include the analysis of certain metabolites or molecules that participate in the antioxidant response such as glutathione, ascorbate, malondialdehyde, among others. There is a long way to go in the study of nanoparticles for their application in agriculture, as well as a large number of questions to be resolved such as: how is it that organic acids or phytosiderophores favor the release of ions from the nanoparticle? Is there really competition in the absorption and accumulation between the essential elements and the nanoparticles present in the medium? Does the application of nanoparticles improve the quality of the fruits?, among others.

Conflict of interest

The authors declare that there are no conflicts of financial or personal interest.

Financial interest

The authors declare they have no financial interests.

Acknowledgements

Leura-Vicenciothanks CONACYT for the post-doctoral scholarship granted to finance the project.

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