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Zinc Oxide Nanoparticles Boosts Phenolic Compounds and Antioxidant Activity of *Capsicum annuum* L. during Germination

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Abstract: The effects of zinc oxide nanoparticles on seed germination and seedling growth of *Capsicum annuum* L. were determined in this research. Total phenols content, total flavonoids, and condensed tannins, as well as 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacity was determined. Results indicated that treatment with zinc oxide nanoparticles (ZnO-NPs) improved seed germination rate during the first seven days. The seed vigor germination increased 123.50%, 129.40% and 94.17% by treatment with ZnO-NPs suspensions at 100, 200 and 500 ppm, respectively. The morphological parameters tested revealed that ZnO-NPs treatments did not significantly affect plumule development, but they had a significant impact ($p \leq 0.01$) on radicle length. Suspensions at 100, 200 and 500 ppm of ZnO-NPs inhibited seedling radicle growth and promoted accumulation of phenolic compounds, with a phytotoxic effect in this organ. Results suggested that zinc oxide nanoparticles influence seed vigor and seedling development and promoted the accumulation of desirable phenolic compounds in the radicle.

Keywords: ZnO nanoparticles; total phenols; total flavonoids; condensed tannins; growth; pepper

1. Introduction

Nanotechnology has drawn the attention of researchers in recent years. The term "nanotechnology" may be defined as the design, synthesis, manipulation and application of atomic or molecular aggregates with a dimension between 1 and 100 nm [1]. The engineering methodology and processing that produce metallic nanoparticles (NPs) alter their physical and chemical properties, as well as their reactivity, due to its small size and high surface volume ratio [2]. This new nanomaterial technology has been applied to modern and sustainable agriculture practices as innovative synthetic pesticides and potential fertilizers in the new green revolution [3].

Consequently, development of a variety of nanoparticles (NPs) based on metals such as iron (Fe), copper (Cu), silver (Ag), zinc (Zn), and metal-oxide polymers such as zinc oxide (ZnO) and titanium

dioxide (TiO_2) for agricultural uses has created potential nanofertilizers [4]. Since ZnO plays a vital role in physiological and anatomical responses, zinc oxide nanoparticles (ZnO NPs) are mostly usedin agricultural applications [5]. ZnO participates in regulation of hormone metabolism: it modifies auxin levels through tryptophan biosynthesis, and it is essential for activation of several enzymes, such as superoxide dismutase and dehydrogenases [6]. ZnO-NPs could potentially be applied as bacteriostatic agents to control the spread and infection of pathogens [7], as well as to enhance seed germination, seedling vigor index, plumule and radicle lenght [8]. In recent years, the seeds germination promoting action of ZnO-NPs employing various plant species have been confirmed [9]. However, some authors have reported phytotoxic effects of ZnO-NPs [10].

Phytotoxicity is a main concern in the application of nanomaterials during seed germination and seedling growth [11], because of seed germination is particularly sensitive to stress conditions. Application of ZnO-NPs as nanofertilizers can produce toxic changes in biological activity related to metal-based NPs, such as production of reactive oxygen species (ROS) [12]. NPs induce oxidative stress in tissues, which may trigger their metabolism and result in increased production of secondary metabolites and improved antioxidant activity [13]. These phytochemicals are responsible of the neutralization of toxic free radicals and prevention of excessive oxidation reactions [14]. However, the phytotoxicity mechanism of ZnO-NPs during germination and seedling growth has not been established [15].

Recent tests on seed germination and growth of plants treated with different NPs types and concentrations have shown their possible short-term phytotoxicity [16]. However, no studies has specifically addressed the effects of ZnO-NPs on the biosynthesis of different types of secondary metabolites and their biological effect on seed germination and early growth of *Capsicum annuum* L. seedlings. Thus, this research employed *Capsicum annuum* L. as a model to define the effects of ZnO-NPs on seed germination, seedling growth, biosynthesis of phenolic compounds (total phenols, flavonoids, and condensed tannins), and DPPH antioxidant capacity of extracts from seedlings exposed to ZnO-NPs.

2. Materials and Methods

2.1. Plant Material

The *Capsicum annuum* L. variety employed in this research was San Luis (Hydro Environment, Tlalpan, Mexico). This variety produces large fruits (12 to 15 cm long on average). The plant is very vigorous, and its canopy provides excellent coverage. The harvest is semi-concentrated and uniform, and it is recommended for fresh market consumption and dehydration processing.

2.2. Characterization of ZnO-NPs

ZnO-NPs were purchased from Nanostructured and Amorphous Materials Inc. (Houston, TX, USA). NPs were characterized morphologically and structurally by transmission electron microscopy (TEM), high resolution transmission electron microscopy (HRTEM) and selected area diffraction (SAED) using a FEI-TITAN 80–300 kV microscope operated at a 300 kV acceleration voltage (FEI-TITAN 80–300 kV, Fisher Scientific, Hillsboro, OR, USA). TEM and HRTEM micrographs were processed by Fast Fourier transformation software (Digital Micrograph 3.7.0, Gatan Software, Pleasanton, CA, USA).

2.3. Treatments

ZnO-NP suspensions were prepared at 100, 200 and 500 ppm based on the studies carried out by Sheteiwy et al. [17] and Salah et al. [18]. To ensure uniform dispersion, all suspensions were sonicated with a Q500 sonicator (Qsonica Newtown, CT, USA) for 25 min at 120 volts-3 amps, and 50 to 60 Ghz. Preparation process of the suspensions with ZnO-NPs was carried out based on a methodology already established by Reddy et al. [19].

2.4. Seed Germination and Seedling Growth

Seeds were counted in lots of 100 per treatment, and placed in a Petri dish (15 cm × 20 cm), above two layers of filter paper (Whatman, 90 mm diameter) using tweezer. Four treatments were applied as ZnO-NPs suspensions, 0 ppm (T1) (control, treated with distilled water), 100 ppm (T2), 200 ppm (T3), 500 ppm (T4). Ten milliliters of each ZnO-NPs suspension treatment were taken with a pipette (Pipetman Classic 1–10 mL) and added to the seeds for imbibition. The Petri dishes were then placed for 72 h in a growth chamber (Equitec model EGCS 3S, 301 3SHR, Equitek Guadalajara, Mexico) at 25 °C ± 1 °C under a 16 h:8 h light:dark photoperiod.

Once the seed imbibition period was completed, four replications of 25 seeds were planted per replication for each treatment on a sheet of Anchor paper (Seedburo Equipment Company, Des Plaines, IL, USA). The paper was moistened with distilled water, and the seeds placed horizontally on the paper, ensuring that the embryo was oriented downwards. Then another sheet of paper was moistened to cover the seeds. Subsequently, the sheets were folded into a roll, about 4 cm wide. Once rolled, the sheets were placed in polyethylene terephthalate baskets (20 cm × 45 cm). The baskets were placed back into an EGCHS 301 test chamber (Equitec, Madrid, Spain) at 25 °C and 75% relative humidity for 14 days.

The bioassay followed the International Seed Testing Association (ISTA) protocols [20], with some modifications. Germination vigor (V%) was calculated as percentage of normal seedlings from the total number. Germination rate (G%) was determined as percentage of germinated seeds from the total seed number. Both variables were recorded at 7 and 14 days after sowing. A normal seedling was considered when it had at least a 2.0 cm radicle and plumule length. Plumule length was measured from the radicle-hypocotyl intersection to the base of the cotyledon, while radicle length was measured from the base of the hypocotyl to the apex of the radicle. Dry biomass weight per replication was determined 14 days after planting with an analytical balance and expressed in mg seedling^{-1} according to Corral-Diaz et al. [21].

2.5. Extraction of Free Phenolic Compounds

Dry biomass accumulation was used to quantify phenolic compounds and to measure the antioxidant activity according to Lopez-Contreras et al. [22]. Dry plumule and radicle tissues were milled and sieved to obtain a particle size $\leq$0.5 mm (mesh 35). One hundred milligrams of sample were homogenized with 3 mL of 80% methanol in a screw cap culture tube, and stirred for 1 h at 200 rpm. After that, samples were centrifuged at 4500× *g*, supernatants were recovered and stored at −20 °C until they were used for phenolics and antioxidant capacity analysis.

2.6. Total Phenols Content

For this procedure, 0.2 mL of the extract were taken, and 2.6 mL of distilled water and 0.2 mL of Folin–Ciocalteu reagent were added. After 5 min, 2 mL of 7% Na_2CO_3 were added and the solution stirred for 30 s. The solution was placed in the dark for 90 min, and the absorbance at 750 nm was measured. Total phenols content was reported in milligrams of gallic acid equivalent per kilogram of sample (mgGAE kg^{-1}), calculated from gallic acid calibration curve from 0 to 200 mg L^{-1}.

2.7. Total Flavonoid Content

Determination of total flavonoid content was based on the reaction of the $AICI_3$-$NaNO_2$-NaOH complex, where 0.2 mL of the extract were taken, and 3.5 mL of distilled water were added. Then 0.15 mL of 5% $NaNO_2$, 0.15 of 10% $AlCl_3$ and 1 mL of 1 M NaOH were added too, at 5 min intervals each. The reaction was left for 15 min and after that, the absorbance at 510 nm was measured. Total flavonoid content was reported in milligrams of catechin equivalent per kilogram of sample (mgCatE kg^{-1}), calculated from catechin calibration curve from 0 to 200 mg L^{-1}.

2.8. Condensed Tannins Content

Condensed tannins content was determined by the vanillin-H_2SO_4 complex reaction. From the extract, 0.25 mL were taken and 0.65 mL of 1% vanillin were added; afterwards, 0.65 mL of 25% H_2SO_4 were added (vanillin and H_2SO_4 were dissolved in methanol). The reaction was carried out for 15 min at 30 °C, and the absorbance at 500 nm was measured. Condensed tannins content was reported in milligrams of catechin equivalent per kilogram of sample (mgCatE kg^{-1}), calculated from catechin calibration curve from 0 to 200 mg L^{-1}.

2.9. Determination of DPPH Antioxidant Capacity

Antioxidant capacity was measured by reduction of the absorbance of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical in the presence of the extract. A 60 µM DPPH working solution was prepared in 80% methanol and its absorbance was 0.95 at 517 nm. The samples were tested by mixing 0.5 mL of the extract with 1.5 mL of the DPPH working solution. The reaction was allowed to continue for 30 min and its absorbance reading taken. Antioxidant capacity was reported in millimols of Trolox equivalent per kilogram of sample (mmolTE kg^{-1}), based on previously built calibration curve with Trolox at concentrations from 0 to 500 mmol L^{-1}.

2.10. Statistical Analysis

The experiment was setup using a completely randomized design with four treatments and four replications. The general linear model was $Y_{ij} = \mu + \alpha_i + e_{ij}$; where Y_{ij} = the response variable; μ = general mean; α_i = effect of ZnO-NPs concentrations; e_{ij} = experimental error. The experimental unit was a roll (folded Anchor paper) with 25 seeds. Results were reported as mean ± standard deviation of four samples. The statistically significant difference between samples was analyzed with a one-way ANOVA and treatment means were compared with a Tukey Test ($p \leq 0.05$) using SPSS statistical package version 21.0 (SPSS Inc., Chicago, IL, USA)

3. Results

3.1. Characterization of ZnO-NPs

Figure 1a shows a typical TEM image for a ZnO-NPs sample. The morphology of NPs is quasi-spherical, as the micrograph shows. These NPs appear well dispersed within the TEM grid. Figure 1b shows a characteristic HRTEM image of a nanoparticle. In this image, lattice stripes are clearly observed with a spacing of 2.81 Å, that correspond to the lattice planes of a hexagonal zinc oxide structure. Figure 1c shows the size distribution of the NPs calculated from more than 300 particles.

Most of the particles in this sample (~75%) have diameters between 12 and 24 nm, and ~30% are larger than 12 nm and smaller than 20 nm. Figure 1d shows a common SAED pattern of ZnO sample. This pattern shows irregular rings associated with planes (100), (002), (102) and (110) of the crystal structure of zinc oxide. The SAED pattern was associated to a crystalline phase of zinc oxide.

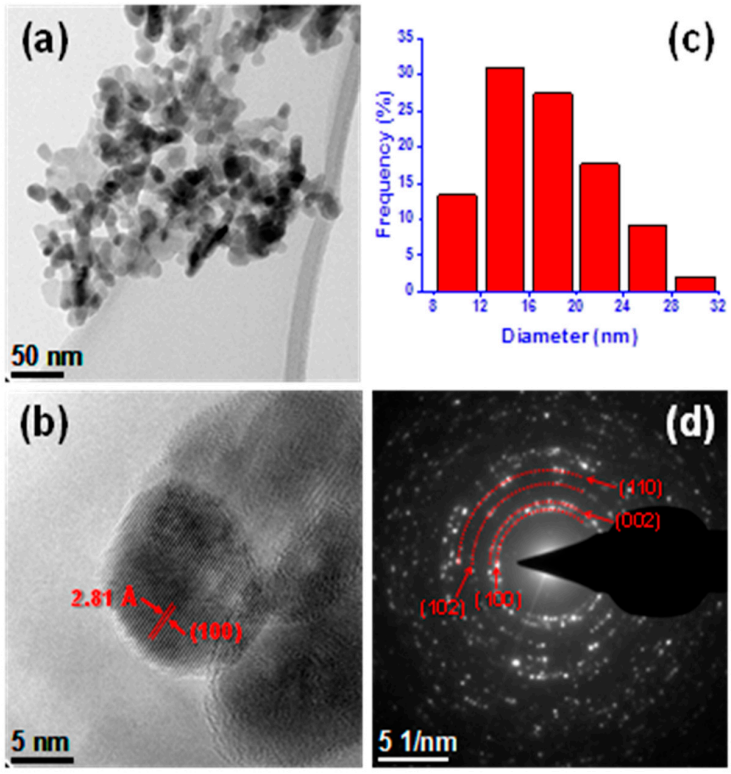

Figure 1. The micrographs show a zinc oxide nanoparticles (ZnO NP) sample. (**a**) Typical transmission electron microscopy (TEM) image of a nanoparticle, (**b**) Typical high resolution transmission electron microscopy (HRTEM) micrograph of a nanoparticle, (**c**) Size distribution of NPs (**d**) Typical selected area diffraction (SAED) pattern obtained for the area observed in panel (**a**).

3.2. Effect of ZnO-NPs on Germination and Vigor

The Figure 2B shows that germination percentage had no significant statistical differences ($p \leq 0.596$). However, germination vigor on the seventh day showed significant effects ($p \leq 0.05$): vigor at 100 and 200 ppm was 50.66% and 52.00%, respectively (Figure 2A). These values increased over the control treatment (0 ppm) by 123.5% and 129.4%, respectively. At a 500-ppm concentration, the germination vigor decreased by 15.4% in comparison to the highest performing treatment (200 ppm). These results indicate that ZnO-NPs application at 100 ppm and 200 ppm increase the emergence speed of the plumule and radicle during the first seven days. However, this response depends on concentration, but at 500 ppm of ZnO-NPs a decrease in the vigor was observed.

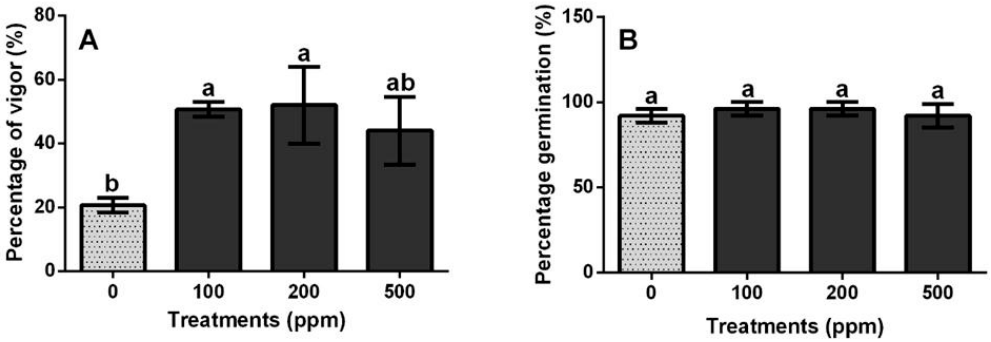

Figure 2. Vigor (**A**) and germination (**B**) percentages in *Capsicum annuum* L. seeds treated with ZnO-zinc oxide naoparticles (ZnO-NPs) at different concentrations (0, 100, 200 and 500 ppm). The bars represent the mean ($n = 4$) ± standard deviation. Different letters are statistically different (Tukey, $p \leq 0.05$) and represent the differences between treatments.

3.3. Effect of ZnO-NPs on Seedling Growth

The Table 1 shows the effect of ZnO-NPs on variables associated to seedling growth. Plumule length was not significantly affected ($p \leq 0.724$). However, radicle length responded significantly ($p \leq 0.01$) to ZnO-NPs doses: as the concentration increased, radicle length decreased by 50.0% (100 ppm), 53.9% (200 ppm) and 54.5% (500 ppm). The same trend was observed for dry biomass weight. Even though the treatments were statistically similar ($p \leq 0.155$), dry biomass accumulation gradually decreased from 100 to 500 ppm (Table 1). The 500-ppm treatment caused the highest biomass reduction with 8.5% to the control treatment (0 ppm). This result indicates that radicle growth is highly affected by ZnO-NPs treatments (Figure 3).

Table 1. Effect of zinc oxide naoparticles (ZnO-NPs) on plumule and radicle length, and fresh seedling weight of *Capsicum annuum* L.

Treatments (ppm)	Plumule Length (cm)	Radicle Length (cm)	Dry Seedling Biomass (mg Seedling^{-1})
0	3.29 ± 0.37 [a]	11.32 ± 2.48 [a]	45.73 ± 3.79 [a]
100	3.65 ± 0.30 [a]	5.66 ± 1.40 [b]	44.13 ± 0.88 [a]
200	3.46 ± 0.39 [a]	5.22 ± 1.65 [b]	42.87 ± 4.57 [a]
500	3.41 ± 0.30 [a]	5.15 ± 1.47 [b]	41.84 ± 5.02 [a]

Values are the average of four replications. Means (n = 4) ± standard deviation. Different letters are statistically different (Tukey, $p \leq 0.05$) and represent the differences between treatments.

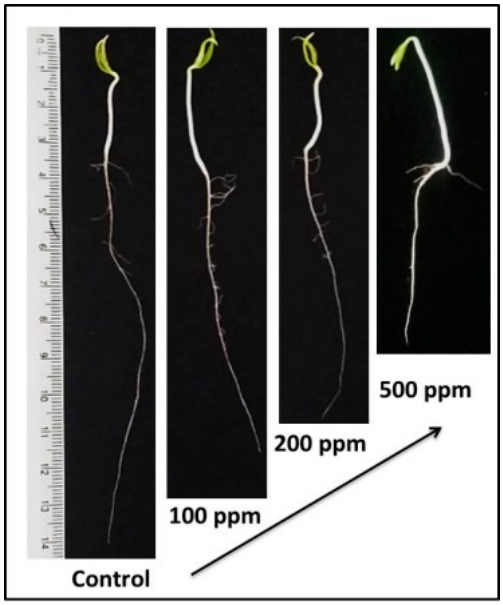

Figure 3. Effect of zinc oxide naoparticles (ZnO-NPs) on growth of *Capsicum annuum* L. seedlings. Each seedling represented an experimental unit.

3.4. Effect of ZnO-NPs on Phenolic Compounds Content and DPPH Antioxidant Activity

The results indicate that the phenolic compounds content in the plumule were not affected by the application of ZnO-NP to the seeds (Figure 4). On the contrary, there was a significant effect ($p \leq 0.01$) in the accumulation of phenolic compounds in the radicle since the increase of ZnO-NPs from 100 to 500 ppm, stimulated the accumulation of total phenols from 350.84 ± 23.99 (100 ppm), 493.80 ± 2.86 (200 ppm) and 539.11 ± 159.70 mgGAE kg^{-1} (500 ppm) (Figure 4A), exceeding the accumulation in the control treatment (0 ppm) in 148.5%, 249.9% and 282%, respectively.

This behavior was also observed for the content of total flavonoids and condensed tannins (Figure 4B,C), in radicles exposed to ZnO-NPs. Plants treated with 500 ppm ZnO-NP accumulated the

highest concentration of total flavonoids and condensed tannins with 331.84 ± 25.34 mg CatE kg^{-1} and 191.95 ± 2.86 mg CatE kg^{-1}, respectively, and exceeded for 164.4% and 627.7% the control treatment.

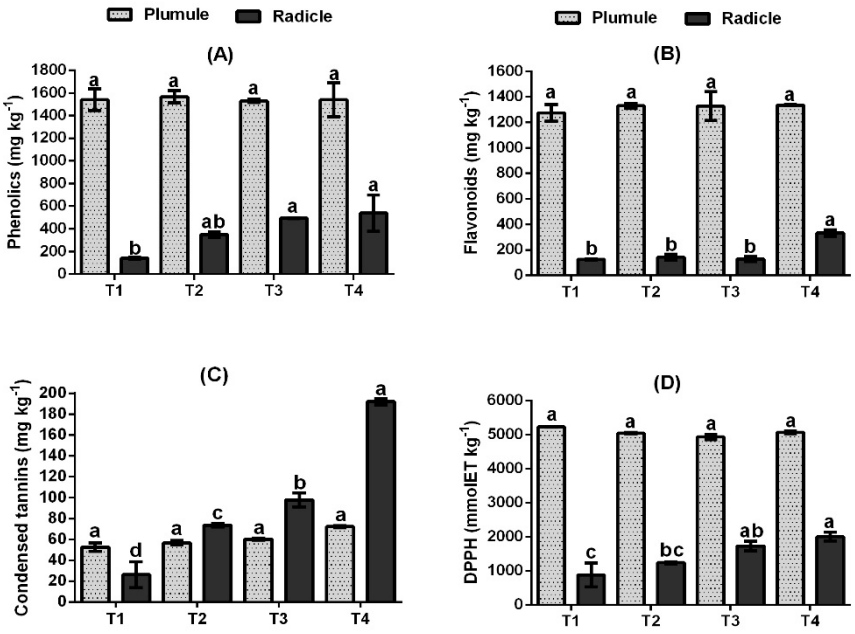

Figure 4. Phenolic content in *Capsicum annuum* L. seedlings treated with ZnO NPs. (**A**) Total phenols, (**B**) Flavonoids, (**C**) Condensed tannins (**D**) Antioxidant capacity (DPPH) (millimole equivalents of Trolox per kilogram of sample (mmolET kg^{-1}). Values are the average of four repetitions. Means (n = 4), bars represent the standard deviation of the mean; Different letters are statistically different (Tukey, $p \leq 0.05$) and represent the differences between treatments.

Figure 4D shows the results for the antioxidant capacity determined by the DPPH method. The DPPH antioxidant capacity values were 4932 ± 73.85 mmolET kg^{-1} to 5231 ± 15.23 in plumule and there were not statistical differences among treatments. On the other hand, the antioxidant capacity of the extracts from the radicle showed significant differences ($p \leq 0.01$). The higher values were found in the 500 ppm of ZnO-NPs treatment, with 2002.14 ± 127.88 mmol TE kg^{-1}, and this treatment was 129.1% higher than the control treatment (0 ppm). This behavior is similar to the one observed with total phenolic compounds in the radicle; the highest concentration was obtained with the 500 ppm ZnO-NPs treatment.

4. Discussion

Increased emergence rate of shoots is caused by an enlarged concentration of indole acetic acid (IAA), as a result of water and nano nutrients filling the space between a selective permeable membrane under the seed coat and the intracellular space in the parenchyma of the seed coat. In turn, these events may increase seed vigor in the first days of germination [23]. Additionally, the beneficial effects of ZnO-NPs during germination leads to hormone biosynthesis, especially auxins and gibberellins, that promotes the degradation of seed reserves and increased seed vigor [24]. The improvement of characteristics associated to physiological quality of the seed may be attributed to NPs inducement of photosensitization reactions and photo-generation of active oxygen such as superoxide and hydroxide anions. These reactions stimulate the ion penetration and promote water and oxygen imbibition, necessary for rapid germination [25]. Studies carried out in seeds and seedling of ragi (finger millet) showed the effects of ZnO-NPs as an improvement in vigor of 45.54% with treatments of 1000 ppm was reported, while at 1500 ppm a significant reduction of this parameter was observed [26]. Similar results were found in *Capsicum annuum* L. seeds exposed to 0.75 g of ZnO-NPs, increasing vigor index significantly (67%) [27]. In this study, concentrations of ZnO-NPs in a range of 100 to 500 ppm affected

negatively the root growth and also led to an increase in non-enzymatic antioxidant tests, total phenols, flavonoids and condensed tannins. The response and accumulation of these compounds in the root was depended on the concentration of ZnO-NPs. Similar results were reported applying ZnO-NPs to seeds of *Brassica nigra*, obtaining a negative effect in the root growth and resulted in an increase of 79% of DPPH radical scavenging activity and an increase in phenolic compounds was also obtained [9].

In potato plants, the application of ZnO-NPs increased the total phenolic compounds the increase was of 1%, 20% and 22%, respectively, at concentrations of 100, 300 and 500 ppm, respectively [28]. Similar results, in the concentration of phenolic compounds were detected in an extracellular medium which increased 127.5% and 22.1%, respectively, in *Arthrospira platensis* (*cyanobacteria*) and *Haematococcus pluvialis* (*microalgae*) after treatments with 100 mg L^{-1} of TiO_2NP. The increases were associated with oxidative stress due to the production of hydrogen peroxide [29].

Although it has been demonstrated that the application of NPs ZnO affects the physiology and biochemistry of plants, there are still generalized debates and ambiguities regarding the effects on the secondary metabolism of the plant [15]. Mainly, the effect of NPs on the phenolic compounds, since these molecules play important roles in the yield and adaptation of the plant, in response to biotic and abiotic stresses [30]. Therefore, it is a priority to understand the integral functioning of the secondary metabolism of the plant, in response to the application of NPs as a possible generator of oxidative stress.

The effect of ZnO-NPs during germination and seedling growth has been reported previously, however, results report positive effects, as well as phytotoxic effects. Response variability is mainly due to NP nature, size, concentration and genotype of the evaluated species [31]. In the present study, application of ZnO-NPs affected radicle development. It must be mentioned that radicle growth index is widely used as an indicator of phytotoxicity, since it is a highly sensitive to stress conditions [32]. The radicle is the first structure of the seed embryo and is therefore the first tissue exposed to excessive concentrations of ZnO-NPs [12]. For this reason, the adverse effects observed were more evident in radicles than in shoots. This suggests that radicle elongation was more sensitive to ZnO-NPs toxicity than shoot elongation, the differential sensitivity might be explained by direct exposure of radicles to ZnO-NPs suspensions [33].

The variation in radicle growth could be caused by the presence of ZnO-NPs or dissolved zinc ions interfering with radicle biochemistry and physiology. This interference promotes variation in radicle length and dry biomass accumulation. Studies in radish, lettuce, cucumber and arabidopsis have mentioned inhibition and variation in radicle growth and are agree with our results [34–36].

This inhibition might be attributed to two main effects: (1) a chemical toxicity dependent on release of (toxic) ions, such as penetration of nano-size particles and dissolution of Zn^{2+} ions from ZnO; (2) stress or stimulus caused by the surface, size or shape of the particles [36]. Toxicity at high concentrations is in accordance to the Shelford´s tolerance law. Reduction in radicle length and plant biomass might be a consequence of toxicity caused by nanoparticle absorption by the plant from the media and intra or intercellular accumulation [37].

Studies using ZnO-NPs reported phytotoxicityin germination of corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) seeds [38]. The results indicated inhibition of radicle development by the presence of ZnO-NPs. Additionally, the potential toxicity of iron (Fe) NPs using three types particle sizes (1 to 20 nm) on germination of barley and flax was studied [39]. Iron NPs affected germination, but shoot growth was more sensitive to them. On the other hand, some seedlings subjected to high concentrations of CuO NPs suffered a reduction in relative growth rate, modified gene expression, and alter the generation of reactive oxygen species [40].

In this study, the results indicate that ZnO-NPs treatments affected only the morphology of the radicle, due to a phytotoxic effect that caused oxidative stress and resulted in a greater activation of secondary metabolism in this tissue [41]. The link between ROS and secondary signaling messengers that lead to transcriptional regulation of the secondary plant metabolism should be considered, as it plays a vital role in the plant response to NPs treatment [15].

Greater accumulation of secondary metabolites in the radicle, is the result of increased ion release from ZnO-NPs (Zn^{2+}), their rapid absorption and solubility during germination and accumulation on the radicle surface, and NPs entrance into the radicle cells that generate excessive ROS accumulation [42]. The effect of ZnO-NPs on radicles can be explained in two ways: (1) the role of zinc in biochemical process, and (2) the role of ROS [9]. Although zinc is an important element for many metabolic processes, at high concentration it interferes with the cell division and induces oxidative stress [43].

A common consequence of damaging ROS levels is damage to cellular macromolecules, including membrane lipids, which leads to cell death and decreases shoot growth [44]. Metallic NPs can also damage other macromolecules such as DNA and affect cell division [45]. In addition, it was affirmed before that nanoparticles trigger alterations in Ca^{2+} and ROS levels involved in cell signaling and complex morphological, physiological and biochemical modifications at the organism level [46].

Plants activate enzymatic and non-enzymatic antioxidant defense mechanisms to eliminate excess of ROS that mitigate the effects of oxidative stress caused by ZnO-NPs. Activation of secondary plant metabolism is the main defense mechanism, and synthesis of phenolic compounds is crucial [17]. Phenolic compounds play a prominent role in ROS detoxification and their concentration may vary significantly [47] and they also act as electron donors in detoxification mechanisms in organelle structures [48].

During the stress caused by heavy metals, phenolic compounds act as metal chelators and on the other hand, can directly remove molecular species of active oxygen, mainly due to their redox properties, which can play an important role in the absorption and neutralization of free radicals, the extinction of singlet and triplet oxygen or the decomposition of peroxides [49]. Therefore, increases in antioxidant activity of plants exposed to NPs, is mainly due to the increase in phenolic compounds, which are powerful scavengers of ROS and are also capable of inhibiting enzymes that produce free radicals [50]. However, its antioxidant action lies mainly in its chemical structure. The main classes of phenolic compounds are defined according to their carbon skeleton (phenolic rings and chemical groups attached to them) in phenolic acids, flavonoids, stibenes and lignans, being the first two the most common in nature. Several thousands of phenolic compounds have been identified in plants and although is almost impossible to know the nature of all the phenolics present in a single sample, it is desirable to know the main classes of phenolic compounds present in it [51]. These compounds have several functions in plants, one of the main ones is the improvement of the phenylpropanoid metabolism and the content of phenolic compounds depends on the different environmental factors and stress conditions [52]. For example, the synthesis of isoflavones and some other flavonoids is induced when plants are infected or injured, most of them have antimicrobial activity. On the contrary, the induction and biosynthesis of phenolic compounds is related to the stress caused by heavy metals, this may explain the discrepancy of our results, since the greatest accumulation occurred in the phenolic compounds [53].

General chelating ability of phenolic compounds is probably related to the high nucleophilic character of the aromatic rings rather than to specific chelating groups within the molecule [54]. However, when the cellular concentration of ROS increases due to biotic and abiotic stresses, the balance of antioxidant defense systems in plants can be inhibited or modulated to avoid the oxidative stress that causes lipid peroxidation [55], this explains the maximum accumulation of phenols, flavonoids and tannins in the radicles exposed to the highest concentration (500 ppm).

It has been reported that high concentrations of Zn can increase phenol content in all parts of the *Coriandrum sativum* L., including leaves and stems, but mainly in radicles [56]. Radicle serve as physical and chemical barriers to biotic and abiotic stressors and provide antioxidants to scavenge excessive ROS production [57]. These compounds might act in synergy, and that could explain the trends observed in phenolic compounds and antioxidant activities [58]. Similar patterns have been obtained for *Stevia rebaudiana* Bertoni [59], also for the antioxidant activity of *Salvia officinalis* L. [60] and in the radicle of *Brassica nigra* [9]. Based on the above results, our study pioneers in the evaluation

of ZnO-NPs phytotoxicity in *Capsicum annuum* L. and it serves as guide to measure effects on early seedling growth.

5. Conclusions

This study reveals the effect of ZnO-NPs at different concentrations on germination, vigor and early growth of *Capsicum annuum* L. seedlings. All concentrations ZnO-NPs (100, 200 and 500 ppm) treatments, significantly inhibited the radicle development due to a phytotoxic effect and led to increased accumulation of phenolic compounds and antioxidant activity in the radicle. Changes induced by ZnO-NPs in the physiology and biochemistry of plants may bring production of desirable secondary metabolites. However, it is necessary to evaluate the effects of ZnO-NPs to understand the specific changes they cause in plants in future studies.

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