Sanchez-Perez DM *et al.* (2025)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 53, Issue 1, Article number 14103
DOI:10.15835/nbha53114103
Research Article

The application of biosynthesized ZnO nanoparticles enhances the morphological and physiological indices of serrano pepper plants

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Abstract

This research aimed to evaluate the effectiveness of the biosynthesized spherical zinc oxide nanoparticles (BZnONPs) applications on morphological and physiological indices of serrano pepper plants cv. 'Chiser-522'. The treatments applied by foliar spray every 15 days from 20 days after transplantation consisted of 10, 20, 30, 40, and 50 ppm of BZnONPs and control (distilled water). A completely randomized design was used with six treatments and fifteen replicas per treatment. Morphological and physiological characteristics such as crop growth rate (CGR), net assimilation rate (NAR), leaf area index (LAI), photosynthetic pigments, and phytochemical compounds were evaluated. The results indicated that the plants treated with 30 and 40 ppm of BZnONPs had higher height, thicker stems, longer roots, and higher CGR, NAR, and LAI than control. Their amounts of photosynthetic pigments and antioxidant compounds were also increased compared to those of the other treatments. Therefore, we conclude that BZnONPs are promising technology that significantly influences chili pepper cultivation's physiological and morphological development at low-level exposures.

Keywords: antioxidants; bioactive compounds; chili plants; plant physiology

Introduction

Chili peppers are one of the most critical cultivars in Mexico, with an economic impact of 20 million dollars per year. In 2023, Mexico produced 237 million tons of green chili, giving it fourth place in global chili production (Hernández-Pérez *et al*., 2020; Ministry of Agriculture and Rural Development, 2023). Jalapenos

Received: 05 Sep 2024. Received in revised form: 12 Dec 2024. Accepted: 16 Dec 2024. Published online: 21 Feb 2025.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

and serrano's are among the most consumed chilies. The 'Chiser-522' is a free pollination variety of 'Chile Soledad' or 'Serrano Delgado', developed by the Genetic Improvement Program of INIFAP (Ramirez-Meraz *et al*., 2019). This variety presents with vigorous plants 90 to 130 cm in height, an intermediate cycle of 80 days to flowering and 110 days to the beginning of harvest, and an average yield of 20.5 t/ha under fertilization. The fruit is uniform and brightly emerald-colored, with an average mass, size, and diameter of 4.7 g, 8.2 cm, and 1.1 cm, respectively. The 'Chiser-522' variety has resistance to a bacterial stain (*Xanthomonas campestris* pv *vesicatoria*) and a fungal pathogen (*Oidiopsis taurica*) (Ramírez-Seañez *et al*., 2023). Therefore, this variety has a high productive potential, and studying its farming conditions and practices is essential to improve it.

In recent years, nanotechnology has gained importance in agriculture because it provides multiple benefits, including supplementing plants with the nutrients necessary for their growth and development (Cerqueira *et al*., 2017). However, one limitation of nanomaterials is their production method, which involves using chemical substances as precursors and reducing/stabilizing agents that often generate toxic and pollution-creating waste (Akbar *et al*., 2020). To replace these harmful substances, the so-called green or biological synthesis that uses plant or microbial extracts arose as a promising alternative to obtaining nanomaterials (Rad *et al*., 2019; Selim *et al*., 2020; Sharma *et al*., 2021). One of the nanomaterials most used in recent years for agricultural applications is zinc oxide (ZnONPs) (Asmat-Campos *et al*., 2022; Matinise *et al*., 2017; Salama *et al*., 2019). Zinc is an essential micronutrient to the crop that takes a crucial role in the physiological and anatomical responses of the plant (Benáková *et al*., 2017; Kloubert and Rink, 2015). Recent studies (Jaithon *et al*., 2024; Landi *et al*., 2020; Ogunyemi *et al*., 2019) show that ZnONPs obtained by biosynthesis (BZnONPs), compared to those obtained by other methods, generate more significant growth-promoting action in plants due to their participation in the regulation of hormonal metabolism (Abdelaziz *et al*., 2022; Nile *et al.*, 2022). BZnONPs modify auxin levels through tryptophan biosynthesis (Abdellatif *et al*., 2022) and are essential for activating several enzymes, such as superoxide dismutase and dehydrogenases (García-López *et al*., 2018).

Furthermore, this nanomaterial increases the amount of bioactive compounds such as phenols and flavonoids (Salih *et al*., 2021), which are responsible for the non-enzymatic defense of the plant against adverse environments (Elsheery *et al.*, 2020). Increasing the concentration of these compounds generates more notable protection against oxidative damage to cells (Chen *et al*., 2018; Sun *et al*., 2020; Zoufan *et al*., 2020), which could lead to increased crop growth and yields. These multiple benefits could be due to the fact that biosynthesized nanoparticles are more biocompatible with plants, according to our previous work, in which nanoparticles obtained by different methods were compared (Sánchez-Pérez *et al*., 2023). In other research, high concentrations (1000 mg L^{-1} and 2000 mg L^{-1}) of zinc sulfate and ZnONPs (Ø = 12-24 nm) were foliarly applied onto habanero pepper plants (*Capsicum chinense* Jacq.) under greenhouse conditions and showed that ZnONPs concentration of 1000 mg L^{-1} improved plant height, stem diameter, and chlorophyll content, and increased fruit biomass and yield accumulation, whereas, application of 2000 mg L^{-1} negatively affected plant growth but increased fruit quality, capsaicin content, dihydrocapsaicin, and Scoville Heat Units. In contrast, applying zinc salts had minimal influence on plant development and fruit production and quality (García-López *et al*., 2019). A recent study on the synergetic action of arbuscular mycorrhiza fungus inoculation, foliar application of zinc oxide and selenium nanoparticles (ZnO-NPs and Se-NPs) on chili (*Capsicum annuum* L.) exhibited better transpiration rate, chlorophyll, stomatal conductance, and photosynthetic rate, as well as higher vegetative growth, productivity, flowering, mineral components, antioxidant enzymes, and nitrogen metabolism enzymes (Sayed *et al*., 2024). In addition, the shape of the nanoparticles can influence the young chili plant (hybrid bell pepper seeds RZ F1 (35-171)) parameters, as levels of chlorophyll and phenolic compounds content in leaves were shown to be greater upon the application of spherical nanoparticles at 100 mg L^{-1}, vs hexagonal nanoparticles (Magdaleno García *et al*., 2023).

Most of the literature is focused on the effect of nanoparticles on yield and plant biomass. However, the plant physiological indices and harvest index are little known. Thus, this research was conducted to test the

hypothesis that the spherical zinc oxide nanoparticles would positively influence the morphological and physiological indices.

Materials and Methods

Plant material and treatments

The experiments were carried out during two spring-summer cycles, 2022 and 2023, from March until June, at the Technological Institute of Torreón (ITT, 7.5 km Old Torreón-San Pedro Highway, Municipality of Torreón, Coahuila, Mexico, 25°36′37″ North, 103°22′33″ West, an altitude of 1150 meters above sea level). The spherical BZnONPs were obtained by green synthesis with a 70-80 nm size using *Larrea tridentata* extract at 20 mg mL^{-1} as a reducing solution and as precursor hexahydrate zinc nitrate (Sigma-Aldrich, Saint Louis, MI, USA). Reaction conditions were pH of 13, 70 °C, and four-hour stirring (Sánchez-Pérez *et al*., 2023a). The seeds of Soledad pepper CHISER-522 variety were donated by the Regional Research Center of the Northeast (CIRNO) of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP for its acronym in Spanish). The seeds were sown in a 200-cavity polystyrene germination tray with peat moss organic substrate (Premier, Teoloyucan Estado De México, Mexico). Transplanting into plastic pots (Ø20 cm and 6 kg capacity) was carried out 17 days after sowing when the seedlings had at least two true leaves and an average height of 15 cm. A mixture of peat moss, sand, and perlite was used as a plant substrate, with a 40:40:20 v/v ratio. During the development of the crop, a Steiner nutrient solution was applied (Steiner, 1961). The pots were placed in a mixed greenhouse with low brick walls, casement windows, plastic cover, and shade mesh located in ITT. The temperature varied from 22 °C to 24 °C during the day and from 17 °C to 20 °C at night. Relative humidity was maintained at a 65 % – 75 % range and radiation fluctuated from 700 w/h to 800 w/h.

The BZnONPs solution was applied as a foliar spray every 15 days from 20 days after transplantation (20 DAT) until harvest (120 DAT). Each plant was treated with 700 mL of the treatment solution. The nanoparticle suspensions were prepared, just prior to application to plant foliage, in deionized water and homogenized with a probe sonicator (Fisher scientific®) for 30 minutes. A completely randomized experimental design was used with five treatments and a control: Control – 0 ppm, T1 – 10 ppm, T2 – 20 ppm, T3 – 30 ppm, T4 – 40 ppm and T5 – 50 ppm. Fifteen replications were carried out, resulting in 90 experimental units (each plant is considered an experimental unit).

Determination of the morphological variables

At 110 DAT, in the fruiting stage of the crop, the plant height was determined with the help of a tape measure from the ground level to the end of the stem. The stem thickness was measured using a Vernier (Mitutoyo® model 500-197-30). To measure the roots' length in cm, the randomly chosen plants were removed from the pots and washed with distilled water to remove the soil. The roots were placed on a flat surface and measured from the beginning of the root zone until the end of the longest root by using a tape measure. The root volume was determined by the displacement method (Burdett, 1979). The root system was introduced to a 500 mL beaker filled to half its capacity, and the amount of the water moved upwards was measured to result in root volume expressed in cm^3.

Measurement of the physiological efficiency of the crop

For the physiological efficiency of the crop, four destructive samplings were carried out, collecting three replicates per treatment at 50, 80, 110, and 140 DAT, following a known methodology (Orozco-Vidal *et al*., 2016; Radford, 1967). Vegetative (leaves and stems) and reproductive (flowers and fruits) organs were separated from each plant. Then, the organs were placed in brown paper bags and dried at a constant temperature of 65 °C for 72 h to obtain the weight of the dry matter (Sedano-Castro *et al*., 2005).

The growth indices were obtained from the dry weights. First, the Crop Growth Rate (CGR), which indicates the accumulation of biomass per unit of time (Equation 1) g m^{-2} day^{-1}, was calculated as follows:

$$CGR=\frac{(W_2-W_1)}{A(T_2-T_1)}, \quad \text{Eq 1}$$

where W_1 is the total dry weight of sample 1, W_2 is the total dry weight of sample 2, A is the sampling area equal to $1m^2$, T_1 is sampling date 1, and T_2 is sampling date 2.

To determine an estimation of the photosynthetic efficiency of the plant, the Net Assimilation Rate (NAR) (g m^2 day^{-1}) was computed by using the following equation:

$$NAR=\frac{(W_2-W_1)(\ln AF_2-\ln AF_1)}{(T_2-T_1)(AF_2-AF_1)}, \quad \text{Eq 2}$$

where W_1 is the total dry weight sampling 1, W_2 is the total dry weight sampling 2, AF_1 is the leaf area sampling 1, AF_2 is the leaf area sampling 2, T_1 is sampling date 1, and T_2 sampling date 2.

Finally, the Leaf Area Index (LAI), which represents the leaf area per unit of soil area (m^2 m^{-2}), was calculated as follows:

$$LAI=\frac{A_f \cdot n}{A_s}, \quad \text{Eq 3}$$

where A_f is the calculated leaf area per plant (m^2), n is the number of plants present per square meter, and A_s is the site area (m^2).

Biomass partitioning was determined at the end of the experiment (120 DAT) using the total dry weight of the vegetative (roots, stems, and leaves) and reproductive (fruits) organs as 100% and determining the percentage according to the organ weight. The harvest index (HI) was also evaluated by dividing the fruit's dry weight by the plant's total weight (roots+stems+leaves+fruit).

Evaluation of the photosynthetic pigments

The total amount of chlorophyll and carotenes was determined by the Lichtenthaler method (Lichtenthaler, 1987) by crushing 1 g of sample (leaves and stems) in 5 mL of pure acetone (Jalmek, NL, Mexico). The resulting mashed plant matter/solvent was filtered and diluted to a constant volume of 10 mL of pure acetone. Absorbance was recorded at 662 nm, 645 nm, and 470 nm with a Jenway 7305 UV-Vis (TEquipment, Long Branch, NJ, USA). The chlorophyll content was calculated according to Lichtenthaler's equations:

$$\text{Chlorophyll } a \text{ concentration } (C_a) = 11.24A_{662} - 2.04A_{645} \quad \text{Eq 4}$$

$$\text{Chlorophyll } b \text{ concentration } (C_b) = 20.13A_{645} - 4.19A_{662} \quad \text{Eq 5}$$

$$\text{Total Chlorophyll} = 7.05A_{662} + 18.08A_{645} \quad \text{Eq 6}$$

$$\text{Carotenes} = (1000A_{470} - 1.9C_a - 63.14C_b)/214 \quad \text{Eq 7}$$

Content of bioactive compounds

Extract preparation: 1 g of fresh sample (leaves and stems) was ground in 10 mL of 80% methanol (Thermo Scientific, USA) and left at constant stirring for 24 h at 70 rpm at room temperature. The extract was centrifuged at 5000 rpm for 5 minutes, and the supernatant was used in subsequent analyses.

The total phenolics were quantified by the Folin-Ciocalteu method (Vernon L. Singleton, Rudolf Orthofer, 1999). 300 µL of the extract, 1080 mL of deionized water (CTR scientific, NL, Mexico), and 120 µL of Folin-Ciocalteu (Sigma-Aldrich, Saint Louis, MI, USA) reagent were added and stirred in a vortex for 10 seconds and left to react in the dark. After 10 min, 0.9 mL of Na_2CO_3 (Sigma-Aldrich, Saint Louis, MI, USA)

at 7.5% (w/v) was added and vortexed for 10 seconds. Samples were placed at room temperature for 30 min. Finally, the absorbance was measured at 765 nm in a Jenway 7305 UV-Vis spectrophotometer (TEquipment, Long Branch, NJ, USA). The standards were prepared with gallic acid (GA) (Sigma-Aldrich, Saint Louis, MI, USA), and results are reported in mg of GA equivalent per 100 g fresh weight (mg GA 100 gFW^{-1}).

The total flavonoids was determined by the aluminium chloride colorimetric assay (Zhishen *et al.*, 1999). 250 µL of the extract was mixed with 1.25 mL of deionized water and 75 µL of 5% $NaNO_2$ (CTR scientific, NL, Mexico). After 5 min, 150 µL of 10% $AlCl_3$ was added. After 6 min, 500 µL of 1 M NaOH (CTR scientific, NL, Mexico) and 275 µL of deionized water were added and stirred vigorously. The absorbances of all samples were measured at 510 nm on a Jenway 7305 UV-Vis spectrophotometer (TEquipment, Long Branch, NJ, USA). The standards were prepared with catechin (Sigma-Aldrich, Saint Louis, MI, USA) dissolved in absolute ethanol, and the results are expressed in mg of catechin per 100 g fresh weight (mg Cat 100 gFW^{-1}).

The antioxidant capacity of the plant extracts was estimated by the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) (Sigma-Aldrich, Saint Louis, MI, USA) assay according to the methodology described by Brand-Williams (Brand-Williams *et al*., 1995). 50 µL of the extract was mixed with 1950 µL of DPPH solution (0.025 mg mL^{-1} in ethanol). After 30 min, the absorbance of the samples was read at 517 nm on a Jenway 7305 UV-Vis spectrophotometer (TEquipment, Long Branch, NJ, USA). The standard curve was prepared with Trolox (Sigma-Aldrich, Saint Louis, MI, USA). The results are expressed in M equivalent of Trolox at 100 g fresh weight (Meq Trolox 100 gFW^{-1}). Analyses were performed in triplicate.

Statistical analysis

All results are the mean values of three replications in a completely randomized design. The data were evaluated by analyzing the variance and comparison of mean values with the Tukey's test ($p \leq 0.05$) using the statistical package Statistical Analysis® System Institute (SAS) version 9.4.

Results

Morphological variables

Plants height

The effect of applying ZnO nanoparticle solution on the plants' height at 110 DAT is shown in Figure 1. The best treatment resulting in the highest average plant height was achieved under the application of the 30-ppm solution, obtaining an average height of 96.00 ± 3.12 cm, an increase of 62% with respect to the control (59.00 ± 2.23 cm). In the same way, the treatment with 40 ppm also shows a significantly increased height compared to the control, in which the plants had an average height of 90.00 ± 1.25 cm, exceeding the control by 50%. However, the plants treated with 10 ppm, 20 ppm, and 50 ppm of BZnONPs are statistically indistinguishable from the control.

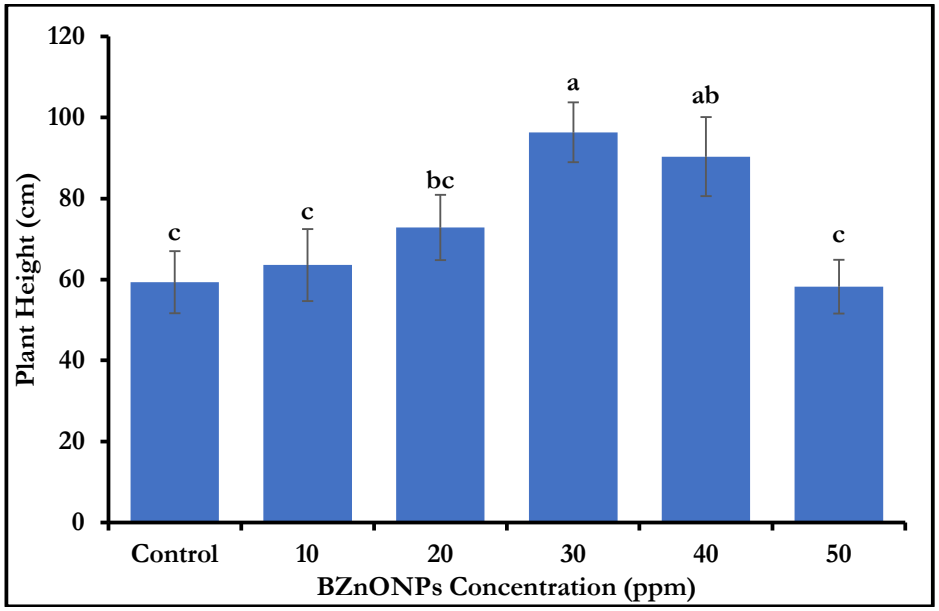

Figure 1. Effect of BZnONPs treatments at different concentrations on the height of the *Capsicum annuum* plants

*The values are the average of three replicas ± SD. According to Tukey's test, values with different letters indicate significant differences ($p < 0.05$)

Stem diameter

In all cases, as represented in Figure 2, the plants treated with BZnONPs had a larger stem diameter than those in which the biosynthesized nanoparticles were not applied. Nonetheless, concerning the control, the most statistically significant results are the 20 ppm, 30 ppm, and 40 ppm treatments, showing an increment of 12%, 17%, and 8%, respectively.

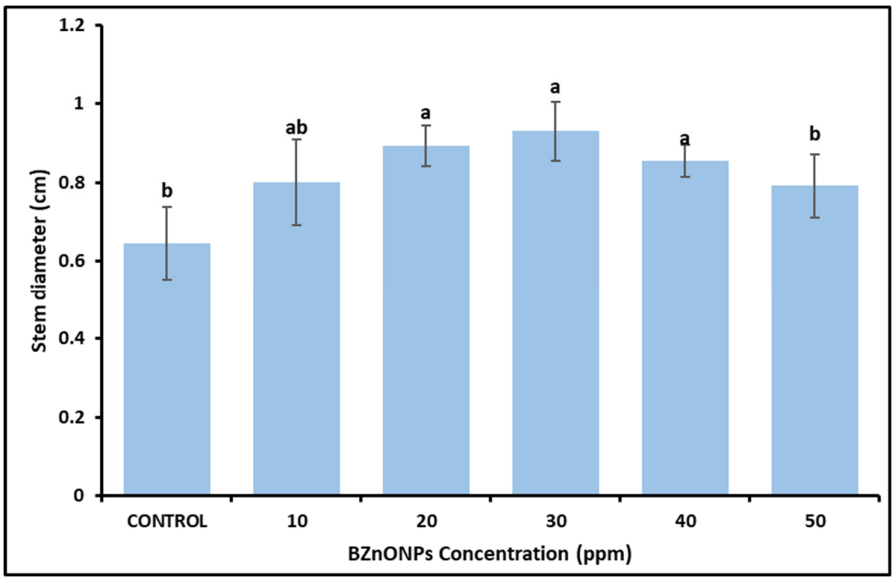

Figure 2. Effect of BZnONPs treatments at different concentrations on the stem diameter of the *Capsicum annuum* plants

*The values are the average of three replicas ± SD. According to Tukey's test ($p < 0.05$), values with different letters indicate a significant difference.

Root length and volume

Plants treated with BZnONPs showed longer and more voluminous roots (Figures 3 and 4) than the control. The longest roots grew under 30 ppm BZnONPs application with a 42% increase in length over the control. In the case of root volume measurement, the best treatment was 50 ppm, 37.5% higher than the control, indicating that the higher concentration of BZnONPs promotes more voluminous root development.

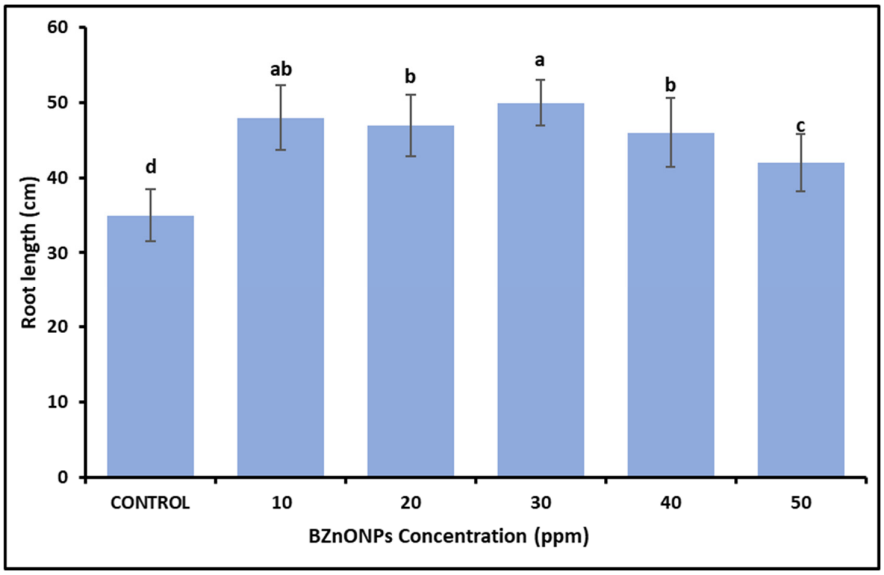

Figure 3. Effect of BZnONPs treatments at different concentrations on the length of *Capsicum annuum* plants

*The values are the average of three replicas ± SD. According to Tukey's test ($p < 0.05$), values with different letters indicate significant differences.

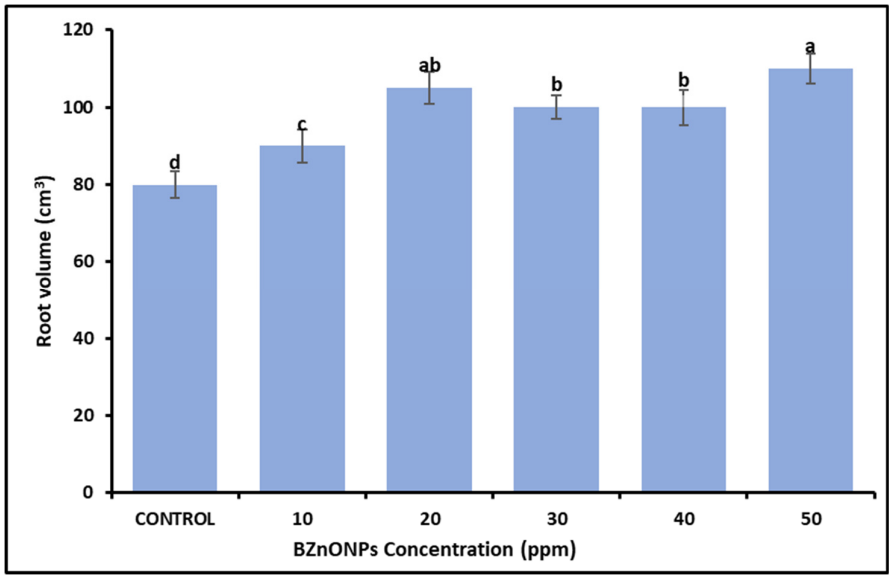

Figure 4. Effect of BZnONPs treatments at different concentrations on the root volume of *Capsicum annuum* plants

*The values are the average of three replicas ± SD. According to Tukey's test ($p < 0.05$), values with different letters indicate significant differences.

Physiological efficiency

Crop Growth Rate (CGR) and Net Assimilation Rate (NAR)

The results for the variable crop growth rate and net assimilation rate during the different phenological stages of the growth are detailed in Tables 1 and 2. The plant's response to the nanoparticles depends strongly on the phenological stage at which BZnONPs are applied. For CGR, in the beginning stage of growth (50-80 DAT), the most significant results were achieved for 50 ppm treatment. Then, when moving forward to 80-110 DAT, the highest value for CGR was given in plants treated with 30 ppm. During the last growth stage (110-140 DAT), the best treatment was 40 ppm. A similar trend was observed for NAR, where at the beginning of the cycle (50-80 DAT), the plants treated with 50 ppm of BZnONPs presented a greater assimilation rate. Meanwhile, in the middle (80-110 DAT) and end of the cycle (110-140 DAT), the highest efficiency was presented in the plants treated with 20 ppm and 30 ppm, respectively.

Table 1. Effect of BZnONPs treatments at different concentrations on Crop Growth Rate (CGR) measured at 50, 80, 110, and 140 DAT of *Capsicum annuum* plants

DAT	Crop Growth Rate (CGR)					
	Control	10	20	30	40	50
50-80	2.06 ± 0.20 e	3.22 ± 0.10 d	3.39 ± 0.30 c	5.15 ± 0.30 ab	4.94 ± 0.30 b	5.43 ± 0.20 a
80-110	8.46 ± 0.40 cd	8.60 ± 0.21 cd	11.57 ± 0.70 b	13.89 ± 0.40 a	9.17 ± 0.40 c	6.02 ± 0.10 d
110-140	0.14 ± 0.02 e	0.20 ± 0.01 d	0.60 ± 0.01 b	0.53 ± 0.03 c	1.33 ± 0.01 a	1.00 ± 0.60 ab
50-140	3.73 ± 0.01 d	4.00 ± 0.03 cd	5.18 ± 0.03 b	6.52 ± 0.01 a	4.86 ± 0.02 c	4.15 ± 0.03 cd

The values are the average of three replicas ± SD. According to Tukey's test ($p < 0,05$), values with different letters indicate significant differences.

Table 2. Effect of BZnONPs treatments at different concentrations on Net Assimilation Rate (NAR) measured at 50, 80, 110, and 140 DAT of *Capsicum annuum* plants

DAT	Control	10	20	30	40	50
50-80	2.06 ± 0.20 e	3.22 ± 0.10 d	3.39 ± 0.30 c	5.15 ± 0.30 ab	4.94 ± 0.30 b	5.43 ± 0.20 a
80-110	8.46 ± 0.40 cd	8.60 ± 0.21 cd	11.57 ± 0.70 b	13.89 ± 0.40 a	9.17 ± 0.40 c	6.02 ± 0.10 d
110-140	0.14 ± 0.02 e	0.20 ± 0.01 d	0.60 ± 0.01 b	0.53 ± 0.03 c	1.33 ± 0.01 a	1.00 ± 0.60 ab
50-140	3.73 ± 0.01 d	4.00 ± 0.03 cd	5.18 ± 0.03 b	6.52 ± 0.01 a	4.86 ± 0.02 c	4.15 ± 0.03 cd

The values are the average of three replicas ± SD. According to Tukey's test ($p < 0,05$), values with different letters indicate significant differences.

Leaf Area Index (LAI)

Figure 5 (lines are shown as visual guides) shows the dynamic changes in the leaf area index upon the influence of BZnONPs treatments. At 50 DAT, there is no significant difference between treatments. When LAI is evaluated at the 80 DAT point, it significantly increases for all the treatments (10 ppm to 50 ppm) but the control. However, at 110 DAT, the plants treated with 30 ppm developed a higher leaf area index, which had an increment of 62 % with respect to the control, and this behavior extends to 140 DAT, exhibiting 64 % higher LAI compared to the control.

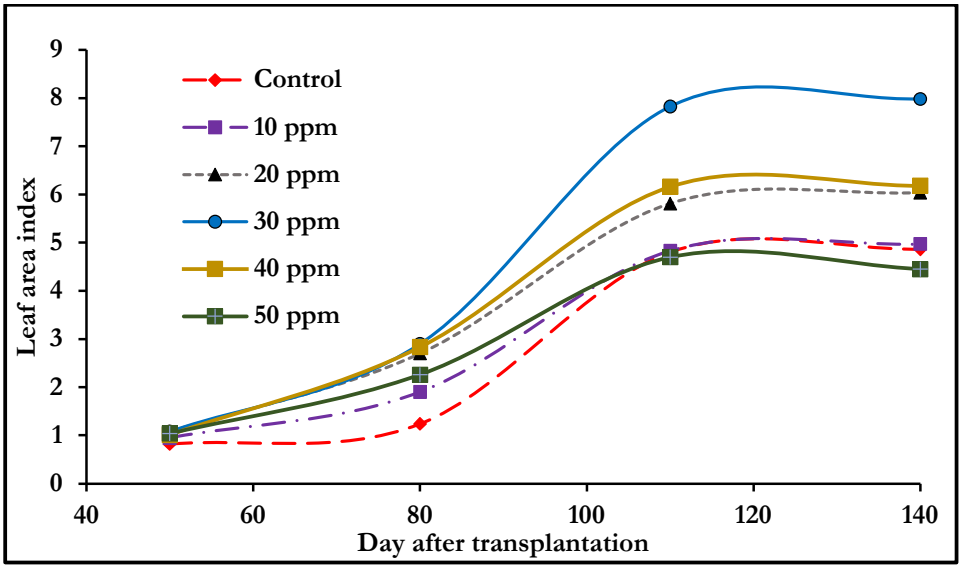

Figure 5. Effect of ZnONP treatments at different concentrations on leaf area index, measured at 50, 80, 110, and 140 DDT, of *Capsicum annuum*. Lines connecting the data are drawn to guide the eye

Biomass partitioning and harvest index

The effects on mass distribution upon the ZnO nanoparticles' application are shown in Table 3. In general, fruit carried the most mass in all treatments. However, comparing each dry mass type, that is, roots, stems, leaves, and fruit, it can be observed that the plants treated with the concentration of 30 ppm generated a higher biomass percentage in the roots, stems, and leaves, with respect to the control. In the treatments with 40 ppm and 50 ppm of BZnONPs, the distribution of the mass is concentrated mainly in the fruit, with ~90 % dry mass (which is 4% more than the control). This directly impacts the harvest indices, where the plants treated with 40 ppm of BZnONPs showed the highest harvest index. The harvest index determines how plant biomass is converted to fruit yield and, consequently, predicts financial revenue from the crops. Thus, 40 ppm or 50 ppm treatment promises the best chili fruit harvest.

Table 3. Effect of different concentrations of BZnONPs treatments on dry mass distribution and harvest index of *Capsicum annuum* plants

Treatments	Dry mass distribution (%)				HI
	Roots	Stems	Leaves	Fruit	
Control	3.09 ± 0.02 b	5.36 ± 0.12 ab	4.74 ± 0.09 a	86.78 ± 2.14 b	0.87 ± 0.02 c
10	3.13 ± 0.13 b	5.07 ± 0.09 b	3.73 ± 0.12 c	88.06 ± 2.09 ab	0.88 ± 0.01 b
20	4.01 ± 0.08 ab	5.72 ± 0.06 a	4.29 ± 0.14 b	85.97 ± 3.12 bc	0.86 ± 0.03 d
30	4.24 ± 0.07 a	5.75 ± 0.21 a	4.65 ± 0.24 a	85.48 ± 3.16 bc	0.85 ± 0.01 e
40	2.75 ± 0.12 c	4.19 ± 0.14 c	2.87 ± 0.18 d	90.17 ± 2.65 a	0.91 ± 0.02 a
50	2.67 ± 0.11 c	4.28 ± 0.18 c	3.08 ± 0.17 c	89.96 ± 3.21 a	0.89 ± 0.01 a

The values are the average of three replicas ± SD. According to Tukey's test ($p < 0,05$), values with different letters indicate significant differences within each parameter (column).

Photosynthetic pigments

Chlorophyll

The results for the total chlorophyll (Figure 6) content indicate that with 30 ppm of BZnONPs, plants generate a higher concentration of chlorophyll (15.82±4.23 mg gFW^{-1}) than the control (10.14±2.12 mg gFW^{-1}). However, at concentrations of 50 ppm, plants have lower chlorophyll production, statistically indifferent to the control.

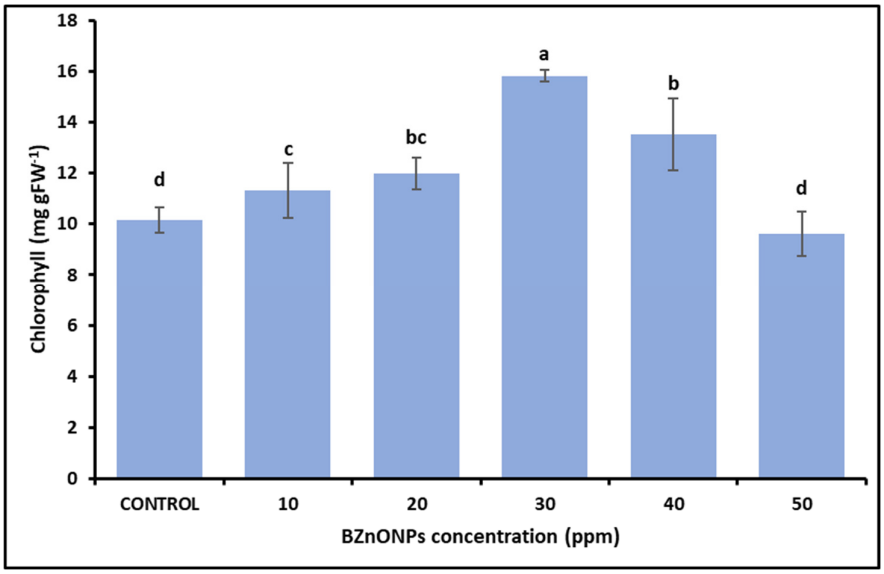

Figure 6. Effect of BZnONPs treatments at different concentrations on chlorophyll content in *Capsicum annuum*

*The values are the average of three replicas ± SD. According to Tukey's test, values with different letters indicate significant differences ($p < 0.05$).

Carotenes

The BZnONPs treatments also induced the generation of a higher concentration of carotenes (Figure 7). In this evaluation, the highest carotenes value was presented in the plants treated with 30 ppm, which increased the carotenes synthesis by 53% compared to the control. However, unlike the chlorophyll content, the plants treated with 50 ppm BZnONPs did not show a significant decrease, even presenting an increase of 22 % with respect to the control.

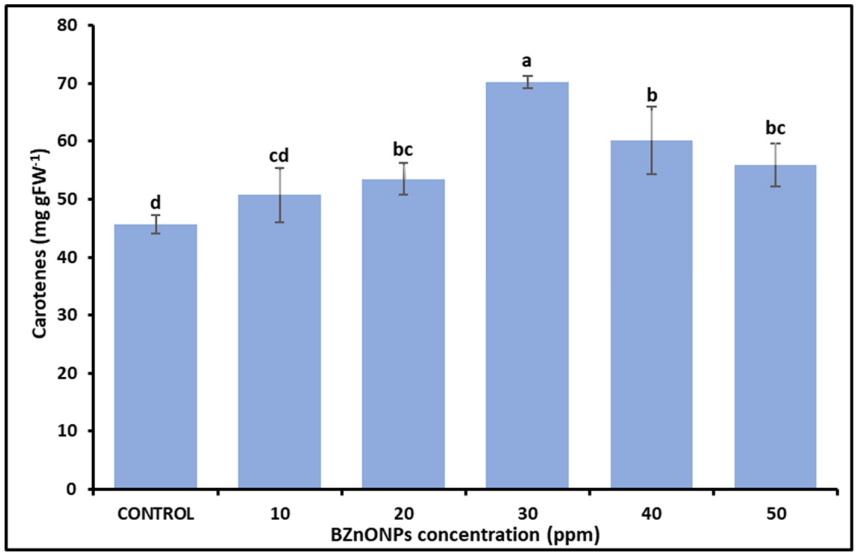

Figure 7. Effect of BZnONPs treatments at different concentrations on carotene content in *Capsicum annuum*

*The values are the average of three replicas ± SD. According to Tukey's test, values with different letters indicate significant differences ($p < 0.05$).

Phytochemical compounds

The antioxidant activity

The antioxidant activity (Figure 8) showed a steady increase depending on the concentration of BZnONPs from 10 ppm to 40 ppm, with the plants of the latter treatment having the highest antioxidant capacity with 78.41 ± 5.21 Meq Trolox 100 gFW^{-1}, surpassing the control by 51 % (51.66 ± 4.23 Meq Trolox 100 gFW^{-1}).

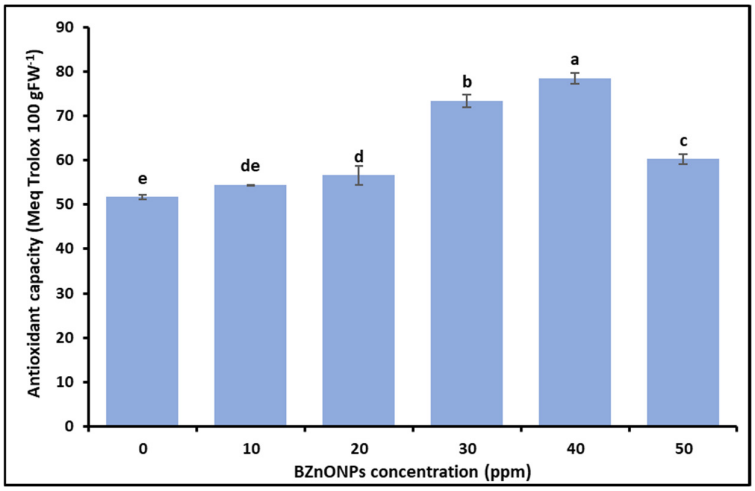

Figure 8. Effect of BZnONPs treatments at different concentrations on antioxidant capacity in *Capsicum annuum*

*The values are the average of three replicas ± SD. According to Tukey's test, values with different letters indicate significant differences ($p < 0.05$).

Total phenol

The results of total phenols (TP) showed a statistically significant difference ($P \leq 0.05$) with all of the foliar applications of BZnONPs in the chili pepper crop. 350 % higher levels of phenols were recorded for the 30 ppm and 40 ppm doses compared to the control treatment (Figure 9).

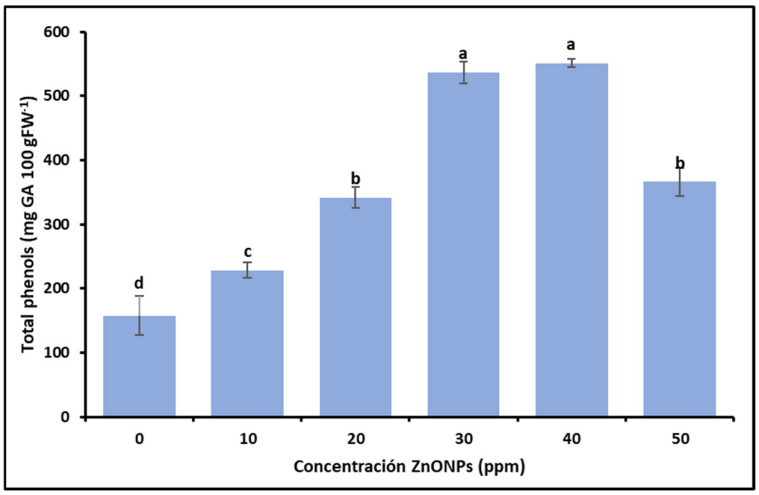

Figure 9. Effect of BZnONPs treatments at different concentrations on total phenol content in *Capsicum annuum*

*The values are the average of three replicas ± SD. According to Tukey's test, values with different letters indicate significant differences ($p < 0.05$).

Flavonoids

The highest concentration of flavonoids (Figure 10) was measured in plants treated with 50 ppm, in which a concentration of 53 ± 2.43 mg CAT 100 gFW^{-1} was presented, which is 190 % higher than the control. In general, the plants treated with BZnONPs contained a greater amount of flavonoids.

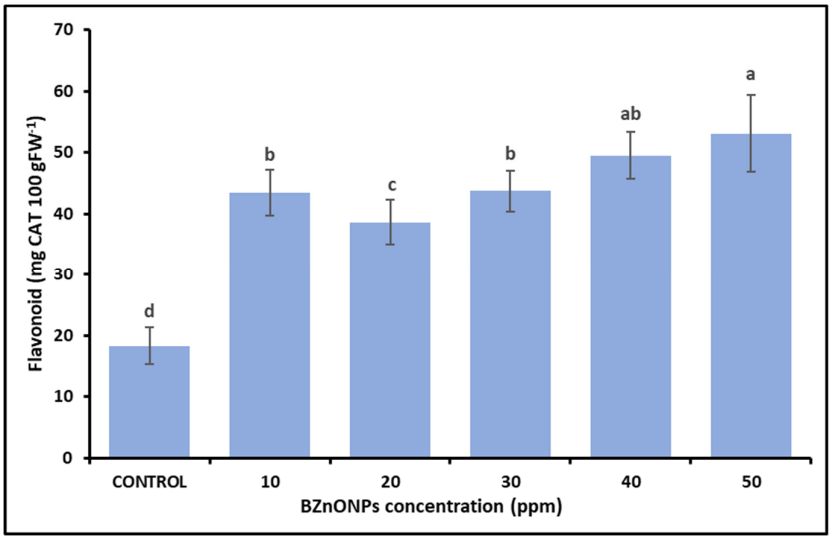

Figure 10. Effect of BZnONPs treatments at different concentrations on the flavonoid content in *Capsicum annuum*

*The values are the average of three replicas ± SD. According to Tukey's test, values with different letters indicate significant differences ($p < 0.05$).

Discussion

The results obtained here show that BZnONPs application significantly influences *Capsicum annuum* plants' growth. The foliar application of 30 ppm and 40 ppm increases the height and stem diameter of the plants, which may occur because zinc is involved in the biosynthesis of endogenous growth hormones, such as auxins and gibberellins (Suganya *et al*., 2020), which are responsible for cell division and cause an increase in plant growth (Al-dhalimi and Al-ajeel, 2020). Also, zinc is required in the production of biomass because this microelement has a crucial function in many enzymes involved in the photosynthetic process, as well as in the integrity and maintenance of plant cell membranes (Burman *et al*., 2013; Nandal and Solanki, 2021).

Applying chemically synthesized ZnONPs at 100 mg L^{-1} concentration resulted in a 25% increase in plant height and a 15% increase in stem diameter in hybrid bell pepper RZ F1 (35-171) in the early stages of plant growth (Magdaleno García *et al*., 2023). Here, we report increases of 62% in plant height and 17% in stem diameter at 110DAT under 30 mg L^{-1} concentration of biosynthesized ZnONPs, which suggests that biosynthesized nanoparticles are more compatible with growing plants and allow better zinc assimilation (Sánchez-Pérez *et al*., 2023).

Burman *et al*. found that foliar application of 1.5 mg L^{-1} and 10 mg L^{-1} of ZnONPs on chickpea plants (*Cicer arietinum*) for 15 days increased the height and dry biomass (Burman *et al*., 2013). Some studies have highlighted that applying zinc nanofertilizer in the form of low-dose foliage spray is very effective in promoting vegetative growth (Gonmei *et al*., 2022; Pejam *et al*., 2021; Sun *et al*., 2020). In this way, BZnONPs are transported through the plant and participate in metabolic processes, increasing the synthesis of tryptophan,

one of the precursors of indoleacetic acid, which is essential in the increment of plant growth and its development (Sourati *et al*., 2022).

On the other hand, the leaf area index, LAI, is a dynamic indicator of crop growth and development (Xiao *et al*., 2023) closely related to biomass and crop yield. Therefore, the higher CGR values presented by plants treated with nanoparticles suggest a higher rate of their metabolic processes (Ahmed *et al*., 2021; Orozco-Vidal *et al*., 2016), which indicates a more significant accumulation of dry matter in a given time in a unit of surface, as seen in current research upon treatment with the higher BZnONPs concentrations (Table 1). The increase in NAR values is attributed to greater photosynthetic efficiency with the treatments and is related to the high LAI of plants. Moreover, we observed that plants treated with 30 ppm presented an LAI increase of 64% at 110 DAT, maintaining this behavior up to 140 DAT, which can be attributed to the nanoparticles improving metabolic processes (Sun *et al*., 2020). In young plants, which could generate resistance to senescence even in the final stage of the crop, this could cause the plants to produce more photo assimilates and obtain greater yields. In addition, the reproductive stage of the crop is extended because, with the impulse in LAI, plants receive a more significant amount of radiation (Chapepa *et al*., 2020) when the leaves are optimally ripe to integrate and export a more considerable amount of photo assimilates to the organs of interest, i.e., the fruit. Consequently, the mass distribution and the harvest indices under the 40 ppm and 50 ppm ZnONP concentrations are the highest, indicating how plant biomass is converted to fruit yield (S. Sharma *et al*., 2021) and, therefore, predicts financial revenue from the crops.

The increase in chlorophyll levels can be attributed to zinc involvement in the chlorophyll synthesis and protection of chloroplast structure (Hussain *et al*., 2021; Li *et al*., 2020; Roosta *et al*., 2018), suggesting that the higher the zinc concentration in the plant, the higher the chlorophyll concentration could be obtained. The high total chlorophyll content is essential as it is the primary photosynthetic photoreceptor (Agati *et al*., 2020). Therefore, an increased concentration of chlorophylls will raise the content of photo assimilates and, consequently, lead to elevated end biomass (Puccinelli *et al*., 2023). However, in plants treated with the highest concentration of BZnONPs, a dramatic decrease in total chlorophyll concentration is observed. This decrease may occur because zinc accumulation causes damage to the subcellular organization (Rajput *et al*., 2021), specifically in the chloroplast, generating its rupture (Sathiyabama, 2019). Similar increases in chlorophyll levels were reported in *Capsicum frutescens* under hydroponic growth (ZnONPs at 25 and 75 mg L^{-1}) (Al-Zuhairi *et al*., 2020), hybrid bell pepper RZ F1 (35-171) (chemically synthesized ZnONPs at 100 mg L^{-1}) (Magdaleno García *et al*., 2023), and other plants like wheat (*Triticum aestivum*), where a reduction in chlorophyll concentration was observed with the increase in the concentration of ZnONPs (Alsuwayyid *et al*., 2022).

The application of BZnONPs also significantly affected the concentration of carotenoids below 30 ppm because, under controlled concentrations, zinc generates stress in the plant, which causes the activation of the non-enzymatic defense system (Ibrahim and Mahmoud, 2021; Kaur and Garg, 2021). However, at higher concentrations and prolonged exposure, zinc can cause phytotoxicity (Song and Lee, 2016); as can be seen in this study, when concentrations of 40 ppm and 50 ppm are used, the content of carotenoids is statistically similar to that of the control. Higher and more prolonged exposure to zinc levels can cause various plant metabolic disorders (Kaur and Garg, 2021). Applying 40 ppm ZnONPs solution increased the concentrations of total phenols, flavonoids, and antioxidant capacity in *Capsicum annuum*, as reported in our previous work in seedlings (Sánchez-Pérez *et al.*, 2023). Similarly, *Stevia rebaudiana* plants treated with CuO and ZnO showed that NPs generate toxic free radicals, increasing stress levels and enhancing all antioxidant activities (Javed *et al*., 2017). Chili peppers are often consumed as superfoods, providing antioxidants such as polyphenols and flavonoids. However, these levels are rarely reported in the growing plants but rather in the harvested fruit (Hareem *et al*., 2024; Khanema *et al*., 2024; Lahbib *et al*., 2023). Our group is in the process of publishing results relating to fruit analyses.

Therefore, these nanomaterials have great potential to be novel abiotic promoters that effectively induce the biosynthesis of the secondary metabolite. After penetrating plant cells, zinc oxide nanoparticles interact with the cell's components, molecules, organelles, and intracellular structures (Paramo *et al*., 2020). The interaction between nanoparticles and target cell organelles, such as chloroplasts and mitochondria, leads to chemical and physical changes (Chen *et al*., 2015). ZnONPs can cause stress and produce excess reactive oxygen species, subsequently affecting cellular structures, organelles, DNA, proteins, carbohydrates, lipids, and secondary metabolites in plants (Li *et al*., 2020; Tariverdizadeh *et al*., 2021).

Conclusions

The chili plants treated with a foliar application of 30 ppm and 40 ppm of BZnONPs had improved their morphological characteristics. They presented higher height, thicker stems, longer roots, and improved CGR, NAR, LAI, and HI than the other treatments. In addition, the BZnONPs intervened in the synthesis of photosynthetic pigments; this caused a higher content of photoassimilated compounds and, therefore, larger plants and a superior amount of bioactive compounds, generating plants more resistant to bio-agent and abiotic stress. Thus, biosynthesized zinc oxide nanoparticles emerge as an alternative treatment to improve plants' morphological and physiological conditions. These NPs in controlled concentrations can be used as abiotic inducers to produce plants with a high content of secondary metabolites, which can protect the plant and improve its metabolism even in the final stages of production.

Authors' Contributions

Conceptualization: EF-L, JAO-V, JEM, and SYM-G; Formal analysis: DMS-P, PY-C, and COP-V; Investigation: DMS-P; Methodology: DMS-P, JAO-V, JEM, and SYM-G; Project administration: JEM, and SYM-G; Validation: DMS-P, RIR-B, COP-V, and JEM; Writing - original draft: DMS-P; Writing - review and editing: DMS-P, EF-L, PY-C, RIP-B, COP-V, JEM and SYM-G

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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