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Research Article

Biofortification with ZnO NPs as nanofertilizers to improve sustainable commercial and phytochemical quality in basil plants

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Abstract

Biofortification is the process of developing a crop with bioavailable micronutrients in its edible parts. This has been done using nanofertilizers, since they can be used to feed plants in a gradual and controlled manner. Therefore, the aim of this work was to evaluate the effect of foliar application of ZnO NPs in different concentrations on the commercial and phytochemical quality of the basil (*Ocimum basilicum* L.) crop, as it is one of the most important aromatic plants used for chemical and pharmacological properties. Four concentrations of ZnO NPs (5, 10, 15 and 20 mg L^{-1}) and a control treatment under a completely randomized design, were evaluated. The results show statistical differences in morphological parameters (leaf and stem fresh weight, height, number of leaves, leaf area and dry weight) with a slight tendency to increase on the treated basil plants mainly at concentration of 20 mg L^{-1}. The highest chlorophyll content (5.54 μg g^{-1} FW) was obtained for the control treatment, whereas the lowest one (4.14 μg g^{-1} FW) was observed for the 20 mg L^{-1} treatment. However, carotenoid content in the leaves was markedly higher than the control, the control had the concentration of 0.84 μg g^{-1} FW, while the treatment with 20 mg L^{-1} ZnO NPs registered a value of 1.08 μg g^{-1} FW. The highest total phenolic, flavonoid, antioxidant capacity and vitamin C content was obtained for 20 mg L^{-1} ZnO NPs. Finally, basil plants treated with ZnO NPs could stimulate enzymatic activity, as demonstrated in this study. Detailed studies are suggested to understand the mechanism of action of nanoscale materials.

Keywords: biofortification; hydroponics; mineral elements; nanotechnology; *Ocimum basilicum* L.; zinc

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Introduction

Micronutrient malnutrition is known to affect more than half of the world's population and it is considered to be among the most serious global challenges to humankind (Singh *et al*., 2016). The enrichment of food with health-related compounds and mineral elements could, therefore, be considered a strategy to fight undernourishment or to deal with specific nutritional needs (Buturi *et al*., 2021). The most practical and sustainable method for addressing the nutritional issue is biofortification, which involves enhancing the nutrients in common crops (Srivastav *et al*., 2022). It can be carried out via agronomic techniques, transgenic technology, or plant breeding (Jha and Warkentin, 2020).

Nanomaterials can be used in agriculture for different approaches, such as in the biostimulation of crops, nano-fertilizers, nano-pesticides, or nano-carriers of other compounds or molecules of interest. Nanotechnology is the latest technology for precision agriculture, this uses improved materials to add value to agriculture, by exploiting their nanoscale properties (Elemike *et al*., 2019). Nanoparticles (NPs) have an average size of <100 nm with high surface-to-volume ratio, whose functionality are dependent on their size and concentration (Janmohammadi *et al*., 2016).

Zinc (Zn^{2+}) is an essential micronutrient for plants and is involved in many biological functions, such as growth promoter, regulator of tryptophan biosynthesis and, consequently, in the metabolic pathway of auxin biosynthesis (García-López *et al*., 2018). Zn^{2+} is an essential element for human nutrition. The Recommended Dietary Allowances (RDA) for zinc is 11 mg day^{-1} for adult males and 8 mg day^{-1} for adult females. Deficiency of zinc has many consequences including a weak immune system, recurrent infections, mental illness, and retarded growth and fertility (Roohani *et al*., 2013).

Zinc oxide nanoparticles (ZnO NPs) have been investigated for their antifungal, antibacterial, and nutritional properties (Rossi *et al*., 2019). Several reports suggest that ZnO NPs improve growth and development in lettuce (Galindo-Guzmán *et al*., 2022), habanero peppers (García-López *et al*., 2019), melon (Rivera *et al*., 2021) and other crops. NPs can affect plant growth by releasing toxic ions, hindering biochemical processes, and inducing imbalance in reactive oxygen species (ROS). An appropriate amount of ROS plays a key role in plant development, cell division, and gene expression. Foliar application of NPs can activate the production of non-enzymatic (proline, phenolic compounds) and enzymatic antioxidants (CAT, POD, SOD) to alleviate stress (Hong *et al*., 2021).

Ocimum basilicum L., also called basil the "king of the herbs", it is an annual and herbaceous plant cultivated extensively in Iran and many other countries with a long history of traditional use and adapted to different gastronomic cultures (Abbasifar *et al*., 2020). The culinary, medicinal and industrial importance of this plant led to affirm its chemical and pharmacological properties (Singh *et al*., 2018). Therefore, it is pertinent to conduct research on the effects of Zn biofortification through nanotechnology on basil plants. Based on the above, the objective of this research was to evaluate the effects of foliar application of ZnO NPs in different concentration and to determine its effect on yield, commercial and nutraceutical quality in basil plants grown under a NFT hydroponic system.

Materials and Methods

Plant and nanoparticles material

Genovese basil seeds (*Ocimum basilicum*) by the Red Fox Organics company (Florida, USA) were germinated in agricultural phenolic foam trays and transplanted three weeks later in a NFT hydroponic system, when they presented two to three true leaves.

The size of the applied ZnO NPs was from 20 to 60 nm, with a purity of 97%, white in color and with semispherical and polygonal structural shape. ZnO NPs were donated by Center for Research Applied

Chemistry (Coahuila, México), and they were synthesized through controlled precipitation (Ramírez-Barrón *et al*., 2019), using the chemical hydrolysis method.

Application of treatments

The experiment was carried out in a shadow house of the Technological Institute of Torreón (ITT), during the spring-summer agricultural cycle of 2021. The ITT is in the municipality of Torreón, Coahuila, México between coordinates 25° 36′ 37″ N - 103° 22′ 33″ W. The shade mesh is a 2 mm thick galvanized steel support structure with 1.25″ and 1.5″ square profiles and anti-insect mesh (crystal color) with threads 25 x 25-inch, 720-gauge, UV-treated polyethylene with diffuse light and 30% shade.

For basil plants four treatments and one control for spray application of ZnO NPs were used (Table 1), each concentration was dissolved in deionized water and applied immediately after preparation. The treatments consisted of three foliar applications using a cylindrical atomizer with an output of 0.14 mL and for a better adherence, a non-ionic surfactant - adherent (INEX-A) was added at a dose of 1-2 mL L^{-1} of spray water. The foliar applications were made during the crop cycle, the first application was at 15, the second at 30 and the third at 60 days after transplantation. Approximately 30 mL of solution was used for each individual plant, enough to cover the entire surface. The foliar applications were made in the morning and without the presence of wind. Steiner nutrient solution (Steiner, 1961) was applied at a pH value of 5.5-6 and an electrical conductivity of 1.5-2 dS m^{-1}.

Table 1. Description of the treatments on basil cultivated in the hydroponic system

Treatment	Concentration ZnO NPs (mg L^{-1})
T1	Control (only deionized water)
T2	5
T3	10
T4	15
T5	20

Steiner nutrient solution (Steiner, 1961) was applied at a pH value of 5.5-6 and an electrical conductivity of 1.5-2 dS m^{-1}.

Variables evaluated

Morphological characteristics

The plants were removed from the hydroponic system, for this, the harvest of the basil plants was carried out at 65 days after transplantation when the leaves had reached their commercial maturity. The cut was made in the morning. The fresh weight of the leaf and stem were immediately measured using a digital weighing scale. Other measured traits were the plant height, leaf number, and leaf area. The leaves were kept flat for scanning in the leaf area meter (area meter LI300®, LAM 1300 series). Senescent leaves and those with broken or missing leaflets were not included. The leaf area was reported in cm^2.

Then, in order to measure their dry weight, the aforementioned samples were kept in an oven at 70 °C for 48 h.

Determination of pigment contents

Chlorophyll and carotenoids were detected in the solution by suspending 500 mg fresh leaves in ethanol at 95% by the method of Lichtenthaler and Wellburn (1983). The homogenate was centrifuged at 1500 × g for 20 min and the supernatant was collected. The absorbance readings were then recorded at 665, 649 and 470 nm on a Jenway 7305 UV-visible spectrophotometer. The content was reported according to equations (1-4):

$$\text{Chlorophyll (a)} = 13.95\ A_{665} - 6.88\ A_{649} \quad (1)$$

$$\text{Chlorophyll (b)} = 24.96\ A_{649} - 7.32\ A_{665} \quad (2)$$

$$\text{Total chlorophyll} = Chl_{(a)} + Chl_{(b)} \quad (3)$$

$$\text{Carotenoids} = (1000\ A_{470} - 2.05\ Chl_{(a)} - 114.8\ Chl_{(b)}) / 245 \quad (4)$$

Extract preparation for phytochemical compounds

To obtain the extracts, 2 g of fresh leaves were mixed in 10 mL ethanol at 80% with constant centrifugation in a rotary shaker for 24 h at 20 rpm at 5 °C. Subsequently, extracts were centrifuged at 3000 rpm for 5 min, and the supernatant was extracted for its subsequent analysis.

Phytochemical compounds

Total phenols were determined according to the method of Singleton et al. (1999); 300 µL of the extract were used and 1080 mL of water were added in a test tube, to then add 120 µL of Folin-Ciocalteu reagent stirring in a vortex for 10 s. After 10 min, 0.9 mL of Na_2CO_3 at 7.5% (w/v) were added and stirred for 10 s. The samples were placed at room temperature for 30 min. Finally, the absorbance at 765 nm was measured in a Jenway 7305 UV-Vis spectrophotometer. The standard was prepared with gallic acid (GA), and the results were expressed in equivalent mg GA 100 g^{-1} of fresh weight.

Total flavonoids were determined according to the method of Hidalgo et al. (2019); 250 µL of ethanolic extract were taken, mixed with 1.25 mL of water and 75 µL of $NaNO_2$ at 5%. After 5 min 150 µL of $AlCl_3$ (aluminum chloride-1-Ethyl-3-methylimidazolium chloride) were added. Subsequently, 500 µL of NaOH 1 M and 275 µL of water were added and vigorously stirred; the absorbances of all samples were measured in a Jenway 7305 UV-Vis spectrophotometer at 510 nm. The standard was prepared with quercetin dissolved in absolute ethanol, and the results were expressed in mg QE 100 g^{-1} of fresh weight.

Total antioxidant capacity was measured according to the method of Hsu et al. (2003); using the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. A DPPH solution was prepared in ethanol at 0.025 mg mL^{-1} concentration; 50 µL of ethanolic extract were mixed with 1950 µL of DPPH solution; after 30 min the samples absorbance was read in a Jenway 7305 UV-Vis spectrophotometer at 517 nm. The results were expressed in µM equivalent in Trolox 100 g^{-1} of fresh weight.

Vitamin C

The vitamin C content was determined with the methodology reported by Padayatt *et al*. (2001), for this 10 g of fresh weight of leaves were placed in a mortar and triturated with 10 mL of hydrochloric acid 2% (v/v), then the mixture was filtered and made up to 100 mL with distilled water in an Erlenmeyer flask. Subsequently 10 mL of the diluted were taken and titrated with 2,6-dichlorophenol (1 X 10^{-3} N) until the solution reached pink. The vitamin C content was determined using the equation (5):

$$\text{Vitamin C (mg 100 g FW)} = \frac{\text{(mL used of 2,6-dichlorophenol) (0.088) (total volume) (100)}}{\text{(volume of the aliquot) (weight of sample)}} \quad (5)$$

Extraction for antioxidant enzyme assays

Crude extract was prepared with 100 g of fresh leaves previously washed, disinfected and dried. The sample was homogenized in 50 mL of 0.1 M potassium phosphate buffer (pH 7.0) as extraction medium. The homogenate was kept at 4 °C for 24 h, then it was filtered to eliminate vegetable residues and the supernatant was centrifuged at 4000 rpm for 20 min at 4 °C, discarding the precipitate and leaving the supernatant, which represents the crude extract containing the enzyme.

Enzymatic activity

The catalase (CAT 1.11.1.6) enzymatic activity was measured according to the method of Aebi (1983). CAT activity was measured spectrophotometrically (Jenway 7305 UV-Vis) at room temperature by monitoring the decrease in absorbance at 240 nm resulting from the H_2O_2 decomposition. Extinction coefficient ($\varepsilon 240$ = 43.6 $M^{-1}cm^{-1}$) and protein content (Bradford, 1976) were used to calculate enzymatic

activity. The activity was expressed in $U.mg^{-1}$ of protein, where one unit (U) of catalase activity was defined as the amount of enzyme that caused an absorbance change of 0.001 per min under assay conditions.

The peroxidase (POD 1.11.1.7) enzymatic activity was measured using guaiacol as the hydrogen donor. POD activity was measured spectrophotometrically (Jenway 7305 UV-Vis) by monitoring the increase in absorbance at 470 nm resulting from the oxidation of guaiacol by H_2O_2. Extinction coefficient ($\varepsilon 470 = 5.57$ $mM^{-1}cm^{-1}$) and protein content (Bradford, 1976) were used to calculate enzymatic activity. The activity was expressed in $U.mg^{-1}$ of protein, where one unit (U) of enzyme activity was defined as 0.001 change in absorbance per min, under assay conditions (Onsa *et al*., 2004).

The polyphenol oxidase (PPO 1.14.18.1) enzymatic activity was measured according to the method of Laminkanra (1995). PPO activity was measured spectrophotometrically (Jenway 7305 UV-Vis) at room temperature by monitoring the increase in absorbance at 420 nm resulting from the decomposition of catechol. Extinction coefficient ($\varepsilon 420 = 3450$ $M^{-1}cm^{-1}$) and protein content (Bradford, 1976) were used to calculate enzymatic activity. The activity was expressed in $U.mg^{-1}$ of protein, where one unit (U) of enzyme activity was defined as 0.001 change in absorbance per min, under assay conditions (Oktay *et al*., 1995; Alici and Arabaci, 2016).

Determination of minerals in leaves

For nitrogen determination (N), the samples were digested using the Kjeldahl method (Plank, 1992), involves the transformation of organic N to ammonium (NH_4^+) by digesting the sample with concentrated sulfuric acid (H_2SO_4) and then measuring the amount of NH_4^+ produced. The concentration of N was expressed as a percentage.

Phosphorus (P) was determined by the ammonium metavanadate (NH_4VO_3) colorimetric method in an absorption range of 430 nm against a K_2HPO_4 curve. In total, 3.5 mL of distilled water, 500 L of the stock solution, and 1 mL of phosphorus reagent were added to the test tubes. Each tube was vortexed and allowed to stand for one hour. At the end, the reading was measured spectrophotometrically (Jenway 7305 UV-Vis). The concentration of P was expressed as a percentage.

Total contents of K^{+1}, Ca^{+2}, Mg^{+2}, Cu^{+2}, Fe^{+2}, Zn^{+2} and Mn^{+2} were determined after digestion of the sample with nitric acid 65%. Dried leaves samples were weighed into digestion tubes and 10 mL of nitric acid were added. Tubes were heated in an infrared digestion apparatus. Nitric acid was added to complete digestion as needed. The solution was allowed to dry when contents of the tubes were clear. The residue was dissolved with enough nitric acid and lanthanum solution in order to reach a final concentration of 1% HNO_3 +0.5% lanthanum 99.99% when taken to the volume of the volumetric flask employed. The solution obtained was then used to determine potassium, calcium, magnesium, copper, iron, zinc and manganese by Flame Atomic Absorption Spectrometry (F-AAS) using a Thermo Scientific iCE. Blanks and calibration standards were read for quality assurance purposes. Results were expressed as mg element kg $dry\ weight^{-1}$ as referred by Kawashima and Valente-Soares (2003).

Statistical analysis

All data presented here are the mean values of five replicates. The data of the variables were determined by analysis of variance and mean comparison test using the Tukey test ($P \leq 0.05$) with the statistical package SAS (Statistical Analysis System Institute) version 9.4.

Results

Morphological characteristics of basil plants

The use of ZnO NPs modified the morphological characteristics of basil plants (Table 2). The results of the variance analysis showed significant differences ($p < 0.05$) on each of these quality variables. We could

observe that the concentration of the 20 mg L^{-1} showed the highest fresh weight of leaf (296 g), fresh weight of stem (33 g), plant height (53 cm), leaf number (926) and leaf area (7583 cm^2). A relative increase in the variables is observed in basil plants treated with NPs-ZnO as the concentration of ZnO NPs applied increases, more than control treatment.

Table 2. Effects of foliar application of ZnO NPs on morphological characteristics of basil plants

ZnO NPs (mg L^{-1})	Fresh weight of leaf (g)	Fresh weight of stem (g)	Plant height (cm)	Leaf number	Leaf area (cm^2)
Control	166 ± 5.20 d	18 ± 5.20 c	45 ± 1.73 b	593 ± 47.25 c	1501 ± 2.32 e
5	232 ± 68.45 c	16 ± 1.82 b	47 ± 1.00 b	763 ± 32.14 b	2943 ± 29.26 d
10	253 ± 1.90 b	25 ± 1.46 b	45 ± 1.00 b	820 ± 69.28 ab	3526 ± 67.83 c
15	281 ± 3.60 a	31 ± 1.70 a	48 ± 1.52 b	863 ± 32.14 ab	5952 ± 62.82 b
20	296 ± 2.64 a	33 ± 1.37 a	53 ± 1.00 a	926 ± 46.18 a	7583 ± 27.83 a
CV	3.19	5.40	2.70	5.97	1.04
R^2	0.98	0.95	0.88	0.89	0.99

Values followed by same literal at same column are not significant according to Tukey ($p < 0.05$)

The dry weight of basil plants showed significant differences ($p < 0.05$) when they were treated with ZnO NPs compared to the control. It is observed that the treated basil plants were heavier than the control treatment; the dry biomass reached 31.21 g in those plants treated with ZnO NPs in concentration 15 mg L^{-1} and 33.05 g in for those treated with 20 mg L^{-1}, while the control treatment only reached 18 g; this corresponds to an increase of 73% and 84%, respectively (Figure 1).

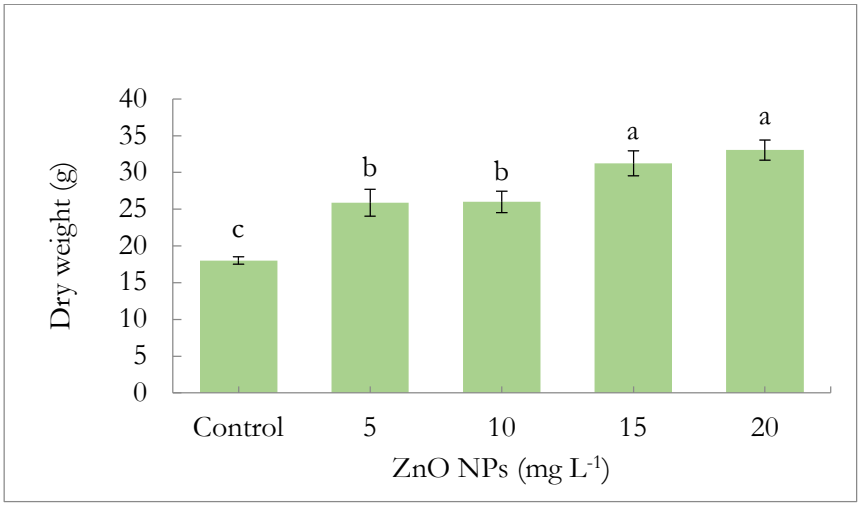

Figure 1. Dry weight of leaves of basil plants after treatment with ZnO NPs
Means with the same literal are not significant according to Tukey ($p < 0.05$)

Pigment contents

According to the Tukey test, the ZnO NPs had a significant effect on total chlorophyll and carotenoid contents ($p < 0.05$) (Figure 2a-b). The highest chlorophyll content (5.54 μg g^{-1} FW) was obtained for the control treatment, whereas the lowest one (4.14 μg g^{-1} FW) was observed for the 20 mg L^{-1} treatment. However, carotenoid content in the leaves was markedly higher than the control, the control had the concentration of 0.84 μg g^{-1} FW, followed by 0.98, 0.95, 0.96 μg g^{-1} FW, while the treatment with 20 mg L^{-1} ZnO NPs registered a value of 1.08 μg g^{-1} FW.

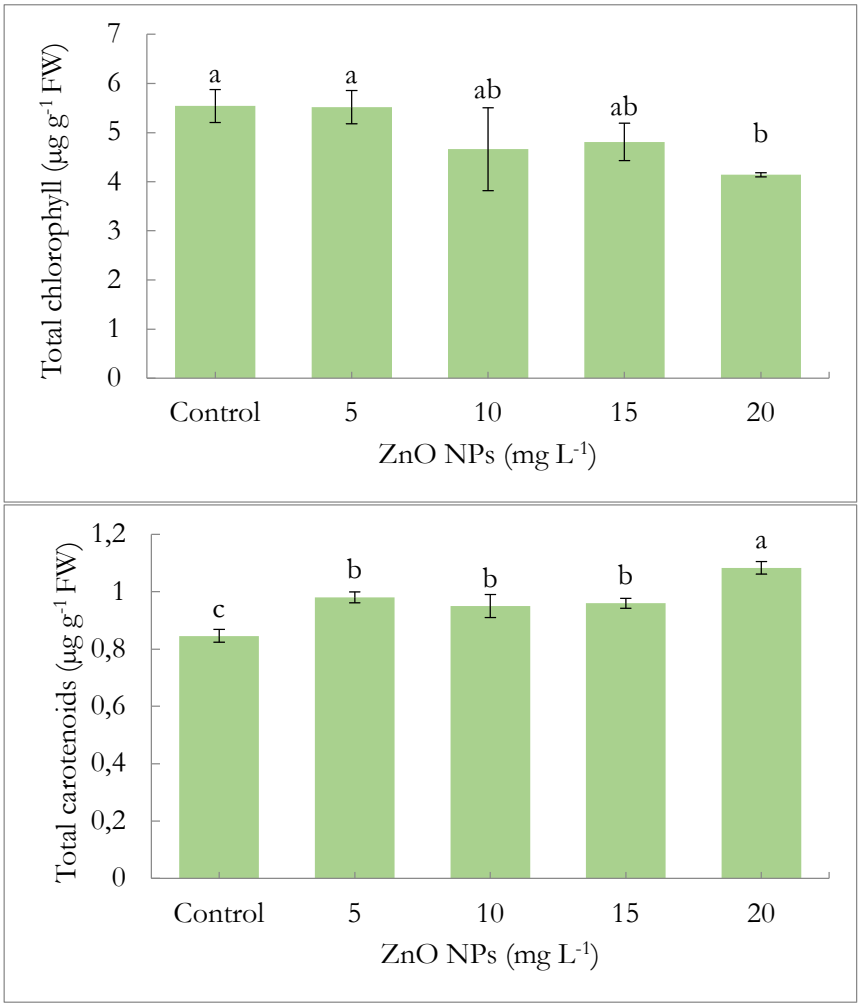

Figure 2. Pigment contents of leaves of basil plants after each treatment of ZnO NPs
Total chlorophyll (a) and total carotenoids (b). Means with the same literal are not significant according to Tukey ($p < 0.05$)

Phytochemical compounds

The foliar spray of ZnO NPs modified the biosynthesis of phytochemical compounds such as total phenols and flavonoids as well as the antioxidant capacity in the basil plants (Figure 3a-c). The results showed significant differences ($p < 0.05$) compared to the control treatment. The highest concentration of the aforementioned was observed for the 20 mg L^{-1} treatment obtaining 40.52 mg of gallic acid 100 g^{-1} FW, 4.39 mg of quercetin 100 g^{-1} FW and 0.05 Meq Trolox 100 g^{-1} FW.

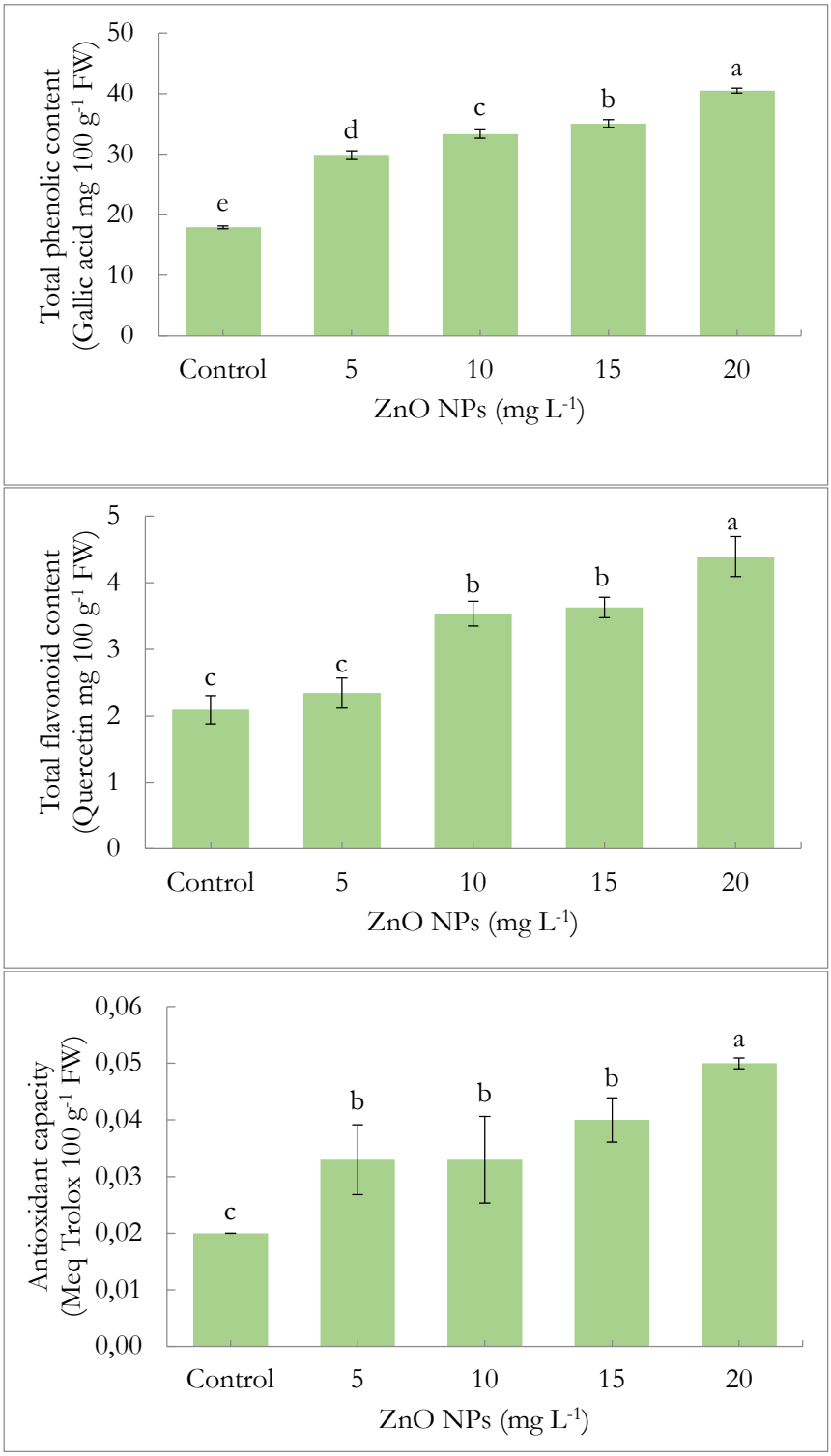

Figure 3. Phytochemical compounds of leaves of basil plants after each treatment of ZnO NPs
Total phenolic content (a), total flavonoid content (b) and antioxidant capacity (c). Means with the same literal are not significant according to Tukey ($p < 0.05$)

Vitamin C

The analysis of variance for vitamin C showed significant differences ($p < 0.05$) in plants treated with ZnO NPs, different concentrations affected the basil plants differently. An increase in the content of vitamin C was observed as the concentration of ZnO NPs increased. The highest concentration was obtained with 20 mg L^{-1} with a mean of 13.20 mg 100 g^{-1} FW, this value is 400% higher than the control treatment.

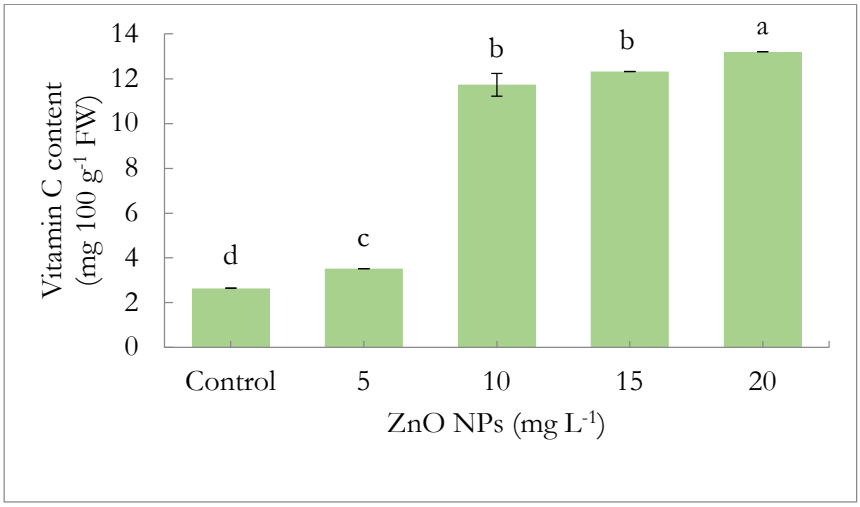

Figure 4. Vitamin C content of leaves of basil plants after each treatment of ZnO NPs
Means with the same literal are not significant according to Tukey ($p < 0.05$)

Enzymatic activity

The results obtained clearly indicate that the application of ZnO NPs induced a higher content of enzymatic compounds in basil plants (Figure 5a-c). Statistically significant differences were observed in all evaluated enzymes ($p < 0.05$). Regarding the CAT, POD and PPO enzymes, an increase in activity was observed with at 20 mg L^{-1} ZnO NPs by 33%, 88% and 128%, respectively, compared to the control.

Determination of minerals in leaves

The effects aforementioned were consistent with the mineral content of the basil plants (Table 3). The total concentration of N showed a significant increase in those plants treated with 10-15 mg L^{-1} ZnO NPs in comparison to the control treatment. Likewise, an increase in the absorption of P was observed in plants treated with 5-10 mg L^{-1} ZnO NPs. On the other hand, the highest concentration of K^{+1} was observed in the control treatment, whereas it had the lowest content of Ca^{+2}. Otherwise, Mg^{+2} content in the leaves did not show significant differences ($p < 0.05$).

The results found for the Cu^{+2}, Fe^{+2}, Zn^{+2} and Mn^{+2} content in the plant tissue showed statistical differences between the treatments. There was a higher concentration of all these elements in the leaves of plants treated with 20 mg L^{-1} ZnO NPs.

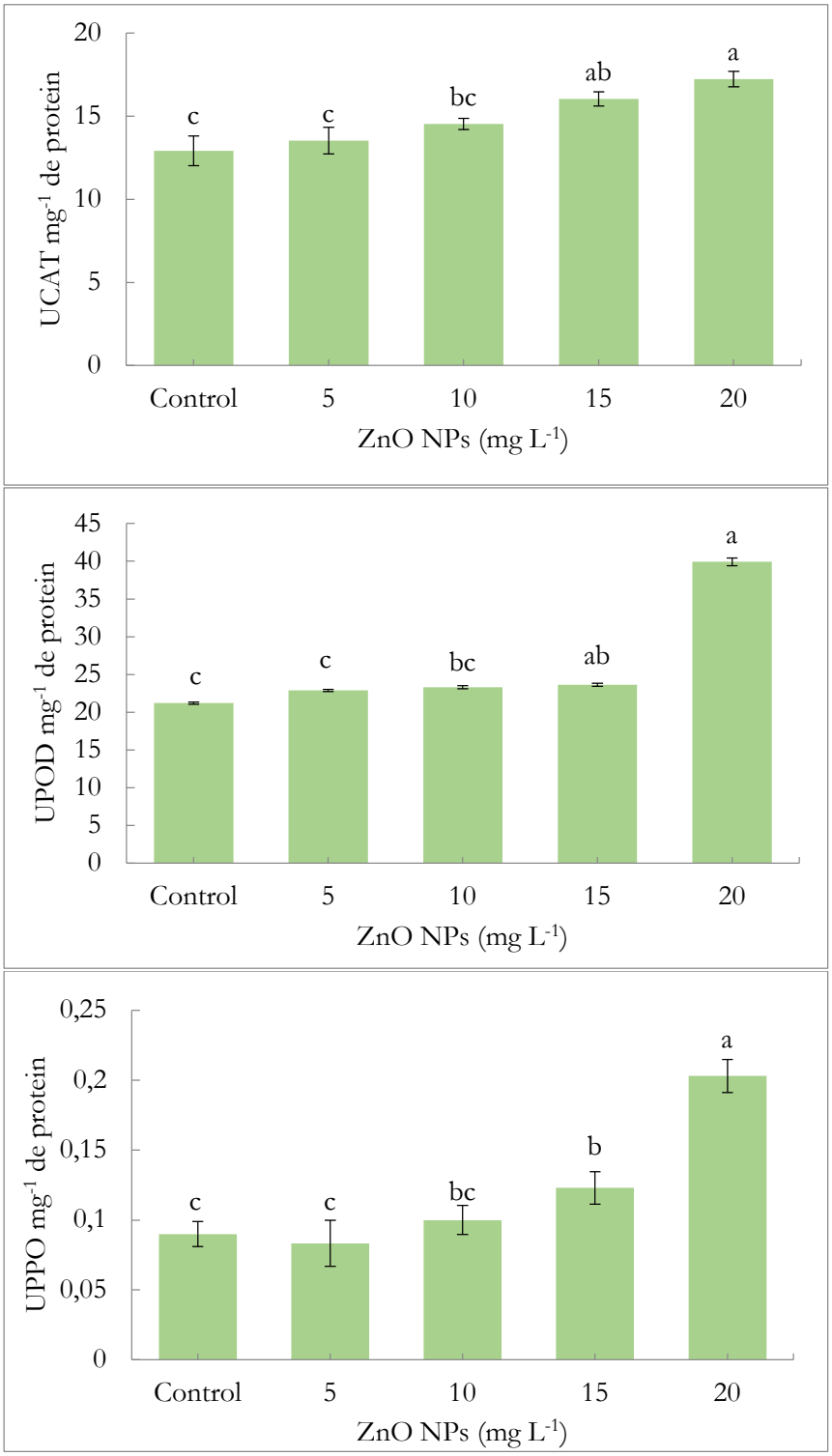

Figure 5. Enzymatic activity of leaves of basil plants after each treatment of NPs-ZnO. Catalase (a), peroxidase (b) and polyphenol oxidase (c)

Means with the same literal are not significant according to Tukey ($p < 0.05$)

Table 3. Effects of foliar application of ZnO NPs on the mineral content of basil plants

ZnO NPs (mg L^{-1})	N	P	K	Ca	Mg
			%		
Control	3.96 ± 0.05 b	0.50 ± 0.05 d	4.78 ± 0.17 a	2.78 ± 0.26 b	0.80 ± 0.05 a
5	3.95 ± 0.03 b	0.84 ± 0.01 a	3.67 ± 0.16 b	3.83 ± 0.13 a	0.84 ± 0.01 a
10	4.27 ± 0.06 a	0.84 ± 0.02 ab	3.83 ± 0.13 b	3.69 ± 0.05 a	0.80 ± 0.00 a
15	4.27 ± 0.01 a	0.57 ± 0.00 c	2.88 ± 0.15 c	3.86 ± 0.20 a	0.80 ± 0.11 a
20	3.89 ± 0.03 b	0.77 ± 0.00 d	2.92 ± 0.08 c	3.50 ± 0.09 a	0.75 ± 0.02 a
ZnO NPs (mg L^{-1})	**Cu**	**Fe**	**Zn**	**Mn**	
			mg kg^{-1}		
Control	14.05 ± 0.50 c	692.70 ± 2.84 d	26.35 ± 0.32 e	177.12 ± 1.49 c	
5	15.77 ± 0.64 b	606.64 ± 2.31 f	26.91 ± 2.81 d	182.81 ± 0.65 b	
10	14.00 ± 0.24 c	723.13 ± 0.13 c	27.82 ± 0.52 c	161.26 ± 0.49 d	
15	15.39 ± 0.40 b	861.09 ± 3.27 b	30.36 ± 0.20 b	189.38 ± 1.32 a	
20	17.44 ± 0.05 a	978.56 ± 1.57 a	32.47 ± 0.15 a	186.63 ± 1.86 a	

Values followed by same literal at same column are not significant according to Tukey ($p < 0.05$)

Discussion

Limited studies have been carried out to date to determine the effects of ZnO NPs on plant growth and productivity (Faizan *et al*., 2020). It is well recognized that ZnO NPs affect crop development and yield and that it accumulates in plant tissue, including in the edible portions. In the present work, the application of ZnO NPs promoted the development of basil plants.

Regarding the morphological characteristics, Zn is needed to synthesize tryptophan, which leads to IAA (a heteroauxin) synthesis by activating tryptophan synthetase (Solanki, 2021). Several reports have shown the positive effect of using Zn in increasing growth parameters of sweet basil (El-Kereti *et al*., 2013) and bean plants (KhavariNejad *et al*., 2014). Improvement in the growth characteristics is due to fact that zinc is an essential element needed for the normal and healthy growth of plants. When the supply of plant with available zinc is inadequate, crop yields are reduced, and the quality of crop products is frequently impaired (Muhammad, 2011).

In addition, positive effects of the ZnO NPs application were reported on leaf chlorophyll content of peanut plants (Prasad *et al*., 2012) indicating that Zn has key roles in chlorophyll synthesis in plants (Abbasifar *et al*., 2020). This is due to zinc acts as a structural and catalytic component of proteins, enzymes and as co-factor for normal development of pigment biosynthesis (Balashouri 1995). The significant role in the metabolism of nitrogen and protection of sulfhydryl groups cause synthesized chlorophyll, in the presence of zinc, completion and the formation of chlorophyll is facilitated (Mohsenzadeh and Moosavian, 2017). However, Zn like other metals, in large quantities is toxic to many plants, and the degradation of chlorophyll in these circumstances is evident. These results demonstrate that ZnO NPs drastically decrease the chlorophyll content, it seems that perhaps the reduction in the amount of chlorophyll is due to the prevention or degradation of the precursors of these pigments (Mohsenzadeh and Moosavian, 2017). The chlorophyll content is considered an important index of the total amount of the light harvesting complex and electron transport components, it is positively related to the photosynthetic rate (Li *et al*., 2019), so it can be used as an indicator to measure the degree of stress caused by NPs. The photosynthesis of chloroplasts is altered, which causes oxygen to become an electron acceptor and reactive oxygen species to be produced (Yan *et al*., 2021). Otherwise, carotenoids are antioxidant compounds soluble in plant cells. These compounds are produced

through a non-enzymatic route which operates to reduce oxidative damage to the plant, they are present in plast of plant tissues, they are also responsible of protecting photosynthetic tissues, especially chlorophyll (Galindo-Guzmán *et al*., 2022). According to the results obtained in this study, it seems that certain amount of zinc induces oxidative stress and causes synthesis of carotenoids.

In the case of the phytochemical compounds, a higher concentration of total phenols flavonoids and antioxidant capacity was observed in those treated with ZnO NPs.

It has been suggested that Zn significantly influences the expression of phenolic biosynthesis pathway genes (Song *et al*., 2015), in the use of carbon to produce phenolic compounds in the cycle of shikimic acid and acetate. Our results are similar with those reported for other researchers who found that phenolic content of plants was significantly enhanced by application of ZnO NPs fertilizers. For example, in basil plant (Abbasifar *et al*., 2020), in orejona lettuce (Fortis-Hernández *et al*., 2022) and habanero pepper (García-López *et al*., 2018).

For flavonoid compounds, they act as antioxidant agents, among them there are antimicrobial compounds, UV protectors, insect protectors (Hernández *et al*., 2022). The application of zinc increased concentration of flavonoids in leaves as in this study as in reports for Spanish lavender (Vojodi Mehrabani *et al*., 2017) and pepper (García-Gómez *et al*., 2017).

In our study, we found significant differences in antioxidant capacity (DPPH method) as a result of the application of ZnO NPs. This mean that the antioxidant capacity may depend on the abundance of metal ions as it has been reported in previous studies (Sida-Arreola *et al*., 2017; Preciado-Rangel *et al*., 2021; Fortis-Hernández *et al*., 2022; Hernández *et al*., 2022).

An increase in the content of vitamin C could be observed as the concentration of ZnO NPs increased, this can be attributed to the fact that ascorbic acid protects cells from oxidative damage, leads to the regeneration of vitamin C (Zahedi *et al*., 2020).

At cellular level, oxidative stress is caused when varied abiotic stress-provoked generations of ROS (such as singlet oxygen, $^1O_2$; superoxide, $O_2^{\cdot-}$; hydrogen peroxide, H_2O_2; hydroxyl radical, $OH^{-}\cdot$) exceeds the pace of their metabolism (Anjum *et al*., 2016). Among major enzymatic antioxidants, catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7) and polyphenol oxidase (PPO; EC 1.14.18.1) are representative heme enzymes meant for metabolizing stress-provoked ROS and controlling their potential impacts on cellular metabolism and functions. The application of ZnO NPs could stimulate enzymatic activity, as demonstrated in this study.

On the other hand, the effect of ZnO NPs aforementioned was confirmed with evaluation of the mineral content in the basil plants. The total concentration of Cu^{+2}, Fe^{+2}, Zn^{+2}, and Mn^{+2} showed a significant increase in comparison to the control treatment. Likewise, an increase in the absorption of N was observed, since Zn is related to the metabolism of N in the plant and it is correlated with the activity of the enzyme nitrate reductase (Preciado-Rangel *et al*., 2021). Zn deficiency or toxicity has been shown to inhibit the enzyme nitrate reductase, leading a decrease in N content and a decrease in the incorporation of N in amino acids and proteins (Luna *et al*., 2000; Sutter *et al*., 2002). The P is a structural element in nucleic acids and plays a key role in energy transfer as a component of adenosine phosphates, and it is also essential for transfer of carbohydrates in leaf cells; K^{+1} affects loading of sucrose and the rate of mass flow-driven solute movement within the plant; Ca^{+2} is important for cell wall and membrane stabilization, osmoregulation and as second messenger allowing plants to regulate developmental processes in response to environmental stimuli; Mg^{+2} is a component of chlorophyll and is required for photosynthesis and protein synthesis (López *et al*., 2020).

Conclusions

In this work, different doses of ZnO NPs were applied to the basil crop. The application of ZnO NPs improves commercial and phytochemical quality of basil plants, especially in the treatments with the highest concentration of 20 mg L^{-1} ZnO NPs, it was a higher production of beneficial metabolites such as carotenoids, phenols, flavonoids, antioxidant capacity, vitamin C and enzymatic activity for this crop. In addition, it was possible to observe that the aforementioned variables were increasing in relation to the applied doses, surpassing the control treatment. Detailed studies are suggested to understand the mechanism of action of nanoscale materials.

Authors' Contributions

Conceptualization, MFH; Methodology, MGG; Investigation and experimentation CVDR; Software, MFH, CVDR; validation, EFL, MGG, PPR and APGG; Writing and editing, EFL, MFH, APGG and MGG; Supervision, MFH and PPR; project administration, MFH.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

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

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