

## EFFECTS OF ZINC OXIDE NANOPARTICLES ON YIELD, NUTRACEUTICAL QUALITY, AND ANTIOXIDANT ACTIVITY IN JALAPEÑO PEPPER

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### ABSTRACT

Nanotechnology has emerged as a promising tool in modern agriculture, particularly through the use of metallic nanoparticles with biostimulant and biofortifying properties. This field study evaluated the effects of foliar application of zinc oxide nanoparticles (ZnO-NPs) on jalapeño pepper (*Capsicum annuum* L.). Treatments consisted of five foliar concentrations (0, 50, 100, 150, and 200 mg L<sup>-1</sup>), and the assessed variables included morphophysiological traits, yield components, biophysical fruit characteristics, nutraceutical quality, antioxidant activity, and zinc bioaccumulation. ZnO-NPs significantly enhanced yield-related traits, increasing total yield (48.34%), fruit length (13.91%), firmness (25.18%), and chlorophyll content (35.33%). Improvements in nutraceutical quality were also observed, including protein content (225%), flavonoids (82.19%), total phenols (118.41%), vitamin C (27.77%), capsaicin (80.21%), and total antioxidant capacity (12.12%). Catalase and peroxidase activities increased by 12.12% and 13.52%, respectively. Zinc concentration in the fruit increased by 55%, confirming a biofortifying effect. Overall, foliar application of ZnO-NPs modulated physiological, yield, and nutraceutical responses in jalapeño pepper plants. Concentrations between 100 and 150 mg L<sup>-1</sup> were identified as the optimal range for practical foliar application under field conditions

**Keywords:** *Capsicum annuum*; nanotechnology; biofortification; bioactive compounds; zinc oxide nanoparticles; antioxidant activity.

## INTRODUCTION

Jalapeño pepper (*Capsicum annuum* L.) is a horticultural crop of great economic, social, and cultural importance in Mexico and one of the most in-demand vegetables in international markets due to its culinary value and nutraceutical attributes (Sánchez-Toledano et al., 2023). Several studies have documented its functional properties, which are associated with bioactive compounds such as capsaicin, flavonoids, polyphenols, and vitamin C, conferring antioxidant, anti-inflammatory, antidiabetic, and anticancer effects (García-Vásquez et al., 2023). However, despite these qualities, the content of essential micronutrients such as zinc (Zn) in its fruits is often limited, reducing its value as a dietary source of this vital element for human health.

This low Zn concentration is largely attributed to its limited availability in agricultural soils, particularly those with alkaline pH, high carbonate content, or elevated phosphorus levels, which reduce Zn solubility and plant uptake (Preciado et al., 2021). This limitation not only affects plant development but also compromises the nutritional quality of the fruits, posing a risk to vulnerable populations that rely heavily on plant-based foods. Zinc deficiency is estimated to affect nearly one-third of the global population, with children under five years of age being the most susceptible due to their high nutritional demands (Praharaj et al., 2021). In plants, Zn plays a crucial role in cellular metabolism, being a structural component of various enzymes and essential for photosynthesis and carbohydrate metabolism (Cakmak et al., 2017).

To address this issue, the foliar application of ZnO-NPs has emerged as a sustainable agronomic strategy to enhance the nutritional quality of plant-based foods and help reduce micronutrient deficiencies in vulnerable populations. ZnO-NPs possess catalytic, physical, nutritional, and antimicrobial properties, along with high penetration capacity in foliar tissues and efficient absorption (Faizan et al., 2021). In addition to supplying Zn, ZnO-NPs act as elicitors, activating secondary metabolic pathways involved in the synthesis of bioactive compounds, thereby increasing the content of polyphenols, flavonoids, and vitamin C in fruits (Páramo et al., 2020; Sánchez-Pérez et al., 2023).

However, the physiological response of plants to ZnO-NPs depends on their physicochemical characteristics, such as size, morphology, synthesis method, and applied concentration (Bandeira et al., 2020). High doses may induce phytotoxic effects, including structural alterations, oxidative

stress, and reduced growth (Palacio-Márquez et al., 2021). Therefore, it is crucial to define safe and effective doses that maximize benefits without causing toxicity.

In this context, and given the importance of the jalapeño pepper and its biofortification potential, the present study evaluated the effect of foliar application of ZnO-NPs on yield, nutraceutical content, and Zn bioaccumulation in fruits. Specifically, it aimed to determine the optimal foliar concentration of ZnO nanoparticles to improve yield, nutraceutical quality, and Zn bioaccumulation in jalapeño pepper fruits, contributing to sustainable production and nutritional improvement.

## MATERIALS AND METHODS

### Growing conditions and plant material

The experiment was carried out at the experimental field of the Universidad Politécnica de la Región Laguna, located in San Pedro de las Colonias, Coahuila, Mexico (25°46'57.8" N, 103°11'30.1" W). The region has a semi-arid, warm climate typical of the Comarca Lagunera, with an average annual temperature of 22°C (range: 16.1–38.5°C) and an average yearly precipitation of 258 mm. The soil texture is clay loam. It showed a moderately alkaline pH of 7.92, an electrical conductivity of 3.02 dS m<sup>-1</sup>, and an organic matter content of 1.25%. Available nitrogen and phosphorus levels were 17.14 and 13.82 mg kg<sup>-1</sup>, respectively. Extractable potassium, calcium, and magnesium were 535.85, 6594.94, and 714.67 mg kg<sup>-1</sup>, while sodium reached 587.97 mg kg<sup>-1</sup>. Micronutrient levels (Fe, Cu, Zn, and Mn) ranged from 0.26 to 3.28 mg kg<sup>-1</sup>, indicating low micronutrient availability. The irrigation water had a neutral pH but moderate to high salinity; therefore, leaching fractions were used to prevent salt buildup in the root zone. Transplanting occurred 55 days after sowing, when seedlings had four true leaves and were 12–15 cm tall. The jalapeño pepper variety 'Orizaba' (Harris Moran®) was used. Plants were spaced 30 cm within rows and 75 cm between rows, resulting in a planting density of 35,500 plants per hectare. Fertilization followed INIFAP recommendations, applying 160–80–00 (N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O). Drip irrigation was scheduled based on the crop's water needs.

### Treatments and experimental design

The ZnO nanoparticles were supplied by Compañía Mexicana Investigación y Desarrollo de Nanomateriales S.A. de C.V. The nanoparticles had an average size of less than 50 nm, a purity of 99.7%, and a density of 5.61 g cm<sup>3</sup>. The treatments were prepared using a ZnO

NPs stock solution at a concentration of 1,000 mg L<sup>-1</sup>. From this stock, five working doses (0, 50, 100, 150, and 200 mg L<sup>-1</sup>) were obtained by dilution in distilled water, and each dose was prepared in a one-liter flask before application. A randomized complete block design was used, with five treatments and six replicates, for a total of 30 experimental units. Each experimental unit measured 4 m in length and 10 m in width, for an area of 40 m<sup>2</sup>. Foliar applications were performed using a 5 L manual sprayer, with each concentration of ZnO NPs diluted in 5 L of distilled water. Five applications were carried out: the first one 15 days after transplanting, followed by four additional applications every 15 days. In all formulations, a carrier ion and a non-toxic commercial surfactant (INEX-A<sup>®</sup>, 0.02% v:v) were included. All applications were conducted between 7:00 and 8:00 AM to ensure uniform coverage.

### Yield and fruit characteristics

Fruits were harvested 85 days after transplanting at commercial maturity. Total fruit weight per plot was recorded using a digital scale (Torrey<sup>®</sup>, Mexico; max. 5 kg), and yield was extrapolated to kg ha<sup>-1</sup>. Fruit length and diameter were measured using a digital caliper (Truper<sup>®</sup>, Mexico) and expressed in centimeters.

### Fruit firmness

Fruit firmness was evaluated using a penetrometer (model FH20000, Exttech<sup>®</sup>, USA) equipped with an 8 mm diameter probe. Six replicates were considered per treatment, and eight fruits were selected from each replicate. Each fruit was placed on a firm, level surface and punctured at two distinct points, recording the average value of both measurements. Results were expressed as maximum compression force in Newtons (N).

### Chlorophyll content

Chlorophyll content was determined following the method of Lichtenthaler and Wellburn (1983). Fresh leaf tissue (500 mg) was suspended in 95% ethanol, homogenized, and centrifuged at 1500 × g for 20 min. Absorbance readings were taken at 665, 649, and 470 nm using a UV-Vis spectrophotometer (Jenway 7305). Chlorophyll concentrations were calculated using the following equations:

- Chlorophyll a = 13.95 × A<sub>665</sub> – 6.88 × A<sub>649</sub>
- Chlorophyll b = 24.96 × A<sub>649</sub> – 7.32 × A<sub>665</sub>
- Total chlorophyll = Chlorophyll a + Chlorophyll b

### Preparation of extracts for non-enzymatic antioxidants

To evaluate bioactive compounds (total phenols, flavonoids, and antioxidant capacity), 2 g of fresh sample were extracted with 10 mL of 80% ethanol. The mixture was agitated for 24 h at 200 rpm and 5 °C, then centrifuged at 3,000 rpm for 5 min. This extraction procedure, which uses 80% ethanol at low temperature and subsequent centrifugation, selectively isolates non-enzymatic antioxidant compounds. The use of an alcohol-based solvent and cold conditions prevents the extraction and activity of antioxidant enzymes, ensuring that the resulting extract contains only non-enzymatic bioactive antioxidant compounds. The supernatant was stored at –20 °C for further analysis.

### Total flavonoids

Total flavonoid content was determined according to the method of Lamaison and Carnet (1990). A 250 μL aliquot of the extract was mixed with specific reagents (NaNO<sub>2</sub>, AlCl<sub>3</sub>, and NaOH), and absorbance was measured at 510 nm. Quercetin was used as the standard, and results were expressed as mg of quercetin equivalents (QE) per 100 g of fresh weight (FW).

### Total phenolic

Total phenolic content was determined using the Folin–Ciocalteu method adapted by Singleton et al. (1999). A 50 μL aliquot of the extract was mixed with distilled water and 10% (v/v) Folin–Ciocalteu reagent, followed by 7.5% (w/v) sodium carbonate solution. After 2 h of reaction, absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of FW.

### Vitamin C content

Vitamin C content was determined according to the method described by Padayatt *et al.* (2001). Fresh tissue (10 g) was homogenized with 10 mL of 2% HCl (v/v), filtered, and adjusted to 100 mL with distilled water. Subsequently, 10 mL of the diluted extract was titrated with 2,6-dichlorophenolindophenol (1 × 10<sup>-3</sup> N) until a persistent pink color appeared. The final result was expressed as mg per 100 grams of FW.

### Capsaicin content

Capsaicin concentration was determined in physiologically mature fruits following the method of Bennet and Kirby (1965). Quantification was performed by spectrophotometry at 286 nm using a Bio-145025 Biomate-5 spectrophotometer (Thermo Electron Corporation). A calibration

curve was prepared using standard capsaicin (Sigma, Co) in the range of 0.5 to 1.5 mg mL<sup>-1</sup>. Results were expressed as mg g<sup>-1</sup> FW of capsaicin.

### Antioxidant capacity

Antioxidant capacity was assessed using the *in vitro* DPPH method described by Brand-Williams et al. (1995). A DPPH solution (0.025 mg mL<sup>-1</sup> in ethanol) was mixed with 50  $\mu$ L of the sample and 1950  $\mu$ L of the DPPH solution. After 30 minutes of reaction, absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Measurements were performed in triplicate, using ethanol as a blank. Trolox was used as the standard, and results were expressed as  $\mu$ M Trolox equivalents per 100 g<sup>-1</sup> FW.

### Preparation of extracts for enzymatic activity and total protein

Fresh plant tissue (100 g) was washed, disinfected, and homogenized with 50 mL of 0.1 M potassium phosphate buffer using 30 s blending cycles for 3 min. The homogenate was kept at 4 °C for 24 h, filtered through gauze, and centrifuged at 4000 rpm for 20 min at 4 °C. The resulting extract was used to quantify catalase, peroxidase, and total protein content.

### Enzymatic activity (catalase and peroxidase)

Catalase activity (EC 1.11.1.6) was determined following the method of Aebi (1983) by measuring the decrease in absorbance at 240 nm due to the decomposition of H<sub>2</sub>O<sub>2</sub>. The extinction coefficient  $\epsilon_{240} = 43.6$  /M-cm was used, and protein concentration was determined using the Bradford method (1976). Results were expressed as U mg<sup>-1</sup> protein. Peroxidase activity (EC 1.11.1.7) was evaluated using guaiacol as a substrate, measuring the increase in absorbance at 470 nm caused by H<sub>2</sub>O<sub>2</sub>-induced oxidation. The extinction coefficient  $\epsilon_{470} = 5.57$  /mM-cm<sup>-1</sup> was applied, and protein content was determined using the Bradford method (1976), with results expressed as U mg<sup>-1</sup> protein (Onsa et al., 2004).

### Protein content

Soluble protein content was determined using the Bradford method (Bradford, 1976). This assay quantifies only the proteins extracted in the phosphate buffer, corresponding to the soluble protein fraction. Therefore, results were expressed as mg of soluble protein per g of FW.

### Zinc content

Zinc concentration in jalapeño pepper fruits was determined by atomic absorption spectrophotometry (VARIAN-SPECTR AA 3110, Palo Alto, CA, USA), following the AOAC

method (1990). Results were expressed as mg kg<sup>-1</sup> dry weight (DW).

### Statistical analysis

Data were subjected to Bartlett's test to assess homogeneity of variance, and normality was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk *W* tests. Analysis of variance (ANOVA) was then performed, and treatment means were compared using Tukey's test at a significance level of  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

### Yield and biophysical fruit quality

Foliar application of ZnO-NPs had a positive effect on yield, firmness, and fruit length in jalapeño pepper. The 100 mg L<sup>-1</sup> treatment was the most effective, resulting in a 48.34% increase in yield compared to the control (Table 1), suggesting a dose-dependent biostimulant effect. This increase is attributed to the essential role of Zn as a cofactor in key physiological processes such as photosynthesis, cellular respiration, and the synthesis of nucleic acids, proteins, and carbohydrates (Marschner and Rengel, 2012; Ramzan et al., 2020). Additionally, Zn is involved in auxin biosynthesis via the tryptophan pathway, promoting cell division and elongation, which enhances vegetative growth and yield (Faizan et al., 2021).

However, concentrations above 100 mg L<sup>-1</sup> did not further improve yield, likely due to phytotoxic effects. High Zn levels can induce oxidative stress by increasing the production of reactive oxygen species (ROS), altering membrane permeability (Lin et al., 2012), and reducing K<sup>+</sup> content due to competition for similar binding sites, thereby disrupting nutrient balance and inhibiting growth (Sturikova et al., 2018; Barrmeda-Medina et al., 2014). Moreover, excessive Zn may reduce hydraulic conductivity, negatively affecting photoassimilate transport and tissue water content, ultimately leading to lower fresh biomass (Barceló and Poschenrieder, 1990; Sturikova et al., 2018).

Regarding fruit quality, firmness responded positively to ZnO-NP application. The 100 mg L<sup>-1</sup> dose increased firmness by 20.85% compared to the control (Table 1). This effect is associated with the role of Zn in maintaining cell wall integrity by promoting the synthesis of structural components such as cellulose, hemicellulose, and pectins, which contribute to mechanical resistance (García et al., 2019; Sharifan et al., 2021). Similar results have been reported in other crops, such as grape (Abou et al., 2021) and bell pepper (Magdaleno et al.,



**Table 1. Effect of foliar application of zinc oxide nanoparticles on yield and commercial quality of jalapeño pepper fruits.**

ZnO NPs mg mL <sup>-1</sup>	Yield ton ha <sup>-1</sup>	Firmness N	Fruit length mm	Chlorophyll µg mg <sup>-1</sup> FW
Control	21.06c	12,18c	51.24b	26.40b
50	23.58bc	16.86b	52.96b	35.88ab
100	32.43a	20.85a	58.37a	40.53a
150	27.01b	16.51b	56.28a	29.95b
200	26.59b	16.70b	56.29a	28.66b

Means with the same letters are not significantly different according to Tukey's test ( $p \leq 0.05$ ).

2023), where ZnO-NPs treatments also enhanced fruit firmness.

### Fruit size and chlorophyll content

Fruit size, measured as length, also increased significantly with the application of ZnO-NPs. The 100 mg L<sup>-1</sup> treatment again showed the best performance, reaching a fruit length of 58.37 mm, which represents a 13.91% increase compared to the control (Table 1). This result aligns with findings by Ucan et al. (2023) in cucumber, where ZnO application significantly increased fruit size. Zinc enhances the activation of enzymes that improve photosynthetic efficiency and redox balance, promoting cell growth and biomass accumulation in reproductive organs (Faizan et al., 2018; Mahmood et al., 2021).

Similarly, chlorophyll content in jalapeño pepper leaves increased significantly with the application of ZnO-NPs, with the highest value observed at 100 mg L<sup>-1</sup>, representing a 35.33% increase compared to the control. This finding is consistent with results reported by Méndez et al. (2016) and Magdaleno-García et al. (2023) in different *Capsicum* varieties, where chlorophyll index also increased with 50 and 100 mg L<sup>-1</sup> ZnO treatments. Zinc is essential for the biosynthesis of light-harvesting complex (LHC) proteins, which support the formation and stability of photosynthetic pigments (Wang et al., 2021), thereby enhancing photosynthetic potential and ultimately improving crop growth and productivity.

### Non-enzymatic antioxidants

Foliar application of ZnO nanoparticles led to an increase in the accumulation of non-enzymatic antioxidant compounds, including flavonoids, total phenols, vitamin C, capsaicin, and total antioxidant capacity. The enhancement of these bioactive compounds is highly desirable due to their strong antioxidant properties and their

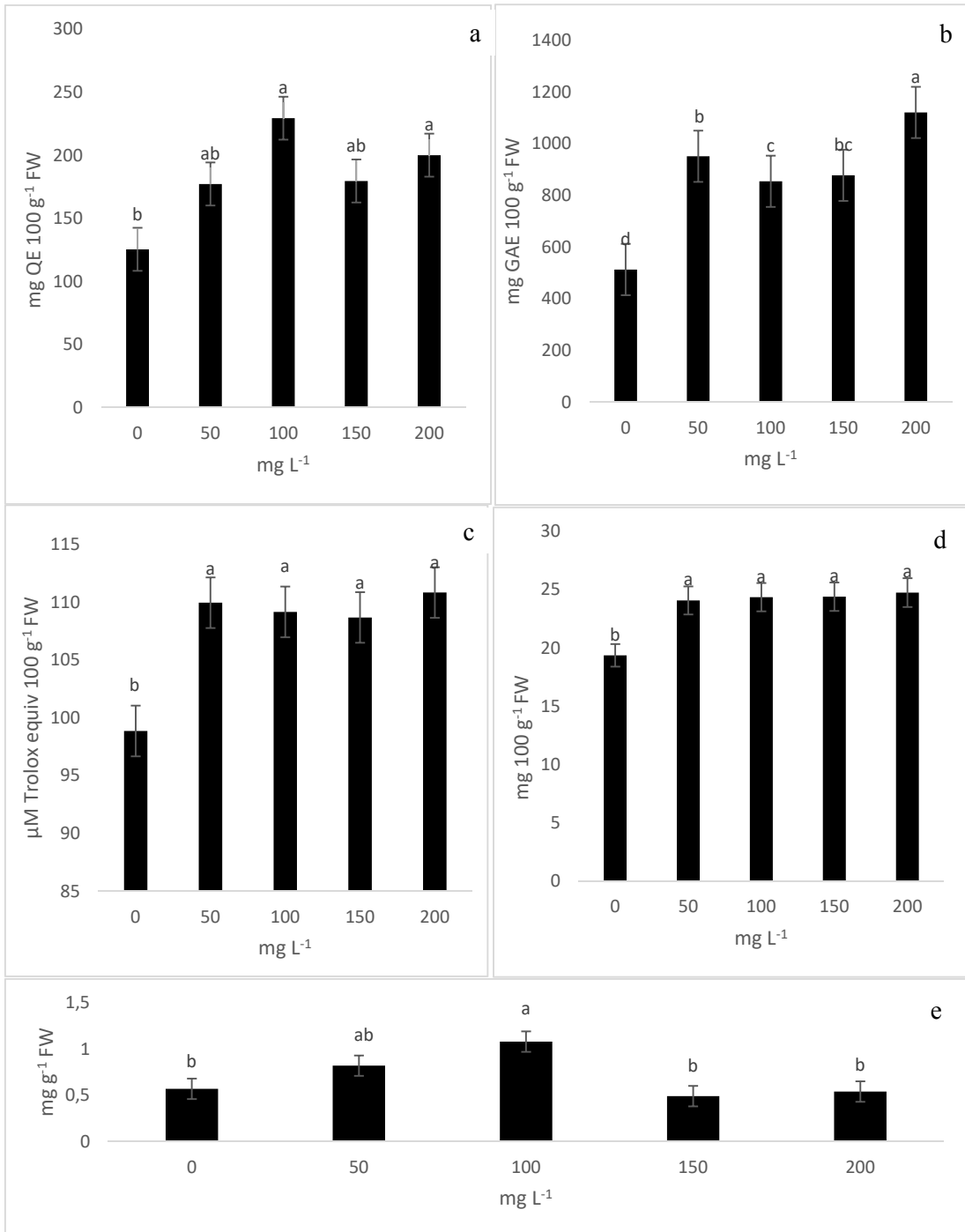
association with the prevention of cardiovascular, neurodegenerative, and inflammatory diseases (Guo et al., 2024; Minocha et al., 2022).

The 100 mg L<sup>-1</sup> ZnO-NPs treatment resulted in an 82.19% increase in flavonoid content compared to the control (Fig. 1a). Zinc has been reported to act as a cofactor for enzymes in the phenylpropanoid pathway, particularly phenylalanine ammonia-lyase (PAL), which plays a key role in flavonoid biosynthesis (García et al., 2018; Patel et al., 2020). These findings are consistent with those reported by Rivera et al. (2021) and Iziy et al. (2019).

Furthermore, the 200 mg L<sup>-1</sup> ZnO-NPs treatment led to a 118.41% increase in total phenolic compounds compared to the control (Fig. 1b). Similar effects have been documented in grapevine (Guillén et al., 2023) and radish (Galindo et al., 2023), and are attributed to the controlled generation of ROS, which activate secondary metabolic pathways such as the phenylpropanoid pathway (Marslin et al., 2017). These metabolites not only function as antioxidants but also serve as protective agents against abiotic and biotic stress conditions (Ferreira et al., 2021).

The highest antioxidant capacity was observed with the 200 mg L<sup>-1</sup> ZnO-NPs treatment, showing a 12.12% increase compared to the control (Fig. 1c). This result is consistent with findings in bell pepper (Uresti et al., 2021) and melon (Rivera et al., 2021) and is attributed to the elicitor effect of ZnO-NPs, which stimulate the biosynthesis of antioxidant enzymes such as SOD, CAT, and POD, thereby strengthening cellular defense mechanisms (Sánchez-Pérez et al., 2023).

Vitamin C content also increased significantly with the 200 mg L<sup>-1</sup> treatment, reaching a 27.77% increase compared to the control (Fig. 1d). Zinc acts as a cofactor for ascorbate oxidase, an enzyme involved in the biosynthesis of this vitamin (Zahedi et al., 2020; Bedlovičová et al., 2020). These findings are in agreement with those of Hong et al. (2021) and Magdaleno et al. (2023), who reported



**Fig. 1.** Effect of foliar application of zinc oxide nanoparticles on (a) flavonoids, (b) phenols, (c) antioxidant capacity, (d) vitamin C, and (e) capsaicin content in jalapeño pepper fruit. Bars represent the mean of six replicates, with error bars indicating the standard error of the mean. Means with different letters are statistically different according to Tukey's test ( $p \leq 0.05$ ).

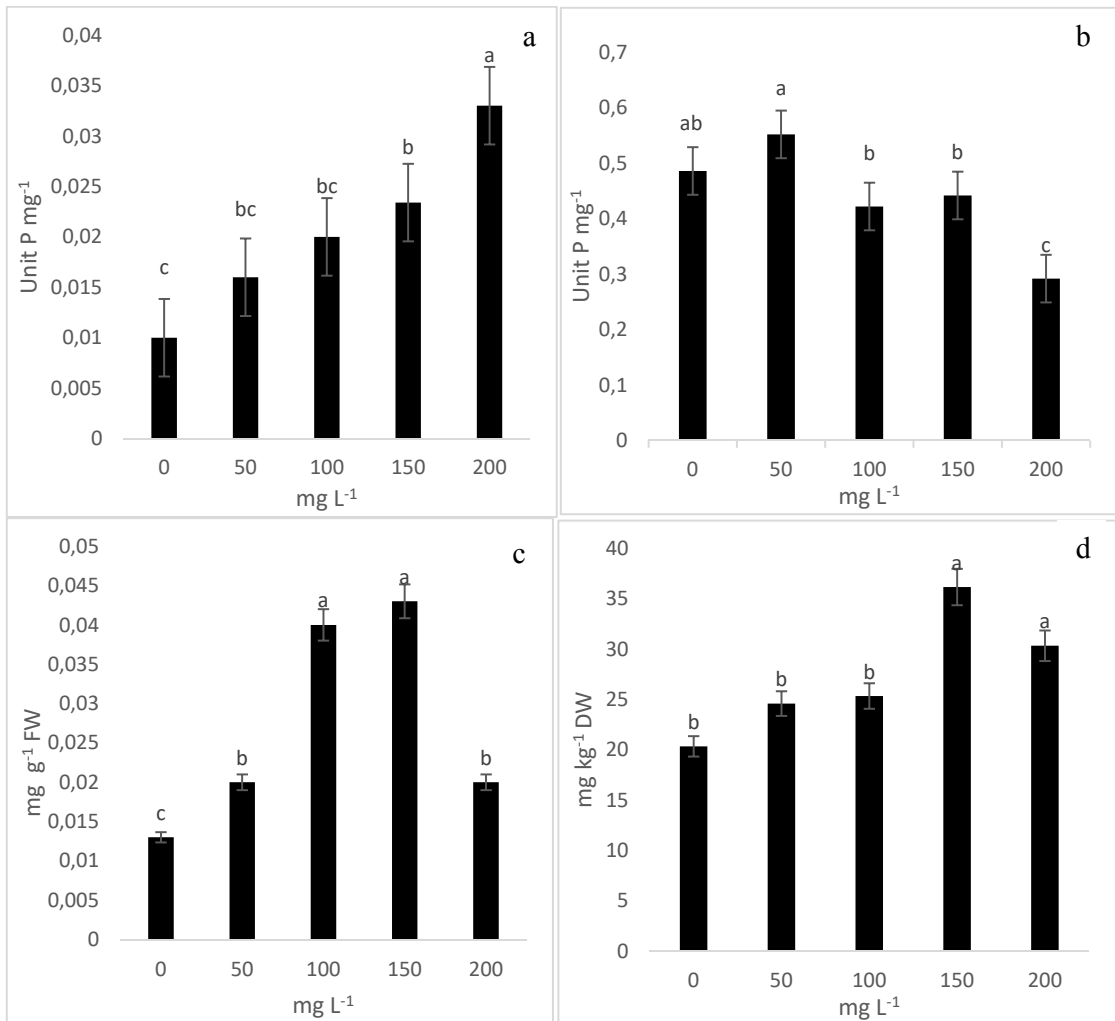
significant increases in vitamin C content in habanero and bell pepper fruits following foliar ZnO-NP application. The accumulation of vitamin C enhances oxidative stress tolerance and provides functional benefits such as immune system support and prevention of respiratory diseases (Hemilä et al., 2023; Wang et al., 2024).

The highest capsaicin concentration was recorded with the 100 mg L<sup>-1</sup> treatment, showing an 80.21% increase compared to the control (Fig. 1e). Zinc may stimulate metabolic pathways such as the shikimic acid pathway, which is a precursor to capsaicin biosynthesis (Solanki, 2021; Chen et al., 2020). However, this effect appears to be concentration-dependent, as higher doses led to a reduction in capsaicin content, possibly

due to excessive oxidative stress impairing its biosynthesis. Similar results were reported by García et al. (2019), who observed a 19.3% increase in capsaicin content in habanero peppers treated with ZnO-NPs.

#### Enzymatic antioxidants

Foliar application of ZnO nanoparticles significantly influenced the activity of antioxidant enzymes, as well as protein content and zinc content in jalapeño pepper fruits. Catalase activity showed a significant increase with ZnO-NPs treatments, reaching its highest value at 200 mg L<sup>-1</sup>, which represented a 12.12% increase compared to the control (Fig. 2a). This pattern suggests a direct dose-dependent response,



**Fig 2.** Effect of foliar application of zinc oxide nanoparticles on (a) catalase, (b) peroxidase, (c) protein, and (d) zinc content in jalapeño pepper fruit. Bars represent the mean of six replicates, with error bars indicating the standard error of the mean. Means with different letters are statistically different according to Tukey's test ( $p \leq 0.05$ ).

consistent with findings by García et al. (2018) in pepper and Dulta et al. (2022) in Carica papaya.

The increase in catalase activity can be explained by the role of Zn as a catalytic cofactor and regulator of redox balance. Catalase is a key enzyme in the detoxification of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), mitigating damage caused by ROS and protecting plant cells from oxidative stress (Nandi et al., 2019). From a nutraceutical perspective, catalase activity has been associated with the prevention of gastrointestinal disorders such as ulcers, and with reduced risk of cancer and other chronic diseases related to oxidative stress (Tandon et al., 2004; Monacelli et al., 2023).

Peroxidase activity increased with the 50 mg L<sup>-1</sup> ZnO-NPs treatment, showing a 13.52% rise compared to the control. However, at higher concentrations, activity declined, indicating an inverse dose-dependent pattern (Fig. 2b). This behavior is consistent with findings by Iziy et al. (2019) in purslane and Faizan et al. (2018) in tomato. ZnO-NPs can induce controlled ROS production, activating defense mechanisms such as peroxidase, which catalyzes peroxide breakdown and protects cellular integrity (Ghosh et al., 2016; Ruiz et al., 2021). Nevertheless, excessive ZnO-NPs concentrations may lead to ROS overproduction, surpassing the plant's antioxidant capacity and reducing peroxidase activity (Kumar et al., 2016).

Protein content in fruits responded positively to the foliar application of ZnO-NPs, with a 225% increase observed at 100 mg L<sup>-1</sup> compared to the control (Fig. 2c). Zinc acts as a cofactor in multiple antioxidant enzymes, enhancing metabolic efficiency and the accumulation of biomolecules, including proteins (Salehi et al., 2021). Similar results have been reported by García et al. (2017) in bean and tomato, and by Faizan et al. (2021) in tomato, attributing the increase to improved cellular metabolism and Zn-induced oxidative stress protection.

Zinc concentration in fruits increased with the application of ZnO-NPs, reaching its peak at 150 mg L<sup>-1</sup>, with a 55% increase relative to the control (Fig. 2d). These results are consistent with those reported by Rivera et al. (2021) in melon. This effect is attributed to the high surface reactivity and bioavailability of nanoparticles, which facilitate foliar absorption and translocation to the fruits (Nandi et al., 2019). Once incorporated into plant tissues, Zn participates in key physiological processes, acting as an enzymatic cofactor in protein synthesis, gene regulation, and activation of antioxidant pathways (Afzal et al., 2022; Jian et al., 2019). Moreover, Zn is essential for the activity of superoxide dismutase (SOD), contributing to redox balance and cellular antioxidant defense.

From a public health perspective, Zn biofortification represents an effective strategy to combat micronutrient deficiencies, strengthen the immune system, and reduce the incidence of respiratory and chronic diseases. Studies by Prasad et al. (2009) and Song et al. (2009) have shown that adequate dietary Zn levels are associated with inflammation modulation, infection resistance, and cancer prevention.

## CONCLUSIONS

The response of jalapeño pepper to foliar application of zinc oxide nanoparticles was dose-dependent. Low concentrations (50–100 mg L<sup>-1</sup>) significantly improved yield and biophysical fruit quality, whereas intermediate concentrations (150–200 mg L<sup>-1</sup>) enhanced nutraceutical quality, enzymatic activity, and zinc accumulation in the fruit. These findings highlight the importance of carefully adjusting ZnO-NP doses to optimize both productivity and nutritional quality. In summary, foliar application of ZnO-NPs at moderate doses (100–150 mg L<sup>-1</sup>) optimized both productivity and nutraceutical properties in jalapeño pepper, confirming its potential as a sustainable biofortification strategy. Therefore, the use of ZnO-NPs represents a viable agronomic approach to increase crop yield and improve the nutritional value of jalapeño pepper fruits, with direct implications for sustainable agriculture and public health.

## Author contributions

The authors declare active participation in the bibliographic review by Alondra Y. Estrada-Navarro and Juan A. Torres-Rodriguez; in the development of the methodology: Daniel Ruiz-Juarez and J. Guadalupe Luna-Ortega; in the discussion of the results: Juan J. Réyes-Perez and Pablo Preciado-Rangel; and in the review and approval of the final version of the article: Pablo Preciado-Rangel and Manuel Fortis-Hernandez.

## LITERATURE CITED

- Aebi HE. 1983. Catalase. In *Methods of Enzymatic Analysis*, Bergmeyer HU Ed., pp. 273–286. Verlag Chemie, Weinheim.
- Afzal S, Aftab T and Singh NK. 2022. Impact of zinc oxide and iron oxide nanoparticles on absorption, translocation and physiological effects in *Oryza sativa* L. *Journal of Plant Growth Regulation* 41:1445–1461. <https://doi.org/10.1007/s00344-021-10388-1>
- AOAC. 1990. *Official Methods of Analysis*, 15th Ed. Association of Official Analytical Chemists, Washington, DC.



- Bandeira M, Giovanela M, Roesch-Ely M, Devine DM and da Silva Crespo J. 2020. Green synthesis of zinc oxide nanoparticles: A review of the synthesis methodology and mechanism of formation. *Sustainable Chemistry and Pharmacy* 15:100223. <https://doi.org/10.1016/j.scp.2020.100223>
- Barceló J and Poschenrieder C. 1990. Plant water relations as affected by heavy metal stress: A review. *Journal of Plant Nutrition* 13:1–37. <https://doi.org/10.1080/01904169009364057>
- Barrameda-Medina Y, Montesinos-Pereira D, Romero L, Blasco B and Ruiz JM. 2014. Role of GSH homeostasis under Zn toxicity in plants with different Zn tolerance. *Plant Science* 227:110–121. <https://doi.org/10.1016/j.plantsci.2014.07.010>
- Bedlovičová Z, Strapáč I, Baláž M and Salayová A. 2020. A Brief Overview on Antioxidant Activity Determination of Silver Nanoparticles. *Molecules* 25(14):3191. <https://doi.org/10.3390/molecules25143191>
- Bennet DJ and Kirby GW. 1965. Colorimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16(3):144–158. <https://doi.org/10.5344/ajev.1965.16.3.144>
- Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72(1–2):248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Brand-Williams W, Cuvelier ME and Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology* 28:25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Cakmak I, McLaughlin MJ and White P. 2017. Zinc for better crop production and human health. *Plant and Soil* 411(1):1–4. <https://doi.org/10.1007/s11104-016-3166-9>
- Chen S, Lin R, Lu H, Wang Q, Yang J, Liu J and Yan C. 2020. Effects of phenolic acids on free radical scavenging and heavy metal bioavailability in *Kandelia obovata* under cadmium and zinc stress. *Chemosphere* 249:126341. <https://doi.org/10.1016/j.chemosphere.2020.126341>
- Dulta K, Koşarsoy Ağçeli G, Chauhan P and Kumar V. 2022. Ecofriendly synthesis of zinc oxide nanoparticles by *Carica papaya* leaf extract and their applications. *Journal of Cluster Science* 33(2):603–617. <https://doi.org/10.1007/s10876-020-01962-w>
- Faizan M, Bhat JA, Chen C, Alyemeni MN, Wijaya L, Ahmad P and Yu F. 2021. Zinc oxide nanoparticles (ZnO-NPs) induce salt tolerance by improving the antioxidant system and photosynthetic machinery in tomato. *Plant Physiology and Biochemistry* 161:122–130. <https://doi.org/10.1016/j.plaphy.2021.02.002>
- Faizan M, Faraz A, Yusuf M, Khan ST and Hayat S. 2018. Zinc oxide nanoparticle-mediated changes in photosynthetic efficiency and antioxidant system of tomato plants. *Photosynthetica* 56(2):678–686. <https://doi.org/10.1007/s11099-017-0717-0>
- Ferreira SS and Antunes MS. 2021. Re-engineering plant phenylpropanoid metabolism with the aid of synthetic biosensors. *Frontiers in Plant Science* 12:701385. <https://doi.org/10.3389/fpls.2021.701385>
- Galindo-Guzmán M, Fortis-Hernández M, Preciado-Rangel P and Galindo-Guzmán AP. 2023. ZnO nanoparticles synthesized by chemical precipitation to increase germination and bioactive compounds in sprouts of *Raphanus sativus* L. *AgroProductividad* 16(2). <https://doi.org/10.32854/agrop.v16i2.2414>
- García-Gómez C, Obrador A, González D, Babín M and Fernández MD. 2017. Comparative effect of ZnO NPs, ZnO bulk and ZnSO<sub>4</sub> in the antioxidant defences of two plant species growing in two agricultural soils under greenhouse conditions. *Science of the Total Environment* 589:11–24. <https://doi.org/10.1016/j.scitotenv.2017.02.153>
- García-López JI, Niño-Medina G, Olivares-Sáenz E, Lira-Saldivar RH, Barriga-Castro ED, Vázquez-Alvarado R, Rodríguez-Salinas PA and Zavala-García F. 2019. Foliar application of zinc oxide nanoparticles and zinc sulfate boosts the content of bioactive compounds in habanero peppers. *Plants* 8(8):254. <https://doi.org/10.3390/plants8080254>
- García-López JI, Zavala-García F, Olivares-Sáenz E, Lira-Saldivar RH, Barriga-Castro ED, Ruiz-Torres NA, Ramos-Cortez E, Vázquez-Alvarado R and Niño-Medina G. 2018. Zinc oxide nanoparticles boost phenolic compounds and antioxidant activity of *Capsicum annuum* L. during germination. *Agronomy* 8(10):215. <https://doi.org/10.3390/agronomy8100215>

- García-Vásquez R, Vera-Guzmán AM, Carrillo-Rodríguez JC, Pérez-Ochoa ML, Aquino-Bolaños EN, Alba-Jiménez JE and Chávez-Servia JL. 2023. Bioactive and nutritional compounds in fruits of pepper (*Capsicum annuum* L.) landraces conserved among indigenous communities from Mexico. *AIMS Agriculture and Food* 8(3):832-850. <https://doi.org/10.3934/agrfood.2023044>
- García JFA, Romero CPA y Reyes DA. 2018. Presencia de carotenoides en chile ancho y pasilla (*Capsicum annuum* L.) en muestras de 10 años y recientes. *Polibotánica* 46(1):259–272. <https://doi.org/10.18387/polibotanica.46.17>
- Ghosh M, Jana A, Sinha S, Jothiramajayam M, Nag A, Chakraborty A, Mukherjee A and Mukherjee A. 2016. Effects of ZnO nanoparticles on plants: cytotoxicity, genotoxicity, dysregulation of antioxidant defenses, and cell cycle arrest. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 807:25–32. <https://doi.org/10.1016/j.mrgentox.2016.07.006>
- Guillén-Enríquez RR, Sánchez-Chávez E, Fortis-Hernández M, Márquez-Guerrero SY, Espinosa-Palomeque B and Preciado-Rangel P. 2023. ZnO nanoparticles improve bioactive compounds, enzymatic activity and zinc concentration in grapevine. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 51(4):13377. <https://doi.org/10.15835/nbha51413377>
- Guo X, Li Q, Luo T, Xu D, Zhu D, Li J, Han D and Wu Z. 2024. Zinc Oxide Nanoparticles Treatment Maintains the Postharvest Quality of Litchi Fruit by Inducing Antioxidant Capacity. *Foods* 13(21):3357. <https://doi.org/10.3390/foods13213357>
- Hemilä H and Chalker E. 2023. Vitamin C reduces the severity of common colds: a meta-analysis. *BMC Public Health* 23(1):2468.
- Iziy E, Majd A, Vaezi-Kakhki MR, Nejadstattari T and Kazemi Noureini S. 2019. Effects of zinc oxide nanoparticles on enzymatic and nonenzymatic antioxidant content, germination, and biochemical and ultrastructural cell characteristics of *Portulaca oleracea* L. *Acta Societatis Botanicorum Poloniae* 88(4):3639. <https://doi.org/10.5586/asbp.3639>
- Jian L, Bai X, Zhang H, Song X and Li Z. 2019. Promotion of alfalfa metal growth and accumulation by co-inoculation with *Sinorhizobium* and *Agrobacterium* under copper and zinc stress. *PeerJ* 7:e6875. <https://doi.org/10.7717/peerj.6875>
- Kumar S and Trivedi PK. 2016. Heavy metal stress signaling in plants. In *Plant Metal Interaction*, Ahmad P Ed., pp. 585–603. Elsevier. <https://doi.org/10.1016/B978-0-12-803158-2.00025-4>
- Lamaison JL and Carnat A. 1990. The Amount of Main Flavonoids in Flowers and Leaves of *Crataegus Monogyna* Jacq. and *Crataegus Laevigata* (Poiret) DC. (Rosacea). *Pharmaceutica Acta Helvetiae* 65:315-320.
- Lichtenthaler HK and Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 11(5):591–592. <https://doi.org/10.1042/bst0110591>
- Lin YF and Aarts MGM. 2012. The molecular mechanism of zinc and cadmium stress response in plants. *Cellular and Molecular Life Sciences* 69:3187–3206. <https://doi.org/10.1007/s00018-012-1089-z>
- Magdaleno-García G, Juárez-Maldonado A, Betancourt-Galindo R, González-Morales S, Sánchez-Vega M, Cabrera-de la Fuente M y Méndez-López A. 2023. Aplicación de nanopartículas de óxido de zinc y cloruro de sodio en chile pimienta. *Ecosistemas y Recursos Agropecuarios* (Núm. Esp. III):e3679. <https://doi.org/10.19136/era.a10nIII.3679>
- Mahmood T, Rana RM, Ahmar S, Saeed S, Gulzar A, Khan MA, Wattoo FM, Wang X, Branca F, Mora-Poblete F, Mafrá GS and Du X. 2021. Effect of Drought Stress on Capsaicin and Antioxidant Contents in Pepper Genotypes at Reproductive Stage. *Plants* 10(7):1286. <https://doi.org/10.3390/plants10071286>
- Marschner P and Rengel Z. 2012. *Marschner's Mineral Nutrition of Higher Plants*, 3rd Ed. Elsevier. <https://doi.org/10.1016/B978-0-12-384905-2.00012-1>
- Marslin G, Sheeba CJ and Franklin G. 2017. Nanoparticles alter secondary metabolism in plants via ROS burst. *Frontiers in Plant Science* 8:832. <https://doi.org/10.3389/fpls.2017.00832>
- Méndez-Argüello B, Lira-Saldívar RH y Servín-Garcidueñas LE. 2016. Promoción del crecimiento en plantas de *Capsicum annuum* por nanopartículas de óxido de zinc. *Nova Scientia* 8(2):140–156.
- Minocha T, Birla H, Obaid AA, Rai V, Sushma P, Shivamallu C and Singh SK. 2022. Flavonoids as promising neuroprotectants and their therapeutic potential against Alzheimer's disease. *Oxidative Medicine and Cellular Longevity* 2022:6038996. <https://doi.org/10.1155/2022/6038996>

- Monacelli F, Saleh EAM, Al-Dolaimy F, Baymakov S, Ullah MI, Khlewee IH, Bisht YS and Alsaalamy AH. 2023. Oxidative stress affects the beginning of the growth of cancer cells through a variety of routes. *Pathology Research and Practice* 249:154664. <https://doi.org/10.1016/j.prp.2023.154664>
- Nandi A, Yan LJ, Jana CK and Das N. 2019. Role of catalase in oxidative stress-and age-associated degenerative diseases. *Oxidative Medicine and Cellular Longevity* 2019(1):9613090. <https://doi.org/10.1155/2019/9613090>
- Onsa GH, Saari N, Selamat J and Bakar J. 2004. Purification and characterization of membrane-bound peroxidases from Metroxylon sagu. *Food Chemistry* 85(3):365–376. <https://doi.org/10.1016/j.foodchem.2003.07.013>
- Padayatty SJ, Daruwala R, Wang Y, Eck PK, Song J, Koh WS and Levine M. 2001. Vitamin C: from molecular actions to optimum intake. In *Handbook of Antioxidants*, pp. 136-165. CRC Press.
- Palacio-Márquez A, Ramírez-Estrada CA, Gutiérrez-Ruelas NJ, Sánchez E, Ojeda-Barrios DL, Chávez-Mendoza C and Sida-Arreola JP. 2021. Efficiency of foliar application of zinc oxide nanoparticles versus zinc nitrate complexed with chitosan on nitrogen assimilation, photosynthetic activity, and production of green beans (*Phaseolus vulgaris* L.). *Scientia Horticulturae* 288:110297. <https://doi.org/10.1016/j.scienta.2021.110297>
- Paramo LA, Feregrino-Pérez AA, Guevara R, Mendoza S and Esquivel K. 2020. Nanoparticles in Agroindustry: Applications, Toxicity, Challenges, and Trends. *Nanomaterials* 10(9):1654. <https://doi.org/10.3390/nano10091654>
- Patel KV, Nath M, Bhatt MD, et al. 2020. Nanofomulation of zinc oxide and chitosan zinc sustain oxidative stress and alter secondary metabolite profile in tobacco. *Biotech* 10:477. <https://doi.org/10.1007/s13205-020-02469-x>
- Praharaj S, Skalicky M, Maitra S, Bhadra P, Shankar T, Brestic M, Hejnak V, Vachova P and Hossain A. 2021. Zinc Biofortification in Food Crops Could Alleviate the Zinc Malnutrition in Human Health. *Molecules* 26(12):3509. <https://doi.org/10.3390/molecules26123509>
- Prasad AS. 2009. Zinc: Role in immunity, oxidative stress and chronic inflammation. *Current Opinion in Clinical Nutrition and Metabolic Care* 12(6):646–652. <https://doi.org/10.1097/MCO.0b013e3283312956>
- Preciado-Rangel P, Campos-Ortiz A, Chávez ES, Reyes-González A, Ruiz-Espinoza F, Ojeda-Barrios DL and Hernández-Montiel L. 2021. Zinc biofortification improves yield, nutraceutical quality, and antioxidant capacity in lettuce. *Tropical and Subtropical Agroecosystems* 24:1–12.
- Ramzan Y, Hafeez MB, Khan S, Nadeem M, Batool S and Ahmad J. 2020. Biofortification with zinc and iron improves the grain quality and yield of wheat crop. *International Journal of Plant Production* 14(3):501–510. <https://doi.org/10.1007/s42106-020-00100-w>
- Rivera-Gutiérrez RG, Preciado-Rangel P, Fortis-Hernández M, Betancourt-Galindo R, Yescas-Coronado P y Orozco-Vidal JA. 2021. Nanopartículas de óxido de zinc y su efecto en el rendimiento y calidad de melón. *Revista Mexicana de Ciencias Agrícolas* 12(5):791–803.
- Ruiz-Torres N, Flores-Naveda A, Barriga-Castro ED, Camposeco-Montejo N, Ramírez-Barrón S, Borrego-Escalante F, Niño-Medina G, Hernández-Juárez A, Garza-Alonso C, Rodríguez-Salinas P and García-López JL. 2021. Zinc Oxide Nanoparticles and Zinc Sulfate Impact Physiological Parameters and Boosts Lipid Peroxidation in Soil Grown Coriander Plants (*Coriandrum sativum*). *Molecules* 26(7):1998. <https://doi.org/10.3390/molecules26071998>
- Salehi H, De Diego N, Rad AC, Benjamin JJ, Trevisan M and Lucini L. 2021. Exogenous application of ZnO nanoparticles and ZnSO<sub>4</sub> distinctly influence the metabolic response in *Phaseolus vulgaris* L. *Science of the Total Environment* 778:146331. <https://doi.org/10.1016/j.scitotenv.2021.146331>
- Sánchez Toledano BI, Camarena Gómez DMJ, López Santiago MA and Cuevas Reyes VC. 2023. Consumer Preferences of Jalapeño Pepper in the Mexican Market. *Horticulturae* 9(6):684. <https://doi.org/10.3390/horticulturae9060684>
- Sánchez-Pérez DM, Márquez-Guerrero SY, Ramírez-Moreno A, Rodríguez-Sifuentes L, Galindo-Guzmán M, Flores-Loyola E and Marszalek JE. 2023. Impact of biologically and chemically synthesized zinc oxide nanoparticles on seed germination and seedlings' growth. *Horticulturae* 9:1201. <https://doi.org/10.3390/horticulturae9111201>
- Sharifan H, Noori A, Bagheri M and Moore JM. 2021. Postharvest spraying of zinc oxide nanoparticles enhances shelf-life qualities and zinc concentration of tomato fruits. *Crop and Pasture Science* 72(9):753–762. <https://doi.org/10.1071/CP21191>

- Singleton VL, Orthofer R and Lamuela-Raventós RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology* 299:152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Solanki M. 2021. The Zn as a vital micronutrient in plants. *Journal of Microbiology, Biotechnology and Food Sciences* 11(3):e4026-e4026. <https://doi.org/10.15414/jmbfs.4026>
- Song Y, Leonard SW, Traber MG and Ho E. 2009. Zinc deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. *The Journal of Nutrition* 139(9):1626–1631. <https://doi.org/10.3945/jn.109.106369>
- Sturikova H, Krystofova O, Huska D and Adam V. 2018. Zinc, zinc nanoparticles, and plants. *Journal of Hazardous Materials* 349:101–110. <https://doi.org/10.1016/j.jhazmat.2018.01.040>
- Tandon R, Khanna HD and Dorababu M. 2004. Oxidative stress and antioxidants status in peptic ulcer and gastric carcinoma. *Indian Journal of Physiology and Pharmacology* 48(1):115–118.
- Ucan-Tucuch O, Betancourt-Galindo R, Juárez-Maldonado A, Sánchez-Vega M, Sandoval-Rangel A y Méndez-López A. 2023. Efecto de maltodextrina y nanopartículas de óxido de zinc sobre biomasa y rendimiento en pepino. *Ecosistemas y Recursos Agropecuarios (Núm. Esp. III):e3699*. <https://doi.org/10.19136/era.a10nIII.3699>
- Uresti-Porras J, Fuente M, Benavides-Mendoza A, Olivares-Sáenz E, Cabrera R and Juárez-Maldonado A. 2021. Effect of grafting and nano ZnO on the nutraceutical and mineral content of pepper. *Plants* 10(12):2793. <https://doi.org/10.3390/plants10122793>
- Wang K, Zhao L, Luo H, Deng C, Gong L and Chen Z. 2024. Association of serum vitamin C levels with Asthma in adults: results of NHANES 2003–2006 and mendelian randomization study. *BMC Pulmonary Medicine* 24:4. <https://doi.org/10.1186/s12890-023-02821-w>
- Wang P and Grimm B. 2021. Connecting chlorophyll metabolism with accumulation of the photosynthetic apparatus. *Trends in Plant Science* 26(5):484–495. <https://doi.org/10.1016/j.tplants.2020.12.005>
- Zahedi SM, Moharrami F, Sarikhani S and Padervand M. 2020. Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress. *Scientific Reports* 10:1–18. <https://doi.org/10.1038/s41598-020-74273-9>

