Fortis-Hernández M *et al.* (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 4, Article number 12969
DOI:10.15835/nbha50412969
Research Article

Commercial and phytochemical quality in biofortified 'Orejona' lettuce with zinc oxide nanoparticles

Manuel FORTIS-HERNÁNDEZ[1], José D. GARCÍA-DELGADO[1], Pablo PRECIADO-RANGEL[1], Radames TREJO-VALENCIA[2], Alberto SÁNCHEZ-ESTRADA[3]*, Judith FORTIZ-HERNÁNDEZ[3]

[1]*Tecnológico Nacional de México, Campus Instituto Tecnológico de Torreón, División de Estudios de Postgrados e Investigación; Carretera Torreón – San Pedro, km 7.5, Ejido Ana, 27170 Torreón, Coahuila, Mexico; fortismanuel@hotmail.com; jose_jdgd@outlook.com; ppreciador@yahoo.com.mx*

[2]*Tecnológico Nacional de México,Instituto Tecnológico de Minatitlán, Blvd. Institutos Tecnológicos S/N, Col. Buena Vista Norte, Minatitlán, Veracruz 96848, México; radamestv@hotmail.com*

[3]*Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD, A.C.), Gustavo Enrique Astiazarán Rosas 46, Hermosillo, Sonora, 83304, México; aestrada@ciad.mx (*corresponding author); jfortiz@ciad.mx*

Abstract

Crop biofortification is a recent strategy based on the production of plants rich in micronutrients for human consumption. The biofortification of plants with minerals is considered one of the least expensive and most efficient ways to improve the commercial and nutritional quality of horticultural products, in addition to improving crop yields. The objective of the research was to evaluate commercial and phytochemical quality, physiological parameters, and zinc concentration in lettuce leaves (*Lactuca sativa* L.) after foliar application of zinc oxide nanoparticles (NPsZnO) produced under a hydroponic system. The experiment was carried out with six treatments (0, 5, 10, 15, 20, and 25 mgL^{-1} NPsZnO) of five replicates each, under a completely randomized design. Five applications every 15 days of each concentration of NPsZnO were made through the crop cycle. The results show no statistical differences in physiological parameters (height, number of leaves, leaf size, crown perimeter, fresh and dry weight), but that do show a slight tendency to increase on the treated lettuce mainly at concentrations of 20 and 25 mgL^{-1}. A positive correlation was found between the phytochemical variables (phenolics and total flavonols) and the concentration of NPsZnO. Even though there was not a clear correlation between NPsZnO concentration and the variables of commercial quality; Zn content in the plant tissue was improved, thereby obtaining a biofortified product for the final consumer.

Keywords: foliar application; *Lactuca sativa* L.; phenolics compounds; flavonoids; zinc

Abbreviations: ABTS: 2, 2'- Azino-bis (3 – Etil Benzotiazolin)-6-ammonium sulfonate; Cla: chlorophyll a, Clb: chlorophyll b, Clt: total chlorophyll; CP: crown perimeter; Cx+c: total carotenoids; DAT: days after transplantation; DW: dry weight; FW: fresh weight; H: plant height; LS: leaf size; L-H: length of the leaf; NL: number of leaves; NS: nutrient solution; NPs: nanoparticles; NPsZnO: nanoparticles; POD: Peroxidase activity; PVC: polyvinylchloride; TEAC: trolox equivalent antioxidant activity; W-H: widest part of the same leaf

Received: 28 Oct 2022. Received in revised form: 14 Nov 2022. Accepted: 21 Nov 2022. Published online: 29 Nov 2022.
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.

Introduction

The biofortification of crops allows the production of micronutrient-rich plants for human consumption. Zinc (Zn) is an essential element for human nutrition. Symptoms of Zn deficiency include stunting, diarrhea, and pneumonia in children, contributing significantly to infant mortality (White and Broadley, 2011). It is estimated that the diet of one third of the world's population is deficient in Zn (Maxfield *et al*., 2021). Also, Zn is an essential micronutrient for the growth and development of vascular plants and is associated with a wide range of metabolic pathways in plants (Castillo-González *et al*., 2018)

The Biofortification has the purpose of increasing the bioavailability of mineral elements through genetic and agronomic pathways. However, the first pathway has several disadvantages because of limited genetic variation and slow movement of the trait (Hirschi, 2009) besides this could be ineffective if there are insufficient mineral elements present (White and Broadley, 2011), while unfortunately, the potential for genetic modifications has political and economic limitations (Watanabe *et al*., 2005).

The radical fertilization as a pathway agronomic is a polluting agent for the environment and limits the availability of other minerals (Hirschi, 2009). While foliar fertilization is a widely used and effective strategy used by producers to complement fertilization, remedy nutritional deficiencies, stimulate development, and improve yield and quality (Otálora *et al*., 2018) and exhibits a very rapid plant response (Meier *et al*., 2020). This can be utilized for all types of crops (Dávila-Rangel *et al*., 2020) and is an excellent possibility to explore as a pathway for biofortification.

However, foliar fertilizers have reception challenges, and their efficacy may be compromised due to the differential between solute size and cell wall pores size and synergistic and antagonistic relationships between nutrients (Rietra *et al*., 2017). Nanotechnology has developed small particles with a high surface-to-volume ratio called nanoparticles (NPs) that are enriched with microelements (Dimkpa *et al*., 2013), whose functionality are dependent on their size and concentration (Janmohammadi *et al*., 2016). Nano-fertilizers may improve the solubility and dispersion of insoluble nutrients in soil, reduce nutrient immobilization and increase bioavailability (Janmohammadi *et al*., 2016). Other benefits of NPs are ameliorative roles against abiotic stress, additional antioxidant capacity, and a higher concentration of phytochemicals, which result in better nutraceutical quality (Kumar *et al*., 2021).

The impact of NPs on living organisms, especially plants, has drawn attention, because the NPs contain agrochemicals that can help distribute pesticides and fertilizers in a controlled way. This has been considered a high specificity procedure with minimal environmental impact (Lira *et al*., 2018). NPsZnO have been investigated for their antifungal, antibacterial, and nutritional (microelement) properties (Rossi *et al*., 2019) and alleviate the bioavailability of heavy metals (Sharifan *et al*., 2019, 2021). Lettuce is a suitable example for effectively increasing the Zn content and improving commercial quality and nutraceutical properties in this way (Preciado-Rangel *et al*., 2021).

The mechanism by which they present these characteristics is through the biosynthesis of antioxidant compounds such as phenolic compounds, and flavonoids (Hatami and Nagdhi, 2019), additionally being a cofactor in the enzymes of the antioxidant system, such as peroxidase (Castillo-González *et al*., 2018). As plant growth promoter, Zn is an important regulator of tryptophan biosynthesis and, consequently, in the metabolic pathway of auxin biosynthesis (García-López *et al.*, 2018) by inhibiting the uptake of metal pollutants (Sharifan *et al*., 2021).

There are relevant results in the application of NPsZnO (10 and 500 mg L^{-1}) that have shown an increase in both nutraceutical quality and improvement of development and yield of tomato, cucumber, habanero pepper, and melon, among other crops (Méndez-Argüello *et al*., 2016; García-López *et al*., 2019; Rivera-Gutiérrez *et al*., 2021). On the other hand, there is existing data that suggests that NPsZnO can inhibit

development at different stages (Ma *et al*., 2010), subsequently reducing plant biomass and yield (Xu *et al*., 2018) and causing phytotoxicity (Chen *et al*., 2018).

It is therefore pertinent to conduct research on the effects of Zn biofortification through nanotechnology on high-demand products such as lettuce. This would increase the nutritional level of lettuce as well as increase its yield and quality (Sapkota *et al*., 2019).

Based on the above, this research was developed to evaluate the effects of foliar application of NPsZnO in different concentrations and to determine its effect on yield, commercial, and nutraceutical quality in lettuce cultivation under a hydroponic system.

Materials and Methods

Plant and nanoparticles material

Seeds of "orejona" lettuce (*Lactuca sativa* L.) of the Vita® brand (California, USA) were germinated and transplantation 3 weeks later, when presenting the first five true leaves, in the hydroponic system.

NPsZnO were between 20 and 60 nm in size, with a purity of 97%, white in colour, and with a hemispherical and polygonal structural shape. NPsZnO were synthesized through controlled precipitation (Ramírez-Barrón *et al*., 2019), using the chemical hydrolysis method. Next, 530.4 g of zinc acetate $(CH_3CO_2)_2Zn$ and 24 L of ethanol were poured into a 200 L reactor with a provided boiler with a temperature controller. The solution was heated to 80 °C for 3 hrs. Subsequently, 0.22 M NaOH solutions were added to the reactor. It was stirred for 1 hr to achieve the precipitation of the NPs. The precipitate was washed several times with distilled water for further characterization by transmission electron microscopy to determine the particle size of the material obtained.

Application of treatments

The experiment was carried out during the autumn-winter agricultural cycle (2020-2021). Lettuce cultivation was carried out under a shade anti aphid mesh structure in the experimental field of the Torreón Technological Institute, located in the Torreón municipality, Coahuila, Mexico between coordinates 25°36'37" North and 103°22'33" West, at an altitude of 1150 m.a.s.l.

Five treatments and the control were evaluated (Table 1). The treatments consisted of foliar application using industrial atomizers (Lion tools, LY, United Kingdon) to apply fine calibrated droplets (50 μm). Approximately 50 mL of solution was used for each individual lettuce, enough to cover the entire surface of the plant until dripping. Tween 20®, (Sinaloa, Mexico) was added as an adherent to the mixture at a concentration of 2 $mL \cdot L^{-1}$. Five foliar applications were carried out. The first was applied 15 DAT and one every 15 days thereafter, and the last one was applied ten days before the harvest.

Hydroponic system and nutrient solution: Floating film NFT hydroponic system was used with a structure of two sections of 6 PVC (polyvinylchloride) tubes 6 m long and 10 cm diameter with holes of 10 cm in diameter with a separation of 30 cm.

Table 1. Treatments evaluated in the hydroponic lettuce experiment

Treatment (T)	Concentration NPsOZn ($mg\ L^{-1}$)
T1	Control (deionized water)
T2	5
T3	10
T4	15
T5	20
T6	25

The NS used was the universal Steiner (1961), which was adjusted and modified based on the analysis of the water utilized for solution preparation and the purity of the fertilizers used. The NS was applied according to the needs of the crop considering the adjustment to a pH value of 5.5 – 6 and an EC of 1.5 – 2.0 dSm^{-1}. The nutritive solution was composed of: 11.95, 1.02, 1.2, 1.49, 1.06 and 11.55 $mmoles \cdot L^{-1}$ of $NO3^{-}$, $H2PO4^{2-}$, $SO4^{2-}$, Ca^{2+}, Mg^{2+} and K^{+}, respectively, with 0.005, 0.0025, 0.0005, 0.0005, 0.0025, 0.003 and 0.0005 gL^{-1} of Fe, Mn, Zn, B, Cu, Mg and Mo, in the same order.

Plant height, number of leaves, crown perimeter, leaf size, fresh weight and dry weight

In each harvested plant was used to measure H, CP and NL; H reading was taken from the level of the agricultural foam to the leaf apex and the middle of crown both reported in cm and NL in head of lettuce was recorded by quantifying all the leaves present on the day of harvest.

The LS was determined in three leaves per lettuce, measuring the length of the leaf (L-H) from its base to the apex and the widest part of the same leaf (W-H), and the ratio L-H/W-H was calculated to define the shape of the leaves. Furthermore, the leaf area was calculated using the ellipse area formula (A= L-H/2 * W-H/2 *π). Lettuce head FW and DW (g) were estimated on a gravimetric balance (Sartorius, USA). Lettuce heads harvested were defoliated, weighed (fw), dehydrated on kraft paper, and placed in a drying oven (Yamato DX600, Osaka, Japan) at 70 °C until constant weight (dw).

Compounds extraction

Sampled outer leaves of the harvested lettuce were stored at -80 °C and lyophilized, after which, 0.2 g of lyophilized leaves were weighed and pulverized in a grinder, placed in the dark in falcon tubes covered with aluminium. 10 ml an 80% methanol – 20% water mixture (JT Baker, USA) was added and homogenized for 3 minutes with a homogenizer ultraturax (IKA, Germany). Then, the samples were sonicated (Branson SFX250, Germany) for one hour at room temperature (in total darkness). After sonication, they were centrifuged at room temperature at 35,000 g for 15 minutes (Sorvall ST8R, USA) and the supernatant was filtered through Whatman No. 1 paper. The filtrate was used to measure total phenols and total flavonoids, chlorophyll a*, b*, total carotenoids (β carotene + xanthophylls), and antioxidant activity.

Total phenol content

Total phenol content was measured according to Singleton and Rossi (1965) with slight modifications. A solution was prepared to mix 200 µL folin Ciocalteu concentrate (Sigma-Aldrich, USA), 750 µL of $NaCO_2$ (JT Baker, USA) at 20%, 100 µL of extract, and 3.9 mL of H_2O. Subsequently, it was left to stand for 1.5 h in total darkness and was measured in a spectrophotometer (Hach 6,500, Germany) at 765 nm. For the blank, the same mixture was used, substituting the extract for 80% methanol extracting solution. A standard curve of gallic acid (Sigma-Aldrich, USA) was made. The results are reported as mg of gallic acid 100 g^{-1} of dry weight.

Total flavonoid content

Total flavonoids were measured by a modified method (Huang *et al.,* 2018). Solution 1 was prepared by mixing 0.3 mL of 5% $NaNO_3$ and 4 mL of bidistilled water and solution 2 was prepared by mixing 2 mL of 1 M NaOH and 2.4 mL of water. Subsequently, 1 mL of the extract was added to solution 1 (for the blank, the extract was replaced by 80% methanol), it was allowed to stand for 5 minutes and 0.3 mL of 10% $AlCl_3$ was added, mixed gently, and left to stand for 1 minute. Finally, 4.4 ml of solution 2 was added to that mixture and shaken vigorously. The absorbance was immediately read in a spectrophotometer (Hach 6,500, Germany) at 425 nm. A standard curve was made with the quercetin standard (Sigma-Aldrich, USA) and the results were reported as mg of quercetin 100 g^{-1} dw.

Content of total chlorophyll, chlorophylls a, b, and total carotenoids (xanthophylls + β-carotene)

An aliquot was taken from the extract to measure the concentration of chlorophylls and total carotenoids according to Lichtenthaler *et al*. (2013) with slight modifications. The aliquot was measured using a spectrophotometer (Hach 6500, Germany) at different wavelengths: With the equations reported by Lichtenthaler *et al*. (2013) and adapted for measuring in 80% methanol were calculated: Cla= 15.65*A(6652.2) - 9.16*A(652.4), Clb = 39.04*A(6652.2) – 15.28*A(652.4), TCl= Cla+Clb, Cx+ c= (1,000*A(470) – 1.63*Ca – 104.96*Cb) / 221. The result obtained was in $\mu g mL^{-1}$, later it was adjusted to $\mu g g^{-1}$ fw. Also, the ratio of chlorophyll a/b was calculated, as well as total chlorophylls/total carotenoids.

Antioxidant activity

The antioxidant activity was estimated by TEAC method. 50 µL of the extract was added to 1 mL of diluted ABTS*+ solution (2, 2'- Azino-bis (3 – Etil Benzotiazolin)-6-ammonium sulfonate, Sigma-Aldrich, USA) prepared according to Márquez *et al*. (2014). The disappearance of ABTS*+ was determined by measuring the decrease of absorbance at 734 nm for 5 min, in the same way, we proceeded for the reference where the sample was replaced by grade ethanol (Merck®, USA). For the calculation of the antioxidant capacity, the change in absorbance was measured in five dilutions of the sample as reference and the corresponding percentage of inhibition was calculated using the following equation: Inhibition % = 1-((As-Ab)/(Ar)) *100 where: As=sample absorbance, Ab= blank absorbance, Ar= reference absorbance. A simple regression curve was made to determine CI50 (concentration 50% inhibition). A curve was made of the inhibition percentage versus concentration of the trolox solutions and calculated the CI50 in µmoles of trolox L^{-1} of solvent. With the CI50 of the sample expressed as mg of fresh sample L^{-1} of solvent and the CI50 of the trolox in µmol of trolox L^{-1} of solvent, is calculated and expressed as µM trolox equivalent antioxidant capacity (µM trolox 100 g^{-1} fw) by the equation of TEAC =CI50 trolox/CI50 sample), where: CI50 of trolox = concentration (moles of trolox L^{-1} solvent) to which the antioxidant trolox inhibits 50% of the free radicals present. CI50 of the sample = concentration (mg of sample L^{-1} solvent) to which the antioxidants of the sample inhibit 50 % of the free radicals present.

Peroxidase activity (POD)

Peroxidase enzyme extraction was performed by homogenizer 100 mg of the lyophilized sample with 5 ml of cold 100 mM Tris HCl (Sigma Aldrich USA) extraction buffer containing 1 % polyvinylpyrrolidone (Sigma-Aldrich, USA). The mixing was adjusted to a pH of 7.1 and centrifuged for 15 min at 6,500 g and 4 °C.

The assay was done at room temperature in the dark according to Flurkey and Jen (1978), with slight modifications: the assay was mixed 2.6 mL of 100 mM Tris-HCl buffer (Sigma-Aldrich USA) pH 7.1, 0.25 ml of 0.1 M guaiacol, 0.1 ml of 0.25% H_2O_2 (Sigma-Aldrich, USA), and 0.05 ml of the sample extract (supernatant). The absorbance change at 470 nm for 3 minutes was determined. The tests were done. Extinction coefficient ($5,570 * 10^{-6}$) reported by Rodríguez *et al.* (2006) and protein content (Bradford, 1976) were used to calculate enzymatic activity. The activity was expressed in $U \cdot mg^{-1}$ of protein, where one unit of enzymatic activity is equal to the formation of 1 µmol of tetraguaicol$\cdot min^{-1}$.

Zinc content in plant tissue

The determination of zinc concentration in plant tissue was carried out using the Mckean (1993) method. A sample of 3.0 g of dry plant tissue was placed in crucibles inside a digital muffle (Model LEF-203S-0 LabTech®) at 600 °C for 4 hours until total calcination, then they were cooled at room temperature in a desiccator (low relative humidity). 10 mL of HCl (Sigma-Aldric, USA) were added to the crucible with the ashes. After the reaction, the content was filtered through pore size ≤ 3 µm filter paper; 50 µL of deionized water was added to each filtrate. Zinc concentration was determined using the GBC Xplor AA atomic absorption equipment (Germany) and Zn content was reported as mg of Zn kg^{-1} fw. Concentration was

calculated using fitted curve calibration (r^2=0.9983) and two blanks, one with a reference sample and the other for the equipment blank.

Experimental design

The experiment was analysed with a completely randomized design considering six treatments with five replicates per treatment, giving a total of 30 experimental units, the mean comparison test was done by the DMS method ($p < 0.05$), using the statistical package SAS Version 9.3 (2009).

Results

Plant height, number of leaves, crown perimeter, leaf size shape of leaves, fresh weight and dry weight

Results of quality variables agreed with that specified in the Official Mexican Standard (NOM-FF-51-1992) (DOF, 1983). H ranged 20 to 30 cm, while NL were 40 to 60 leaves, CP was 40 to 50 cm, average long size of leaves of 20 to 30 cm and 10 to 20 cm wide, with a typical obovate shape and fresh weight of approximately between 0.8 and 1.0 kg on average at harvest. No statistical differences ($p < 0.05$) were found on each of these variables (H, NL, and CP) neither between the treatments nor concerning the control. We could only observe that T4 showed the lowest height (23.4 cm) and in the opposite case, T3 showed the highest height (25.33 cm) and T1 (22.66 cm), while lettuces treated had 2.8 to 6.5 leaves more than T1. Previous results were directly proportional to the amount of dry biomass at the end of the experiment. Treated harvested lettuces with NPsZnO presented 4 and 8 % more crown perimeter than T1, only T4 was similar to the control (data not shown).

The leaf size measured in their components L-L and W-L and the shape of the leaf (L-L/W-L), as well as the FW and DW, maintain a similar pattern of increasing values in the lettuce treated with NPsZnO. T1 lettuces showed L-L of 20.73 cm and W-L of 13.13 cm, T5 was higher than T1 by almost 1 cm in length, while T2 was 6.23 cm longer (Table 2). On the other hand, only the T3 had smaller leaf width (1.17 cm) than the control. According to the value of the L-L/W-L ratio, the lettuces show an obovate shape, with T3 being the most elongated and T5 the widest. Although there were no significant differences in L-L and W-L of the leaf, the calculated area showed significant differences between treatments and concerning the T1, in which the T1 showed a smaller area of 213.7 cm^2, followed by the treatment with T3 (230.70 cm2), in contrast to the treatments of T2 and T5 exceeded 304 cm^2, with the largest leaf area (Table 2).

The FW and DW did not show significant differences, however, it is observed that the treated lettuces were heavier both fresh and dry than the control lettuces; this is reflected by the fact that the treatments exceeded 0.9 kg fw compared to the 0.738 kg fw of T1. The dry biomass reached 59.67 g in T4 and 76 g in T5, while T1 only reached 57 g. Both in FW and DW, the treated lettuces were superior between 24 and 34% for FW and between 4-33% for DW (Table 2).

Table 2. Leaf size and shape, fresh and dry weight of hydroponically produced lettuce exposed to different foliar-applied concentrations of NPsZnO

Treatments (mg L^{-1})	Large (cm)	Width (cm)	L/W[y]	Area (cm^2)	Fresh weight (Kg)	Dry weight (g)
T1	20.73 ± 3. 99 a	13.13 ± 1.83 a	1.58	213.8 ± 5.73 d	0.74 ± 0.262 a	57.00 ± 14.17 a
T2	26.96 ± 3. 45 a	14.36 ± 0.58 a	1.88	304.0 ± 11.57 a	0.92 ± 0.179 a	62.05 ± 1. 93 a
T3	24.56 ± 3.04 a	11.96 ± 2.05 a	2.05	230.7 ± 7.25 c	1.00 ± 0.247 a	63.39 ± 6. 65 a
T4	23.25 ± 1.06 a	15.05 ± 1.06 a	1.54	274.5 ± 10.88 b	0.93 ± 0.348 a	59.67 ± 6.02 a
T5	21.66 ± 2. 92 a	17.90 ± 1.78 a	1.21	304.5 ± 14.08 a	0.92 ± 0.246 a	76.00 ± 5. 29a
T6	25.23 ± 1. 56 a	14.13 ± 1.72 a	1.79	280.0 ± 14.80 b	0.95 ± 0.138 a	63.00 ± 9. 84a

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

[y]L= Large, W= Width; Leaf shape: >1 obovate, =1 round, <1 oval.

Total phenol content

The content of total phenols in the lettuce leaves shows significant differences ($P \leq 0.05$). A relative increase in the concentration of total phenols is observed in lettuce leaves treated with NPsZnO as the concentration of NPsZnO applied increases, it is observed that the treatment with the lowest concentration of T2 presented values similar to T1 (266 mg of gallic acid 100 g^{-1} fw) until reaching the highest concentration in T6 with 519 mg of gallic acid·100 g^{-1} fw (Figure 1a), 52.63%, more than T1.

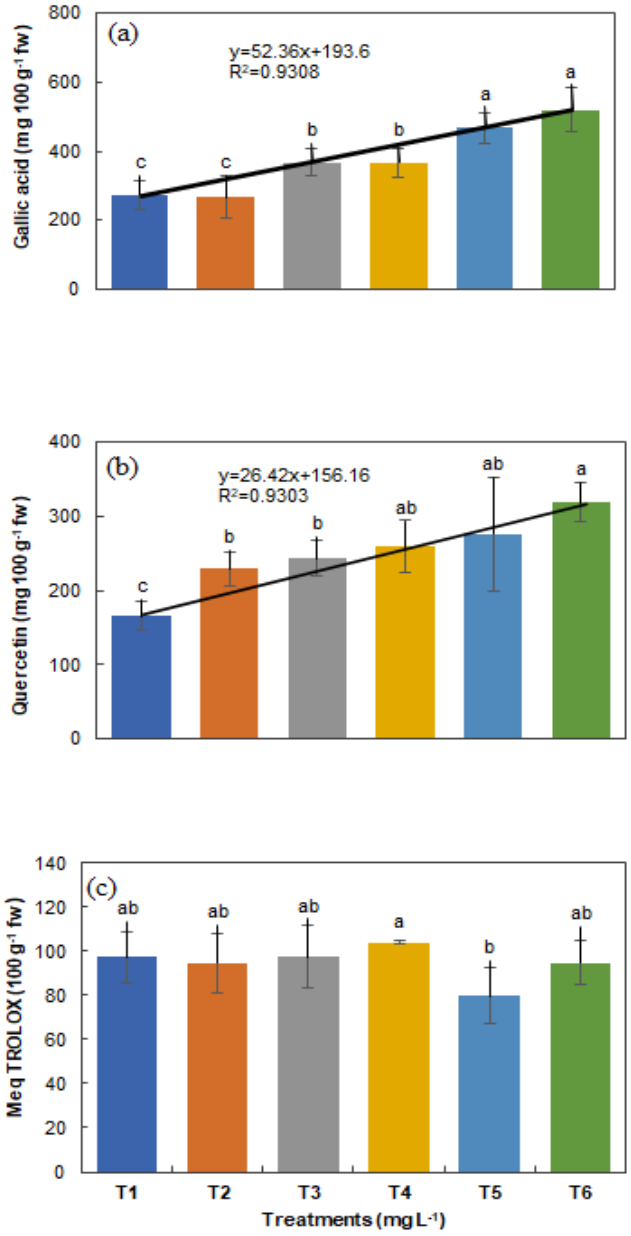

Figure 1. (A) Content of total phenols, (B) total flavonoids and (C) Antioxidant capacity in hydroponically produced lettuce leaves exposed to different concentrations of NPsZnO

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

Total flavonoid content

The content of flavonoids in plant tissue showed a statistical difference. An increase proportional to the concentration of the NPsZnO applied is observed. In Figure 1b, it can be seen that the plants evaluated with T6 had 319.11 mg100 gr^{-1} fw content of total flavonoids; 48.1% more than T1, while T2, T3, T4 and T5 were statistically similar to T6.

Antioxidant capacity

The antioxidant capacity measured as meq trolox presents only significant differences between T5 and T4, in addition, in this variable, a similar pattern of proportionality is not observed as in the other nutraceutical variables, that is, an increase in the concentration of NPs does not increase the antioxidant capacity even though it increased total phenols and the flavonoids concentration (Figure 1c).

Chlorophyll content and total carotenoids

According to the DMS analysis, there were significant differences ($p < 0.05$) among treatments in TCl, Cla and Clb content. T6 treatment had the highest concentration of TCl with a value of 32.73 $\mu g.g^{-1}$ dw, followed by T1 and T6 with levels of 25.82 and 23.44 $\mu g.g^{-1}$ dw respectively, while T2, T3, and T4 registered lower values with 15.33, 15.89 and 20.83 $\mu g g^{-1}$ dw respectively (Table 3). The content of Cla and Clb showed a similar pattern to TCl. Contrary to what is commonly reported, the concentration of Clb was higher than Cla in all treatments. The Cla/Clb ratio was similar in each of the treatments, with values between 0.42 and 0.55, except T4, which reported a value of approximately 0.28.

The carotenoids show statistical differences between treatments as well as TCl in the extreme treatments (T1, T5, and T6). The highest concentration of Cx+c obtained was 3.54, 2.84, and 3.51 $\mu g g^{-1}$ dw respectively, while T2, T3, and T4 treatments reached 2.57, 2.37 and 2.65 $\mu g g^{-1}$ dw respectively (Table 3). However, the Clt/ Cx+c ratio remained homogeneous in all the treatments with values between 6 and 9, except T5 that presented a value of 11.50.

Table 3. Content of total chlorophyll, chlorophylls a* and b* as well as total carotenoids in lettuce leaves harvested produced hydroponically and exposed to different concentrations of foliar-applied NPsZnO

Treatments (mg L^{-1})	Cla ($\mu g\ g^{-1}$dw)	Clb ($\mu g\ g^{-1}$dw)	TCl ($\mu g\ g^{-1}$dw)	Cla/Clb	Cx+c ($\mu g\ g^{-1}$dw)	Ct/ Cx+c
T1	9.23 ± 0.70 b	16.61 ± 0.33 b	25.82 ± 0.53 b	0.555	3.54 ± 0.20 a	7.29
T2	4.56 ± 0.16 e	10.76 ± 0.11 c	15.33 ± 0.25 c	0.423	2.57 ± 0.12 b	9.74
T3	5.64 ± 0.37 d	10.24 ± 0.17 c	15.89 ± 0.24 c	0.550	2.37 ± 0.30 b	6.69
T4	4.52 ± 0.29 e	16.31 ± 1.53 b	20.83 ± 1.33 b	0.277	2.65 ± 0.23 b	7.86
T5	11.39 ± 1.13 a	21.34 ± 0.29 a	32.73 ± 1. 93 a	0.533	2.84 ± 0.73 ab	11.50
T6	7.56 ± 0.70 c	15.87 ±0.16 b	23.44 ± 0.22 b	0.477	3.51 ± 0.33 a	6.67

Cla = chlorophyll a, Clb = chlorophyll b, TCl = total chlorophyll, Cx+c = total carotenoids.
Values followed by same literal at same column are not significant according to DMS ($p < 0.05$).

Peroxidase activity

POD variable shows statistical differences between the treatments. In Figure 2, it can be seen that the plants of T3, T4 and, T5 showed the highest peroxidase enzymatic activity by producing 0.42, 0.40 and, 0.53 µMol of tetraguaicol·mg^{-1} protein, 54.72% and 69.82% more activity than T1 and T2 respectively (Figure 2).

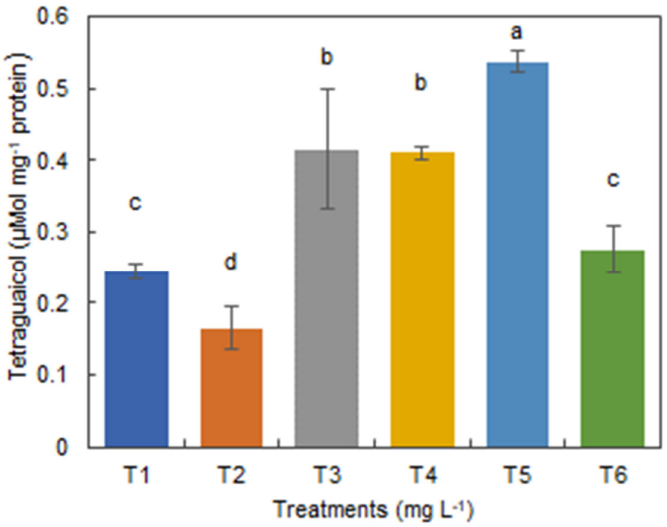

Figure 2. Enzymatic activity of peroxidase of lettuce leaves produced hydroponically, exposed to different concentrations of NPsZnO applied foliar
Means with the same literal are not significant according to DMS ($p < 0.05$)

Zinc content in plant tissue

The results obtained for the variable zinc content in the plant tissue found no statistical differences between the treatments, however, there was 25% more concentration of the zinc content in the leaves in T5 and T6 concerning T1, while T2 had the lowest concentration of the zinc even below T1 (Figure 3).

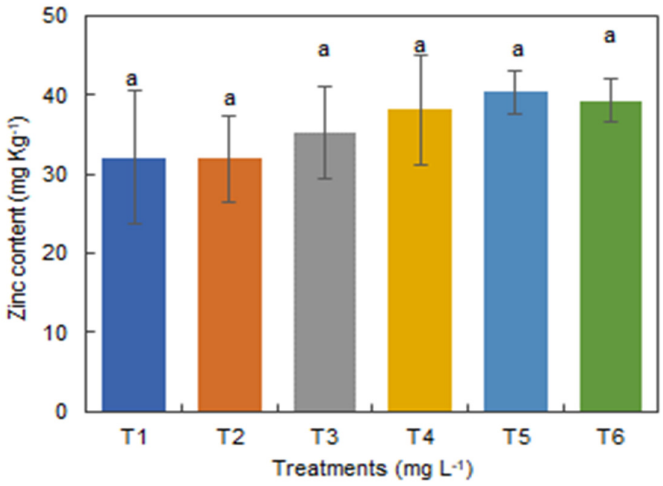

Figure 3. Effect of NPsZnO on the Zn content in the plant tissue of lettuce produced in a hydroponic system
Means with the same literal are not significant according to DMS ($p < 0.05$).

Discussion

NPsZnO applications have shown positive effects on the commercial quality and nutraceutical quality of some horticultural species (Goswani and Mathur, 2019). In the present work, the application of NPsZnO slightly promoted the growth and development of lettuce reflected in the area leaf and biomass accumulation

(FW and DW); this increase coincides with that reported by Song and Kim (2020) in lettuce and carrots treated with NPsZnO applied to the substrate in concentrations of 1 to 100 $mg kg^{-1}$ of the substrate. Méndez-Argüello *et al*. (2015 and 2016) promoted the growth and increased of dry biomass by foliar-applied NPsZnO 15 days after germination in chili plants.

The NL in the lettuce treated with NPsZnO increased as it did on an onion crop that registered a greater number of leaves due to the fortnightly application of NPsZnO at concentrations between 10 and 40 $\mu g mL^{-1}$ (Laware and Shilpa, 2014) which indicates that development was also stimulated through greater foliar sprouting and consequently a greater leaf area (Zhao *et al*., 2014) (Figure 1A-1C).

This could be partly responsible for the effect observed on the growth and development of lettuce plants due to NPsZnO indirectly promoting cell division and cell elongation due to the effect of the biosynthesis stimulation of auxin, through the precursor tryptophan, a phytohormone that plays a role in the process of cell division and elongation (García-López *et al*., 2018).

In general, the nutraceutical characteristics were positively affected by the application of NPsZnO, which was proportional to the concentration of the applied treatments (Table 2), as was observed in *Capsicum chinense*, with the increase of total phenols, total flavonoids, condensed tannins, and DPPH antioxidant capacity (García-López *et al*., 2019). A higher concentration of total phenols and flavonoids was observed in those treated with NPsZnO. Some studies have shown that metal nanoparticles can act as elicitors that stimulate the production of secondary metabolites, among which are polyphenols (Hatami and Nagdhi, 2019), as has been proven in different studies and species such as *Cucumis melo* (Shah *et al*., 2021) and *Brassica oleracea* var. Italic (Awan *et al*., 2021).

Despite the increase in phenolic compounds and flavonoids, it was not reflected in an increase in antioxidant capacity in a clear way, and even in some treatments, it was slightly inhibited. Generally, at a higher concentration of polyphenols, the antioxidant capacity increases, however, our results did not present this asseveration but coincided with what was reported by García-López *et al*. (2018) in which they did not observe changes in the response of antioxidant capacity to the stimulation of NPsZnO in habanero pepper. This lack of correlation could have been because phenolic acids were used to protect cellular components from possible damage by the presence of NPs causing oxidative stress (Večeřová *et al*., 2019). In lettuce, phenolic compounds act as substrates for enzymatic oxidations responsible for browning, which reduces the visual quality of fresh products (Singh *et al*., 2018), very common in outer leaves (Figure 1a-1c).

The higher content of Clb over Cla found in the lettuces is not uncommon, since it coincides with what has been reported in others research (Sapkota *et al*., 2019), which could be explained as a natural characteristic of the lettuces and/or the cultivation system used (use of shade mesh) reinforced by the low values obtained in the concentration of chlorophyll (Cla, Clb, and TCl) and the Cla/Clb ratio (Table 3).

This ratio is an indicator of the functionality of the pigment in the adaptation/acclimatization process, since Cla is present in the reaction centers of photosystems I and II, while Clb is found exclusively in the antenna pigments, which means that at a lower Cla/Clb ratio elongation of the antenna system of photosystem II occurs due to the shading of the leaves, which in turn is reinforced by the fact that the values of the Clt/Cx+c ratio exceeded the 5.2 (Table 3) normal range for leaves that are under shade (Lichtenthaler *et al*., 2013) a common situation in most of the inner leaves of lettuce and/or the shading of the mesh used in the cultivation system. Therefore, the exposure of lettuce to the applied NPsZnO concentrations did not demonstrate neither physiological stimulus nor phytotoxicity affecting the chlorophyll content or carotenoids content of the treated plants.

The application of NPsZnO could stimulate POD activity enzyme as part of the antioxidant system, which acts by converting H_2O_2, providing protection against oxidative damage (García-López, 2019; Raliya *et al*., 2017), and reducing damage to the cell membrane. It can be seen from Figure 2 that the effect of NPsZnO on POD activity increased first in T3, T4 and T5 and then effect decreased with an increase in NPsZnO (T6). It was demonstrated that an increased concentration of NPsZnO would improve the antioxidant activity of

lettuce by enhancing the activity of the POD enzyme. However, at a concentration of 25 mg L^{-1} in 5 applications, the effect of NPsZnO could be inhibited and the enhancement of POD enzyme activity would be ineffective (Liang *et al*., 2021).

The effective assimilation of Zn is confirmed by observing an increase in the concentration in the foliar tissue as the applied dose of the NPsZnO increases (Figure 3), coinciding with a similar increase in the sorghum crop (Raliya *et al*., 2017) and beans (Sida-Arreola *et al*., 2017).

Conclusions

The results suggest that the biofortification of Zn through NPsZnO by foliar route presented a slight increase in Zn in lettuce leaves, especially in the treatments with the highest concentration of NPsZnO, which led an increase in the levels of antioxidant compounds (phenols and flavonoids), POD enzyme activity and consequently a higher nutraceutical quality.

The commercial and visual quality of the lettuces remained unchanged even when there was a greater accumulation of fresh and dry biomass in the lettuces treated with NPsZnO.

Detailed studies are suggested to understand the mechanism of action of nanoscale materials. Additionally, an evaluation of its use in agriculture must be holistic in order to determine its most effective and efficient usage.

Authors' Contributions

The contributions of authors to the manuscript the authors are mentioned by initials: Conceptualization MFH, ASE and JDGD; Investigation and experimentation MFH, JDGD, ASE and JFH; Data analysis MFH, ASE, JDGD, PPR and RTV; Funding acquisition MFH, ASE and JFH; Methodology MFH, PPR, ASE, RTV and JFH; Project administration MFH and ASE; Resources MFH and ASE; Writing - original draft ASE and MFH; Writing - review and editing ASE, MFH, PPR, JFH and RTV. 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.

References

Awan S, Shahzadi K, Javad S, Tariq A, Ahmad A, Ilyas S (2021). A preliminary study of influence of zinc oxide nanoparticles on growth parameters of *Brassica oleracea* var *italic*. Journal of the Saudi Society of Agricultural Sciences 20(1):18-24. *https://doi.org/10.1016/j.jssas.2020.10.003*

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*

Castillo-González J, Ojeda-Barrios D, Hernández-Rodríguez A, González-Franco AC, Robles-Hernández L, López-Ochoa GR (2018). Zinc metalloenzymes in plants. Interciencia 43(4):242-248. *https://www.redalyc.org/articulo.oa?id=33957240004*

Chen J, Dou R, Yang Z, You T, Gao X, Wang L (2018). Phytotoxicity and bioaccumulation of zinc oxide nanoparticles in rice (*Oryza sativa* L.). Plant Physiology and Biochemistry 130:604-612. *https://doi.org/10.1016/j.plaphy.2018.08.019*

Dávila-Rangel IE, Trejo-Téllez LI, Ortega-Ortiz H, Juárez-Maldonado A, González-Morales S, Companioni-González B, ... Benavides-Mendoza A (2020). Comparison of iodide, iodate, and iodine-chitosan complexes for the biofortification of lettuce. Applied Sciences 10(7):2378. *https://doi.org/10.3390/app10072378*

Dimkpa CO, Latta DE, McLean JE, Britt DW, Boyanov MI, Anderson AJ (2013). Fate of CuO and ZnO nano- and microparticles in the plant environment. Environmental Science & Technology 47(9):4734-4742. *https://doi.org/10.1021/es304736y*

Flurkey WH, Jen JJ (1978). Peroxidase and polyphenol oxidase activities in developing peaches. Journal of Food Science 43(6):1826-1828. *https://doi.org/10.1111/j.1365-2621.1978.tb07424.x*

García-López JI, Niño-Medina G, Olivares-Sáenz E, Lira-Saldivar RH, Barriga-Castro ED, Vázquez-Alvarado R, ... 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-Saldívar RH, Díaz Barriga-Castro E, Ruiz-Torres, NA, Ramos-Cortez E, Vázquez-Alvarado R, Niño-Medina G (2018). Zinc oxide nanoparticles boosts phenolic compounds and antioxidant activity of *Capsicum annuum* L. during germination. Agronomy 8(10):215. *https://doi.org/10.3390/agronomy8100215*

Goswami P, Mathur J (2019). Positive and negative effects of nanoparticles on plants and their applications in agriculture. Plant Science Today 6(2):232-242. *https://doi.org/10.14719/pst.2019.6.2.502*

Hatami M, Naghdi Badi H, Ghorbanpour M (2019). Nano-elicitation of secondary pharmaceutical metabolites in plant cells: A Review. Journal Medicinal Plants 18(71):6-36. *https://doi.org/10.29252/jmp.3.71.6*

Hirschi KD (2009). Nutrient biofortification of food crops. Annual Review of Nutrition 29:401-421. *https://doi.org/10.1146/annurev-nutr-080508-141143*

Huang R, Wu W, Shen S, Fan J, Chang Y, Chen S, Ye X (2018). Evaluation of colorimetric methods for quantification of citrus flavonoids to avoid misuse. Analytical Methods 10(22):2575-2587. *https://doi.org/10.1039/c8ay00661jIziy*

Janmohammadi M, Amanzadeh T, Sabaghnia N, Dashti S (2016). Impact of foliar application of nano micronutrient fertilizers and titanium dioxide nanoparticles on the growth and yield components of barley under supplemental irrigation. Acta Agriculturae Slovenica 107(2):265-276. *https://doi.org/10.14720/aas.2016.107.2.01*

Kumar A, Singh IK, Mishra R, Singh A, Ramawat N, Singh A (2021). The role of zinc oxide nanoparticles in plants: A critical appraisal. In: Sharma N, Sahi S (Eds). Nanomaterial biointeractions at the cellular, organismal and system levels. Springer Nature, Switzerland pp 249-267. *https://doi.org/10.1007/978-3-030-65792-5_10*

Laware SL, Shilpa R (2014). Effect of titanium dioxide nanoparticles on hydrolytic and antioxidant enzymes during seed germination in onion. International Journal Current Microbiology Applied Science 3(7):749-760.

Liang Z, Pan Z, Li W, Kou E, Kang Y, Lei B, Song S (2021). Dose-dependent effect of ZnO quantum dots for lettuce growth. ACS Omega 6(15):10141-10149. *https://doi.org/10.1021/acsomega.1c00205*

Lichtenthaler HK, Babani F, Navrátil M, Buschmann C (2013). Chlorophyll fluorescence kinetics, photosynthetic activity, and pigment composition of blue-shade and half-shade leaves as compared to sun and shade leaves of different trees. Photosynthesis Research 117(1):355-366. *https://doi.org/10.1007/s11120-013-9834-1*

Lira-Saldivar RH, Méndez-Argüello B, De los Santos-Villarreal G, Vera-Reyes I (2018). Nanotechnology potential in sustainable agriculture. Acta Universitaria Multidisciplinary Scientific Journal 28(2):9-24. *https://doi.org/10.15174/au.2018.1575*

Ma X, Geiser-Lee J, Deng Y, Kolmakov A (2010). Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Science of the Total Environment 408:3053-3061. *https://doi.org/10.15174/au.2018.1575*

Márquez CJ, Otero CM, Rojano BA, Osorio JA (2014). Antioxidant activity and phenolic compounds concentration of tree tomato (*Cyphomandra betacea* S.) in postharvest. Temas Agrarios 19:173-184.

Maxfield L, Shukla S, Crane JS (2021). Zinc Deficiency. StatPearls, Treasure Island, FL.

Mckean SJ (1993). Manual de análisis de suelos y tejido vegetal: una guía teórica y práctica de metodologías [Soil and plant tissue analysis manual: a theoretical and practical guide to methodologies]. International Center for Tropical Agriculture (CIAT), Palmira, Colombia.

Meier S, Moore F, Morales A, González ME, Seguel A, Meriño-Gergichevich C, ... Mejías J (2020). Synthesis of calcium borate nanoparticles and its use as a potential foliar fertilizer in lettuce (*Lactuca sativa*) and zucchini (*Cucurbita pepo*). Plant Physiology and Biochemistry 151:673-680. *https://doi.org/10.1016/j.plaphy.2020.04.025*

Méndez-Argüello B, Lira-Saldívar RH, Ruíz-Torres NA, Cárdenas-Flores A, Ponce-Zambrano R, Vera-Reyes I, ... De los Santos G (2015). Influencia de nanopartículas de óxido de zinc puras y dopadas con plata en el crecimiento y producción de biomasa en plántulas de chile [Influence of pure and silver-doped zinc oxide nanoparticles on growth and biomass production in chili seedlings]. Aguilar-González CN, Regalado-González C, Escalante-Lozada A (Eds). XVI Congreso Nacional de Biotecnología y Bioingeniería, Sociedad Mexicana de Biotecnología y Bioquímica. Guadalajara, Jalisco, México, 2015 pp IIO22.

Méndez-Argüello B, Vera-Reyes I, Mendoza-Mendoza E, García-Cerda LA, Puente-Urbina BA, Lira-Saldívar RH (2016). Growth promotion of *Capsicum annuum* plants by zinc oxide nanoparticcles. Nova Scientia 8(17):140-156.

Norma Oficial Mexicana NOM-FF-51-1982.1983. Productos alimenticios no industrializados para uso humano hortalizas en estado fresco lechuga [Non-industrialized food products for human use fresh vegetables lettuce]. *http://www.dof.gob.mx/nota_detalle.php?codigo=4791830&fecha=13/01/1983*

Otálora G, Piñero MC, López-Marín J, Varó P, del Amor FM (2018). Effects of foliar nitrogen fertilization on the phenolic, mineral, and amino acid composition of escarole (*Cichorium endivia* L. var *latifolium*). Scientia Horticulturae 239:87-92. *https://doi.org/10.1016/j.scienta.2018.05.031*

Preciado-Rangel P, Campos-Ortiz A, Chávez ES, Reyes-González A, Ruiz-Espinoza F, Ojeda-Barrios D, Hernández-Montiel L (2021). Zinc biofortification improves yield, nutraceutical quality and antioxidant capacity in lettuce. Tropical and Subtropical Agroecosystems 24:122. *http://dx.doi.org/10.56369/tsaes.3844*

Raliya RV, Saharan V, Dimkpa C, Biswas P (2017). Nanofertilizer for precision and sustainable agriculture: current state and future perspectives. Journal of Agricultural and Food Chemistry 66(26):6487-6503. *https://doi.org/10.1021/acs.jafc.7b02178*

Ramírez-Barrón SN, Sánchez-Valdés S, Puente-Urbina BA, Martínez-Montemayor S, Esparza-González SC, Betancourt-Galindo R (2019). Preparation of a Pressure Sensitive Adhesive (PSA) with the ZnO Nanoparticles Incorporation. Study of its physicochemical and antimicrobial properties. Revista Mexicana de Ingeniería Biomédica 40(1):1-10. *http://dx.doi.org/10.17488/RMIB.40.1.5*

Rietra RP, Heinen M, Dimkpa CO, Bindraban PS (2017). Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. Communications in Soil Science and Plant Analysis 48(16):1895-1920. *https://doi.org/10.1080/00103624.2017.1407429*

Rivera-Gutiérrez RG, Preciado-Rangel P, Fortis-Hernández M, Betancourt-Galindo R, Yescas-Coronado P, Orozco-Vidal JA (2021). Zinc oxide nanoparticles and their effect on melon yield and quality. Revista Mexicana de Ciencias Agrícolas 12(5):791-803. *https://doi.org/10.29312/remexca.v12i5.2987*

Rodríguez Y, Fernández A, Peteira B, Fernández F, Solórzano E (2006). Inducing defence enzymes in two rice (*Oryza sativa*) varieties by *G. mosseae* arbuscular mycorrhizal fungus. Revista Colombiana de Biotecnología 8(2):35-49.

Rossi L, Fedenia LN, Sharifan H, Ma X, Lombardini L (2019). Effects of foliar application of zinc sulfate and zinc nanoparticles in coffee (*Coffea arabica* L.) plants. Plant Physiology and Biochemistry 135: 160-166. *https://doi.org/10.1016/j.plaphy.2018.12.005*

Sapkota S, Sapkota S, Liu Z (2019). Effects of nutrient composition and lettuce cultivar on crop production in hydroponic culture. Horticulturae 5(2):72. *https://doi.org/10.3390/horticulturae5040072*

SAS. 2009. JMP User Guide. Release 8. SAS Institute Inc., Cary, North Carolina, US, pp 513.

Shah AA, Aslam S, Akbar M, Ahmad A, Khan WU, Yasin NA, ... Ali S (2021). Combined effect of *Bacillus fortis* IAGS 223 and zinc oxide nanoparticles to alleviate cadmium phytotoxicity in *Cucumis melo*. Plant Physiology and Biochemistry 158:1-12. *https://doi.org/10.1016/j.plaphy.2020.11.011*

Sharifan H, Ma X (2021). Foliar application of Zn agrichemicals affects the bioavailability of arsenic, cadmium and micronutrients to rice (*Oryza sativa* L.) in flooded paddy soil. Agriculture 11(6):505. *https://doi.org/10.3390/agriculture11060505*

Sharifan H, Ma X, Moore JM, Habib MR, Evans C (2019). Zinc oxide nanoparticles alleviated the bioavailability of cadmium and lead and changed the uptake of iron in hydroponically grown lettuce (*Lactuca sativa* L. var *Longifolia*). ACS Sustainable Chemistry & Engineering 7(19):16401-16409. *https://doi.org/10.1021/acssuschemeng.9b03531*

Sida-Arreola JP, Sánchez E, Ojeda-Barrios DL, Ávila-Quezada GD, Flores-Córdova MA, Márquez-Quiroz C, Preciado-Rangel P (2017). Can biofortification of zinc improve the antioxidant capacity and nutritional quality of beans?. Emirates Journal of Food and Agriculture 29(3):237-241. *https://doi.org/10.9755/ejfa.2016-04-367*.

Singh B, Suri K, Shevkani K, Kaur A, Kaur A, Singh N (2018). Enzymatic browning of fruit and vegetables: A review. Enzymes in Food Technology 63-78. *https://doi.org/10.1007/978-981-13-1933-4_4*

Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enolology and Viticululture 16(3): 144-158.

Song U, Kim J (2020). Zinc oxide nanoparticles: a potential micronutrient fertilizer for horticultural crops with little toxicity. Horticulture, Environment, and Biotechnology volume 61(3):625-631. *https://doi.org/10.1007/s13580-020-00244-8*

Steiner A (1961). A universal method for preparing nutrient solutions of a certain desired compositions. Plant and Soil 15(2):134-154.

Večeřová K, Večeřa Z, Mikuška P, Coufalík P, Oravec M, Dočekal B, ... Urban O (2019). Temperature alters susceptibility of *Picea abies* seedlings to airborne pollutants: The case of CdO nanoparticles. Environmental Pollution 253:646-654. *https://doi.org/10.1016/j.envpol.2019.07.061*

Watanabe KN, Sassa Y, Suda E, Chen CH, Inaba M, Kikuchi A (2005). Global political, economic, social and technological issues on transgenic crops-review. Plant Biotechnology 22(5):515-522. *https://doi.org/10.5511/plantbiotechnology.22.515*

White PJ, Broadley MR (2011). Physiological limits to zinc biofortification of edible crops. Frontiers in Plant Science 2:80. *https://doi.org/10.3389/fpls.2011.00080*

Xu J, Luo X, Wang Y, Feng Y (2018). Evaluation of zinc oxide nanoparticles on lettuce (*Lactuca sativa* L.) growth and soil bacterial community. Environment Science Pollution Research 25(6):6026-6035. *https://doi.org/10.1007/s11356-017-0953-7*

Zhao L, Peralta-Videa JR, Rico CM, Hernandez-Viezcas JA, Sun Y, Niu G, ... Gardea-Torresdey JL (2014). CeO2 and ZnO nanoparticles change the nutritional qualities of cucumber (*Cucumis sativus*). Journal Agricultural and Food Chemistry 62:2752-2759. *https://doi.org/10.1021/jf405476u*

Notes:

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.