ORIGINAL RESEARCH PAPER

Effects of zinc oxide nanoparticles on enzymatic and nonenzymatic antioxidant content, germination, and biochemical and ultrastructural cell characteristics of *Portulaca oleracea* L.

Elham Iziy[1], Ahmad Majd[2]*, Mohammad Reza Vaezi-Kakhki[3], Taher Nejadsattari[1], Sakineh Kazemi Noureini[3]

[1] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Biology, Faculty of Life Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran
[3] Department of Biology, Faculty of Sciences, Hakim Sabzevari University, Sabzevar, Iran

* Corresponding author. Email: majda.iautnb@gmail.com

Abstract

This report focuses on the application of zinc oxide nanoparticles (ZnO NPs) carrying phycomolecule ligands as a novel plant growth promoter aimed at increasing the crop productivity of purslane (*Portulaca oleracea* L.). Experiments were performed under controlled greenhouse conditions using a completely randomized design with nine replications. Purslane seeds were treated with four concentrations of ZnO NPs (0, 10, 100, and 500 mg L^{-1}) and four concentrations of bulk ZnO (0, 10, 100, and 500 mg L^{-1}). The ultrastructural characteristics of the leaves of the plants treated with of 500 mg L^{-1} ZnO NPs were determined using transmission electron microscopy (TEM). The results indicated that the treatment with ZnO NPs increased the content of chlorophyll *a* and chlorophyll *b*, carotenoids, and total phenolic and flavonoid compounds significantly more than the treatment with bulk ZnO. Our findings also showed that the application of high concentrations of ZnO NPs is the most effective strategy to considerably induce the antioxidant capacity and enzymes of purslane plants. Furthermore, the seed germination percentage and sprout growth rates were significantly higher in the plants treated with 500 mg L^{-1} of ZnO NPs (100% ±0.00), compared to the control plants (93.33% ±1.66). The TEM images revealed the concentration of ZnO NPs and cell membrane rupture, as well as a deformation in the shape of chloroplasts and a decrease in their number in the plants treated with 500 mg L^{-1} ZnO NPs, compared to the control plants. Owing to their toxicity, high concentrations of ZnO NPs lead to oxidative stress in plants. Thus, our findings provide a new alternative strategy for increasing crop productivity, i.e., the application of ZnO NPs as a novel plant growth booster, in comparison with the bulk ZnO treatment.

Keywords

zinc nanoparticles; photosynthetic pigments; peroxidase; catalase

Introduction

Purslane (*Portulaca oleracea* L.) belongs to the family Portulacaceae. It is the eighth most commonly known herb in the world [1]. It grows in cultivated fields, gardens, and waste places. Purslane has a long history of use as human food [1], livestock feed [2], and medical expenses [3]. Recent studies have shown that purslane has a higher nutritional value than most cultivated vegetables, and it has thus been placed on the

list of powerful foods. Powerful foods are natural foods with a high nutritional content. The leaves and stems of this plant are edible and are used as a vegetable in many parts of the world [4,5]. Important scientific discoveries are being made on this medicinal plant, as it has been designated as a global elixir by the World Health Organization (WHO). It is the most common medicinal plant used by this organization [6]. Usually, the aerial parts of this plant, including the leaves, stems, and seeds, are used. Owing to its antioxidant properties and high nutrient levels, purslane is referred to as the "power food of the future" [7,8].

Zinc (Zn) is an essential mineral nutrient that acts as an important biotic limiting factor toward plant growth, development, fertility, and protection. It plays critical roles in the activities of enzymes belonging to six different classes (hydrolases, transferases, oxidoreductases, lyases, isomerases, and ligases) and has many functions in regulatory proteins. Considering the basic roles of Zn in human health, the bio-fortification of crops with essential nutrients and the modification of their fertility have got much attention. Hence, modern agricultural research needs to develop novel formulations of nutrient solutions, fertilizers, and pesticides [9].

The nanoparticles of metal oxides have specific physiochemical properties (thermal, surface, electrical, and optical), different from their bulk counterparts. Among different metal oxide nanoparticles, the most commercially utilized ones in different industries are zinc oxide, titanium dioxide, silver oxide, cerium dioxide, and copper oxide nanoparticles [10]. The usage of zinc oxide nanoparticles (ZnO NPs) at appropriate concentrations (based on the plant species, developmental stage, and treatment method) improved the growth, physiology, and protection of different plant species, such as *Pisum sativum* [11], *Zea mays* [12], and *Leucaena leucocephala* [13], but caused phytotoxicity in *Arabidopsis* [14] and *Oryza sativa* [15] at high doses. Therefore, further precise studies are required to clarify the feasible effectiveness or phytotoxicity of ZnO NPs.

With the advent of nanotechnology, the increase of nanoparticle concentrations in the environment is undeniable and has a huge impact on the industry, society, and environment [3,6,16]. Like most nanoparticles, ZnO NPs are toxic to living organisms. However, owing to their toxicity, these nanoparticles can be used as antibacterial [17], antifungal [18], antiviral [19], and antialgae [20] materials. The mechanism of nanoparticle toxicity is generally triggered by the induction of oxidative stress cause by free radical formation [21]. In a few studies on the toxicity of nanoparticles in plants, the results indicate the disruption of germination and plant growth [22]. The defense mechanism of plants involves counteracting the damage caused by oxidative stress, removal of active oxygen species, and reduced physiological activity of antioxidant enzymes and antioxidants. The antioxidants produced by nonenzymatic mechanisms in plants include vitamin E, ascorbate, glutathione, flavonoids, carotenoids, and the like [23,24].

However, owing to the medicinal [25] and antioxidative [26] effects of the phenols and flavonoids of purslane on human health [27], as well as the role of nanoparticles in plant defense, Zn nanoparticles can be effectively used to increase the concentration of these useful compounds in plants. Accordingly, the purpose of this research was to evaluate the effects of ZnO NPs on the enzymatic and nonenzymatic antioxidant content, germination, and biochemical and ultrastructural cell characteristics of *P. oleracea*.

Material and methods

Preparation, experimental design, and treatments

This research was conducted by a completely randomized design with nine replications in each treatment in a factorial layout at the Medicinal Herbs Research Center, Hakim Sabzevari University, Iran. The treatments were performed with four different concentrations of ZnO NPs (0, 10, 100, and 500 mg L^{-1}) and four different concentrations of bulk ZnO (0, 10, 100, and 500 mg L^{-1}). The exposure concentrations of ZnO NPs were selected from the initial experiments based on the root elongation assay. The seeds were first sterilized with hypo sodium chloride (5%) for 10 min and then washed superficially with distilled water five times. The seeds were exposed to ZnO NPs (size

≤50 nm; purity: 99.94 wt%; specific surface area: 31 m² g⁻¹; Sigma-Aldrich Co. LLC., USA) and bulk ZnO (Sigma-Aldrich) suspensions (in ultrapure water) for 2 h on a rotary shaker at 20°C. Subsequently, 160 seeds were rinsed with ultrapure water and transferred onto a wet filter paper in Petri dishes of 10-cm diameter and placed in a growth chamber for 5 days at 25 ±2°C for seed germination and growth. During the experimental period, the number of germinated seeds was recorded daily. Germination percentage, germination rate, average roots and stem lengths, and seedling dry weight were measured. The germination percentage and rate were determined using the formulas described by El-Keblawy and Al-Ansari [28].

For the pot experiment, the purslane seeds were planted in pots with a volume of 4 kg. The seeds were grown under natural conditions, with the temperature approximately 25–27°C at night and 31–35°C during the day. Twenty-one-day-old seedlings were treated with four concentrations of ZnO NPs (0, 10, 100, and 500 mg L⁻¹) and four concentrations of bulk ZnO (0, 10, 100, and 500 mg L⁻¹). All treatments were performed by adding suspensions of different concentrations to the soil of the pots. Plants were harvested 1 week after the last treatment for the biochemical analysis.

Plants were grouped as follows: C – control (0 mg L⁻¹ ZnO NPs or bulk ZnO), 10 mg L⁻¹ ZnO NPs, 100 mg L⁻¹ ZnO NPs, 500 mg L⁻¹ ZnO NPs, 10 mg L⁻¹ bulk ZnO, 100 mg L⁻¹ bulk ZnO, 500 mg L⁻¹ bulk ZnO.

Biochemical analyzes

The content of photosynthetic pigments, such as chlorophyll *a*, *b* and carotenoids, was estimated using the method described by Arnon [29]. The content levels of chlorophyll *a*, *b* and carotenoids were determined using the equations proposed by Lightenthaler [30] and expressed as mg g⁻¹ fresh weight (FW).

The flavonoid content was estimated using the equations proposed by Krizek et al. in 1998 [31] and expressed as mg g⁻¹ FW.

Total soluble phenols in the leaf extracts were determined using the Folin-Ciocalteu reagent procedure [32], with gallic acid as the standard compound.

The antioxidant assay was performed using the ferric reducing antioxidant power (FRAP) method. The total antioxidant capacity was measured according to Benzie and Strain [33].

The enzymes were extracted according to Chen et al. [34]. Enzymes were extracted at 4°C in a mortar and pestle using 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM Na_2-EDTA and 0.5 mM ascorbic acid as an extraction buffer. The homogenate was centrifuged for 15 min at 4°C, and the resulting supernatants were separated as enzyme extracts.

The peroxidase enzyme was assayed as described by Dimkpa et al. [35]. The peroxidase activity (EC 1.11.1.7) was assayed using a reaction mixture comprising acetate buffer (0.2 M, pH 4.8), 3% H_2O_2 and 0.04 M benzidine in 50% (v/v) methanol. The reaction was started by adding the enzyme extract. The peroxidase activity was expressed as ΔA min⁻¹ g⁻¹ FW.

The catalase activity (EC 1.11.1.6) was determined as a decrease in absorbance at 240 nm [36] and expressed as ΔA min⁻¹ g⁻¹ FW.

Transmission electron microscope (TEM)

Potential cellular and subcellular damages, including NP localization, in the leaf upon exposure to ZnO NPs were investigated using TEM (Zeiss EM900) and compared with the control leaf samples. The leaf samples were prefixed in 2% (v/v) glutaraldehyde for 2 h, washed in 0.1 M phosphate buffer (pH 7.2), postfixed in 1% osmium tetraoxide for 2 h, dehydrated in ethanol, and then infiltrated and embedded in epoxy resin. Subsequently, the samples were imaged by TEM, and the image analysis was performed using ImageJ. For TEM analysis, we chose to investigate only the samples treated with the highest concentrations (500 mg L⁻¹) of ZnO NPs and bulk ZnO because of the challenges in locating small particles and identifying potential cellular damages associated with the exposure to low concentrations of NPs.

Statistical analyses

Data analyses were carried out with the R statistical software version 3.5.1. The mean values of each treatment group were submitted to variance analysis by the Duncan's test at the level of 5% of probability.

Results

Seedling growth characteristics

The statistical results showed that the root length increased in the groups treated with both ZnO NPs and bulk ZnO compared to the control group. Significant changes were observed only at the concentration of 500 mg L^{-1} of ZnO NPs ($p < 0.001$) and at the concentration of 10 mg L^{-1} of bulk ZnO ($p < 0.01$). The highest stem length was observed in the group treated with 500 mg L^{-1} of ZnO NPs, and it was significantly higher than that observed in the control group ($p < 0.05$). The lowest stem length was observed in the group treated with 10 mg L^{-1} of bulk ZnO, but changes were not significant in comparison to the control group. The fresh seedling weight was reduced in all groups treated with ZnO NPs and bulk ZnO, compared to the control group. This reduction was significant in all groups ($p < 0.001$), except for the group treated with 500 mg L^{-1} of bulk ZnO. Furthermore, the dry seedling weight decreased in all treatment groups, except for the group treated with 500 mg L^{-1} of bulk ZnO; however, the changes were not significant in comparison to the control group. The germination percentages were higher in the ZnO-NPs groups than in the control group. In contrast, the germination percentages in the groups treated with 10 and 500 mg L^{-1} of bulk ZnO decreased compared to the control group, and only the germination percentage of the group treated with 100 mg L^{-1} of bulk ZnO was equal to that of the control group. However, the changes were not significant in comparison to the control group. An increase in germination rate was observed in the groups treated with 100 and 500 mg L^{-1} of ZnO NPs compared to the control group. In contrast, the germination rate in the groups treated with 10 and 500 mg L^{-1} of bulk ZnO decreased compared to the control group, and only the germination rate of the group treated with 100 mg L^{-1} of bulk ZnO was equal to that of the control group. However, the changes were not significant in comparison with the control group (Tab. 1).

Tab. 1 Effects of ZnO NP and bulk ZnO treatments on germination properties in *Portulaca oleracea* L.

Treatments	Stem length (mm)	Root length (mm)	Seedling fresh weight (g)	Seedling dry weight (g)	GP%	GR
Control	5.33 ±0.57 [ab*]	20.00 ±1.00 [c]	0.0049 ±0.00 [b]	0.00012 ±0.00 [bc]	93.33 ±2.88 [ab]	6.10 ±0.21 [a]
ZnO NPs 10 mg L^{-1}	5.66 ±1.52 [ab]	18.66 ±1.52 [bc]	0.0038 ±0.00 [a]	0.00006 ±0.00 [a]	95.00 ±0.00 [ab]	5.70 ±1.01 [a]
ZnO NPs 100 mg L^{-1}	6.33 ±0.57 [bc]	17.66 ±0.57 [b]	0.0039 ±0.00 [a]	0.00007 ±0.00 [ab]	95.00 ±0.00 [ab]	6.26 ±0.06 [a]
ZnO NPs 500 mg L^{-1}	7.66 ±0.57 [c]	15.33 ±1.15 [a]	0.0040 ±0.00 [a]	0.00007 ±0.00 [ab]	100.00 ±0.00 [b]	6.66 ±0.00 [a]
Bulk ZnO 10 mg L^{-1}	4.33 ±0.57 [a]	24.00 ±1.00 [d]	0.0039 ±0.00 [a]	0.00007 ±0.00 [ab]	91.66 ±7.63 [ab]	5.90 ±0.50 [a]
Bulk ZnO 100 mg L^{-1}	5.66 ±0.57 [ab]	20.00 ±1.00 [c]	0.0040 ±0.00 [a]	0.00008 ±0.00 [abc]	93.33 ±2.88 [ab]	6.10 ±0.32 [a]
Bulk ZnO 500 mg L^{-1}	6.00 ±1.00 [b]	20.00 ±1.00 [c]	0.0046 ±0.00 [b]	0.00013 ±0.00 [c]	86.66 ±10.40 [a]	5.62 ±0.77 [a]

Data are presented as mean ±*SD*. * Significant differences in the mean value of each treatment group are represented by different lowercase letters based on the Duncan's test ($p < 0.05$, $n = 9$).

Photosynthetic pigments content

In comparison with the untreated control, the groups treated with ZnO NPs and bulk ZnO exhibited an increase in the chlorophyll *a* and *b* content, where the highest increase was recorded in the group treated with 500 mg L^{-1} ZnO NPs significantly ($p < 0.001$). However, the changes observed with 10 mg L^{-1} ZnO NPs and bulk ZnO were

not statistically significant. Moreover, the statistical analysis of the results showed that in comparison with the control group, the groups treated with ZnO NPs and bulk ZnO exhibited an increase in their carotenoid content, where the highest significant increase was recorded in the group treated with 500 mg L^{-1} of ZnO NPs ($p < 0.001$). However, the changes observed with 10 mg L^{-1} of ZnO NPs and bulk ZnO were not statistically significant (Tab. 2).

Flavonoid content, phenolic content, and total antioxidant capacity

The treatment with ZnO NPs and bulk ZnO increased the leaf flavonoid content of the plants, wherein the highest increase was recorded in the group treated with 500 mg L^{-1} of ZnO NPs. Significant changes were observed for all concentrations of ZnO NPs and bulk ZnO ($p < 0.001$). The phenolic content of leaf tissues was influenced by ZnO NPs and bulk ZnO treatments. Compare to the control, all treatments led to an increase in total soluble phenolic content of the leaves, and the highest increase was observed in the group treated with 500 mg L^{-1} ZnO NPs. Significant changes were observed for all concentrations of ZnO NPs and bulk ZnO, except for 10 mg L^{-1} bulk ZnO ($p < 0.001$). In comparison with the control group, the total antioxidant capacity was altered in all ZnO NP- and bulk ZnO-treated groups, with the highest increase recorded in the group treated with 500 mg L^{-1} of ZnO NPs. Significant changes were observed for all concentrations of ZnO NPs and only one concentration (500 mg L^{-1}) of bulk ZnO ($p < 0.001$) (Tab. 2).

Tab. 2 Effects of ZnO NP and bulk ZnO treatments on the physiological properties of *Portulaca oleracea* L.

Treatments	Chl*a* (mg g^{-1} FW)	Chl*b* (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	Flavonoid (µg g^{-1} FW)	Total phenolics (µg g^{-1} FW)	Total antioxidant capacity (mg dL^{-1} vitamin C)
Control	0.66 ±0.0231 [a]	0.070 ±0.0036 [a]	0.332 ±0.0049 [a]	0.16 ±0.002 [a]	32.11 ±0.007 [a]	13.58 ±0.146 [a]
ZnO NPs 10 mg L^{-1}	0.77 ±0.0237 [b]	0.077 ±0.0013 [ab]	0.349 ±0.0015 [a]	0.66 ±0.014 [c]	45.78 ±1.159 [d]	19.67 ±0.310 [c]
ZnO NPs 100 mg L^{-1}	0.95 ±0.0319 [c]	0.091 ±0.0024 [c]	1.079 ±0.0185 [c]	0.83 ±0.016 [e]	52.08 ±0.070 [d]	21.68 ±0.729 [c]
ZnO NPs 500 mg L^{-1}	1.89 ±0.0687 [e]	0.182 ±0.0031 [e]	2.243 ±0.0982 [e]	1.76 ±0.051 [g]	57.08 ±0.056 [e]	37.19 ±1.440 [d]
Bulk ZnO 10 mg L^{-1}	0.74 ±0.0142 [ab]	0.071 ±0.00026 [a]	0.338 ±0.0042 [a]	0.54 ±0.020 [b]	33.34 ±0.067 [b]	13.84 ±0.037 [a]
Bulk ZnO 100 mg L^{-1}	0.87 ±0.0320 [c]	0.083 ±0.0023 [bc]	0.935 ±0.0210 [b]	0.71 ±0.034 [d]	39.94 ±0.280 [b]	14.00 ±0.020 [a]
Bulk ZnO 500 mg L^{-1}	1.08 ±0.107 [d]	0.115 ±0.0110 [d]	1.444 ±0.0375 [d]	1.09 ±0.023 [f]	45.41 ±1.774 [c]	19.74 ±0.640 [b]

Data are presented as mean ±*SD*. * Significant differences in the mean value of each treatment group are represented by different lowercase letters based on the Duncan's test ($p < 0.05$, $n = 9$).

Antioxidant enzyme activity

The application of ZnO NPs and bulk ZnO considerably induced the activity of peroxidase (POX) in the roots, shoots, and leaves of the treated plants, with the highest activity recorded in the plants treated with 500 mg L^{-1} ZnO NPs, compared to the control ($p < 0.001$) (Tab. 3). In contrast to POX, a decrease in the activity of catalase (CAT) was observed in the roots, shoots, and leaves of the purslane seedling treated with 500 mg L^{-1} ZnO NPs. Similarly, the bulk ZnO treatment affected the catalase activity in the roots, shoots, and leaves of the purslane seedlings, with the highest activity observed in the leaves of the seedlings treated with 500 mg L^{-1} bulk ZnO, compared to the control ($p < 0.001$) (Tab. 3).

TEM analysis of leaf ultrastructure

Evaluation of several TEM images of the leaf ultrastructure revealed that ZnO NPs affect certain cell parameters and cause changes in intracellular organelles. Among various organelles, the chloroplasts appeared to be the most sensitive to ZnO NPs.

Tab. 3 Effects of ZnO NP and bulk ZnO treatments on antioxidant enzymes in *Portulaca oleracea* L.

Treatment	CAT activity (ΔA min^{-1} g^{-1} FW)			POX activity (ΔA min^{-1} g^{-1} FW)		
	Root	Stem	Leaf	Root	Stem	Leaf
Control	1.70 ±0.008 [a]	1.43 ±0.003 [b]	3.11 ±0.001 [d]	0.052 ±0.000 [a]	0.066 ±0.0018 [a]	0.067 ±0.0005 [a]
ZnO NPs 10 mg L^{-1}	1.72 ±0.006 [b]	1.46 ±0.007 [c]	2.86 ±0.027 [c]	0.053 ±0.0003 [b]	0.068 ±0.0003 [ab]	0.085 ±0.0022 [d]
ZnO NPs 100 mg L^{-1}	1.82 ±0.010 [d]	1.50 ±0.005 [e]	2.81 ±0.003 [b]	0.055 ±0.0003 [c]	0.070 ±0.0005 [b]	0.100 ±0.0008 [f]
ZnO NPs 500 mg L^{-1}	1.70 ±0.003 [a]	1.39 ±0.014 [a]	2.54 ±0.019 [a]	0.057 ±0.0008 [d]	0.083 ±0.0019 [d]	0.101 ±0.0001 [g]
Bulk ZnO 10 mg L^{-1}	1.71 ±0.004 [a]	1.47 ±0.010 [a]	3.12 ±0.003 [f]	0.052 ±0.0005 [d]	0.067 ±0.0012 [a]	0.072 ±0.0011 [a]
Bulk ZnO 100 mg L^{-1}	1.74 ±0.009 [c]	1.51 ±0.006 [d]	3.12 ±0.001 [d]	0.053 ±0.0001 [b]	0.067 ±0.000 [ab]	0.082 ±0.0005 [c]
Bulk ZnO 500 mg L^{-1}	1.70 ±0.008 [a]	1.43 ±0.003 [b]	3.42 ±0.020 [e]	0.054 ±0.0003 [b]	0.075 ±0.0028 [c]	0.088 ±0.0015 [e]

Data are presented as mean ±*SD*. * Significant differences in the mean value of each treatment group are represented by different lowercase letters based on the Duncan's test ($p < 0.05$, $n = 9$).

A comparison of the chloroplasts of the treated and untreated control plants showed that they were oval and stretched in the control plants (Fig. 1A), but were more or less spherical and swollen in the plants treated with ZnO NPs and bulk ZnO that it's more invasive in ZnO NPs treatments (Fig. 1B–D). Furthermore, the thylakoids, which were well ordered in the control plants (Fig. 1A), were less consistent and dilated in the treatment plants (Fig. 1B,D). In addition, both ZnO NPs and bulk ZnO affected the starch content of chloroplasts, reducing the number and size of starch grains in the bulk ZnO treatment (Fig. 1D) and increasing the number and size of starch grains in the ZnO NP treatment (Fig. 1B). The cell membrane and apoplast space were also affected by ZnO NPs and bulk ZnO. In the ZnO NP treatment, the cellular membrane collapsed and the cells wrinkled, widening the apoplastic space (aps = 308 ±133 nm) (Fig. 1C).

Discussion

Seedling growth characteristics

The application of ZnO NPs improved the seedling growth indexes. Furthermore, in response to the ZnO NP treatments, the stem length increased significantly compared to the control group. Similar studies conducted by Venkatachalam et al. [37], Prasad et al. [38], and Tarafdar et al. [39] also showed the positive effects of ZnO NPs on stem length in young buds. The zinc element likely stimulates the shoot and shortening root. In Lin and Xing's study [40] on *Lolium perenne*, significant effects of ZnO NPs were observed on the epidermis and root cortex, with NP internalization in the endodermal and vascular tissues [41]. A high dose of ZnO NPs produced a greater effect, whereas the equivalent amount of bulk ZnO produced a moderate effect.

It is known that the Zn metal acts as a cofactor in many plant enzymes and is involved in the production and synthesis of proteins in plants. Another prominent role of zinc is in the synthesis of the amino acid tryptophan as a prerequisite for the production of the hormone auxin, which causes an increase in the longitudinal shoot growth and a decrease in root length [42].

Unlike the study conducted by Venkatachalam et al. [37], which showed an increase in the fresh and dry weights of the cotton seedlings treated with ZnO NPs when compared with the control group, our results showed a decrease in the fresh and dry weights of the seedlings in the treated groups compared to the control group. It can be said that the fresh and dry weight loss of the roots and stems in the present study was probably due to the disruption of the biosynthesis and transfer of growth regulators, such as gibberellic acid and auxin, in the plants treated with both ZnO NPs and bulk ZnO. This result is consistent with the studies on the *Brassica napus* plant conducted by Mousavi Kohi et al. [43]. Furthermore, Rao and Shekhawat's study on the toxicity effects of 200, 500, 1,000, and 1,500 mg L^{-1} of ZnO NPs on the growth and metabolism of *B. juncea* showed a decrease in the fresh and dry weights of plants in a dose-dependent manner [44].
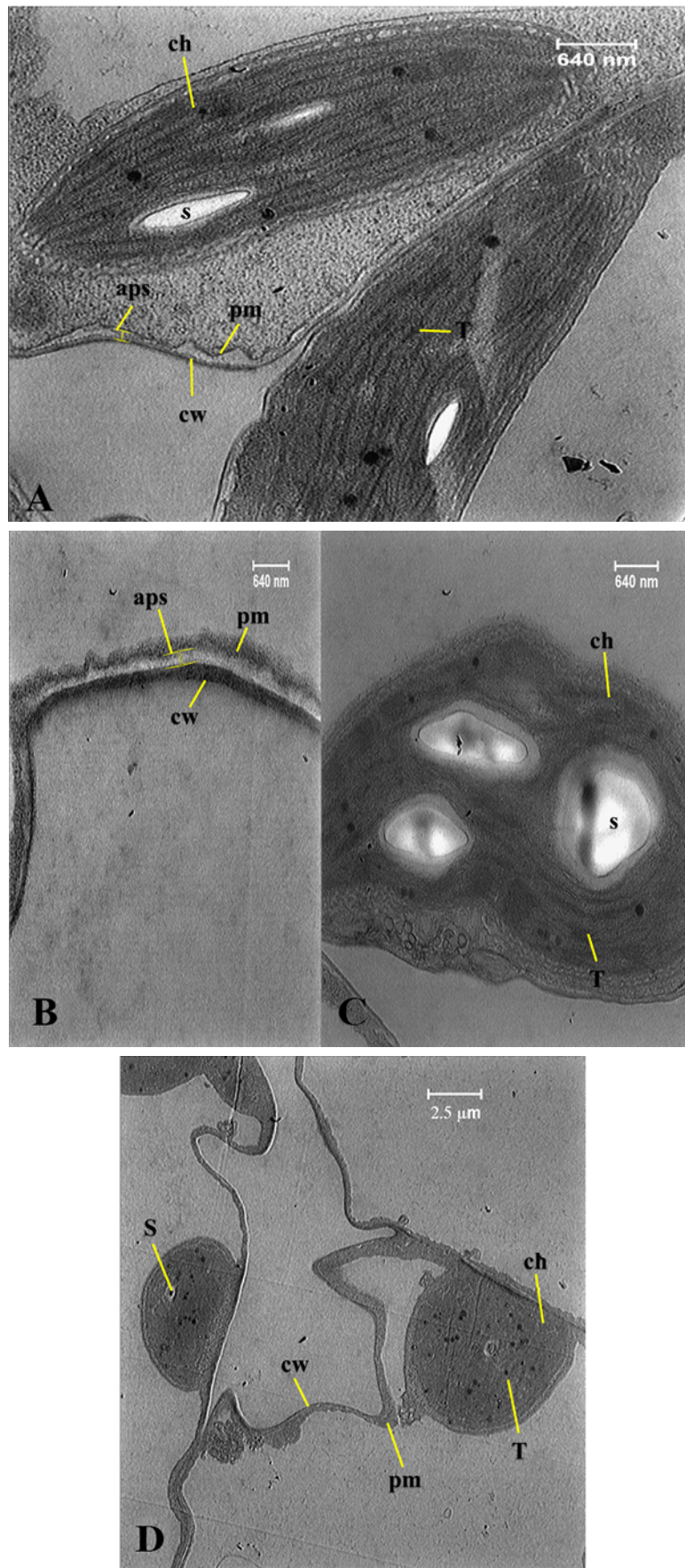
Fig. 1 Effect of treatments on the plant leaf's ultrastructure in *Portulaca oleracea* L. (**A**) Control. (**B,C**) ZnO NPs 500 mg L⁻¹. (**D**) Bulk ZnO 500 mg L⁻¹. ch – chloroplast; T – thylakoid; S – starch; cw – cell wall; pm – plasma membrane; aps – apoplastic space.

Several reports have stated that ZnO NPs affect seed germination in various plants. Bandyopadhyay et al. [45], who studied the plants of alfalfa, and Venkatachalam et al. [37], who studied the plants of *Gossypium hirsutum* L., observed an increased in the percentage and rate of germination upon treatment with ZnO NPs. Our results also showed a significant increase in the percentage and rate of germination of the group treated with 500 mg L^{-1} of ZnO NPs, compared to the control group. The reason for increased germination percentage in nanoparticle-treated plants could be the increased absorption of nanoparticles into the seed coat and, consequently, the increase in water absorption, light, and activity of the rubisco enzyme [46]. Light activates the PIL5 protein destruction pathway (in conjunction with the bHLH protein phytochrome) and leads to an increase in the biosynthesis and degradation of GA [47], which, in turn, results in rapid seeding growth in *P. oleracea*.

Photosynthetic pigments content

Our results showed an increase in the content of chlorophyll *a* and *b* and carotenoids in all groups treated with ZnO NPs and bulk ZnO in comparison with the control plants. However, the content of these photosynthetic pigments was higher in the treatment with ZnO NPs than with bulk ZnO, and the greatest increase was observed in the plants treated with 500 mg L^{-1} ZnO NPs. Our findings are consistent with the results of Venkatachalam et al. [37], Prasad et al. [38], and Tarafdar et al. [39], who studied the effect of ZnO NPs on the content of photosynthetic pigments in cotton, peanut, and millet, respectively. In plants, the deficiency of Zn causes chlorosis in the leaves, followed by the appearance of necrotic white stains in the older leaves. The presence of chlorosis indicates a plant's requirement for the Zn element in the biosynthesis of chlorophyll. The addition of Zn affects chlorophyll biosynthesis directly as well as indirectly. In the indirect method, the addition of Zn leads to more nitrogen uptake and contributes to nitrogen metabolism. Since nitrogen is an essential part of the chlorophyll molecule, it can be said that zinc indirectly affects the chlorophyll content of a plant. In contrast, some studies have reported a decrease in the amount of chlorophyll under different concentrations of ZnO NPs in pumpkin, wheat, and maize plants, suggesting that presence of Zn in the root environment prevented the absorption of iron and the resulting deficiency of iron might have led to the disruption of chlorophyll biosynthesis [48].

Flavonoid and phenolic content and total antioxidant capacity

The results showed that the flavonoid and phenolic content and total antioxidant capacity were increased in all groups treated with ZnO NPs and bulk ZnO, compared to the control plants. However, the amounts of nanoparticles treatments were higher than those of bulk ZnO, and the greatest increase was observed in the plants treated with 500 mg L^{-1} of ZnO NPs. In addition, the results prove the hypothesis that the response of plants to nanoproducts might be different from their response to bulk compounds. Differences in plant behavior in response to nanomaterials might be attributed to the differences in the physicochemical characteristics of these compounds, which influence the uptake, biomolecular interactions, signaling cascades, and biological systems of nanoparticles. Recent scientific experiments have shown that nanoparticles are more bioactive agents than the bulk metal because of their unique physicochemical properties [49,50]. Differential behavior of plants in response to metal nanoparticles and bulk metal has been related to their surface and quantum effects, which in turn, influence their chemical reactivity and interaction with target biomolecules, like DNA, lipids, proteins and enzymes, and other cellular components [51].

Our results are consistent with the findings of Ushahra et al. [52] regarding the effect of nanoparticles on the antioxidant capacity of *Eruca sativa*. Of course, Lin and Aarts [53] showed that the antioxidant capacity decreases under very high concentrations of nanoparticles, because the Zn, as a heavy metal, is toxic to many plants at too high concentrations. Some findings indicate that the reason for the decrease in antioxidant capacity is the excessive production of free radicals, which, in turn, reduces growth and antioxidant activity [54]. The flavonoid and phenolic compounds are secondary

metabolites and have a protective and antioxidant role [55,56]. It has also been reported that in plants treated with high concentrations of heavy metals, the flavonoid and phenolic compounds are activated and protect the plant. Phenolic compounds have the ability to chelate heavy metals [55,57]. It was demonstrated that the flavonols contained in the vacuole can be used in the flavonol-peroxidase cleansing system to purge the active oxygen species, especially H_2O_2 [58]. The activity of such systems in plants seems necessary because it provides optimal conditions for plant growth. Our results contradict the findings of Javed et al. [54] who studied the effect of ZnO NPs on the phenolic and flavonoid content of *Stevia rebaudiana* and showed a decrease in the phenol and flavonoid content of the plants treated with 100 and 1,000 mg L^{-1} of nanoparticles, compared to the control plants. The reason for this decrease in the phenolic and flavonoid content under high concentrations of nanoparticles is the imbalance between antioxidant activity and oxidative stress, which results in a reduction in antioxidant activity, including the reduction of phenolic and flavonoids content. However, the current study showed that no such toxicity symptoms observed in the purslane plants treated with ZnO NPs coated with phycomolecules, even at higher doses (500 mg L^{-1}). The likely reasons are the growth and biochemical changes induced by ZnO NPs or bulk ZnO particles partly because of the toxic effects of Zn ion dissolution, root exudates, or the physical interaction of ZnO particles with roots and structural induction:

- The hydrodynamic diameter of nanoparticles is too large to cross the cell wall pores (5 to 20 nm in diameter). Therefore, the selective permeability of the cell wall [59] or the presence of plasmodesmata (40 to 50 nm in diameter) allows the nanoparticles to pass through the cell wall.
- Although the absorption of bulk ZnO particles by the roots should be naturally lower than that of the ZnO NPs, it has been shown by some studies that Zn accumulation in the roots of the plants treated with bulk ZnO particles is more than in the roots of the plants treated with ZnO NPs [60].
- The inhibitory effects of the bulk ZnO treatments are much higher than those of the ZnO NP treatments on the growth of *P. oleracea* L. These results are consistent with the results of Mousavi Kohi et al., who studied the inhibitory effects of the bulk ZnO and ZnO NP treatments in *B. napus* [43].

Antioxidant enzyme activity

The findings of the current study clearly indicated that the activity of the peroxidase enzyme was the highest in the leaf and the lowest in the root. However, at high concentrations, ZnO NPs were more effective in increasing the peroxidase activity than the bulk ZnO particles. The highest activity of peroxidase was observed in the leaves of the plants treated with 500 mg L^{-1} ZnO NPs. Hereby, it is manifested that metal nanoparticles affect the peroxidase activity more efficiently than their bulk counterparts. Interestingly, the ZnO NP treatment mitigated heavy metal (Cd and Pb) toxicity in *L. leucocephala* by enhancing the photosynthetic pigment content, reducing lipid peroxidation, and inducing antioxidant enzymes [13].

In contrast, the activity of the catalase enzyme in the leaf was generally higher than in the root and stem. However, its activity decreased in the leaves of the plants treated with ZnO NPs and slightly increased in the leaves of the plants treated with bulk ZnO.

Numerous researchers have studied the effect of ZnO NPs and bulk ZnO on the activity of antioxidant enzymes in different parts of plants. For example, Bandyopadhyay et al. [45] studied the effect of ZnO NPs and bulk ZnO on the activity of peroxidase and catalase in alfalfa roots, stems and leaves. Chen et al. [61] studied the effect of ZnO NPs on the activity of peroxidase and catalase in the roots and stems of rice plants. Venkatachalam et al. [37] and Hernandez-Viezcasa et al. [62] studied the effect of ZnO NPs on the activity of peroxidase and catalase in the leaves of cotton and *Prosopis juliflora-velutina*, respectively. Krishnaraj et al. [63] studied the effect of oxidizing nanoparticles on the activity of peroxidase and catalase in *Bacopa monnieri*. The results of all these studies are consistent with our results. Reports show that heavy metals, including Zn, produce reactive oxygen species (ROS), which are deleterious to plant cells. A regulatory defense mechanism increases the levels of antioxidant enzymes, including peroxidase, catalase, and superoxide dismutase, in order to protect

plant cells under oxidative stress conditions. It has also been shown that the catalase enzyme involved in the removal of excess H_2O_2 in the cell [64]. The reduced activity of catalase in the leaf of a plant treated with 500 mg L^{-1} ZnO NPs might be due to the loss of a defense system, which resulted in decreased antioxidant activity. It has been demonstrated that nanoparticles stimulate ROS production by inhibiting the expression of the genes encoding antioxidant enzymes, including catalase and peroxidase and by destroying the H_2O_2 collecting system [61].

Ultrastructural study

The easiest way to determine the condition of nanoparticles in plant tissues is through transient electron microscopy. Zhou et al showed that increasing the concentration of copper nanoparticles increases the intracellular accumulation of copper [65]. The results of Sharma et al. [66], who showed that increasing the concentration of silver nanoparticles increased the intracellular accumulation of silver [66], are consistent with the results of these studies. Unlike animals, algae, fungi, and plants have a cell wall that prevents the entry of nanoparticles. Only the nanoparticles smaller than the cell wall pores can pass through the cell wall. The accumulation of high concentrations of nanoparticles in plant cells are caused by the additional absorption processes of the cell wall and membrane, including ion exchanges, facilitated release, and active transfer [67]. Electron microscopic images show the damaged membrane, cell wall, and chloroplasts in the leaf cells of the plants with ZnO NPs, compared to the leaf cells of the control group. This damage is probably due to the high accumulation of ZnO NPs and formation of oxidative stress, which in turn prevents the growth of purslane plants. After transferring nanoparticles to cells, they are transmitted from one cell to another via plasmodesmata [68]. Sometimes the accumulation of nanoparticles might occur as a result of the blockage of pores and canals [68]. The application of ZnO NPs has been shown to upregulate the expression of key antioxidant stress-responsive enzymes in *G. hirsutum* [37] and *O. sativa* [15]. Contradictorily, exposure to ZnO NPs in wheat enhanced the generation of ROS and adversely changed the antioxidant defense systems, consequently leading to the destruction of the crucial cellular structure, especially membranes, and growth inhibition [69]. Hence, more research is needed to make a more accurate evaluation.

Conclusion

The application of ZnO NPs at a concentration of 500 mg L^{-1} increased the content of some phytochemicals, such as flavonoids and phenolics, and total antioxidant capacity and modified seed germination and seedling growth parameters. In conclusion, it seems that owing to the high consumption of nanoproducts, the likelihood of the entry of these nanoparticles into the food chain, especially through plant-derived foods/ drugs, is inevitable. This study provides a valuable theoretical basis and insight into the potential advantage and toxicity of ZnO NPs in the pharmaceutically important plant, purslane. Moreover, a comparison of the ZnO NPs with bulk ZnO particles was also presented. The current study provides valuable data and evidence for increasing crop productivity by increasing the concentration of useful compounds, such as antioxidants and secondary metabolites, through the application of ZnO NPs in *P. oleracea*. However, more convincing studies are required to illustrate the elusive ecotoxicological impacts of these nanoproducts. The nanoforms of Zn were found more effective than its bulk counterparts in influencing plant growth and physiology. An increase in the production of secondary metabolites might help in the production of new effective metabolites, probably applicable for therapeutics proposes.

References

1. AnastáCio A, Carvalho IS. Accumulation of fatty acids in purslane grown in hydroponic salt stress conditions. Int J Food Sci Nutr. 2013;64:235–242. https://doi.org/10.3109/09637486.2012.713915

2. Naik VV, Karadge BA. Effect of NaCl and Na_2SO_4 salinities and light conditions on seed germination of purslane (*Portulaca oleracea* Linn.). Journal of Plant Stress Physiology. 2017:1–4. https://doi.org/10.19071/jpsp.2017.v3.3142

3. Biswas P, Wu CY. Nanoparticles and the environment. Journal of the Air and Waste Management Association. 2005;55:708–746. https://doi.org/10.1080/10473289.2005.10464656

4. Odhav B, Beekrum S, Akula U, Baijnath H. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. J Food Compost Anal. 2007;20:430–435. https://doi.org/10.1016/j.jfca.2006.04.015

5. Santiago-Saenz YO, Hernández-Fuentes AD, Monroy-Torres R, Cariño-Cortés R, Jiménez-Alvarado R. Physicochemical, nutritional and antioxidant characterization of three vegetables (*Amaranthus hybridus* L., *Chenopodium berlandieri* L., *Portulaca oleracea* L.) as potential sources of phytochemicals and bioactive compounds. Journal of Food Measurement and Characterization. 2018;12:2855–2864. https://doi.org/10.1007/s11694-018-9900-7

6. Simonet BM, Valcárcel M. Monitoring nanoparticles in the environment. Anal Bioanal Chem. 2009;393:17. https://doi.org/10.1007/s00216-008-2484-z

7. Dkhil MA, Moniem AEA, Al-Quraishy S, Saleh RA. Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. J Med Plant Res. 2011;5:1589–1593.

8. Shahidi F, Zhong Y. Novel antioxidants in food quality preservation and health promotion. European Journal of Lipid Science and Technology. 2010;112:930–940. https://doi.org/10.1002/ejlt.201000044

9. Encalada MA, Hoyos KM, Rehecho S, Berasategi I, de Ciriano MGÍ, Ansorena D, et al. Anti-proliferative effect of *Melissa officinalis* on human colon cancer cell line. Plant Foods Hum Nutr. 2011;66:328–334. https://doi.org/10.1007/s11130-011-0256-y

10. Rastogi A, Zivcak M, Sytar O, Kalaji HM, He X, Mbarki S, et al. Impact of metal and metal oxide nanoparticles on plant: a critical review. Front Chem. 2017;5:78. https://doi.org/10.3389/fchem.2017.00078

11. Mukherjee A, Sun Y, Morelius E, Tamez C, Bandyopadhyay S, Niu G, et al. Differential toxicity of bare and hybrid ZnO nanoparticles in green pea (*Pisum sativum* L.): a life cycle study. Front Plant Sci. 2016;6:1242. https://doi.org/10.3389/fpls.2015.01242

12. Subbaiah LV, Prasad TNVKV, Krishna TG, Sudhakar P, Reddy BR, Pradeep T. Novel effects of nanoparticulate delivery of zinc on growth, productivity, and zinc biofortification in maize (*Zea mays* L.). J Agric Food Chem. 2016;64:3778–3788. https://doi.org/10.1021/acs.jafc.6b00838

13. Venkatachalam P, Jayaraj M, Manikandan R, Geetha N, Rene ER, Sharma N, et al. Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in *Leucaena leucocephala* seedlings: a physiochemical analysis. Plant Physiol Biochem. 2017;110:59–69. https://doi.org/10.1016/j.plaphy.2016.08.022

14. Wang X, Yang X, Chen S, Li Q, Wang W, Hou C, et al. Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in *Arabidopsis*. Front Plant Sci. 2016;6:1243. https://doi.org/10.3389/fpls.2015.01243

15. Salah SM, Yajing G, Dongdong C, Jie L, Aamir N, Qijuan H, et al. Seed priming with polyethylene glycol regulating the physiological and molecular mechanism in rice (*Oryza sativa* L.) under nano-ZnO stress. Sci Rep. 2015;5:14278. https://doi.org/10.1038/srep14278

16. Chow JC, Watson JG, Savage N, Solomon CJ, Cheng YS, McMurry PH, et al. Nanoparticles and the environment. J Air Waste Manag Assoc. 2005;55:1411–1417. https://doi.org/10.1080/10473289.2005.10464743

17. Salamanca-Buentello F, Persad DL, Martin DK, Daar AS, Singer PA. Nanotechnology and the developing world. PLoS Med. 2005;2:e97. https://doi.org/10.1371/journal.pmed.0020097

18. Bhati-Kushwaha H, Malik C. Applications of nanotechnology in the field of medicine. LS: International Journal of Life Sciences. 2013;2:14–25. https://doi.org/10.5958/j.2319-1198.2.1.003

19. Malmsten M. Antimicrobial and antiviral hydrogels. Soft Matter. 2011;7:8725–8736. https://doi.org/10.1039/c1sm05809f

20. Lin D, Xing B. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environ Pollut. 2007;150:243–250. https://doi.org/10.1016/j.envpol.2007.01.016

21. Handy RD, Owen R, Valsami-Jones E. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology. 2008;17:315–325. https://doi.org/10.1007/s10646-008-0206-0

22. Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS. Nanoparticulate material delivery to plants. Plant Sci. 2010;179:154–163. https://doi.org/10.1016/j.plantsci.2010.04.012

23. Ma X, Geiser-Lee J, Deng Y, Kolmakov A. Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Sci Total Environ. 2010;408:3053–3061. https://doi.org/10.1016/j.scitotenv.2010.03.031

24. Sheykhbaglou R, Sedghi M, Shishevan MT, Sharifi RS. Effects of nano-iron oxide particles on agronomic traits of soybean. Not Sci Biol. 2010;2:112. https://doi.org/10.15835/nsb224667

25. Lee WM, An YJ, Yoon H, Kweon HS. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): plant agar test for water-insoluble nanoparticles. Environ Toxicol Chem. 2008;27:1915–1921. https://doi.org/10.1897/07-481.1

26. Wang H, Kou X, Pei Z, Xiao JQ, Shan X, Xing B. Physiological effects of magnetite (Fe_3O_4) nanoparticles on perennial ryegrass (*Lolium perenne* L.) and pumpkin (*Cucurbita mixta*) plants. Nanotoxicology. 2011;5:30–42. https://doi.org/10.3109/17435390.2010.489206

27. Chalenko G, Gerasimova N, Vasyukova N, Ozeretskovskaya O, Kovalenko L, Folmanis G, et al. Metal nanoparticles induce germination and wound healing in potato tubers. Dokl Akad Nauk. 2010;435(1):428–430. https://doi.org/10.1134/S0012496610060165

28. El-Keblawy A, Al-Ansari F. Effects of site of origin, time of seed maturation, and seed age on germination behavior of *Portulaca oleracea* from the Old and New worlds. Can J Bot. 2000;78:279–287. https://doi.org/10.1139/b00-001

29. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 1949;24:1. https://doi.org/10.1104/pp.24.1.1

30. Lichthentaler H. Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. In: Colowick, SP, Kaplan NO, editors. Methods in enzymology. Vol. 148. San Diego, CA. Academic Press; 1987. p. 350–382. https://doi.org/10.1016/0076-6879(87)48036-1

31. Krizek DT, Britz SJ, Mirecki RM. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cv. New Red Fire lettuce. Physiol Plant. 1998;103:1–7. https://doi.org/10.1034/j.1399-3054.1998.1030101.x

32. Nicholson RL, Hammerschmidt R. Phenolic compounds and their role in disease resistance. Annu Rev Phytopathol. 1992;30:369–389. https://doi.org/10.1146/annurev.py.30.090192.002101

33. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70–76. https://doi.org/10.1006/abio.1996.0292

34. Chen J, Xiao Q, Wang C, Wang WH, Wu FH, He BY, et al. Nitric oxide alleviates oxidative stress caused by salt in leaves of a mangrove species, *Aegiceras corniculatum*. Aquat Bot. 2014;117:41–47. https://doi.org/10.1016/j.aquabot.2014.04.004

35. Dimkpa CO, McLean JE, Latta DE, Manangón E, Britt DW, Johnson WP, et al. CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. Journal of Nanoparticle Research. 2012;14:1125. https://doi.org/10.1007/s11051-012-1125-9

36. Pereira G, Molina SMG, Lea P, Azevedo RA. Activity of antioxidant enzymes in response to cadmium in *Crotalaria juncea*. Plant Soil. 2002;239:123–132. https://doi.org/10.1023/A:1014951524286

37. Venkatachalam P, Priyanka N, Manikandan K, Ganeshbabu I, Indiraarulselvi P, Geetha N, et al. Enhanced plant growth promoting role of phycomolecules coated zinc oxide nanoparticles with P supplementation in cotton (*Gossypium hirsutum* L.). Plant Physiol Biochem. 2017;110:118–127. https://doi.org/10.1016/j.plaphy.2016.09.004

38. Prasad T, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, et al. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. J Plant Nutr. 2012;35:905–927. https://doi.org/10.1080/01904167.2012.663443

39. Tarafdar J, Raliya R, Mahawar H, Rathore I. Development of zinc nanofertilizer to enhance crop production in pearl millet (*Pennisetum americanum*). Agricultural Research. 2014;3:257–262. https://doi.org/10.1007/s40003-014-0113-y

40. Lin D, Xing B. Root uptake and phytotoxicity of ZnO nanoparticles. Environ Sci Technol. 2008;42:5580–5585. https://doi.org/10.1021/es800422x

41. Nel AE, Mädler L, Velegol D, Xia T, Hoek EM, Somasundaran P, et al. Understanding biophysicochemical interactions at the nano–bio interface. Nat Mater. 2009;8:543. https://doi.org/10.1038/nmat2442

42. Farokh P, Mahmoodzadeh H, Satari T. Response of seed germination of safflower to UV-B radiation. Res J Environ Sci. 2010;4:70–74. https://doi.org/10.3923/rjes.2010.70.74

43. Kouhi SMM, Lahouti M, Ganjeali A, Entezari MH. Comparative effects of ZnO nanoparticles, ZnO bulk particles, and Zn^{2+} on *Brassica napus* after long-term exposure: changes in growth, biochemical compounds, antioxidant enzyme activities, and Zn bioaccumulation. Water Air Soil Pollut. 2015;226:364. https://doi.org/10.1007/s11270-015-2628-7

44. Rao S, Shekhawat G. Toxicity of ZnO engineered nanoparticles and evaluation of their effect on growth, metabolism and tissue specific accumulation in *Brassica juncea*. J Environ Chem Eng. 2014;2:105–114. https://doi.org/10.1016/j.jece.2013.11.029

45. Bandyopadhyay S, Plascencia-Villa G, Mukherjee A, Rico CM, José-Yacamán M, Peralta-Videa JR, et al. Comparative phytotoxicity of ZnO NPs, bulk ZnO, and ionic zinc onto the alfalfa plants symbiotically associated with *Sinorhizobium meliloti* in soil. Sci Total Environ. 2015;515:60–69. https://doi.org/10.1016/j.scitotenv.2015.02.014

46. Yang F, Hong F, You W, Liu C, Gao F, Wu C, et al. Influence of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach. Biol Trace Elem Res. 2006;110:179–190. https://doi.org/10.1385/BTER:110:2:179

47. Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI, Choi G. Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in *Arabidopsis*. Plant J. 2006;47:124–139. https://doi.org/10.1111/j.1365-313X.2006.02773.x

48. Connell SL, Al-Hamdani SH. Selected physiological responses of kudzu to different chromium concentrations. Can J Plant Sci. 2001;81:53–58. https://doi.org/10.4141/P00-029

49. Asgari-Targhi G, Iranbakhsh A, Ardebili ZO. Potential benefits and phytotoxicity of bulk and nano-chitosan on the growth, morphogenesis, physiology, and micropropagation of *Capsicum annuum*. Plant Physiol Biochem. 2018;127:393–402. https://doi.org/10.1016/j.plaphy.2018.04.013

50. Moghaddasi S, Fotovat A, Khoshgoftarmanesh AH, Karimzadeh F, Khazaei HR, Khorassani R. Bioavailability of coated and uncoated ZnO nanoparticles to cucumber in soil with or without organic matter. Ecotoxicol Environ Saf. 2017;144:543–551. https://doi.org/10.1016/j.ecoenv.2017.06.074

51. Mehrian SK, De Lima R. Nanoparticles cyto and genotoxicity in plants: mechanisms and abnormalities. Environ Nanotechnol Monit Manag. 2016;6:184–193. https://doi.org/10.1016/j.enmm.2016.08.003

52. Ushahra J, Bhati-Kushwaha H, Malik C. Biogenic nanoparticle-mediated augmentation of seed germination, growth, and antioxidant level of *Eruca sativa* Mill. varieties. Appl Biochem Biotechnol. 2014;174:729–738. https://doi.org/10.1007/s12010-014-1068-y

53. Lin YF, Aarts MG. The molecular mechanism of zinc and cadmium stress response in plants. Cellular and Molecular Life Sciences. 2012;69:3187–3206. https://doi.org/10.1007/s00018-012-1089-z

54. Javed R, Usman M, Yücesan B, Zia M, Gürel E. Effect of zinc oxide (ZnO) nanoparticles on physiology and steviol glycosides production in micropropagated shoots of *Stevia rebaudiana* Bertoni. Plant Physiol Biochem. 2017;110:94–99. https://doi.org/10.1016/j.plaphy.2016.05.032

55. Posmyk M, Janas K, Kontek R. Effect of anthocyanin-rich red cabbage extract on cytological injury induced by copper stress in plant and animal tissues. Ochrona Środowiska i Zasobów Naturalnych. 2007;33:50–56.

56. Wianowska D, Maksymiec W, Dawidowicz AL, Tukiendorf A. The influence of heavy

metal stress on the level of some flavonols in the primary leaves of *Phaseolus coccineus*. Acta Physiol Plant. 2004;26:247–254. https://doi.org/10.1007/s11738-004-0014-y

57. Posmyk M, Kontek R, Janas K. Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotoxicol Environ Saf. 2009;72:596–602. https://doi.org/10.1016/j.ecoenv.2008.04.024

58. Yamasaki H, Sakihama Y, Ikehara N. Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_2O_2. Plant Physiol. 1997;115:1405–1412. https://doi.org/10.1104/pp.115.4.1405

59. Mendil D, Uluözlü ÖD, Hasdemir E, Tüzen M, Sari H, Suicmez M. Determination of trace metal levels in seven fish species in lakes in Tokat, Turkey. Food Chem. 2005;90:175–179. https://doi.org/10.1016/j.foodchem.2004.03.039

60. Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T, et al. Antibacterial properties of nanoparticles. Trends Biotechnol. 2012;30:499–511. https://doi.org/10.1016/j.tibtech.2012.06.004

61. Chen J, Liu X, Wang C, Yin SS, Li XL, Hu WJ, et al. Nitric oxide ameliorates zinc oxide nanoparticles-induced phytotoxicity in rice seedlings. J Hazard Mater. 2015;297:173–182. https://doi.org/10.1016/j.jhazmat.2015.04.077

62. Hernandez-Viezcas J, Castillo-Michel H, Servin A, Peralta-Videa J, Gardea-Torresdey J. Spectroscopic verification of zinc absorption and distribution in the desert plant *Prosopis juliflora-velutina* (velvet mesquite) treated with ZnO nanoparticles. Chem Eng J. 2011;170:346–352. https://doi.org/10.1016/j.cej.2010.12.021

63. Krishnaraj C, Jagan E, Ramachandran R, Abirami S, Mohan N, Kalaichelvan P. Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. plant growth metabolism. Process Biochem. 2012;47:651–658. https://doi.org/10.1016/j.procbio.2012.01.006

64. Singh VP, Srivastava PK, Prasad SM. Nitric oxide alleviates arsenic-induced toxic effects in ridged *Luffa* seedlings. Plant Physiol Biochem. 2013;71:155–163. https://doi.org/10.1016/j.plaphy.2013.07.003

65. Zhou D, Jin S, Li L, Wang Y, Weng N. Quantifying the adsorption and uptake of CuO nanoparticles by wheat root based on chemical extractions. Journal of Environmental Sciences. 2011;23:1852–1857. https://doi.org/10.1016/S1001-0742(10)60646-8

66. Sharma P, Bhatt D, Zaidi M, Saradhi PP, Khanna P, Arora S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl Biochem Biotechnol. 2012;167:2225–2233. https://doi.org/10.1007/s12010-012-9759-8

67. Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao AJ, et al. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology. 2008;17:372–386. https://doi.org/10.1007/s10646-008-0214-0

68. Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresdey JL. Interaction of nanoparticles with edible plants and their possible implications in the food chain. J Agric Food Chem. 2011;59:3485–3498. https://doi.org/10.1021/jf104517j

69. Tripathi DK, Mishra RK, Singh S, Singh S, Singh VP, Singh PK, et al. Nitric oxide ameliorates zinc oxide nanoparticles phytotoxicity in wheat seedlings: implication of the ascorbate–glutathione cycle. Front Plant Sci. 2017;8:1. https://doi.org/10.3389/fpls.2017.00001