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Nanoparticle Treatments Based on Zinc Oxide and *Moringa oleifera* Leaf Extracts Alleviate Salinity Stress in Faba Bean (*Vicia faba* L.)

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Abstract

Salinity stress limits crop growth and productivity, including legumes in various regions worldwide. The impact of foliar-applied zinc nanoparticles (ZnNPs) and combined zinc nano-loaded with moringa extracts (ZnONPs) on salt tolerance in faba beans (cultivar, Giza-716) grown under saline soil (50 and 100 mM NaCl) was investigated. *Moringa oleifera* extract has been used as a chelating agent to synthesize zinc oxide nanoparticles. The crystalline structure, morphology, and chemical composition of ZnO nanoparticles were studied using various characterization techniques, including UV-visible spectroscopy (UV), Fourier Transform Infrared Analysis (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). Morphological, chemical, and biochemical parameters of plants at 60 and 90 days after sowing were assessed. Salinity stress caused a remarkable reduction in growth traits, photosynthetic pigments and proline levels of the faba bean. Foliar spray with ZnNPs and ZnONPs on faba bean grown under saline soils promoted plant growth parameters (*i.e.*, shoot length, numbers of leaves, relative water content, shoot and roots fresh and dry weights), photosynthetic pigments (Chl a, b, total chlorophyll, and carotenoids), proline and mineral elements (Na^+, K^+, Ca^{2+}, and Zn^{2+}) compared to control. However, at 100 mM NaCl, there were no significant variations in the mentioned parameters. This study suggested that there is potential for foliar spraying with ZnNPs and ZnONPs in improving growth parameters, photosynthesis efficiency and biochemical aspects of faba bean plants under saline conditions.

Keywords

Faba Bean, Green Synthesis, ZnO Nanoparticles, Salinity Stress, Proline

1. Introduction

Faba bean, also known as the broad bean, field bean, or horse bean, is a significant leguminous crop worldwide due to its nutrient-rich seeds or fresh green fruits used as human food or animal feed [1]. Faba beans are a source of protein, carbohydrate, folic acid, vitamin C, dietary fibre, macro, and microelements such as Ca, K, Mg, Na, P, S, Al, B, Ba, Fe, Co, Ga, Li, Mn, Ni, Cr, Pb, Sr, Cu, Zn, and antioxidants [2].

Salinity is one of the most limiting abiotic stresses that impacts agricultural plant quality and quantity worldwide [3]. Salinity stress causes a combination of ionic and osmotic stresses in plants, leading to cellular, molecular, and physiological deterioration, as well as reduced food uptake and photosynthetic performance [4]. Salt stress produces plenty of reactive oxygen species (ROS), which degrades biomolecules, including proteins, lipids, and nucleic acids, as well as other enzymatic activities, and even causes the cell membrane system to deteriorate [5]. Salinity has become a critical environmental challenge affecting plant productivity in dry and semi-arid regions [6]. Salt significantly influences cell growth and expansion, plant membrane irregularity, ion toxicity, metabolic function change, germination mechanism, photosynthetic activity, leaf, shoot, and root lengths [7].

Generally, high salinity limits water absorption from the soil, causing the accumulation of sodium (Na^+) and chloride (Cl^-), which induces physiological drought and oxidative stress in plants [8]. Salt tolerance is generally expressed by activating cellular pathways, including stress hormones, antioxidant enzymes, and osmoprotectant metabolites like amino acids and carbohydrates [9]. Faba bean is relatively sensitive to soil salinity, which significantly reduces yield in regions with saline soils [10]. Application of exogenous plant growth regulators, fertilizers and osmoprotectants mitigate salt-induced losses [11].

Zinc is one of the most critical elements necessary for efficient crop growth. It activates over 300 enzymes; it also helps directly enhance photosynthesis by partaking in carbohydrate metabolism processes [12]. Zinc deficiency is among the most frequent abiotic stress indicators symptoms exhibited by grown plants in salt and calcareous soils [13]. Furthermore, Zn deficiency causes physiological stress in plants, mainly due to disturbances in various enzymatic systems, decreased plant growth and crop yield, photosynthetic inhibition, and increased ROS levels in several plants [14].

Moringa oleifera Lam. (MLE) is high in potassium, cytokinin, minerals, carbohydrates, vitamins, proteins, flavonoids, and antioxidant compounds [15]. MLE leaf extract has been utilized as a plant natural growth stimulant in various

crops, enhancing plant growth biomass production and inducing tolerance to salt stresses [16].

Nanotechnology can significantly affect global food production, food safety, and food security. Nanotechnology is also widely used in agriculture as a promising technique for improving plant growth and yields [17]. Nanotechnology has been widely utilized to produce fertilizers due to its ability to homogenize the distribution of nutrients [18]. It is also preferred as they are environmentally benign [19]. Adsorbed nanoparticles gradually penetrate plant tissues and easily enter plant cells through the shoot and root, such as the cuticle, epidermis, stomata, hydathodes, stigma, root tips, lateral cortex plants, root junctions, bark, and other plant surfaces [20]. ZnONPs improved shooting, plantlet regeneration, and somatic embryogenesis by enhancing proline synthesis, peroxidase, superoxide dismutase, catalase activity and improving abiotic and biotic stress tolerance [21]. Therefore, our research aims to study the effect of ZnONPs nanoparticles on vegetative growth, several physiological traits, photosynthetic pigments, proline accumulation, and ion contents of faba bean cultivar under saline conditions, based on employing natural chemicals transferred into plant tissues via nanoparticles which maybe reduce the effect of stress on plants grown under salinity.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Field experiments were conducted in a greenhouse at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya. Faba beans were grown five months from December 2020 to May 2021. The faba bean cultivar "Giza-716" were obtained from Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt. The seeds were selected according to the similarity in size and colour. The selected seeds were washed with distilled water, sterilized in 1% (v/v) sodium hypochlorite for approximately 2 min and after that, rinsed with distilled water (dH_2O) and left to dry at room temperature overnight. The seeds were then placed on sterilized and moisturized filter paper in a petri dish and stratified at 4˚C for 4 days. Finally, seeds were then sown in plastic pots (30 × 50 cm) containing 92.52% sand, 5.48% silt, and 3.0% clay, with a pH 7.8 and EC.1.2 $dS{\cdot}m^{-1}$. In the greenhouse with the mean day/night temperature and relative humidity of 29˚C ± 4˚C, 38% ± 5%, and 17˚C ± 2˚C, 50% ± 5%, respectively. The experiment was laid out in a Randomization Complete Blocks Design (RCBD) with three replicates.

Experimental Treatments

The experiment consisted of two salinity levels (50 and 100 mM NaCl) and a 0 mM control. The salinity levels were obtained by adding appropriate amounts of dry NaCl (Merck, India) to dH_2O. All plants were irrigated daily with tap water for one week. The treatments were applied 21 days after sowing when all seeds had germinated. Consequently, the salt treatments were re-applied to each pot in

14 days intervals for 120 days. Foliar application of zinc oxide nanoparticles (ZnONPs; 50 $Mg{\cdot}L^{-1}$) and zinc nanoparticles (ZnNPs; 50 $Mg{\cdot}L^{-1}$) were done twice a week from the 30th day after sowing.

2.2. Preparation of *Moringa oleifera* Leaf Extract

Fresh *M. oleifera* leaves were collected from the JKUAT garden. The leaves were separated from the stems, washed with distilled water, and air-dried to eliminate any residue detritus. Then, phytochemical components were extracted from the dried leaves using dH_2O (500 $g{\cdot}L^{-1}$ dry material). According to [22].

2.3. Synthesis of ZnO-NPs

ZnONPs were synthesized using zinc nitrate, as described by [23]. During the preparation, 4.735 g zinc nitrate Zn $[NO_3]_2{\cdot}6H_2O$ (Loba-Chemie, India) was dissolved in 50 mL of dH_2O and stirred for 45 min. Then, *M. oleifera* leaf extract solution (10 mL) was added dropwise into the zinc nitrate solution while vigorous stirring at 80˚C for 3 h to allow the formation of ZnONPs. The solution eventually turned cloudy yellow. Finally, the solution was centrifuged at 12,000 rpm for 10 minutes and washed with distilled water to clear any impurities or absorbed ions. Then the product was dried in an oven at 70˚C for 48 hours.

2.3.1. Sample Characterizations

The synthesized ZnO-NPs were characterized by UV-visible spectrophotometer (Jenway-6800, Shimadzu, Japan) in a wavelength range between 200 - 600 nm. The X-ray diffractometer (XRD) was used to study the surface morphology, size and crystalline nature of ZnO NPs It produced diffractions at a scanning rate of 20/min in the 2 to 500 nm wavelength at room temperature with a CuKa radiation set at 40 kV and 20 mA. The Fourier transform infrared (FT-IR) spectra were recorded on the JascoFT-IR5300 model spectrophotometer in KBr pellets. Additionally, the particle size and characterization of the samples were carried out by high-resolution scanning electron microscopy (SEM) (JCM-7000 Neo Scope™ Benchtop SEM; JEOL, Japan).

2.3.2. Data Collection

Nine plants were randomly selected from each plot 60 and 90 days after sowing (DAS). Plant growth parameters such as height (cm), the number of leaves/plants (shoots, roots, fresh, and dry weight), photosynthetic activity, and biochemical parameters were assessed.

2.4. Measurement of the Growth Parameters

The growth of plants exposed to salt treatments was assessed “30 - 60 days” after treatment. Three replicates taken for each treatment were used to calculate the mean of each measurement. The measurements taken included length of the shoot (SFW), number of leaves, and root fresh weight (RFW). The freshly harvested samples were packed and preserved in an aerated oven for 2 days at 70˚C.

After that, the samples were wholly desiccated, and the root dry weight (RDW) and shoot dry weight (SDW) were assessed.

2.5. Leaf Relative Water Content

Relative water content (RWC) was determined according to [24]. And calculated using the following formula:

$$\mathrm{RWC} = \left(\mathrm{FW} - \mathrm{DW}\right)/\left(\mathrm{TW} - \mathrm{DW}\right) \times 100 \tag{1}$$

where FW = fresh weight. TW = Turgid weight. DW = Oven dry weight.

2.6. Determination of Photosynthetic Pigments

The chlorophyll contents (Chl a, Chl b and carotenoids) in fresh leaves was measured using spectrophotometry [25]. Fresh leaves were taken from the middle of five primary leaves (60 and 90 days after sowing). 1 gm fresh tissue was ground in a mortar with 20 mL (80% v/v) acetone (Loba-Chemie, India) 0.5 g calcium carbonate (Loba-Chemie, India). Then the extract was collected in a conical flask after being filtered via No. 2 filter paper. The filtrate was contained in a standard flask with % acetone added to make volume up to 20 ml. The extract's optical density (OD) was measured using a spectrophotometer at 645, 663, and 470 nm wavelengths. Chlorophyll a, chlorophyll b and total chlorophyll were assessed based on the following equations:

$$\mathrm{Chl\ a}\left(\mathrm{mg}\cdot\mathrm{g}^{-1}\ \mathrm{FW}\right) = \left(12.21\times \mathrm{A663} - 2.81\times \mathrm{A645}\right)\times V/\left(1000\times W\right) \tag{2}$$

$$\mathrm{Chl\ b}\left(\mathrm{mg}\cdot\mathrm{g}^{-1}\ \mathrm{FW}\right) = \left(20.13\times \mathrm{A645} - 5.03\times \mathrm{A663}\right)\times V/\left(1000\times W\right) \tag{3}$$

$$\mathrm{Total\ Chl}\left(\mathrm{mg}\cdot\mathrm{g}^{-1}\ \mathrm{FW}\right) = \left(17.90\times \mathrm{A645} + 8.08\times \mathrm{A663}\right)\times V/\left(1000\times W\right) \tag{4}$$

$$\begin{aligned}&\mathrm{Carotenoids}\left(\mathrm{mg}\cdot\mathrm{g}^{-1}\ \mathrm{FW}\right)\\ &= \left[\left(1000\times \mathrm{A470} - 3.27\times \mathrm{Chl\ a} - 104\times \mathrm{Chl\ b}\right)/227\right]\times V/\left(1000\times W\right)\end{aligned} \tag{5}$$

where V is the volume of 80% (v/v) acetone (mL), and W is the fresh weight (FW) of the sample (g).

2.7. Proline Content

Proline content in leaves after the anthesis period (60 and 90 days after sowing) was estimated using the modified [26]. Briefly, 0.5 g of fresh leaf tissue was crushed and ground in a mortar with 10 ml of sulfosalicylic acid. The homogenate was filtered through Whatman No 2-filter paper; 2 ml of the extract was mixed with 2 ml of ninhydrin acid, 2 ml of glacial acetic acid and incubated for 1 h at 100˚C in a water bath until the emergence of red colour. Then the tube was let to cool at room temperature, and 4 mL of toluene was added to the solution; after that, 2 mL of the coloured red layer from the tube was used to measure the OD 520 nm with a spectrophotometer. Proline concentration is calculated using a standard curve and fresh weight mmol proline ($\mathrm{g}\cdot\mathrm{FW}^{-1}$). The sulfosalicylic acid, ninhydrin, and glacial acetic acid were purchased (Loba-Chemie, India).

2.8. Mineral Ion Content

Fresh leaves were dried at 35˚C for 48 h. Approximately 0.3 g of leaves were grounded to powder form and burned at 500˚C using a muffle furnace. The ash was treated with 10 ml of an acid mixture containing HNO_3: $HClO_4$ (2:1 v/v) and digested for 1 to 2 h until the red NO_2 fumes ceased. After the complete digestion, the colourless digests (2 - 3 ml) volume was made up to 20 ml by distilled water then filtered through Whatman No.1 filter paper. The nitric acid and perchloric acid were purchased (Loba-Chemie, India). The solution's aliquots were used to determine ions, viz., Na^+, K^+, Ca^{2+}, and Zn^{2+} by inductively coupled plasma atomic absorption spectrometry (Optima 2000 DV, Perkin Elmer, USA). The content was determined following the earlier procedure [27].

2.9. Statistical Analysis

Data on plant growth and biochemical analysis were subjected to a two-way analysis of variance (ANOVA) to determine the treatment effects. The interaction between the factors was selected, and data could not be pooled whenever there was a significant interaction. The means were separated using the least significant difference test using SPSS 10 for Windows, 2001 (SPSS Inc., USA). The differences were considered significant at $P < 0.05$.

3. Results

3.1. Physical and Chemical Properties of Soil Mixture

Soil Properties after Harvesting

Irrigating faba beans plants with saline water (50 - 100 mM NaCl) significantly reduced K^+, HCO_3 and SO_4 levels while increasing Na^+, Cl^-, pH, and EC concentrations in soil. After harvesting, no carbonate was detected in the soil solution. Salinity levels increased the Na^+ concentration in the soil (**Table 1**).

3.2. UV-Vis Absorption Spectroscopy Analysis

The absorption spectrum of the green synthesized had an absorption peak at 370 nm. While ZnNPs showed excitation absorption (371 nm). At 370 nm, sharp bands of zinc colloids were observed, indicating that the zinc ion is efficiently reduced by *M. oleifera* extract. The absorption peak at 370 nm indicates the

Table 1. Physical and chemical properties of the soil before and after the field experiments.

Sample ID	PH %	E. C. mmohs/cm^3	Soil content of cations (meq/L.)		Soil content of anion (meq/L.)			
			K ppm	Na ppm	HCO_3	CL	CO_3	SO_4
0 mM	5.6	0.133	TRAC	TRAC	4.50	7.00	0.00	5.92
50 mM	6.3	0.669	153	296	3.20	35.60	0.00	3.10
100 mM	6.6	3.27	192	314	1.75	44.37	0.00	1.70

presence of blue-shifted absorption spectra in ZnONPs compared to the bulk value (377 nm), as shown in Figure 1.

3.3. FTIR

FTIR was used to determine the different functional groups contained in synthesized nanoparticles. The peaks were used to identify the functional groups present in ZnO nanoparticles, as shown in Figure 2. Peaks were found at 3486, 2405, 1651, 1459, 1160, 890, and 770 cm^{-1}. The fingerprint area of zinc oxide nanoparticles is 1700 - 800 cm^{-1} bandwidth. The peak at 3486 cm^{-1} corresponds to N-H stretching of protein secondary amides, while the peak at 2405 cm^{-1} is due to C-H stretching of protein methyl groups. The peak at 1651 cm^{-1} is produced by -CO stretching the amide-I band of proteins, while at 1459 cm^{-1} is

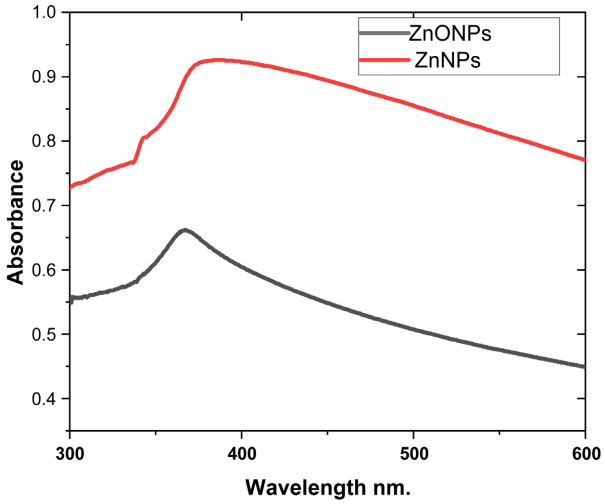


Figure 1. UV-Vis absorption spectra of ZnO nanoparticle. (ZnNPs, and ZnONPs).

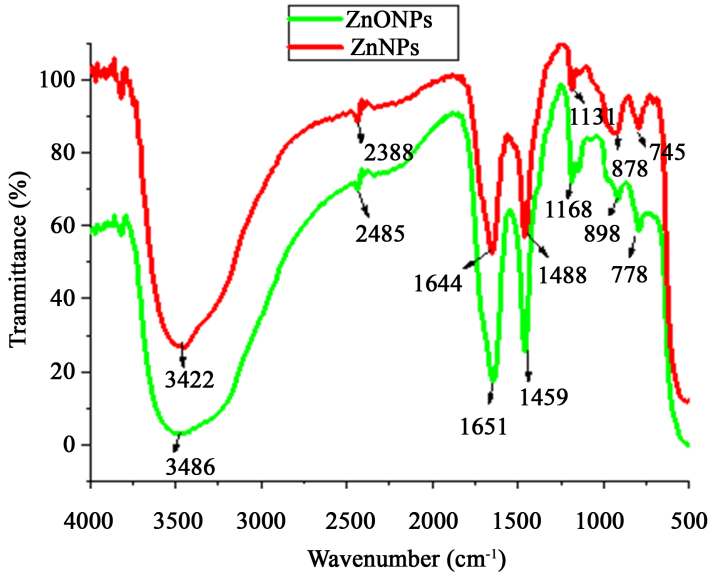


Figure 2. FTIR spectra of ZnNPs, and ZnONPs.

produced by C-N stretching vibrations of aromatic amines [28]. The presence of C-O stretching vibration of alcohol and C-H vibration of the CH=CH of the ethylene system is shown by the bands at 890 and 770 cm^{-1}, respectively. These proteins can also bind zinc oxide nanoparticles and function as capping agents, increasing their stability. The C-OH group of phenols is responsible for the peak at 1160 cm^{-1}, indicating the function of polyphenols such as terpenoids and flavonoids, which may also operate as bio-reducing agents. As a result, proteins serve as both stabilizing and reducing agents. Zn-O nanoparticles are responsible for the peak at 770 cm^{-1}. These functional groups have been observed on the surface of ZnNPs produced from leaf extracts of moringa plants; this result agrees with the results reported by [29].

3.4. XRD Analysis

The structure and phase purity of the samples were identified from XRD patterns, as shown in (**Figure 3(A)** and **Figure 3(B)**). The sharp diffraction peaks

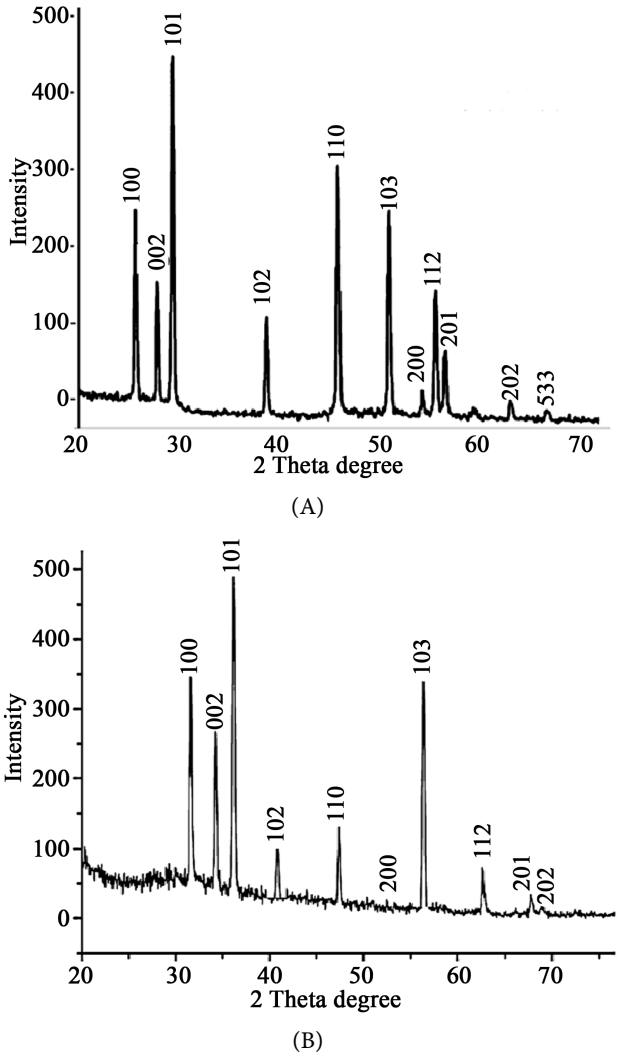


Figure 3. XRD patterns of ZnO nanoparticles. (A) ZnNPs, and (B) ZnONPs.

were observed at 2θ values 26.3˚, 29.1˚, 36.33˚, 39.29˚, 45.33˚, 51.52˚, 53.50˚, 52.50˚, 56.23˚, 64.84˚, and 67.79˚ degrees. Diffraction peaks of XRD are very well matched with the hexagonal wurtzite structure by comparison with the data from JCPDS card No. 89 - 1397. All the reflection peaks obtained were corresponding to (100), (002), (101), (102), (110), (103), (200), (112), (201), (202), and (533) diffraction lattice planes respectively which confirm the hexagonal wurtzite structure for the synthesized nanoparticles. This pattern follows the standard peaks displayed by the International Centre for Diffraction Data.

3.5. SEM

The size, shape and surface morphology of the ZnNPs and ZnONPs nanoparticles were determined using SEM analysis, and SEM images were shown in (Figure 4(A) and Figure 4(B)). The synthesized products are spherical and crystalline in structure according to detailed structural characteristics, with diameters around 198 - 213 nm, respectively. The SEM results revealed that the size and shape of the nanoparticles were affected by different precursors.

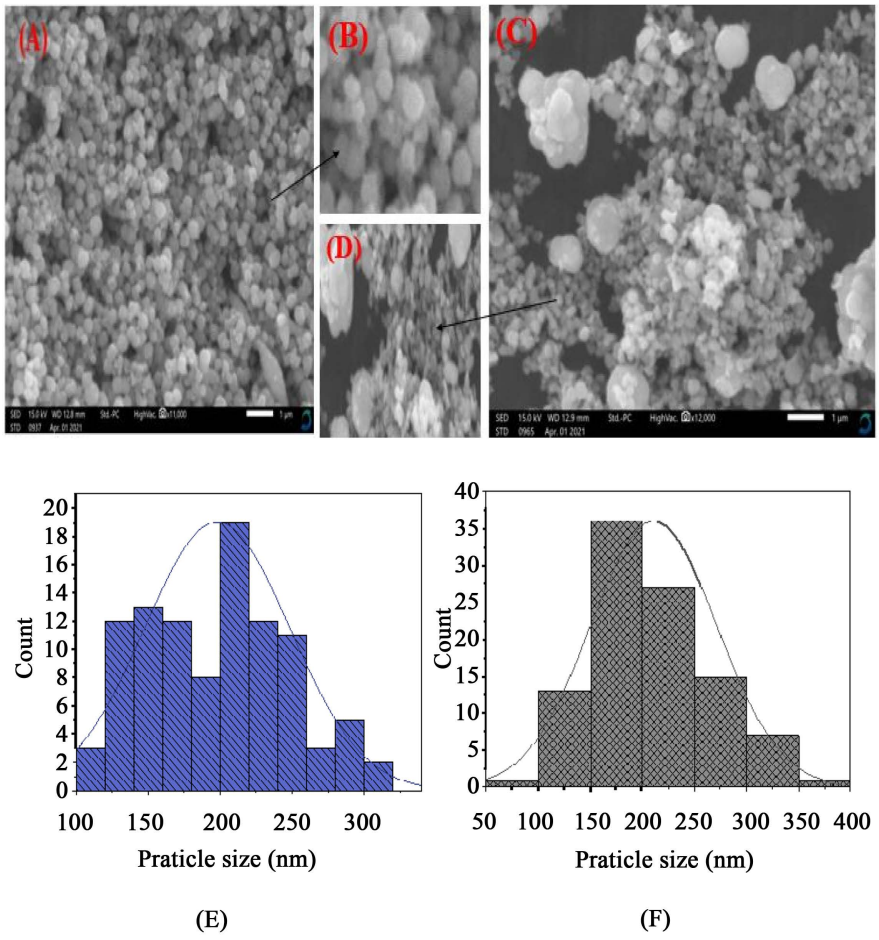
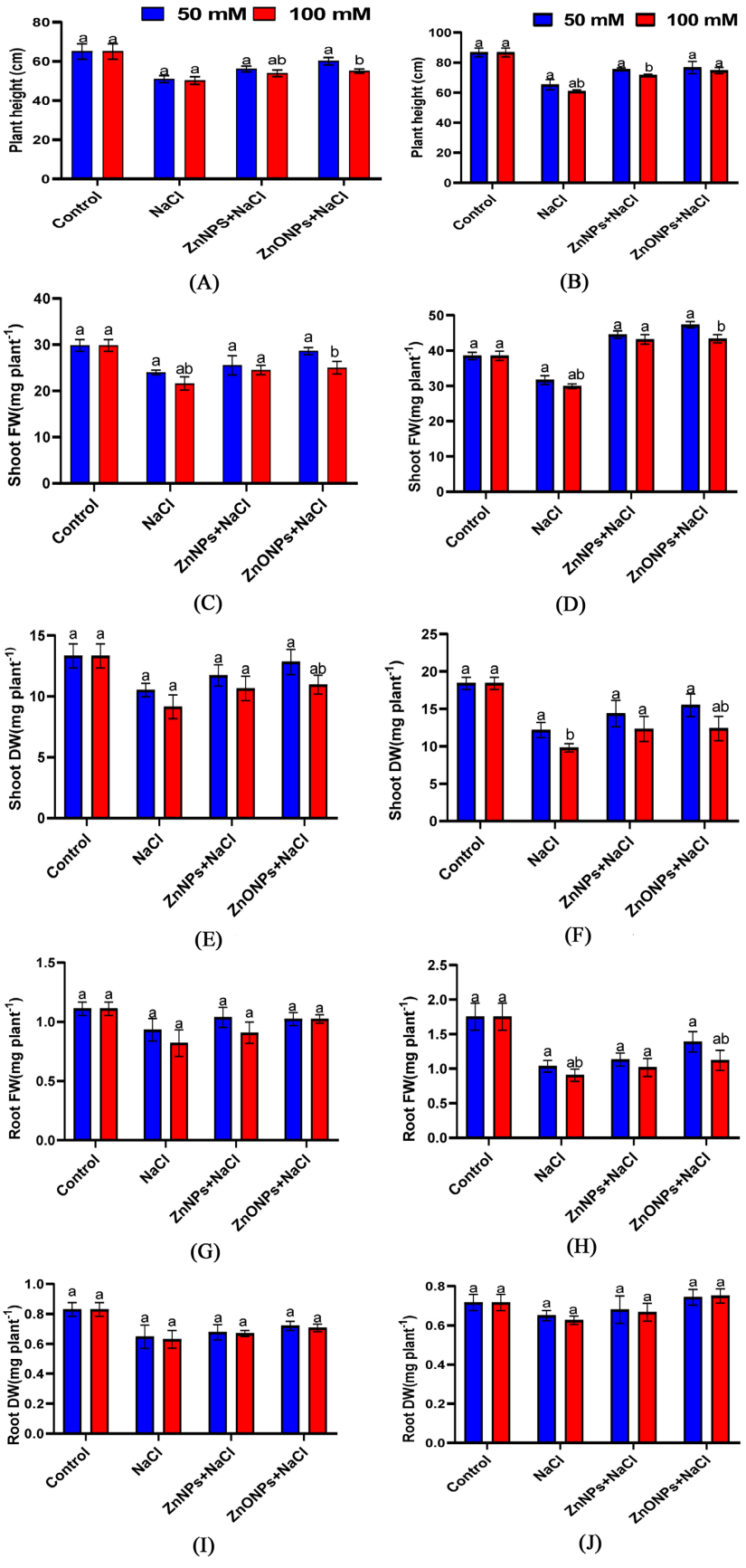


Figure 4. SEM images of green synthesized ZnNPs (A), its zoomed image (B) and particle size distribution (E); SEM image of ZnONPs (C), its zoomed image (D) and particle size distribution (F).

3.6. Effects of Foliar Applications of ZnNPs, and ZnONPs on Growth Parameters of Faba Bean

The growth responses of the faba bean cultivar under salinity conditions were evaluated by measuring growth-related parameters to assess their salt-tolerant capacity. The bean leaf number, plant height, and total DW of cultivar decreased with the increasing salinity levels. Specifically, when increasing NaCl concentration from 50 and 100 mM NaCl levels, the growth-related parameters, such as plant height, leaf number, WC, shoot, root, FW, and DW, was reduced; the results are shown in (**Figures 5(A)-(N)**). In comparison with the control and salinity levels (50 and 100 mM NaCl), was reduced plant height by (78.28%, 80.85%, 75.24% and 73.03%) at 60 and 90 days, respectively (**Figure 5(A)** and **Figure 5(B)**). However, there was a significant increase in plant height compared to salt-stressed plants when ZnNPs and ZnONPs treatments were applied (110.26%, 107.37%, 117.92%, 109.62%, 115.58%, 117.36%, 117.57% and 112.79%) at 60 and 90 days, respectively (**Figure 5(A)** and **Figure 5(B)**). Furthermore, the shoot FW decreased at salinity (50 and 100 mM NaCl) as compared to control plants by (80.32%, 76.34%, 82.21%, and 77.68%) at 60 and 90 days, respectively (**Figure 5(C)** and **Figure 5(D)**). Foliar spray of ZnNPs and ZnONPs cause a noticeable increment in SFW compared to salt-stressed plants by (106.46%, 119.36%, 113.89%, 115.74%, 140.65%, 149.50%, 144.19%, and 144.83%) at 60 and 90 days, respectively (**Figure 5(C)** and **Figure 5(D)**). On the other hand, the salt-stressed faba bean plants showed a considerable decrease in shoot dry (SDW) than the non-stressed plants (**Figure 5(E)** and **Figure 5(F)**). The treating faba beans with ZnNPs and ZnONPs increase the plant SDW compared to salt-stressed plants by (111.31%, 116.52%, 121.86%, 119.80%, 118.06%, 125.71%, 127.17%, and 126.32%) at 60 and 90 days, respectively (**Figure 5(E)** and **Figure 5(F)**). However, salt stress reduced the root FW, and the highest reduction was recorded at 100 mM NaCl than control plants (**Figure 5(G)** and **Figure 5(H)**). In contrast, the results showed the application of ZnNPs and ZnONPs caused a significant increase in root FW in plants grown under salt treatments compared to Salt-stressed plants by (110.75%, 104.75%, 109.67%, 124.39%, 109.70%, 113.33%, 134.95%, and 124.44%) at 60 and 90 days, respectively (**Figure 5(G)** and **Figure 5(H)**). In comparison with the untreated control sample, there was decreased significantly root DW (RDW) in salt-stressed plants (50 and 100 mM NaCl) levels (**Figure 5(I)** and **Figure 5(J)**). In contrast, the root DW significantly increased in plants treated with ZnNPs and ZnONPs compared to salt-stressed ones (104.68%, 106.34%, 112.50%, 111.11%, 104.61%, 106.45%, 113.84%, and 120.96%) at 60 and 90 days, respectively (**Figure 5(I)** and **Figure 5(J)**). Moreover, a significant decrease in leaf number was observed in 50 and 100 mM NaCl levels, respectively, compared to untreated control plants (**Figure 5(K)** and **Figure 5(L)**). Foliar application of ZnNPs and ZnONPs led to a significant increase in the leaf number of faba beans compared to salt-stressed plants by (131.51%, 133.51%, 142.08%, 139.70%, 125.11%, 120.79%, 141.44%, and 140.73%) at 60 and 90 days, respectively (**Figure 5(K)** and **Figure 5(L)**). The water content (WC%) of faba bean


50 mM
100 mM
Plant height (cm)
Shoot FW(mg plant-1)
Shoot DW(mg plant-1)
Root FW(mg plant-1)
Root DW(mg plant-1)
Control
NaCl
ZnNPS+NaCl
ZnONPs+NaCl
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)

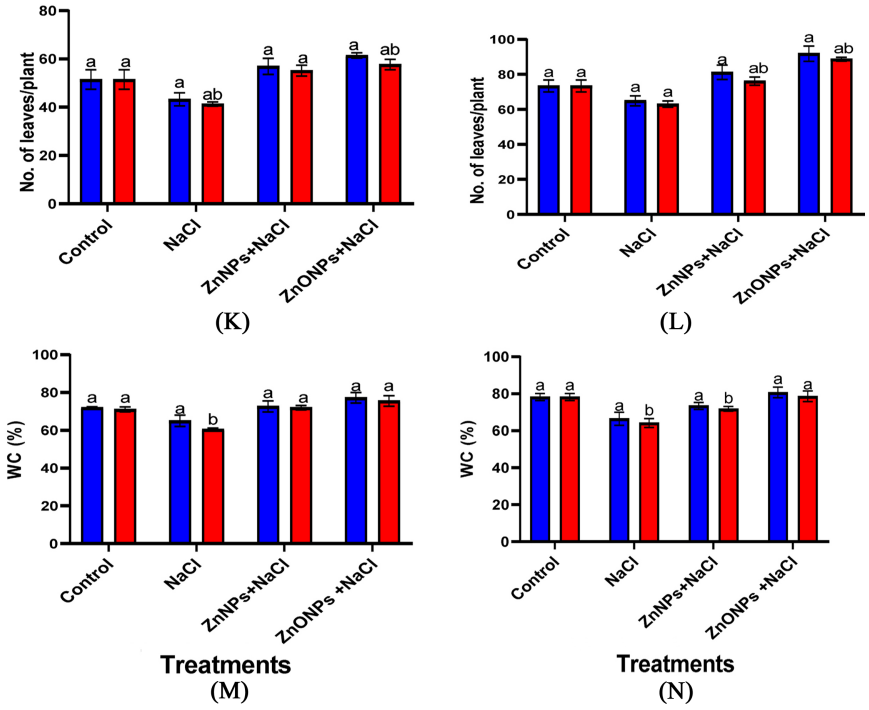


Figure 5. Effects of different levels of salinity stress (50 and 100 mM NaCl), single and combined treatment on growth characteristics of Faba bean, such as ((A) and (B)) plant height (cm); ((C) and (D)) shoot FW; ((E) and (F)) shoot DW; ((G) and H); root FW ((I) and (J)) root DW; ((K) and (L)) No. of leaves/plant; and ((M) and (N)) WC%, at 60 and 90 days, respectively. The values are the means of three replicates ± standard error.

significantly decreased in salt-stressed plants compared to control plants (**Figure 5(M)** and **Figure 5(N)**). However, the foliar application of ZnNPs and ZnONPs in salt-stressed plants significantly increased the water content (WC%) in the leaf of faba beans compared to untreated salt-stressed plants by (111.66%, 119.06%, 118.69%, 124.91%, 110.53%, 111.85%, 121.42%, and 122.36%) at 60 and 90 days, respectively (**Figure 5(M)** and **Figure 5(N)**).

3.7. Exogenous Application of ZnNPs, and ZnONPs on Leaf Pigments in Faba Bean under Salt Stress

To evaluate the roles of foliar application with ZnNPs and ZnONPs on the photosynthetic pigments under NaCl stress, the levels of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) in salt challenged faba bean leaves were determined In comparison with the control sample, there was a significant decrease in chl a content by (71.42%, 85.71%, 67.74% and 51.61%), chl b content by (85.93%, 89.84%, 78.43% and 83.33%), total chl content by (85.17%, 91.83%, 65.07% and 73.01%), and carotenoids content by (78.78%, 86.36%, 10.32% and 134.42%) in the plants exposed to 50 and 100 mM NaCl stresses, at 60 and 90 days, respectively, the results are shown in (**Figures 6(A)-(H)**). In contrast, spraying of ZnNPs and ZnONPs protection photosynthetic pigments from salinity-induced harmful impacts, as evident by the observed enhanced contents of chl a by (175.32%, 172.22%, 206.66%, 177.77%,

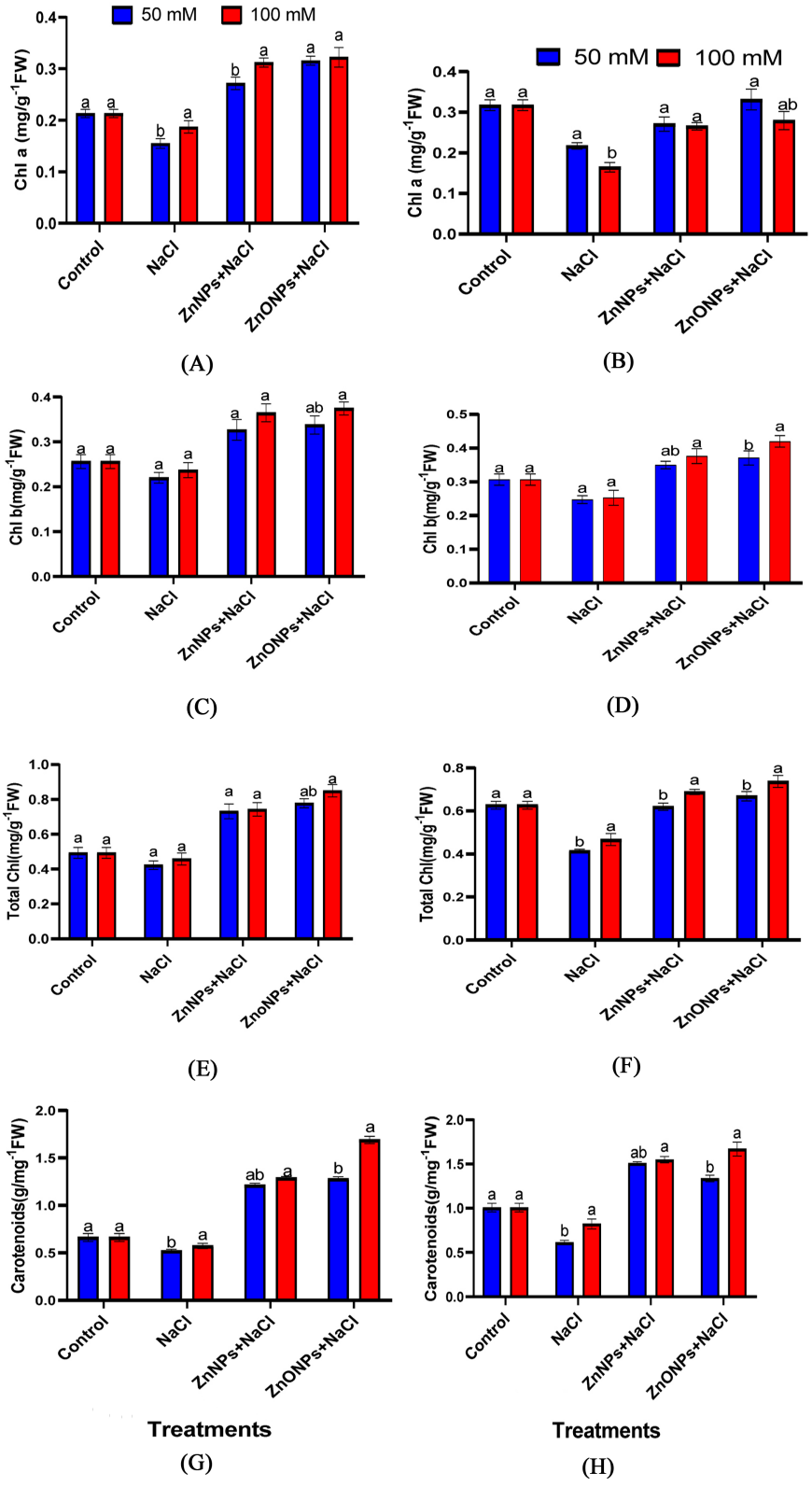


Figure 6. Effects of different levels of salinity stress (50 and 100 mM NaCl), single and combined Treatment on Pigments contents of faba bean. Chl a ((A) and (B)), Chl b ((C) and (D)), total Chl ((E) and (F)) and total Carotenoids ((G) and (H)) at 60 and 90 days, respectively. The values are the means of three replicates ± standard error.

128.57%, 162.50%, 157.14% and 168.75%) at 60 and 90 days, respectively (Figure 6(A) and Figure 6(B)), chl b by (145.45%, 156.52%, 149.94%, 160.86%, 141.66%, 146.82%, 154.16% and 162.69%) at 60 and 90 days, respectively (Figure 6(C) and Figure 6(D)), total chl by (173.80%, 164.44%, 183.33%, 186.66%, 148.78%, 147.82%, 160.97% and 158.69%) at 60 and 90 days, respectively (Figure 6(E) and Figure 6(F)), and carotenoids by (232.69%, 224.56%, 244.23%, 294.73%, 245.90%, 187.80%, 218.03% and 202.43%) at 60 and 90 days, respectively, in salt-treated plants (50 and 100 mM NaCl) when compared with only salt-stressed plants (Figure 6(G) and Figure 6(H)).

3.8. Effects of ZnNPs, and ZnONPs on Proline Content of Faba Bean under Salt Stress

NaCl treatment significantly increased free proline contents, whereas 100 mM Treatment significantly decreased. Intervening the salt stress with ZnNPs and ZnONPs treatment greatly enhanced the proline accumulation at 60 and 90 days, respectively, are shown in (Figure 7(A) and Figure 7(B)). The proline concentration increased at the salinity level of 100 mM NaCl, then decreased at 50 mM NaCl compared to control plants. When plants were under salt stress, the proline level increased in NP untreated plants at 100 mM NaCl compared to control plants. However, plants that were under salt stress; a 50 mM treatment increased proline accumulation) compared to control plants. However, the ZnNPs and ZnONPs, when applied to plants, significantly increased proline accumulation content than the salt-stressed plants at 60 and 90 days, respectively (Figure 7(A) and Figure 7(B)).

3.9. Effects of ZnNPs, and ZnONPs Application on Mineral Ion Contents in Faba under Salt Stress

Among the mineral ions, Na^+ contents of shoot gradually increased with increasing salinity in salinity-treated plants. The Na^+ contents of the shoot were

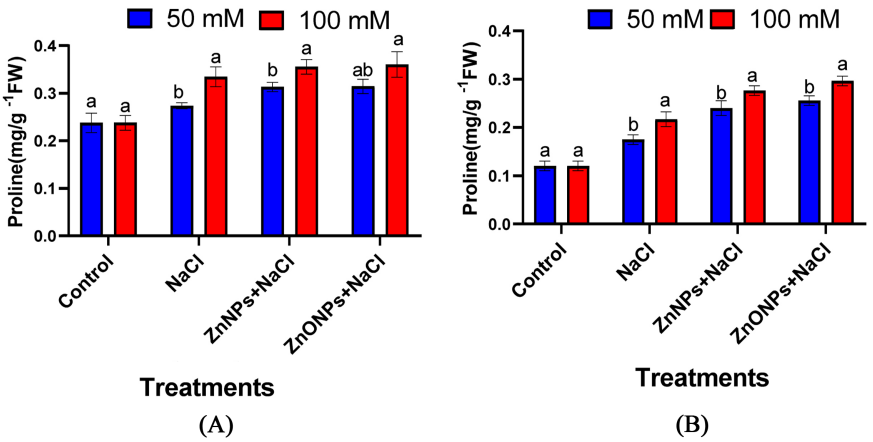


Figure 7. Effects of different levels of salinity stress (50 and 100 mM NaCl), single and combined Treatment on Proline of Faba bean. (A) at 60, (B) 90 days. The values are the means of three replicates ± standard error.

also higher than control plants in the combined treatment of ZnNPs + NaCl and ZnONPs + NaCl compared to salt-stressed plants at 60 and 90 days, respectively, are shown in (**Figure 8(A)** and **Figure 8(B)**). Plants treated with 50 mM NaCl had higher Na^+ concentrations in their shoots than control plants (245.45% and 337.50%) at 60 and 90 days, respectively. In addition, compared to the control, plants treated with 100 mM showed higher Na^+ concentrations in their shoots (336.36% and 309.52%) at 60 and 90 days, respectively. The concentration of Na^+ was reduced in the shoot of plants treated with ZnNPs + 50 mM NaCl and ZnONPs + 50 mM NaCl compared to 50 mM NaCl plants by (66.66%, 62.96%, 67.76% and 74.07%) at 60 and 90 days, respectively. Moreover the amount of Na^+ was increased significantly in the shoot of plants treated with ZnNPs + 100 mM NaCl and ZnONPs + 100 mM NaCl compared to the content in 100 mM NaCl plants (64.86%, 62.16%, 119.23% and 138.46%) at 60 and 90 days, respectively. On the other hand, salinity levels (50 and 100 mM NaCl) significantly decreased K^+ contents in the shoot of faba bean plants versus non-stress plants by (75.83%, 63.88%, 81.55% and 71.11%) at 60 and 90 days, respectively, are shown in (**Figure 8(C)** and **Figure 8(D)**). Intervening by foliar application of ZnNPs and ZnONPs for plants in both salinity levels, the accumulation of K^+contents in the shoot compared to only salt-stressed plants significantly improved (196.29%, 200.43%, 207.40%, 143.47%, 130.55%, 131.25%, 163.88% and 137.50%) at 60 and 90 days, respectively. In comparison with the untreated control plants and salt-stressed plants grown under (50 and 100 mM NaCl) levels showed a decrease of Ca^{2+} contents by (73.75%, 67.50%, 66.66% and 53.33%) at 60 and 90 days, respectively, are shown in (**Figure 8(E)** and **Figure 8(F)**). Further, foliar application of ZnNPs and ZnONPs to the plants significantly improved the accumulation of Ca^{2+} contents in the shoot, compared to only salt-stressed faba bean plants (114.28%, 140%, 128.57%, 160%, 120%, 137.50%, 130% and 150%) at 60 and 90 days, respectively. Furthermore, salinity significantly increased the Zn^{2+} content in the shoot of faba bean plant treated with salt (50 and 100 mM NaCl) compared to the non-stress plants by (336.36%, 309.09%, 412.50%, and 402.50%) at 60 and 90 days, respectively, are shown in (**Figure 8(G)** and **Figure 8(H)**). Besides, plants sprayed with ZnNPs and ZnONPs also significantly increased Zn^{2+} contents in the shoot compared to only salt-stressed plants (116.21%, 120.58%, 113.51%, 123.52%, 121.21%, 115.62%, 130.30% and 128.12%) at 60 and 90 days, respectively.

4. Discussion

In the present study, the green synthesis methods of nanoparticle biosynthesis represent an easy, eco-friendly, and cost-effective process to prepare nanoparticles by zinc nitrate solution with moringa extract. *M. oleifera* leaf extracts demonstrated potential for NPs synthesis in structural and optical investigations due to the quantum confinement effect, which confirmed the synthesis of efficient ZnONPs using UV, FTIR, XRD, and SEM analysis; also, the wavelength

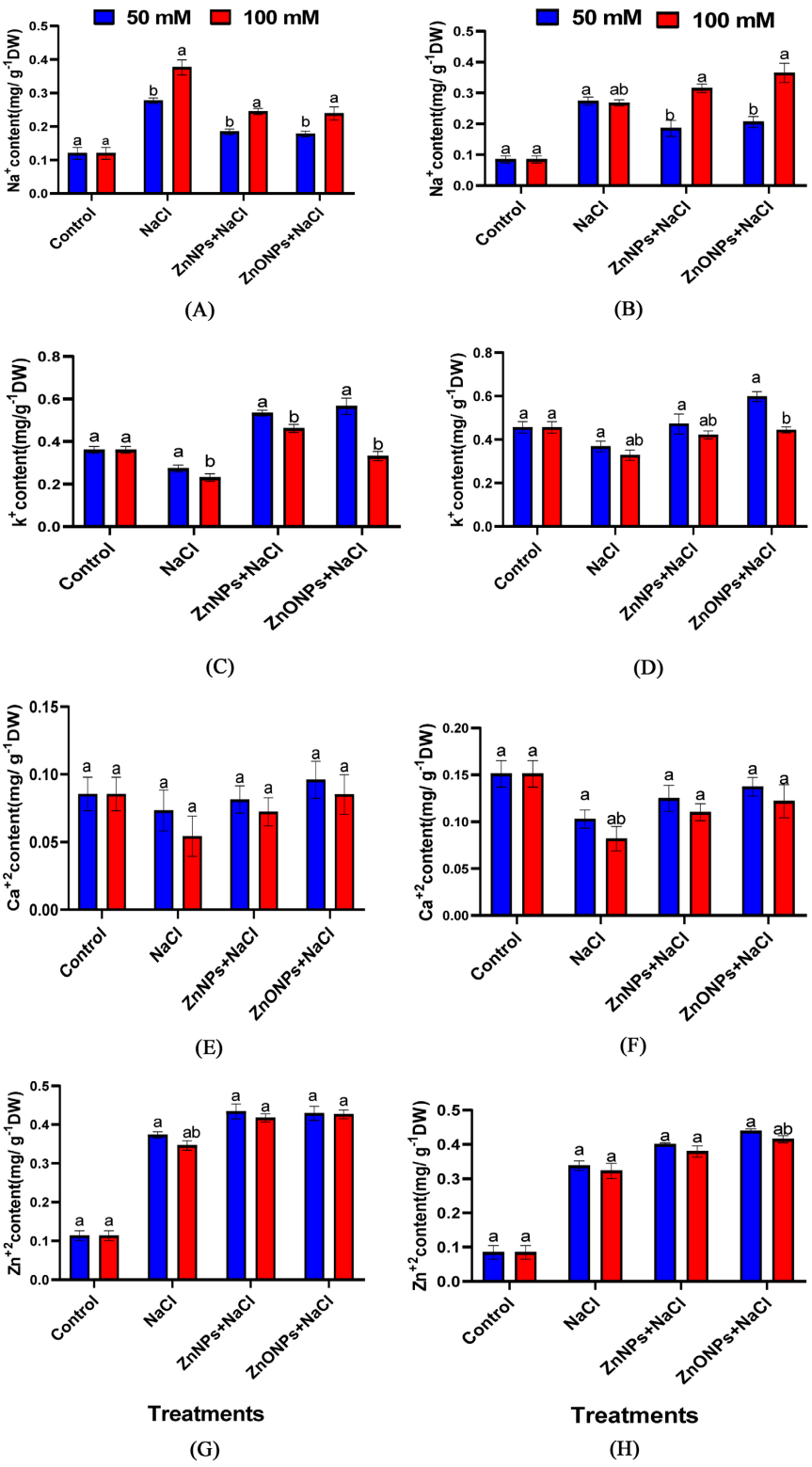


Figure 8. Effects of different levels of salinity stress (50 and 100 mM NaCl), single and combined treatment on primary mineral ion contents in shoot tissues of Faba bean. ((A) and (B)) shoot Na^{+}; ((C) and (D)) shoot K^{+}; ((E) and (F)) shoot Ca^{2+}; and ((G) and (H)) Zn^{2+} shoot at 60 and 90 days, respectively. The values are the means of three replicates ± standard error.

of the 370 nm absorption peak confirms the presence of ZnONPs which is in agreement with the earlier study [30]. In addition to the biomolecule absorption bands utilized as reduction and stabilization (capping agents) in *M. oleifera* leaf extract, FTIR absorption confirms the existence of ZnONPs [31]. The wide peaks in the XRD data indicate the crystallinity and purity of the nanoparticles in the samples [32]. The SEM pictures highlight the size, shape, and surface morphology of the ZnONPs, as shown in **Figure 4**. Structural characterizations show that the produced products are spherical and crystalline in structure. SEM pictures indicated that plant-derived NPs are entirely pure, implying that the plant has a significant ability to synthesize ZnONPs [33].

Salinity affects physiological, morphological and biochemical plant operations involving seed germination, plant growth, water and nutrient intake [34]. In this study, high salinity levels negatively influenced faba bean plants, as demonstrated by substantial reductions in growth parameters such as shoot height, root length, number of leaves, FW, DW of shoot and root of salt-stressed plants compared to the control group. These findings corroborate [35], which found that faba bean morphological and growth indices significantly reduced under salt stress. The reduction in plant height and number of flowers could be due to the detrimental impact of salinity stress. Salinity is a critical abiotic factor limiting growth and crop production [36]. Increasing salinity in irrigation water may have caused a reduction in faba bean biomass and growth parameters (plant height, number of leaves plants, shoots, roots, fresh and dry weight). [37] reported that the effect of salinity on faba bean plant development might be attributable to various reasons, including severe osmotic stress and ion toxicity.

The current study investigates the effect of foliar application of nanoparticles with ZnNPs and ZnONPs on the growth parameter attributes of faba bean plants grown under salt stress (**Figure 5**). In agreement with our results, [38] demonstrated that the zinc treatment boosted jojoba and maize plants' growth and yield indices. Application of ZnO nanoparticles was used to restore most of the growth parameters in winter wheat, resulting in enhanced chlorophyll content, shoot height, and grain production with unchanged plant biomass [39]. Moreover, [40] observed that reducing salt stress in sunflower plants treated with ZnONPs was more significant than in plants treated with dissolved ZnO alone. Additionally, foliar application of nanoparticles may reduce salt stress on the cells, enhancing plant growth, photosynthetic pigments, proline content, and grain yield of wheat and corn plants [41].

The influence of salt stress on chlorophyllase activity, which lowers chlorophyll synthesis or negatively affects the quantity and structure of chloroplasts, might explain the decrease in chlorophyll concentrations [42]. Exposing faba beans to salinity caused a significant reduction in the content of chlorophyll a, b, total chlorophyll, and carotenoids in stressed plants compared to the control (**Figure 6**). This finding was in line with the results of [43], who showed that salinity stress reduced the photosynthesis pigment, growth and yield of faba bean plants. The decrease in chlorophyll could be due to reactive oxygen species (ROS)

causing damage to chlorophyll, which means the plant didn't collect enough light and photosynthesis decreased or stopped [44]. This decrease might also be due to chlorophyll deterioration, reduced chlorophyll biosynthesis, and thylakoid membrane stability [45].

The content of chlorophyll a, b, total chlorophyll, and carotenoids in salt-stressed faba beans were significantly improved with ZnONPs as a foliar application compared with the control. Similarly, [46] found that salt-tolerant pistachio plants had increasing or constant chlorophyll levels under salinity conditions, whereas salt-sensitive plants had decreased chlorophyll contents. Furthermore, [47] reported that the plant biomass, root and shoot lengths, chlorophyll and protein contents, and phosphatase enzyme activity were significantly increased when ZnONPs was applied to Gossypium hirsutum, cluster bean, *Cucumis sativus*, *Cicer arietinum*, *Brassica napus*, *Raphanus sativus*, and *Vigna radiata*. Therefore, it's probable that the decrease in chlorophyll contents under salt stress is attributable to increased pigment degradation or reduced pigment synthesis, as well as disruption of enzyme activity involved in pigment production [48]. [49] suggested that foliar application of ZnONPs exposure in cotton plants significantly increased growth rate, biomass, photosynthetic pigment levels, protein content, antioxidant enzyme activity, and increased the expression level of SOD and POX isoenzymes, while MDA production reduced.

Proline accumulation is a sensitive physiological marker of a plant's reaction to different abiotic stress, including salinity, and it contributes to membrane stability in many crops' salt tolerance mechanisms [50]. Proline is also an antioxidant and a stress-related signalling molecule in plants under salinity stress [51]. Our findings also suggest that salinity caused a significant increase in proline accumulation in faba bean plants compared to non-stressed plants and results are consistent with previous research on several crops (tomato, faba bean, and chickpea) [52] [53]. In addition, results might have been a significant effect of foliar spray of ZnNPs and ZnONPs to stressed plants caused a further increase in proline content compared with the untreated stressed plants. These findings supported by [54] stated that ZnONPs promoted proline synthesis and enhanced abiotic stress tolerance of bananas. Also, [55] found that ZnONPs on tomato plants improved antioxidant systems and accelerated proline accumulation, which might provide plants more stability and improve photosynthetic efficiency.

Our findings showed that compared to non-stressed plants, salinity significantly increased Na^+ concentration while decreasing K^+, Ca^{2+}, and Zn^{2+} levels in faba bean leaves. In the shoot of the faba bean, Na^+ concentration was significantly increased with increasing salt concentration in single and combined treatments compared to their salt-stressed plants. The gradual increase in the Na^+ content has also been documented under saline conditions [56]. [57] reported that the poor growth performance of salinized plants was closely correlated with ionic toxicity due to an overabundance of toxic Na^+ in the cells and a significant decrease in beneficial ions, namely K^+, Ca^{2+}, and Mg^{2+} contents in faba bean,

which could have occurred due to cell membrane damage, ion leakage, and disruption in essential ion uptakes.

In plants exposed to water and salt stress, K^+ is related to the accumulation of osmolytes and increased antioxidant components [58]. One of the most critical functions of K^+ as a crucial nutrient in terrestrial ecosystems is as a significant contributor to water and solute transport K^+ is an essential cation in vacuolar and cellular growth because of its high mobility [59]. The reduction in K^+ concentration in plant tissue might be explained by interactions between Na^+ and K^+ at uptake sites in the roots, the impact of Na^+ on K^+ transport into the xylem, or uptake process inhibition. [60]. Ca^{2+} content declines under salinity stress conditions. Ca^{2+} is one of the most critical ions that stabilize the membrane structure and function, whereas K^+ is an important cationic osmolyte [61]. In this study, the decrease in Ca^{2+} contents of shoot under salinity and combined treatments of NaCl in Faba bean might be attributable to the reduction in Ca^{2+} availability and a limitation of Ca^{2+} absorption and transport to growth tissue under salinity [62]. Zinc plays a significant role in chlorophyll synthesis, pollen function, and fertilization and stabilizes proteins, membranes, and DNA-binding proteins such as Zn fingers [63]. Moreover, the foliar application of ZnONPs treatments considerably enhanced the nutritional content of Sweet Basil Plant leaves, including N, P, K, Fe, Zn, and Cu, compared to control leaves [64]. These improvements in plant growth, physiological traits, and quality with foliar application of ZnONPs might be attributable to 1) enhancing nutrient usage efficiency. 2) reducing soil toxicity caused by fertilizer overdosage. 3) increasing antioxidant enzyme activity, shielding plants from the harmful effects of reactive oxygen species [65].

5. Conclusion

Nanotechnology is one of the most recent technical developments in agriculture, and it is one of the most promising since it is both environmentally friendly and low-cost. Zinc oxide nanoparticles with varied particle sizes have been successfully synthesized utilizing *Moringa oleifera* extract and confirmed using various techniques (*i.e.* XRD, SEM, FTIR and UV-Vis spectroscopy). The foliar application of zinc oxide nanoparticles (ZnONPs) mitigated the adverse effects of salinity conditions that might be employed in faba bean plant irrigation. Also, ZnONPs enhanced faba bean growth parameters and chemical properties such as (Chl a, b, Total Chl), Carotenoids, Proline, and mineral contents.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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