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Salt stress mitigation in chickpea seedlings: a comparative study of zinc oxide nano and bulk particles

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Abstract: Nanotechnology plays a vital role in enhancing plant tolerance to salt stress; however, comparative studies on zinc oxide bulk particles (ZnO bulk) and zinc oxide nanoparticles (ZnO NPs) in this context remain unexplored. Since zinc (Zn) is an essential micronutrient involved in enzyme activation, photosynthesis, and antioxidant responses, it is important to understand how ZnO bulk and ZnO NPs influence chickpea growth under salt stress. This study investigated the morphological and physiological responses of chickpea seedlings treated with ZnO bulk (50 mg/L) and ZnO NPs (50 mg/L) under varying salt concentrations (20, 40, 80, and 120 mmol/L). Salt stress significantly inhibited chickpea growth, reducing the relative growth rate, net assimilation rate, total chlorophyll content, and potassium (K) and zinc ion levels while increasing sodium (Na), chlorine (Cl), malondialdehyde (MDA), and proline content. However, the application of ZnO bulk and ZnO NPs improved these parameters, mitigating the negative effects of salt stress. Furthermore, exogenous ZnO bulk and ZnO NPs to salt-stressed (20, 40, 80, and 120 mmol/L) chickpea resulted in decreased malondialdehyde content by 30, 32, 47, 34%, and 58, 31, 48, 47%, proline content by 4, 6, 1.6, 4% and 22, 21, 22, 28%, respectively, in comparison to the control. Notably, ZnO bulk and ZnO NPs enhanced antioxidant enzyme activities, including superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, and glutathione reductase. These findings suggest that foliar application of ZnO bulk and ZnO NPs helps alleviate salt stress in chickpeas, promoting better growth and physiological performance under saline conditions.

Keywords: crop; electrical conductivity; environment; ion toxicity; soil

Chickpea (*Cicer arietinum* L.), a key grain legume crop, boasts a worldwide production of 17.2 million tons (FAO 2018). This crop serves as a critical provider of protein and essential amino acids, making it a dietary staple in many regions (Jukanti et al. 2012). However, chickpeas are notably sensitive to salt, with their growth declining when soil electrical conductivity (EC) exceeds 6 dS/m (Flowers et al. 2010). Globally, excessive soil salinity slashes annual chickpea yields by 8–10% (Flowers et al. 2010). Salinity ranks among the most severe abiotic stressors, undermining crop productivity worldwide (Ibrahimova et al. 2025). Over 80% of the planet's land is affected by salt degradation, with 20% occurring in irrigated areas and 2% in arid regions (Flowers et al. 2010, Machado and Serralheiro 2017, Asif et al. 2018).

Factors such as unsustainable irrigation practices, sodium chloride residues in water, and rising groundwater levels are poised to exacerbate salinity issues in the years ahead (Deinlein et al. 2014). Soil salinity increases due to the consequence of climate change-related effects such as increased evaporation and salt deposition in the soil (Zhu et al. 2015). Toxic effects

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of salt on plants include osmotic stress, oxidative stress, Na^+ toxicity, and disruption of ionic equilibrium (Bartels and Sunkar 2005). Excessive salinity hampers plant growth and development through shoot-independent and shoot-dependent ion stressors triggered by salt exposure (Roy et al. 2014). A drop in external osmotic potential further undermines plant growth and biomass production (Puniran-Hartley et al. 2014). The Na^+ signal transduction system, vital for detoxifying cells and preserving ion balance, is especially susceptible to the harmful effects of ion toxicity (Zhu 2002). Meanwhile, the presence of K^+ ions in the cytoplasm is indispensable for protein synthesis, enzymatic functions, and ribosome activity (Shabala and Cuin 2008). Consequently, maintaining a stable Na^+/K^+ ratio becomes a critical determinant of salt stress tolerance, with salt-resistant plants distinguished by their ability to keep this cellular balance intact (Zhu 2003).

Like all living organisms, plants depend on the micronutrient zinc (Zn), which supports membrane structure, cell division, chlorophyll production, and the efficiency of photosynthetic processes while also contributing to enzyme activity (Stefanov et al. 2019, Abbasifar et al. 2020). Zn is particularly important in chlorophyll biosynthesis, as it stabilises chloroplast structure and activates key enzymes such as carbonic anhydrase. Moreover, Zn plays a vital role in activating antioxidant enzymes like catalase, superoxide dismutase, and peroxidases, which help neutralise reactive oxygen species (ROS) generated during salt stress, thus preserving cellular integrity and metabolic functions (Singh et al. 2022).

Interest in using nanomaterials in many applications, such as nanofertilisers, has increased in recent years. Due to their unusually large surface areas and surface charges, nanoparticles (NP) are more reactive than their bulk form (Dimkpa et al. 2019). This is because NP are so small that their dimensions are below 100 nm in at least one dimension. Recent studies have shown that NPs, particularly when used as nanofertilisers, not only enhance nutrient uptake efficiency (Seghatoleslami and Forutani 2015, Vishekaii et al. 2019) but also play active roles in mitigating abiotic stresses by modulating physiological and biochemical pathways (Dilnawaz et al. 2023a). For example, ZnO NPs can reduce oxidative damage in crops under stress (Zahedi et al. 2020). Moreover, NPs have been shown to improve photosynthetic performance, even under adverse climatic conditions, from cellular to canopy levels (Dilnawaz et al. 2023b). Specifically, studies have shown that ZnO NPs reduce malondialdehyde (MDA) levels, a marker of oxidative stress, while simultaneously enhancing the activity of antioxidant enzymes such as catalase and superoxide dismutase in stressed *Leucaena leucocephala* (Lam.) de Wit plants, thereby bolstering their resilience (Zahedi et al. 2020). Green pea and sugar beet research (Sun et al. 2020) also found outcomes that were comparable to these ones.

How chickpeas affected by salt stress respond to foliar application of zinc oxide bulk particles (ZnO bulk) and zinc oxide nanoparticles (ZnO NPs) is unknown. In light of this, we hypothesised that chickpeas would have a differential response to ZnO bulk and ZnO NPs concentrations and that optimising its dose might mitigate the effects of salt stress. Therefore, the purpose of this study was to investigate whether or not chickpeas could benefit from foliar applications of ZnO bulk and ZnO NPs in order to reduce the negative effects of salt stress.

MATERIAL AND METHODS

Plant materials and experimental treatment. The experiment was conducted in a climate-controlled greenhouse at Imam Abdulrahman Bin Faisal University, Saudi Arabia. Seeds of *Cicer arietinum* L. cv. Avarodhi, sourced from Altuajri (Saudi Arabia), were surface sterilised using 4% sodium hypochlorite for 5 min and thoroughly rinsed with sterile distilled water. Three uniform seedlings were transplanted into each 17 cm × 20 cm plastic pot filled with 2.5 kg of a peat moss and perlite mixture (2:1, *v*/*v*), selected for its neutral pH (6.5–7.0) and low electrical conductivity (0.5 dS/m), ensuring optimal drainage and aeration. Before treatment, plants were irrigated twice a week with deionised water. Greenhouse conditions were maintained at a photoperiod of 16/8 h (light/dark) with day/night temperatures of 22 ± 2 °C/16 ± 2 °C, relative humidity of 60–70%, and photosynthetically active radiation (PAR) of ~300 μmol/m^2/s, monitored using a LI-COR light sensor (model LI-190R, LI-COR Biosciences, Nebraska, USA). Foliar application of ZnO bulk (50 mg/L) and ZnO NPs (50 mg/L) was performed on days 7, 14, and 21 after planting. Salt stress treatments (NaCl at 0, 20, 40, 80, and 120 mmol/L) were initiated on day 20 and applied three times per week *via* irrigation. Each treatment combination was replicated in three independent trials, and plants were harvested at 30 days of age for physiological and biochemical analyses.

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Synthesis and characterisation of ZnO nanoparticles. ZnO nanoparticles were synthesised following Ezealisiji and Xavier (2020). 10 g of air-dried lemon leaves were ground into a fine powder, mixed with 100 mL of double-distilled water, and heated at 60 °C for 2 h. The cooled extract was filtered and stored. Concurrently, a 0.0015 mol/L zinc nitrate solution in 200 mL water was prepared and blended with the extract at 80 °C for 6 h until a faint yellow colour appeared, signalling ZnO NP formation. The precipitate was washed, dried at 60 °C for 8 h, and calcined at 350 °C for 2 h. A transmission electron microscope (TEM) (JEM-2100CX, Amagasaki , Japan) analysed ZnO nanoparticle size and shape, showing 30–50 nm particles with spherical or irregular forms. Selected area electron diffraction (SAED) displayed rings consistent with the wurtzite hexagonal structure, verifying crystallinity.

Relative growth rate. Biomass production was used to calculate the relative growth rate (RGR) (Singh and Singh 1994), which was defined as the per cent increase in plant dry weight over a certain period as a percentage of the plant's initial dry weight and expressed as (g/day).

Net assimilation rate. The net assimilation rate (NAR) was computed by taking the total dry matter generated throughout the treatment period and dividing it by the cumulative duration during which the leaf surface was exposed to sunlight, following the method of Singh and Singh (1994). This metric, known as the NAR, quantifies the efficiency of biomass production relative to light exposure and is expressed in units of grams per square meter per day ($g/m^2/day$), providing a standardised measure of photosynthetic productivity over time.

Total chlorophyll content. Photosynthetic pigments were extracted using the technique described by Arnon (1949), a widely recognised approach in plant physiology. A 0.25 g portion of leaf tissue was homogenised in 5 mL of 80% (*v*/*v*) acetone at room temperature (~20 °C) to ensure effective pigment dissolution. The homogenate was centrifuged a 3 000 rpm (approximately 1 000 × *g*) for 10 min at 4 °C to separate the pigment-rich supernatant from the cellular debris. The absorbance of this supernatant was measured at 663 nm and 645 nm with a spectrophotometer, providing the data needed to compute the total chlorophyll concentrations.

Determination of key elements. Na, K, Cl, and Zn concentration in leaves was measured using Forster et al. (1994). Dried leaf tissue was analysed for these ion contents using a Kjeldahl digestion apparatus with perchloric acid ($HClO_4$) and nitric acid (HNO_3). Flame photometry was used to quantify the level of these ions present.

Proline determination. The proline concentration was determined following the protocol established by Bates et al. (1973). A 0.5 g leaf tissue sample was homogenised with 10 mL of 3% sulfosalicylic acid to extract the proline content. The resulting mixture was then filtered using Whatman No. 40 filter paper to isolate the liquid components from the solid residue. Next, 2 mL each of ninhydrin solution and glacial acetic acid were added to the reaction mixture in test tubes. This combination was heated at 95 °C for approximately 1 h to facilitate the reaction, after which it was rapidly cooled in an ice bath to halt the process. To separate the chromophore, the mixture was extracted with 10 mL of toluene, with the aqueous phase being distinguished from the toluene layer through 1–2 min of continuous airstream circulation. The absorbance of the resulting coloured toluene phase was then measured at 520 nm using a spectrophotometer after allowing it to air-dry at room temperature for about 2–3 min to stabilise the readings.

Determination of MDA content. The malondialdehyde concentration was evaluated using the colourimetric procedure established by Heath and Packer (1968), a method widely adopted for detecting lipid peroxidation products. The process started with 0.5 g of plant material being homogenised in 5 mL of a 5% (*w*/*v*) trichloroacetic acid (TCA) solution to extract the MDA effectively. After centrifugation at 4 000 × *g* for 10 min at 4 °C, the supernatant was retrieved, free of solid debris, for the next phase. This supernatant was combined with 2 mL of a TCA mixture infused with 0.67% thiobarbituric acid (TBA), initiating a reaction highlighting MDA presence through colour change. The solution was incubated at 100 °C in a water bath for 30 min to develop the colour fully, then cooled rapidly on ice. Absorbance readings of a sample portion were obtained at 450, 532, and 600 nm *via* spectrophotometer, allowing the MDA content to be quantified in millimoles per gram of fresh weight (mmol/g FW).

Extraction and measurement of antioxidant enzyme activities. The isolation of antioxidant enzymes was performed using a technique adapted from Mukherjee and Choudhuri (1983). Freshly harvested leaves (0.5 g) were homogenised in 10 mL of phosphate buffer (pH 7.0) to extract the enzymes. The

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resulting mixture was centrifuged at 4 °C for 10 min at 15 000 × *g* to separate the supernatant, which was immediately used for enzyme activity assays at room temperature (~ 20 °C).

SOD activity. The activity of superoxide dismutase (SOD) was assessed using the nitro-blue-tetrazolium (NBT) reduction assay, as described by Chen and Wang (2006). The reaction mixture, totalling approximately 3 mL, consisted of 50 µL of enzyme extract, 150 µL of riboflavin (0.013 mol/L), 2.5 mL of methionine (0.013 mol/L), 250 µL of NBT (0.063 mol/L), and 50 µL of phosphate buffer (0.050 mol/L, pH 7.8). Absorbance was recorded at 560 nm using a spectrophotometer to quantify the enzyme's ability to inhibit NBT reduction.

CAT activity. Catalase (CAT) activity was determined following the procedure outlined by Aebi (1984). A 40 mL reaction mixture was prepared by combining the enzyme extract with 0.016 mL of 30% hydrogen peroxide (H_2O_2) and a 0.01 mol/L phosphate buffer (pH 7.0). The rate of H_2O_2 decomposition was measured by monitoring absorbance at 240 nm with a spectrophotometer (LKB-Biochrom 4050, Massachusetts, USA), reflecting CAT's catalytic efficiency.

APX activity. The ascorbate peroxidase (APX) activity was evaluated using the method developed by Nakano and Asada (1981). A reaction mixture was prepared by adding 20 µL of enzyme extract to a solution containing 0.1 mol/L potassium phosphate buffer (pH 7.0), 0.0005 mol/L ascorbate, 0.0001 mol/L EDTA, and 0.0010 mol/L H_2O_2. APX activity was calculated based on ascorbate oxidation, using an extinction coefficient of 2.8×10^3 L/mol/cm, with absorbance changes tracked over time.

GPX activity. Glutathione peroxidase (GPX) activity was measured using a protocol adapted from Elia et al. (2003). The reaction's optical density was determined at 340 nm, and the activity was quantified using an extinction coefficient of 6.62×10^3 L/mol/cm, reflecting the enzyme's role in reducing peroxide levels.

GR activity. The activity of glutathione reductase (GR) was analysed following the method proposed by Carlberg and Mannervik (1975). The enzyme's capacity to regenerate reduced glutathione was assessed, with results expressed in units per gram of fresh weight (U/g FW) protein, providing insight into its protective function against oxidative stress.

Statistical analysis. Data were analysed using analysis of variance (ANOVA) performed in Minitab 17 software (Massachusetts, USA). Results were reported as means ± standard error (SE) with a sample size of three (n = 3). The least significant difference (*LSD*) test was applied, and bars labelled with different letters indicated statistically significant differences at a probability level of $P \leq 0.05$.

RESULTS

In Figure 1, transmission electron microscopy (TEM) revealed zinc oxide nanoparticles applied to chickpea (*Cicer arietinum* L. cv. Avarodhi) seedlings under salt stress conditions (0, 20, 40, 80, and 120 mmol/L).

Under salt stress, the foliar application of ZnO bulk and ZnO NPs had a significant ($P \leq 0.001$) effect on the chickpea's relative growth rate, net assimilation rate, and total chlorophyl (*Chl*) content. In this manner, salt stress (20, 40, 80, and 120 mmol/L) decreased relative growth rate by 22, 65, 78, and 91%, net assimilation rate by 13, 31, 62, and 92%, and total chlorophyll content by 17, 20, 75, and 86%, compared to fully irrigated plants without salt stress (Figure 2).

By applying ZnO bulk and ZnO NPs to the leaves, these growth traits got a lot better, and the changes were even more noticeable with ZnO bulk and ZnO NPs. Exogenous application of ZnO bulk and ZnO NPs reduced the negative effects of salt stress on chickpea relative growth rate, net assimilation rate, and total *chl* content. This means that spraying ZnO bulk and ZnO NPs on salt-stressed plants gave better results than spraying ZnO bulk and ZnO NPs on salt-stressed plants that did not have ZnO bulk and ZnO applied.

Ion content raised or lowered significantly ($P \leq 0.001$) in salt-stressed (20, 40, 80, and 120 mmol/L) chickpea plants. For example, Na and *Chl* content increased by 77, 88, 94, 94% and 18, 57, 81, 88%, while K and Zn

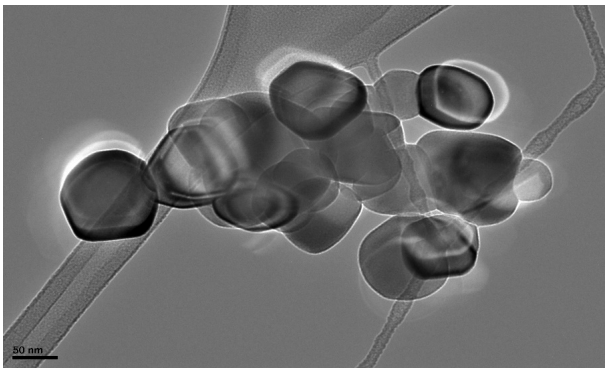

Figure 1. Transmission electron microscope image of zinc oxide nanoparticles

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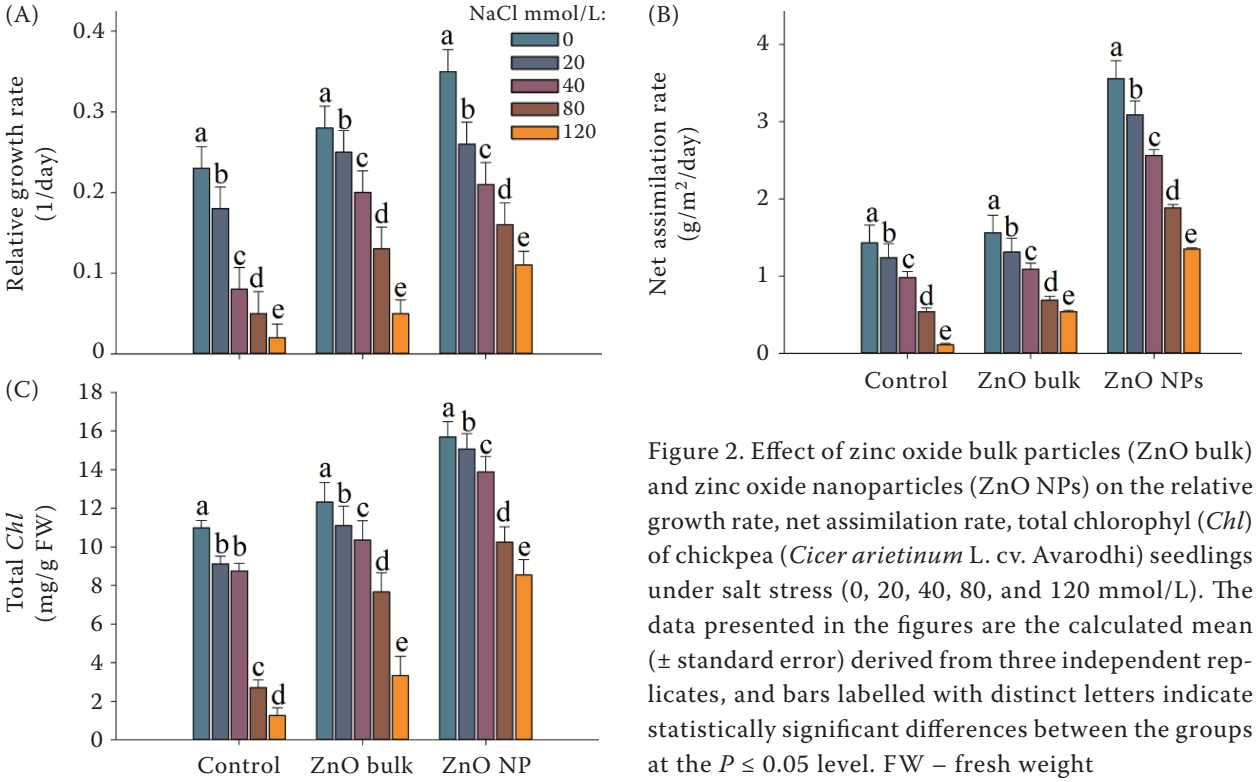

Figure 2. Effect of zinc oxide bulk particles (ZnO bulk) and zinc oxide nanoparticles (ZnO NPs) on the relative growth rate, net assimilation rate, total chlorophyl (*Chl*) of chickpea (*Cicer arietinum* L. cv. Avarodhi) seedlings under salt stress (0, 20, 40, 80, and 120 mmol/L). The data presented in the figures are the calculated mean (± standard error) derived from three independent replicates, and bars labelled with distinct letters indicate statistically significant differences between the groups at the $P \leq 0.05$ level. FW – fresh weight

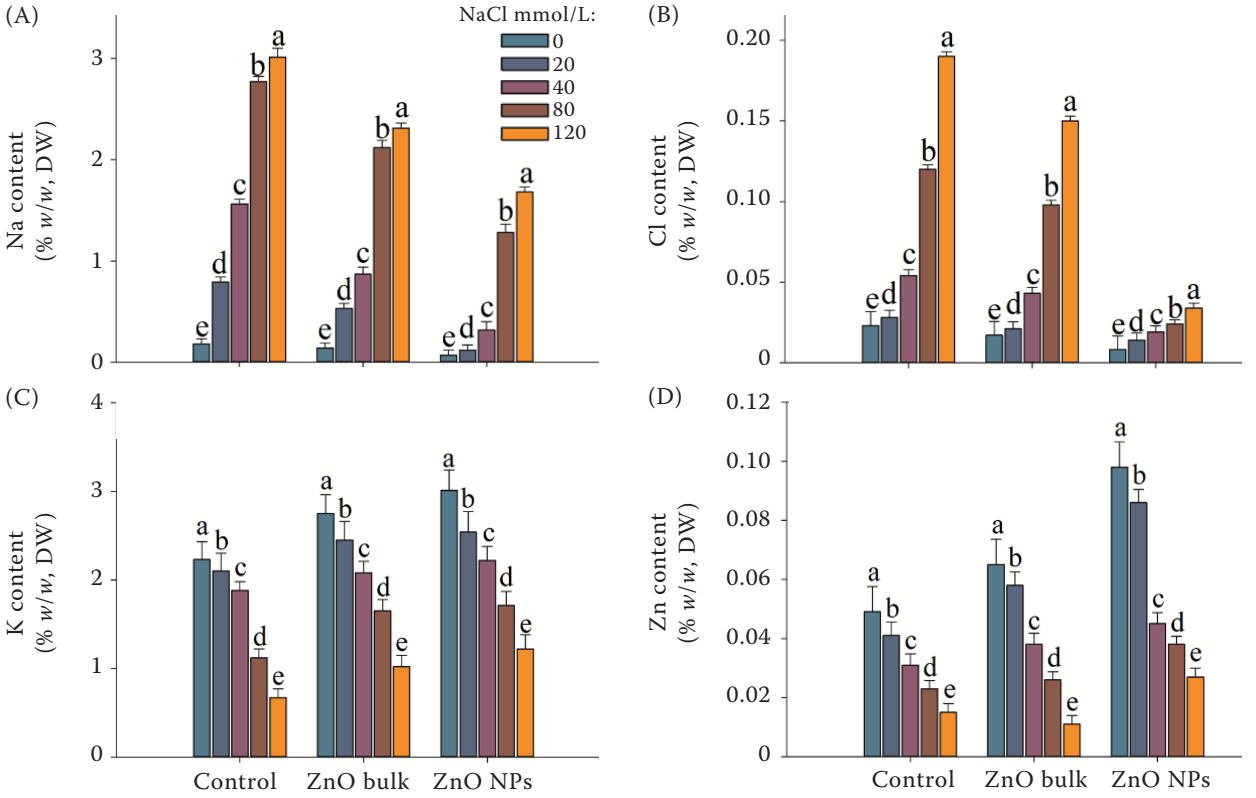

Figure 3. Effect of zinc oxide bulk particles (ZnO bulk) and zinc oxide nanoparticles (ZnO NPs) on the Na, Cl, K and Zn content of chickpea (*Cicer arietinum* L. cv. Avarodhi) seedlings under salt stress (0, 20, 40, 80, and 120 mmol/L). The data presented in the figures are the calculated mean (± standard error) derived from three independent replicates, and bars labelled with distinct letters indicate statistically significant differences between the groups at the $P \leq 0.05$ level. DW – dry weight

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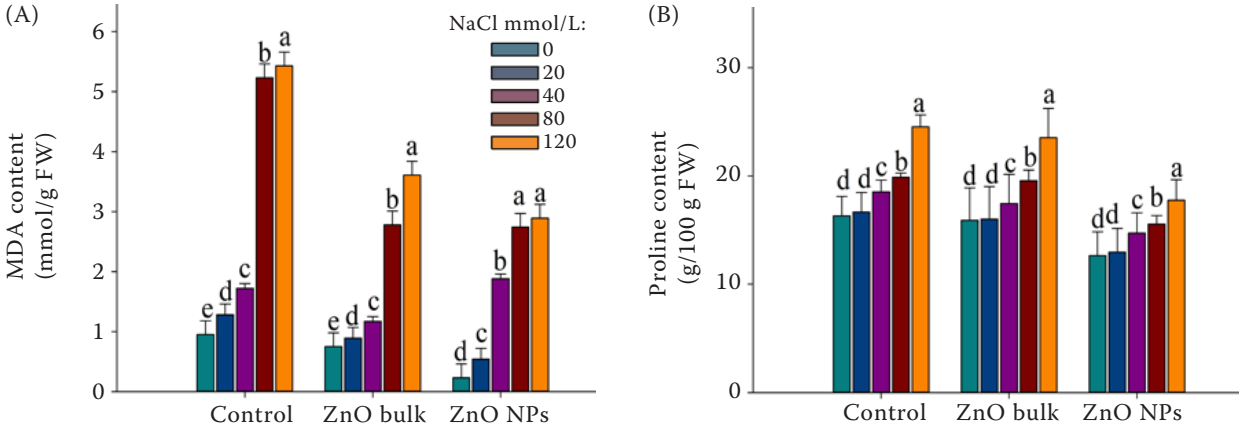

Figure 4. Effect of zinc oxide bulk particles (ZnO bulk) and zinc oxide nanoparticles (ZnO NPs) on the malondialdehyde (MDA) and proline content of chickpea (*Cicer arietinum* L. cv. Avarodhi) seedlings under salt stress (0, 20, 40, 80, and 120 mmol/L). The data presented in the figures are the calculated mean (± standard error) derived from three independent replicates, and bars labelled with distinct letters indicate statistically significant differences between the groups at the $P \leq 0.05$ level. FW – fresh weight

content decreased by 6, 16, 50, 70% and 2, 2, 43, 63%, respectively, compared to control (Figure 3).

However, when ZnO bulk and ZnO NPs were applied to chickpea seedlings, Na and Chl levels dropped while K and Zn levels were improved (Figure 2). The salt treatments (20, 40, 80, and 120 mmol/L) increased the MDA content in chickpea seedlings by 26, 45, 82, and 83%, respectively, compared to the control seedlings, which was statistically significant ($P \leq 0.001$). But when ZnO bulk and ZnO NPs were applied, the MDA content were dropped significantly, compared to the salt-stressed (20, 40, 80, and 120 mmol/L) chickpea plants (Figure 4).

When compared to the level of proline found in salt-stressed chickpea plants that were not exposed to ZnO bulk and ZnO NPs, the salt treatments of 20, 40, 80, and 120 mmol/L significantly ($P \leq 0.001$) enhanced the proline content by 2, 12, 18, and 33%, respectively. In spite of this, the proline concentration in chickpea plants that had been treated with ZnO bulk and ZnO NPs was considerably ($P \leq 0.001$) lower (Figure 2).

SOD, CAT, and APX activities were elevated in salt-stressed (20, 40, 80, and 120 mmol/L) chickpea plants by 14, 33, 61, 64, 10, 29, 52, 53%, and 6, 13, 35, 39%, respectively, compared to those in untreated plants. However, exposure of chickpea plants to ZnO bulk and ZnO NPs increased SOD activity by 18, 14, 10, 5% and 37, 40, 43, 41%, CAT activity by 16, 5, 3, 7% and 33, 25, 29, 29%, APX activity by 15, 16, 18, 14% and 50, 49, 49, 46%, respectively, in comparison to control (Figure 5).

In response to salt stress (20, 40, 80, and 120 mmol/L), the activity of GPX and GR increased by 3, 12, 28, 30% and, 11, 27, 55, and 59%, respectively, compared to that of control plants. In addition, the exogenous application of ZnO bulk and ZnO NPs dramatically boosted GPX and GR activities ($P \leq 0.001$) (Figure 6).

DISCUSSION

Salt stress ranks among the foremost constraints on crop productivity in irrigated agricultural systems, where excessive salinity in soil and water hampers plant growth and development, ultimately presenting a significant challenge to global food security (Deinlein et al. 2014, Stefanov et al. 2019). Increasing salinity in semi-arid countries, such as Saudi Arabia, amplifies these risks to agricultural sustainability. Several physio-biochemical processes and nutrient deficits could be impacted by salt stress, limiting plant growth. As a result, considerable yield losses may result from these stresses. Nanoparticles have recently been widely used to prevent yield losses (Dilnawaz and Misra 2023). The formulation of nanoparticles like ZnO bulk and ZnO NPs is considered fertiliser and can potentially provide salt stress tolerance. Soil salinity has been shown to inhibit chickpea plant growth in the current studies (Figure 7).

Salt stress significantly reduced the relative growth and net assimilation rates compared to the control plants. This decline in net assimilation is primarily due to salt-induced osmotic and ionic stress, which impairs stomatal conductance, reduces CO_2 availability, and disrupts chloroplast function, ultimately suppressing photosynthetic efficiency. Similar reduc-

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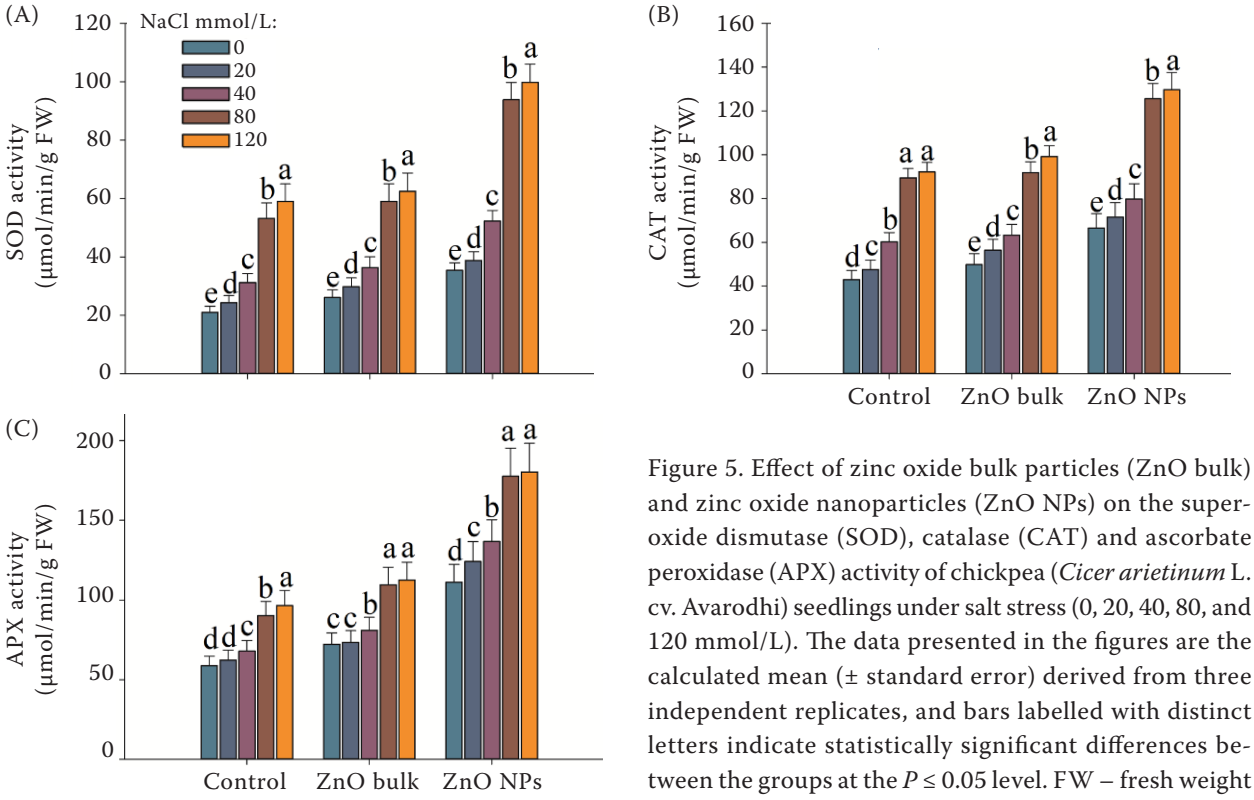

Figure 5. Effect of zinc oxide bulk particles (ZnO bulk) and zinc oxide nanoparticles (ZnO NPs) on the superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activity of chickpea (*Cicer arietinum* L. cv. Avarodhi) seedlings under salt stress (0, 20, 40, 80, and 120 mmol/L). The data presented in the figures are the calculated mean (± standard error) derived from three independent replicates, and bars labelled with distinct letters indicate statistically significant differences between the groups at the $P \leq 0.05$ level. FW – fresh weight

tions in net assimilation rate under NaCl stress have been observed in *Phaseolus vulgaris* (salt sensitive) and *Sesbania aculeata* (salt tolerant) (Ashraf and Bashir 2003), corroborating our findings.

Salt stress reduced chlorophyll concentration, likely due to the destabilisation of membrane protein complexes (Misra et al. 1997, Alharbi et al. 2021). This decline was similarly reported in chickpea seedlings exposed to salinity. Applying ZnO bulk and ZnO NPs to salt-treated soybean plants resulted in a rise in total *Chl* levels. This conclusion is in line with previous findings, which showed that increasing the amount of exogenously provided ZnO NPs in sugarcane during chilling stress improved their levels of chlorophyll content (Elsheery et al. 2020). Na^+ is primarily taken up by root cells through ion diffusion mechanisms, particularly *via* non-selective cation channels driven by electrochemical gradients. Higher external salt

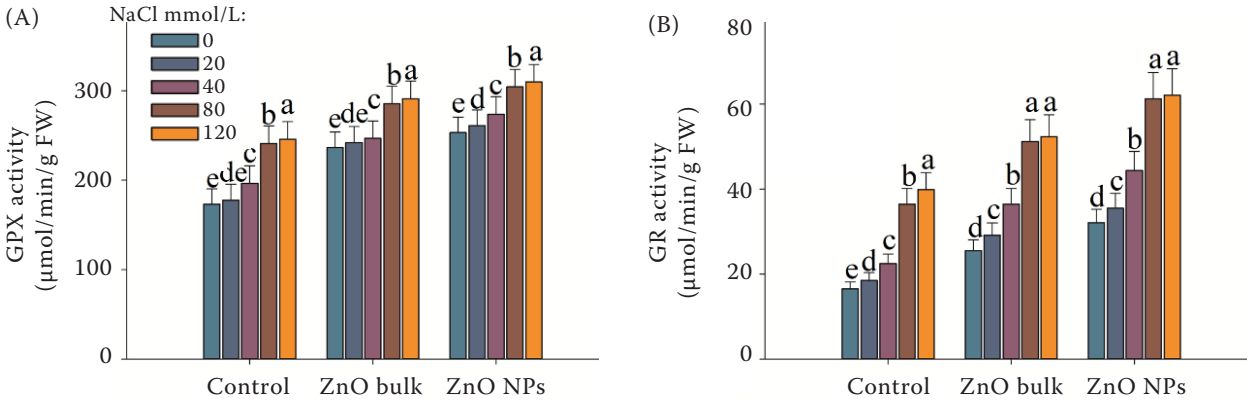

Figure 6. Effect of zinc oxide bulk particles (ZnO bulk) and zinc oxide nanoparticles (ZnO NPs) on the glutathione peroxidase (GPX) and glutathione reductase (GR) activity of chickpea (*Cicer arietinum* L. cv. Avarodhi) seedlings under salt stress (0, 20, 40, 80, and 120 mmol/L). The data presented in the figures are the calculated mean (± standard error) derived from three independent replicates, and bars labelled with distinct letters indicate statistically significant differences between the groups at the $P \leq 0.05$ level. FW – fresh weight

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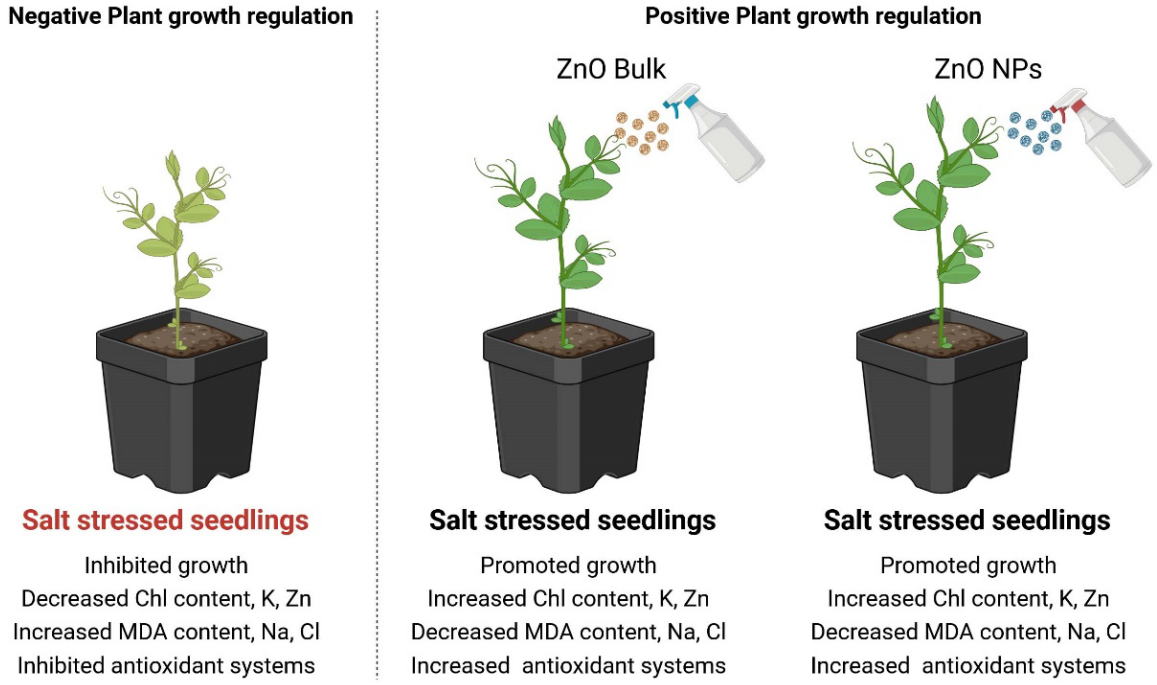

Figure 7. A model figure demonstrates how zinc oxide bulk particles (ZnO bulk) and zinc oxide nanoparticles (ZnO NPs) contribute to improved plant growth and physiological conditions under salt stress. Chl – chlorophyl; MDA – malondialdehyde

availability promotes greater Na^+ and Cl^- accumulation in plant tissues (Britto and Kronzucker 2015, Joshi et al. 2022), explaining this study's elevated shoot ion concentrations (Figure 3). In this study, the chickpea plants that had been treated with salt had more sodium and chlorine (Figure 3). This could be because plants have too much salt in them. Both ZnO bulk and ZnO NPs lower the amount of Na and Cl in the water. This could be because chickpea roots can only absorb Na^+. Our results showed that K and Zn levels were lower in plants that had been grown in salty conditions. Due to declines in active transport, transpiration flux, and membrane permeability, a lack of water in the soil makes it harder for nutrients to move around the roots and be taken up by the plant (Dimkpa et al. 2017). When ZnO NP was added to either the soil or the leaves of rice plants, the amount of nutrients (K and Zn) enhanced (Singh et al. 2022), which is what occurred in this study. So, our results showed that salt stress adversely affects chickpeas' nutritional content. However, outside ZnO bulk and ZnO NPs help reduce these detrimental consequences. Previous research has shown that ZnO NPs can increase the amount of macronutrients and micronutrients in rice (Singh et al. 2024) and maize (Seleiman et al. 2023). An osmotic adjustment system is responsible for maintaining cell homeostasis. This process ultimately results in the production of organic osmolytes, such as proline, which protects cells from the harmful effects of salt stress (Mohamed et al. 2019, Singh et al. 2022). A rise in the amount of the amino acid proline is indicative of an osmotic stress response due to the role it plays in the process of osmotic adjustment in plant cells. Accumulation of proline, which acts as an osmoprotectant, ascorbic acid, and glutathione, which act as antioxidants, defend plant cells from the damaging effects of salt stress by bringing the osmotic pressure of the cytosol and the vacuole into equilibrium with that of the surrounding environment (El Moukhtari 2020). In chickpea plants subjected to salt stress, the notable buildup of proline points to a disruption in water homeostasis induced by high salinity levels. Research on sweet pepper has similarly documented a rise in proline content as a reaction to salt exposure, a finding corroborated by Abdelaal et al. (2020), suggesting that proline serves as a protective osmolyte in response to such stress. However, in chickpea seedlings facing saline conditions, both ZnO bulk and ZnO NPs demonstrated a remarkable ability to inhibit proline synthesis, suggesting that these treatments alleviate the osmotic pressure and associated water deficits that typically increase proline accumulation.

MDA is a marker for oxidative stress that is synthesised as a byproduct of the lipid peroxidation

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reaction (Alharbi et al. 2021). The chickpea seedlings were subjected to salt stress, which resulted in an increased formation of reactive oxygen species (ROS), which in turn caused oxidative damage to lipids and an increase in MDA levels. ZnO bulk and ZnO NPs decline MDA and H_2O_2 content during salt stress in chickpea seedlings, which indicates that ZnO bulk and ZnO NPs. MDA was decreased in stressed *Leucaena leucocephala* owing to ZnO nanoparticles (Zahedi et al. 2020). Results were also revealed in green peas and sugar beet (Sun et al. 2020), which were comparable to these results.

Cellular osmoprotectants (Hasan et al. 2020, 2021a, b) and antioxidant enzyme systems (Hasan et al. 2021c) are responsible for maintaining the appropriate level of balance between the generation of ROS and their elimination. Plants have evolved a comprehensive antioxidant system that consists of enzymatic and non-enzymatic components (Sen 2019). This system was developed in order to avoid oxidative damage from occurring under stress conditions. The enzyme superoxide dismutase catalyses the dismutation of superoxide anions to dioxygen (O_2) and hydrogen peroxide, making it a key element of the antioxidant defence system (Abdulmajeed et al. 2021). CAT is the major antioxidant enzyme that converts H_2O_2 into H_2O and O_2, combined with APX and POX (Garg and Manchanda 2009). Changes in the expression level of these enzymes are one of the ways in which plants react to oxidative stress (Varjovi et al. 2015). Mango seedlings that were exposed to salt stress exhibited higher levels of SOD, CAT, and POX activity than non-stressed plants (Srivastav et al. 2010). In the present study, salt stress decreased the SOD, CAT, APX, GPX and GR activity in chickpea seedlings, which indicated a reduced antioxidant defense system. Overexpression of these enzymes and enhancement of their activity to regulate the translocation of ions in salt-stressed plants has been achieved by the utilisation of several techniques, one of which is the application of nanoparticles (Alabdallah et al. 2021, Alabdallah and Hasan 2021).

NPs enhance plant growth under stress and trigger a suite of defence mechanisms. These mechanisms include the upregulation of antioxidant enzyme systems and alteration in stress-responsive gene expression. These multifaceted mechanisms improve cellular homeostasis and enhance stress tolerance (Dilnawaz and Misra 2023). ZnO bulk and ZnONPs enhanced antioxidant enzyme activities in chickpea seedlings, aligning with previous studies linking Zn availability to redox homeostasis. However, ZnO NPs demonstrated superior performance, likely due to their higher surface area and enhanced bioavailability, facilitating more efficient uptake and systemic distribution of Zn within plant tissues. This led to a more pronounced increase in relative growth rate, net assimilation rate, and total chlorophyll content, reflecting improved photosynthetic efficiency and metabolic function. Mechanistically, applying ZnO NPs more effectively reduced Na^+ and Cl^- accumulation, possibly by stabilising membrane integrity and modulating ion transport systems under salt stress. Furthermore, ZnO NPs significantly lowered MDA and proline levels, suggesting improved oxidative and osmotic stress tolerance. In contrast, ZnO bulk forms, while beneficial, showed comparatively moderate effects likely due to lower solubility and limited mobility in plant tissues. These findings highlight the distinct and enhanced action of ZnO NPs in mitigating salt-induced physiological damage through improved nutrient uptake, ion balance, and antioxidant defence. Thus, ZnO NPs represent a promising nano-enabled strategy to enhance plant resilience and productivity under saline conditions.

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