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EVALUATION OF THE IMPACT OF ZINC AND IRON NANOPARTICLES ON GROWTH OF WHEAT (*TRITICUM AESTIVUM* L.) UNDER SALT STRESS CONDITIONS

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Abstract

Soil salinity adversely impacts plant growth, physiological functions, and yield by disrupting water uptake, nutrient balance, and metabolic activities. ZnO and FeO nanoparticles can improve plant growth under saline environments. Zn contributed to chloroplast membrane stabilization, enhanced chlorophyll biosynthesis, and activation of photosynthetic enzymes, while Fe acted as a cofactor in chlorophyll formation and supported electron transport processes. Both nutrients synergistically improved antioxidant defence by upregulating the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), thereby reducing lipid peroxidation and protecting membranes from oxidative damage. Moreover, Zn and Fe supplementation regulated osmolyte accumulation and ion homeostasis, leading to improved water relations and nutrient uptake under saline conditions. Overall, the combined application of Zn and Fe effectively mitigated salt-induced oxidative stress, sustained metabolic functions, and significantly enhanced wheat growth and biomass production. A hydroponic experimental study was carried out in the glass house of SARC, ISES University of Agriculture, Faisalabad to compare the efficacy of ZnO and FeO NPs in alleviating salinity stress across wheat genotypes. Four wheat varieties (Akbar-2019, FSD-08, Arooj and Lasani-08) were used from which two were salt tolerant and two were salt sensitive. Synthesized the nanoparticles of ZnO and FeO and then characterized them by SEM, XRD and FTIR. Treatments were T_1: Control**,** T_2: 40 mM NaCl + ZnO (50 ppm)**,** T_3: 60 mM NaCl + ZnO (50 ppm)**,** T_4: 80 mM NaCl + ZnO (50 ppm)**,** T_5: 100 mM NaCl + ZnO (50 ppm)**,** T_6: 150 mM NaCl + ZnO (50 ppm)**,** T_7: 40 mM NaCl + FeO (20 ppm)**,** T_8: 60 mM NaCl + FeO (20 ppm)**,** T_9: 80 mM NaCl + FeO (20 ppm)**,** T_{10}: 100 mM NaCl+ FeO (20 ppm)**,** T_{11}: 150 mM NaCl+ FeO (20 ppm)**.** After 40 days of transplanting plants were harvested and data was recorded. According to the results increasing NaCl concentrations (40-150 mM) progressively reduce shoot and root length, shoot and root fresh weight across all varieties. Arooj consistently shows the highest fresh shoot weight under all treatments, including salinity stress, indicating better tolerance. Lasani-2008 shows the greatest reduction under high salinity. Zinc and iron concentration also more in Arooj and Akbar-2019 as compare to Lasani and Fsd-2008.

Key words: Zinc; Iron; Salinity; Biofortication; Nanoparticles; Wheat

Introduction

Wheat (*Triticum aestivum* L.) is a cornerstone of global food security, contributing significantly to caloric intake and serving as a staple crop for millions. However, its productivity is increasingly challenged by abiotic stresses, notably soil salinity, which impairs plant growth, nutrient uptake, and yield. Salinity affects approximately 20% of cultivated land worldwide, with projections indicating further expansion due to climate change and irrigation practices (Anon., 2023). To address these challenges, innovative agricultural technologies, such as nanotechnology, have emerged as promising tools to enhance crop resilience and productivity. Nanoparticles (NPs), particularly zinc oxide (ZnO) and iron oxide (FeO), have garnered attention for their ability to improve nutrient delivery, mitigate stress, and enhance physiological processes in plants (Sun *et al*., 2024).

Zinc and iron are essential micronutrients critical for plant growth, enzymatic functions, and stress tolerance. Zinc plays a pivotal role in protein synthesis, membrane integrity, and antioxidant defense, while iron is vital for chlorophyll synthesis, photosynthesis, and electron transport. However, their availability in saline soils is often limited due to high pH and ionic imbalances. The application of ZnO and FeO NPs offers a novel approach to overcome these limitations by providing a controlled release of nutrients, improving uptake efficiency, and reducing toxicity compared to conventional fertilizers (Dimkpa, 2022).

This study focuses on the application of ZnO and FeO NPs on salt-sensitive and salt-tolerant wheat varieties under controlled temperature and humidity. Salt-sensitive varieties, such as those susceptible to ionic and osmotic stress, often exhibit reduced growth and yield under saline conditions, while salt-tolerant varieties employ mechanisms like ion exclusion and osmolyte accumulation to maintain productivity (Munns & Tester, 2024).

ZnO NPs enhanced photosynthetic efficiency and antioxidant capacity in wheat under drought stress However, the application of NPs in saline environments, particularly for wheat, remains underexplored. Furthermore, the interaction between NP properties (size, concentration, and coating) and plant genotype-specific responses under salinity stress warrants investigation (Faizan, 2023).

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Micronutrient nanoparticles, particularly zinc (Zn) and iron (Fe), play a pivotal role in enhancing salt stress tolerance in wheat through their involvement in osmotic adjustment and reactive oxygen species (ROS) regulation. Under salinity, osmotic imbalance restricts water uptake, but the application of Zn and Fe nanoparticles facilitates the accumulation of compatible solutes such as proline, soluble sugars, and glycine betaine, which collectively lower cellular osmotic potential and help maintain turgor pressure. In addition, Zn nanoparticles regulate ion transporters to sustain a favourable K^+/Na^+ ratio, while Fe nanoparticles contribute to ion equilibrium through their involvement in membrane stabilization and Fe-dependent enzymes (Gupta *et al*., 2024). These effects are further complemented by improved aquaporin-mediated water transport, ensuring efficient osmotic balance. Alongside this, Zn and Fe nanoparticles activate a robust antioxidant defence system that mitigates oxidative stress by enhancing the activity of superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), and ascorbate peroxidase (APX). Zn primarily functions as a cofactor for SOD, accelerating the conversion of superoxide radicals to hydrogen peroxide, while Fe is crucial for CAT and POD activities that detoxify hydrogen peroxide into water and oxygen. Consequently, the combined action of these nanoparticles reduces lipid peroxidation, lowers malondialdehyde (MDA) levels, stabilizes photosynthetic membranes, and limits chloroplast-derived ROS production. Collectively, these mechanisms highlight the dual contribution of micronutrient nanoparticles in maintaining osmotic stability and mitigating oxidative damage, thereby supporting improved growth and physiological performance of wheat under salt stress (Hasan *et al*., 2025).

Nanoparticles (NPs) have emerged as promising tools in agriculture due to their exceptional physicochemical properties including high surface-area-to-volume ratios, tuneable reactivity, and the ability to control release kinetics of encapsulated substances. These qualities make them superior to many traditional bulk fertilizer materials for nutrient delivery, allowing for enhanced uptake efficiency, reduced losses, and more precise targeting of micronutrients essential for plant health and yield. In wheat, where micronutrient deficiencies (e.g., zinc, iron) and abiotic stresses such as salinity are major constraints, using nanoparticles can directly improve nutrient translocation and bioavailability under challenging soil conditions (Singh *et al*., 2025).

Moreover, nanoparticles have been shown to alleviate multiple abiotic stresses. In saline environments, they help maintain ion homeostasis (by reducing Na^+ accumulation and improving K^+/Na^+ balance), enhance antioxidant defence systems (e.g., catalase, peroxidase activities), and promote osmotic adjustment through compounds like proline. These stress-mitigating effects are often accompanied by maintained or improved physiological traits (chlorophyll content, relative water content) and even improved nutritional quality of grains ("Nano fortification"). Thus, integrating nanoparticles into crop management does not merely protect yield under stress but also contributes to producing crops with better nutritional profiles and less environmental impact (Farooq *et al*., 2023).

This study seeks to fill these knowledge gaps by evaluating the agronomic and physiological impacts of ZnO and FeO NPs on wheat, with implications for sustainable agriculture in salt-affected regions.

Objectives of the study were

- Synthesis and characterization of nanoparticles of zinc and iron
- To evaluate the effect of zinc oxide (ZnO) nanoparticles on the growth performance of wheat under salinity stress.
- To assess the impact of iron oxide (FeO) nanoparticles on wheat growth under salinity stress.

Methodology

Method for Preparation of nanoparticles of ZnO: Zinc oxide nanoparticles were prepared by co- precipitation technique (Asenjo *et al*., 2001) in Lab-138 SARC, Faisalabad. The Procedure proposed by Asenjo *et al*., (2001) was followed with different modifications. Briefly explained here, freshly prepared NaOH solution was slowly added to the solution of ZnSO4.7H2O in drop wise manner at 2:1 ratio respectively. Resulting milky white mixture was stirred for 12 hours on magnetic stirrer. Prepared ZnO precipitate was filtered through Whatman No. 42 filter paper and then was thoroughly washed with deionized water. Washing and filtration was done at least thrice to completely wash the precipitates Afterward precipitate was oven dried in forced air oven at 105°C. Dried precipitates were ground in a pestle and mortar and calcinate at 550°C for two hours. Balanced reaction equation is as follows:

$$^{2}\mathrm{NaOH+ZnSO_4.7H_2O} \longrightarrow \mathrm{ZnO(ppt) + Na_2SO_4 + 8\ H_2O}$$

Methods for the preparation of iron NPs: One of the most used techniques is co-precipitation from aqueous solutions. In the co-precipitation method, a basic solution containing ferric and ferrous ions is mixed in a 1:2 ratio at a high temperature. This process makes it simple to manage the form and size of the nanoparticles by first adjusting the various salts, such as sulphates, nitrates, and chlorides. Secondly, using the variation in the ratio of Fe^{2+}/Fe^{3+} and, thirdly, by adjusting the solutions pH level and temperature. A black suspension is eventually acquired. The precipitated particles can be separated from the suspension using an external magnetic field, then they can be washed repeatedly with ethanol and three times with distilled water. A moderate oxidant and an aqueous solution of Fe (II) salt react to produce spherical nanoparticles (NP) with a diameter of 30–100 nm. The concentration of cations, the existence of counterions, and the pH of the solution are the variables that determine the phase and size of the particles. The mean size of the particles is mostly controlled by changes in pH and ionic strength (from 15 nm to 2 nm). NPs typically combine to lower surface energy and because of their huge surface-area-to-volume ratio (Girardet *et al*., 2024).

Characterization: Characterization of nanoparticles (Zinc and Iron) was done by Scanning Electron Microscope (SEM), X-Ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR).

Hydroponic study: A hydroponic experimental study was carried out in the glass house of SARC, ISES University of Agriculture, Faisalabad.

Wheat nursery planting: Sand was first washed in 0.05 N hydrochloric acid (HCl), then the acid was removed by washing with tap water. Sand that has been acid-washed was then be placed to iron trays after being cleaned with distilled water. In these trays, healthy seeds from 20 different wheat varieties were planted.

At two leave stage healthy seedling of wheat varieties were transplanted into thermo pole sheets with foam plugs over half-strength Hoagland's solution (Hoagland & Arnon, 1950) in tubs with pH adjusted daily at 6.5 ± 0.5 using 1 N HCl or NaOH. Each tub had three replications arranged in a completely randomized design. The solution was continuously aerated for 8 hrs with pump.

The treatment plan is T_1: Control**,** T_2: 40 mM NaCl + ZnO (50 ppm)**,** T_3: 60 mM NaCl + ZnO (50 ppm)**,** T_4: 80 mM NaCl + ZnO (50 ppm)**,** T_5: 100 mM NaCl + ZnO (50 ppm)**,** T_6: 150 mM NaCl + ZnO (50 ppm)**,** T_7: 40 mM NaCl + FeO (20 ppm)**,** T_8: 60 mM NaCl + FeO (20 ppm)**,** T_9: 80 mM NaCl + FeO (20 ppm)**,** T_{10}: 100 mM NaCl+ FeO (20 ppm)**,** T_{11}: 150 mM NaCl+ FeO (20 ppm)**.**

Foliar spray of zinc (50ppm) and iron (20 ppm) chemically synthesized nanoparticles 2-3 times was carried out after twenty days of transplanting at vegetative growth stage using a fine mist sprayer.

Plants were harvested 40 days after transplantation. Data on shoot and root lengths and fresh weights was taken using a meter rod and weight balance, respectively. To determine the levels of Na^+, K^+, Zn and Fe. Plant samples were dried at 65°C plus 5°C in a forced air oven before being weighed to determine their dry weight. Na^+ and K^+ were measured by flame photometry. Zn and Fe were measured by Atomic Adsorption Spectrophotometry.

Ionic parameters: The concentration of Na^+, K^+ ions was determined by using flame photometer (PFP7-Jenway, UK). For the determination of zinc and iron a calibrated Atomic Absorption Spectrophotometer (such as PerkinElmer Analyst or similar models) was used. Wavelengths used for measurement: Zinc (Zn): 213.9 nm and Iron (Fe): 248.3 nm. The digested plant samples were aspirated into the flame of the AAS, and absorbance was recorded. The concentrations of Zn and Fe were quantified based on standard calibration curves and expressed as mg/kg (ppm) (Ryan *et al.*, 2001).

Statistical Analysis

Treatments of the trial were applied according to the CRD. Data was analyzed according to the techniques defined by (Steel *et al.*, 1997). Statistics 8.1 software was used for analysis ensuring robust and reliable evaluation of the experimental results. Least significance test was employed for pairwise comparison at significance level $p<0.05$ (Anova Tables 1-2).

Results

Characterization of ZnO nanoparticles

Scanning electron microscope (SEM): Electron microscopes have a characteristics very narrow electron beam that gives micrograph a higher depth of field and enables it to yield a characteristic three-dimensional appearance. This three-dimensional appearance makes it easy to understand the surface morphology and structure of the particles. SEM image of the synthesized ZnO-NPs is depicted in Fig. 1. This micrograph confirms that the ZnO particles are in the nano size range. It can also be seen that the particles are spherical in shape.

X-Ray diffraction (XRD): XRD analysis was done to determine the purity and crystalline size of synthesized ZnO-NPs. X-Ray diffraction pattern of ZnO-NPs is represented in Fig. 2.

All the diffraction peaks show sharp peak intensities that indicate the prepared material has good crystalline nature and consist of particles in nanorange. It also confirmed the purity of synthesized ZnO-NPs as there were no peakes other than ZnO ones. Results are also in accordance with the ones presented by (Sahu *et al.*, 2023).

Fourier transform infrared spectroscopy (FTIR): The synthesized iron and zinc oxide nanoparticles were analysed by FTIR spectroscopy technique in order to find out the functional groups present in the particles.

ZnO nanoparticles were confirmed by the presence of a strong absorption band in the 400-600 cm^{-1} region (Zn–O bond). The broad O–H and H–O–H peak indicates surface-adsorbed water or hydroxyl groups, which was common for nanoparticles exposed to air. Minor peaks elsewhere may be due to synthesis byproducts or environmental contaminants. This FTIR spectrum is typical for ZnO nanoparticles and confirms their successful synthesis and the presence of characteristic functional groups (Fig. 3).

Iron Oxide (FeO)

Scanning electron microscope (SEM): The particles in the image likely clustered together due to magnetic interactions or van der Waals forces, a common behaviour for iron oxide nanoparticles. SEM image of the synthesized FeO-NPs is depicted (Fig. 4) at a magnification of 20 kX. This micrograph confirmed that the FeO particles were in the nano size range. The nanoparticles appear aggregated into larger clumps rather than being uniformly dispersed. This aggregation can result from synthesis conditions, drying processes, or magnetic attraction between particles. Some particles show faceted or angular shapes, while others are more rounded, indicating possible variations in crystal structure.

X-Ray diffraction (XRD)

Iron Oxide (FeO): Similarly, the X-ray diffraction pattern of iron oxide nanoparticles was illustrated in Figure 5. Peaks were found in a diffractogram at different angles of which indicated that the synthesized iron oxide nanoparticles were crystalline phase. All the diffraction peaks showed sharp peak intensities that indicates the prepared material has good crystalline nature and consist of particles in nano range. It also confirmed the purity of synthesized FeO-NPs as there were no traces of peaks recorded other than FeO ones.

Fourier transform infrared spectroscopy (FTIR): The FTIR spectrum of the synthesized iron oxide (FeO) nanoparticles reveals key functional groups that confirm their successful formation and highlight their potential application in wheat biofortification. A broad absorption band was observed around 3200–3600 cm^{-1} corresponds to O–H stretching vibrations, indicating the presence of hydroxyl groups and adsorbed water molecules on the nanoparticle surface.

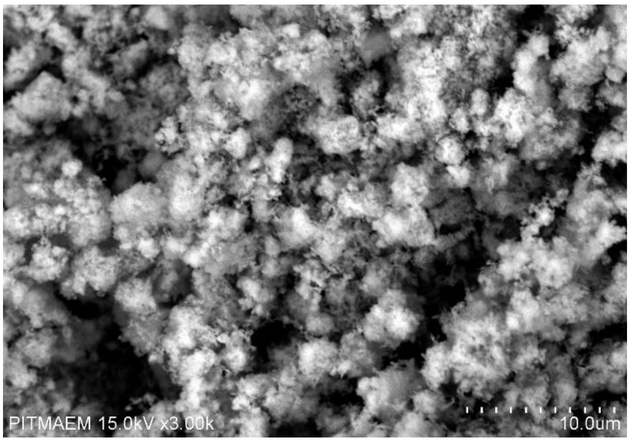


Fig. 1. SEM micrograph of ZnO-NPs.

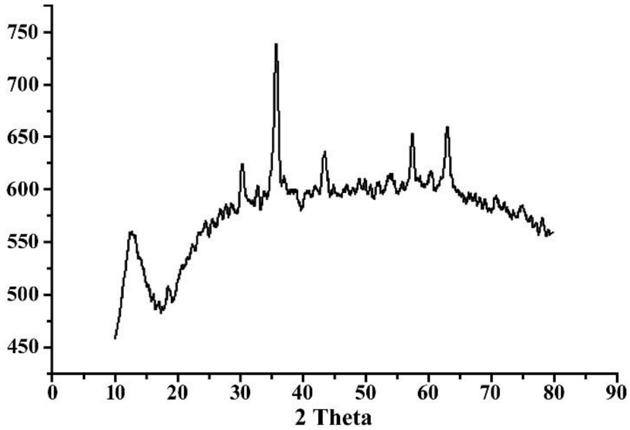


Fig. 2. XRD analysis of ZnO NPs.

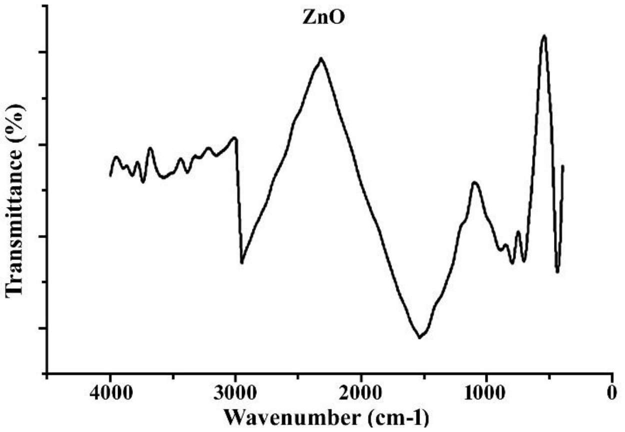


Fig. 3. FTIR of ZnONPs.

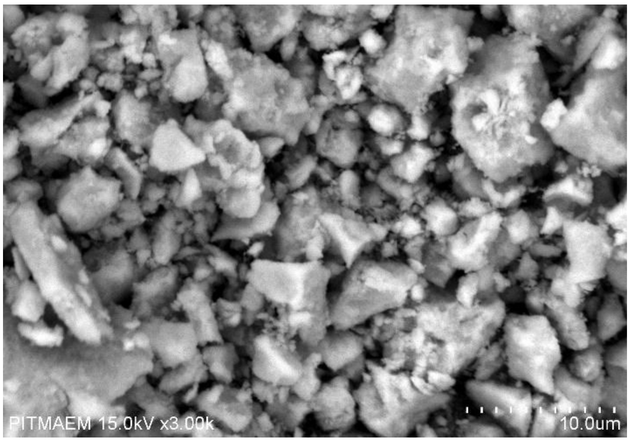


Fig. 4. SEM image of FeO.

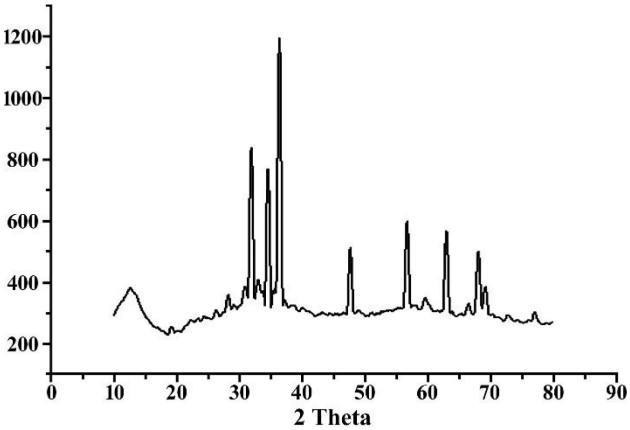


Fig. 5. XRD analysis of FeO NPs.

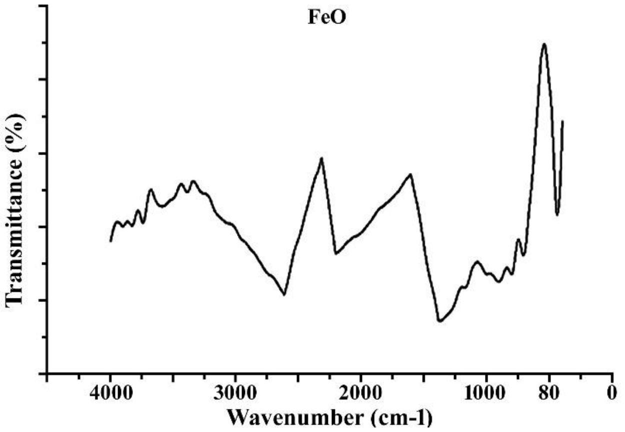


Fig. 6. FTIR of FeONPs.

Growth parameters

Shoot length (cm): Shoot length variations in four wheat varieties under different salinity and nanoparticle treatments are shown in Fig. 7. Increasing NaCl concentrations (40-150 mM) progressively reduce shoot length across all varieties, with Lasani-2008 showing the highest sensitivity (55% at 150 mM NaCl). 50ppm partially counters salt stress at ≤80 mM NaCl (e.g., Akbar-2019 maintains control-equivalent shoot length at 40 mM NaCl + ZnO). Effectiveness declines at ≥100 mM NaCl, consistent with studies showing ZnO NPs lose efficacy under extreme salinity. ZnO consistently outperforms FeO (20 ppm) in protecting shoot growth. For instance at 80 mM NaCl, ZnO-treated Lasani-2008 shows 40.67 cm vs. 38.67 cm with FeO. Even under extreme salinity (150 mM NaCl), ZnO application (50 ppm) partially preserved shoot length in Lasani-2008 (35 cm) compared to FeO (27.67 cm). Arooj exhibited the highest control shoot length (61 cm) but severe sensitivity to salinity (43.66 cm at 100 mM NaCl + 50 ppm ZnO). In contrast, Lasani-2008 showed moderate tolerance, retaining 35 cm shoot length at 150 mM NaCl + ZnO.

Root length: The effects of combined salt (NaCl) stress and nanoparticle treatments (ZnO and FeO) on root length across four wheat varieties are shown (Fig. 8). Increasing NaCl concentrations consistently reduced root length in all varieties, particularly at ≥ 60 mM. In Lasani-2008 root length was dropped from 32.33 cm (control) to 14.66 cm at 150 mM NaCl + ZnO. Similar trends were observed in FSD-2008 (32.33 cm - 18.33 cm). This aligns with findings that salt stress inhibits root elongation by disrupting ion homeostasis and inducing oxidative damage. Root were lengths increased or remained stable compared to control, especially in FSD-2008 (36.33 cm) and Lasani-2008 (40.33 cm), suggesting a positive effect of Zn and Fe on root growth under non-stress conditions. Arooj consistently produced the longest roots under most treatments, indicating better root growth resilience. FSD-2008 and Lasani-2008 showed more pronounced reductions under salt stress, especially with FeO treatment.

The data in Fig. 8 shows that ZnO and FeO nanoparticles can enhance root length in wheat varieties, with ZnO being more effective under salt stress conditions. Increasing NaCl concentrations reduce root length, but ZnO helps mitigate this effect better than FeO, consistent with recent studies on nanoparticle-mediated stress tolerance in plants.

Figure 9 presents the fresh shoot weight of four wheat varieties (Akbar-2019, FSD-2008, Arooj, Lasani-2008) under different treatments involving salinity stress (NaCl at various concentrations) combined with either ZnO (50 ppm) or FeO (20 ppm) applications, along with a control (no treatment). All varieties show their highest fresh shoot weight under control conditions, with Arooj (33.66 g) and Lasani-2008 (31.33 g) having notably higher weight than Akbar-2019 (26.33 g) and FSD-2008 (21.66 g). This indicates the baseline growth potential without stress.

The variation in chlorophyll content (SPAD units) among 4 wheat varieties Akbar-19, FSD-08, Arooj, and Lasani-08 subjected to 12 different treatments (T1–T12) is presented in Fig. 10. Across all treatments, the variety Arooj consistently exhibited the highest chlorophyll content, ranging approximately from 36 to 48 SPAD units, indicating superior photosynthetic pigment retention. Akbar-19 followed, with moderately high chlorophyll levels in most treatments, whereas FSD-08 generally showed the lowest values, particularly under T6–T10, where chlorophyll content was dropped below 30 SPAD units. Lasani-08 maintained intermediate values between Akbar-19 and FSD-08.

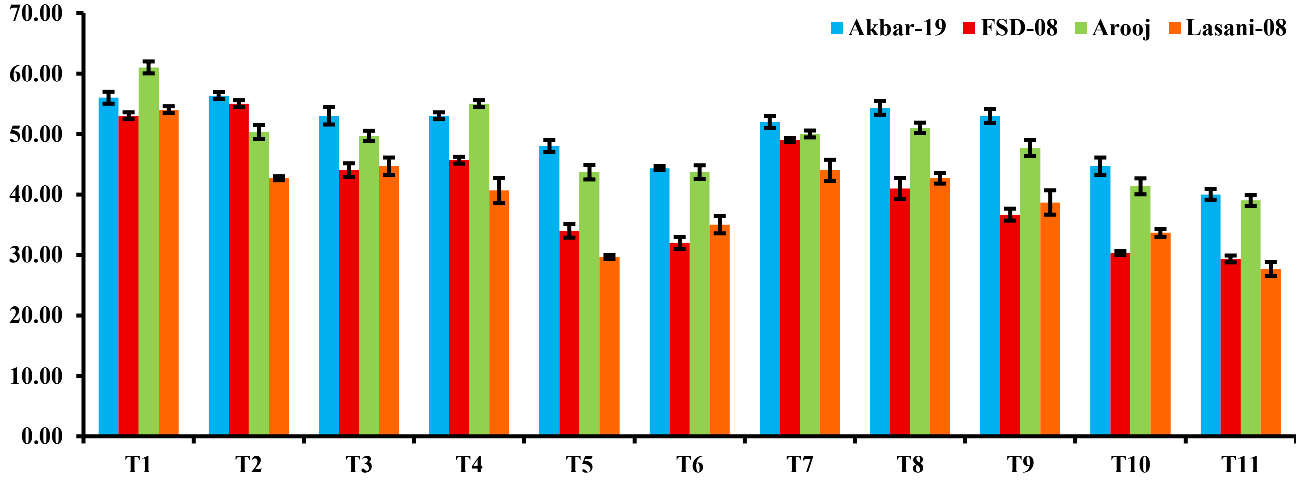
Ionic parameters: Sodium (Na^+) concentration in the shoots of four wheat varieties (Akbar-2019, FSD-2008, Arooj, Lasani-2008) under different treatments involving salinity (NaCl) levels and micronutrient applications (ZnO and FeO) is shown (Table 1). Na^+ content is lowest across all varieties, ranging from about 20 to 39 ppm, indicating baseline sodium accumulation without stress or supplementation. Slight increases or similar Na^+ levels compared to control, suggesting micronutrient application alone does not significantly increase Na^+ accumulation. Na^+ content in shoots increases progressively with higher salinity levels across all varieties and treatments. For example, at 150 mM NaCl + 50 ppm ZnO, Na^+ reaches 46.33 ppm in Akbar-2019 and up to 52 ppm in Lasani-2008. Generally, treatments with 20 ppm FeO result in slightly higher Na^+ accumulation than those with 50 ppm ZnO at the same salinity level. For instance, at 150 mM NaCl, FeO-treated plants have Na^+ contents of 42 to 52.66 ppm, slightly higher than ZnO-treated plants (46.33 to 52 ppm). FSD-2008 and Lasani-2008 tend to accumulate more Na^+ in shoots under salt stress compared to Akbar-2019 and Arooj, indicating varietal variation in sodium uptake or exclusion mechanisms. The table 1 shows that increasing salinity leads to increased Na^+ accumulation in wheat shoots, with variations depending on variety and micronutrient treatment. FeO tends to cause slightly higher Na^+ accumulation than ZnO under salt stress. These results highlight the importance of managing sodium uptake and micronutrient nutrition to improve salt tolerance in wheat.

The control treatment shows baseline K content ranging from about 14.93 to 17.03 mg/g across varieties. NaCl treatments at various concentrations (40 to 150 mM) combined with ZnO or FeO show variable K content, sometimes higher or similar to control, depending on the variety and treatment. For example, Lasani-2008 shows increased K content with 60 mM NaCl + 20 ppm FeO (17.8 ± 0.90) compared to control (15.96 ± 1.58), indicating a possible positive interaction under salt stress with FeO. Similarly, Akbar-2019 maintains relatively high K content under 40 mM NaCl + 50 ppm ZnO (17.76 ± 0.81) compared to control (17.03 ± 0.29). In case of varieties akbar-2019 generally shows higher K content across treatments compared to other varieties. FSD-2008 tends to have lower K content in control but shows improvement under ZnO and FeO treatments. Arooj and Lasani-2008 show variable responses, with Lasani-2008 particularly responsive to FeO under salt stress. The data suggests that potassium content in wheat grains varies with variety and treatment. Micronutrient applications (ZnO, FeO) combined with moderate salt stress (NaCl) can influence K content positively or negatively depending on the variety. Akbar-2019 appears more resilient in maintaining higher K content under these treatments. This aligns with recent research showing varietal differences in K uptake and the complex interplay between K, Na, and micronutrients under salinity stress in wheat (Table 2).

The above given table 03 presents the K/Na ratio in four wheat varieties (Akbar-2019, FSD-2008, Arooj, Lasani-2008) under different treatments involving salt stress (NaCl at various concentrations) combined with foliar applications of zinc oxide (ZnO) and iron oxide (FeO) at specified ppm levels.

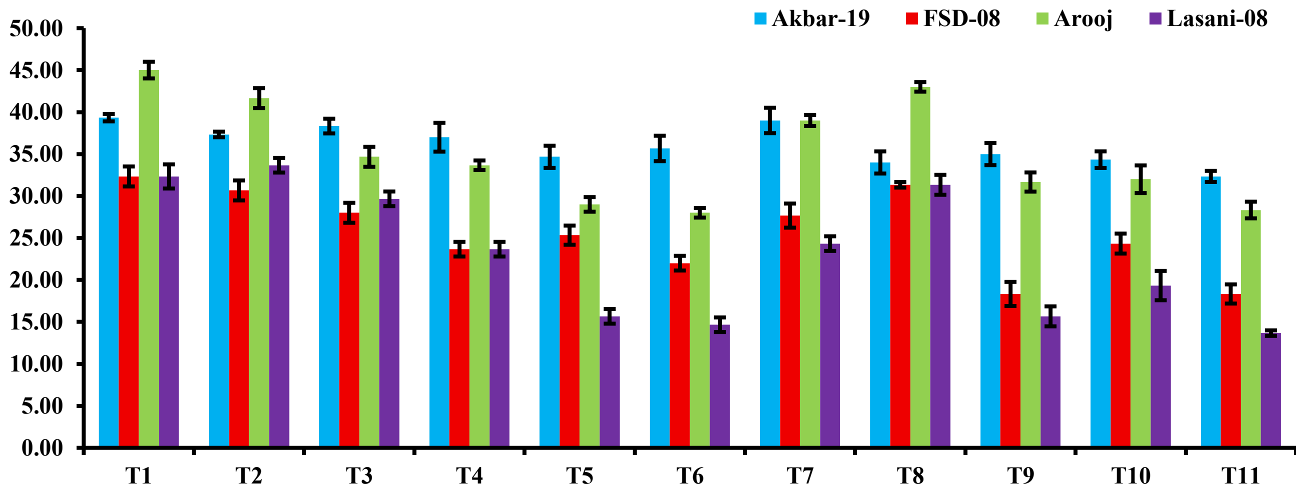
The control treatment (no salt or micronutrient application) shows the baseline K/Na ratios for each variety, with FSD-2008 having the highest ratio (0.76) and Arooj the lowest (0.32). Under salt stress treatments (40 mM to 150 mM NaCl), the K/Na ratio generally increases across all varieties, indicating higher Na^+ accumulation relative to K^+, which is typical under saline conditions due to Na^+ uptake and K^+ displacement. The application of ZnO (50 ppm) and FeO (20 ppm) along with salt stress tends to modulate the K/Na ratio.

For Akbar-2019, the K/Na ratio increases progressively with higher salt concentration, reaching up to 2.85 (150 mM NaCl + 50 ppm ZnO). FSD-2008 maintains relatively high Na/K ratios across treatments, with slight increases under higher salt concentrations. Arooj and Lasani-2008 show moderate increases in Na/K ratio with salt stress but also benefit from micronutrient treatments, which help maintain lower ratios compared to salt stress alone. Generally, FeO treatments at 20 ppm tend to result in slightly higher K/Na ratios compared to ZnO at 50 ppm under the same salt stress levels, suggesting differential effects of these micronutrients on ion homeostasis. The increase in K/Na ratio with salt concentration reflects the typical salt stress response, where Na^+ accumulation disrupts K^+ uptake, but micronutrient applications (Zn and Fe) may help in maintaining better ion balance and improving salt tolerance by enhancing antioxidant defenses and ion homeostasis mechanisms. The table 3 shows that increasing salt concentrations raise the K/Na ratio in all wheat varieties, indicating salt stress impact. However, foliar applications of ZnO and FeO can modulate this effect, helping to maintain better ion balance.



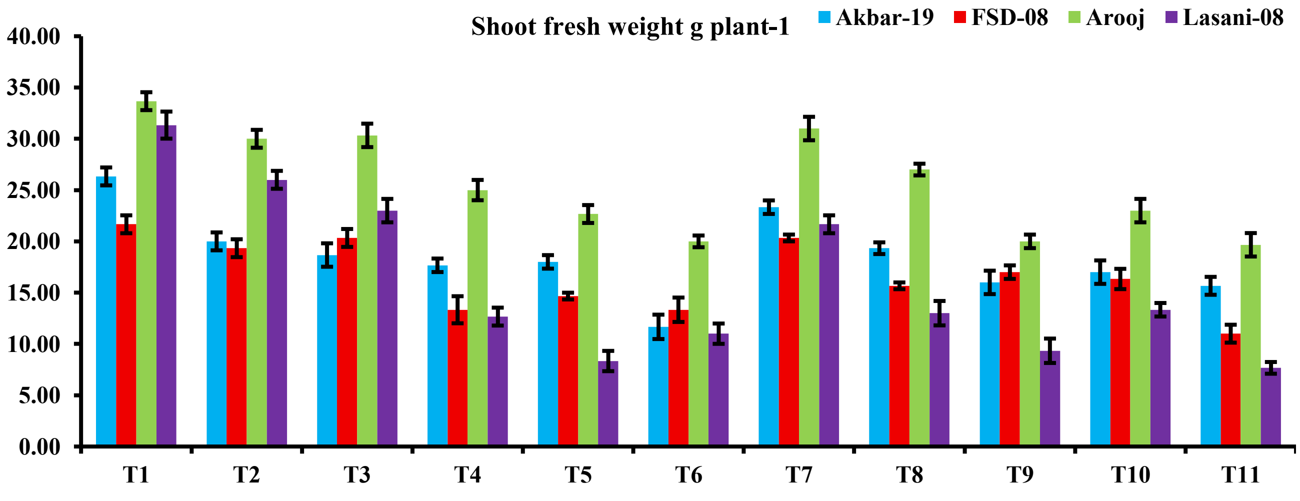
T1 =control, T2 = 40 mM NaCl+50ppm ZnO, T3= 60 mM NaCl +50 ppm ZnO, T4= 80 mM NaCl +50 ppm ZnO, T5= 100 mM NaCl +50 ppm ZnO, T6= 150 mM NaCl +50 ppm ZnO, T7= 40 mM NaCl +20ppm FeO, T8= 60 mM NaCl +20 ppm FeO, T9= 80 mM NaCl +20 ppm FeO, T10= 100 mM NaCl +20 ppm FeO, T11= 150 mM NaCl +20 ppm FeO.

Fig. 7. Shoot length (cm) variations in four wheat varieties under different salinity and nanoparticle treatments.



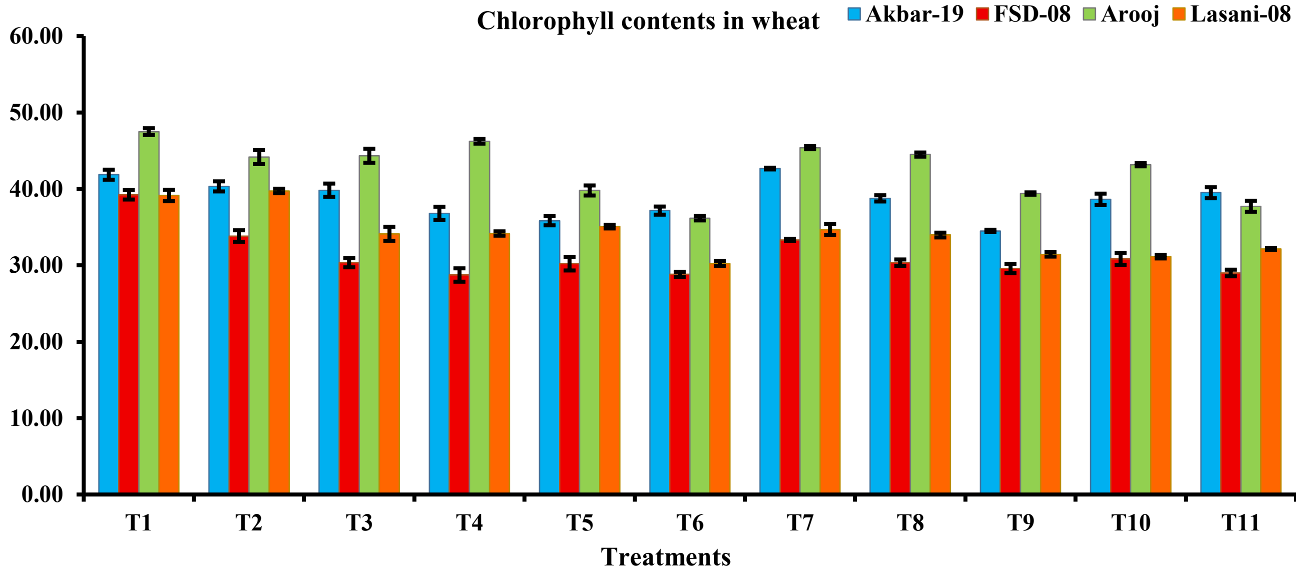
T1 =control, T2 = 40 mM NaCl+50ppm ZnO, T3= 60 mM NaCl +50 ppm ZnO, T4= 80 mM NaCl +50 ppm ZnO, T5= 100 mM NaCl +50 ppm ZnO, T6= 150 mM NaCl +50 ppm ZnO, T7= 40 mM NaCl +20ppm FeO, T8= 60 mM NaCl +20 ppm FeO, T9= 80 mM NaCl +20 ppm FeO, T10= 100 mM NaCl +20 ppm FeO, T11= 150 mM NaCl +20 ppm FeO

Fig. 8. Root length (cm) variations in four wheat varieties under different salinity and nanoparticle treatments.



T1 =control, T2 = 40 mM NaCl+50ppm ZnO, T3= 60 mM NaCl +50 ppm ZnO, T4= 80 mM NaCl +50 ppm ZnO, T5= 100 mM NaCl +50 ppm ZnO, T6= 150 mM NaCl +50 ppm ZnO, T7= 40 mM NaCl +20ppm FeO, T8= 60 mM NaCl +20 ppm FeO, T9= 80 mM NaCl +20 ppm FeO, T10= 100 mM NaCl +20 ppm FeO, T11= 150 mM NaCl +20 ppm FeO

Fig. 9. Fresh Shoot weight (g/pot) variations in four wheat varieties under different salinity and nanoparticle treatments.



T1 =control, T2 = 40 mM NaCl+50ppm ZnO, T3= 60 mM NaCl +50 ppm ZnO, T4= 80 mM NaCl +50 ppm ZnO, T5= 100 mM NaCl +50 ppm ZnO, T6= 150 mM NaCl +50 ppm ZnO, T7= 40 mM NaCl +20ppm FeO, T8= 60 mM NaCl +20 ppm FeO, T9= 80 mM NaCl +20 ppm FeO, T10= 100 mM NaCl +20 ppm FeO, T11= 150 mM NaCl +20 ppm FeO

Fig. 10 Chlorophyll contents (SPAD value) variations in four wheat varieties under different salinity and nanoparticle treatments.

Table 1. Na (ppm) concentration in shoot variations in four wheat varieties under different salinity and nanoparticle treatments.

Treatment	**Na (ppm) concentration in shoot**			
	Varieties			
	Akbar-2019	**FSD-2008**	**Arooj**	**Lasani-2008**
Control	23.33 ± 0.88	38.66 ± 0.88	20.33 ± 0.67	20 ± 1.16
40 mM NaCl + 50ppm ZnO	32 ± 1.16	34.33 ± 1.16	22.66 ± 1.20	30 ± 0.88
60 mM NaCl + 50ppm ZnO	29 ± 0.58	37.33 ± 1.16	22 ± 1.16	36 ± 1.16
80 mM NaCl + 50 ppm ZnO	29 ± 0.58	42 ± 0.88	22.33 ± 1.16	37.66 ± 1.16
100 mM NaCl + 50ppm ZnO	29.66 ± 0.88	46 ± 0.67	24 ± 0.67	42 ± 1.16
150 mM NaCl + 50 ppm ZnO	37.66 ± 0.88	41.66 ± 0.88	32 ± 1.53	42 ± 1.16
40 mM NaCl + 20 ppm FeO	37.6 ± 0.88	47.33 ± 1.16	30.66 ± 1.16	46 ± 1.2
60 mM NaCl + 20 ppm FeO	38.33 ± 0.33	47.66 ± 0.67	41 ± 1.16	46 ± 1.16
80 mM NaCl + 20 ppm FeO	41.66 ± 1.16	50 ± 1.16	42 ± 1.16	49.66 ± 1.2
100 mM NaCl + 20 ppm FeO	46.33 ± 0.33	48.66 ± 0.88	44 ± 1.16	52 ± 1.16
150 mM NaCl + 20 ppm FeO	42 ± 1.00	52 ± 1.77	46 ± 1.16	52.66 ± 0.67

LSD Genotype = 2.5, Treatments = 2.6, genotype*Treatment = 1.5

Note: Each value is presented as a mean ± standard deviation, indicating the average result and the variability among replicates. LSD pairwise comparison for treatments and wheat genotypes at $p<0.05$. * Critical value for comparison in least significance difference test

Table 2. K (ppm) concentration in wheat shoot variations in four wheat varieties under different salinity and nanoparticle treatments.

Treatment	**K (ppm) contents in wheat**			
	Varieties			
	Akbar-2019	**FSD-2008**	**Arooj**	**Lasani-2008**
Control	17.03 ± 0.29	14.93 ± 1.07	15.93 ± 0.26	15.96 ± 1.58
Control	17.76 ± 0.81	15.1 ± 0.99	16.03 ± 0.53	15.4 ± 1.26
40 mM NaCl + 50ppm ZnO	15.16 ± 0.44	15.5 ± 0.35	15.06 ± 1.91	17.19 ± 0.14
60 mM NaCl + 50ppm ZnO	17.53 ± 0.81	15.3 ± 0.95	16.5 ± 0.81	15.33 ± 0.78
80 mM NaCl + 50 ppm ZnO	15.76 ± 1.47	15.56 ± 0.33	14.96 ± 1.49	17.8 ± 0.90
100 mM NaCl + 50ppm ZnO	16.96 ± 0.97	16.16 ± 0.41	16.63 ± 1.57	15.17 ± 0.55
150 mM NaCl + 50 ppm ZnO	15.3 ± 1.48	16.3 ± 0.87	15.43 ± 0.85	17.68 ± 0.81
40 mM NaCl + 20 ppm FeO	16.4 ± 1.46	15.66 ± 0.52	16.4 ± 1.78	15.62 ± 1.59
60 mM NaCl + 20 ppm FeO	16.76 ± 0.13	16.23 ± 1.30	16.8 ± 0.80	16.95 ± 0.97
80 mM NaCl + 20 ppm FeO	16.3 ± 0.76	15.68 ± 0.59	15.9 ± 1.67	15.75 ± 1.34
100 mM NaCl + 20 ppm FeO	16.4 ± 0.50	16.36 ± 1.73	17.23 ± 0.44	15.75 ± 1.34

LSD Genotype = 1.5, Treatments = 0.9, genotype*Treatment = 3.6

Note: Each value is presented as a mean ± standard deviation, indicating the average result and the variability among replicates. LSD pairwise comparison for treatments and wheat genotypes at $p<0.05$. * Critical value for comparison in least significance difference test

Table 3. K/Na ratio variations in four wheat varieties under different salinity and nanoparticle treatments.

Treatment	K/Na ratio			
	Varieties			
	Akbar-2019	FSD-2008	Arooj	Lasani-2008
Control	0.73 ± 0.02	0.56 ± 0.02	0.55 ± 0.01	0.52 ± 0.02
40 mM NaCl + 50ppm ZnO	0.42 ± 0.03	0.40 ± 0.01	0.35 ± 0.02	0.39 ± 0.01
60 mM NaCl + 50ppm ZnO	0.38 ± 0.03	0.43 ± 0.00	0.44 ± 0.03	0.41 ± 0.02
80 mM NaCl + 50 ppm ZnO	0.36 ± 0.02	0.33 ± 0.00	0.38 ± 0.02	0.34 ± 0.02
100 mM NaCl + 50ppm ZnO	0.32 ± 0.03	0.32 ± 0.03	0.32 ± 0.09	0.31 ± 0.04
150 mM NaCl + 50 ppm ZnO	0.78 ± 0.05	0.76 ± 0.08	0.70 ± 0.09	0.68 ± 0.05
40 mM NaCl + 20 ppm FeO	0.74 ± 0.04	0.63 ± 0.05	0.52 ± 0.02	0.50 ± 0.03
60 mM NaCl + 20 ppm FeO	0.40 ± 0.02	0.40 ± 0.13	0.36 ± 0.05	0.37 ± 0.05
80 mM NaCl + 20 ppm FeO	0.81 ± 0.01	0.63 ± 0.02	0.51 ± 0.03	0.47 ± 0.02
100 mM NaCl + 20 ppm FeO	0.40 ± 0.01	0.42 ± 0.04	0.36 ± 0.02	0.38 ± 0.02
150 mM NaCl + 20 ppm FeO	0.34 ± 0.02	0.34 ± 0.02	0.30 ± 0.01	0.29 ± 0.02

LSD Genotype = 1.56, Treatments = 0.90, genotype*Treatment = 3.12

Note: Each value is presented as a mean ± standard deviation, indicating the average result and the variability among replicates. LSD pairwise comparison for treatments and wheat genotypes at $p<0.05$. * Critical value for comparison in least significance difference test

Table 4. Zinc concentration (ppm) in shoot variations in four wheat varieties under different salinity and nanoparticle treatments.

Treatment	Zinc concentration (ppm) in shoot			
	Varieties			
	Akbar-2019	FSD-2008	Arooj	Lasani-2008
Control	20.66 ± 0.17	11.73 ± 0.13	10.5 ± 0.31	9.66 ± 0.13
40 mM NaCl + 50ppm ZnO	23.36 ± 0.19	9.83 ± 0.12	9.83 ± 0.15	9.56 ± 0.15
60 mM NaCl + 50ppm ZnO	23.2 ± 0.15	10.3 ± 0.30	19.96 ± 0.03	9.91 ± 0.11
80 mM NaCl + 50 ppm ZnO	10.63 ± 0.32	9.2 ± 0.15	10.2 ± 0.15	10.58 ± 0.22
100 mM NaCl + 50ppm ZnO	10.96 ± 0.20	9.96 ± 0.03	10.63 ± 0.32	12.53 ± 0.51
150 mM NaCl + 50 ppm ZnO	10.63 ± 0.33	10.33 ± 0.15	10 ± 0.10	12.66 ± 0.10
40 mM NaCl + 20 ppm FeO	11.46 ± 0.26	11.03 ± 0.03	10.83 ± 0.17	13.3 ± 0.10
60 mM NaCl + 20 ppm FeO	9.63 ± 0.32	15.4 ± 0.31	6.1 ± 0.38	10.53 ± 0.11
80 mM NaCl + 20 ppm FeO	9.96 ± 0.03	16.19 ± 0.01	6.63 ± 0.32	10.98 ± 0.03
100 mM NaCl + 20 ppm FeO	11.26 ± 0.32	9.56 ± 0.09	9.53 ± 0.13	10.43 ± 0.17
150 mM NaCl + 20 ppm FeO	11.8 ± 0.40	9.96 ± 0.03	9.91 ± 0.06	11.35 ± 0.05

LSD Genotype = 1.56, Treatments = 0.90, genotype*Treatment = 3.12

Note: Each value is presented as a mean ± standard deviation, indicating the average result and the variability among replicates. LSD pairwise comparison for treatments and wheat genotypes at $p<0.05$. * Critical value for comparison in least significance difference test.

Table 5. Iron concentration (ppm) in shoot variations in four wheat varieties under different salinity and nanoparticle treatments

Treatment	Iron concentration (ppm) in shoot			
	Varieties			
	Akbar-2019	FSD-2008	Arooj	Lasani-2008
Control	30.5 ± 0.29	25.86 ± 0.07	26.2 ± 0.35	26.66 ± 0.33
40 mM NaCl + 50ppm ZnO	18.16 ± 0.33	25.5 ± 0.25	26.5 ± 0.29	26.33 ± 0.33
60 mM NaCl + 50ppm ZnO	19 ± 0.29	24.5 ± 0.29	28.83 ± 0.33	24.66 ± 0.33
80 mM NaCl + 50 ppm ZnO	16.33 ± 0.17	25.86 ± 0.19	30.16 ± 0.44	15.66 ± 0.33
100 mM NaCl + 50ppm ZnO	16.66 ± 0.17	24.23 ± 0.15	27.33 ± 0.44	13.66 ± 0.33
150 mM NaCl + 50 ppm ZnO	16.33 ± 0.33	25.3 ± 0.15	20.83 ± 0.6	15.66 ± 0.33
40 mM NaCl + 20 ppm FeO	35.66 ± 0.44	35.5 ± 0.29	35.03 ± 0.32	23.66 ± 0.33
60 mM NaCl + 20 ppm FeO	36.66 ± 0.44	34 ± 0.29	38.66 ± 0.33	24.66 ± 0.67
80 mM NaCl + 20 ppm FeO	35.76 ± 0.43	34.06 ± 0.07	39 ± 0.58	25.66 ± 0.33
100 mM NaCl + 20 ppm FeO	35.76 ± 0.15	33.16 ± 0.17	38.33 ± 0.33	26 ± 0.58
150 mM NaCl + 20 ppm FeO	35.23 ± 0.12	34.16 ± 0.17	36 ± 0.58	24.33 ± 0.88

LSD Genotype = 1.56, Treatments = 0.90, genotype*Treatment = 3.12

Note: Each value is presented as a mean ± standard deviation, indicating the average result and the variability among replicates. LSD pairwise comparison for treatments and wheat genotypes at $p<0.05$. * Critical value for comparison in least significance difference test

ANOVA Table 1.

Treatment	Shoot length (cm)			
	Akbar-2019	**FSD-2008**	**Arooj**	**Lasani-2008**
Control	56 ± 0.99	53 ± 0.57	61 ± 0.99	54 ± 0.57
40 mM NaCl+ 5 0ppm ZnO	56.33 ± 1.44	55 ± 1.14	50.33 ± 0.87	42.67 ± 1.43
60 mM NaCl + 50 ppm ZnO	53 ± 0.99	44 ± 1.14	49.66 ± 1.18	44.67 ± 0.33
80 mM NaCl + 50 ppm ZnO	53 ± 0.99	45.66 ± 0.32	55 ± 0.57	40.67 ± 1.75
100 mM NaCl + 50 ppm ZnO	48 ± 1.14	34 ± 0.98	43.66 ± 1.31	29.67 ± 2.01
150 mM NaCl + 50 ppm ZnO	44.33 ± 0.87	32 ± 0.57	43.66 ± 0.87	35 ± 1.14
40 mM NaCl + 20ppm FeO	52 ± 0.57	49 ± 0.57	50 ± 0.57	44 ± 2.06
60 mM NaCl + 20 ppm FeO	54.33 ± 0.33	41 ± 0.98	51 ± 1.14	42.67 ± 1.44
80 mM NaCl + 20 ppm FeO	53 ± 1.14	36.66 ± 1.75	47.66 ± 0.87	38.67 ± 0.87
100 mM NaCl + 20 ppm FeO	44.66 ± 1.44	30.33 ± 0.32	41.33 ± 1.31	33.67 ± 0.65
150 mM NaCl + 20 ppm FeO	40 ± 1.14	29.33 ± 1.74	39 ± 0.98	27.67 ± 1.43

LSD Genotype = 1.56, Treatments = 0.90, Genotype*Treatment = 3.12

Note: Each value is presented as a mean ± standard deviation, indicating the average result and the variability among replicates

LSD pairwise comparison for treatments and wheat genotypes at $p<0.05$. * Critical value for comparison in least significance difference test

Treatment	Fresh shoot weight (g/pot)			
	Varieties			
	Akbar-2019	**FSD-2008**	**Arooj**	**Lasani-2008**
Control	26.33 ± 0.87	21.66 ± 0.87	33.66 ± 0.87	31.33 ± 1.32
40 mM NaCl + 50ppm ZnO	20 ± 1.14	19.33 ± 0.87	30 ± 1.14	26 ± 1.14
60 mM NaCl + 50 ppm ZnO	23.33 ± 0.66	20.33 ± 1.32	31 ± 0.99	21.66 ± 0.87
80 mM NaCl + 50 ppm ZnO	18.66 ± 0.66	20.33 ± 0.33	30.33 ± 0.87	23 ± 0.99
100 mM NaCl + 50 ppm ZnO	19.33 ± 1.19	15.66 ± 1.19	27 ± 0.57	13 ± 0.99
150 mM NaCl + 50 ppm ZnO	17.66 ± 0.66	13.33 ± 0.33	25 ± 1.14	12.66 ± 0.87
40 mM NaCl + 20ppm FeO	17 ± 0.57	16.33 ± 0.33	23 ± 0.57	13.33 ± 1.19
60 mM NaCl + 20 ppm FeO	18 ± 1.14	14.6 6 ± 0.66	22.66 ± 0.66	8.33 ± 1.19
80 mM NaCl + 20 ppm FeO	16 ± 1.14	17 ± 0.99	20 ± 1.14	9.33 ± 0.66
100 mM NaCl + 20 ppm FeO	11.66 ± 0.87	13.33 ± 0.87	20 ± 1.14	11 ± 0.57
150 mM NaCl + 20 ppm FeO	15.66 ± 0.87	11 ± 0.57	19.66 ± 1.19	7.66 ± 1.19

LSD Genotype = 2.5 Treatments =1.6 , Genotype *Treatment = 2.06

Note: Each value is presented as a mean ± standard deviation, indicating the average result and the variability among replicates

LSD pairwise comparison for treatments and wheat genotypes at $p<0.05$. * Critical value for comparison in least significance difference test

Treatment	Root length (cm)			
	Varieties			
	Akbar-2019	**FSD-2008**	**Arooj**	**Lasani-2008**
Control	39.33 ± 0.44	32.33 ± 1.19	45 ± 0.99	32.33 ± 1.44
40 mM NaCl + 50ppm ZnO	37.33 ± 0.87	30.66 ± 1.19	41.66 ± 1.19	33.66 ± 0.87
60 mM NaCl + 50 ppm ZnO	38.33 ± 1.32	28 ± 1.14	34.67 ± 0.87	29.66 ± 0.87
80 mM NaCl + 50 ppm ZnO	37 ± 1.51	23.66 ± 1.44	33.66 ± 0.66	23.66 ± 0.87
100 mM NaCl + 50 ppm ZnO	34.66 ± 1.32	25.33 ± 1.44	29 ± 1.14	15.66 ± 1.19
150 mM NaCl + 50 ppm ZnO	35.66 ± 0.66	22 ± 1.14	28 ± 0.99	14.66 ± 0.33
40 mM NaCl + 20ppm FeO	34 ± 1.71	31.33 ± 0.87	43 ± 0.57	31.33 ± 0.87
60 mM NaCl + 20 ppm FeO	39 ± 1.51	27.66 ± 0.87	39 ± 0.57	24.33 ± 0.87
80 mM NaCl + 20 ppm FeO	34.33 ± 1.32	24.33 ± 0.33	32 ± 0.57	19.33 ± 1.19
100 mM NaCl + 20 ppm FeO	35 ± 0.99	18.33 ± 1.19	31.66 ± 1.65	15.66 ± 1.75
150 mM NaCl + 20 ppm FeO	32.33 ± 1.19	18.33 ± 1.19	28.33 ± 1.19	13.66 ± 0.87

LSD Genotype= 1.55, Treatments= 0.89, genotype*Treatment= 3.10

Note: Each value is presented as a mean ± standard deviation, indicating the average result and the variability among replicates

LSD pairwise comparison for treatments and wheat genotypes at $p<0.05$. * Critical value for comparison in least significance difference test

The Zinc content in shoots varies by variety, highest in Akbar-2019 (20.66 mg/kg) and lowest in Lasani-2008 (9.66 mg/kg). Generally, increases Zn content compared to control, notably in Arooj (20.96 mg/kg vs. 10.5 controls) and Akbar-2019 (22.33 vs. 20.66). At moderate salt stress (40 mM NaCl), Zn content remains high or increases in some varieties with ZnO or FeO, e.g., Akbar-2019 maintains high Zn (~23 ppm). At higher salt stress (60-150 mM NaCl), Zn content in shoots generally decreases across varieties and treatments, indicating salt stress reduces Zn accumulation. FeO treatments at higher NaCl levels tend to maintain slightly higher Zn content compared to ZnO treatments in some cases, e.g., Lasani-2008 shows higher Zn with FeO at 80 mM NaCl (13.3 mg/kg) than with ZnO (12.66 ppm). Akbar-2019 consistently shows higher shoot Zn content across treatments, while Arooj and Lasani-2008 have lower Zn under salt stress (Rasheed *et al.*, 2024).

ANOVA Table 2.

Analysis of variance table for shoot length

Source	DF	SS	MS	F	P
V001	2	12.4	6.205		
V002	10	4246.5	424.652	122.99	0.0000
V003	3	1831.0	610.343	176.78	0.0000
V002*V003	30	3839.3	127.977	37.07	0.0000
Error	86	296.9	3.453		
Total	131	10226.2			

Grand mean 46.727; CV 3.98

Analysis of variance table for root length

Source	DF	SS	MS	F	P
V001	2	0.69	0.347		
V002	10	1804.56	180.456	48.33	0.0000
V003	3	1713.36	571.119	152.94	0.0000
V002*V003	30	2548.23	84.941	22.75	0.0000
Error	86	321.14	3.734		
Total	131	6387.98			

Grand mean 32.295; CV 5.98

Analysis of variance table for shoot fresh weight

Source	DF	SS	MS	F	P
V001	2	0.42	0.212		
V002	10	1814.73	181.473	67.98	0.0000
V003	3	1113.36	371.119	139.02	0.0000
V002*V003	30	2626.73	87.558	32.80	0.0000
Error	86	229.58	2.669		
Total	131	5784.81			

Grand mean 20.871; CV 7.83

Analysis of variance table for chlorophyll contents

Source	DF	SS	MS	F	P
V001	2	34.14	17.0715		
V002	10	367.48	36.7481	3.01	0.0026
V003	3	133.83	44.6099	3.65	0.0156
V002*V003	30	1274.38	42.4792	3.48	0.0000
Error	86	1050.19	12.2115		
Total	131	2860.02			

Grand mean 38.776; CV 9.01

Discussion

ZnO nanoparticles enhance shoot growth under salt stress, with efficacy depending on wheat variety and salinity intensity. These results corroborate recent evidence on ZnO's role in improving stress resilience and nutrient fortification (Adil *et al*., 2022).

Recent studies emphasize the importance of root system architecture (RSA) in wheat's response to abiotic stresses like salinity and drought. Root length is a critical trait for water and nutrient uptake, influencing overall plant performance under stress. A study on wheat root traits under water stress showed that root length and root system depth were significantly decreased under stress, with genotypic variation affecting the extent of reduction (Cope *et al*., 2024). This aligns with the observed reduction in root length with increasing NaCl concentrations in reflecting salt-induced stress (Fig. 8). Micronutrient supplementation, particularly Zn and Fe, can improve root growth and stress tolerance by enhancing nutrient uptake and physiological functions. Varietal differences in root length under stress conditions are consistent with findings that wheat genotypes vary in RSA traits, influencing their adaptability to stress environments. Arooj's relatively longer roots under salt stress suggest it may possess better genetic traits for root growth maintenance under salinity. Salt stress typically reduces root growth by causing osmotic and ionic toxicity, limiting cell elongation and division. ZnO's relatively better performance than FeO under salt stress may be due to zinc's role in stabilizing membranes and enzymes involved in root growth and stress response (Guo *et al*., 2025).

The observed decline in fresh shoot weight with increasing salinity aligns with previous studies showing that salinity reduces wheat shoot biomass significantly (Khan *et al*., 2024). The mitigating effect of micronutrient foliar sprays (ZnO and FeO) on salinity stress is supported by research indicating that Zn and Fe can enhance plant growth and stress tolerance by improving physiological and biochemical processes (Khan *et al*., 2021). Recent studies also highlight that integrated nutrient management (e.g., biochar, press mud, micronutrients) can enhance wheat fresh weight and overall growth under stress conditions, corroborating the positive effects seen with ZnO and FeO foliar applications here (Manzoor *et al*., 2021). The treatment with ZnO 50 ppm + FeO 20 ppm generally increased fresh root weight across all varieties compared to control. For example in Akbar-2019 it was increased from 10 to 13.5 g, and Lasani-2008 from 8.33 to 10 g. This aligns with studies showing that ZnO and FeO nanoparticles enhance plant growth traits and root biomass by improving nutrient uptake and photosynthesis efficiency (Mahmoud *et al*., 2019).

Chlorophyll content, a vital physiological trait is directly linked to photosynthetic efficiency and plant productivity. The observed varietal differences in chlorophyll content highlight the role of genetic makeup in maintaining photosynthetic pigments under varying environmental conditions. Arooj, which maintained the highest chlorophyll levels across treatments, appears to possess greater inherent tolerance to salinity stress, likely due to more efficient osmotic adjustment, better ion homeostasis, or protective antioxidant mechanisms.

The high chlorophyll contents (Fig. 10) observed in T1 and T2 across all varieties suggest that these treatments either involved lower salinity stress or optimal nanoparticle application rates that enhanced chlorophyll biosynthesis and reduced chlorophyll degradation. Nanoparticles such as ZnO and FeO are known to play a role in chlorophyll metabolism. zinc being essential for chlorophyll synthesis through its role in carbonic anhydrase and tryptophan synthesis, and iron being a cofactor for key enzymes in chlorophyll biosynthesis. Thus, their optimal supply could have supported pigment maintenance even under salinity conditions (Gupta *et al*., 2024).

The increase in shoot Na^+ content with rising NaCl concentration reflects greater uptake and translocation of sodium under salt stress, which can be toxic and affect plant growth. Micronutrient treatments (ZnO and FeO) influence Na^+ accumulation differently, possibly by affecting plant ion transport or stress tolerance mechanisms. Varietal differences suggest genetic variation in Na^+ exclusion or compartmentalization, which is important for breeding salt-tolerant wheat. Higher Na^+ in shoots correlates with salt stress severity, consistent with known plant responses where Na^+ accumulation disrupts cellular functions and reduces growth. Akbar-2019's improved tolerance to abiotic stresses including salinity is supported by its better shoot and root growth parameters under saline conditions compared to FSD-2008 (Tariq *et al*., 2024). Differences in nutrient content and stress responses among these varieties are linked to their genetic traits and physiological adaptations, as seen in grain quality and yield studies (Dawood *et al*., 2021). Micronutrient applications like Zn and Fe have been shown to mitigate salt stress effects by improving antioxidant activities and nutrient balance in wheat (Ahmad *et al*., 2024). Na accumulation in roots increases with salinity and varies by wheat variety and micronutrient treatment. Akbar-2019 shows better control of Na uptake under salt stress, supporting its suitability for saline environments, while Lasani-2008 accumulates more Na, indicating lower tolerance. ZnO and FeO treatments modulate Na content differently, with ZnO possibly aiding in Na exclusion. These findings align with recent research on wheat varietal responses to salinity and micronutrient-mediated stress mitigation. Potassium is critical for wheat growth and yield, and its uptake can be influenced by external factors such as salinity and micronutrient supplementation (Zn, Fe) (Ranjeet *et al*., 2025). Studies indicate that moderate NaCl levels can sometimes stimulate K uptake in wheat, especially when combined with micronutrients, due to complex ion interactions and improved nutrient use efficiency. Wheat varieties differ in their potassium use efficiency and response to salinity and nutrient treatments, which aligns with the observed varietal differences in table (Wang *et al*., 2018).

K/Na ratio is an important physiological indicator reflecting the balance between sodium (Na^+) and potassium (K^+) ions in plants. Maintaining a low K/Na ratio is crucial for salt tolerance because high Na^+ disrupts cellular functions, while K^+ is essential for enzyme activation, osmotic regulation, and overall plant growth under salt stress (Fathalli *et al*., 2025).

Salt stress causes increased Na^+ uptake and decreased K^+ content, raising the K/Na ratio, which negatively affects plant growth and physiological functions. Exogenous application of micronutrients like Zn and Fe can alleviate salt stress effects by improving antioxidant enzyme activities and maintaining better K^+ retention, thus lowering the K/Na ratio or mitigating its increase. Maintaining a higher K^+/Na^+ ratio is associated with improved salt tolerance in plants, as K^+ is critical for enzymatic activities and osmotic balance under stress conditions. The differential response among varieties indicates genetic variability in salt tolerance and nutrient use efficiency, which is important for breeding salt-tolerant wheat cultivars (Peng *et al*., 2025).

Zinc application (ZnO) enhances Zn accumulation in shoots, consistent with findings that Zn fertilization improves Zn concentration in plant tissues, boosting nutritional quality. Salt stress (NaCl) negatively affects Zn uptake and accumulation, likely due to ionic imbalance and reduced nutrient absorption under salinity. This is reflected in the reduced shoot Zn content at higher NaCl concentrations (Smaoui *et al*., 2024). FeO application alongside ZnO can modulate Zn uptake under salt stress, possibly by mitigating oxidative stress or improving nutrient balance, as Fe is known to interact with Zn uptake and plant stress responses. arietal differences in Zn accumulation align with genotypic variability reported in literature, where some genotypes are more efficient in Zn uptake and translocation (Rasheed *et al*., 2024). The decrease in Zn content with increasing salt stress also aligns with research showing that salinity reduces micronutrient availability and uptake in plants (Smaoui *et al*., 2024).

Akbar-2019 zinc-biofortified wheat variety released in 2019, known for higher zinc content and better agronomic traits compared to traditional varieties. FSD-2008 a commonly grown variety in Pakistan used as a comparison standard. Arooj and Lasani other wheat varieties with varying zinc uptake abilities. Studies show that wheat varieties with vigorous root systems (like Akbar-2019) have higher Zn uptake and translocation to grains. Root system architecture (RSA) is a critical factor influencing Zn acquisition, especially under stress conditions. Varieties with weaker RSA (FSD-2008) tend to have lower Zn content under stress (Noor *et al*., 2024).

Conclusion

Salinity stress severely hampers wheat production by disrupting water uptake, ion homeostasis, and nutrient assimilation, ultimately reducing growth and yield. The present findings demonstrate that the application of zinc and iron nanoparticles can effectively alleviate these adverse effects by enhancing physiological performance and promoting plant vigor, particularly in salt-tolerant wheat varieties. From a practical perspective, integrating Zn and Fe nanoparticles into wheat cultivation under saline conditions offers a viable strategy to maintain productivity on salt-affected lands, which are expanding globally due to climate change and poor irrigation practices. This approach not only improves crop resilience but also provides farmers with a cost-effective and sustainable management option to stabilize wheat yields in marginal environments. By adopting such nanotechnology-based interventions, wheat production in saline soils can be significantly strengthened, ensuring food security in salt-prone regions. The present study demonstrates that the application of Zn and Fe nanoparticles effectively mitigated the adverse effects of salt stress in wheat by improving physiological and biochemical responses. Mechanistically, nanoparticles contributed to the maintenance of ion homeostasis by restricting toxic Na^+ accumulation while enhancing K^+ retention, thereby sustaining cellular metabolic activities under salinity. Furthermore, enhanced activities of antioxidant enzymes such as SOD, POD, and CAT suggest that nanoparticles significantly strengthened the plant's antioxidative defense system, reducing oxidative damage caused by salt-induced ROS. In addition, the nanoparticles promoted the accumulation of compatible osmolytes (such as proline), which stabilized proteins and membranes and contributed to osmotic adjustment under stress.

Future directions: For future directions, large-scale field trials are essential to validate the effectiveness of these nanoparticles under diverse agro-climatic conditions and to assess their long-term impact on soil health and crop performance. Moreover, combining nanoparticle application with other soil amendments such as organic matter, biochar, or gypsum could further enhance salt tolerance by improving soil structure, nutrient availability, and microbial activity. Such integrated approaches can provide farmers with sustainable, cost-effective, and scalable solutions to stabilize wheat yields in saline environments and contribute to global food security.

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