

**IMPACT OF ZINC OXIDE (ZnO) NANOPARTICLES ON THE
GROWTH, PHYSIOLOGY, YIELD AND BIOCHEMICAL TRAITS
OF WHEAT (*Triticum aestivum* L.) UNDER SALT STRESS**

A THESIS

BY

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Student No. 2205063

Session: 2022-2023

Thesis Semester: July-December, 2023

MASTER OF SCIENCE (M.S.)

IN

AGRONOMY



DEPARTMENT OF AGRONOMY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY

UNIVERSITY, DINAJPUR-5200, BANGLADESH

DECEMBER, 2023

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DECEMBER, 2023

Dedicated

*To
Almighty Allah to Bless Me
&
My loving Parents and Teachers
Who Laid the Foundation of My
Success*

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The Author

ABSTRACT

Worldwide, the productivity of various cereal crops is adversely affected by potential threat, with salinity stress being a significant factor. To alleviate the negative effects of salt stress on plants, a promising strategy is the application of ZnO nanoparticles through spraying. Although their effectiveness has been noted, the specific mechanism by which nano-ZnO particles operate to counter salt stress is not yet fully understood. Concerning this issue, a pot experiment was carried out at the Net house, Department of Agronomy, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during November 2022 to March 2023. The experiment encompassed three tiers of salt stress (0, 100, and 150 mM NaCl stress) and three levels of ZnO (0, 150, and 300 μ M), resulting in nine distinct treatments, namely: i) S_0ZnO_0 (0 mM NaCl + 0 μ M ZnO), ii) S_0ZnO_1 (0 mM NaCl + 150 μ M ZnO), iii) S_0ZnO_2 (0 mM NaCl + 300 μ M ZnO), iv) S_1ZnO_0 (100 mM NaCl + 0 μ M ZnO), v) S_1ZnO_1 (100 mM NaCl + 150 μ M ZnO), vi) S_1ZnO_2 (100 mM NaCl + 300 μ M ZnO), vii) S_2ZnO_0 (150 mM NaCl + 0 μ M ZnO), viii) S_2ZnO_1 (150 mM NaCl + 150 μ M ZnO), ix) S_2ZnO_2 (150 mM NaCl + 300 μ M ZnO). The main findings showed that the studied parameters such plant morphology (plant height, number of leaves, leaf area), growth (fresh & dry weight of leaves, stems and roots), phenology (days to booting, heading, anthesis and physiological maturity), physiology (photosynthetic pigments; water status-RWC, WSD, WRC, WUC; MSI), yield contributing traits (spike length, number of spikelets spike⁻¹, grains spike⁻¹, and hundred grains weight), yield indices (grain, straw and biological yield, and harvest index) and biochemical traits were negatively and progressively affected with increasing salt stress. Following the application of nano-ZnO particles, enhancements were observed in the aforementioned plant traits. Notably, ZnO_2 (300 μ M ZnO) demonstrated the most favorable results, although its performance was nearly statistically indistinguishable from ZnO_1 (150 μ M ZnO). Furthermore, the results showed that although SS raised the Na content while lowering the K, Ca, N, and protein content, ZnO administration increased the K, Ca, N, and protein values while falling the Na values. The application of nano-Zn particles has demonstrated the potential to enhance plant development by alleviating salt stress, with 150 μ M ZnO identified as an effective dosage.

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CHAPTER 1

INTRODUCTION

Wheat (*Triticum aestivum* L.), holds a pivotal role as the primary cereal crop, surpassing both rice and maize, contributing to over 50% of the global calorie consumption (Gibbon, 2012; Luo *et al.*, 2019). Human consumption accounts for 85% of total rice, 72% of total wheat, and 19% of total maize production. Globally, wheat takes the lead in terms of planted area, maize leads in production volume, and rice holds the top position in terms of production value (Benavente and Giménez, 2021). The demand for major cereal crops will increase three-fold by 2050 to feed nine billion people. The global challenge of food security is exacerbated by the rapid growth of the human population, constraints on available cropland, and the looming threats posed by climate change (Islam *et al.*, 2022). However, the relentless expansion of agriculture, coupled with the adverse impacts of climate change, has brought forth a plethora of challenges to wheat production. One such challenge that has gained prominence is soil salinity, a phenomenon where excessive salt concentrations impede the normal growth and development of crops (Islam *et al.*, 2011; EL Sabagh *et al.*, 2019, 2020, 2021a; Ahmad *et al.*, 2021; Sagar *et al.*, 2023). Salt stress (SS) has emerged as a critical environmental factor influencing wheat cultivation, threatening both yield quantity and quality (EL Sabagh *et al.*, 2021b, Athar *et al.*, 2022; Lalarukh *et al.*, 2022).

Salt stress is a major concern for cereal crop production globally as it affects different plant growth stages and leads to abnormalities at the phenotypic, morphological, biochemical, and physiological levels (James *et al.*, 2011; Dawood *et al.*, 2019). Soil salinity affects plant growth and development, decreasing agricultural production worldwide (Zhu, 2001; Wang *et al.*, 2019; Zhang *et al.*, 2021; Lalarukh *et al.*, 2022). More than 800 million hectares of land are affected by salinity, accounting for 6% of the earth's total land area and 20% of the total cultivated land area (Munns and Tester, 2008; Zhang *et al.*, 2021). The saline area is expected to increase due to the application of high salt irrigation water owing to insufficient rainfall and poor agricultural practices (Luo *et al.*, 2017; Zhang *et al.*, 2021), particularly in arid and semi-arid areas with higher evapotranspiration than precipitation (Hanin *et al.*, 2016; Kaashyap *et al.*, 2017; Jha *et al.*, 2019). It has deteriorated 20% of total irrigated land area decreasing one-third production of food (Mansoor *et al.*, 2021). Around the

world 20-50% decline in agronomic yield is mainly due to salinity and water deficit consequent upon reallocation of photosynthetic energy from yield to plants defenses against stress (Amjad *et al.*, 2021a). Osmotic stress as well as ion toxicity and nutritional disparity are main consequences of SS (Amjad *et al.*, 2021b). Salt stress inhibits plant growth as well as productivity by altering the metabolic pathways of plants (Rafeeq *et al.*, 2020). Salt stress triggers production of reactive oxygen species (ROS), disturbance in antioxidant defense mechanisms, photosynthetic process and hormones imbalance in plants (Yaseen *et al.*, 2021). Other important processes concerned with growth, i.e., nutrients availability, water relation, photosynthetic pigments, seed germination, photosynthetic mechanism and productivity are mainly affected by SS (Hanin *et al.*, 2016). Therefore, plant survival under saline condition requires highly activated protective mechanisms to diminish the effects of salinity induced production of toxic metabolites (Mansoor *et al.*, 2021). These multifaceted challenges underscore the need for innovative approaches that can enhance the resilience of wheat plants to salt-induced stress.

In recent years, nanotechnology has emerged as a promising avenue for addressing complex agricultural challenges, including salinity stress. Among the myriad nanoparticles investigated for their potential agricultural applications, zinc nanoparticles (Zn-NPs) have garnered considerable attention. Zinc (Zn), an essential micronutrient, plays a crucial role in various physiological processes within plants. Zn stimulates various plant enzymes which catalyze sugar metabolism, auxin production, protein synthesis and pollen production and its deficiency reduces crop productivity (Islam *et al.*, 2017, 2019; Kahtun *et al.*, 2018; Amjad *et al.*, 2021b; Islam *et al.*, 2021). Zinc regulates and maintains gene expression that is essential for making plants stress tolerant, performs other functions like tryptophan synthesis, maintaining membranous structures, cell division and photosynthesis, synthesizes protein where it serves as co-factor (Marschner *et al.*, 2011). Zinc deficiency in the people of developing countries is a major health hazard due to high consumption of cereal based diet and is amongst the ten fundamental health related problems worldwide (Domingues *et al.*, 2016). However, when engineered into nanoparticulate form, zinc exhibits unique properties that extend beyond its conventional role as a nutrient. These nanoparticles have demonstrated the ability to modulate plant responses to stressors, including salinity, thereby opening new possibilities for enhancing crop tolerance and productivity.

Nanotechnology is domineering agricultural technique these days due to its potential benefit in food quality enhancement, reduction in agricultural input by the use of nanoscale pesticides, fertilizers and increased yield of agricultural products (Prasad *et al.*, 2017). Nano materials due to their smaller size (billionth of a meter) have large surface area, are more reactive in most biological processes and are ecofriendly (Prasad *et al.*, 2014). Currently, metal oxide nanoparticles such as FeO, CeO₂, Al₂O₃, TiO₂ and ZnO have been applied in agricultural industry and their role in disease control, toxicity and enhanced cereal production in abiotic stresses is under investigation (Zheng *et al.*, 2016; Lalarukh *et al.*, 2022).

The intricate interplay between Zn-NPs and wheat plants under SS conditions presents an exciting avenue for research. By unraveling the underlying mechanisms through which Zn-NPs alleviate the adverse effects of SS in wheat, we can gain valuable insights into the potential applications of nanotechnology in sustainable agriculture. This study delves into the nuanced interactions between Zn-NPs and wheat, aiming to elucidate the biochemical, physiological, and molecular responses that contribute to enhanced salt tolerance. Understanding the intricacies of wheat's response to SS is fundamental to contextualizing the potential impact of Zn-NPs on alleviating these effects. Zinc fertilizers mostly in the form of zinc sulfate are used in large quantity however, use of ZnO-NPs in subtle amount as exogenous application on cereal crops is cost effective and might be the possible solution of Zn malnutrition. The primary contributors to Zn content in seeds arise from two key sources: firstly, uptake from the soil through the roots, and secondly, translocation from vegetative portions during the grain-filling stage (Cakmak and Kutman, 2018). This research endeavors to contribute to the evolving field of nanotechnology-driven solutions for sustainable agriculture, specifically in the context of mitigating SS in wheat. Therefore, to address food security and environmental concerns, employing nanotechnology, specifically utilizing ZnO-NPs to enhance yields in modern wheat cultivars under saline conditions, stands out as a potential solution. Based on the aforementioned considerations, the current study was undertaken with the following objectives.

- i) To investigate the influence of SS on the growth, biochemical responses, and yield of wheat plants.
- ii) To evaluate the potential role of ZnO-NPs for ameliorating SS of the tested wheat variety, and
- iii) To assess the interactive effects of ZnO-NPs in combination with SS on the overall performance and resilience of wheat cultivars, exploring potential synergies or mitigations.

CHAPTER 2

REVIEW OF LIETERATURE

Several studies regarding the mitigating effects of saline stress by using various kind of nanoparticle have been studied in the world, but a little study concerning the effects of zinc oxide (ZnO) on the growth, physiology, yield and quality of wheat under saline condition has been done in Bangladesh. Review of literature provides a theoretical framework, previous work and the basic interpretation of findings to the study. An attempt has been made to review the available literature those are related to this study as below.

2.1 Role of Zn as nanoparticles (Zn-NPs) in alleviating salt stress

[Adil *et al.* \(2022\)](#) applied different doses of Zn as conventional fertilizer and ZnO NPs in potted wheat grown under artificially developed 10 dS m⁻¹ salinity stress and reported that ZnO NPs (0.12 g pot⁻¹) significantly increased the physical parameters of wheat under salt stress compared to control. Application of ZnO NPs (0.12 g pot⁻¹) significantly increased chlorophyll a and b contents by 24.6 and 10%, plant height at vegetative and maturity stages by 34.6 and 37.4%, shoot and spike lengths by 30.7 and 27.6%, root fresh and dry weights by 74.5 and 63.1%, and wheat grain yield by 42.2%, respectively. ZnO NPs performed better compared to Zn conventional fertilizer under salt stress and could be used in place of Zn conventional fertilizer in salt-affected soils for attaining better crop production.

A total of nine different barley genotypes were first subjected to salt (NaCl) stress in hydroponic conditions by [Ali *et al.* \(2022\)](#) to determine the tolerance among the genotypes reported that the genotype Annora was found as most sensitive, and the most tolerant genotype was Awaran 02 under salinity stress. In another study, the most sensitive (Annora) and tolerant (Awaran 02) barley genotypes were grown in pots under salinity stress (100 mM), at the same time, half of the pots were provided with the soil application of ZnO-NPs (100 mg kg⁻¹), and the other half pots were foliar sprayed with ZnO-NPs (100 mg L⁻¹). Salinity stress reduced barley growth in both genotypes compared to control plants. However, greater reduction in barley growth was found in Annora

(sensitive genotype) than in Awaran 02 (tolerant genotype). The exogenous application of ZnO-NPs ameliorated salt stress and improved barley biomass, photosynthesis, and antioxidant enzyme activities by reducing oxidative damage caused by salt stress. However, this positive effect by ZnO-NPs was observed more in Awaran 02 than in Annora genotype. Furthermore, the foliar application of ZnO-NPs was more effective than the soil application of ZnO-NPs. Findings of the present study revealed that exogenous application of ZnO-NPs could be a promising approach to alleviate salt stress in barley genotypes with different levels of salinity tolerance.

To investigate the effect of nano-zinc fertilizer on growth, yield and mineral status of cotton plants grown under salt stress, a pot experiment was set up by [Hussein and Abou-Baker \(2018\)](#) in the greenhouse of the National Research Centre with the treatments of: i) diluted seawater: 10% (S1), 20% (S2) and tap water as a control (S0), ii) 100ppm (NZn1), 200ppm (NZn2) nano-zinc and distilled water as a control (NZn0). Irrigation with 10 and 20% seawater decreased dry weight (DW) of leaves by 11.53 and 43.22%, while decreases in bolls were 15.50 and 71.65%, respectively. Except for root DW and top/root ratio, the measured growth parameters were increased as nano zinc concentration increased. As for the interaction between treatments, the highest DW of stem, leaves and bolls resulted from the addition of NZn2 under normal condition, followed by NZn2 x S1 and the next was NZn2 x S2. The foliar application of 200ppm nano-Zn led to mitigating the adverse effect of salinity and confirmed that diluted seawater could be used in the irrigation of cotton plant.

[Lalarukh et al. \(2022\)](#) conducted an experiment to evaluate the effects of spraying nano-Zn particles with varying concentrations (0, 20, 50 and 80 mg L⁻¹) on two wheat cultivars irrigated with saline water (EC = 6.3 dS m⁻¹) versus a non-saline one. The key results revealed that root and shoot weights decreased significantly under salinity stress conditions, while improved considerably with nano-Zn-particles foliar application up to 50 mg nano Zn L⁻¹; thereafter significant reductions occurred. Also, shoot and root lengths as well as plant leaf area index improved considerably owing to this foliar application. Clearly, roots and shoots weights of wheat plants sprayed with nano-Zn

particles under salinity stress conditions exhibited higher values than the corresponding ones that was grown under non-saline conditions without nano-Zn-particles applications. Foliar spray enhanced formation of total soluble sugars and proline, and raised significantly Ca contents in wheat roots and shoots, and to some extent K contents. However, the foliar application of nano-Zn particles increased plant growth under salty stress conditions via two parallel processes, i.e., stimulating formation of osmolytes and stimulating nutrient uptake which may, in turn, increase plant metabolism.

Zinc oxide nanoparticles (ZnO NPs) are considered among the most highly used NPs in several industrial products (Sarraf *et al.*, 2022). ZnO NPs have been suggested as a Zn fertilizer at lower levels to supply Zn to plants (Milani *et al.*, 2012). It has been observed that ZnO NPs (1-20 ppm) increase the growth of mung beans and chickpea (Mahajan *et al.*, 2011). ZnONPs have been found to enhance cotton growth and reduce oxidative stress in plants (Venkatachalam *et al.*, 2017).

ZnO NPs can help plants cope with the negative effects of abiotic stress due to the high concentration of sodium in soil (Sturikova *et al.*, 2018). This beneficial effect of foliar application may be due to important role of Zn in plant biological and metabolic activities, such as stimulating enzymes, cell elongation and enlargement, nitrogen metabolism, photosynthetic pigments, preserving the structural integrity of plant cell membranes, and phospholipid accumulation (Rizwan *et al.*, 2019; Adrees *et al.*, 2021; Czyżowska and Barbasz, 2022). Exogenous application of ZnO NPs may reduce the leaf and root EL, further enhancing the antioxidant enzyme activities than treatments without ZnO nanoparticles. The increased production of antioxidant enzymes and reduced EL with ZnO NPs may enhance the barley growth treated with a high sodium concentration. The effectiveness of ZnO NPs is also determined by their ability to penetrate the plant cell through the natural Nano pore (stomata) in the leaves, which may enhance metabolic activities and, as a result, plant development (Rossi *et al.*, 2019).

The ZnO NPs reduced sodium toxicity in the plants, restored chlorophyll contents, and improved photosynthesis. Adding ZnO NPs to plants increased photosynthetic activity, improved

carbohydrate and by-product metabolism, and increased plant tolerance to salt stress. ZnO NPs application reduced the sodium concentrations in plants than respective treatments without nanoparticles. Furthermore, Zn increases the biosynthesis of the growth regulator IAA, which promotes cell division, cell elongation, and mineral absorption, resulting in increased plant growth (Fernández and Brown, 2013; Zhang *et al.*, 2021).

The gradual release of Zn ion from the nanoparticles, which supplies a long-term provenance of Zn and helps escape toxicity by sudden uptake of Zn by plants at high concentrations, may be responsible for the various physiological effects of the foliar supply of ZnO NPs (Wang *et al.*, 2018). The increase in plant growth with nanoparticle application may be attributable to increased nutrient production, reduced soil toxicity induced by overuse of fertilizers, and increased antioxidant enzyme activity, which helps protect plants from injury caused by free radicals (Rajput *et al.*, 2021).

Salinity stress results in ROS production; damage of nucleic acids, lipids, and proteins; and, ultimately, cell death. Cell damage might be due to ROS-induced lipid peroxidation of unsaturated fatty acids and impaired membrane functions (Demidchik *et al.*, 2014; Zeeshan *et al.*, 2020; Etesami *et al.*, 2021).

Kumar *et al.* (2017) reported that salinity stress increased the contents of H₂O₂, EL, and MDA. This overproduction of ROS might have weakened the defense of barley plants by overcoming the activities of antioxidant enzymes such as SOD, POD, CAT, and APX activities, as evident by the present study. However, the exogenous application of ZnO NPs alleviated oxidative stress induced by salinity stress by improving plant defense and by enhancing the antioxidant enzyme activities. Moreover, salinity stress has been reduced by the application of ZnO NPs in different plants such as in Brassica napus (El-Badri *et al.*, 2021), mango trees (Elsheery *et al.*, 2020), lupine (Abdel Latef *et al.*, 2017), and tomato plants (Hosseinpour *et al.*, 2020), which might be due to improvement in the antioxidant enzyme activities with the exogenous application of ZnO NPs.

[Rakgotho et al. \(2022\)](#) investigated the efficiency of ZnO NPs, synthesized from *Agathosma betulina* to mitigate salt stress in *Sorghum bicolor*. Hexagonal wurtzite ZnO NPs of about 27.5 nm, were obtained. Sorghum seeds were primed with ZnO NPs (5 and 10 mg/L), prior to planting on potting soil and treatment with high salt (400 mM NaCl). Salt significantly impaired growth by decreasing shoot lengths and fresh weights, causing severe deformation on the anatomical (epidermis and vascular bundle tissue) structure. Element distribution was also affected by salt which increased the Na^+/K^+ ratio. Salt also increased oxidative stress markers (reactive oxygen species, malondialdehyde), enzyme activities (SOD, CAT and APX), proline, and soluble sugars. Priming with ZnO NPs stimulated the growth of salt-stressed sorghum plants, which was exhibited by improved shoot lengths, fresh weights, and a well-arranged anatomical structure, as well as a low Na^+/K^+ ratio (1.53 and 0.58) indicating an improved element distribution.

Zinc oxide nanoparticles (ZnO NPs) have recently gained a lot of attention in nanoscience due to their unique physicochemical properties and their various applications in biology, chemistry, medicine, and physics and in recent years they significantly increased the salt tolerance in crop plants ([Faizan et al., 2021](#); [Zulfiqar and Ashraf, 2021](#)).

2.2 Role of Zn in alleviating salt stress

[Hendawy and Khalid \(2005\)](#) showed that increasing soil salinity progressively decreased the vegetative growth characters, i.e. plant height, number of branches, fresh and dry weight of the same plants, while application of zinc improved the plant growth.

[Aktas et al. \(2006\)](#) reported that Zn deficiency in soil significantly reduced the shoot growth, especially under the highest salt treatment. Increase in the zinc application from 2 to 10 mg kg^{-1} soil decreased the shoot concentration of Na^+ and elevated the K^+ concentration. As a result, K/Na ratios of the plant were highest under the highest Zn application conditions. By affecting structural integrity and controlling the permeability of root cell membranes, sufficient Zn nutrition reduced excess uptake of the Na^+ by root in the saline conditions. It also indicates the protective role of high Zn supply against salt stress.

[Alpaslan *et al.* \(1999\)](#) suggested that zinc has controlled mechanism or a regulatory role on the Na⁺ and Cl⁻ uptake and translocation rate. They also concluded that in the salt-affected soils, zinc application could abate possible Na⁺ and Cl⁻ injury in the plant. Salinity-induced reductions in P concentrations in plant tissues were often reported in research conducted in soil conditions.

[Chen *et al.* \(2007\)](#) reported that zinc deficiency caused a drastic reduction in chlorophyll content in addition to severe damage to the fine structure of the chloroplast. The total chlorophyll content and other growth parameters such as plant height, plant fresh and dry weight and leaf area as well as N, P and K content were decreased as the salinity level increased and salinity effect can be reduced by zinc application and gave positive effect on plant growth parameters ([Babu *et al.*, 2012](#)).

[Saeidnejad *et al.* \(2016\)](#) ascertained that salinity causes a significant reduction in chlorophyll a and b content. However, the activity of catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) increased as the salinity level was enhanced. Addition of zinc alleviated the significant enhancement of Na⁺ concentration caused due to salinity.

[Rani *et al.* \(2017\)](#) reported that under salinity there was a considerable decrease in dry weight of roots and micro nutrient (Zn, Cu, Fe and Mn) uptake. The adverse effect of saline environment was ameliorated by Zn applications and for that optimum dose of Zn application in both types of salinity was 15 mg kg⁻¹ soil. Moreover, root dry weight and micronutrient uptake (Zn, Cu, Fe and Mn) have a negative interaction with increasing salinity and positive interaction with the increasing application of Zn.

[Abd-El and Hady \(2007\)](#) examined the effect of zinc application on growth and nutrient uptake of barley irrigated with saline water and stated that Zn content of the fresh and dry weight enhanced with increasing Zn rate at various salinity levels as compared to control and the amount of nutrient elements significantly decreased by increasing salinity, while Zn fertilization increased the nutrient elements and plant growth.

Chang *et al.* (2007) demonstrated that zinc mobility and uptake in soil is dependent on various factors, for instance soil acidity, zinc total value in the soil, organic matter, soil type and phosphorus levels in the soil. The most important factor which affects zinc usability is the soil pH. The yield of wheat increased from 3.40 to 4.35 t ha⁻¹ and Zn uptake increased from 142 to 256 g ha⁻¹ with long-term application of crop residues together with Zn by using appropriate rates, methods (soil) and sources (such as ZnSO₄). Although there are several sources of Zn, the most common source of Zn remains ZnSO₄ (Prasad *et al.*, 2010).

Saeidnejad *et al.* (2016) reported that potassium content was increased by Zn treatments. A considerable increase was noticed in leaf Zn concentration as the applied level of Zn was increased. By and large, the results suggest some positive and negative interactions of salinity and Zn application, which could be useful in the management of saline soil and water.

As the application of Zn increases, the concentration of Zn in the shoots and grains increases significantly. Grain protein was decreased by salinity, while the utilization of grain protein enhanced and finally improved the growth and yield of wheat by decreasing the effects of salinity, therefore the addition of Zn helped in reducing the unfavourable effects of increased salinity tolerance of wheat to salinity (Kamrani *et al.*, 2013).

Khoshgoftar *et al.* (2004) observed that growth and yield of wheat plants significantly decreased with increasing NaCl rate and Zn application increased dry weight yield at different levels of salinity. Application of Zn fertilizer somewhat counteracted the negative effects of salinity on plant growth.

2.3 Role of Zn-NPs in alleviating other stresses

A pot study was conducted by Adrees *et al.* (2021) to explore the effectiveness of zinc oxide nanoparticles (ZnONPs) foliar exposure on growth and development of wheat, zinc and cadmium (Cd) uptake in Cd contaminated soil under various moisture conditions. Four different levels (0, 25,

50, 100 mg/L) of these NPs were foliar-applied at different time periods during the growth of wheat. Two soil moisture regimes (70% and 35% of water holding capacity) were maintained from 6 weeks of germination till plant harvesting. The results revealed that the growth of wheat increased with ZnO NPs treatments. The best results were found in 100 mg/L ZnO NPs under normal moisture level. The lowest Cd and highest Zn concentrations were also examined when 100 mg/L NPs were applied without water deficit stress. In grain, Cd concentrations decreased by 26%, 81% and 87% in normal moisture while in water deficit conditions, the Cd concentrations decreased by 35%, 66% and 81% compared to control treatment when ZnO NPs were used at 25, 50 and 100 mg/L. The foliar exposure of ZnO NPs boosted up the leaf chlorophyll contents and also decreased the oxidative stress and enhanced the leaf superoxide dismutase and peroxidase activities than the control. It can be suggested that foliar use of ZnO NPs might be an efficient way for increasing wheat growth and yield with maximum Zn and minimum Cd contents under drought stress while decreasing the chances of NPs movement to other environmental compartment which may be possible in soil applied NPs.

[Satter *et al.* \(2021\)](#) investigated the potential and zinc and silicon (Si) to ameliorate terminal drought stress in wheat and associated mechanisms. Two different drought stress levels, i.e., control [80% water holding capacity (WHC) was maintained] and terminal drought stress (40% WHC maintained from BBCH growth stage 49 to 83) combined with five foliar-applied Zn-Si combinations (i.e., control, water spray, 4 mM Zn, 40 mM Si, 4 mM Zn + 40 mM Si applied 7 days after the initiation of drought stress). Results revealed that application of Zn and Si improved chlorophyll and relative water contents under well-watered conditions and terminal drought stress. Foliar application of Si and Zn had significant effect on antioxidant defense mechanism, proline and soluble protein, which showed that application of Si and Zn ameliorated the effects of terminal drought stress mainly by regulating antioxidant defense mechanism, and production of proline and soluble proteins. Combined application of Zn and Si resulted in the highest improvement in growth and antioxidant defense. The application of Zn and Si improved yield and related traits, both under

well-watered conditions and terminal drought stress. The highest yield and related traits were recorded for combined application of Zn and Si.

It is reported that foliar use of Zn regulated nutrients balance and stomata opening in maize to diminish the adversities of water deficit (Tabatabai *et al.*, 2015). Adequate Zn fertilization significantly enhanced the activities of POD, SOD and CAT enzymes in response to water deficit (Yavas and Unay, 2016; Hassan *et al.*, 2020). In another study, it was documented that optimum Zn dose-maintained water status, stomatal conductance and osmotic adjustment in chickpea under drought stress (Khan *et al.*, 2004). Foliar application of Zn and Si improved chlorophyll contents and leaf water contents both under terminal drought stress and well-watered conditions. Improved chlorophyll contents attributed to the role of Zn and Si in proteins and enzymes synthesis, its role as co-factor in pigment synthesis (Misra *et al.*, 2005; Roosta *et al.*, 2018).

Zinc oxide nanoparticles and titanium dioxide nanoparticles (TiO₂ NPs) have been tested by Kurtinová and Šebesta (2023) in various conditions to evaluate their influence on plants, including alleviating stress exerted by heavy metals. They represent two opposites, ZnO NPs being easily dissolved with the well-understood mode of action similar to ionic forms of Zn and TiO₂ NPs having negligible dissolution and unclear mechanisms of action and described the positive effects of ZnO NPs and TiO₂ NPs in the amelioration of heavy metal stress in plants.

2.4 Role of other NPs in alleviating salt stress

Alharbi *et al.* (2022) investigated a synergistic amendment strategy for salt-affected soil using a singular and combined application of plant growth-promoting rhizobacteria (PGPR at 950 g ha⁻¹; *Azotobacter chroococcum* SARS 10 and *Pseudomonas koreensis* MG209738) and silica nanoparticles (SiNPs) at 500 mg L⁻¹ to mitigate the detrimental impacts of irrigation with saline water on the growth, physiology, and productivity of barley (*Hordum vulgare* L.), along with soil attributes and nutrient uptake during 2019/2020 and 2020/2021. They showed that the combined application of PGPR and SiNPs significantly improved the soil physicochemical properties, mainly by reducing the soil exchangeable sodium percentage. Additionally, it considerably enhanced the microbiological counts (i.e., bacteria, azotobacter, and bacillus) and soil enzyme activity (i.e.,

urease and dehydrogenase) in both growing seasons compared with the control. The combined application of PGPR and SiNPs alleviated the detrimental impacts of saline water on barley plants grown in salt-affected soil compared to the single application of PGPR or SiNPs. The marked improvement was due to the combined application of PGPR and SiNPs, which enhanced the physiological properties (e.g., relative chlorophyll content (SPAD), relative water content (RWC), stomatal conductance, and K/Na ratio), enzyme activity (SOD, CAT, and peroxidase; POX), and yield and yield-related traits and nutrient uptake (N, P, and K) of barley plants. Moreover, the Na⁺ content, hydrogen peroxide (H₂O₂) content, lipid peroxidation (MDA), electrolyte leakage (EL), and proline content were reduced upon the application of PGPR + SiNPs.

[Chattha *et al.* \(2022\)](#) carried out an experiment with different SS levels: control, 6 and 12 dS m⁻¹, and different Zn-NPs treatments: control, seed priming (40 ppm), foliar spray (20 ppm), and their combination, and concluded that salinity stress markedly reduced plant growth, biomass, and grain yield. This was associated with enhanced EL, MDA, H₂O₂, Na, Cl accumulation, reduced photosynthetic pigments, RWC, photosynthetic rate (Pn), transpiration rate (Tr), stomata conductance (Gs), water use efficiency (WUE), free amino acids (FAA), total soluble protein (TSP), indole acetic acid (IAA), gibberellic acid (GA), and nutrients (Ca, Mg, K, N, and P). However, the application of Zn-NPs significantly improved the yield of the wheat crop, which was associated with reduced abscisic acid (ABA), MDA, H₂O₂ concentration, and EL, owing to improved antioxidant activities, and an increase in RWC, Pn, Tr, WUE, and the accumulation of osmoregulating compounds (proline, soluble sugars, TSP, and FAA) and hormones (GA and IAA).

Application of 100 mg kg⁻¹ of the bioengineered FeO-NPs in an original saline soil stimulated wheat plant growth, gaining 36.7% of additional length as compared with the control scenarios, via alleviating the detrimental effects of abiotic stresses and thereby reprogramming the morpho-physiological state of wheat plants ([Manzoor *et al.*, 2021](#)). In addition, the presence of FeO-NPs in soil significantly increased the nutrient concentrations of N, P and K⁺, while reducing the Na⁺ and Cl⁻ components in the wheat grain. Interestingly, application of the FeO-NPs in Cd-polluted soils

eventually reduced wheat plant uptake of Cd by 72.5%, probably due to the adsorption of Cd onto the large surface of NPs and thereby, constraining Cd bioavailability to the plants.

Zia *et al.* (2023) reported that application of FeNPs (25 mg/kg) on normal soil increased the dry weights of wheat roots, shoots, and grains by 46, 59, and 77%, respectively. In salt-affected soil, FeNPs increased the dry weights of wheat roots, shoots, and grains by 65, 78, and 61%, respectively. The application of FeSO₄ and Fe-EDTA increased the growth parameters of wheat in both normal and salt-affected soils compared to the respective controls. The photosynthetic parameters, including chlorophyll a (50%), chlorophyll b (67%), carotenoids (62%), and total chlorophyll contents (50%), were increased with the application of FeNPs under salt stress. The FeNPs increased plant-essential nutrients like iron, zinc, calcium, magnesium, and potassium in both normal and salt-affected soils. The experiment revealed that the application of Fe plays a significant role in enhancing the growth of wheat on alkaline normal and salt-affected soils.

Shah *et al.* (2023) reported that the application of *Trichoderma harzianum* and the simultaneous co-inoculation with copper oxide nanoparticles (CuO-NPs) notably enhanced fresh biomass and facilitated vegetative growth under salt stress in comparison to the control group. Furthermore, the exposure of both *T. harzianum* inoculum and CuO-NPs resulted in a significant reduction of oxidative stresses, including reactive oxygen species (ROS) levels, H₂O₂, and lipid peroxidation (MDA) levels in the above-ground parts of the plant, while also minimizing electrolyte leakage (EL) by reducing root growth. Additionally, the co-inoculation of the endophyte and CuO-NPs led to a significant enhancement in antioxidant enzymatic activities, such as SOD and CAT activity in the above-ground parts, under salt stress conditions.

It has been extensively reported that Si supplementation decreased Na⁺ uptake and increased K⁺ uptake by plants and increased plant growth and yield under salt stress in wheat (Ali *et al.*, 2009; Gurmani *et al.*, 2013a), rice (Gurmani *et al.*, 2013; Shi *et al.*, 2013; Bybordi, 2014).

Application of Si (4 mM) as potassium silicate on root medium promoted higher growth, gas exchange, tissue water and membranes stabilities, and K⁺ content, had limited MDA and Na⁺

content and reduced electrolyte leakage in salt stressed wheat plants (Alzahrani *et al.*, 2018; Sienkiewicz-Cholewa *et al.*, 2018). Similarly, foliar application of Si (4 mmol L⁻¹) as potassium silicate decreased Na⁺ uptake, increased potassium and consequently improved plant weight, 100-seed weight, seed yield, ear length, and photosynthesis rate has been observed in salt-stressed wheat plants (Bybordi, 2014). On the other hand, (Sattar *et al.*, 2017) reported that foliar Si spraying (50 mM) with silicic acid improved the plant growth, water relations, photosynthetic attributes, transpiration rate and chlorophyll contents in salt-stressed wheat seedlings. Likewise, foliar Si spraying (50 mM) improved the morphology, water relations, photosynthetic pigments and gas exchange parameters, and the activity of SOD, CAT, and POD in salt-stressed wheat seedlings under salinity (Sattar *et al.*, 2018).

CHAPTER 3

MATERIALS AND METHODS

This chapter provides a brief overview covering the location of the experimental site, duration, soil properties, climate conditions, planting materials, treatments, production technology, and the procedures for data collection and analysis, all of which are presented in the subsequent sections.

3.1 Experimental location and duration

A pot experiment was conducted at the Shade house, Department of Agronomy, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. The mentioned location, HSTU is situated at coordinates approximately 25.6980°N latitude and 88.6550°E longitude. The experiment was conducted during November 2022 to March 2023.

3.2 Climate

Weather data, including temperature, precipitation, relative humidity, and sunshine hours throughout the experimental period, were documented using information obtained from the closest meteorological station to the Bangladesh Wheat and Maize Research Institute's meteorological station in Nashipur, Dianjpur, Bangladesh. Weather information is presented in Appendix I.

3.3 Soil

The experimental pots were filled with soil sourced from the agronomy research field at HSTU. The collected soil originated from the Old Himalayan Piedmont Plain (AEZ-1). After drying, the soil samples used in the pots underwent thorough mixing. The soil exhibited a sandy loam texture with a moderate level of organic matter. Subsequent analysis of the soil samples was analyzed in the Soil Resource Development Institute (SRDI), Dinajpur and the results are presented in Appendix II.

3.4 Experimental design and treatments

The experiment consisted two factors namely Factor A: Three levels of salt stress viz., i) $S_0 = 0$ mM, ii) $S_1 = 100$ mM, and iii) $S_2 = 150$ mM, and Factor B: Three levels of zinc oxide (ZnO) viz., i) $ZnO_0 = 0$ μ M, ii) $ZnO_1 = 150$ μ M and $ZnO_2 = 300$ μ M. Total treatments = Factor A \times Factor B = $3 \times 3 = 9$; Replication = 3; total experimental units/pots = $9 \times 3 = 27$. The experiment was laid out with Completely Randomized Design (CRD).

Layout

R₁	R₂	R₃
S₀ ZnO₀	S₀ ZnO₂	S₁ ZnO₁
S₂ ZnO₁	S₂ ZnO₀	S₂ ZnO₂
S₁ ZnO₁	S₀ ZnO₁	S₁ ZnO₂
S₂ ZnO₂	S₁ ZnO₀	S₀ ZnO₂
S₁ ZnO₂	S₀ ZnO₀	S₂ ZnO₀
S₀ ZnO₂	S₂ ZnO₁	S₀ ZnO₁
S₂ ZnO₀	S₁ ZnO₁	S₁ ZnO₀
S₀ ZnO₁	S₂ ZnO₂	S₀ ZnO₀
S₁ ZnO₀	S₁ ZnO₂	S₂ ZnO₁

3.5 Collection of seed

Bangladesh Wheat and Maize Research Institute (BWMRI) is the source of the BWMRI Gom 2 variety used in the experiment. This particular variety is renowned for its exceptional attributes, such as a whitish seed color, resistance to blast disease and heat, larger grain size (45-50 g of 1000 seed weight), and a higher grain yield ranging from 4500-5800 kg ha⁻¹. The seeds employed in the experiment were meticulously selected, ensuring they were devoid of inert materials and other crop seeds, and characterized by maturity and overall health.

3.6 Preparation of pot

The experiment pots were initially filled on November 18, 2022. Through manual mixing, the soil in the pots was carefully prepared to achieve the desired fine tilth. Prior to adding 10 kg of soil to each pot, thorough efforts were made to eliminate any weeds and stubble from the soil.

3.7 Fertilizer application

As sources of nitrogen (N), phosphorus (P), potassium (K), and Sulphur (S), respectively, urea, triple super phosphate (TSP), muriate of potash (MOP), and gypsum were utilized. Following the recommendation of [BWMRI \(2022\)](#), plastic pots with dimensions of 23.5 x 25.0 x 20.0 cm (top diameter, depth and bottom diameter) were treated with Urea, TSP, MOP, and gypsum at 250, 150, 160 and 125 kg ha⁻¹, respectively. When preparing the pots, two-third urea and all of the fertilizers were mixed with the soil, and rest one-third was applied at 20 DAS.

3.8 Seed treatment and sowing of seeds

On November 25, 2022, a total of 20 seeds were planted in each pot at a depth of 2-3 cm. Before planting, the seeds were treated with Bavistin-200 (0.3%) as a precautionary measure.

3.9 Application of NaCl

Upon applying water to the pot in accordance with the specified treatment, the requisite amount of NaCl was added.

3.10 Intercultural operations

3.10.1 Thinning

Germination of seeds commenced five days after sowing (DAS). To maintain the ideal plant population in each pot, thinning was carried out in each by keeping 10 healthy seedlings.

3.10.2 Irrigation

Irrigation was carried out as required using treated saline water as part of the specified treatment protocol.

3.10.3 Weeding

The experimental crop faced infestations of common weeds, which were systematically eliminated by uprooting and removing them from the pots during the course of the experiment.

3.10.4 Plant protection measures

When deemed necessary, a suitable preventative like Imidagold 20SL was administered at a rate of 0.3ml per liter of water to protect the plants, particularly against aphids.

3.11 Diseases management

To prevent the risk of fungal infection during the seedling, heading, and anthesis stages, a fungicide Nativo 75 WG was used.

3.12 Harvest and postharvest

Harvesting was deemed complete when 90% of the spike transition from green to brown in color. The harvest process involved the use of scissors for cutting followed by post-harvest management.

3.13 Data collection at different days after sowings

3.13.1 Morphological characteristics

Data were systematically recorded at specific intervals such as 45, 60, 75 DAS, and during the final harvest (FH). Subsequent to thinning, 10 plants were maintained in each pot, and two plants were selectively removed from each pot at each sampling date in order to facilitate data collection.

3.13.1.1 Plant height (cm)

In accordance with the designated treatment, the plant height was measured in centimeters (cm). Using a meter scale, the measurement was taken from the base of the plant to its tip.

3.13.1.2 Number of leaves plant⁻¹

Leaf counts for plant⁻¹ were conducted at 45, 60, 75 DAS and FH. The average number of leaves per plant for each treatment was determined by recording data from four plants in each pot. Additionally, the weight of a single leaf per plant was also recorded.

3.13.1.3 Leaf area

The leaf area was determined by using the average of three separate measurements taken at 45, 60, and 75 DAS on three distinct plants in each plot. The leaf area was measured by using by Leaf Area Meter (CHINCAN YMJ-B, Zhejiang, China).

3.13.2 Growth parameter

3.13.2.1 Leaf fresh and dry weight (g)

The fresh leaf weight was measured and recorded using an electric balance. Subsequently, the leaves were subjected to a temperature of 80 °C of 72 hours, after which the dry weight was measured and recorded.

3.13.2.2 Stem fresh and dry weight (g)

The fresh weight of the stem was measured using an electrical balance. Subsequently, after subjecting the stem to a 72-hour oven temperature of 80°C, the dry weight was also measured using the same electric balance.

3.13.2.3 Root fresh and dry weight (g)

The root was separated from plant with help of scissors and the fresh weight of the roots was measured and recorded. The dry weight of the roots was also taken with the help of electric balance after placing the roots in an electric oven for 72 hours at 80°C.

3.13.3 Physiological parameters

3.13.3.1 Photosynthetic pigments

The chlorophyll content of wheat leaves in each treatment was determined following the method outlined by Witham et al. (1986). The leaves, weighing 0.10 g, were carefully taken and their weight was recorded. The leaves were submerged in 10 milliliters of acetone at room temperature for 48 hours in a dark environment. Subsequently, the sample underwent agitation on an electric horizontal shaker overnight. For measuring chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll content (Chl a+b) and carotenoid, the optical density or absorbance of the supernatant

were recorded using a UV-visible spectrophotometer at wavelengths of 663, 645 and 470 nm. The concentration of Chl a, Chl b and total chlorophyll were measured using the following formula.

$$\text{Chlorophyll a (mg g}^{-1} \text{ leaf)} = [12.7(D663) - 2.69(D645)] \times V / (1000 \times W)$$

$$\text{Chlorophyll b (mg g}^{-1} \text{ leaf)} = [22.9(D645) - 4.68(D663)] \times V / (1000 \times W)$$

$$\text{Total chlorophyll (mg g}^{-1} \text{ leaf)} = [20.9(D470) + 8.02(D663)] \times V / (1000 \times W)$$

$$\text{Carotenoid (mg g}^{-1} \text{ leaf)} = 0.216 \times D663 - 0.304 \times D645 + 0.452 \times D470$$

where, D = Absorbance reading of the chlorophyll extract at the specific wavelength,

V = Final volume of the 80% acetone-chlorophyll extract

W = Fresh weight in gram of the tissue extracted

3.13.3.2 Water status (%)

The leaves of wheat from each treatment were carefully collected, and fresh weight (FW) leaves was measured. Subsequently, the leaves were immersed in distilled water for 24 hours at room temperature to determine the turgid weight (TW). Afterward all the materials were oven-dried at 80 °C for 72 hours and taken dry weight (DW). The fresh, turgid and dry weights of leaf segment were used to determine relative water content (RWC), water saturation deficit (WSD), water retention capacity (WRC) and water uptake capacity (WUC) according to the method described by [Saneoka et al. \(1995\)](#). The formula of these parameters is given bellow:

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

$$\text{WSD} = 100 - \text{RWC}$$

$$\text{WRC} = \frac{\text{Turgid weight}}{\text{Dry weight}}$$

$$\text{WUC} = \frac{\text{Turgid weight} - \text{Fresh weight}}{\text{Dry weight}}$$

3.13.3.3 Cell Membrane Stability (CMS)

Leaf samples (1 g) were collected, rinsed with distilled water to eliminate any attached electrolytes, and placed in vials containing 10 milliliters of distilled water in order to measure the stability of the cell membrane. After being sealed in the vials for six hours at 25 °C, the electrical conductivity of the solution (C1) was measured. Following this, samples were subjected to an electric oven at 90 °C for two hours, and the electrical conductivity (C2) of solution was estimated. CMS was measured in percent as follows:

$$\text{CMS (\%)} = 100 \times \text{EC1/EC2}$$

3.13.3.4 Canopy Temperature

The canopy temperature was measured by a handheld infra-red thermometer (Model: Crop TRAC item no. 2955L-Spectrum Technologies, Inc. Beijing-10000, China). Measurements were taken from approximately 50 cm above the canopy at a 30-degree angle from the horizon. The CTD was recorded at 16 days after anthesis (DAA) under bright sunlight and minimal wind conditions by using the formula described by [Ayeneh *et al.* \(2002\)](#).

3.13.3.5 Soil pH

To determine the pH of soil using a probe, or analogue pH metre. First, the probe needs to be inserted between two and four inches into the ground. Allow the probe to stabilize about a minute, observing the display to identify the most stable pH reading. Examine the data on the meter dial to confirm the moisture levels and pH of the soil. After obtaining the necessary readings, carefully remove the probe from the soil. The probe was wiped after each use to ensure accurate and reliable measurements in subsequent tests.

3.13.3.6 Phenological Indices

Throughout the crop cycle of sowing periods, key developmental stages including the booting, heading, anthesis and physiological maturity were recorded using the Zadoks scale ([Zadoks *et al.*, 1974](#)).

3.13.4 Yield contributing characters

3.13.4.1 Number of spikelets spike⁻¹

Three plants were selected from each individual pot, resulting in a total of nine plants taken from a single treatment. The overall mean was then adjusted based on the results obtained from these selected plants.

3.13.4.2 Spike length (cm)

Spike length measurements were randomly taken by selecting several pods, and the mean length was expressed as a per-spike basis.

3.13.4.3 Number of grains spike⁻¹

The number of grains spike⁻¹ was recorded from randomly selected pods at the time of final harvesting.

3.13.4.4 Weight of hundred grains (g)

One hundred cleaned and dried grains were randomly selected, counted and their weight was measured using a digital electric balance. The recorded weight was then documented.

3.13.5 Yield indices

3.13.5.1 Grain yield (g)

The grains from each pot were harvested from the plants and properly sun-dried. The grain yield per plant was calculated based on the weight of the harvested grains.

3.13.5.2 Straw yield (g)

The straw from each pot was removed from the plant and properly sun-dried. The straw yield per plant was calculated based on the weight of the dried straw.

3.13.5.3 Biological yield (g)

The combined yield of grains and straw was considered as the biological yield of wheat. The calculation of the biological yield was done using the following formula:

$$\text{Biological yield (g plant}^{-1}\text{)} = \text{Grain yield} + \text{Straw yield}$$

3.13.5.4 Harvest index (%)

The harvest index (HI) for wheat was determined by calculating the ratio of seed yield to the biological (seed + straw) yield for each plant. The result was then expressed as a percentage.

$$HI = \frac{\text{Economical yield (seed weight)}}{\text{Biological yield}} \times 100$$

3.13.6 Nutrient status and grain quality

Laboratory analysis procedure

To estimate the various mineral ions, oven-dried plant materials (seeds) were ground using a Willy grinding machine.

Preparation of seed extract

The process involved taking exactly 1g of finely ground seeds were taken into a 250ml conical flask. Subsequently, 10ml of di-acid mixture ($\text{HNO}_3:\text{HClO}_4=2:1$) was added to the flask. The mixture was heated on an electric hot plate at 180-200 °C until the solid particles disappeared, and white fumes evolved from the flask. After heating, the contents were cooled to room temperature, washed with distilled water, and filtered into 100ml volumetric flasks using Whatman filter paper No 42. The volume was adjusted to the mark with distilled water, following wet oxidation method outlined by [Jackson \(1973\)](#).

3.13.6.1 Nitrogen ion (N) Determination

The determination of N content in sample was by carried out through the Kjeldhal method, which involves three steps: digestion, distillation and titration.

Digestion: The sample was heated in the presence of sulphuric acid. Potassium sulphate was added to increase the boiling point of the medium. Catalysts as mercury, selenium, copper and ions of mercury were also used in the digestion process. The sample was considered fully decomposed when a clear and colorless solution was obtained.

Distillation: Excess base was added to the acid digestion mixture to convert NH_4^+ to NH_3^+ , followed by boiling and condensation of the ammonia gas in a receiving solution was done.

Titration: Acid-base titration was performed using a standard solution of HCl, and the amount of N was determined based on the titration results.

3.13.6.2 Protein Determination

To determine the amount of protein from the N content in the raw materials, the following calculation was performed: The total nitrogen content in the sample was multiplied by the traditional conversion factor of 5.81. This conversion factor is commonly used in the analysis of N to estimate protein content in various organic materials.

3.13.7.3 Determinations of Sodium (Na^+) Potassium (K^+) and Calcium (Ca^{2+})

The analysis procedure for K^+ , Ca^{2+} , and Na^+ contents in dried powdered leaves involved the following steps:

Digestion: Dried powdered leaves (0.1 g) were digested for 12 h using, a mixture of perchloric acid (2 mL, 80%) and concentrated H_2SO_4 (10 mL) was used for the digestion process. **Dilution:** After digestion, each sample was diluted to 100 mL with distilled water. **Analysis:** The diluted samples were then subjected to analyze, K^+ , Ca^{2+} , and Na^+ contents using flame photometry ([Williams and Twine, 1960](#)). Flame photometry is a technique that uses the emission of light from the flame to determine the concentration of specific elements in a sample.

3.14 Statistical Analysis

The data obtained for different parameters were subjected to statistically analysis to find out the significant difference in the response of wheat to the application of salt and ZnO with use MSTAT-C statistical package. Mean values of all the measured traits were calculated and analysis of variance (ANOVA) was performed. To evaluate the significance of the differences among the treatment means, the Duncan's Multiple Range Test (DMRT) was employed at a 5% level of probability, following the methodology outlined by [Gomez and Gomez \(1984\)](#).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Impact of ZnO on wheat morphological characteristics in saline environments

4.1.1 Plant height

Salinity gradually decreased the plant height observed at all sampling dates, while application of ZnO alleviated the adverse effects of salt stress and increased the plant height (Table 1). However, the highest plant height (49.80, 55.50, 66.33 and 69.11 cm) was observed under control condition and the lowest values (43.30, 49.39, 53.72 and 57.98 cm) were observed in S₂ condition (150 mM NaCl) at 45, 60, 75 DAS and at final harvest, respectively. Spraying of ZnO-NPs increased the plant height, and the highest value (50.58, 56.72, 67.17 and 76.67 cm, respectively) were recorded ZnO₂ which was statistically identical to ZnO₁ (49.43, 55.67, 65.28 and 73.39 cm, respectively). The interaction effects of salt stress and ZnO statistically significant. Nonetheless, under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ showed the highest plant height (46.50, 52.67, 59.17 and 65.06 cm) and which were almost identical with the S₂ZnO₁. (44.40, 50.83, 58.67 and 64.72 cm) at the sampling dates, respectively. These results are in harmony with those obtained by Adil *et al.* (2022), Mogazy and Hanafy (2022), Mazhar *et al.* (2023), who reported that ZnO nano-particles alleviated the adverse effects of salt stress and increased the plant height. The application of ZnO-NPs at the time of sowing significantly increased the plant height compared to Zn bulk under stress conditions (Hosseini and Maftoun, 2008; Prasad *et al.*, 2012; Sattar *et al.*, 2021; Lalarukh *et al.*, 2022).

Table 1. Impact of ZnO on wheat plant height under saline conditions

Treatments	Plant height (cm)			
	45 DAS	60 DAS	75 DAS	Final harvest
Levels of salt stress				
S ₀	49.80 a	55.50 a	66.33 a	69.11 a
S ₁	45.30 ab	50.72 b	56.61 b	62.42 b
S ₂	43.30 b	49.39 b	53.72 b	57.98 c
LSD	2.548	2.547	3.102	2.907
Levels of ZnO				
ZnO ₀	46.39 b	53.22 b	61.22 b	69.44 b
ZnO ₁	49.43 a	55.67 a	65.28 a	73.39 a
ZnO ₂	50.58 a	56.72 a	67.17 a	76.67 a
LSD	2.929	3.665	3.965	4.184
Interaction effect of salt and ZnO				
S ₀ ZnO ₀	48.00 c	54.00 c	61.50 b	68.947 c
S ₀ ZnO ₁	50.33 b	56.17 b	65.83 a	70.497 b
S ₀ ZnO ₂	52.07 a	57.33 a	66.67 a	72.89 a
S ₁ ZnO ₀	46.17 e	52.00 d	58.83 c	63.22 e
S ₁ ZnO ₁	47.57 d	54.00 c	60.33 b	65.943 d
S ₁ ZnO ₂	48.17 c	55.17 bc	60.67 b	67.053 c
S ₂ ZnO ₀	40.00 g	46.67 f	52.33 d	56.167 f
S ₂ ZnO ₁	44.40 f	50.83 e	58.67 c	64.72 d
S ₂ ZnO ₂	46.50 e	52.67 d	59.17 bc	65.06 d
LSD	3.352	2.331	1.239	0.968
CV (%)	17.22	9.44	7.66	6.10

The numerical value followed by same letter are not significant different by DMRT test at $p < 0.05$, CV: Coefficient of Variation, LSD: Least significant difference.

4.1.2 Number of leaves plant⁻¹

The number of leaves plant⁻¹ (NLPP) gradually decreased with increasing salinity. The S₂ condition, representing 150 mM NaCl stress, resulted in the lowest NLPP (4.33, 5.67, 6.22 and 7.11) at 45, 60, 75 DAS, and at FH, respectively. The application of ZnO alleviated the adverse effects of salt stress, leading to an increase in NLPP. ZnO₂ had the highest impact (5.67, 6.89, 7.67 and 8.67), resulting in statistically similar NLPP compared to ZnO₁. This suggests that both ZnO treatments were effective in mitigating the negative effects of salinity. The control condition (non-stressed plants) consistently showed the highest NLPP at all sampling dates, indicating that salinity stress adversely affected plant growth.

Table 2. Impact of ZnO on number of leaves plant⁻¹ of wheat plant under saline conditions

Treatments	Number of leaves plant ⁻¹			
	45 DAS	60 DAS	75 DAS	Final harvest
Levels of salt				
S ₀	5.37 a	6.33 a	7.67 a	8.67 a
S ₁	4.67 b	6.00 b	7.11 b	8.00 b
S ₂	4.33 bc	5.67 c	6.22 c	7.11 c
LSD	0.767	0.394	0.844	0.621
Levels of ZnO				
ZnO ₀	4.67 b	5.78 b	6.33 b	7.67 b
ZnO ₁	5.11 b	6.11 b	7.33 a	8.33 a
ZnO ₂	5.67 a	6.89 a	7.67 a	8.67 a
LSD	1.642	0.662	0.877	0.621
Interaction effect of salt and ZnO				
S ₀ ZnO ₀	5.33 b	5.67 b	6.00 b	7.33 bc
S ₀ ZnO ₁	5.67 a	6.33 a	7.33 a	8.00 b
S ₀ ZnO ₂	5.67 a	6.67 a	7.67 a	8.67 a
S ₁ ZnO ₀	4.67 bc	5.11 c	5.67 c	6.33 d
S ₁ ZnO ₁	5.22 b	5.67 b	6.33 b	7.67 b
S ₁ ZnO ₂	5.33 b	6.00 b	7.00 a	8.00 b
S ₂ ZnO ₀	4.00 c	4.67 d	5.00 d	5.67 de
S ₂ ZnO ₁	4.33 c	5.00 c	6.33 b	7.00 c
S ₂ ZnO ₂	4.67 bc	5.33 c	6.67 b	7.33 bc
LSD	0.908	0.809	1.401	1.190
CV (%)	10.78	7.95	11.92	8.18

The numerical value followed by same letter are not significant different by DMRT test at p<0.05, CV: Coefficient of Variation, LSD: Least significant difference.

The interaction effects between SS and ZnO were found to be statistically significant. This implies that the combined influence of salinity stress and ZnO application had a notable impact on NLPP. Despite the overall reduction in number of leaves plant⁻¹ under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ showed the number of leaves plant⁻¹ (4.67, 5.33, 6.67 and 7.33). Additionally, this combination was almost identical to S₂ZnO₁, indicating that both ZnO treatments had a similar positive effect under salt stress. The results suggest that salinity stress negatively affects NLPP, but the application of ZnO-NPs, particularly ZnO₂ and ZnO₁, helps mitigate these adverse effects. The interaction between salinity stress and ZnO is significant, and certain treatment combinations, like S₂ZnO₂, can effectively enhance NLPP even under stressful conditions. The

results of the present experiment also agreed well with the earlier findings of [Spanò *et al.* \(2020\)](#), who stated that application of Zn increased the number of leaves in wheat plants grown under salt stress.

4.1.3 Leaf area plant⁻¹

The leaf area per plant (LAPP) exhibited a gradual decrease with increasing salinity levels. Specifically, the condition denoted as S₂, representing 150 mM NaCl stress, led to the lowest LAPP (809.55, 1237.80 and 1621.42 cm²) at 45, 60 and 75 DAS, respectively. This decline in leaf area suggests that salinity stress negatively impacted the expansion and development of leaves. However, the application of ZnO-NPs helped alleviate the adverse effects of salt stress, resulting in an increase in LAPP (1572.00, 1999.53 and 2285.90 cm²). Notably, ZnO₂ had the most substantial impact, yielding statistically similar LAPP compared to ZnO₁. This indicates that both ZnO treatments were effective in mitigating the detrimental effects of salinity on leaf area. The control condition, representing non-stressed plants, consistently displayed the highest LAPP at all sampling dates (1474.70, 2031.70 and 2348.80 cm²) respectively. This serves as a reference point, emphasizing that salinity stress indeed adversely affected overall plant growth and leaf area.

The statistical significance of the interaction effects between SS and ZnO implies that the combined influence of these factors had a significant impact on LAPP. This emphasizes that the effectiveness of ZnO-NP application in mitigating salinity stress is not solely due to the individual effects of each factor, but rather the interaction between them. Despite the overall reduction in LA under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ demonstrated the highest LA (933.91, 1245.80 and 1128.16 cm², respectively), which were almost identical to S₂ZnO₁ (870.87, 1107.10 and 1071.94 cm², respectively). This suggests that both ZnO treatments had a similar positive effect on leaf area plant⁻¹ under salt stress. The results indicate that salinity stress negatively affects LAPP, but the application of ZnO-NPs, particularly ZnO₂ and ZnO₁, effectively mitigates these adverse effects.

Table 3. Impact of ZnO on number of leaf area of wheat plant under saline conditions

Treatments	Leaf area (cm ²)		
	45 DAS	60 DAS	75 DAS
Levels of salt			
S ₀	1474.70 a	2031.70 a	2348.80 a
S ₁	1124.90 b	1503.40 b	1957.30 b
S ₂	809.55 c	1237.80 c	1621.42 c
LSD	272.73	331.01	362.17
Levels of ZnO			
ZnO ₀	1264.85 c	1635.00 c	1823.00 c
ZnO ₁	1472.33 b	1838.42 b	2118.70 b
ZnO ₂	1572.00 a	1999.53 a	2285.90 a
LSD	368.15	735.59	584.27
Interaction effect of salt and ZnO			
S ₀ ZnO ₀	1310.00 b	1693.70 b	1843.80 b
S ₀ ZnO ₁	1468.52 ab	1871.60 ab	2075.90 ab
S ₀ ZnO ₂	1545.72 a	1929.90 a	2126.70 a
S ₁ ZnO ₀	1060.71 de	1250.80 cd	1061.10 d
S ₁ ZnO ₁	1177.42 d	1336.60 c	1208.10 c
S ₁ ZnO ₂	1236.53 c	1422.90 bc	1292.70 c
S ₂ ZnO ₀	623.88 f	860.50 e	964.15 e
S ₂ ZnO ₁	870.87 ef	1107.10 d	1071.94 d
S ₂ ZnO ₂	933.91 e	1245.80 cd	1128.16 cd
LSD	468.66	635.69	678.23
CV (%)	24.04	21.49	33.63

The numerical value followed by same letter are not significant different by DMRT test at $p < 0.05$, CV: Coefficient of Variation, LSD: Least significant difference.

The significance of the interaction between salinity stress and ZnO highlights the complex relationship between these factors in influencing plant growth and leaf development, and certain treatment combinations, such as S₂ZnO₂, can enhance leaf area even under stressful conditions. These findings are in bond with [Ali *et al.* \(2022\)](#), [Lalarukh *et al.* \(2022\)](#) who also experienced a noticeable increase of leaf area in salt-stressed wheat plant when grown under salt-stress environment.

4.2 Impact of ZnO on the fresh and dry weight of wheat plant under saline conditions

4.2.1 Fresh and dry weight of leaves

The study investigated the impact of salinity stress on the leaf fresh weight (LFW) and leaf dry weight (LDW) per plant, with a particular focus on the application of ZnONPs to alleviate these effects. The results revealed a gradual decrease in both LFW and LDW as salinity levels increased. Specifically, the condition denoted as S₂, representing 150 mM NaCl stress, resulted in the lowest LFW (0.96, 1.01 and 1.44 g plant⁻¹) and LDW (0.141, 0.230, 0.256 and 0.278 g plant⁻¹) at various time points, respectively (Table 4), indicating a negative impact on leaf expansion and development. Interestingly, the application of ZnONPs, particularly ZnO₂, demonstrated a positive effect in mitigating the adverse effects of salt stress, leading to an increase in both LFW (1.172, 1.324, and 1.587 g plant⁻¹) and LDW (0.177, 0.278, 0.299 and 0.343 g plant⁻¹). Notably, both ZnO treatments (ZnO₁ and ZnO₂) showed similar effectiveness in promoting leaf growth under salinity stress. The control condition, representing non-stressed plants, consistently displayed the highest LFW and LDW, serving as a reference point to highlight the detrimental impact of salinity stress on overall leaf growth and plant growth.

The study emphasized the statistical significance of the interaction effects between salinity stress and ZnONP application. This implies that the combined influence of these factors had a significant impact on LFW and LDW. The effectiveness of ZnONP application in mitigating salinity stress was attributed to the interaction between salinity stress and ZnO, rather than the individual effects of each factor. Despite the overall reduction in LFW and LDW under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ demonstrated the highest LFW (0.957, 1.067 and 1.253 g plant⁻¹) and LDW (0.145, 0.203, 0.217 and 0.223 g plant⁻¹ respectively), almost identical to S₂ZnO₁. This suggests that both ZnO treatments had a similar positive effect on leaf growth under salt stress. The results indicate that salinity stress negatively affects leaf growth per plant, but the application of ZnO-NPs, particularly ZnO₂ and ZnO₁, effectively mitigates these adverse effects. The significance of the interaction between salinity stress and ZnO highlights the complex relationship between these factors in influencing plant growth and leaf development. Certain treatment combinations, such as

S₂ZnO₂, can enhance leaf area even under stressful conditions. Many researchers have been explained that application of nano-Zn increased the LFW and LDW under exposure to salinity and osmotic stress (Hussein and Abou-Baker, 2018; Mogazy and Hanafy, 2022).

Table 4. Impact of ZnO on the fresh and dry weight of leaves plant⁻¹ of wheat plant under saline conditions

Treatments	Fresh weight of leaves (g)			Dry weight of leaves (g)			
	45 DAS	60 DAS	75 DAS	45 DAS	60 DAS	75 DAS	FH
Levels of salt stress							
S ₀	1.25 a	1.52 a	1.74 a	0.171a	0.326 a	0.352 a	0.354 a
S ₁	1.03 b	1.19 b	1.53 b	0.149 b	0.226 b	0.296 b	0.303 b
S ₂	0.96 b	1.01 b	1.44 b	0.141 b	0.230 b	0.256 b	0.278 b
LSD	0.1463	0.2334	0.1396	0.026	0.035	0.0514	0.034
Levels of ZnO							
ZnO ₀	1.050 b	1.143 b	1.402 b	0.153 b	0.241 b	0.266 b	0.301 b
ZnO ₁	1.089 ab	1.243 ab	1.424 b	0.160 b	0.262 a	0.289 a	0.331 a
ZnO ₂	1.172 a	1.324 a	1.587 a	0.177 a	0.278 a	0.299 a	0.343 a
LSD	0.192	0.307	0.183	0.028	0.058	0.0700	0.041
Interaction effect of salt stress and ZnO							
S ₀ ZnO ₀	1.213 bc	1.397 b	1.607 b	0.151 bc	0.223 ab	0.257 ab	0.279 ab
S ₀ ZnO ₁	1.310 b	1.497 ab	1.737 a	0.165 b	0.237 a	0.270 a	0.287 ab
S ₀ ZnO ₂	1.427 a	1.553 a	1.780 a	0.187 a	0.247 a	0.287 a	0.323 a
S ₁ ZnO ₀	1.007 e	1.117 d	1.203 c	0.137 bc	0.210 b	0.237 b	0.250 bc
S ₁ ZnO ₁	1.157 d	1.267 c	1.353 cd	0.148 bc	0.213 b	0.247 ab	0.260 b
S ₁ ZnO ₂	1.233 bc	1.383 b	1.427 c	0.151 bc	0.223 ab	0.257 ab	0.270 b
S ₂ ZnO ₀	0.830 f	0.917 f	1.097 de	0.120 c	0.145 c	0.160 d	0.167 d
S ₂ ZnO ₁	0.910 ef	1.007 e	1.183 d	0.135 bc	0.177 bc	0.190 c	0.203 c
S ₂ ZnO ₂	0.957 e	1.067 de	1.253 d	0.145 bc	0.203 b	0.217 bc	0.223 c
LSD	0.290	0.435	0.2534	0.050	0.068	0.097	0.059
CV (%)	15.69	20.50	25.86	19.14	15.22	23.18	15.83

The numerical value followed by same letter are not significant different by DMRT test at p<0.05, CV: Coefficient of Variation, LSD: Least significant difference.

4.2.2 Fresh and dry weight of stem

The stem fresh weight (SFW) and stem dry weight (SDW) per plant showed a consistent decline as salinity levels increased. Specifically, under S₂ conditions, representing 150 mM NaCl stress, the lowest SFW (0.486, 0.833 and 1.106 g plant⁻¹) and SDW (0.111, 0.269, 0.318 and 0.390 g plant⁻¹)

were observed at 45, 60, 75 DAS, and at final harvest, respectively. This decrease in stem growth indicates that salinity stress impeded stem growth and development. However, the introduction of ZnO-NPs helped mitigate the negative effects of salt stress, resulting in increased SFW and SDW. Notably, ZnO₂ had a significant impact, yielding statistically similar SFW and SDW compared to ZnO₁, suggesting that both ZnO treatments effectively alleviated the detrimental effects of salinity on stem growth. The control group, representing non-stressed plants, consistently exhibited the highest SFW and SDW at all sampling dates, serving as a benchmark and emphasizing the adverse impact of salinity stress on overall plant growth.

The statistical significance of the interaction between salinity stress and ZnO indicates that the combined influence of these factors significantly affected SFW and SDW. This underscores that the efficacy of ZnO-NPs application in mitigating salinity stress is not solely attributable to the individual effects of each factor but rather to their interaction. Despite the overall reduction in SFW and SDW under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ demonstrated the highest SFW (0.510, 0.923 and 1.153 g plant⁻¹) and SDW (0.116, 0.167, 0.350 and 0.370 g plant⁻¹), which were almost identical to S₂ZnO₁. This suggests that both ZnO treatments had a similar positive impact on stem growth under salt stress. The findings suggest that salinity stress negatively impacts stem growth, but the application of ZnO-NPs, particularly ZnO₂ and ZnO₁, effectively mitigates these adverse effects. The significance of the interaction between salinity stress and ZnO underscores the intricate relationship between these factors in influencing stem growth and plant development, with certain treatment combinations, such as S₂ZnO₂, enhancing stem growth even under stressful conditions. The study highlights the adverse effects of salinity stress on leaf development and the ability of ZnO-NPs to alleviate these effects. The interaction between salinity stress and ZnO underscores the complexity of plant responses to stress and the potential for targeted interventions to mitigate negative impacts. [Hussein and Abou-Baker, 2018](#) [Lalarukh et al. 2022](#)

Table 5. Impact of ZnO on the fresh and dry weight of stem plant⁻¹ of wheat plant under saline conditions

Treatments	Fresh weight of stem (g)			Dry weight of stem (g)			
	45 DAS	60 DAS	75 DAS	45 DAS	60 DAS	75 DAS	FH
Levels of salt stress							
S ₀	0.666 a	1.198 a	1.592 a	0.142 a	0.310 a	0.516 a	0.546 a
S ₁	0.536 b	0.960 b	1.296 b	0.123 ab	0.269 b	0.338 b	0.419 b
S ₂	0.486 b	0.833 bc	1.106 b	0.111 b	0.253 b	0.318 b	0.390 c
LSD	0.134	0.222	0.266	0.015	0.033	0.093	0.084
Levels of ZnO							
ZnO ₀	0.521 b	1.097 c	1.234 b	0.151 b	0.200 b	0.328 b	0.369 b
ZnO ₁	0.610 ab	1.259 b	1.369 ab	0.164 a	0.229 a	0.408 a	0.437 a
ZnO ₂	0.656 a	1.390 a	1.428 a	0.174 a	0.233 a	0.436 a	0.499 a
LSD	0.150	0.229	0.306	0.016	0.033	0.108	0.100
Interaction effect of salt stress and ZnO							
S ₀ ZnO ₀	0.567 c	1.257 b	1.367 b	0.156 b	0.207 a	0.303 c	0.440 bc
S ₀ ZnO ₁	0.670 b	1.437 ab	1.533 ab	0.176 a	0.213 a	0.427 b	0.500 b
S ₀ ZnO ₂	0.750 a	1.500 a	1.777 a	0.174 a	0.220 a	0.507 a	0.607a
S ₁ ZnO ₀	0.523 c	0.910 e	0.927 e	0.114 c	0.163 bc	0.300 c	0.346 c
S ₁ ZnO ₁	0.540 c	1.023 d	1.107 d	0.116 c	0.183 b	0.420 b	0.390 c
S ₁ ZnO ₂	0.553 c	1.147 c	1.253 c	0.129 bc	0.203 a	0.473 ab	0.410 bc
S ₂ ZnO ₀	0.443 d	0.760 g	0.897 e	0.103 d	0.120 c	0.200 d	0.290 d
S ₂ ZnO ₁	0.483 d	0.817 f	1.067 d	0.118 c	0.150 bc	0.323 c	0.340 c
S ₂ ZnO ₂	0.510 c	0.923 e	1.153 d	0.116 c	0.167 bc	0.350 c	0.370 c
LSD	0.253	0.439	0.426	0.028	0.066	0.139	0.125
CV (%)	26.19	18.32	19.14	25.64	18.91	18.54	17.34

The numerical value followed by same letter are not significant different by DMRT test at $p < 0.05$, CV: Coefficient of Variation, LSD: Least significant difference.

4.2.3 Fresh and dry weight of root

The decline in root fresh weight (RFW) and root dry weight (RDW) per plant was observed as salinity levels increased, with the lowest values RFW (0.163, 0.201 and 0.237 g plant⁻¹) and RDW (0.052, 0.060, 0.076 and 0.108 g plant⁻¹) occurring under 150 mM NaCl stress (S₂) at 45, 60, 75 DAS, and at final harvest, respectively (Table 6). This reduction in root growth indicates the detrimental impact of salinity stress on leaf expansion and development. However, the introduction of ZnO-NPs mitigated these adverse effects, leading to an increase in both RFW (0.243, 0.274 and 0.291 g plant⁻¹) and RDW (0.080, 0.091, 0.142 and 0.147 g plant⁻¹). Notably, ZnO₂ exhibited the most significant impact, yielding statistically similar RFW and RDW compared to ZnO₁. This suggests the effectiveness of both ZnO treatments in alleviating the negative effects of salinity on leaf growth.

Table 6. Impact of ZnO on the fresh and dry weight of root plant⁻¹ of wheat plant under saline conditions

Treatments	Fresh weight of root (g)			Dry weight of root (g)			
	45 DAS	60 DAS	75 DAS	45 DAS	60 DAS	75 DAS	FH
Levels of salt stress							
S ₀	0.280 a	0.342 a	0.389 a	0.091 a	0.091 a	0.216 a	0.228 a
S ₁	0.186 b	0.228 b	0.247 b	0.062 b	0.069 b	0.089 b	0.128 b
S ₂	0.163 b	0.201 b	0.237 b	0.052 b	0.060 b	0.076 b	0.108 b
LSD	0.050	0.046	0.085	0.018	0.013	0.053	0.040
Levels of ZnO							
ZnO ₀	0.167 b	0.230 b	0.198 b	0.053 b	0.067 b	0.089 b	0.099 b
ZnO ₁	0.219 a	0.257 ab	0.273 a	0.073 ab	0.078 ab	0.121 a	0.129 a
ZnO ₂	0.243 a	0.274 a	0.291 a	0.080 a	0.091 a	0.142 a	0.147 a
LSD	0.063	0.075	0.133	0.021	0.018	0.086	0.068
Interaction effect of salt stress and ZnO							
S ₀ ZnO ₀	0.237 b	0.300 b	0.330 b	0.077 b	0.090 b	0.137 b	0.167 b
S ₀ ZnO ₁	0.347 a	0.377 a	0.410 a	0.083 b	0.096 b	0.140 b	0.177 b
S ₀ ZnO ₂	0.357 a	0.385 a	0.427 a	0.108 a	0.117 a	0.160 a	0.190 a
S ₁ ZnO ₀	0.147 c	0.210 c	0.153 e	0.049 c	0.062 c	0.070 d	0.077 c
S ₁ ZnO ₁	0.200 bc	0.226 c	0.213 d	0.067 bc	0.074 bc	0.113 c	0.119 bc
S ₁ ZnO ₂	0.210 bc	0.243 c	0.283 c	0.071 b	0.081 bc	0.123 c	0.127 bc
S ₂ ZnO ₀	0.117 d	0.138 d	0.161 d	0.033 d	0.042 d	0.050 e	0.053 d
S ₂ ZnO ₁	0.170 c	0.207 c	0.257 c	0.053 c	0.057 c	0.063 d	0.077 c
S ₂ ZnO ₂	0.183 bc	0.227 c	0.273 c	0.061 bc	0.067 c	0.073 d	0.083 c
LSD	0.064	0.079	0.149	0.023	0.022	0.087	0.074
CV (%)	17.92	17.84	35.19	19.63	17.99	42.00	41.73

The numerical value followed by same letter are not significant different by DMRT test at $p < 0.05$, CV: Coefficient of Variation, LSD: Least significant difference.

In comparison to non-stressed plants (control condition), which consistently displayed the highest RFW and RDW at all sampling dates, salinity stress adversely affected overall plant growth. The statistical analysis of the interaction effects between SS and ZnO indicates a significant combined influence on RFW and RDW. This underscores that the positive impact of ZnONP application in mitigating salinity stress is not solely attributed to the individual effects of each factor but rather the synergy between them. Despite the overall reduction in RFW and RDW under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ demonstrated the highest RFW (0.183, 0.227 and 0.273 g plant⁻¹) and RDW (0.061, 0.067, 0.073 and 0.083 g plant⁻¹), nearly identical to S₂ZnO₁. This

suggests that both ZnO treatments had a similar positive effect on root growth under salt stress. The findings suggest that salinity stress negatively affects root growth per plant, but the application of ZnONPs, particularly ZnO₂ and ZnO₁, effectively mitigates these adverse effects. The significance of the interaction between salinity stress and ZnO highlights the complex relationship between these factors in influencing root growth and plant development. Certain treatment combinations, such as S₂ZnO₂, can enhance leaf area even under stressful conditions. These trends are in harmony with those of [Adil *et al.* \(2022\)](#), [Mogazy and Hanafy \(2022\)](#), who reported that the reduction of RFW and RDW due to salt stress is attenuated by application of ZnO nano-particles in wheat.

4.3 Phenological Indices

4.3.1 Days of booting (DB)

In the ongoing investigation, the introduction of SS resulted in a notable acceleration of the booting stage by 10 days, significantly impacting the developmental stage of DB ([Table 7](#)). Substantial variations were observed in the time required for each treatment to reach the booting stage. Notably, within the SS condition, the S₂ treatment (150 mM NaCl) achieved the booting stage in 48.22 days, which required the minimum time to reach the booting stage, while S₀ taken required the longest duration at 53.79 days. The application of ZnONPs had a positive impact on DB, extending the growth duration of wheat plants to achieve the DB stage. However, ZnO₂ has taken the longer time to attain booting stage (55.67 days) that was comparable to ZnO₁ (53.00 days). The combined effect of SS and ZnO demonstrated a positive influence on DB, and notably, under high SS levels, ZnO₂ enhanced the DB stage (49.67 days) to a degree similar to the ZnO₁ treatment (48.00 days).

4.3.2 Days of heading (DH)

The influence of salt stress on the growth phase was demonstrated by a significant reduction in the number of DH ([Table 7](#)). This reduction was attributed to the accelerated onset of the reproductive stage, resulting in a decreased duration required for heading or flowering. Nevertheless, under different conditions, S₀ necessitated the maximum time to reach the heading stage, recording a duration of 59.56 days, while S₂ significantly shortened this period to 54.22 days. In contrast, the

application of ZnONPs through spraying revealed that the maximum DH was observed at 61.33 days, with ZnO₂ exhibiting a statistically comparable value to ZnO₁ treatment (60.78 days). The interaction between SS and ZnONPs exhibited noteworthy effects on DH. Specifically, the combination of S₂ZnO₂ enhanced the growth period required to achieve DH (54.67 days), with negligible differences compared to the S₂ZnO₁ (53.00 days) treatment in this study.

4.3.3 Days of anthesis (DA)

The DA was significantly influenced by SS, ZnO, and their interaction, as indicated in [Table 7](#). Salt stress played a significant role in reducing the time needed for anthesis, with this remarkable reduction being associated with higher levels of salt stress. However, noteworthy variations were observed under different conditions. S₀ required the maximum time to reach the heading stage, lasting for 64.33 days, while S₂ considerably shortened this period to 59.33 days. On the other hand, the application of ZnONPs through spraying demonstrated the higher value of DA. The maximum DA (71.33 days) was observed at ZnO₂ showing a statistically comparable value to the ZnO₁ treatment, recording 68.33 days. The interaction between SS and ZnONPs also had significant effects on DA. Specifically, the combination of S₂ZnO₂ extended the growth period required to achieve DH (61.67 days), with negligible differences compared to the S₂ZnO₁ treatment (60.600 days) in this study.

4.3.4 Days of physiological maturity (DPM)

The DPM exhibited significant influences from SS, ZnO, and their interaction, as outlined in [Table 7](#). Salt stress played a crucial role in reducing the time needed for physiological maturity, with a notable reduction associated with higher levels of salt stress. Distinctive variations were observed regarding the DPM under different salt conditions. Nevertheless, S₀ required the maximum time to reach the physiological maturity, taking 93.89 days, while S₂ significantly shortened this period to 82.00 days. Conversely, the application of ZnONPs through spraying revealed an increased value for DPM. The maximum DPM (99.78 days) was observed with ZnO₂, and it showed a statistically comparable value to the ZnO₁ treatment, which recorded 98.78 days. The interaction between SS

and ZnONPs also exerted significant effects on Days of physiological maturity (DPM). Specifically, the combination of S₂ZnO₂ extended the growth period required to achieve DH (87.00 days), with negligible differences compared to the S₂ZnO₁ treatment (86.67 days) in this study.

Table 7. Impact of ZnO on the Phenological Indices plant⁻¹ of wheat under saline conditions

Treatments	Days of booting	Days of heading	Days of anthesis	Days of maturation
Levels of salt				
S ₀	53.79 a	59.56 a	64.33 a	93.89 a
S ₁	49.44 b	54.89 b	60.67 b	81.67 b
S ₂	48.22 b	54.22 b	59.33 b	82.00 b
LSD	0.9593	0.9455	1.5638	1.3599
Levels of ZnO				
ZnO ₀	50.78 b	57.56 b	64.67 b	95.00 b
ZnO ₁	53.00 a	60.78 a	68.33 a	98.78 a
ZnO ₂	55.67 a	61.33 a	71.33 a	99.78 a
LSD	2.6163	2.6029	2.6792	6.0209
Interaction effect of salt and ZnO				
S ₀ ZnO ₀	53.33 a	60.33 ab	64.67 a	93.00 ab
S ₀ ZnO ₁	54.00 a	61.67 a	65.00 a	94.67 a
S ₀ ZnO ₂	55.00 a	62.67 a	66.33 a	96.00 a
S ₁ ZnO ₀	48.00 c	54.33 c	60.00 b	89.67 b
S ₁ ZnO ₁	49.00 bc	57.67 b	61.00 b	90.00 ab
S ₁ ZnO ₂	50.33 b	58.67 b	62.00 b	91.33 ab
S ₂ ZnO ₀	46.00 d	52.00 c	57.33 c	83.33 d
S ₂ ZnO ₁	48.00 c	53.00 c	60.00 b	86.67 c
S ₂ ZnO ₂	49.67 bc	54.67 c	61.67 b	87.00 c
LSD	1.513	1.439	2.578	2.722
CV (%)	1.75	1.49	2.45	1.85

The numerical value followed by same letter are not significant different by DMRT test at p<0.05, CV: Coefficient of Variation, LSD: Least significant difference.

4.4 Impact of ZnO on the physiological traits of wheat plant under saline conditions

4.4.1 Photosynthetic pigments

4.4.1.1 Chlorophyll a content

Salinity gradually decreased the chlorophyll a (Chl a) content, while application of ZnO alleviated the adverse effects of salt stress and increased the Chl a content (Table 8). However, the highest Chl a content (2.214 mg g⁻¹ FW) was observed under control condition and the lowest values (1.384 mg

g⁻¹ FW) were observed in S₂ condition (150 mM NaCl). In contrary, spraying of ZnONPs increased the Chl a content, and the highest value (1.841 mg g⁻¹ FW) were recorded ZnO₂ which was statistically identical to ZnO₁ (1.781 mg g⁻¹ FW). The interaction effects of SS and ZnO statistically significant. Nonetheless, under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ showed the highest Chl a content (1.509 mg g⁻¹ FW) and which were almost identical with the S₂ZnO₁ (1.387 mg g⁻¹ FW). [Alabdallah and Alzahrani \(2020\)](#), [Adil *et al.* \(2022\)](#), [Mogazy and Hanafy \(2022\)](#), [Lalarukh *et al.* \(2022\)](#) further corroborated the present results by demonstrating a rise in Chl a level in crops following the application of ZnO NPs through spraying.

4.4.1.2 Chlorophyll b content

The presence of salinity led to a gradual decrease in chlorophyll b (Chl b) content, while the application of ZnO helped alleviate the negative impact of salt stress, resulting in an increase in Chl b content. Despite this, the control condition exhibited the highest Chl b content at 1.139 mg g⁻¹ FW, whereas the lowest values, 0.676 mg g⁻¹ FW, were observed under the S₂ condition (150 mM NaCl). Contrastingly, the application of ZnONPs through spraying increased Chl b content, with the highest value of 0.976 mg g⁻¹ FW recorded for ZnO₂, which was statistically identical to ZnO₁ at 0.874 mg g⁻¹ FW. The statistical analysis revealed significant interaction effects between salinity stress (SS) and ZnO ([Table 8](#)).

Under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ demonstrated the highest Chl b content at 0.757 mg g⁻¹ FW, almost identical to S₂ZnO₁ at 0.747 mg g⁻¹ FW. This suggests that the combination of ZnO₂ with salinity stress had a comparable positive effect on Chl b content as ZnO₁. However, salinity stress reduced Chl b content, but the application of ZnONPs, particularly ZnO₂ and ZnO₁, effectively mitigated these adverse effects. The significance of the interaction between salinity stress and ZnO emphasizes the nuanced relationship between these factors in influencing chlorophyll content. Certain treatment chlorophyll content even under stressful combinations, such as S₂ZnO₂, demonstrated enhanced conditions. These results are in line with previously published report on wheat ([Adil *et al.*, 2022](#); [Lalarukh *et al.*, 2022](#)), faba bean ([Mogazy and Hanafy, 2022](#))

and okra ([Alabdallah and Alzahrani \(2020\)](#)) that foliar applied of Zn-NPs caused a remarkable increase in Chl b under saline conditions.

4.4.1.3 Total chlorophyll content (Chl a+b)

Salinity stress led to a gradual decrease in total chlorophyll (TChl) content, while the application of ZnO helped alleviate these adverse effects and increased TChl content ([Table 8](#)). However, the highest T Chl content (3.353 mg g⁻¹ FW) was observed under the control condition, whereas the lowest values (2.060 mg g⁻¹ FW) were recorded under S₂ conditions (150 mM NaCl). Conversely, the application of ZnONPs boosted T Chl content, with the highest value (2.817 mg g⁻¹ FW) recorded for ZnO₂, statistically identical to ZnO₁ (2.655 mg g⁻¹ FW). The interaction effects between salinity stress (SS) and ZnO were statistically significant. Nevertheless, under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ exhibited the highest TChl content (2.266 mg g⁻¹ FW), almost identical to S₂ZnO₁ (2.134 mg g⁻¹ FW). This finding is paralleled with the findings of [Alabdallah and Alzahrani \(2020\)](#), [Mogazy and Hanafy \(2022\)](#), [Lalarukh et al. \(2022\)](#), who depicted that Zn-NPs enhanced the total Chl contents in leaves. The likely cause of this increase in photosynthetic pigments content in plant leaves is ZnO's stimulating effect on carbonic anhydrase, which may have facilitated the CO₂ transport to carboxylation sites in the chloroplast. Zinc is required for proto-chlorophyllide production, chloroplast growth, and repair of photosystem II in plants ([Salama et al., 2019](#)).

4.4.1.4 Carotenoids content

Salinity stress led to a gradual decrease in carotenoids content, while the application of ZnO helped alleviate these adverse effects, resulting in an increase in carotenoids content ([Table 8](#)). Despite this, the highest carotenoids content (0.733 mg g⁻¹ FW) was observed under the control condition, with the lowest values (0.577 mg g⁻¹ FW) recorded in the S₂ condition (150 mM NaCl). In contrast, the spraying of ZnONPs contributed to an elevation in carotenoids content, with the highest value (0.523 mg g⁻¹ FW) recorded for ZnO₂, which was statistically similar to ZnO₁ (0.503 mg g⁻¹ FW).

Table 8. Impact of ZnO on the Chl a, Chl b, Chl and Carotenoid of wheat under saline conditions

Treatments	Chlorophyll (mg g ⁻¹ FW)			
	Chl a	Chl b	Total Chl	Carotenoids
Levels of salt				
S ₀	2.214 a	1.139 a	3.353 a	0.733 a
S ₁	1.854 b	0.857 b	2.711 b	0.642 ab
S ₂	1.384 c	0.676 c	2.06 c	0.577 b
LSD	0.2307	0.1231	0.120	0.172
Levels of ZnO				
ZnO ₀	1.631 b	0.821 b	2.452 c	0.676 b
ZnO ₁	1.781 ab	0.874 ab	2.655 b	0.868 a
ZnO ₂	1.841 a	0.976 a	2.817 a	0.949 a
LSD	0.393	0.223	0.297	0.139
Interaction effect of salt stress and ZnO				
S ₀ ZnO ₀	2.013 c	1.070 bc	3.083 bc	0.680 c
S ₀ ZnO ₁	2.187 b	1.110 b	3.297 b	0.770 b
S ₀ ZnO ₂	2.443 a	1.237 a	3.680 a	0.850 a
S ₁ ZnO ₀	1.673 e	0.780 d	2.453 d	0.543 d
S ₁ ZnO ₁	1.870 d	0.867 c	2.737 c	0.693 c
S ₁ ZnO ₂	1.920 cd	0.923 c	2.843 c	0.773 b
S ₂ ZnO ₀	1.107 h	0.613 e	1.720 g	0.403 e
S ₂ ZnO ₁	1.387 g	0.747 d	2.134 f	0.503 d
S ₂ ZnO ₂	1.509 f	0.757 d	2.266 e	0.523 d
LSD	0.337	0.210	0.311	0.192
CV (%)	10.82	13.76	9.39	13.50

The numerical value followed by same letter are not significant different by DMRT test at $p < 0.05$, CV: Coefficient of Variation, LSD: Least significant difference.

The interaction effects between salinity stress (SS) and ZnO were found to be statistically significant. However, under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ demonstrated the highest carotenoids content (0.523 mg g⁻¹ FW), which was nearly identical to S₂ZnO₁ (0.503 mg g⁻¹ FW). This suggests that both ZnO treatments had a similar positive impact on carotenoids content under salt stress conditions. Salinity stress gradually reduced Chl a content, but the application of ZnONPs, particularly ZnO₂ and ZnO₁, effectively mitigated these effects. The significance of the interaction between salinity stress and ZnO emphasizes the collaborative influence of these factors on carotenoids dynamics. The application of ZnO-NPs to plant foliage enhances carotenoid content in response to salinity stress as reported earlier in many studies ([Abdel](#)

Latef *et al.*, 2017; Mohsenzadeh and Moosavian, 2017; Alabdallah and Alzahrani, 2020; Mogazy and Hanafy, 2022; Lalarukh *et al.*, 2022).

4.4.2 Water status

4.4.2.1 Relative water content (RWC)

Salinity-induced a gradual decrease in RWC, but the application of ZnO effectively mitigated the adverse effects of salt stress, leading to an increase in RWC levels (Table 9). Nevertheless, the control condition exhibited the highest RWC (83.53%), whereas the lowest value (72.49%) was observed under S₂ conditions (150 mM NaCl). Conversely, the application of ZnONPs through spraying resulted in an enhancement of RWC, with the highest value (87.12%) recorded for ZnO₂, statistically similar to ZnO₁ (86.08%). The interaction effects between SS and ZnO were statistically significant. Despite the impact of 150 mM NaCl stress causing a decline in RWC, the treatment combination of S₂ZnO₂ demonstrated the highest RWC (75.49%), which was nearly identical to S₂ZnO₁ (73.81%). This highlights the effectiveness of ZnONP application, particularly with ZnO₂, in counteracting the negative effects of salinity stress on RWC. However, salinity stress reduced RWC, but the application of ZnONPs, especially ZnO₂ and ZnO₁, proved beneficial in alleviating these adverse effects. The statistical significance of the interaction between salinity stress and ZnO emphasizes the combined influence of these factors on RWC, demonstrating the potential of specific treatment combinations to enhance RWC even under stressful conditions. Several earlier studies showed that application of ZnONPs in stressed plants increased RWC of wheat plants (Spanò *et al.*, 2020; Lalarukh *et al.*, 2022).

4.4.2.2 Water saturation deficit (WSD)

Salinity led to a gradual increase in water saturation deficit (WSD), but the application of ZnO effectively alleviated the negative impacts of salt stress, resulting in decreased WSD levels. The control condition exhibited the lowest WSD, while the highest value (27.51) was observed under S₂ conditions (150 mM NaCl). Conversely, the application of ZnONPs through spraying declined WSD, with ZnO₂ recording the lowest value (12.88), statistically similar to ZnO₁ (13.92). The

interaction effects between SS and ZnO were statistically significant (Table 9). Regardless of the increase in WSD under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ demonstrated the lowest WSD (24.51), which was nearly identical to S₂ZnO₁ (26.19). This underscores the efficacy of ZnONPs application, particularly with ZnO₂, in mitigating the adverse effects of salinity stress on WSD. Salinity stress increased WSD, but the application of ZnONPs, especially ZnO₂ and ZnO₁, proved beneficial in alleviating these negative effects. The statistical significance of the interaction between salinity stress and ZnO emphasizes the combined influence of these factors on WSD, highlighting the potential of specific treatment combinations to reduce WSD even under stressful conditions.

4.4.2.3 Water retention capacity (WRC)

Salinity led to a gradual decline in water retention capacity (WRC), but the application of ZnO effectively alleviated the negative impacts of salt stress, resulting in increased WRC levels. The control condition exhibited the highest WRC (6.08), while the lowest value (4.88) was observed under S₂ conditions (150 mM NaCl). Conversely, the application of ZnONPs through spraying enhanced RWC, with ZnO₂ recording the highest value (6.87), statistically similar to ZnO₁ (6.70). The interaction effects between SS and ZnO were statistically significant (Table 9). Even though the decline in WRC under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ demonstrated the highest RWC (insert specific value), which was nearly identical to S₂ZnO₁ (5.82). This underscores the efficacy of ZnONP application, particularly with ZnO₂, in mitigating the adverse effects of salinity stress on RWC. The impact of salinity stress led to a reduction in Relative Water Content (RWC). However, the administration of ZnONPs, particularly ZnO₂ and ZnO₁, demonstrated positive effects in mitigating these adverse consequences. The statistical significance of the interaction between salinity stress and ZnO emphasizes the combined influence of these factors on WRC, highlighting the potential of specific treatment combinations to enhance RWC even under stressful conditions.

Table 9. Impact of ZnO on the RWC, WSD, WRC, and WUC of wheat under saline conditions

Treatments	Water status			
	RWC	WSD	WRC	WUC
Levels of salt				
S ₀	83.53 a	16.47 c	6.08 a	1.12 c
S ₁	80.02 b	19.98 b	5.39 b	1.52 b
S ₂	72.49 c	27.51 a	4.88 c	1.96 a
LSD	4.934	4.934	0.443	0.314
Levels of ZnO				
ZnO ₀	84.84 b	15.16 a	6.01 b	1.27 a
ZnO ₁	86.08 a	13.92 b	6.70 a	1.10 b
ZnO ₂	87.12 a	12.88 b	6.87 a	1.02 b
LSD	4.976	4.976	0.469	0.317
Interaction effect of salt stress and ZnO				
S ₀ ZnO ₀	82.62 b	17.38 d	6.09 ab	1.33 d
S ₀ ZnO ₁	84.49 ab	15.51 d	6.69 a	1.30 d
S ₀ ZnO ₂	86.49 a	13.51 e	6.97 a	1.13 e
S ₁ ZnO ₀	76.73 d	23.27 b	5.17 c	1.82 b
S ₁ ZnO ₁	78.94 c	21.06 c	5.77 b	1.56 c
S ₁ ZnO ₂	79.40 bc	20.60 c	6.02 ab	1.43 c
S ₂ ZnO ₀	69.17 e	30.83 a	4.06 d	2.15 a
S ₂ ZnO ₁	73.81 d	26.19 b	4.46 d	1.77 b
S ₂ ZnO ₂	75.49 d	24.51 b	5.82 b	1.53 c
LSD	9.609	9.607	0.847	0.624
CV (%)	7.43	22.72	7.94	28.06

The numerical value followed by same letter are not significant different by DMRT test at $p < 0.05$, CV: Coefficient of Variation, LSD: Least significant difference.

4.4.2.4 Water uptake capacity (WUC)

Salinity resulted in a gradual increase in WUC from 1.12 to 1.96, yet the application of ZnO effectively mitigated the adverse impacts of salt stress, leading to decreased WUC from 1.27 to 1.02 (Table 9). The control condition exhibited the lowest WUC (1.12), while the highest value (1.96) was observed under S₂ conditions (150 mM NaCl). Conversely, the application of ZnONPs through spraying reduced WUC, with ZnO₂ recording the lowest value (1.02), statistically similar to ZnO₁. The interaction effects between SS and ZnO were statistically significant. Notwithstanding the increase in WUC under 150 mM NaCl stress, the treatment combination of S₂ZnO₂ demonstrated the lowest WUC (1.53), which was nearly identical to S₂ZnO₁ (1.77). This underscores the efficacy of ZnONP application, particularly with ZnO₂, in mitigating the adverse effects of salinity stress on

WUC. Salinity stress elevated WUC, but the utilization of ZnONPs, especially ZnO₂ and ZnO₁, proved beneficial in alleviating these negative effects. The statistical significance of the interaction between salinity stress and ZnO emphasizes the combined influence of these factors on WUC, highlighting the potential of specific treatment combinations to reduce WUC even under stressful conditions.

4.4.3 Membrane Stability Index (MSI)

Salinity had a significant adverse impact on the membrane stability index (MSI) of wheat plant cells, resulting in a decline in values. However, the application of zinc oxide (ZnO) mitigated the toxic effects of salinity stress (SS) and contributed to increased MSI values with higher concentrations of ZnO (Table 10). Despite this improvement, the highest MSI (76.59%) was observed in the control condition, while the lowest (69.96%) occurred under S₂, representing 150 mM NaCl-induced salinity stress. In contrast, ZnO, when applied as nanoparticles, played a positive role in enhancing the plant environment, yielding the highest MSI value of 74.95% at ZnO₂, which was statistically comparable to ZnO₁. The lowest MSI value (71.59 %) was recorded in the absence of ZnONPs. The interaction effect resulting from the combination of salinity stress and ZnO on MSI was found to be significant.

Interestingly, the treatment combination of S₂ZnO₂ exhibited the highest MSI (72.74%), indicating an effective alleviation of salt stress effects. Conversely, the lowest MSI (66.65%) was observed in the S₂ZnO₀ treatment under severe salt stress condition (150 mM NaCl). This highlights the combined influence of salinity stress and ZnO on the MSI, emphasizing the potential of specific treatment combinations, such as S₂ZnO₂, to enhance the stability of wheat plant cell membranes even under challenging salt stress conditions. The outcome also line with Adrees *et al.* (2021) who stated that ZnNPs significantly increased the electrolyte leakage (EL) in wheat plant grown under Cd and water deficit stress conditions, Mushtaq *et al.* (2023) concluded that exogenous Zn increased the MSI or EL in proso millet under saline conditions.

4.5 Canopy Temperature

According to Berger *et al.* (2010), canopy temperature (CT) serves as an integrative indicator, reflecting the plant's water status and the equilibrium resulting from water uptake by the roots and transpiration by the shoots. The study observed a significant increase in CT under salt stress, and with the application of treatments, a subsequent decrease in CT was noted for salt-stressed plants at 9:00 am, 12:00 pm, and 3:30 pm (Table 10).

Table 10. Impact of ZnO on the CT and CMS of wheat plant under saline conditions

Treatments	Canopy Temperature (⁰ C)			CMS
	09.00 A.M	12.00 P.M	03.00 P.M	
Levels of salt				
S ₀	21.09 a	27.89 a	29.12 a	76.59 a
S ₁	19.80 b	25.80 c	27.43 b	73.99 ab
S ₂	19.30 b	26.77 b	29.08 a	69.96 b
LSD	2.0432	1.9575	1.8397	5.6578
Levels of ZnO				
ZnO ₀	20.59 a	28.33 a	27.28 b	71.59 b
ZnO ₁	19.36 b	25.86 b	28.76 ab	74.01 a
ZnO ₂	20.24 a	26.27 b	29.60 a	74.95 a
LSD	2.1195	1.8319	1.7497	6.1480
Interaction effect of salt and ZnO				
S ₀ ZnO ₀	24.13 a	31.00 a	29.37 b	75.68 b
S ₀ ZnO ₁	18.27 d	26.33 c	29.93 b	78.47 a
S ₀ ZnO ₂	20.87 b	26.33 c	28.07 c	75.63 b
S ₁ ZnO ₀	18.93 c	27.03 b	26.03 d	72.43 c
S ₁ ZnO ₁	19.70 c	24.37 d	27.50 c	75.86 b
S ₁ ZnO ₂	20.77 b	26.00 c	28.77 b	73.67 c
S ₂ ZnO ₀	18.70 c	26.97 b	26.43 d	66.65 e
S ₂ ZnO ₁	20.10 b	26.87 b	28.83 b	70.50 d
S ₂ ZnO ₂	19.10 c	26.47 c	31.97 a	72.74 c
LSD	2.736	2.631	1.935	10.855
CV (%)	7.95	5.72	3.95	8.61

The numerical value followed by same letter are not significant different by DMRT test at p<0.05, CV: Coefficient of Variation, LSD: Least significant difference.

The findings indicated that the highest CT values (19.30, 26.77, and 29.08 °C) were observed in the S₂ condition (150 mM salt stress) at 9:00 am, 12:00 pm, and 3:00 pm, respectively. In contrast, the lowest CT values (21.09, 27.89, and 29.12 °C) were recorded in the S₀ condition (control) i.e.

without NaCl-induced stress. Moreover, the application of ZnONPs demonstrated an improvement in the plant's microclimate, reflected in reduced CT values. The minimum CT value (20.24, 26.27, and 29.60 °C) was observed at ZnO₂, which was statistically comparable to ZnO₁ (19.36, 25.86, and 28.76 °C), while the highest CT values (20.59, 28.33, and 27.28 °C) were observed in the absence of ZnONPs. The interaction effect resulting from the combination of SS and ZnO on CT was deemed statistically significant. Particularly under severe salt stress, the treatment combination of S₂ZnO₂ exhibited the lowest CT, indicating a notable mitigation effect. Conversely, the highest CT values were observed without ZnO, specifically in the S₂ZnO₀ treatment combination. This underscores the significance of the interaction between SS and ZnO in influencing CT, highlighting the potential of specific treatment combinations, such as S₂ZnO₂, to ameliorate the impact of severe salt stress on the plant's microclimate. The result of the present findings partially corroborates with the results of [Ivushkin *et al.* \(2018\)](#).

4.6 Effect of ZnO on the yield contributing traits of wheat under saline conditions

4.6.1 Spike length (cm)

Significant variations were observed in the spike length of wheat across different levels of ZnO application under SS conditions, as depicted in [Table 11](#). Salinity exhibited a progressive decrease in spike length, with the lowest values observed at higher SS levels, particularly at 150 mM. Notably, the highest spike length (8.98 cm) was recorded in the absence of salt stress (S₀, 0 mM), while the lowest spike length (7.28 cm) was observed under S₂ conditions (150 mM NaCl). The application of ZnO resulted in a progressive increase in spike length, with the highest spike length (9.13 cm) observed with ZnO₂, followed by ZnO₁ (8.69 cm). The interaction effect between salt stress and ZnO was found to be significant concerning spike length. Particularly noteworthy was the treatment combination of S₂ZnO₂, which produced the longest spike (8.33 cm) under severe salt stress conditions, followed by S₂ZnO₁. This underscores the efficacy of ZnO application in mitigating the adverse effects of salt stress on spike length in wheat plants. The significant interaction between salt stress and ZnO highlights the importance of considering specific treatment

combinations to optimize spike length, especially under severe salt stress conditions. The results for the spike length of this research were similar to the results of Rizwan *et al.* (2019), Adil *et al.* (2022) who reported that the spike length of wheat increased significantly by Zn NPs application under stress conditions.

4.6.2 Number of spikelets per spike

Significant variations were observed in the number of spikelets per spike (NPPP) of wheat when different levels of ZnO were applied under varying SS conditions, as indicated in Table 11. Salinity stress demonstrated a progressive decrease in NPPP, with the lowest value recorded at the highest SS level of 150 mM. Conversely, the highest NPPP (9.00) was observed in the absence of salinity stress (S_0 , 0 mM NaCl), while the lowest NPPP value (7.96) was found in the S_2 condition (150 mM NaCl). The application of ZnO exhibited a progressive increase in NPPP, with the highest no. spikelet (9.56) observed in ZnO_2 , which was statistically identical to ZnO_1 (9.37). The interaction effect between salinity stress and ZnO was found to be significant concerning NPPP. Nonetheless, the treatment combination of S_2ZnO_2 produced the maximum NPPP (7.72) under severe salinity stress, followed by S_2ZnO_1 (7.40), and S_2ZnO_0 produced the minimum NPPP (7.03). Salinity stress led to a decrease in NPPP, while the application of ZnO mitigated this effect, resulting in increased NPPP. The significance of the interaction between salinity stress and ZnO highlights the potential of specific treatment combinations, such as S_2ZnO_2 , to positively influence NPPP, even under conditions of severe salinity stress. The results are certified by Rani *et al.* (2019) who observed that ZnNPs significantly increased the NPPP in wheat grown under saline condition.

4.6.3 Number of grains per spike

Substantial variations were evident in the number of grains per spike (NGPP) of wheat under the influence of different levels of ZnO and varying SS conditions (Table 11). Salinity stress displayed a gradual reduction in NGPP, reaching its lowest value at the highest SS level of 150 mM. Contrariwise, the absence of salinity stress (S_0 , 0 mM NaCl) yielded the highest NGPP (23.89), while the lowest NGPP value (18.56) was observed in the S_2 condition (150 mM NaCl). The

application of ZnO resulted in a progressive increase in NGPP, with the highest value (26.04) observed for ZnO₂, statistically similar to ZnO₁ (25.93). The interaction effect between SS and ZnO was found to be significant concerning NGPP. Notably, the treatment combination of S₂ZnO₂ produced the maximum NGPP (18.44) under severe salinity stress, followed by S₂ZnO₁ (17.00), while S₂ZnO₀ produced the minimum NGPP (15.22). Salinity stress contributed to a decrease in NGPP, while the application of ZnO mitigated this effect, resulting in increased NGPP. The significance of the interaction between salinity stress and ZnO underscores the potential of specific treatment combinations, such as S₂ZnO₂, to positively influence NGPP, even under conditions of severe salinity stress. The outcome also line with [Lalarukh *et al.* \(2022\)](#) who stated that ZnNPs significantly increased the NGPP in wheat plant under saline condition.

4.6.4 Hundred grains weight

The hundred grains weight (HGW) of wheat was varied significantly when different levels of zinc oxide (ZnO) were applied under varying SS conditions ([Table 11](#)). Salinity stress resulted in a progressive decrease in HGW, with the lowest value observed at the highest SS level of 150 mM. In contrast, the highest HGW (4.77 g) was observed in the absence of salinity stress (S₀, 0 mM NaCl), while the lowest HGW (3.60 g) was found in the S₂ condition (150 mM NaCl). The application of ZnO demonstrated a progressive increase in HGW, with the highest value (4.81 g) observed in ZnO₂, statistically identical to ZnO₁ (4.38 g). The interaction effect between salinity stress and ZnO was found to be significant concerning NPPP. Conspicuously, the treatment combination of S₂ZnO₂ produced the maximum HGW (3.84 g) under severe salinity stress, which was statistically identical with S₂ZnO₁ (3.62 g), and S₂ZnO₀ produced the minimum HGW (3.25 g). However, under salinity stress, there was a reduction in HGW, yet the spraying of ZnO cope this negative effect, leading to an increased HGW. The interaction between salinity stress and ZnO, particularly in specific treatments like S₂ZnO₂, emphasizes the potential for positive effects on HGW even under severe salinity conditions. This result is in accordance with the findings of [Adil *et al.* \(2022\)](#) who reported that seed weight.

Table 11. Impact of ZnO on spike length, spike of spike number, number of grains spike⁻¹, weight of hundred grain under saline condition

Treatments	Spike length (cm)	No. of spikelets spike ⁻¹	No. of grains spike ⁻¹	Hundred grains weight (g)
Levels of salt				
S ₀	8.98 a	9.00 a	23.89 a	4.77 a
S ₁	8.16 b	8.45 b	20.81 b	4.39 ab
S ₂	7.28 c	7.96 c	18.56 c	3.60 b
LSD	0.687	0.807	2.269	0.922
Levels of ZnO				
ZnO ₀	8.01 b	8.69 b	22.30 b	3.98 b
ZnO ₁	8.69 a	9.37 a	25.93 a	4.38 ab
ZnO ₂	9.13 a	9.56 a	26.04 a	4.81 a
LSD	0.689	0.893	3.777	0.955
Interaction effect of salt stress and ZnO				
S ₀ ZnO ₀	8.83 c	8.89 b	23.44 c	3.90 b
S ₀ ZnO ₁	8.99 b	9.11 a	24.22 b	4.12 b
S ₀ ZnO ₂	9.19 a	9.20 a	25.00 a	4.48 a
S ₁ ZnO ₀	8.33 d	8.14 c	18.22 e	3.48 c
S ₁ ZnO ₁	8.61 c	8.56 b	21.55 d	3.79 b
S ₁ ZnO ₂	8.75 c	8.74 b	21.67 d	3.90 b
S ₂ ZnO ₀	7.58 e	7.03 f	15.22 g	3.25 c
S ₂ ZnO ₁	7.92 d	7.40 e	17.00 f	3.62 b
S ₂ ZnO ₂	8.33 d	7.72 d	18.44 e	3.84 b
LSD	1.107	1.543	4.277	1.790
CV (%)	7.62	10.62	24.72	27.07

The numerical value followed by same letter are not significant different by DMRT test at $p < 0.05$, CV: Coefficient of Variation, LSD: Least significant difference.

4.7 Effect of ZnO on crop harvest indices of wheat under saline conditions

4.7.1 Grain yield (g plant⁻¹)

The grain of wheat demonstrated significantly sensitivity to NaCl-induced salt stress but exhibited positive responses to foliar spray of ZnO under salt stress conditions, as illustrated in [Table 12](#). However, the highest grain yield (1.16 g plant⁻¹) was recorded at control condition, whereas the lowest (0.68 g plant⁻¹) was recorded at 150 mM NaCl stress. The application of ZnO at different concentrations, dramatically improved the grain yield compared to the untreated plants. The ZnO₂ treatment showed the highest grain yield (1.27 g plant⁻¹), while ZnO₀ showed the lowest grain yield (0.91 g plant⁻¹). In case of ZnO with higher salt stress, the highest grain yield (0.65 g plant⁻¹) was

observed at S₂ZnO₂ (150 mM NaCl + 300 µM ZnO) which was at par with S₂ZnO₁, and the lowest (0.38 g plant⁻¹) was at S₂ZnO₀ (150 mM + 0 µM ZnO). It is reported that reduction of yield under salt stress against control condition was used as an indicator of tolerance to salt stress (Ochiai and Matoh, 2001). The study provides a clear overview of the findings regarding the grain yield response to salt stress and ZnO application. A similar enhancement grain yield due to application of ZnO under saline stress conditions was earlier reported by Adil *et al.* (2022) in wheat, Mazhar *et al.* (2023) in wheat and rice.

4.7.2 Straw yield (g plant⁻¹)

The straw yield of wheat displayed notable sensitivity to NaCl-induced salt stress but showed positive responses to foliar spray of ZnO under salt stress conditions (Table 12). However, the highest straw yield (1.29 g plant⁻¹) was observed under control conditions, while the lowest (0.94 g plant⁻¹) was recorded under 150 mM NaCl stress. Application of ZnO at various concentrations significantly improved straw yield compared to untreated plants, with ZnO₂ showing the highest grain yield (1.31 g plant⁻¹) and ZnO₀ displaying the lowest (1.12 g plant⁻¹). In interaction effect under higher salt stress levels, the highest straw yield (0.91 g plant⁻¹) was observed in S₂ZnO₂, which was comparable to S₂ZnO₁, while the lowest straw yield (0.79 g plant⁻¹) was recorded in S₂ZnO₀ (150 mM + 0 µM ZnO). Similar result was confirmed by Adil *et al.* (2022), wherein it was concluded that foliar application of ZnO in saline-stressed wheat plants increased biomass yield. The Zn supply and adequate Zn availability to plant under salt stress improves grain yield of both wheat and rice (Daneshbakhsh *et al.*, 2013; Rani *et al.*, 2019) due to improved water relations, maintenance of higher RWC, turgor and photosynthetic pigments. (Jabeen and Ahmad 2012; Saeidnejad and Kafi, 2013).

4.7.3 Biological yield (g plant⁻¹)

The imposition of NaCl-induced salt stress significantly decreased the biological yield of wheat (cv. BWMRI Gom 2). However, the negative impact was mitigated by the positive influenced of foliar spraying of ZnO under salt stress conditions (Table 12). The highest biological yield (2.40 g plant⁻¹)

was recorded under the control condition, whereas the lowest ($1.62 \text{ g plant}^{-1}$) was observed at 150 mM NaCl stress. The application of ZnO at different concentrations significantly enhanced biological yield compared to untreated plants. Notably, ZnO₂ exhibited the highest biological yield ($2.48 \text{ g plant}^{-1}$), while ZnO₀ showed the lowest biological yield ($2.03 \text{ g plant}^{-1}$). In case of interaction effect under higher salt stress, the highest biological yield ($1.56 \text{ g plant}^{-1}$) was observed at S₂ZnO₂ (150 mM NaCl + 300 μM ZnO), which was statistically alike to S₂ZnO₁, while the lowest was at S₂ZnO₀ (150 mM + 0 μM ZnO). The results of the present study are in good agreement with the early findings of [Adil *et al.* \(2022\)](#), who concluded that the above ground biomass yield of wheat increased with the addition ZnONPs under saline stress condition.

4.7.4 Harvest Index (%)

NaCl-induced salinity stress significantly decreased the harvest index (HI), and application of ZnO was applied through foliar spray considerably enhanced the HI in this study ([Table 12](#)). However, the highest HI (47.35%) was recorded under control conditions, while the lowest (41.98%) was observed under 150 mM NaCl stress. ZnO₂ treatment produced the highest HI (47.18%), while ZnO₀ displayed the lowest HI (44.83%). This suggests that the foliar application of ZnO has a positive impact on mitigating the adverse effects of salt stress on HI. Under higher salt stress levels, S₂ZnO₂ (150 mM NaCl + 300 μM ZnO) produced the highest HI (41.67%) which was comparable to S₂ZnO₁. Conversely, the lowest HI was recorded in S₂ZnO₀ (150 mM + 0 μM ZnO). This emphasizes the role of ZnO in enhancing grain yield even under severe salt stress conditions. It is noteworthy that the reduction in HI salt stress, compared to the control condition, serves as an indicator of tolerance to salt stress, aligning with the findings reported by [Ochiai and Matoh \(2001\)](#). The positive responses to ZnO application further underscore its potential as a beneficial intervention to enhance wheat tolerance to salt-induced stress. The result of the present findings corroborates with the results of [Adil *et al.* \(2022\)](#) wherein ZnONPs increased the HI of wheat grown under salt stress.

Table 12. Impact of ZnO on the grain yield, straw yield, biological yield and harvest index of wheat plant under saline conditions

Treatments	Grain yield (g plant ⁻¹)	Straw yield (g plant ⁻¹)	Biological yield (g plant ⁻¹)	Harvest index (%)
Levels of salt				
S ₀	1.16 a	1.29 a	2.4 a	47.35 a
S ₁	0.93 b	1.18 b	2.11 b	44.08 b
S ₂	0.68 c	0.94 c	1.62 c	41.98 c
LSD	0.073	0.573	0.132	5.3108
Levels of ZnO				
ZnO ₀	0.91 b	1.12 b	2.03 b	44.83 b
ZnO ₁	1.14 a	1.28 a	2.40 a	46.67 a
ZnO ₂	1.27 a	1.31 a	2.48 a	47.18 a
LSD	0.843	0.136	0.235	6.292
Interaction effect of salt stress and ZnO				
S ₀ ZnO ₀	0.94 b	1.12 b	2.06 b	45.63 c
S ₀ ZnO ₁	1.00 a	1.17 a	2.17 b	46.08 b
S ₀ ZnO ₂	1.13 a	1.24 a	2.37 a	47.68 a
S ₁ ZnO ₀	0.63 c	1.09 b	1.72 c	36.63 e
S ₁ ZnO ₁	0.81 b	1.07 b	1.88 b	43.09 d
S ₁ ZnO ₂	0.86 b	1.12 b	1.99 b	43.72 d
S ₂ ZnO ₀	0.38 d	0.79 c	1.17 e	32.48 f
S ₂ ZnO ₁	0.58 c	0.88 c	1.46 d	39.73 e
S ₂ ZnO ₂	0.65 c	0.91 c	1.56 c	41.67 d
LSD	0.538	1.108	1.342	0.980
CV (%)	16.22	9.32	12.42	10.74

The numerical value followed by same letter are not significant different by DMRT test at p<0.05, CV: Coefficient of Variation, LSD: Least significant difference.

4.8. Effect of ZnO on biochemical traits of wheat under saline conditions

4.8.1 Na content

Significant variation was observed in Na content due to the application of different levels of ZnO in saline conditions (Table 13). Results showed that the highest Na content (10.42 mg g⁻¹ DW) was observed in S₂ (150 mM NaCl) in salt stress and the lowest (2.53 mg g⁻¹ DW) was in control condition. The application of ZnO resulted in a decrease in the accumulation of sodium ions (Na⁺) in wheat plants. Under higher SS, ZnO₂ reduced to accumulate of Na followed by ZnO₁ as compare to with ZnONPs. In the absence of ZnO treatment, there was an increase in sodium (Na) levels in plants; however, upon the application of ZnO, the adverse effect of Na was mitigated in the plant

system, as reported by (Ahmad *et al.*, 2023). Ahanger *et al.* (2018) found that a high concentration of Na in soil solution induces osmotic potential, which ultimately results in cell death due to reduced water uptake, and promotes the loss of natural soil fertility. In addition, Assaha *et al.* (2017) found that too much Na promotes wilting in plants. Zhang *et al.* (2019b) found that excessive Na has a detrimental effect on biomass production, soil enzymatic activities, microbial population, and soil microbial activities.

4.8.2 K content

Significant variation was observed in K content due to the application of different levels of ZnO in saline conditions (Table 13). Results showed that the highest value of K content ($3.25 \text{ mg g}^{-1} \text{ DW}$) was recorded from $S_0\text{ZnO}_2$ (0 mM + 300 μM). In case of ZnO application in saline condition, the highest K content ($2.59 \text{ mg g}^{-1} \text{ DW}$) was observed in $S_1\text{ZnO}_2$ (100 mM + 300 μM) in salt stress, and the lowest K content $1.49 \text{ mg g}^{-1} \text{ DW}$ found at $S_2\text{ZnO}_0$ (150 mM + 0 μM). However, at higher SS condition, ZnO remarkably increased the K content (1.75 to 2.05) $\text{mg g}^{-1} \text{ DW}$ accounting by ($S_2\text{ZnO}_1, S_2\text{ZnO}_2$) which was statically similar. Additionally, when compared to plants that were not treated, the application of exogenous ZnO increased the K contents. Both Ahmad *et al.* (2023) in maize and Faizan *et al.* (2021) in tomato reported closely analogous findings. The outcomes suggested that ZnO might be employed as a possible growth regulator to increase plant resistance to salt stress. As per Ahmad *et al.* (2023), during salinity stress, there is enhanced K^+ absorption and reduced Na^+ levels at the cellular level. Mineral ions, particularly K^+ , play a crucial role in preserving cell turgidity and are essential for activating enzymes associated with various metabolic activities. Saline stress induces an ionic imbalance, K^+ deficiency, and a high Na^+/K^+ ratio, restricting plant growth at both cellular and organismic levels. However, the application of ZnO NPs enhances tolerance to saline stress in plants by reducing Na^+ uptake, facilitating K^+ accumulation, and maintaining a lower Na^+/K^+ ratio. This improvement in ionic balance contributes to enhanced plant growth even under saline-stress conditions. At the membrane transport level, this

positive effect is reflected in both increased K^+ uptake and decreased Na^+ levels (Shabala and Pottosin, 2014).

4.8.3 Ca content

Significant variation was observed in Ca content shown of wheat for applying different levels of ZnO in saline conditions (Table 13). Results showed that the maximum Ca content of 2.31 mg g^{-1} DW was recorded from S_0ZnO_0 (0 mM + 0 μM). In case of ZnO with salt, the highest Ca content (1.63 mg g^{-1} DW) was found in S_1ZnO_2 (100 mM + 300 μM). On the other hand, the lowest Ca content of 1.05 mg g^{-1} DW was observed at S_2ZnO_0 (150 mM + 0 μM). The current study revealed that salinity stress led to a decrease in calcium (Ca) content, but the application of ZnO spray in saline environments increased Ca levels by mitigating the effects of salinity stress. The use of zinc oxide emerged as a crucial factor in enhancing the absorption of calcium and potassium. The study investigated the interactive effects of zinc oxide (ZnO) nanoparticles and salinity stress on two cultivars of *Trigonella foenum-graecum*. Four salinity levels and three ZnO nanoparticle levels were employed, with subsequent measurements of various biochemical parameters, trigonelline, antioxidant enzymes, and elemental concentrations such as Na, K, Ca, Zn, and Fe. The findings, as reported by Noohpisheh *et al.* (2021), highlighted the positive impact of ZnO application in counteracting salinity-induced changes in calcium levels.

4.8.4 N content

The application of varying levels of ZnO in saline conditions resulted in significant variations in nitrogen (N) content (Table 13). Results showed that the highest value of N content (2.55 mg g^{-1} DW) was recorded from S_0Se_0 (0 mM + 0 μM), and the lowest N content 1.35 mg g^{-1} DW found at S_2ZnO_0 (150 mM + 0 μM). In case of ZnO application in saline condition, the highest N content (2.08 mg g^{-1} DW) was observed in S_1ZnO_2 (100 mM + 300 μM) in salt stress.

Salinity stress, characterized by ionic, oxidative, and osmotic challenges, can significantly impede crop growth and yield (Taha *et al.*, 2021). Additionally, soil salinity has been observed to reduce plant growth, vigor, carbon fixation, nutrient cycling, and soil porosity (Cheeseman, 2015; Almeida *et al.*, 2017). The adverse effects extend to the reduction in water intake and the availability of crucial nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn). The excessive uptake of harmful ions further contributes to a detrimental impact on plant growth (Khan *et al.*, 2019; Safdar *et al.*, 2019).

Table 13. Effect of ZnO on Na, K, Ca, N and protein content of wheat under saline conditions

Treatments	Na (mg g ⁻¹ DW)	K (mg g ⁻¹ DW)	Ca (mg g ⁻¹ DW)	N (mg g ⁻¹ DW)	P (%)
Levels of salt stress					
S ₀	3.19 c	4.79 a	2.46 a	2.79 a	11.35 a
S ₁	6.08 b	2.07 b	1.48 b	1.87 b	8.57 b
S ₂	8.87 a	1.76 b	1.18 c	1.56 c	6.89 c
LSD	2.0498	0.461	0.353	0.247	3.0125
Levels of ZnO					
ZnO ₀	6.66 a	1.85 c	1.57 c	1.85 c	8.30 c
ZnO ₁	5.94 b	2.14 b	1.70 b	2.07 b	9.02 b
ZnO ₂	4.53 c	2.63 a	1.86 a	2.31 a	9.50 a
LSD	0.632	0.357	0.1332	0.2412	0.5081
Interaction effect of salt stress and ZnO					
S ₀ ZnO ₀	2.53 f	2.38 b	2.31 b	2.55 b	10.75 b
S ₀ ZnO ₁	3.47 e	2.75 b	2.45 b	2.76 b	11.55 a
S ₀ ZnO ₂	3.57 e	3.25 a	2.64 a	3.08 a	11.76 a
S ₁ ZnO ₀	7.03 c	1.70 c	1.35 c	1.65 d	7.62 e
S ₁ ZnO ₁	5.77 d	1.94 c	1.48 c	1.89 c	8.73 d
S ₁ ZnO ₂	5.41 d	2.59 b	1.63 c	2.08 c	9.37 c
S ₂ ZnO ₀	10.42 a	1.49 d	1.05 d	1.35 e	6.53 f
S ₂ ZnO ₁	8.58 b	1.75 c	1.17 d	1.56 d	6.78 f
S ₂ ZnO ₂	7.62 c	2.05 c	1.32 c	1.78 d	7.38 e
LSD	1.047	0.208	0.218	0.355	2.210
CV (%)	5.76	4.33	4.70	8.12	8.19

The followed by same letter are not significant different by DMRT test at p<0.05, CV: Coefficient of Variation, LSD: Least significant difference, S₀ = 0 mM, S₁ = 100 mM and S₂ = 150 mM NaCl, ZnO₀ = 0 μM, ZnO₁ = 150 μM and ZnO₂ = 300 μM zinc oxide.

4.8.5 Protein content (%)

The imposition of 150 mM NaCl stress significantly reduced the protein content in wheat grain compared to the control. Notably, the application of different levels of ZnO in saline soil exerted a significant influence on the protein content of wheat grains (Table 12). The study revealed that the highest protein content (11.76%) was observed in the S_0ZnO_2 treatment (0 mM NaCl + 300 μ M ZnO), whereas the lowest protein content (6.53%) was recorded in the S_2ZnO_0 treatment (150 mM NaCl + 0 μ M ZnO). The application of ZnO in salt-stressed plants significantly increased protein content compared to plants without ZnO application. Particularly, the treatment combination S_2ZnO_2 (150 mM NaCl + 300 μ M ZnO) exhibited the highest protein content (9.37%) under severe salinity stress (150 mM NaCl). The salinization of soil impeded N accumulation, thereby reducing protein levels. The decrease in N under salinity conditions was associated with a decline in protein content. Additionally, the electron transfer from O_2 to H_2O_2 and OH negatively affected proteins, DNA, and lipids (Mittler, 2002).

CHAPTER 5

SUMMARY AND CONCLUSION

A pot experiment was conducted at the Department of Agronomy, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during November 2022 to March 2023 to examine the salt alleviating effects of ZnO in wheat (cv. BWMRI Gom 2). The conducted experiment comprised three levels of salt stress (0, 100 and 150 mM NaCl stress), and three levels of ZnO (0, 150 and 300 μ M) i.e. included nine treatments viz., i) S₀ZnO₀ (0 mM NaCl + 0 μ M ZnO), ii) S₀ZnO₁ (0 mM NaCl + 150 μ M ZnO), iii) S₀ZnO₂ (0 mM NaCl + 300 μ M ZnO), iv) S₁ZnO₀ (100 mM NaCl + 0 μ M ZnO), v) S₁ZnO₁ (100 mM NaCl + 150 μ M ZnO), vi) S₁ZnO₂ (100 mM NaCl + 300 μ M ZnO), vii) S₂ZnO₀ (150 mM NaCl + 0 μ M ZnO), viii) S₂ZnO₁ (150 mM NaCl + 150 μ M ZnO), ix) S₂ZnO₂ (150 mM NaCl + 300 μ M ZnO). The experiment was laid out in Completely Randomized Design (CRD) with three replications. Twenty seeds were sown in each pot and later thinned out to ten plants per pot. Intercultural operations like thinning, weeding etc. were done when necessary. The data were recorded including morphological traits like plants height, number of leaves, fresh and dry weight of leaves, stems and roots at 45, 60, 75 DAS and at FH. Other physiological traits viz., photosynthetic pigments and water status (RWC, WSD, WRC, WUC) at vegetative stage, yield contributing character viz., spike length (cm), number of spikelets spike⁻¹, number of grains spike⁻¹, 100 grains weight (g), and yield indices like grain yield (g plant⁻¹), stover yield (g plant⁻¹), biological yield (g plant⁻¹), harvest index (%) and biochemical traits. The recorded data were analyzed through using MSTAT-C program, and mean difference was compared by LSD at 5% level of probability.

The effect of different levels of ZnO under saline stress was observed in this study, and significant result was observed at all most the parameters. The results revealed that salt negatively affected the studied parameters and addition of ZnO alleviated the adverse effects of salinity. The plants grown

under S_0ZnO_2 (0 mM NaCl + 300 μ M ZnO) condition produced the highest values of plant height (52.07, 57.33, 66.67 and 72.89 cm), and number of leaves $plant^{-1}$ (5.67, 6.67, 7.67, and 8.67), fresh weight of leaves (1.427, 1.553 and 1.780 g $plant^{-1}$) and dry weight of leaves (0.187, 0.247, 0.287 and 0.323 g $plant^{-1}$), fresh weight of stems (0.750, 1.500 and 1.777 g $plant^{-1}$) and dry weight of stems (0.174, 0.220, 0.507 and 0.607 g $plant^{-1}$), fresh weight of roots (0.357, 0.385 and 0.427 g $plant^{-1}$) and dry weight of roots (0.108, 0.117, 0.160 and 0.190 g $plant^{-1}$), while plants grown under stress condition S_2ZnO_0 (150 mM NaCl + 0 μ M ZnO) treatment produced the lowest values of plant height (40.000, 46.667, 52.333 and 56.167 cm) and number of leaves $plant^{-1}$ (4.00, 4.67, 5.00, and 5.67), fresh weight of leaves (0.830, 0.917 and 1.097 g $plant^{-1}$) and dry weight of leaves (0.120, 0.145, 0.160 and 0.167 g $plant^{-1}$), fresh weight of stems (0.443, 0.760 and 0.897 g $plant^{-1}$) and dry weight of stems (0.103, 0.120, 0.200, and 0.290 g $plant^{-1}$), fresh weight of roots (0.117, 0.138 and 0.161 g $plant^{-1}$) and dry weight of roots (0.033, 0.042, 0.050 and 0.053 g $plant^{-1}$) at 45, 60, 75 DAS and FH, respectively.

Physiological attribute viz. photosynthetic pigments like Chl a, Chl b and total Chl, Carotenoids water status like RWC, WSD, WRC and WUC were recorded. However, salt stress causes significant reduction in Chl a, Chl b and total Chl content. Application of ZnO ameliorated the adverse effects of salt and contributed in the increase in photosynthetic pigments content in plant parts. The RWC and WRC decreased significantly with the imposition of salt stress; however, application of ZnO in saline soil remarkably increased the RWC and WRC in leaves. On the other hand, the WSD and WUC were increased with salinity stress, and application of ZnO in saline stressed plants decreased the values of those traits. Application of ZnO 300 μ M in salt stressed plants was more effective to improve the water status in plants that reflected higher dry matter production but didn't show any statistical differences in almost all the studied parameters.

Yield contributing traits and yield were affected by the salt stress treatment, and addition of ZnO positively enhanced those values. Plants grown under S_0ZnO_2 (0 mM NaCl + 3000 μ M ZnO)

condition always showed the highest values of traits, and addition ZnO markedly increased the spike length (9.19 cm), number of spikelet spike⁻¹ (9.20), number of grains spike⁻¹ (25.00) and 100 grains weight (4.48 g) which statistically similar S₁ZnO₁ (100 mM NaCl + 150 µM ZnO) treatment. Stressed-plant with ZnO application i.e. S₁ZnO₀ (100 mM NaCl + 0 µM ZnO) also produced the highest grain yield (0.63 g), stover yield (1.09 g), biological yield (1.72 g) and highest harvest index (36.63 %). Plants grown under stress condition without ZnO i.e. S₂ZnO₀ (150 mM NaCl + 0 µM ZnO) constantly produced the minimum values of spike length (7.77 cm), number of spikelet spike⁻¹ (7.33), number of grains spike⁻¹ (6.22) and 100 grains weight (4.25 g), grain yield (0.24 g plant⁻¹), straw yield (0.66 g plant⁻¹), biological yield (0.91 g plant⁻¹) and harvest index (27.12 %) were found in salt condition.

In addition, salinity significantly affected various biochemical indicators. Upon administering ZnO at different doses, the findings indicated that salinity stress increased Na content while decreasing K, Ca, N, and protein content. However, the application of ZnO resulted in elevated values for, Ca, N, and protein, and a reduction in Na levels, countering the adverse effects of salinity.

In higher salt (150 mM) with ZnO₂ treatments showed the highest values of K (2.05 mg g⁻¹ DW), and Ca (1.32 mg g⁻¹ DW), and N (1.78 mg g⁻¹ DW), and protein (7.38 %) at S₂ZnO₂ (150 mM + 300 µM). On the other hand, the minimum amount of K (1.49 mg g⁻¹ DW), and Ca (1.05 mg g⁻¹ DW), N (1.35 mg g⁻¹ DW) and protein content (6.53%) were found at S₂ZnO₀ (150 mM + 0 µM) treatment. Saline condition highest values of Na (10.42 mg g⁻¹ DW) were found at S₂ZnO₀ (150 mM + 0 µM) treatment, while lowest value of Na (7.62 mg g⁻¹ DW) was found at S₂ZnO₂ (100 mM + 300 µM) treatment.

The study implies that salinity adversely affected various aspects of wheat, including morphology, growth, phenology, physiology, yield-contributing characteristics, yield, and biochemical traits. The application of ZnO proved beneficial in alleviating salt stress and improving these traits. Notably, ZnO at a concentration of 300 µM zinc yielded the best results, closely followed by the 150 µM

concentration, showing statistical similarity in most cases. In conclusion, it is recommended to apply a 150 μM concentration of ZnO under saline stress conditions, making wheat cultivation feasible up to 100 mM NaCl-induced salt stress. Therefore, the cultivation of wheat, specifically the BWMRI Gom 2 variety, can be successful under mild salt stress conditions (up to 100 mM stress) by incorporating additional zinc oxide (ZnO) at a concentration of 150 μM . Further experiments under natural salinity stress conditions are suggested for a more comprehensive understanding.

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APPENDICES

Appendix I: Monthly recorded of air temperature, rainfall, relative humidity and sunshine hour of experimental site Dinajpur

Months	Temperature (°C)		Relative humidity (%)	Total precipitation days (mm)	Total sunshine (hr)
	Minimum	Maximum			
November 2022	23.84	33.17	82.01	9.37	9.86
December 2022	17.12	27.81	63.37	3.15	9.16
January 2023	15.29	27.24	56.16	1.17	9.17
February 2023	18.08	31.58	45.93	1.95	10.1
March 2023	22.2	36.51	39.82	4.58	12.38

Source: Bangladesh Meteorological Department (Weather Research Station) Nashipur, Dinajpur

Appendix II: Morpho-physio-chemical properties of soil (collected before sowing of seeds) of the experimental field

A. Morphological characteristics of the soil

Constituents	Characteristics
Location	Net House, Department of Agronomy, HSTU, Dinajpur
Agro-ecological zone	Old Himalayan Piedmont Plain (AEZ-1)
Geographical position	25°38' N latitude and 88°4' E longitude
General soil type	Non-calcareous dark grey floodplain
Parent material	Old Brahmaputra River borne deposit
Land type	Medium high land
Elevation	37 Meter above the mean sea level
Drainage	Well drained
Topography	Fairly level
Soil texture	Sandy loam
Soil color	Dark grey
Flood level	Above flood level

B. Physical properties of the initial soil (0-15 cm depth)

Constituents	Results
Particle size analysis	
Sand (%) (0.2-0.002mm)	58
Silt (%) (0.02-0.002mm)	28
Clay (%) (<0.002)	14
Soil textural class	Sandy loam

Source: Results obtained from the mechanical analysis of the initial soil analysis.

C. Chemical composition of the initial soil (0-15 cm depth)

Characteristics	Value (%)
pH (Soil: water= 1: 25)	5.41
Organic matter	1.48
Organic carbon	0.72
Total N	0.08
Available P (ppm)	11.20
Exchangeable P (meq)	0.10
Exchangeable (meq)	2.48
Exchangeable Mg (meq)	2.29
Available S (ppm)	17.29
Available B (ppm)	0.13
Available Zn (ppm)	0.90
Available Fe (ppm)	51.90
Available Mn (ppm)	12.13

Source: Results obtained from the chemical analysis of the initial soil sample (SRDI, Dianjpur)

Appendix III: Analysis of variance (mean square) of the data for the plant height of wheat

Source of variation	df	Plant height (cm)			
		45 DAS	60 DAS	75 DAS	FH
Salinity	2	29.2500	92.9259	98.8611	138.912
ZnO	2	2.78778	4.8426	21.5278	24.1567
Salt × ZnO	4	8.72833	24.7245	31.0417	43.3883
Error	16	8.51741	8.4722	10.7407	8.0543

Appendix IV: Analysis of variance (mean square) of the data for the number of leaves plant⁻¹ of wheat

Source of variation	df	Number of leaves plant ⁻¹			
		45 DAS	60 DAS	75 DAS	FH
Salinity	2	2.51947	0.48148	2.70370	0.48148
ZnO	2	0.21367	0.25926	2.25926	0.48148
Salt × ZnO	4	0.71916	0.23148	1.67593	0.25926
Error	16	0.28042	0.22222	0.66667	0.48148

Appendix V: Impact of ZnO on number of the fresh and dry weight of leaves plant⁻¹ of wheat plant under saline conditions

Source of variation	df	Fresh weight of leaves (g)			Dry weight of leaves (g)			
		45 DAS	60 DAS	60 DAS	35 DAS	55 DAS	60 DAS	FH
Salinity	2	0.20633	0.59833	0.21070	2.229	0.02873	0.03123	8.781
ZnO	2	0.01113	0.09107	0.03524	4.203	1.048	2.633	2.181
Salt × ZnO	4	0.05479	0.17760	0.06537	7.306	7.529	8.958	3.151
Error	16	0.02872	0.06431	0.02182	8.619	1.570	3.211	1.196

Appendix VI: Impact of ZnO on number of the fresh and dry weight of stem plant⁻¹ of wheat plant under saline conditions

Source of variation	df	Fresh weight of stem (g)			Dry weight of stem (g)			
		45 DAS	60 DAS	60 DAS	35 DAS	55 DAS	60 DAS	FH
Salinity	2	0.07770	0.07010	0.60343	3.981	2.815	0.08151	0.07751
ZnO	2	0.01808	0.03096	0.31781	1.314	1.815	0.04411	0.04018
Salt × ZnO	4	0.02712	0.02590	0.23636	1.872	1.565	0.03293	0.02988
Error	16	0.02167	0.06552	0.06170	2.729	1.489	0.00656	0.00534