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RESEARCHARTICLE

Impact of Green-Synthesized Zinc Nanoparticles on Wheat Growth and Morphological Traits under Salt Stress

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Abstract

Wheat *(Triticum aestivum* L.) is an important staple crop that suffers from salinity stress, which limits growth and production worldwide. A study on the effect of green synthesized zinc nanoparticles (ZnNPs) on wheat growth under salt stress conditions was carried out to evaluate their activity against salinity. The study was carried out at Faisalabad University of Agriculture, where different concentrations of salts (0, 60 and 120 mM NaCl) and ZnNPs (0, 100, 150 and 200 mg/L) were applied. They measured the essential parameters like leaf area, chlorophyll, carotenoids and leaf number. According to result the ZnNPs exhibit highly significant mitigation effect of the salt stress at the concentration of 100 mg/L and 150 mg/L. The application of ZnNPs preserved leaf area, chlorophyll content, and carotenoid levels under salt stress at 60 mM and 120 mM NaCl more effectively as compared to the control plant. As a result, green-synthesized ZnNP can be helpful for the betterment of wheat in saline soils which will ultimately contribute towards sustainability.

KEYWORDS
Wheat, Zinc, Nanoparticles, Salinity

1 | INTRODUCTION

Wheat which belongs to the species *Triticum aestivum* L has the highest area under cultivation in the world and is the most important food crop. However, abiotic stresses affect the wheat crop increasingly (Hossain *et al*., 2021). Stress salinity is one of the most major constraints of agricultural productivity (Majeed & Muhammad, 2019). When plants experience salinity stress, it hampers their ability to grow. This happens when the cellular processes are disturbed because the plant are not able to take enough water. Moreover, the ion homeostasis of the plant cell is altered due to salinity stress. Ultimately, the growth is stunted, biomass reduced and lowers the yield of crops. (Arif *et al*., 2021; Shahid *et al*., 2020) The occurrence of salt-affected soils may increase due to global climate change at a faster rate. This may further worsen the condition of the soils affecting the sustainability of wheat grain.

Nanotechnology is gaining popularity for decreasing salinity in recent years according to the research of Singh *et al* (2024). Out of the various nanoparticulate material, it has been observed in the past studies that zinc nanoparticle (ZnNP) can be used to not only promote the plant growth but also enhance stress tolerance, and nutrient uptake (Jafir *et al*., 2024). Green synthesis of ZnNPs represents an environmentally friendly alternative to the use of toxic reagents in conventional chemical synthesis and high-energy processes (Angle, 2024). Zinc nanoparticles made from green synthesis will be safer for the environment and stable in nature and thus could be used in agriculture (Osman *et al*., 2024).

The use of green-synthesized ZnNPs to wheat growing under salt stress improved various growth parameters like root and shoot growth, leaf area, and overall health (Singh *et al*., 2025). According to Rasheed *et al*. (2024), these nanoparticles likely work through several processes like boosting antioxidant enzyme activity, osmoregulation, and ion intake. ZnNPs play a significant role in counteracting the oxidative damage caused by the generation of ROS under saline conditions. ZnNPs also help in maintaining cellular robustness by enhancing the synthesis of stress-related proteins and osmolytes (Rasheed *et al*., 2024).

The objective of this study is to evaluate the effects and morphological characteristics of wheat plants exposed to green synthesized ZnNPs at varied levels of salt stress. Through the assessment of important parameters such as plant height, root length, shoot length, leaf area, and biomass accumulation, this study aims to evaluate zinc nanoparticle (ZnNP) potential to boost the performance of wheat in saline environments for sustainable strategies to enhance crop resilience against salinity.

2 MATERIALS AND METHODS

Study Area and Experimental Setup

The experiment carried out in the University of Agriculture Faisalabad to evaluate the efficacy of green synthesized zinc nanoparticles (ZnNPs) in reducing salt stress in wheat. We got wheat seeds of the SARC-5 kind from the Ayub Agriculture Research Institute, Faisalabad. The method of the study was with Completely Randomized Design (CRD). It was arranged in a two-way factorial (2 way ANOVA) with three replications. We prepared pots with sandy loamy soil and sowed the seeds of wheat in each pot. Stress of salt was done at three levels i.e. 0mM, 50mM and 100mM while five concentrations of ZnNPs (0mg/L, 100mg/L, 150mg/L, 200mg/L) were applied to check the effect on growth of wheat. The experiment tried to see how different concentrations of ZnNPs affected the different morphological characteristics and physiological response of wheat in salt stressed conditions.

Experimental Details

In study 2025, wheat variety SARC-5 was used for wheat seedling growth. Thirty-six pots were taken with 7kg of sandy-loamy soil in each pot for the experiment. The treatments consisted of different levels of salt stress along with different concentrations of ZnNP and three replications for each treatment. Plants were monitored for 40 days post-sowing. The experiment was carried out under laboratory conditions with temperature, humidity and light maintained optimum for the wheat.

Morphological Parameters

Several morphological parameters were measured to assess the growth of wheat plants under varying salt stress levels and ZnNP treatments:

Number of leaves

The total number of leaves per plant was counted, and the average for each treatment was calculated to determine the effect of ZnNPs and salt stress on leaf production.

Root Length (cm)

One plant from each replication was carefully uprooted, and the root length was measured using a measuring scale. The mean root length was calculated across all replicates for each treatment.

Shoot Length (cm)

Similarly, the shoot length was measured from the base of the plant to the tip of the longest shoot, and the mean was calculated from the replicates.

Fresh Root Weight (g)

The roots of one plant from each replication were separated and cleaned. The fresh weight of the roots was measured using a weighing balance.

Fresh Shoot Weight (g)

The shoot portion of one plant from each replication was collected, cleaned, and weighed to determine the fresh weight.

Dry Root Weight (g)

The root of one plant fromeach replication was dried initially at room temperature for a few hours to remove humidity, and then fully dried in an oven at 100°C for 24 hours. After drying, the dry root weight was measured using a weighing balance.

Dry Shoot Weight (g)

The shoot of one plant from each replication was dried using the same procedure as the root, and the dry shoot weight was measured after full dehydration.

Physiological Parameters

To assess the physiological responses of wheat, the following methods were employed:

Leaf Area (cm²)

Two fully expanded leaves from each replicate were carefully uprooted, and the leaf area was calculated using the formula:

$$Leaf\ area\ = Leaf\ length\ x\ Leaf\ width$$

This measurement provided an understanding of how salt stress and ZnNPs affected the leaf's photosynthetic area (Valentinuz, 2002).

Chlorophyll Content (mg/g)

Chlorophyll content was measured using spectrophotometry after extracting chlorophyll from the leaf samples (Palta, 1990). Approximately 0.5 g of fresh leaf tissue was ground in a mortar and pestle, and the extract was obtained by adding 5 ml of 80% acetone. The extract was stored overnight at 10°C and then centrifuged at 1400 rpm for five minutes. The absorbance of the supernatant was measured at wavelengths of 663 nm, 645 nm, and 480 nm for chlorophyll a, chlorophyll b, and carotenoids, respectively, using the following equations:

$$\text{Chl. a}\left(\frac{\text{mg}}{\text{g}}\right) = [12.7(\text{OD}663) - 2.69(\text{OD}645)] \times \frac{V}{1000} \times W$$

$$\text{Chl. b}\left(\frac{\text{mg}}{\text{g}}\right) = [22.9(\text{OD}645) - 4.68(\text{OD}630)] \times \frac{V}{1000} \times W$$

Where OD represents the optical density at specific wavelengths, V is the volume of the extract, and W is the weight of the leaf tissue used.

Statistical Analysis

The data collected from all experiments were analyzed using COSTAT software. A two-way ANOVA was employed to assess the impact of different salt stress levels and ZnNP concentrations on wheat growth. Post-hoc tests were conducted to make pairwise comparisons of the treatment means and identify significant differences (Clark *et al*., 2012).

RESULTS

Growth Parameters Under Different ZnNPs Concentrations and Salt Stress Levels

The experiment's outcomes show how zinc nanoparticles affect wheat growth under salt stress at 0, 60, and 120 mM NaCl concentrations. The experiment was successful, and the results were satisfactory. According to the expectations, the 0 mM NaCl condition showed the highest growth values for all parameters as no salt stress was present. Plants have higher root and shoot length and shoot and root fresh weights in the non-saline treatment, which shows that wheat grows better in absence of salinity.

When salt stress was imposed at 60 mM and 120 mM NaCl there was a marked decline in all growth parameters. The treatment with the highest salt concentration (120 mM NaCl) produced the most significant reduction in wheat growth, as seen in the lowest value for root length, shoot length, shoot fresh weight, root fresh weight, root dry weight and shoot dry weight of all ZnNP treatments. High salt stress harms wheat growth, suggesting that elevated levels of salt have a negative influence (Fig. 1).

The plants respond differently when Zn nanoparticles (ZnNPs) are applied depending on the concentration of ZnNPs and level of salt stress. The result of ZnNP application in 60 mM NaCl showed a better growth as compared to control (0 mg/L ZnNPs). Of the different concentrations of ZnNP (0, 100, 150 and 200 mg/L) tested, the 100mg/L and 150mg/L concentrations were most effective. The adverse effects of salt stress could be minimized through these treatments which could produce relatively better growth over the control under moderate (60mM) and high (120mM) salt stress. Both root and shoot lengths were considerably higher at 100 mg/L and 150 mg/L ZnNPs concentration as compared to the treatment with 0 mg/L ZnNPs under moderate salt stress (60 mM NaCl). Similar trends in shoot fresh weight and root fresh weight were shown by ZnNP-treated plants wherein their fresh weight increased as compared to the control, particularly at 60 mM and 120 mM NaCl concentrations. The application of ZnNPs, especially 100 mg/L and 150 mg/L, positively affected root dry weight and shoot dry weight. ZnNP treatment exhibited better performance than the control under moderate high salt stresses. Although there was a general decline in dry months, however, the performance was better in ZnNP than the control (Fig. 1).

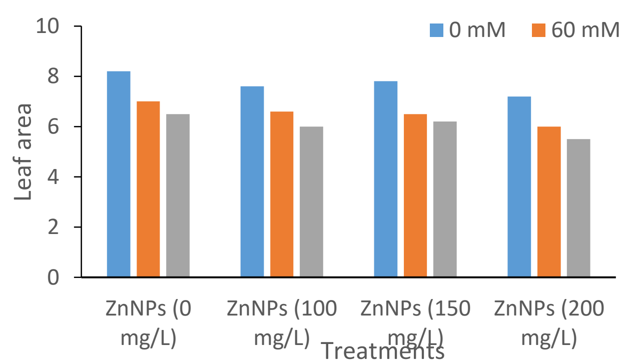

Fig. 2: Leaf Area (cm^2) under different treatments.

Leaf Area (cm^2)

Wheat plants that had no salt stress (0 mM) showed the largest leaf area in all ZnNP treatments. Thus, leaf area analysis showed that. In particular, the 0 mg/L ZnNP treatment at 0 mM salt concentration produced the highest leaf area suggesting that the plants were photosynthetically most active under optimal conditions (no salt stress). As salt stress increased, leaf area was decreased as seen in 60 mM and 120 mM. The leaf area was reduced at 60 mM salt concentration, whereas the highest negative effect on leaf area was noted at 120 mM salt concentration. Out of the ZnNP treatments, the 200 mg/L ZnNP concentration caused the lowest leaf area reduction at high salt stress (120 mM). This suggests that higher ZnNP concentrations could alleviate some of the salt stress negative effects.

Chlorophyll a Content (mg/g)

Both zinc nanoparticles concentrations and salt stress affected chlorophyll a contents. When salt concentration was 0 mM, all ZnNP treatments registered a relatively high chlorophyll a, with the control (0 mg/L ZnNP) showing the highest. As the concentration of salt increased to 60 mM and 120 mM, there was a reduction in chlorophyll a content of all treatments. Chlorophyll a level was higher in 100 mg/L and 200 mg/L ZnNPs treated plant leaves over others under 120 mM salt stress. It is suggested that ZnNPs (100 mg/L and 200 mg/L) may mitigate salt-induced inhibition of chlorophyll synthesis. The chlorophyll a content was lowest for all ZnNP concentrations when 120 mM salts were applied. This confirmed salt stress negatively impacted photosynthetic activity (Fig. 2).

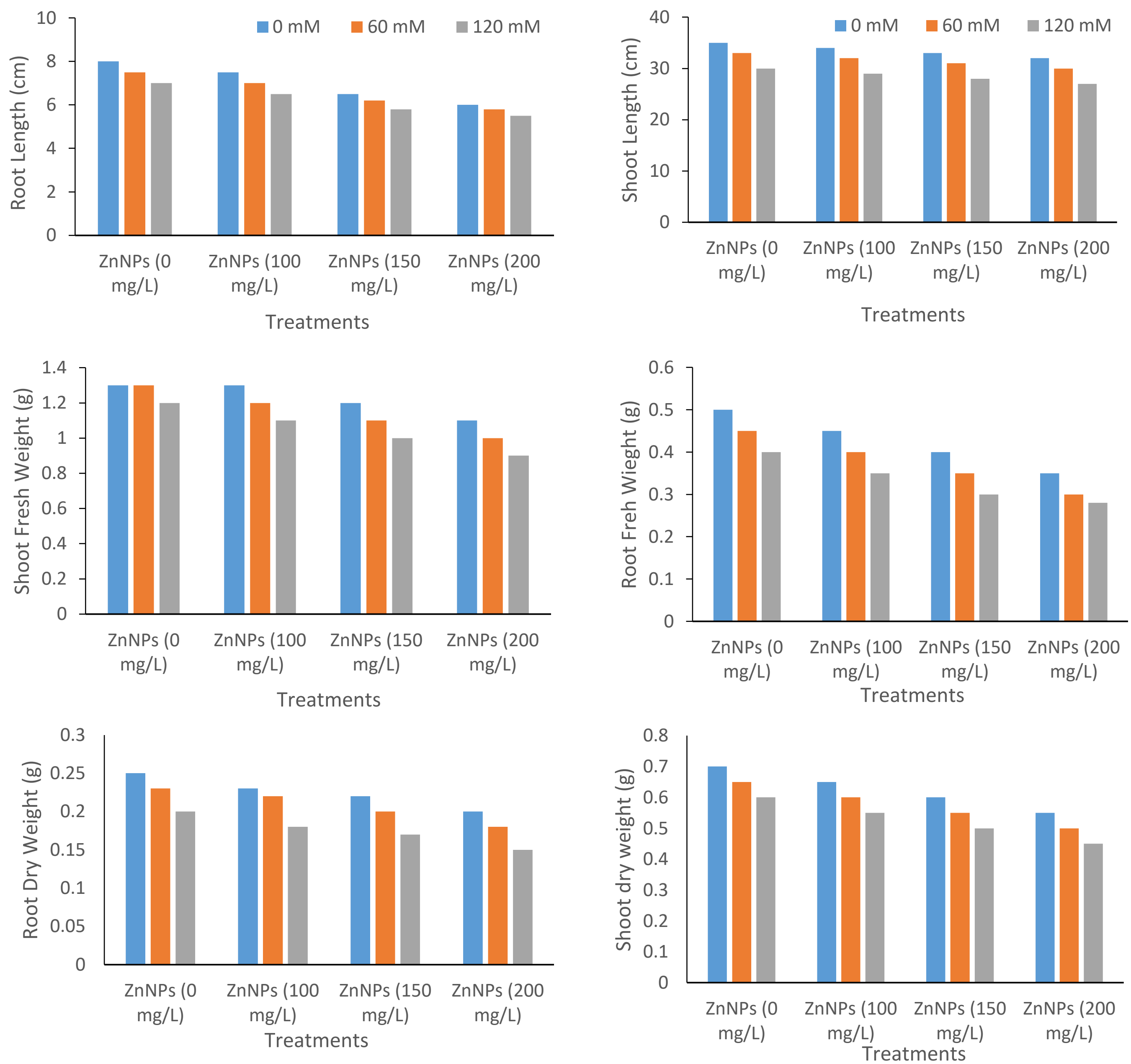

Fig. 1: Effects of Zinc Nanoparticles (ZnNPs) on Wheat Growth Parameters Under Varying Salt Stress Concentrations

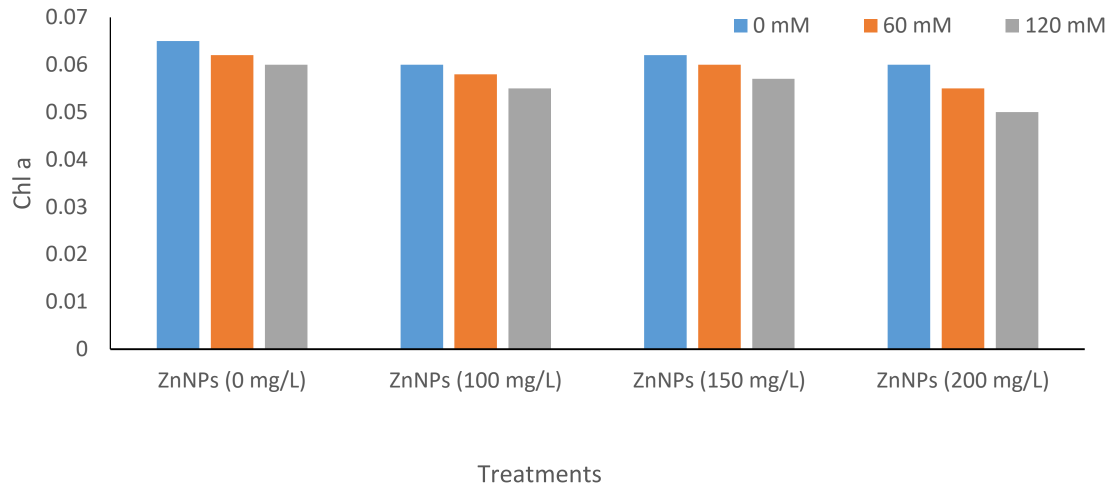

Fig. 2: Chlorophyll a Content (mg/g) under different treatments

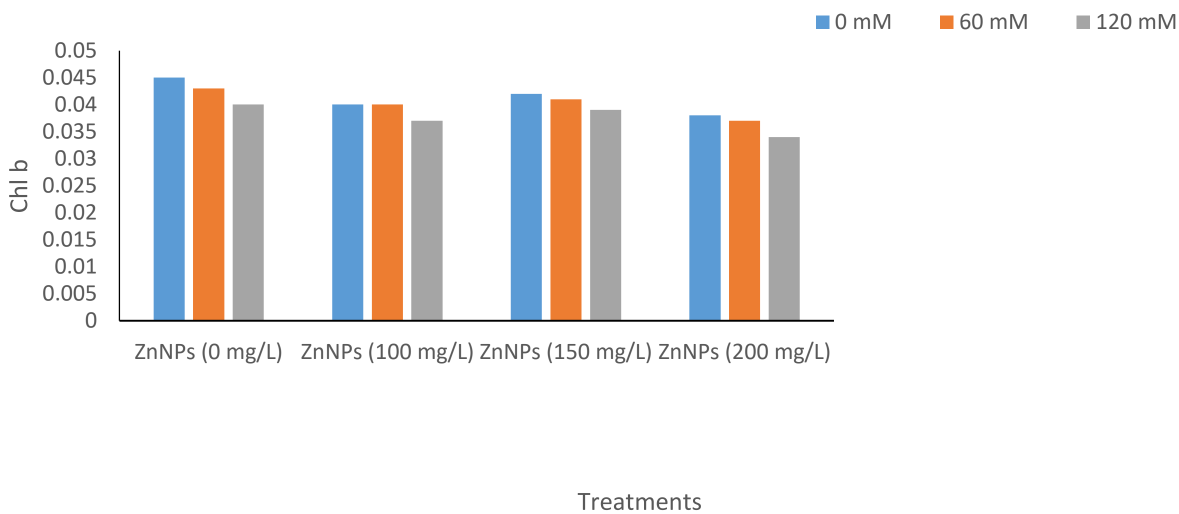

Fig. 3: Chlorophyll b Content (mg/g) under different treatments

Chlorophyll b Content (mg/g)

Chlorophyll b content followed a similar trend to chlorophyll a, with the highest values observed in the 0 mM salt treatment for all ZnNP concentrations. As the salt stress level increased, chlorophyll b content decreased. At 60 mM salt, the decline in chlorophyll b was moderate, but at 120 mM salt, there was a significant reduction. The 100 mg/L ZnNP treatment showed the least decline in chlorophyll b content compared to other ZnNP treatments, indicating that this concentration may provide better protection against salt-induced chlorophyll degradation. In contrast, the 150 mg/L and 200 mg/L ZnNP treatments showed a more pronounced decrease in chlorophyll b content, suggesting that higher ZnNP concentrations might not always be beneficial under high salt stress conditions (Fig. 3).

Carotenoids Content (mg/g)

Carotenoid content, a vital antioxidant that aids in protecting plants from oxidative stress, showed a similar pattern to the chlorophyll contents. At 0 mM salt concentration, carotenoid levels were highest across all ZnNP treatments. However, as salt concentration increased to 60 mM and 120 mM, a decline in carotenoid content was observed. The reduction in carotenoids was most significant under 120 mM salt stress. Interestingly, plants treated with 100 mg/L and 200 mg/L ZnNPs maintained relatively higher carotenoid levels compared to other treatments under salt stress, particularly under 120 mM. This suggests that ZnNPs may help in mitigating the negative effects of salt stress on carotenoid biosynthesis, which could enhance the plant's ability to combat oxidative damage (Fig. 4).

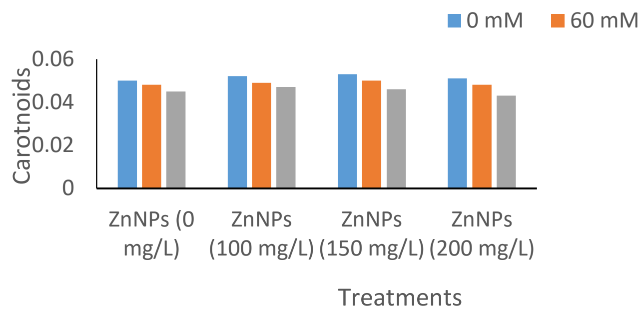

Fig. 4: Carotenoids Content (mg/g) under different treatments

Number of Leaves

The number of leaves was highest in the 0 mM salt treatment across all ZnNP concentrations, indicating that optimal conditions (without salt stress) support the maximum leaf production. With the introduction of salt stress (60 mM and 120 mM), the number of leaves significantly decreased in all ZnNP treatments, with the 120 mM salt treatment showing the greatest reduction in leaf count. However, plants treated with 100 mg/L and 200 mg/L ZnNPs showed a higher number of leaves compared to those treated with 0 mg/L and 150 mg/L ZnNPs, particularly under the 120 mM salt stress. This suggests that ZnNPs, particularly at 100 mg/L and 200 mg/L, may help improve the plant's overall growth and mitigate the negative effects of salt stress on leaf production (Fig. 5).

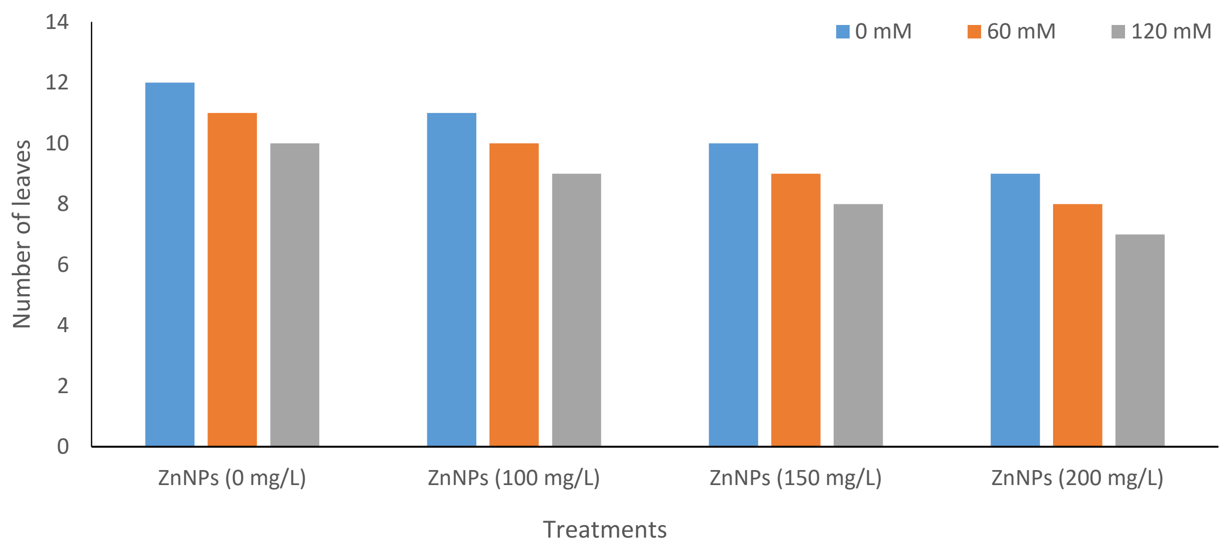

Fig. 5: Number of Leaves under different treatments

4 | DISCUSSION

According to Rakgotho *et al*. (2022), the physicochemical properties of wheat impacted by the green-synthesized zinc nanoparticles (ZnNPs) due to salt stress was remarkable. As per expectations, the 0 mM NaCl (control) treatment gave the highest values for all attributes meaning salt stress was not applied. In optimum conditions (without salt stress), wheat showed the highest photosynthetic activity, which causes a larger leaf area and better overall plant growth. But, with increase in salt concentration from 60 mM to 120 mM NaCl, all the parameters were reduced significantly which confirmed the salt effects inhibitory effect on wheat growth as already being reported (Nadeem *et al*., 2013).

The decrease in leaf area, chlorophyll content, and biomass accumulation under salt stress occurs due to the osmotic stress and generation of reactive oxygen species (ROS) that disrupt cellular process (Arif *et al*., 2020). Salt stress disturbed uptake of ions that cause damage to cell organelles and reduced efficiency of photosynthesis (Mushtaq *et al*., 2020). We can also see this in the decline in the contents of chlorophyll a, chlorophyll b, and carotenoids which help in sustaining the photosynthetic machinery and providing protection against oxidative stress (Behera *et al*. 2002). Increased concentrations of salt, especially at 120 mM NaCl, have negative effects on leaf area, chlorophyll content and growth of the plants.

Using ZnNPs at 100 mg/L and 150 mg/L effectively protected against salt-induced injuries. The findings of the study support the hypothesis of ZnNPs functioning as stress alleviators, underpinning the plant's response to oxidative stress, and boosting ion uptake (Shoukat *et al*., 2024). ZnNPs help to access plant cell membrane and increase absorption of the nutrients along with an improved system of antioxidant defence (Singh *et al*., 2024). The higher concentrations of ZnNP (100 mg/L and 150 mg/L) assisted in the maintenance of higher levels of chlorophyll a, chlorophyll b, and carotenoid contents. Subsequently, it reveals that the use of ZnNPs mitigates the salt-induced reduction in the photosynthetic pigments. This was noticed particularly in 120 mM NaCl treatment, where the ZnNPs showed greater chlorophyll and carotenoid contents compared to the untreated control. A supportive evidence of proposed potential role of ZnNPs in mitigating ROS induced oxidative damage in plant tissue that protect chloroplast integrity and maintain photosynthetic activity. (Seleiman *et al*., 2023)

Notably, the effects of 150 mg/L ZnNPs differed from the effects of 100 mg/L and 200 mg/L ZnNPs. Although, it alleviated the detrimental effects of salt stress on a number of parameters; it also caused a slight decrease in the amount of chlorophyll b at 120 mM salt stress.

The finding implies a dose-related effect. When the concentration of ZnNP particles is unusual, it may cause toxicity to the plants and they may not be able to take full advantage of the beneficial effects of the nanoparticles (Khan *et al*., 2023). Shoukat *et al*. (2024) also found that too many nanoparticles can lead to toxic impacts that are detrimental to the growth of the plant.

ZnNPs also produced promising results via overall plant vigor and leaf number. Plants treated with 100 mg/L and 200 mg/L ZnNPs exhibited a significantly higher number of leaves, which relates directly to both photosynthetic capacity and plant health, than untreated controls under saline stress conditions. In other words, ZnNPs help in making new leaves and enhance the general architecture of plant growth even under stress conditions. Boosting leaf growth is essential for long-term photosynthesis and plant well-being under saline conditions (Thabet, & Alqudah 2024).

The use of ZnNPs, notably at the concentrations of 100 mg/L and 150 mg/L, also improved root and shoot growth. As the zinc oxide impact further increased root and shoot lengths and the fresh and dry weights, it indicates a possible improvement in the ability of the plant to absorb water and nutrients under salt stress. The findings of Jafir *et al*. (2024) support this, who suggested that ZnNPs enhance the root system's growth that helps in the uptake of water and absorption of nutrients, normally restricted under saline conditions.

Also, even though the dry weight of both roots and shoots was somewhat reduced in the high salt condition, the dry weight was always higher for ZnNP-treated plants than controls. ZnNPs are known to enhance the partitioning towards roots and shoots under salt stress. As a result, plant productivity can be maintained through ZnNPs applications. Though ZnNPs helped to recover some of the salt inflicted decline, the overall decline in growth parameters working under 120 mM NaCl treatment indicates that high salt concentration continues to be a major limiting factor for wheat productivity irrespective of ZnNP application.

According to study findings, ZnNPs especially at 100 mg/L and 150 mg/L, could be a potential strategy for improving growth of wheat under salt stress. ZnNPs application improved the chlorophyll content, leaf area, number of leaves and biomass accumulation. Thus, indicating the ameliorative effect of ZnNPs in salinity stressed wheat for improving stress tolerance (Khan *et al*., 2023). This is useful in places where salinity is a big problem for wheat production.

Conclusion

The study's findings indicate that green-synthesized ZnNPs are efficient for improving the growth of wheat in salt-stressed conditions. Based on the results, ZnNPs improve the various physiological traits including chlorophyll content and leaf area as well as carotenoid content and suppress the adverse effects of salt stress on plants. These nanoparticles can be considered as elements of sustainable agricultural practices aiming to increase the tolerance of crops to salinity, especially in saline soils. In order to ensure the real-world use of ZnNPs in the agricultural system, further research is needed to explore the mechanism of their action on wheat and optimize their application for different stress conditions.

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Authors' Contribution: All authors made substantial contributions to the conceptualization and development of the manuscript. FUR and MUHA designed the study and structured the manuscript outline. MKI contributed to the characterization of materials, while MK performed the analytical data evaluation and prepared the associated figures and graphs. MKI, AH and MK conducted the literature review and drafted the relevant sections. All authors contributed to critical writing and manuscript revision, and approved the final version of the manuscript.

Generative AI Statements: The authors declare that this manuscript has been written without the use of generative artificial intelligence tools.

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