Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 08 | 2024
DOI: 10.5281/zenodo.13621995

OPTIMIZATION AND CHARACTERIZATION OF SYNTHESIZED ZnO NANOPARTICLES AND CONVENTIONAL FERTILIZERS FOR ENHANCED MAIZE TOLERANCE TO SALINITY STRESS

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Abstract

Zinc oxide nanoparticles (ZnO NPs) were synthesized via a precipitation method using NaOH as the precipitating agent and $ZnSO_4 \cdot 7H_2O$ as the starting material. The synthesis process was conducted at the Department of Physics, University of Agriculture, Faisalabad. The resulting ZnO NPs were dried at 80°C and calcined at 500°C, yielding monodisperse particles with a wurtzite structure and an average crystallite size of 12 nm. Characterization techniques including UV-Visible absorption spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) confirmed the successful synthesis, revealing spherical morphologies and strong UV absorption properties. The study further explored the application of these ZnO NPs as an innovative nutrient management strategy to enhance maize (*Zea mays* L.) growth under salinity stress—a major challenge in agriculture. Hydroponic experiments were conducted to assess the impact of different Zn concentrations (2, 5, 10, 25 ppm) in both nanoparticle and conventional fertilizer forms under saline conditions (10 mM NaCl). The results demonstrated that ZnO NPs significantly outperformed conventional Zn fertilizers, enhancing biomass accumulation, morphological attributes, chlorophyll content, and water retention in maize. Optimal ZnO NP concentration (10 ppm) provided notable improvements in plant growth while minimizing the toxic effects of excessive zinc. These findings highlight the potential of ZnO NPs as a superior and sustainable approach to mitigating salinity stress in maize, offering valuable insights for future research and practical applications in crop management.

Keywords: Nanoparticles, Conventional Fertilizers, Maize, Salinity Stress, Characterization.

INTRODUCTION

Salinity is a major abiotic stress that poses a significant threat to global crop production, particularly in arid and semi-arid regions (Yadav et al., 2020; Rengasamy, 2010). This stress is primarily caused by the accumulation of salts, such as sodium chloride (NaCl), in the soil and irrigation water, leading to detrimental effects on plant growth and productivity (Choudhary & Kharche, 2018).

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The impact of salinity stress on plants includes ion toxicity, osmotic stress, nutrient imbalances, oxidative stress, and impaired photosynthetic processes (Chourasia et al., 2021; Munns & Tester, 2008). These physiological and biochemical changes result in reduced plant growth, yield, and quality, posing a serious challenge to food security.

Maize (*Zea mays* L.), one of the most important staple crops worldwide, had a global production of approximately 1.1 billion metric tons in 2020 (FAO, 2020). Despite its significance, maize is highly sensitive to salinity stress, which can drastically impair its growth, development, and productivity (Sabagh et al., 2021; Zörb et al., 2019).

As salinity stress continues to expand across agricultural lands, there is an urgent need to develop effective strategies to mitigate its impact on maize cultivation. Several approaches have been proposed to combat salinity stress, including genetic improvement, cultural practices, and chemical treatments. Recently, nanotechnology and nutrient management have emerged as promising solutions.

Nanotechnology involves the application of nanoscale materials to enhance plant growth and stress tolerance, while nutrient management focuses on optimizing the uptake and utilization of essential nutrients by plants (Liu et al., 2021). Among the essential micronutrients, zinc (Zn) plays a crucial role in plant growth and development and has been shown to improve crop tolerance to various stresses, including salinity (Al Murad et al., 2020; Türkan & Demiral, 2009).

Zinc oxide nanoparticles (ZnO NPs) have attracted considerable attention due to their superior properties compared to conventional zinc fertilizers. These include increased solubility, bioavailability, and mobility within plant tissues, as well as enhanced antioxidant capacity and stress response mechanisms (Rajput et al., 2021; Dimkpa & Bindraban, 2018). The application of nano-Zn has the potential to significantly improve maize tolerance to salinity stress by promoting better nutrient uptake and strengthening the plant's defense mechanisms against the adverse effects of salinity.

However, the success of nano-Zn in mitigating salinity stress largely depends on the optimization of Zn doses. Both deficiency and excess of Zn can lead to negative outcomes in plants. Low Zn levels can result in Zn deficiency, which hampers plant growth and reduces stress tolerance (Gao et al., 2005). Conversely, excessive Zn levels can cause toxicity, leading to stunted growth, leaf chlorosis, and impaired physiological functions (Marschner, 2011). Therefore, determining the optimal Zn doses for maize under salinity stress is critical for maximizing crop yield and stress tolerance.

The current study aims to optimize the levels of both conventional and nano-Zn fertilizers to enhance maize crop tolerance to salinity stress. By exploring the effects of different Zn doses on maize growth, yield, and stress tolerance under saline conditions, this research seeks to compare the efficacy of conventional and nano-Zn fertilizers in mitigating salinity stress.

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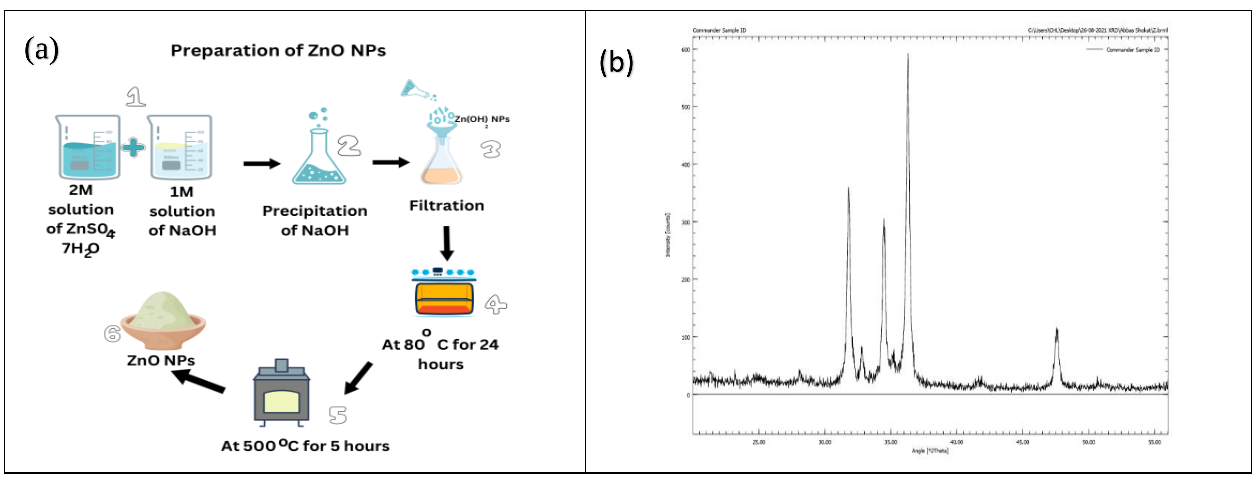
MATERIALS AND METHODS

Synthesis and Characterization of Nanoparticles

Zinc oxide (ZnO) nanoparticles were synthesized using a chemical precipitation method. The synthesis involved the use of 0.1 M sodium hydroxide (NaOH) and 0.05 M zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) as the key reactants. The reaction was maintained at 80°C for 2 hours to facilitate the formation of ZnO nanoparticles (Fig-1a). After the reaction, the resulting precipitate was filtered, washed multiple times with distilled water, and then subjected to calcination at 500°C for 5 hours to obtain the final ZnO nanoparticles (Chen et al., 2011). Post-synthesis, the ZnO nanoparticles were characterized using advanced analytical techniques at the Department of Materials Science, Technical Faculty, Christian-Albrecht University, Kiel, Germany. The characterization included Ultraviolet–Visible (UV-Vis) Spectroscopy (UV-6000, R&M, UK), X-ray Diffraction (XRD) (Oxford Instruments, x-act), and Scanning Electron Microscopy (SEM) (Zeiss, Supra 55VP, Germany). For Transmission Electron Microscopy (TEM) analysis, the ZnO nanoparticles were ground and dispersed in n-Butanol and then prepared on copper lacey TEM grids (FEI, Tecnai G2 F30 S Twin 300kV/FEG, Netherlands) equipped with an Energy Dispersive X-ray (EDX) detector. The XRD analysis confirmed the crystalline nature of the ZnO nanoparticles, revealing an average particle size of approximately 12 nm (Fig-1b). Morphological studies using SEM and TEM indicated that the ZnO nanoparticles were predominantly spherical, with sizes ranging between 10-20 nm (Fig-1e,f). A stabilizing agent, polyvinylpyrrolidone (PVP), was employed to enhance the dispersion stability of the nanoparticles. SEM micrographs, obtained at 50000X magnification, further validated the nanoscale size of the ZnO particles.



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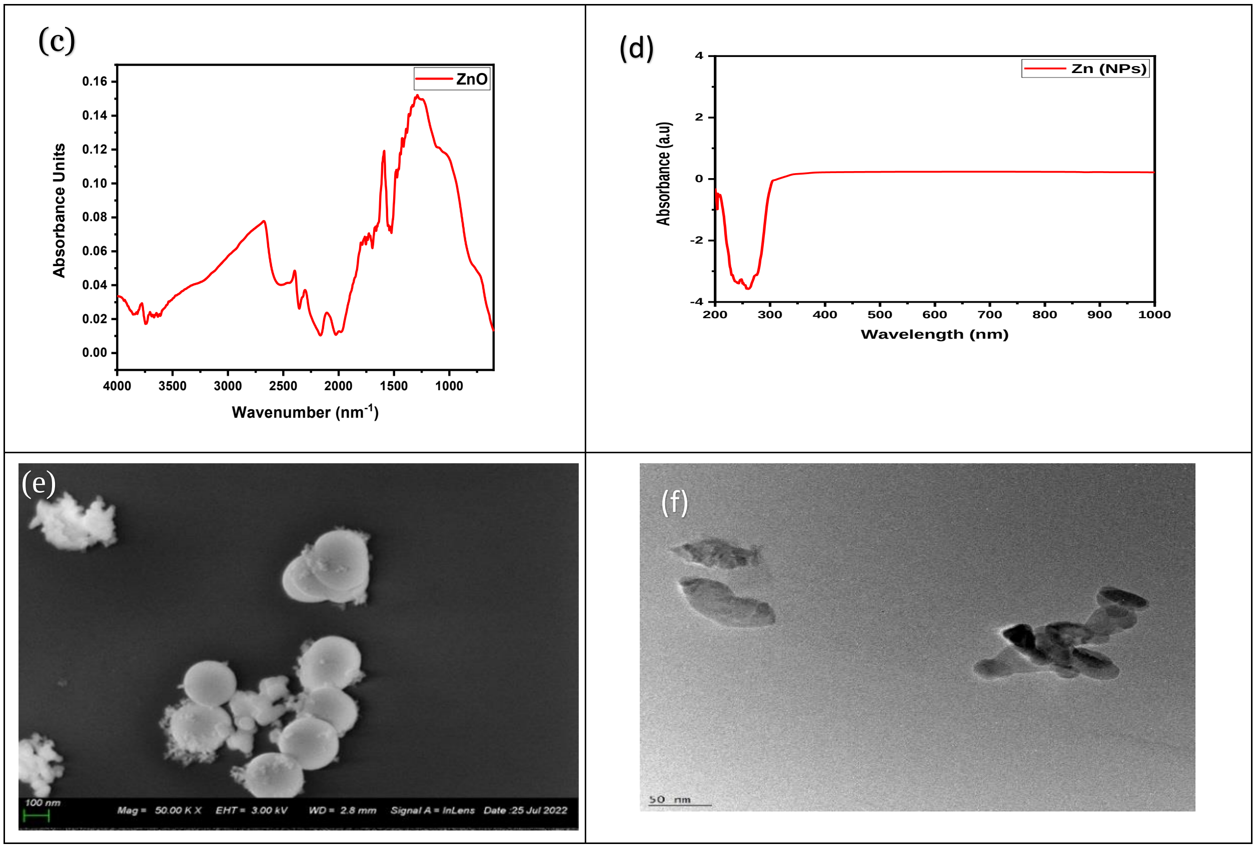

Figure 1: *(top)* Synthesis of ZnO NPs (a) XRD graph (b); *(middle)* FTIR Graph (c) UV VIS graph (d); (*bottom*) SEM micrograph (e) and TEM Micrograph of ZnO NPs (f).

Hydroponic Study Setup and Experimental Conditions

Hydroponic study was conducted at the wire house of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad (31.43° N and 73.06° E) for adjusting the best optimum level for NPs and conven. Fertilizers. Maize plants (FH-1046) seeds were acquired from Ayub Agriculture Research Institute (AARI), Faisalabad. Iron trays (12 × 18 cm) filled with sand were used for the nursery. Healthy and sterilized maize seeds were sown in trays. Optimum moisture level and temperature were maintained for germination. After the two-leaf stage, uniform seedlings were transplanted into 25 L plastic tubs having Hoagland's nutrition solution (Hoagland et al., 1950) (Tab 2). Plants' roots were adjusted in thermopore sheets having holes. Nine treatments were tested against the same salinity levels (control and 10 mM), with three replications for Zn optimization (Tabl-1). Salinity was developed by adding sodium chloride (NaCl) salt in intervals after seven days of transplantation. Artificial aeration was given through air pumps for 6 to 8 hours per day. The pH was maintained at 6-6.5 throughout the experiment. The study included agronomic, physiological, and ionic analyses were determined.

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Chemical Parameters

The samples were saved in the oven at 65 ºC ± 5 ºC for 72 hours for oven drying. Shoot samples were ground using grinding mill. Potassium and Sodium concentration were determined by using flame photometer. All plant samples were prepared by wet digestion method. 0.1g plant sample was taken with the help of weighing balance, then added di-acid (HNO_3: $HClO_4$) with the ratio of 3:1. 3 ml nitric acid solution and 1ml per chloric acid and placed it on hot plate. After complete digestion, made volume of all samples up to 50 ml in volumetric flask. Standards solutions were prepared using salts of NaCL and KCl. After it, run the samples on Flame photometer (Sherwood 410, UK) and noted the absorbance reading (Allison and Richards, 1954). Micro and macronutrients were measured by inductively coupled plasma mass spectroscopy (Agilent 7700, USA) as used by (Wu *et al.*, 2018).

Table 1: Treatment Plan for Zn Optimization

Sr. No.	Treatment (Zn Optimization)
1	Control
2	Zn NPs @ 2 ppm
3	Zn NPs @ 5 ppm
4	Zn NPs @ 10 ppm
5	Zn NPs @ 25 ppm
6	Conven. Zn @ 2 ppm
7	Conven. Zn @ 5 ppm
8	Conven. Zn @ 10 ppm

Table 2: Composition of Hoagland Nutrition Solution

Reagents	Stock Solution g/L (1 M)	1 M stock solution for 25 L Hoagland nutrient solution
Macronutrients		
KNO_3	101.0 g	62.5 mL
KH_2PO_4	136.0 g	12.5 mL
$MgSO_4.7H_2O$	246.0 g	25.0 mL
$Ca(NO_3)_2.4H_2O$	236.0 g	62.5 mL
Micronutrients		
H_3BO_3	2.86 g	12.5 mL
$MnCl_2.4H_2O$	1.81 g	12.5 mL
$CuSO_4.5H_2O$	0.08 g	12.5 mL
$H_2MoO_4.H_2O$	0.02 g	12.5 mL
Fe-EDTA	37.33 g	12.5 mL

Physiological Parameters

Measurement of Relative Water Contents (RWC)

Young leaf samples were first weighed to determine their fresh weight (0.5 g) for the relative water content study. They were then promptly watered to achieve complete turgor (Sairam et al., 2002). Following a 4-hour period, the samples were extracted using distilled water. The turgid leaves were then promptly wiped using filter paper to

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eliminate any surface moisture, and the completely turgid weight (TW) was measured. The dry weight (DW) of these samples was then ascertained by oven drying them for 48 hours at 65°C. Relative water content was calculated using the following formula:

$$RWC = \frac{(FW - DW)}{(TW - DW)} \dots\dots\dots\dots\dots\dots\dots \ (1)$$

Membrane Stability Index (MSI)

Using the procedure described by Sairam et al. (2002), the ions that were released from leaf tissue into distilled water were measured in order to define the membrane stability index. Two groups of test tubes with double-distilled water were filled with fresh leaf samples (0.2 g). After 30 minutes of incubation in a water bath at 40°C, a voltage meter was used to measure the ECs (C1) in one set of test tubes. ECs (C2) was measured after the second set was cooked for 15 minutes at 100°C in boiling water. MSI was quantified using the following formula:

$$MSI = 1 - \frac{C1}{C2} \times 100 \dots\dots\dots\dots\dots\dots\dots \ (2)$$

Statistical Analysis

Treatments of the trials were applied according to CRD under two-way factorial. Data were analyzed according to the techniques defined by (Steel et al., 1997). Statistix 8.1 software was used for analysis and comparison of data. Box plots were made by using using R version 4.0.5 (R Core Team, 2021).

RESULTS (Zn OPTIMIZATION EXPERIMENT)

Agronomic Parameters

The box plots demonstrate that nano Zn at 10 mg/L consistently yielded the best results across various growth parameters, including shoot fresh weight, root fresh weight, shoot length, shoot dry weight, root dry weight, and root length. Under non-saline conditions, the 10 ppm dose led to the highest values, indicated by taller boxes and shorter whiskers, which suggest uniform growth. Conversely, the 25 ppm dose resulted in lower values across these parameters, indicating that it was less effective. This suggests that while higher doses of Zn may provide benefits up to a certain point, 10 ppm is optimal without leading to diminishing returns (Fig-2).

Under saline conditions, the trend remained consistent. Nano Zn at 10 mg/L continued to outperform other treatments, with higher median values and more consistent results across all parameters. Conven. Zn generally showed lower performance across all zinc levels, with nano Zn at 10 mg/L consistently standing out as the most effective treatment in both NS and saline environments. Overall, the data clearly demonstrates that 10 ppm is the optimal dose for nano Zn, providing the best balance of effectiveness across different growth conditions without the drawbacks seen at higher concentrations (Fig-2)

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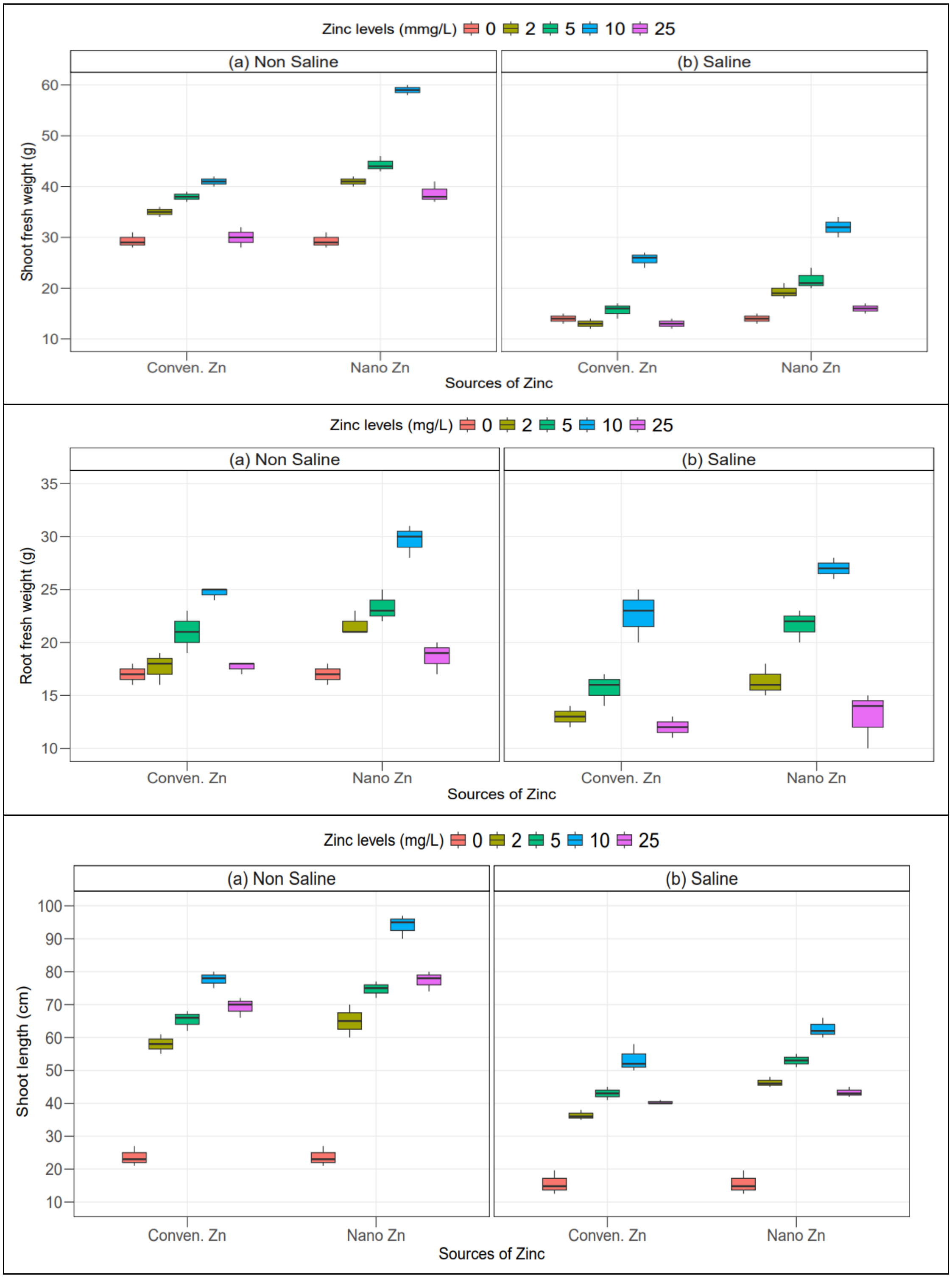

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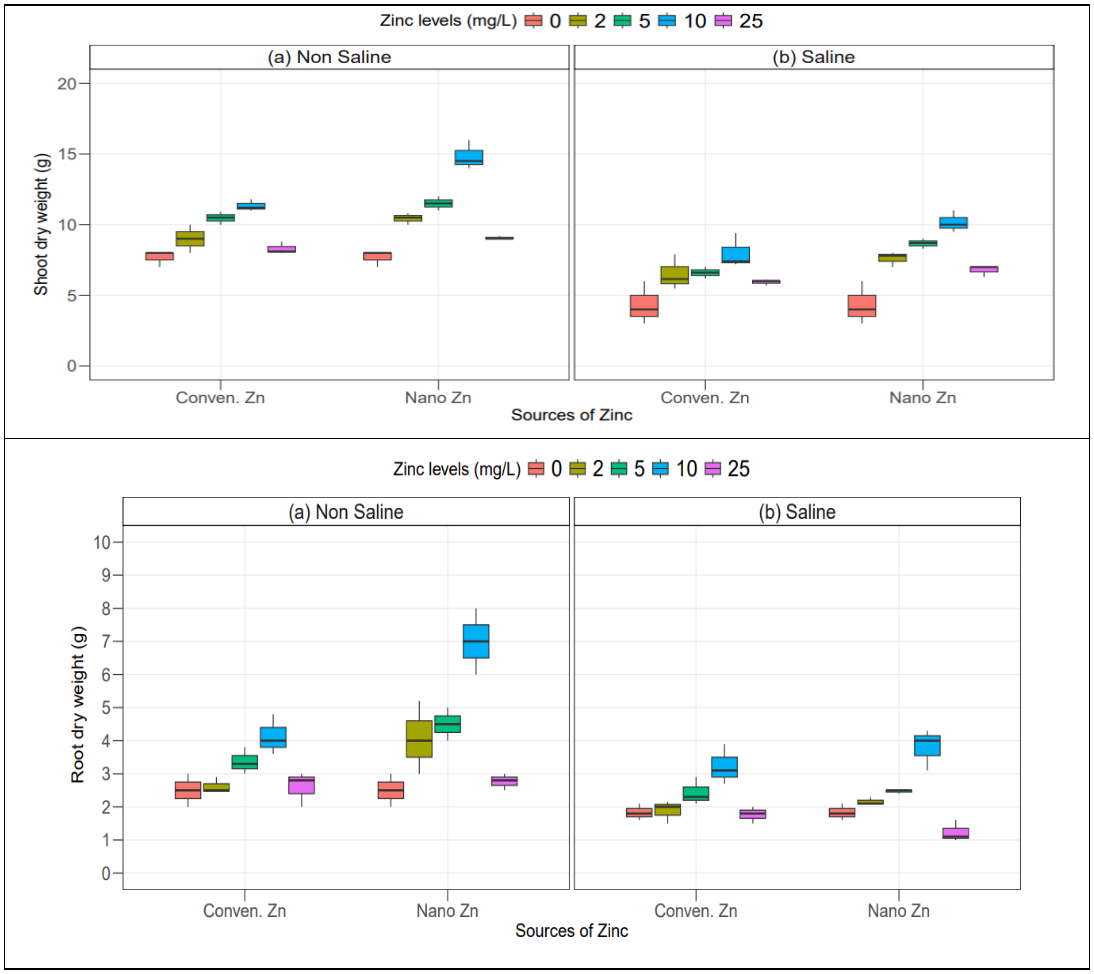

Figure 2: Optimization of Zn doses and their effects on agronomic parameters with nano Zn and conven. Zn under non-saline and saline conditions

Physiological and Chemical Parameters

The box plots illustrate the impact of different zinc levels on several physiological parameters, under both non-saline and saline conditions.

For sodium in shoots, the plots reveal that under NS conditions, both conven. Zn and nano Zn, particularly at lower doses, result in relatively low Na accumulation. The boxes are compact with shorter whiskers, indicating minimal variation among samples. However, under saline conditions, Na content increased significantly across all treatments, especially at the 0 and 2 mg/L levels. Nano Zn at 10 mg/L showed reduced Na accumulation, with the boxes and whiskers positioned lower on the graph, suggesting its efficacy in minimizing Na uptake under stress. The potassium in shoots parameter showed that nano Zn at 10 mg/L consistently resulted in the highest K accumulation across both NS and saline conditions. In the potassium-sodium (K/Na) ratio, nano Zn at 10 mg/L showed the highest values under both NS and saline conditions, with taller boxes

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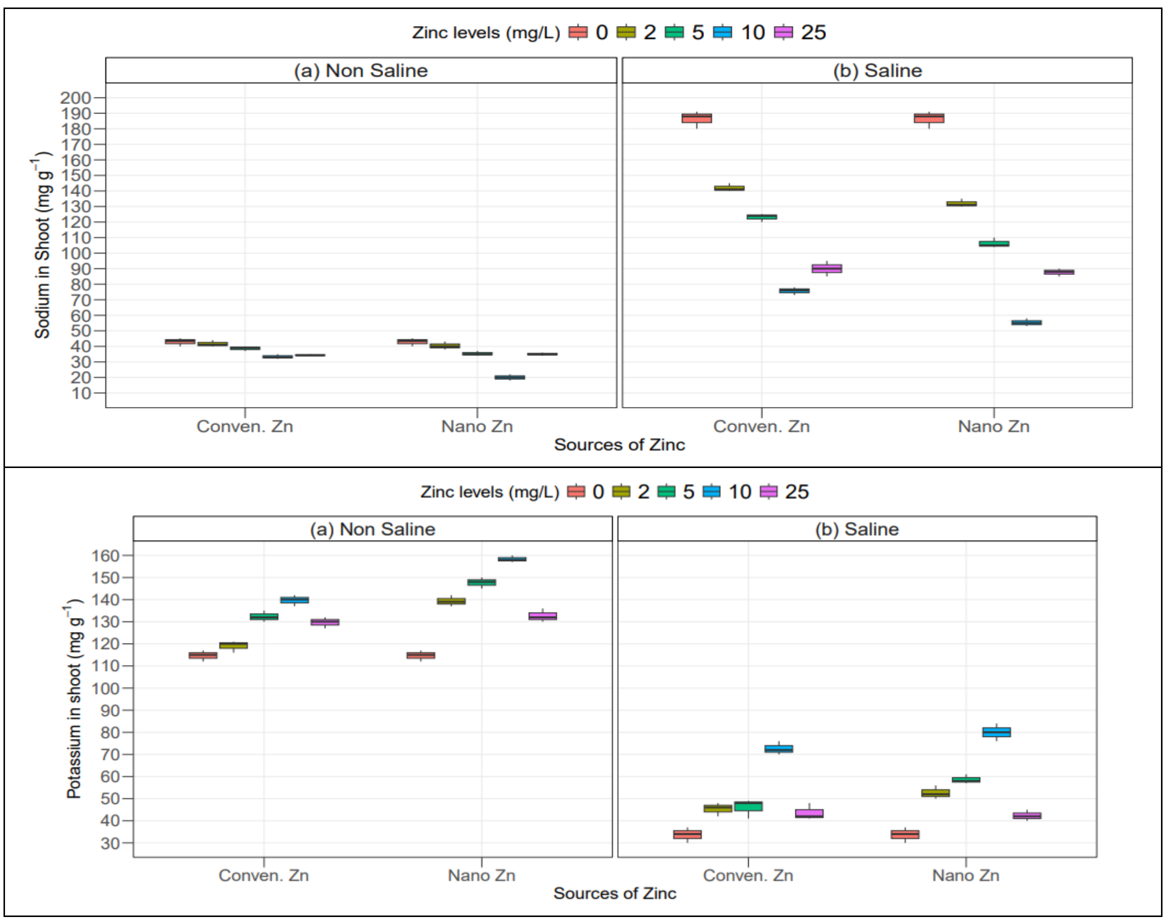
and longer whiskers, highlighting its superior ability to maintain a favorable K/Na balance (Fig-3).

For relative water content, nano Zn at 10 ppm again performed the best, with higher boxes and longer whiskers, indicating better water retention across both conditions. Under saline stress, nano Zn at 10 ppm maintained higher RWC compared to other treatments, with more compact boxes suggesting consistent effects across samples. The membrane stability index results showed that nano Zn at 10 ppm led to the highest MSI values, especially under NS conditions. The boxes at this level were taller and the whiskers longer, indicating enhanced membrane integrity. Under saline conditions, while MSI decreased overall, nano Zn at 10 ppm still outperformed other treatments. Nano Zn at higher levels, particularly 25 ppm, resulted in the highest Zn accumulation. However, 10 ppm also showed strong performance with well-positioned boxes and shorter whiskers under both conditions, indicating good Zn uptake without excessive variability. Overall, the results across these parameters demonstrate that nano Zn at 10 ppm is the most effective treatment, consistently enhancing physiological performance, especially under saline conditions (Fig-3).



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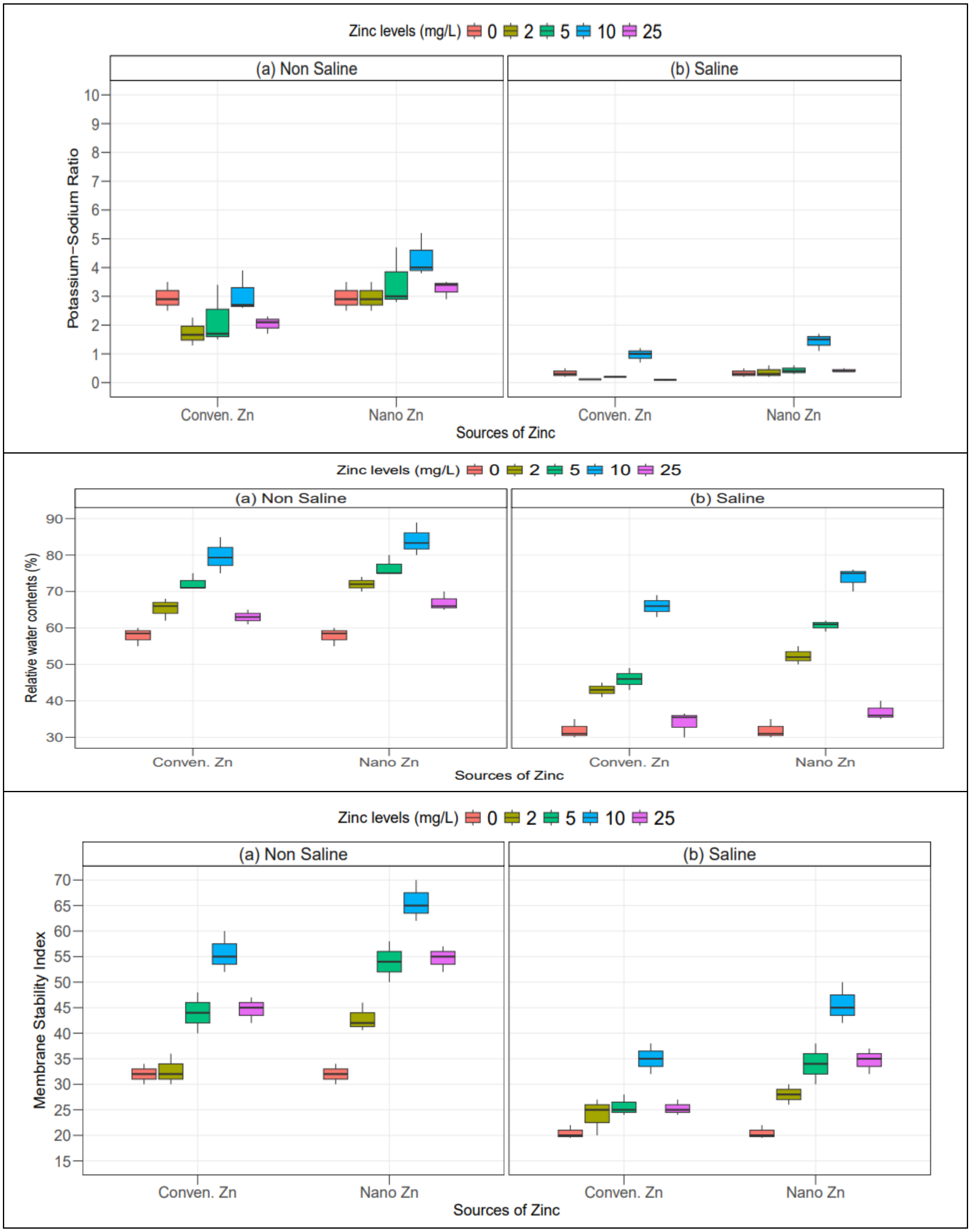

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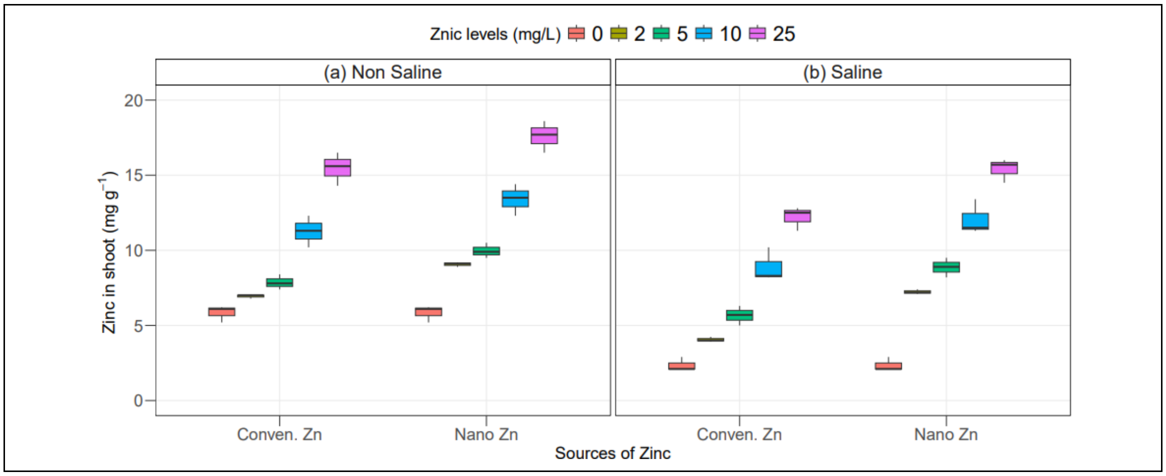

Figure 3: Optimization of zinc doses and their effects on ionic and physiological parameters with nano Zn and conven. Zn under non-saline and saline conditions

DISCUSSION

Climate change and salinization of agricultural land are posing serious challenges to food security on a global scale. Human activities have exacerbated salinization, which has been detrimental to the yield potential of major grain crops (Ondrasek et al., 2011; Hussain et al., 2019). In addition to causing precise ion toxicity, osmotic stress, nutrient imbalances, and reactive oxygen species that may harm cell components, maize is particularly sensitive to salinity stress (Raza et al., 2019; Mansoor et al., 2022).

In this study, zinc (Zn) applications through nanoparticles and conventional fertilizers were considered as potential solutions for enhancing maize crop resistance to salinity stresses. Notably, nano-Zn treatments have shown superior performance compared to conventional forms under both saline and non-saline conditions, particularly at specific concentrations. Based on the results obtained from the Zn optimization experiments, nanoparticles may improve maize crop tolerance to salinity stress. These findings are consistent with previous studies that have provided evidence supporting the use of Zn optimization to mitigate the impact of salinity stress on crops. According to Etesami et al. (2021) and Junedi et al. (2023), salinity negatively impacts agricultural productivity, and effective solutions are needed. Mushtaq et al. (2023) and Nazir & Wahid (2023) have further demonstrated how salinity stress negatively affects cellular structure and function.

This study evaluated the agronomic and physiological parameters of maize growth and development under both saline and non-saline conditions. Nano-Zn at 10 ppm exhibited marked enhancements in agronomic and physiological parameters compared to the control. The results are consistent with previous studies that have shown the potential of zinc for enhancing crop resilience under saline conditions (Junedi et al., 2023).

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Furthermore, chlorophyll content (SPAD), relative water content (RWC), membrane stability index (MSI), and ion balance (K/Na ratio) were also found to be positively affected by zinc applications.

The study confirmed that nano-Zn was more beneficial than conventional Zn, especially at 10 parts per million, under both saline and non-saline conditions. These findings align with existing literature on the importance of Zn as a micronutrient and the advantages of using Zn nanoparticles (Del Buono et al., 2021). Zn is an essential micronutrient responsible for enzyme activation, protein synthesis, and growth regulation in plants (Umair et al., 2020). The smaller size and larger surface area of Zn nanoparticles provide advantages such as enhanced solubility and mobility, leading to improved bioavailability and nutrient use efficiency (Seleiman et al., 2020; Al-Juthery et al., 2021).

This higher bioavailability could explain why Zn nanoparticles performed better than conventional Zn in this study. Under salinity stress, Zn has been reported to be vital in enhancing antioxidant enzyme activity, maintaining membrane integrity, and regulating osmotic mechanisms in plants. There is evidence that these mechanisms may contribute to the improved growth and physiological parameters observed in maize plants treated with Zn nanoparticles (Del Buono et al., 2021).

Nano-Zn possesses enhanced performance due to its ability to penetrate plant cell walls and plasma membranes more effectively than conventional forms. This leads to increased uptake and translocation efficiency of nutrients within the maize plants (Kumar et al., 2021; Souri et al., 2021). This study demonstrated that Zn nanoparticles enhance nutrient uptake and translocation efficiency in maize shoots, thereby mitigating the adverse effects of salinity stress.

These findings align with studies that have shown the potential of nano fertilizers in improving nutrient uptake and translocation efficiency in plants. Nanoparticles provide an efficient delivery system, facilitating the delivery of essential elements to target locations within the plant system (Yadav et al., 2023). By enhancing nutrient availability and utilization, nano fertilizers offer promising solutions to alleviate the detrimental effects of salinity stress and enhance crop resilience.

In conclusion, optimizing Zn levels, particularly through nanoparticles, can be an effective strategy to enhance maize crop tolerance to salinity stress. The superior performance of nanoparticles compared to conventional fertilizers highlights the potential of nanotechnology in advancing sustainable agricultural practices, especially in regions affected by high salinity levels.

However, it is crucial to carefully determine the appropriate dosage to avoid adverse effects and ensure optimal benefits. Continued research in this field will contribute to developing accurate and efficient agronomic practices for enhancing crop resilience and addressing the global challenge of food security in the face of climate change and salinization.

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