Nano-Biofertilizers from Zinc Sulphate and *Nigella* Seed Extract with Bacterial Mediation: Synthesis and Characterization

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This study aimed to synthesize nanoparticles by using zinc sulphate ($ZnSO_4$) with seed extract and bacterial processing. The resulting nano-biofertilizers were used to treat maize crop. Zinc nanoparticles were prepared by green synthesis using extract from *Nigella* seeds. Seed germination experiment was followed under salt stress in sand with three replications. Treatments included control (non-saline), saline, no-fertilizer, biofertilizer, Zn nanoparticles, and complete nano-biofertilizers applied at different concentrations. The rate of emergence was high in control as compared to saline conditions. Results indicated that control (non-saline) conditions were more efficient in stimulating the plant growth and the product had more potential to promote maximum yield in maize crop. Plants with the treatment of nano-biofertilizers gave higher yield as compared to the plants which were treated with nanoparticles or biofertilizers separately. Nanoparticles and biofertilizers both showed variations in plant yield. Characterization and morphological representation of plant samples (Zn-nano, biofertilizers, and nano-biofertilizers) was done by various analyses including SEM, FTIR, and XRD. It was concluded that nanoparticles and biofertilizers in combination can enhance the maize crop's productivity and growth under certain favorable conditions and under salt stress.

DOI: 10.15376/biores.21.1.1968-1989

Keywords: Scanning electron microscopy; Fourier transform infrared; X-ray diffraction; Salt stress; Nano-biofertilizers

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INTRODUCTION

Nutrient management has played a vital role in advancing food security over the past century, particularly under favorable environmental conditions (Rayan *et al*. 2012). However, agricultural development is often constrained by dry and arid climates, especially in inland regions (Roozitalab 2000). Therefore, there is a critical need to achieve sustainable growth in rain-fed agricultural systems, including the production of grain, pastures, grazing lands, and livestock.

Nutrient deficiencies are commonly observed in degraded or sandy soils, where low organic matter content reflects weak soil structure and limited chemical fertility. Additionally, severe deficiencies of nitrogen, phosphorus, sulfur, and other essential nutrients, such as zinc and iron, are also predominant in many soils (Sahrawat and Wani 2013). Low crop yields can be primarily attributed to imbalanced and inadequate fertilizer use, nutrient depletion due to continuous cropping, soil erosion, salinity, and structural degradation. Furthermore, wide gaps between nutrient supply and crop demand along with the low efficiency of applied fertilizers, exacerbate the problem (Rao *et al.* 2010; Rayan *et al*. 2012).

$ZnSO_4$ has been widely used as a Zn source for plants. In such application, nearly 90% of the soluble Zn becomes converted into different types of its insoluble forms in the soil. Many soil bacteria have enzymes or metabolic pathways that can push the zinc into a different chemical state (Anuradha *et al*., 2015). Zinc sulfate ($ZnSO_4$) bulk salt is commonly used as a source of Zn in calcareous soils, and it is highly soluble. Conversely, zinc sulfate can rapidly convert to an insoluble form after complex reactions with soil components. Likewise, zinc hydroxide ($Zn(OH)_2$) has been synthesized and confirmed by XRD, showing distinct reflections that allow it to be differentiated from ZnO in mixed systems (Said *et al.* 2021). In several composite materials produced *via* wet-chemical or low-temperature routes, $ZnO/Zn(OH)_2$ hybrid phases have also been documented, with secondary peaks attributable to crystalline $Zn(OH)_2$ (Altuntasoglu *et al.* 2010). In the long term, this insoluble form of (ZnSO4) turns out to be useless for the plant–soil system, especially in calcareous soils (Yaseen and Hussain 2021). Nanofertilizers that are being used now are getting much attention in the field of agriculture because of their high solubility, availability, diffusion, and reactivity in the soil (El-Saadony *et al.* 2021). Nanoparticles have higher surface area than macroscopic particles, but soluble Zn sulfate would have the highest availability of the ions as compared with their bulk salts (Mukherjee *et al.* 2016).

In the last 10 years, "green" synthesis utilizing plant extracts has become a popular and environmentally acceptable way to make metal and metal-oxide nanoparticles (NPs) instead of using synthetic chemicals. Plant-derived extracts, which are abundant in phytochemicals including phenolic compounds, flavonoids, terpenoids, proteins, sugars, and other bioactive molecules, have been shown to function as effective reducing agents, donating electrons to metal ions and promoting their transformation into zerovalent metals or metal oxides (Singh *et al.* 2018; Khan *et al.* 2018).

Nano-fertilizers, when compared with conventional fertilizers, are formulated with the intent of improving and enhancing the plant nutrition and productivity (Mikkelsen 2018). These modern formulations are typically made up of certain nano-coated materials and by using various nanotechnological methods for enhancing the nutrient's bioavailability and release of essential elements. Three categories of nano-fertilizers include nano-scale fertilizers (nanoparticles containing nutrients), nano-scale additives (conventional fertilizers containing nanoscale additives), and nano-scale inserts (conventional fertilizers composed or packed with nanoparticles). Coating of nano-material can reduce the release of nutrients or porous nano-fertilizer can incorporate a network of channels that prevent nutrient melting (Mikkelsen 2018).

Nanotech or nano science is usually defined as the method of synthesizing different nano coated materials (size not greater than 100 nm), which are applicable in such domains as agriculture, diagnostics, pharmaceuticals, engineering, food security, energy infrastructures, and environmental investigations (Malea *et al*. 2019). Nanomaterials can have many different encouraging impacts among plant yield and their growth parameters such as seedling growth and germination, photosynthetic activity, whole proteins, sugars, nitrogen, biomass, and micronutrients have been noticed in various plants; *e.g.*, spinach (Srivastava *et al*. 2014), mung bean and chickpea (Mahajan *et al*. 2011), *Solanum lycopersicum* (Faizan *et al*. 2018), cucumber (Moghaddasi *et al*. 2017), and common wheat (Zhang *et al*. 2018).

An important trace element is zinc, which has many positive impacts in the biochemical and physiological functioning of the plants. It has a significant role in processes such as chlorophyll and protein synthesis, enzymatic activity, and various metabolic activities (Singh *et al*. 2018). Zinc triggers N_2 activation in leguminous plants *e.g.*, *Phaseolus vulgari*s L. by increasing the number of nodules formed among plant roots (Hemantaranjan and Garg 2015). Zinc deficiency can be a worldwide issue among the crop's productivity (Impa *et al*. 2013). There is a need to emphasize the ability to replenish zinc within the nanoparticles' types *e.g.*, fertilizers (Dimkpa *et al*. 2015; Du *et al*. 2019).

Biofertilizers, which consist of the main constituents among living microorganisms, when interacted with plants, seeds, soil surfaces or rhizosphere, can enhance the overall growth and nutrient absorption in plants (Vessey 2003; Bardi and Malusà 2012; Malusa *et al.,*2016). By speeding up the microbiological processes, biofertilizers can increase the nutrients' supply towards the plants and facilitate easy absorption. By fixing the atmospheric nitrogen, dissolving soluble phosphates, and producing the plant growth promoting nutrients, the soil fertility can be improved (Mazid and Khan 2015). The main aim is to predict the impacts of three types of biofertilizers, which contain algae (alive or dead), nitrogen fixer (*Azotobacter chroococcum*), and P-solubilizer (*Bacillus megaterium*) in plant extensions within the laboratory under the pressure of aluminum.

Biofertilizer usage has led to increased nutrient uptake by plants and yielded higher biomass production. There are interactions between the rhizosphere and plant roots; therefore, the soils that carry bacteria play a major part in promoting growth and yield among plants. The compounds that are extracted by the plant's roots (Römheld 1986; Lévai 2004) and bacterial components (Katznelson and Bose 1959) both are essential to form different mineral components. Bio-fertilizers have economic and environmental interactions, and their long-term usage greatly improves the fertility of the soil (Mahdi *et al*. 2010; Singh *et al*. 2011). Biofertilizers are used in order to enhance the yield of plant by about 10% to 40% and also the protein content, essential amino acids, vitamins, and organic matter (Bhardwaj *et al*. 2014).

A symbiotic N_2 fixing rhizobacterium genus *Rhizobium*, which belongs to the family, Rhizobiaceae, develops mutual relationships by invading the orchids' roots. Related genus types include *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, Rhizobium, and *Sinorhizobium*, and these altogether are named as '*Rhizobia*'. Non-symbiotic rhizobacteria in non-leguminous plants which fixes nitrogen are called '*Diazotrophs*' and form involuntary relations with their host (plants) (Verma *et al*. 2010). More advanced methods can be used with traditional methods to promote multiple performances, analysis of variance, and studies to assess the risks of biofertilizers before eliciting from the biosphere (Sharma *et al*. 2012). The main advantage of using biofertilizers as an integral part of industrial activity is that they provide a modern field emerging nowadays. Only a few countries have used these recently developed fertilizers, and their applications is expected to grow over time (Weekley *et al*. 2012). The exploitation of bio-fertilizers will probably provide various powerful strategies to improve the entire agricultural yield.

The aims of the present research work were basically to select nanoparticles for the synthesis of nano-biofertilizers. Another aim was the selection, optimization, and incorporation of suitable bacteria (for preparation of biofertilizers) for the synthesis of nano-biofertilizers. Pilot-scale synthesis of nano-biofertilizers was conducted with accurate measurements. The purpose of synthesizing these nano-biofertilizers was to examine the effects of nano-biofertilizers application on maize crop and the production rate of the plant. Along with nano-biofertilizers, nanoparticles and biofertilizers were also devised to treat the maize crop and to evaluate the comparative effects, including seed germination proportion and growth parameters of the crop.

EXPERIMENTAL

Materials and Methods

Selection of nanoparticles

1.6 g of zinc sulphate ($ZnSO_4$) was selected because it acts as a precursor to synthesize particles by using the modified protocol described by Tarafder *et al*. (2020).

Preparation of Plant Extract

In order to synthesize the zinc nanoparticles, an extract from *Nigella* seeds was prepared. *Nigella* seeds were obtained from regional market and ground into a fine powder (5 g). About 50 mL of distilled water was added to 5 g of *Nigella* powder. The mixture was heated in a microwave oven at 1000 W for 5 mins, then filtered the plant extract by using Whatman No.1 filter paper and stored for future experimental procedures.

Preparation of Salt Solution

To synthesize the zinc nanoparticles, salt solution was prepared first. For this, about 1.6 g of $ZnSO_4$ was mixed with 100 mL of distilled water (providing 0.1M soln. of $ZnSO_4$).

Synthesis of Nanoparticles

About 10 mL of 0.1M $ZnSO_4$ solution was poured in a biuret. In a conical flask 20 mL of *Nigella* extract was added, and a solution of $ZnSO_4$ was added dropwise to plant extract and shaken continuously to mix thoroughly until the color of the mixture did not change. Falcon tubes (60 mL) were filled with $ZnSO_4$ + Plant extract solution, and the mixture was centrifuged at 600 rpm for 15 mins each time. The supernatant was discarded from falcon tubes, and dense nanoparticles at the bottom of tubes were separated in Petri dishes. The solids were then dried at 60 °C in a drying oven for 60 mins. Later, the dried nanoparticles were stored in airtight bottles for future experimental work.

Evaluation of Prepared Samples

X-Ray diffraction (XRD) (using Bruker model D2-Phaser) and Fourier Transform Infrared Spectrometry (FTIR) analyses of biofertilizers, nanoparticles, and nano-biofertilizers were carried out with the help of Agilent Technologies Cary (630 model) at Food and Biotechnology Research Center PCSIR, Laboratories Complex Lahore, Pakistan. Scanning electron microscopy (SEM) of biofertilizers, nanoparticles, and nano-biofertilizers was performed by using an S-3700N (Hitachi) device at 10 kV with 500 magnification by field emission scanning electron microscope (FESEM) at Dr. Ikram-ul-Haq Institute of Industrial Biotechnology (IIIB), GC University Lahore, Pakistan.

Characterization of Nanoparticles

Optimization of biofertilizers

The biofertilizers used in this work were the product of NIBGE, Faisalabad, Pakistan. They were utilized in the experiment and also for the synthesis of nano-biofertilizers.

Incorporation of Biofertilizers into the Zn-Nanoparticles to Synthesize Nano-biofertilizers

Nano-biofertilizers were synthesized by incorporating ZnNPs in biofertilizers with a specific ratio of 1:50 respective ratio of biofertilizers and nanoparticles. ZnNPs were added in different amounts such as 2, 5, and 10 mg in 100 mg of biofertilizers. Nano-biofertilizers were also given to the crop in the same amounts of 2, 5, and 10 mg compared to biofertilizers in 0.01 mg amounts. All the treatments were given to each experimental plate at the same time.

Table 1. Layout of the Experiment According to CRD with Three Replicates

VoSoToR1	VoSoT1R1	VoSoT2R1	VoSoT3R1	VoSoT4R1	VoSoT5R1	VoSoT6R1	VoSoT7R1
VoSoToR2	VoSoT1R2	VoSoT2R2	VoSoT3R2	VoSoT4R2	VoSoT5R2	VoSoT6R2	VoSoT7R2
VoSoToR3	VoSoT1R3	VoSoT2R3	VoSoT3R3	VoSoT4R3	VoSoT5R3	VoSoT6R3	VoSoT7R3
VoS1ToR1	VoS1T1R1	VoS1T2R1	VoS1T3R1	VoS1T4R1	VoS1T5R1	VoS1T6R1	VoS1T7R1
VoS1ToR2	VoS1T1R2	VoS1T2R2	VoS1T3R2	VoS1T4R2	VoS1T5R2	VoS1T6R2	VoS1T7R2
VoS1ToR3	VoS1T1R3	VoS1T2R3	VoS1T3R3	VoS1T4R3	VoS1T5R3	VoS1T6R3	VoS1T7R3

Notes: Vo is maize cultivar (MMRI-Yellow), So is 0 mM NaCl stress, S1 is 120 mM NaCl stress, To is no-fertilizer treatment, T1 is 2 mg-ZnNPs, T2 is 5 mg-ZnNPs, T3 is 10 mg-ZnNPs, T4 is nano-biofertilizers (2 mg), T5 is nano-biofertilizers (5 mg), T6 is nano-biofertilizers (10 mg), T7 is biofertilizers (0.01 mg)

Seed Germination

Maize cultivar (*Zea mays*, MMRI-Yellow) was obtained from Punjab Seed Corporation, Lahore, Pakistan. Before sowing, the seeds were sterilized for 7 to 10 mins in the normal liquid bleach to remove any contamination. After sterilizing, the seeds were then soaked in water for 24 h and later dried in air. The sandy loam soil was collected from the Lahore vicinity and cleared all the stones, debris, and straw particles. To remove soil, sand was thoroughly washed out with tap water and dried in air for 24 h. Black plates with a diameter of 25 cm and depth of 2.5 cm were washed thoroughly to avoid contamination and filled out with sand.

Completely randomized designs (CRD) along with three replicates were prepared for the experiment to ensure the statistical reliability.

Sowing and Application of Treatments

Seeds were sown in the sand on 18 June 2021. Each replicate contained 22 seeds. Various treatments including nanoparticles, biofertilizers, and nano-biofertilizers were given to seeds in different concentrations mentioned in the layout of the experiment after sowing seeds. About 0 mM NaCl solution was given to 24 plates, and 120 mM saline treatment was given to the other 24 plates.

Germination Record

Germination started after one day of sowing. Data regarding germination was recorded regularly on a daily basis. Germination %age was calculated after experimenting. Germination was recorded for 18 days. The mean emergence time (MET) in days was calculated by using the Eq. 1 as proposed by Benvenuti *et al*. (2001),

$$MET= \sum (n \times g)/N \quad (1)$$

where n is the number of seedlings emerging per day, g is the number of days needed for emergence, and N is the total number of emerged seeds.

The vigor index of seedling was determined using Eq. 2.

$$\text{Vigor Index} = \text{Seedling length (cm)} \times \text{Seed germination (\%)} \quad (2)$$

Growth Analysis

Root and shoot lengths of one plant taken from each replicate were measured from base to tip with the help of a measuring scale. The fresh weight of one plant from each replicate was measured using an electric balance. These plants were taken for fresh weight oven dried at 60 °C for about 24 h approx. After that, their weight was determined. The specimens were then dried for 2 h more and weighed again to verify completeness of drying. The seedling length and leaf length of fresh plants from each replicate were also determined by using the measuring scale. The mean values of each parameter were calculated.

Statistical Analysis

Data were put through ANOVA and comparison of means ($P \leq 0.05$) by using CoStat v6.303.

RESULTS AND DISCUSSIONS

The analyses (SEM, FTIR, XRD) were conducted to interpret the structural determination, distribution of all the samples, and their particle sizes. All the samples were ground into a fine powder before analysis. The analyses were specifically conducted to determine the efficiency of ZnNPs and nano-biofertilizers.

SEM Analysis

This analysis involving the distribution, shape, size, and surface morphologies of the particles considered for control and saline samples was conducted utilizing a FESEM model (Hitachi S-3700N) at 10 kV with 500 magnification mode. Results are presented in Fig. 1.

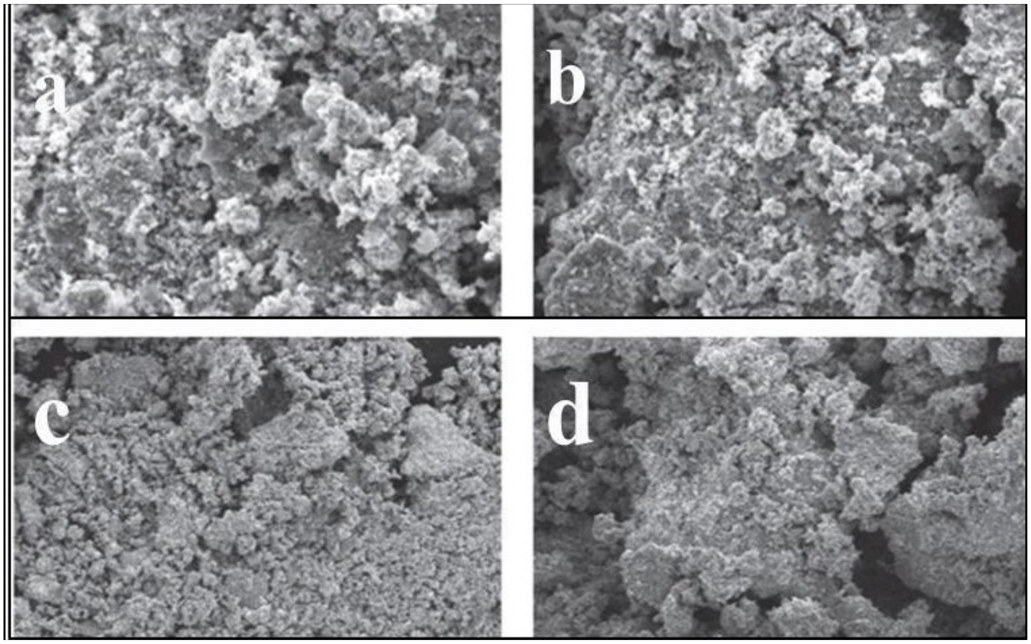

Fig. 1. SEM analysis of (a,b) Zn-Nano, (c) Biofertilizers and (d) Nano-biofertilizers

Particle size was also measured using the Java-based ImageJ Software. The morphology and structural analysis of biofertilizers, nano-biofertilizers, and ZnNPs (which were synthesized by 20% *Nigella* plant extract) was determined by using SEM analysis. After careful and detailed microscopic examination of the nano-materials, the microscopic images demonstrated the formation of networks that were joined together by ZnNPs. SEM analysis revealed the aggregation of nanoparticles, biofertilizers, and Zn nano-biofertilizers, whereas some particles were scattered on the surface. Illustration of these particles justified that the prepared ZnNPs were in accordance with the results from XRD. The images showed that the shape of the prepared ZnNPs (which were individually dispersed while taking measurements) was spherical and size of the particles was 25 nm to 40 nm. The SEM images also indicated the distribution and structure of the biosynthesized ZnNPs. The shape of particles of biofertilizers and nano-biofertilizers was slightly changed. They were in the form of clusters and pentagonal objects. These factors may be important for evaluating the potential effectiveness of the nanoparticles, biofertilizers, and nano-biocomposites in applications related to control and saline treatments.

FTIR Analysis

An FTIR spectrometer (Cary 630 model, Agilent Technologies) was used to detect the functional groups, as indicated by different peaks for ZnNPs, biofertilizers, and nano-biofertilizers, which are displayed in graphs.

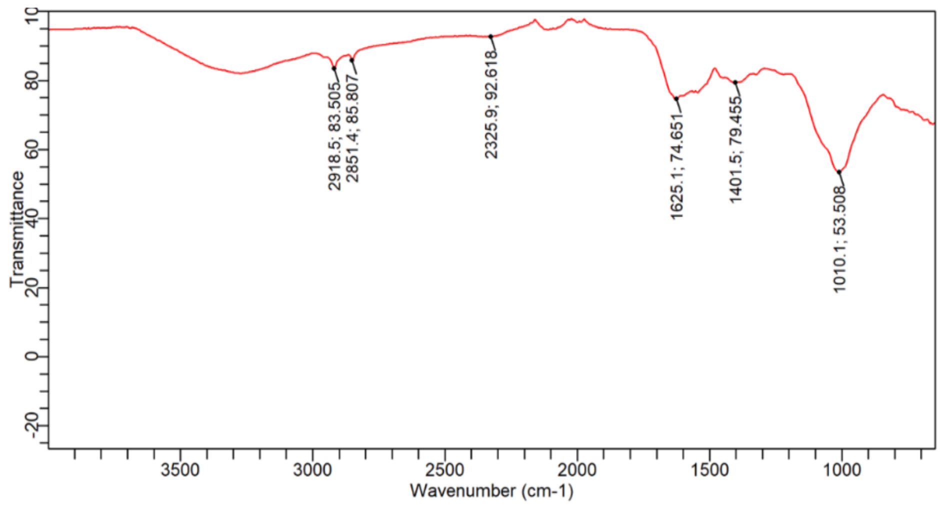

Fig. 2(a). FTIR spectrum of zinc nanoparticles

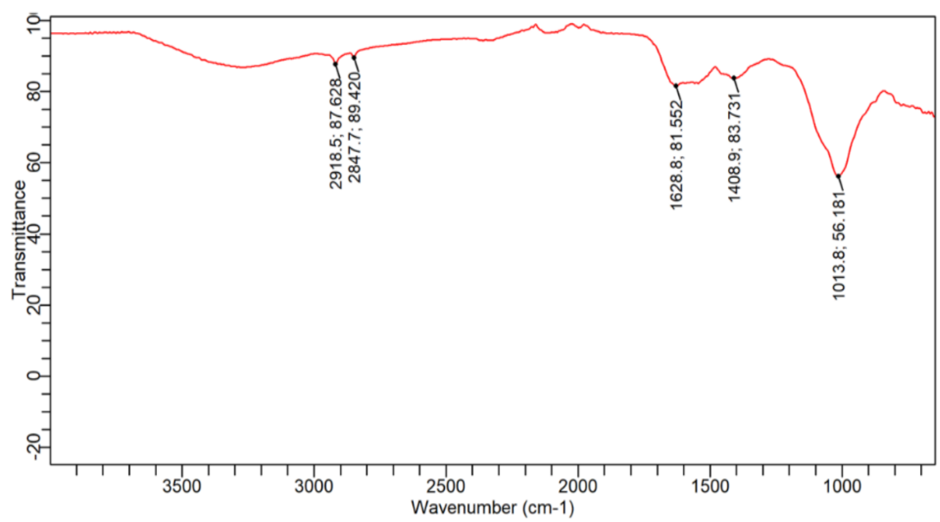

Fig. 2(b). FTIR spectrum of biofertilizers

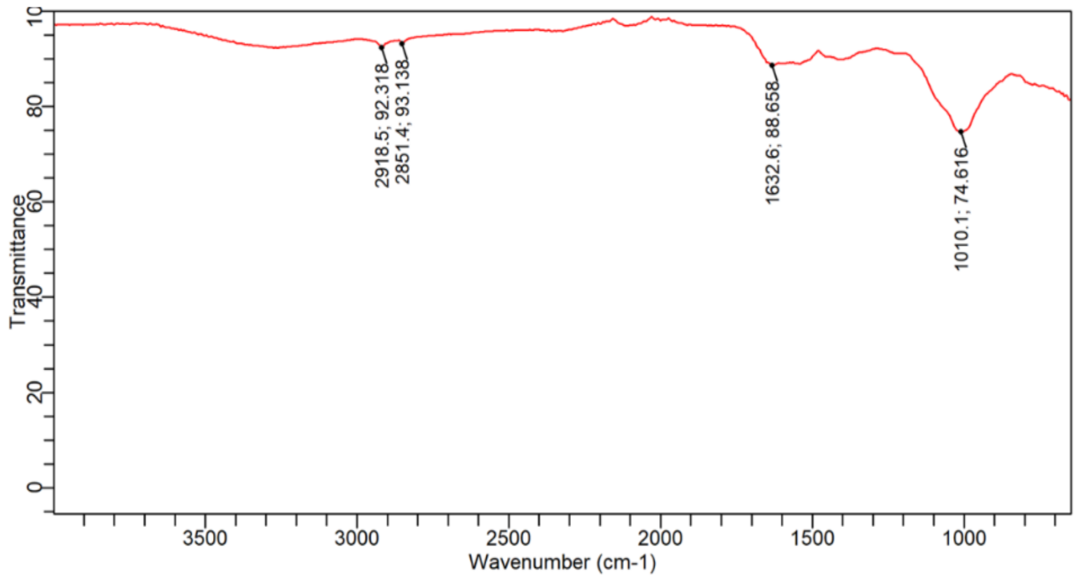

Fig.2(c). FTIR spectrum of nano-biofertilizers

FTIR spectra of the synthesized zinc nanoparticles exhibited prominent absorbance peaks at 1010, 1401, 1625, 2325 and 2851 cm^{-1} with corresponding intensities of 53.5, 79.4, 74.6, 92.6, and 85.8, respectively. The vibration band at 1010 cm^{-1} was attributed to C−O stretching vibration of alcohol/amine group. The peak observed at 1401 cm^{-1} C−H was attributed to the bending vibration of aliphatic chains. The 1625 cm^{-1} band corresponds to the C=C stretching of aromatic compounds, which indicates the presence of phenolic/protein components. The band observed at 2325 cm^{-1} was assigned as the weak band corresponds to the atmospheric CO_2 absorption, and the peak observed at 2851 cm^{-1} represents the aliphatic C−H stretching vibrations.

The results involving nanoparticles indicated that the nanoparticles were capped and stabilized by the oxygen- and nitrogen-containing biomolecules that conferred enhanced stability on the nanoparticles. Results involving the biofertilizers showed peaks at 1013, 1408, 1628, 2847 and 2918 cm^{-1} with the corresponding intensities of 56.2, 83.7, 81.6, 89.4, and 87.6, respectively. The peak observed at 1013 cm^{-1} implies strong C−O stretching vibrations (alcohols, esters, ethers, glycosidic C−O−C of carbohydrates) and partially overlapped with C−N stretching of aliphatic amines.

The peak observed at 1408 cm^{-1} indicates mixed CH_2/CH_3 bending or symmetric stretch of carboxylate anion. The band at 1628 cm^{-1} shows amide I C=C stretching of proteins/peptides. The band at 2847 cm^{-1} represents symmetric aliphatic C−H stretching and band at 2918 cm^{-1} indicates asymmetric aliphatic C−H stretching respectively. For the nano-biofertilizers spectra, the peaks observed were 1010, 1632, 2851, and 2918 cm^{-1} with intensities of 74.6, 88.6, 93.1, and 92.3 respectively. The peak observed at 1010 cm^{-1} shows C−O stretching (alcohols, esters, ethers, glycosidic C−O−C). The band vibration at 1632 cm^{-1} indicates the presence of amide I (C=O stretching of proteins/peptides). The peak observed at 2851 cm^{-1} represents the symmetric aliphatic C−H stretching, and the peak at 2918 cm^{-1} shows the asymmetric aliphatic C−H stretching.

XRD Analysis

X-Ray diffractometer was used to analyze different fertilizers, particles, and nano-sized materials. The nanomaterials were manually dispersed on the glass substrate for the

XRD analysis. Nanoparticles of zinc (ZnNPs, which were prepared from 20% plant extract), biofertilizers, and nano-biofertilizers were examined by using Cu-Kα in XRD to ensure the occurrence of nanoparticles and for the determination of their structures. Peaks of ZnNPs were observed at 11.96°, 14.12°, 21.3°, 31.76°, 41.96°, and 54.48° having intensities of 80 (a.u), 93 (a.u), 51 (a.u), 45 (a.u), 34 (a.u), and 36 (a.u). These peaks were distinctly in accord with the literature and demonstrated that manufactured nanoparticles were crystalline in nature of zinc-based nanoparticles and their composites. Similarly, the diffraction pattern of biofertilizers is illustrated in Fig. 3, and the peaks which were analyzed at 13.6°, 14.84°, 26.44°, and 30.52° demonstrated the shape of biofertilizers which was hexagonal and intensities observed were 90 (a.u), 81 (a.u), 83 (a.u) and 58 (a.u), respectively. In the nano-bio diffractogram, the peak observed at an angle of 26.8° has 72 (a.u) intensity and the shape of the particles was trigonal.

The presence of a few low-intensity peaks indicates that the composite exhibits more amorphic distribution and a relatively low degree of crystallinity. It can be concluded that all of the detected peaks of ZnNPs, biofertilizers, and nano-biofertilizers have spherical, trigonal, and hexagonal configurations. Smaller particle sizes showed broader diffraction peaks because of the destructive interference effects.

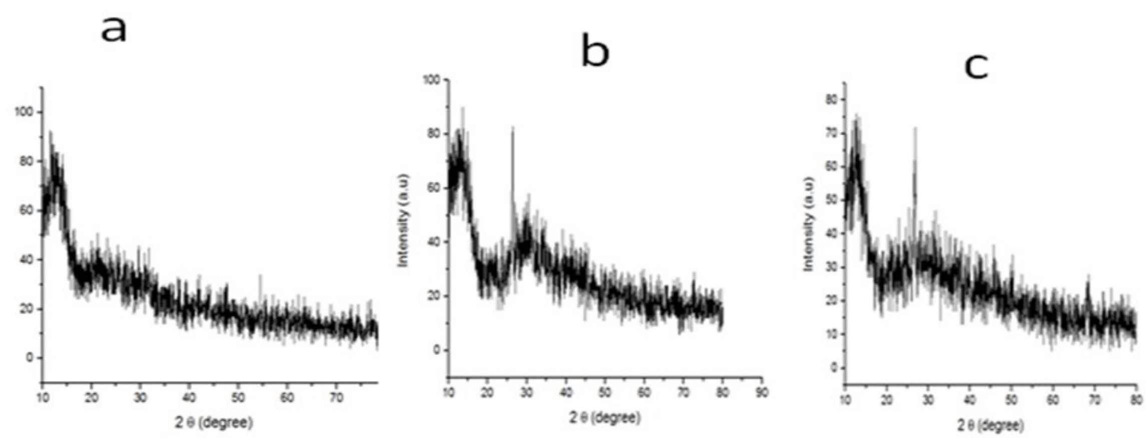

Fig. 3. XRD patterns of (a) Zn NPs; (b) Biofertilizer; and (c) Nano-biofertilizer

GERMINATION MEASUREMENTS

Germination Percentage

The main effects of salinity and treatment were observed as non-significant source of variations. The interactive effects of salinity and treatment also appeared to be non-significant. Comparison of seed germination percentages are given in Fig. 4. Plants gave a positive response when the seeds were treated with 0 mM NaCl and salinized with 120 mM of NaCl soln. Values obtained for means for various treatment conditions were statistically different from each other and from the control, whereas in saline medium they were at par with each other. The LSD value ($P \leq 0.05$) for seed germination was 6.10%.

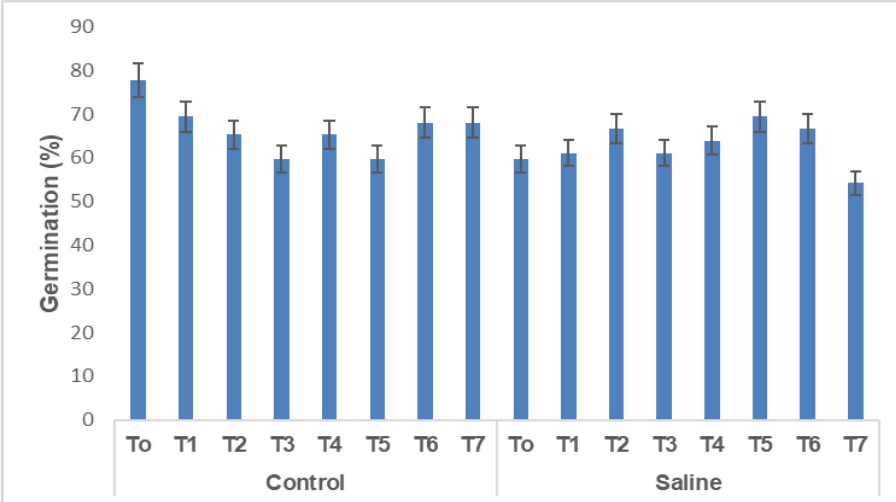

Fig. 4. Germination percentages of maize plants as affected by different treatments of Nanoparticles, Biofertilizers, and Nano-biofertilizers applied under salt stress (Mean± Std. Error). where To is no-fertilizer treatment, T1 is 2 mg-ZnNPs, T2 is 5 mg-ZnNPs, T3 is 10 mg-ZnNPs, T4 is nano-biofertilizers (2 mg), T5 is nano-biofertilizers (5 mg), T6 is nano-biofertilizers (10 mg), T7 is biofertilizers (0.01 mg)

Growth Measurements

Fresh weight

A non-significant source of variations was observed for the effect of salinity, whereas in case of treatment, it was highly significant. Significant sources of variations were found in the interactive effects of salinity and treatment. Data for the fresh weight (g) of plant body are given in Fig. 5. Noticeable values were observed in plant variety when seeds were treated with control NaCl and salinized with NaCl. Seeds showed minimum results when only treated with NaCl. Non-significant values of comparison of means for both control and saline were statistically different. The LSD value ($P \leq 0.05$) for fresh weight was 0.25.

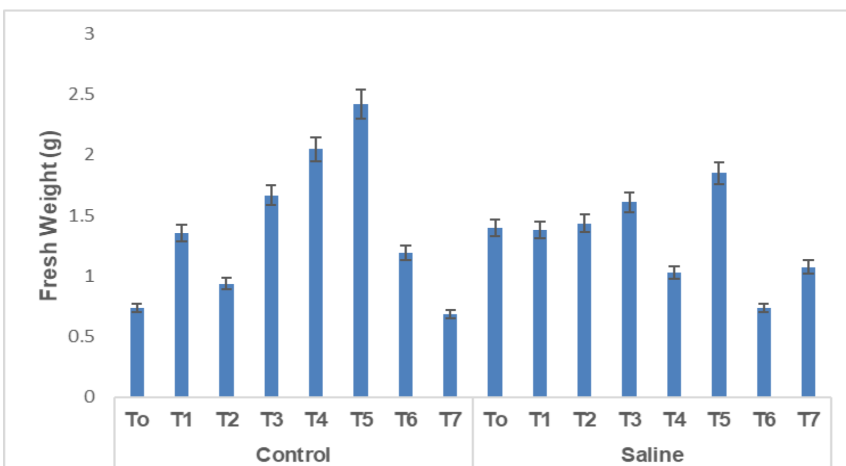

Fig. 5. Fresh weights of maize plants as affected by different treatments of Nano-particles, Biofertilizers, and Nano-biofertilizers applied under salt stress (Mean± Std. Error). Variables: To is no-fertilizer treatment, T1 is 2 mg-ZnNPs, T2 is 5 mg-ZnNPs, T3 is 10 mg-ZnNPs, T4 is nano-biofertilizers (2 mg), T5 is nano-biofertilizers (5 mg), T6 is nano-biofertilizers (10 mg), T7 is biofertilizers (0.01 mg)

Dry weight

The main effects of salinity were shown to be non-significant and the effect of treatments was a moderately significant source of variations. Interactive effects were a significant source of variations when considering salinity and treatment. Dry weight (g) comparative means data is given in Fig. 6.

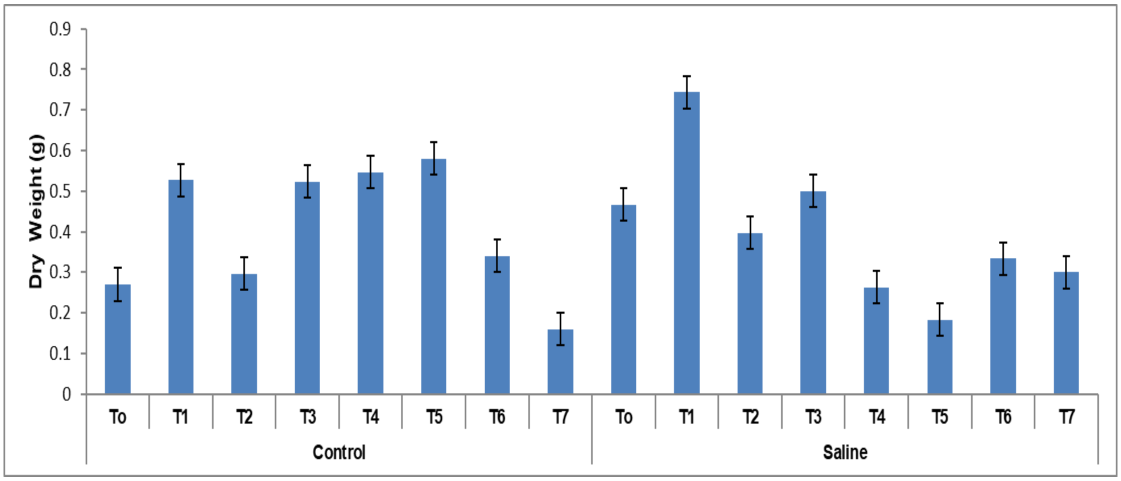

Fig. 6. Dry weights of maize plants as affected by different treatments of Nano-particles, Biofertilizers, and Nano-biofertilizers applied under salt stress (Mean± Std. Error). Where To is no-fertilizer treatment, T1 is 2 mg-ZnNPs, T2 is 5 mg-ZnNPs, T3 is 10 mg-ZnNPs, T4 is nano-biofertilizers (2 mg), T5 is nano-biofertilizers (5 mg), T6 is nano-biofertilizers (10 mg), T7 is biofertilizers (0.01 mg).

Less significant values were observed when seeds were salinized with NaCl only. The values observed when treated with control were significant. The non-significant values of comparison of means for both salinity and control were statistically different. The LSD value ($P \leq 0.05$) for dry weight was 0.09.

Seedling Length

No significant variations in seedling length were found for salinity and treatment. In addition, no statistically significant interactive effects were found for salinity and treatment. Seedling length comparative means data is given in Fig. 7. Highest values were noticed in maize variety when seeds were treated with control and under salt stress. Non-significant values of comparison of means for control were at par with each other and for salinity, statistical differences were observed. For seedling length, the LSD value ($P \leq 0.05$) was 3.08.

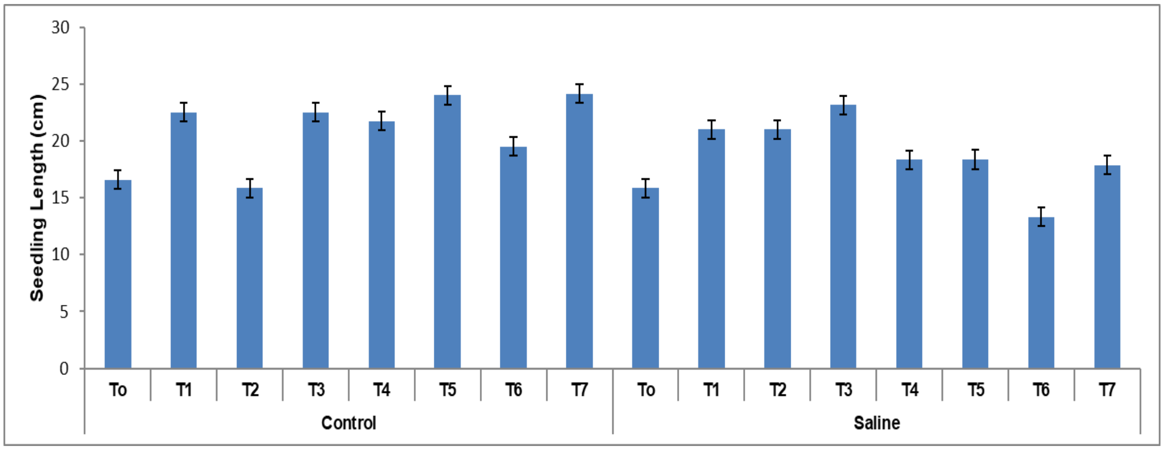

Fig. 7. Seedling lengths of maize plants as affected by different treatments of Nano-particles, Biofertilizers, and Nano-biofertilizers applied under salt stress (Mean± Std. Error). To is no-fertilizer treatment, T1 is 2 mg-ZnNPs, T2 is 5 mg-ZnNPs, T3 is 10 mg-ZnNPs, T4 is nano-biofertilizers (2 mg), T5 is nano-biofertilizers (5 mg), T6 is nano-biofertilizers (10 mg), T7 is biofertilizers (0.01 mg).

Leaf Length

The main effects of salinity and treatment were not significant in the case of leaf length. Likewise, interactive effects were not significant. Leaf length comparative means data are given in Fig. 8.

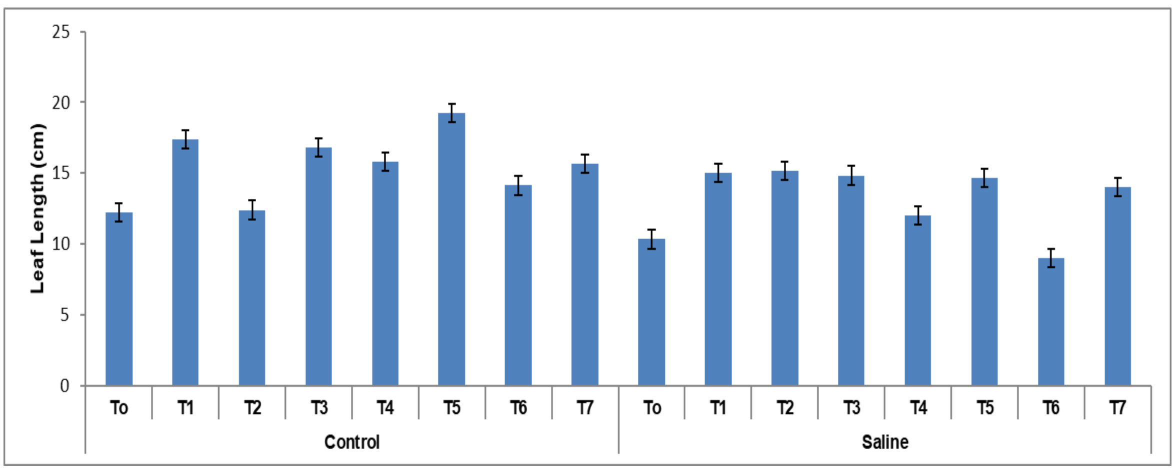

Fig. 8. Leaf length of maize plants as affected by different treatments of Nano-particles, Biofertilizers, and Nano-biofertilizers applied under salt stress (Mean± Std. Error). To is no-fertilizer treatment, T1 is 2 mg-ZnNPs, T2 is 5 mg-ZnNPs, T3 is 10 mg-ZnNPs, T4 is nano-biofertilizers (2 mg), T5 is nano-biofertilizers (5 mg), T6 is nano-biofertilizers (10 mg), T7 is biofertilizers (0.01 mg).

Significant values were observed in maize cultivar (MMRI-Yellow) when seeds were treated with control NaCl and salinity. For both control and salinity, significant values of comparison of means were at par with each other. The LSD value ($P \leq 0.05$) for leaf length was 2.67.

Root Length

Salinity and treatment did not show significant effects for root length. Likewise, the interactions of salinity and treatment were not significant. Root length comparative means data is given in Fig. 9. Highest values were observed in maize variety when seeds were salinized with 120 mM NaCl solution and for the control treatment. Mean values for salinity and control were at par with each other. The LSD value ($P \leq 0.05$) for root length was 1.52.

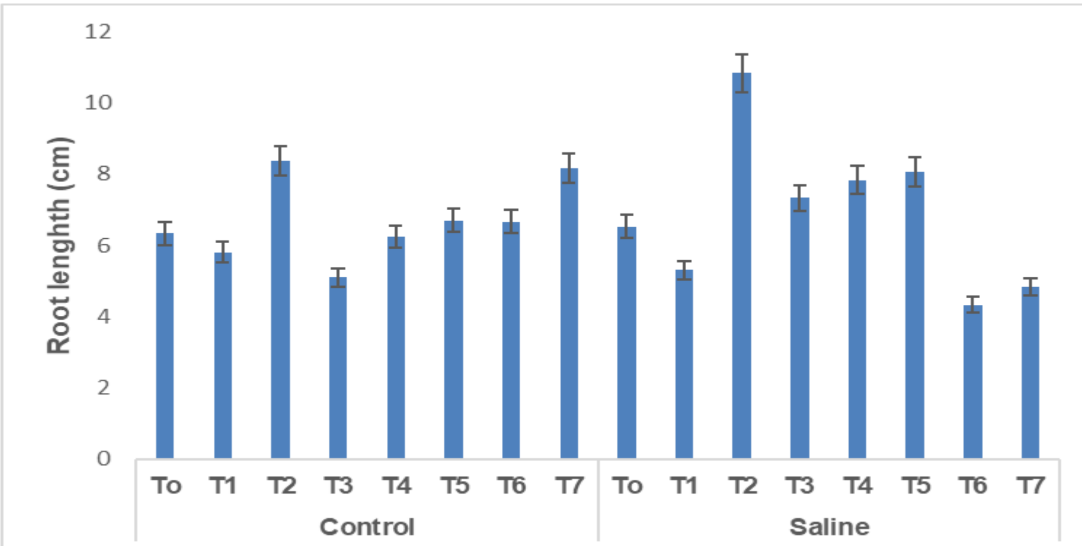

Fig. 9. Root length of maize plants as affected by different treatments of Nano-particles, Biofertilizers, and Nano-biofertilizers applied under salt stress (Mean± Std. Error). To is no-fertilizer treatment, T1 is 2 mg-ZnNPs, T2 is 5 mg-ZnNPs, T3 is 10 mg-ZnNPs, T4 is nano-biofertilizers (2 mg), T5 is nano-biofertilizers (5 mg), T6 is nano-biofertilizers (10 mg), T7 is biofertilizers (0.01 mg).

Shoot Length

The main effects of salinity and treatment did not show significant differences for shoot length. Likewise, interactive effects were not significant. Shoot length comparative means data is given in Fig. 10. The highest values were observed in maize variety MMRI-Yellow when salinized with NaCl solution and treated with control treatment. Means for both control and salinity were not significantly different. The LSD value for shoot length ($P \leq 0.05$) was 3.20.

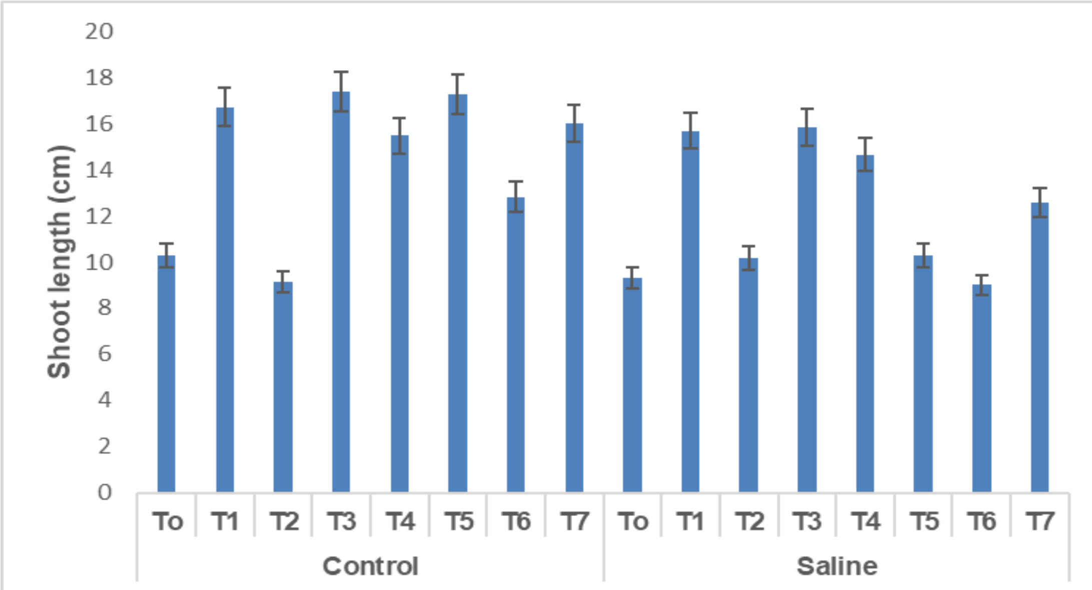

Fig. 10. Shoot length of maize plants as affected by different treatments of Nano-particles, Biofertilizers and Nano-biofertilizers applied under salt stress (Mean± Std. Error). To is no-fertilizer treatment, T1 is 2 mg-ZnNPs, T2 is 5 mg-ZnNPs, T3 is 10 mg-ZnNPs, T4 is nano-biofertilizers (2 mg), T5 is nano-biofertilizers (5 mg), T6 is nano-biofertilizers (10 mg), T7 is biofertilizers (0.01 mg).

Significant values were observed in maize cultivar (MMRI-Yellow) when seeds were treated with control NaCl and salinity. For both control and salinity, significant values of comparison of means were at par with each other. The LSD value ($P \leq 0.05$) for leaf length was 2.67.

Vigor Index

The main effects of salinity and for treatment showed moderately significant differences for vigor index. However, their interaction was not significant. Vigor index comparative means data is shown in Fig. 11. Prominent values were observed among plant variety (MMRI-Yellow) when salinized with NaCl solution and treated with control treatment. The LSD value for vigor index ($P \leq 0.05$) was 5.97.

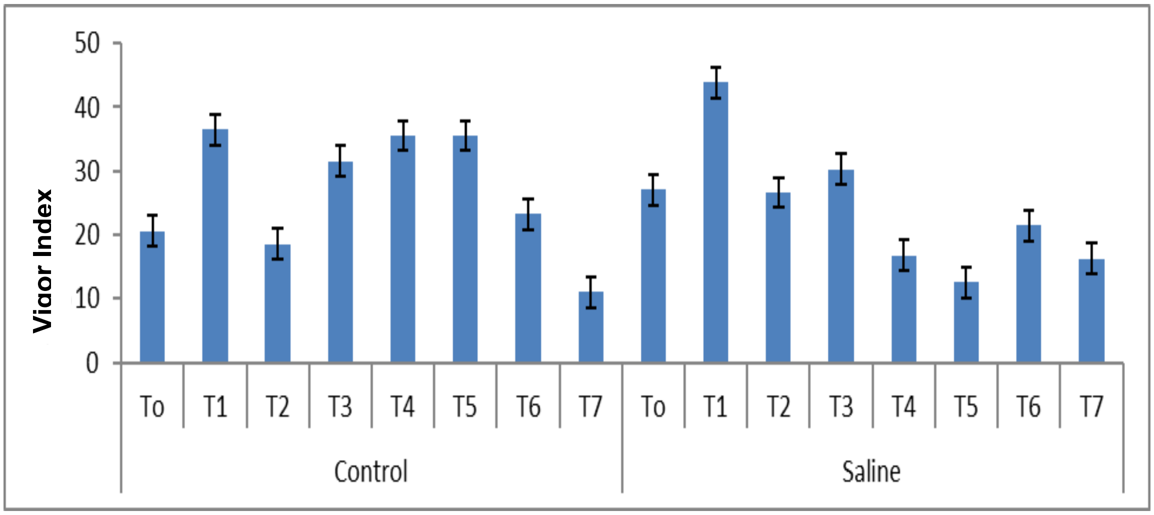

Fig. 11. Vigor index of maize (*Zea mays*) plants as affected by different treatments of Nano-particles, Biofertilizers and Nano-biofertilizers applied under salt stress (Mean± Std. Error). To is no-fertilizer treatment, T1 is 2 mg-ZnNPs, T2 is 5 mg-ZnNPs, T3 is 10 mg-ZnNPs, T4 is nano-biofertilizers (2 mg), T5 is nano-biofertilizers (5 mg), T6 is nano-biofertilizers (10 mg), T7 is biofertilizers (0.01 mg).

DISCUSSION

This study considered the preparation of nano-biofertilizers by using suitable bacteria and the implementation of nano-biofertilizers on maize plant to check their viability. The synthesis of ZnNPs and its use in biofertilizers to make nano-biofertilizers were delineated. Nano-structured particles are suggested for use because they provide new methods to improve the production of crops. Nanoparticles having tiny size and very large surface area are anticipated as an advantageous substance by which to distribute Zn ions in association with fertilizers for plants. Nutrients are necessary for a plant to function normally, especially during their growth and development phase. Plant development gets stunted and agricultural output is depressed when plants are unable to complete their life cycles and perform physiological processes due to a lack of essential nutrients (Kalaji *et al*. 2014). In the entire world, zinc insufficiency is the most prevalent micronutrient deficiency that limits the productivity of agriculture. One of the most efficient ways to enhance agriculture yields globally is through the development and application of fertilizers in nano form. The ZnNPs are considered to be harmless for use with living organisms. Previous studies have shown that these materials can aid in the prevention and protection the plants from diseases due to their antibacterial properties. ZnNPs have also been shown to promote seed germination, plant growth, and other forms of preventative care for various plant ailments (Matinise *et al*. 2017).

In order to figure out the impacts of ZnNPs among agricultural plants, different investigations have been done, and besides affecting plant development and growth, biologically synthesized ZnNPs have been demonstrated to enhance the soil enzyme's activity *e.g.*, phytase, acid phosphatases, and alkaline phosphatases (Ahmed *et al*. 2023). Some past surveys have justified that when fertilizers were given to the crops, they only showed 30% enhancement of productivity, whereas the remaining 70% depends upon the different agricultural aspects and statistics. Nanoparticles can regulate metabolic activities of plants, which affects the viability to stimulate nutrients such as phosphorus in plants (Zahra *et al*. 2015). Nano-biofertilizers are synthesized using different microorganisms, although some nanofertilizers can be made biosynthetically. Moreover, these biosynthetically formulated nanoparticles studied and used worldwide due to its multiple applications in the field of nano-medicine. These biosynthetically formulated nanoparticles are more cost efficient and eco-friendly (Patel and Krishnamurthy 2015).

Previous studies have shown that nanoparticles can be synthesized by using certain physical, chemical, and biological methods. Nanoparticles can also be biosynthesized by using oxidation and reduction processes when considered in small quantities showing minimum deficiencies either *in-vivo* or *in-vitro*. In these processes, substances including proteins, sugars, enzymes, and phytochemicals *e.g.*, flavonoids, phenolics, cofactors and terpenoids, *etc*., can act as reducing or stabilizing agents. There are many investigations that emphasizes the importance and use of biosynthesized nanoparticles in nano-fertilization (Belal and El-Ramady 2016; Dubey and Mailapalli 2016; Mani and Mondal 2016; Chhipa 2017; Khan and Rizvi 2017; Okorie *et al*. 2017). Hence, it can be concluded that by utilizing various microorganisms and plant extracts, it is possible to carry out the biosynthesis of nanofertilizers. The biological approach to the synthesis of nanofertilizers allows for the production of nanoparticles through various biotechnological innovations. With the passage of time, there is an increased emphasis on investigation of the novel methods for the preparation of biological mediated nanofertilizers.

The purpose of this study was to figure out the impacts of nano-fabricated zinc particles and nano-biofertilizers on production, harvest, and different parameters of maize cultivar MMRI-Yellow undergoing salt stress. A seed germination trial experiment was performed in the sand in normal atmospheric conditions to check the efficiency of nano-particles, biofertilizers, and mainly nano-biofertilizers by their treatment with maize seeds.

After sowing seeds, they were treated with salt solution and Hoagland’s solution. This experiment involved fertilizer treatments which were no-fertilizer application, ZnNPs, bio-fertilizers, and a combination of Zn nano-biofertilizers. These fertilizers were given to a maize plant variety (MMRI-Yellow) in different concentrations after sowing the seeds. Plants were watered daily and observed regularly. All the readings were recorded on a daily basis with the plant growth including number of seedlings sprouted each day, shoot and root lengths, dry and fresh weights, vigor index, and seedling length. Germination started after one day and seedlings started to grow. Maximum results were obtained by the plants that were grown in complete nano-biofertilizers. Seeds that were treated with nanoparticles and bio-fertilizers also showed desirable results. The were sufficient yields even in no-fertilizer applications. The analysis of variance (ANOVA) and graphical representation showed that both treatments control and salinity had almost equal interactive effects and yield relative to maize plant, but as compared to salinity, the growth of seeds in the control condition gave better results. After complete emergence and plant growth, different parameters were observed such as leaf length, shoot and root lengths, fresh and dry weights, plant height, and these parameters showed the best outcomes. Characterization of all the three samples (nanoparticles, bio-fertilizers, and Zn nano-biofertilizers) was conducted by means of SEM analysis, FTIR, and X-ray diffraction methods which provided the morphological representation, distribution, and size of the particles.

FTIR spectrometry was mainly used to assess certain characteristics of different potential substances to regulate and stabilize the biosynthetic nanoparticles by undergoing the process of bioreduction. This analysis encompassed certain aspects such as chemical composition, functional groups, atomic structures, and surface chemistry (Ahmadi-

Nouraldinvand *et al*. 2022). SEM analysis serves as a valuable process for visualizing dimensions, morphology, and distribution of nanofibers (Sowmya *et al*. 2019). Additionally, X-Ray diffractometer was used to identify the crystalline structures and phases of the synthesized nano materials (Venkateswaran *et al*. 2022).

CONCLUSIONS

1. It can be concluded that nano-biofertilizers promoted the plant growth and enhanced the nutrient value by utilizing various effects on soil and plants.
2. Nano-materials and biofertilizer components were shown to have interactive benefits; therefore nano-biofertilizers showed a great response in the improvement of the plant growth, yield, and development and quality parameters of crops as they were described in different biological and chemical investigations.
3. Biofertilizers when combined with zinc nanoparticles eventually increased the crop yield by enhancing the photosynthetic activity, efficiency and growth rate which increased the productivity and different growth parameters of the maize crop.

Competing Interests

The authors declare that there are no conflicts of interest.

Availability of Data and Material

All the data generated in this research work has been included in this manuscript.

Acknowledgments:

The authors are thankful to Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R31), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

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Article submitted: July 8, 2025; Peer review completed: October 5, 2025; Revised version received: November 26, 2025; Accepted: November 30, 2025; Published: January 16, 2026.
DOI: 10.15376/biores.21.1.1968-1989