

EFFECT OF BIOSYNTHESED ZINC NANOPARTICLES ON WHEAT (*Triticum aestivum* L.) PRODUCTION

Abstract

The research was carried out at the Green Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad, Karnataka, with a specific emphasis on the biosynthesis of zinc nanoparticles using *Pseudomonas* and actinobacteria. The zinc nanoparticles were biosynthesized and characterized through UV-Visible spectroscopy, Particle Size Analyzer (PSA), Scanning Electron Microscope (SEM), (EDX), X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). After biosynthesis and characterization of the nanoparticles (NPs), a pot experiment was conducted under controlled condition to know the effect of biosynthesized zinc nanoparticles on wheat. Seed priming @ 500 ppm and foliar spraying @ 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through actinobacteria (T₁₂) recorded significantly higher plant height (93.73 cm), number of tillers per plant (4.87), total dry matter production (45.57 g pot⁻¹), number of grains per spike (46.73), grain weight per spike (1.83 g), test weight (42.26 g), grain yield (3.95 g plant⁻¹) and straw yield (5.97 g plant⁻¹) and found on par with seed priming @ 500 ppm and foliar spraying @ 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through *Pseudomonas* (T₅).

Key Words: Actinobacteria, biosynthesis, nanoparticles, seed priming, zinc

1. Introduction

Wheat is a major food crop cultivated globally, providing food for 35 per cent of the world's population (Mohammadi-joo et al. 2015). The most of wheat that is grown on a worldwide is hexaploid, and extensively utilised to produce a variety of baked food products including bread, there is a substantial impact on human health based on the composition and nutritional quality of the wheat. Zn nanoparticles (Zn NPs) are among the top three most manufactured and used engineered nanoparticles (Zhang et al. 2015). ZnO nanoparticles, one of the best source for preventing Zn deficiency and enhancing crop quality and productivity (Dimkpa et al. 2015). ZnNPs have an impact on plant metabolism at the molecular level by activating antioxidants and reductases, as well as influencing the synthesis of plant hormones (Timilsina and Chen 2021).

Nanotechnology may help bring about a new technological revolution in agriculture. Several problems with conventional biofortification could potentially resolved by nanotechnology (Shakiba et al. 2020). The encapsulation of nutrients with nanomaterials results in efficient nutrient absorption by plants, due to the gradual or controlled release of nanoparticles and simple passage through biological barriers by nanoparticles entering the plant vascular system (De La Torre-Roche et al. 2020). In comparison to conventional fertilisers, long-term delivery of plants via nanofertilizers enables enhanced crop growth. As nanofertilizers are added in small amounts, these also prevent soil from becoming burdened with the by-products of chemical fertilisers and reduce the environmental hazards (Silva et al. 2018).

In order to increase productivity and the quality of the food produce, seed priming has been used to synchronise and speed up germination, boost seedling vigour, and increase plant resistance to biotic and abiotic stresses (Acharya et al. 2019). According to recent studies, seed nano-priming can activate several genes during germination, particularly those involved in plant stress resistance (Salama et al. 2019). Using nanotechnology for seed priming is a relatively new field of study; it can be used to target seed biofortification to reduce malnutrition (Nile et al. 2022).

Although applying nutrients to the soil is the most popular method, it has significant drawbacks in terms of the nutrients' availability to the plants, due to the insoluble forms of the inorganic nutrients are fixed in the soil and also prone to leaching by irrigation or rainfall (Alshaal and El-Ramady 2017). Foliar application overcomes these constraints. Additionally,

foliar feeding has demonstrated to be the quickest way to rectify nutrient shortages, increase crop production, and improve crop product quality. It also minimises environmental pollution and optimises nutrient utilisation by using less amount of fertiliser to the soil (Morab et al. 2021).

2. Material and Methods

2.1 Experimental site

Biosynthesis, characterization of zinc nanoparticles were done in Green Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad. At the Microbial Genetics Laboratory, Department of Agricultural Microbiology, UAS, Dharwad, *Pseudomonas* and actinobacterial isolates were collected and screened. The pot experiment was conducted during 2022-2023 at Institute of Agri- Biotechnology (IABT) polyhouse, University of Agricultural Sciences, Dharwad.

2.2 Physio-Chemical properties of the soil

The soil for the pot experiment was collected from the agronomy field near the field laboratory. Around 20 kg of soil was filled in the each pot. The soil sample was drawn before start of experiment from the polybags. The soil was dried, powdered and allowed to pass through 2 mm sieve and was analyzed for physical and chemical properties. The textural class of experimental soil was clayey and pH-7.76; EC-0.27 dS m⁻¹; organic carbon- 0.49 %; available nitrogen- 251.37 kg ha⁻¹; phosphorus- 31.45 kg ha⁻¹; potassium -363.24 kg ha⁻¹; zinc- 0.53 ppm and iron – 6.78 ppm (Table 1).

Table 1. Soil physical and chemical properties of the pot experimental soil

	Properties	Value	Methods employed
I.	Physical properties		
	Particle size analysis		
a.	Coarse sand (%)	6.51	
b.	Fine sand (%)	13.64	
c.	Silt (%)	26.45	

d.	Clay (%)	52.37	International pipette method (Piper, 2002)
e.	Textural class	Clayey	
II.	Chemical properties		
a.	Soil pH (1:2.5 soil: water)	7.76	Potentiometric method (Piper, 2002).
b.	Electrical conductivity (dS m^{-1})	0.27	Conductivity bridge (Piper, 2002)
c.	Organic carbon (%)	0.49	Walkely and Blacks wet oxidation method (Jackson, 1973)
d.	Available nitrogen (kg ha^{-1})	251.37	Alkaline permanganate method (Subbiah and Asija, 1956)
e.	Available P_2O_5 (kg ha^{-1})	31.45	Olsen's method (Jackson, 1973)
f.	Available K_2O (kg ha^{-1})	363.24	Flame photometer method (Jackson, 1973)
g.	DTPA extractable micronutrients (ppm)		
	Zinc (ppm)	0.53	DTPA extractant method (Lindsay and Norvell, 1978)
	Iron (ppm)	6.78	

2.3 Experimental procedure

Wheat seeds of the UAS 334 variety were collected from the Main Agricultural Research Station in Dharwad. The seeds were sown at a rate of 5 seeds per pot. Seeds were primed with biosynthesized zinc nanoparticles solution at 500 ppm, for a period of six hours for respective treatments. Nitrogen, phosphorus, and potassium were applied as urea, diammonium phosphate, and muriate of potash, respectively.

2.4 Treatmental details

The study was carried out using a Completely Randomized Design (CRD), sixteen treatments replicated three times. The experimental details was T₁- seed priming with BS (Bacterial (*Pseudomonas*) synthesized) ZnNPs @ 500 ppm; T₂- foliar spraying with BS ZnNPs @ 500 ppm; T₃- foliar spraying with BS ZnNPs @ 1000 ppm; T₄- foliar spraying with BS ZnNPs @ 1500 ppm; T₅- seed priming @ 500 ppm + foliar spraying @ 500 ppm with BS ZnNPs; T₆- seed priming @ 500 ppm + foliar spraying @ 1000 ppm with BS ZnNPs ; T₇-

seed priming @ 500 ppm + foliar spraying @ 1500 ppm with BS ZnNPs; T₈- seed priming with ABS (Actinobacteria synthesized) ZnNPs @ 500 ppm; T₉- foliar spraying with ABS ZnNPs @ 500 ppm; T₁₀- foliar spraying with ABS ZnNPs @ 1000 ppm; T₁₁- foliar spraying with ABS ZnNPs @ 1500 ppm; T₁₂- seed priming @ 500 ppm + foliar spraying @ 500 ppm with ABS ZnNPs; T₁₃- seed priming @ 500 ppm + foliar spraying @ 1000 ppm with ABS ZnNPs ; T₁₄- seed priming @ 500 ppm + foliar spraying @ 1500 ppm with ABS ZnNPs; T₁₅- RDF (recommended dose of fertilizers-100:75:50, N:P₂O₅:K₂O kg ha⁻¹, respectively) and T₁₆- control (without any fertilizer application). Foliar spraying at panicle initiation stage of the crop is common for all the foliar applied treatments. RDF is common for all the treatments except control.

2.5 Experimental procedure for growth parameters

The plant height, the perpendicular distance from soil level in the pot to the tip of longest fully opened leaf at 30 and 60 DAS and from base of plant to the base of panicle at 90 DAS was measured and expressed in centimeter (cm). The number of tillers were counted separately in each plant and recorded at 60 and 90 DAS. It was expressed as number of tillers per plant. Dry matter production and accumulation was studied for only above ground portions of the plant at harvest stage. The plant was uprooted from the pot and it was air dried and then oven dried at 70 °C till they attained constant weight. It was expressed in grams.

2.6 Experimental procedure for yield and yield components

The spikes from each plant in pot at harvest were used for recording the number of grains per spike. These spikes were threshed separately and number of grains per spike was recorded. The grains threshed for recording the number of grains per spike was used to estimate grain weight per ear spike and was expressed in gram. Randomly, 100 grains from each pot were weighed and then it was multiplied by 10 to get 1000 grain weight. The total yield from each plant was recorded and was presented as grain yield per plant. Straw yield per plant was worked out for respective treatment and was presented as straw yield per plant. The data on grain yield and straw yield were used to calculate the harvest index by using the formula given by Donald (1962).

2.7 Statistical analysis

The data collected from the experiment at various growth stages were subjected statistical analysis following the method given by Gomez and Gomez (1984). The significance level used in the 'F' test was P = 0.01 (1%). The critical difference (CD) at 1%

levels was computed whenever the 'F' test was given significant results. The mean values of treatments were separately subjected to Duncan Multiple Range Test (DMRT) using the corresponding error mean sum of squares and degrees of freedom.

3. Results and Discussion

3.1 Effect of biosynthesized zinc nanoparticles on wheat growth

Seed priming @ 500 ppm and foliar spraying @ 500 ppm with ZnNPs biosynthesized from actinobacteria and *Pseudomonas* resulted significantly higher plant height (93.73 and 92.70 cm, respectively), number of tillers per plant (4.87 and 4.73, respectively) and total dry matter production (45.57 and 44.36 g pot⁻¹, respectively) at harvest and found on par with seed priming @ 500 ppm and foliar spraying @ 1000 ppm with ZnNPs biosynthesized from actinobacteria and *Pseudomonas* (Table 2, 3 and 4). Foliar spraying of ZnONP significantly enhanced the wheat morphophysiological parameters. This improvement might have been caused by increased cell size, cell division, and the remobilization of stored carbohydrates in the plants (Adrees et al. 2021). Foliar application of ZnONPs significantly increased wheat growth and total chlorophyll content. This is because Zn is a crucial structural component of proteins, an enzyme cofactor for metabolic-related enzymes and promoting the expression of genes involved in chlorophyll biosynthesis (Rastogi et al. 2017). The size and shape of the nanomaterial play a critical role in determining easy way passing through the leaves (Ahmad et al. 2023). After foliar application of ZnNPs, wheat growth and chlorophyll content significantly increased. This might be because of the ZnNPs' growth-stimulating effects, which led to an increase in internodal elongation and leaf area and played a crucial role in the biosynthesis of phytohormones, primarily IAA and gibberellins (Zhang et al. 2017; Tahoun et al. 2022). Increase in dry matter production by the application of micronutrients compared to the control, which might be because Zn treatment had a greater impact on plant growth and development during the crop's early growth stages, ultimately leading to an increase in dry matter of the plant (Fathi et al. 2017). The application of zinc nanoparticles enhanced physiological processes by increasing chlorophyll content and the antioxidant activity of peroxidase, catalase, and polyphenol oxidase as a result of increase in fresh and dry weight of the plant (Farnia et al. 2015). Fertilisation using nanoparticles improved plant development and dry matter production in relation to the proper balance of nutrients. Increased dry matter is due to the result of increased stomata control, electron transfer, and enzyme activation which resulted in the growth improvement (Arafat et al. 2023).

3.2 Effect of biosynthesized zinc nanoparticles on yield and yield components in wheat

Seed priming (500 ppm) and foliar spraying at panicle initiation stage (500 ppm) with ZnNPs biosynthesized from actinobacteria and *Pseudomonas* recorded significantly higher number of grains per spike (46.73 and 45.37, respectively), grain weight per spike (1.83 and 1.82 g, respectively) and test weight (42.26 and 42.19 g, respectively) which was on par with combined application of seed priming (500 ppm) and foliar spraying at panicle initiation stage (1000 ppm) with ZnNPs biosynthesized from actinobacteria and *Pseudomonas* (Table 5). Seed priming @ 500 ppm and foliar spraying @ 500 ppm at panicle initiation stage with ZnNPs biosynthesized from actinobacteria and *Pseudomonas* increased the wheat yield per plant by 44.16 and 41.97 per cent, respectively than control (Table 6). Zinc promotes the synthesis of auxin, which improves the absorption of nitrogen and eventually results in enhanced yield and yield characteristics (Waraich et al. 2011). The synthesis of carbonic anhydrase enzyme is increased by Zn treatment, which maximises nutrient intake and grain yield by increasing photosynthetic activity in leaves. Application of ZnONPs raised the chlorophyll content, and the increased chlorophyll content had a beneficial impact on net photosynthesis as well as increasing dry weight and improving yield (Adhikari et al. 2016). ZnONPs application improved the number of tillers, thousand grain weight, panicle length, and number of fertile spikelets (Ghafariyan et al. 2013; Hussain et al. 2018).

Table 2. Plant height of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	Plant height (cm)		
	30 DAS	60 DAS	90 DAS
T ₁ - SP with BS ZnNPs @ 500 ppm	36.93 ^a	78.27 ^b	83.97 ^b
T ₂ - FS with BS ZnNPs @ 500 ppm	32.17 ^b	75.53 ^b	86.30 ^b
T ₃ - FS with BS ZnNPs @ 1000 ppm	31.40 ^b	75.30 ^b	85.37 ^b
T ₄ - FS with BS ZnNPs @ 1500 ppm	32.63 ^b	70.47 ^c	78.20 ^c
T ₅ - SP + FS with BS ZnNPs @ 500 ppm	37.17 ^a	82.73 ^a	92.70 ^a
T ₆ - SP + FS with BS ZnNPs @ 1000 ppm	37.50 ^a	81.67 ^a	90.47 ^a
T ₇ - SP + FS with BS ZnNPs @ 1500 ppm	36.97 ^a	71.40 ^c	80.40 ^c
T ₈ - SP with ABS ZnNPs @ 500 ppm	38.13 ^a	78.43 ^b	84.37 ^b
T ₉ - FS with ABS ZnNPs @ 500 ppm	32.27 ^b	76.30 ^b	87.13 ^b
T ₁₀ - FS with ABS ZnNPs @ 1000 ppm	32.80 ^b	75.87 ^b	85.50 ^b
T ₁₁ - FS with ABS ZnNPs @ 1500 ppm	31.37 ^b	70.63 ^c	79.37 ^c
T ₁₂ - SP + FS with ABS ZnNPs @ 500 ppm	37.53 ^a	83.30 ^a	93.73 ^a
T ₁₃ - SP + FS with ABS ZnNPs @ 1000 ppm	38.30 ^a	81.93 ^a	91.60 ^a
T ₁₄ - SP + FS with ABS ZnNPs @ 1500 ppm	37.27 ^a	72.10 ^c	80.53 ^c
T ₁₅ - RDF (100:75:50, N: P ₂ O ₅ : K ₂ O kg ha ⁻¹)	32.17 ^b	68.97 ^c	78.10 ^c
T ₁₆ - Control	27.43 ^c	63.82 ^d	72.27 ^d
S.Em.±	0.40	0.81	0.84

SP-Seed priming; **FS**-Foliar spraying; **BS**-Bacterial (*Pseudomonas*) synthesized; **ABS**-actinobacterial synthesized; Seed priming @ 500 ppm common for all seed primed treatments; RDF common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.01)

Table 3. Number of tillers per plant in wheat Impact of seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	Number of tillers per plant	
	60 DAS	90 DAS
T ₁ - SP with BS ZnNPs @ 500 ppm	4.67 ^b	3.83 ^b
T ₂ - FS with BS ZnNPs @ 500 ppm	4.50 ^b	3.73 ^b
T ₃ - FS with BS ZnNPs @ 1000 ppm	4.37 ^b	3.67 ^b
T ₄ - FS with BS ZnNPs @ 1500 ppm	3.43 ^a	3.20 ^c
T ₅ - SP + FS with BS ZnNPs @ 500 ppm	5.47 ^a	4.73 ^a
T ₆ - SP + FS with BS ZnNPs @ 1000 ppm	5.30 ^a	4.60 ^a
T ₇ - SP + FS with BS ZnNPs @ 1500 ppm	3.67 ^c	3.27 ^c
T ₈ - SP with ABS ZnNPs @ 500 ppm	4.70 ^b	3.97 ^b
T ₉ - FS with ABS ZnNPs @ 500 ppm	4.57 ^b	3.80 ^b
T ₁₀ - FS with ABS ZnNPs @ 1000 ppm	4.43 ^b	3.70 ^b
T ₁₁ - FS with ABS ZnNPs @ 1500 ppm	3.57 ^c	3.23 ^c
T ₁₂ - SP + FS with ABS ZnNPs @ 500 ppm	5.53 ^a	4.87 ^a
T ₁₃ - SP + FS with ABS ZnNPs @ 1000 ppm	5.37 ^a	4.70 ^a
T ₁₄ - SP + FS with ABS ZnNPs @ 1500 ppm	3.73 ^c	3.30 ^c
T ₁₅ - RDF (100:75:50, N: P ₂ O ₅ : K ₂ O kg ha ⁻¹)	3.40 ^c	3.17 ^c
T ₁₆ - Control	2.37 ^d	2.13 ^d
S.Em.±	0.14	0.09

SP-Seed priming; **FS**-Foliar spraying; **BS**-Bacterial (*Pseudomonas*) synthesized; **ABS**-actinobacterial synthesized; Seed priming @ 500 ppm common for all seed primed treatments; RDF common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.01)

Table 4. Effect of seed priming and foliar spraying with biosynthesized zinc nanoparticles on drymatter production in wheat

Treatment details	Dry matter production (g pot ⁻¹)
	90 DAS
T ₁ - SP with BS ZnNPs @ 500 ppm	36.85 ^b
T ₂ - FS with BS ZnNPs @ 500 ppm	39.15 ^b
T ₃ - FS with BS ZnNPs @ 1000 ppm	37.43 ^b
T ₄ - FS with BS ZnNPs @ 1500 ppm	32.18 ^c
T ₅ - SP + FS with BS ZnNPs @ 500 ppm	44.36 ^a
T ₆ - SP + FS with BS ZnNPs @ 1000 ppm	43.52 ^a
T ₇ - SP + FS with BS ZnNPs @ 1500 ppm	32.63 ^c
T ₈ - SP with ABS ZnNPs @ 500 ppm	36.97 ^b
T ₉ - FS with ABS ZnNPs @ 500 ppm	40.12 ^b
T ₁₀ - FS with ABS ZnNPs @ 1000 ppm	38.92 ^b
T ₁₁ - FS with ABS ZnNPs @ 1500 ppm	32.25 ^c
T ₁₂ - SP + FS with ABS ZnNPs @ 500 ppm	45.57 ^a
T ₁₃ - SP + FS with ABS ZnNPs @ 1000 ppm	43.85 ^a
T ₁₄ - SP + FS with ABS ZnNPs @ 1500 ppm	33.49 ^c
T ₁₅ - RDF (100:75:50, N: P ₂ O ₅ : K ₂ O kg ha ⁻¹)	32.15 ^c
T ₁₆ - Control	16.43 ^d
S.Em.±	0.85

SP-Seed priming; **FS**-Foliar spraying; **BS**-Bacterial (*Pseudomonas*) synthesized; **ABS**-actinobacterial synthesized; Seed priming @ 500 ppm common for all seed primed treatments; RDF common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.01)

Table 5. Yield attributes of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	Yield attributes		
	No of grains per spike	Grain weight per spike (g)	Test weight (g)
T ₁ - SP with BS ZnNPs @ 500 ppm	41.57 ^b	1.67 ^b	40.25 ^b
T ₂ - FS with BS ZnNPs @ 500 ppm	43.13 ^b	1.71 ^b	40.62 ^b
T ₃ - FS with BS ZnNPs @ 1000 ppm	42.40 ^b	1.69 ^b	40.43 ^b
T ₄ - FS with BS ZnNPs @ 1500 ppm	38.43 ^c	1.52 ^c	38.76 ^c
T ₅ - SP + FS with BS ZnNPs @ 500 ppm	45.37 ^a	1.82 ^a	42.19 ^a
T ₆ - SP + FS with BS ZnNPs @ 1000 ppm	45.10 ^a	1.80 ^a	41.87 ^a
T ₇ - SP + FS with BS ZnNPs @ 1500 ppm	39.53 ^c	1.56 ^c	39.12 ^c
T ₈ - SP with ABS ZnNPs @ 500 ppm	41.93 ^b	1.68 ^b	40.32 ^b
T ₉ - FS with ABS ZnNPs @ 500 ppm	43.20 ^b	1.72 ^b	40.79 ^b
T ₁₀ - FS with ABS ZnNPs @ 1000 ppm	42.70 ^b	1.70 ^b	40.57 ^b
T ₁₁ - FS with ABS ZnNPs @ 1500 ppm	38.57 ^c	1.54 ^c	38.94 ^c
T ₁₂ - SP + FS with ABS ZnNPs @ 500 ppm	46.73 ^a	1.83 ^a	42.26 ^a
T ₁₃ - SP + FS with ABS ZnNPs @ 1000 ppm	46.27 ^a	1.81 ^a	42.15 ^a
T ₁₄ - SP + FS with ABS ZnNPs @ 1500 ppm	39.70 ^c	1.58 ^c	39.17 ^c
T ₁₅ - RDF (100:75:50, N: P ₂ O ₅ : K ₂ O kg ha ⁻¹)	37.93 ^c	1.51 ^c	38.52 ^c
T ₁₆ - Control	32.50 ^d	0.85 ^d	35.46 ^d
S.Em.±	0.47	0.02	0.27

SP-Seed priming; **FS**-Foliar spraying; **BS**-Bacterial (*Pseudomonas*) synthesized; **ABS**-actinobacterial synthesized; Seed priming @ 500 ppm common for all seed primed treatments; RDF common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.01)

Table 6. Grain yield, straw yield and harvest index of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	Yield		
	Grain yield (g plant ⁻¹)	Straw yield (g plant ⁻¹)	Harvest index
T ₁ - SP with BS ZnNPs @ 500 ppm	3.21 ^b	5.13 ^b	0.38 ^a
T ₂ - FS with BS ZnNPs @ 500 ppm	3.34 ^b	5.32 ^b	0.39 ^a
T ₃ - FS with BS ZnNPs @ 1000 ppm	3.28 ^b	5.24 ^b	0.38 ^a
T ₄ - FS with BS ZnNPs @ 1500 ppm	2.75 ^c	4.62 ^c	0.37 ^a
T ₅ - SP + FS with BS ZnNPs @ 500 ppm	3.89 ^a	5.93 ^a	0.40 ^a
T ₆ - SP + FS with BS ZnNPs @ 1000 ppm	3.80 ^a	5.81 ^a	0.40 ^a
T ₇ - SP + FS with BS ZnNPs @ 1500 ppm	2.80 ^c	4.67 ^c	0.37 ^a
T ₈ - SP with ABS ZnNPs @ 500 ppm	3.26 ^b	5.17 ^b	0.39 ^a
T ₉ - FS with ABS ZnNPs @ 500 ppm	3.40 ^b	5.38 ^b	0.39 ^a
T ₁₀ - FS with ABS ZnNPs @ 1000 ppm	3.35 ^b	5.26 ^b	0.39 ^a
T ₁₁ - FS with ABS ZnNPs @ 1500 ppm	2.78 ^c	4.64 ^c	0.37 ^a
T ₁₂ - SP + FS with ABS ZnNPs @ 500 ppm	3.95 ^a	5.97 ^a	0.40 ^a
T ₁₃ - SP + FS with ABS ZnNPs @ 1000 ppm	3.87 ^a	5.85 ^a	0.40 ^a
T ₁₄ - SP + FS with ABS ZnNPs @ 1500 ppm	2.82 ^c	4.70 ^c	0.38 ^a
T ₁₅ - RDF (100:75:50, N: P ₂ O ₅ : K ₂ O kg ha ⁻¹)	2.74 ^c	4.61 ^c	0.37 ^a
T ₁₆ - Control	1.25 ^d	2.28 ^d	0.35 ^a
S.Em._±	0.08	0.10	0.008

SP-Seed priming; **FS**-Foliar spraying; **BS**-Bacterial (*Pseudomonas*) synthesized; **ABS**-actinobacterial synthesized; Seed priming @ 500 ppm common for all seed primed treatments; RDF common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.01)

Conclusion

Seed priming and foliar spraying at panicle initiation stage with zinc nanoparticles biosynthesized through actinobacteria recorded significantly higher plant height, number of tillers per plant, total dry matter production, number of grains per spike, grain weight per spike, test weight, grain yield and straw yield. Biosynthesis of nanoparticles using microorganisms is considered to be an environmentally friendly approach. Farmers can replace the conventional zinc and iron source with nano forms to obtain the higher yields, where biosynthesized nanoparticles could be alternative to chemical nanoparticles in terms of high cost and pollution hazards. Seed priming and foliar spraying with biosynthesized zinc nanoparticles recorded significantly higher growth, yield and yield attributing characters.

References

- Acharya, P., Jayaprakasha, G.K., Crosby, K.M., Jifon, J.L., & Patil, B.S. (2019). Green-synthesized nanoparticles enhanced seedling growth, yield, and quality of onion (*Allium cepa* L.). *ACS Sustainable Chemistry & Engineering*, 7(17), 14580-90.
- Adhikari, T., Kundu, S., & Rao, A.S. (2016). Zinc delivery to plants through seed coating with nano-zinc oxide particles. *Journal of Plant Nutrition*, 39(1), 136-146.
- Adrees, M., Khan, Z. S., Hafeez, M., Rizwan, M., Hussain, K., Asrar, M., & Ali, S. (2021). Foliar exposure of zinc oxide nanoparticles improved the growth of wheat (*Triticum aestivum* L.) and decreased cadmium concentration in grains under simultaneous Cd and water deficient stress. *Ecotoxicology and Environmental Safety*, 208, 111627.
- Ahmad, W., Zou, Z., Awais, M., Munsif, F., Khan, A., Nepal, J., & Khan, H. (2023). Seed-primed and foliar oxozinc nanofiber application increased wheat production and zn biofortification in calcareous-alkaline soil. *Agronomy*, 13(2), 400.
- Alshaal, T., & El-Ramady, H. (2017). Foliar application: from plant nutrition to biofortification. *Environment, Biodiversity and Soil Security*, 3(1), 71-83.
- Arafat, A. A., Abdel Fattah, A. K., Abd Elghany, S. H., & Esmaeil, M. A. (2023). Effect of zinc nanoparticles on plant growth and some soil properties. *Asian Soil Research Journal*, 7(1), 9-20.
- De La Torre-Roche, R., Cantu, J., Tamez, C., Zuverza-Mena, N., Hamdi, H., Adisa, I. O., & White, J. C. (2020). Seed biofortification by engineered nanomaterials: a pathway

to alleviate malnutrition?. *Journal of Agricultural and Food Chemistry*, 68(44), 12189-12202.

- Dhaliwal, S. S., Sharma, V., Shukla, A. K., Verma, V., Kaur, M., Shivay, Y. S., & Hossain, A. (2022). Biofortification—A frontier novel approach to enrich micronutrients in field crops to encounter the nutritional security. *Molecules*, 27(4), 1340-77.
- Dimkpa, C. O., McLean, J. E., Britt, D. W., & Anderson, A. J. (2015). Nano-CuO and interaction with nano-ZnO or soil bacterium provide evidence for the interference of nanoparticles in metal nutrition of plants. *Ecotoxicology*, 24, 119-129.
- Donald, C.M. (1962). In search of yield. *Journal of Australian Institute of Agricultural Science*, 28, 171-178.
- Farnia, A., Omid, M. M., & Farnia, A. (2015). Effect of nano-zinc chelate and nano-biofertilizer on yield and yield components of maize (*Zea mays* L.), under water stress condition. *Indian Journal of Natural Sciences*, 5(29), 4614-4624.
- Fathi, A., Zahedi, M., & Torabian, S. (2017). Effect of interaction between salinity and nanoparticles (Fe_2O_3 and ZnO) on physiological parameters of *Zea mays* L. *Journal of Plant Nutrition*, 40(19), 2745-2755.
- Ghafariyan, M. H., Malakouti, M. J., Dadpour, M. R., Stroeve, P., & Mahmoudi, M. (2013). Effects of magnetite nanoparticles on soybean chlorophyll. *Environmental science & technology*, 47(18), 10645-10652.
- Gomez, K.A., & Gomez, A.A. (1984). Statistical Procedure for Agricultural Research, 2nd Ed., A Wiley-International Science Publication, New York (USA). 680.
- Hussain, A., Ali, S., Rizwan, M., ur Rehman, M. Z., Javed, M. R., Imran, M., & Nazir, R. (2018). Zinc oxide nanoparticles alter the wheat physiological response and reduce the cadmium uptake by plants. *Environmental Pollution*, 242, 1518-1526.
- Jangid, B., Srinivas, A., Kumar, M. R., Ramprakash, T., Prasad, T. N. V. K. V., Kumar, A. K., & Dida, V. K. (2019). Influence of zinc oxide nanoparticles foliar application on zinc uptake of rice (*Oryza sativa* L.) under different establishment methods. *International Journal of Chemical Studies*, 7(1), 257-261.
- Mohammadi-joo, S., Mirasi, A., Saeidi-aboeshaghi, R., & Amiri, M. (2015). Evaluation of bread wheat (*Triticum aestivum* L.) genotypes based on resistance indices under field conditions. *International Journal of Biosciences*, 6(2), 331-7.

- Morab, P. N., GV, S. K., Rameshbhai, K. A., & Uma, V. (2021). Foliar nutrition of nano-fertilizers: A smart way to increase the growth and productivity of crops. *Journal of Pharmacognosy and Phytochemistry*, *10(1)*, 1325-1330.
- Nile, S. H., Thiruvengadam, M., Wang, Y., Samynathan, R., Shariati, M. A., Rebezov, M., & Kai, G. (2022). Nano-priming as emerging seed priming technology for sustainable agriculture-recent developments and future perspectives. *Journal of nanobiotechnology*, *20(1)*, 254.
- Rastogi, A., Zivcak, M., Sytar, O., Kalaji, H. M., He, X., Mbarki, S., & Brestic, M. (2017). Impact of metal and metal oxide nanoparticles on plant: a critical review. *Frontiers in chemistry*, *5*, 78.
- Salama, D. M., Osman, S. A., Abd El-Aziz, M. E., Abd Elwahed, M. S., & Shaaban, E. A. (2019). Effect of zinc oxide nanoparticles on the growth, genomic DNA, production and the quality of common dry bean (*Phaseolus vulgaris*). *Biocatalysis and Agricultural Biotechnology*, *18*, 101083.
- Shakiba, S., Astete, C. E., Paudel, S., Sabliov, C. M., Rodrigues, D. F., & Louie, S. M. (2020). Emerging investigator series: polymeric nanocarriers for agricultural applications: synthesis, characterization, and environmental and biological interactions. *Environmental Science: Nano*, *7(1)*, 37-67.
- Silva, S., Arrieta-Cortes, R., Fernandez-Luqueno, F., & Lopez-Valdez, F. (2018). Design and production of nanofertilizers. *Agricultural Nanobiotechnology: Modern Agriculture for a Sustainable Future*, 17-31.
- Tahoun, A. M. A., El-Enin, M. M. A., Mancy, A. G., Sheta, M. H., & Shaaban, A. (2022). Integrative soil application of humic acid and foliar plant growth stimulants improves soil properties and wheat yield and quality in nutrient-poor sandy soil of a semiarid region. *Journal of Soil Science and Plant Nutrition*, *22(3)*, 2857-2871.
- Timilsina, A., & Chen, H. (2021). The Emerging Applications of Zinc-Based Nanoparticles in Plant Growth Promotion. *Nanotechnology in Plant Growth Promotion and Protection: Recent Advances and Impacts*, 45-62.
- Waraich, E. A., Ahmad, R., & Ashraf, M. Y. (2011). Role of mineral nutrition in alleviation of drought stress in plants. *Australian journal of crop science*, *5(6)*, 764-777.

Zhang, R., Zhang, H., Tu, C., Hu, X., Li, L., Luo, Y., & Christie, P. (2015). Phytotoxicity of ZnO nanoparticles and the released Zn (II) ion to corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) during germination. *Environmental Science and Pollution Research*, 22, 11109-11117.

Zhang, T., Sun, H., Lv, Z., Cui, L., Mao, H., & Kopittke, P. M. (2017). Using synchrotron-based approaches to examine the foliar application of ZnSO₄ and ZnO nanoparticles for field-grown winter wheat. *Journal of agricultural and food chemistry*, 66(11), 2572-2579.

UNDER PEER REVIEW