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Biosynthesized Zinc Nanoparticles and their Plant Growth-Promoting Effect on Wheat (*Triticum aestivum L.*) Cultivation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The zinc nanoparticles were biosynthesized using *Pseudomonas* and actinobacteria 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 field experiment was conducted to know the effect of biosynthesized zinc nanoparticles on wheat crop. In wheat seed priming at 500 ppm and foliar spraying at 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through actinobacteria (T_6) increased the plant height (25.75%), number of tillers per meter row length (35.98%), leaf area (25.84%), leaf area index (25.75%), total dry matter production (36.46%), productive spikes per square meter (16.98%), number of grains per spike (27.29%), grain weight per spike (30.71), 1,000 grain weight (10.43%), grain yield (17.58%) and straw yield (14.59%) than seed priming at 500 ppm and foliar spraying at 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through *Pseudomonas* (T_3) and commercial zinc nanoparticles (T_9). Farmers can replace the conventional zinc source with nano forms to obtain the higher yield and yield attributing characteristics, where biosynthesized nanoparticles could be alternative to chemical nanoparticles in terms of high cost and pollution hazards.

Keywords: 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 [1]. 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. Zinc is essential for the synthesis and activation of several hormones (auxin and gibberellin) and enzymes that enhance seed germination per cent and seedling growth. Additionally, zinc plays a important role in biosynthesis of proteins, carbohydrates, lipids, and nucleic acids in plants [2]. Zinc nanoparticles are among the top three most manufactured and used engineered nanoparticles [3]. Zinc nanoparticles, one of the best source for preventing zinc deficiency and enhancing crop quality and productivity [4]. Zinc nanoparticles have an impact on plant metabolism at the molecular level by activating antioxidants and reductases, as well as influencing the synthesis of plant hormones [5]. Zinc can serve as a cofactor for P-solubilizing enzymes like phosphatase and phytase, and zinc nanoparticles boosted their activity in the soil [6].

Nanotechnology may help bring about a new technological revolution in agriculture. Several problems with conventional biofortification could potentially resolved by nanotechnology [7]. It is possible to produce nanofertilizers using nanomaterials because of their high surface-to-volume ratio, gradual and controlled release at target places, and other characteristics [8]. 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 [9]. 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 [10]. Unlike chemical fertilisers, nanofertilizers can be synthesized and applied based on the crop's nutritional needs and the status of the soil's nutrient levels using biosensors [11].

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 [12]. According to recent studies, seed nano-priming can activate several genes during germination, particularly those involved in plant stress resistance [13]. Using nanotechnology for seed priming is a relatively new field of study; it can be used to target seed biofortification to reduce malnutrition [14].

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 [15]. 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 [16].

2. MATERIALS AND METHODS

2.1 Experimental Site

Biosynthesis, characterization and standardization of zinc nanoparticles and lab experiments were done in Green Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad, India. *Pseudomonas* and actinobacterial isolates were collected from the Microbial Genetics Laboratory, Department of Agricultural Microbiology, UAS, Dharwad, India and screened for confirmation. The field study was carried out during the *rabi* season of 2022-23 at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. Geographically Dharwad is situated in the Northern Transition Zone (Zone 8) of Karnataka which lies between 15° 26’ North latitude, 75° 07’ East longitudes with an altitude of 678 m above MSL (mean sea level).

2.2 Physio-Chemical Properties of the Soil

The soil at the experimental site was characterized as deep black (vertisols). Before start of the experiment, composite soil samples were collected from the experimental sites at a depth of 0 to 30 cm. These soil samples were air-dried, powdered, passed through a 2 mm sieve, and then analyzed for their physical and chemical properties. The textural class of experimental soil was clayey and pH-7.79; EC-0.28 dS m^{-1}; organic carbon- 0.51%; available nitrogen- 268.45 kg ha^{-1} ; phosphorus- 35.04 kg ha^{-1}; potassium -342.26 kg ha^{-1} ; zinc- 0.56 ppm and iron - 7.12 ppm (Table 1).

2.3 Experimental Procedure

Wheat seeds of the UAS 334 variety were collected from the Main Agricultural Research Station in Dharwad. The maturity duration was 100-105 days (UAS 334). The net plot size of individual plot is 10 square meters. Row to row 20 cm and plant to plant spacing 10 cm. The seeds were sown at a rate of 150 kg per hectare, evenly distributed in furrows spaced 20.0 cm apart using a wooden marker, and subsequently covered with soil manually. The sowing was taken up on November 21, 2022. 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. Fertilizers (100: 75: 50 kg N: P_2O_5: K_2O kg ha^{-1}) was applied at basal and remaining 50 kg N was top dressed at 30 DAS.

Table 1. Physio-Chemical properties of the experimental soil

	Properties	Value	Methods employed
I.	**Physical properties**		
	Particle size analysis		International pipette method (Piper, 2002).
a.	Coarse sand (%)	6.76	
b.	Fine sand (%)	12.16	
c.	Silt (%)	30.85	
d.	Clay (%)	50.24	
e.	Textural class	Clayey	
II.	**Chemical properties**		
a.	Soil pH (1:2.5 soil: water)	7.79	Potentiometric method (Piper, 2002).
b.	Electrical conductivity (dS m^{-1})	0.28	Conductivity bridge (Piper, 2002).
c.	Organic carbon (%)	0.51	Walkely and Blacks wet oxidation method (Jackson, 1973).
d.	Available nitrogen (kg ha^{-1})	268.45	Alkaline permanganate method (Subbiah and Asija, 1956).
e.	Available P_2O_5 (kg ha^{-1})	35.04	Olsen’s method (Jackson, 1973).
f.	Available K_2O (kg ha^{-1})	342.26	Flame photometer method (Jackson, 1973).
G	DTPA extractable micronutrients (ppm)		
	Zinc (ppm)	0.56	DTPA extractant method (Lindsay and Norvell, 1978).
	Iron (ppm)	7.12	

2.4 Treatmental Details

The study was carried out using a Randomized Complete Block Design (RCBD), twelve treatments replicated three times. The experimental details was T_1- seed priming with BS (Bacterial (*Pseudomonas*) synthesized) ZnNPs at 500 ppm; T_2- foliar spraying with BS ZnNPs at 500 ppm; T_3- seed priming at 500 ppm + foliar spraying at 500 ppm with BS ZnNPs; T_4- seed priming with ABS (actinobacterial synthesized) ZnNPs at 500 ppm; T_5- foliar spraying with ABS ZnNPs at 500 ppm; T_6- seed priming at 500 ppm + foliar spraying at 500 ppm with ABS ZnNPs; T_7- seed priming with commercial ZnNPs at 500 ppm; T_8- foliar spraying with commercial ZnNPs at 500 ppm; T_9- seed priming at 500 ppm + foliar spraying at 500 ppm with commercial ZnNPs; T_{10}- foliar spraying with $ZnSO_4$ at 0.5% ; T_{11}- RDF (recommended dose of fertilizers-100:75:50, N:P_2O_5:K_2O kg ha^{-1}, respectively) and T_{12}-control (without any fertilizer application). Foliar spraying at panicle initiation stage of the crop is common for all the foliar applied treatments. RDF- 100:75:50, N: P_2O_5: K_2O kg ha^{-1} common for all the treatments.

2.5 Experimental Procedure for Growth Parameters

The plant height of five randomly selected plants and tagged plants in each net plot was measured from base of the plant 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 and harvest and it was expressed in centimeters per plant (cm). The destructive plant samples were collected to determine the total dry matter production at 30, 60, 90 DAS and at harvest. Plant samples were collected from second row on either side of the plot to a meter row length at each time. After sampling, the plants were oven dried at 70 °C to a constant weight to determine the total dry matter production and data were expressed in grams (g) meter row length^{-1}.The plant samples per meter row length collected for dry matter production were used for recording the number of tillers at 30, 60, 90 DAS and at harvest. Leaf area is computed by length and width method. It was multiplied by the factor 0.65. Data on leaf area were recorded at 30, 60 and 90 DAS, the leaf area at harvest could not be measured due to complete drying of leaves. It was expressed in dm^2 by following procedure given by Gomez [17].

$$\text{Leaf area } (dm^2) \text{ of each leaf} = L \times W \times K\text{-- (1)}$$

Where,

L = Maximum length of leaf
W = Maximum width of leaf
K = Factor (0.65)

Leaf area index: Leaf area index was calculated by using the formula as suggested by Sestak et al. [18].

$$\text{Leaf area index} = \frac{\text{Leaf area (dm2)}}{\text{Land area occupied by the plant (dm2)}} \text{ ------ -- (2)}$$

2.6 Experimental Procedure for Yield and Yield Attributes

Ten spikes randomly chosen from each plot during harvest to record the number of grains per spike. These selected spikes were individually threshed, and the number of grains per spike was recorded. Grains from the net plot were collected to measure the 1000 grain weight, also expressed in grams (g). The overall biomass yield for each net plot was recorded during harvest. After the threshing process, grains were separated, cleaned, and weighed. The straw yield per net plot was calculated by deducting the total grain weight from the total biomass for the respective treatment. Subsequently, the grain and straw yields of the plots were quantified in kg per hectare (kg ha^{-1}).

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 [19]. The significance level used in the 'F' test was P = 0.01 (1%) and P = 0.05 (5%). The critical difference (CD) at 1% and 5% 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 at 500 ppm and foliar spraying at 500 ppm with zinc nanoparticles biosynthesized by actinobacteria resulted in significantly higher plant height (98.43 cm), number of tillers per meter row length (176.33), leaf area (74.65 dm^2 m row $length^{-1}$), leaf area index (3.32) and total dry matter production (372.24 g m row $length^{-1}$) it could be due to actinobacterial strains released higher number of secondary metabolites which was on par with seed priming at 500 ppm and foliar spraying at 500 ppm with ZnNPs biosynthesized by *Pseudomonas* (98.37 cm, 174.33, 73.94 dm^2 m row $length^{-1}$, 3.29 and 368.37 g m row $length^{-1}$, respectively) and commercial zinc nanoparticles (97.70 cm, 171.33, 72.92 dm^2 m row $length^{-1}$, 3.24 and 365.76 g m row $length^{-1}$, respectively) (Table 2, 3, 4, 5 and 6). Increased plant height and photosynthetically active leaf area by zinc nanoparticles, it might be due to the cause of enhanced dry matter accumulation. It might also attributed to the complimentary effects of other nutrients like magnesium, iron, and sulphur with zinc. The positive improvement in nano foliar spray might be due to the rapid translocation and assimilation of zinc nanoparticles, which further led to the expression of growth-accelerated enzymatic activity and auxin metabolism in plants. Zinc acts as an enzyme activator in plants and is directly involved in the biosynthesis of auxin, which produces more dry matter [20]. Plants may readily absorb the highly soluble zinc sulphate, but has a short retention period in the plant system. However, unlike bulk zinc sulphate, zinc nanoparticles in the nanoscale form is absorbed by plants to a greater extent. These nanoparticles have shown successful in promoting plant growth and development [21]. zinc nanoparticles can be used as a source of zinc in plants to speed up metabolic and enzymatic activities and enhance plant development when used at the optimum concentration [22]. Significant increase in plant height and drymatter production with zinc nanoparticles over the commercial zinc sulphate, is might be due to zinc nanoparticles that help to improve the zinc absorption significantly than commercial zinc sulphate [23]. Because of their substantially decreased proline concentration, zinc nanoparticles maximise zinc availability while reducing abiotic stress on the plant, ensuring maximal development. Zinc nanoparticles also boosted the rate of photophosphorylation to fulfil ATP requirements for other physiological processes of the plants, which might have ultimately helped in increase the crop growth [24]. The primary function of zinc as a nutrient for optimal growth and development, cell elongation, membrane structure, stability, and environmental stress tolerance and protection [25].

3.2 Effect of Biosynthesized Zinc Nanoparticles on Yield and Yield Components in Wheat

Seed priming at 500 ppm and foliar spraying at 500 ppm with zinc nanoparticles biosynthesized by actinobacteria recorded significantly higher productive spikes per square meter (259.33 m^{-2}), number of grains per spike (48.50), grain weight per spike (1.83 g) and test weight (42.35 g) and found on par with seed priming at 500 ppm and foliar spraying at 500 ppm with ZnNPs biosynthesized by *Pseudomonas* (256.33 m^{-2}, 48.23, 1.81 g and 42.17 g, respectively) and commercial zinc nanoparticles (254.67 m^{-2}, 47.90, 1.80 g and 41.94 g, respectively) (Table 7). Seed priming at 500 ppm and foliar spraying at 500 ppm at panicle initiation stage with ZnNPs biosynthesized from actinobacteria, *Pseudomonas* and commercial zinc nanoparticles increased the wheat yield by 17.58, 16.27 and 15.56 per cent, respectively compared to control (Table 8). Armin et al*.* [26] observed that the grain mass increased after the application of zinc nano-fertilizer as compared to the control. Increased individual grain sink strength is indicated by the greater thousand grain weight. Phytohormones, particularly cytokinins play a significant role in increased sink size by encouraging cell proliferation in the early stages of seed filling by Janmohammadi et al*.* [27]. A sufficient zinc supply has enhanced the supply of other nutrients and regulated the overall plant growth and development and resulted in an increase in the number of panicles per square meter. The increase in the number of grains per panicle might have been caused by its stimulation of physiological processes such as photosynthesis, translocation, and assimilation of photosynthates, as well as the formation of more spikelets during the spikelet initiation process, which ultimately led to the formation of more number of grains per panicle [28]. Improvements in biochemical and physiological processes that might be due to zinc which acted as a cofactor for a number of enzymes, finally impacted on better crop growth and yield. Zinc nanoparticles have the capacity to pass through the surface of leaves and release zinc ions across the cuticle due to their extremely smaller size. In addition to that, the highest thousand grain weight suggested that the cytokinin hormone's enhanced activity has resulted in larger individual grain sink size [29]. Application of zinc nanoparticles resulted in the highest grain yield, and this might be because of the smaller size and greater surface area of nano fertilisers, which improved the absorption and translocation of zinc in plant tissue [30]. The increase in overall yield is the result of zinc, which helps in increasing the fertilisation percentage during the blooming stage. This facilitated the transport of photosynthetic byproducts to the pollen grains, enhancing their vitality [31].

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

Treatment details	Plant height (cm)			
	30 DAS	60 DAS	90 DAS	At harvest
T$_1$- SP with BS ZnNPs	38.20^{a}	77.30^{b}	86.67^{b}	89.23^{b}
T$_2$- FS with BS ZnNPs	34.17^{b}	74.13^{b}	89.53^{b}	91.17^{b}
T$_3$- SP + FS with BS ZnNPs	39.23^{a}	82.53^{a}	95.70^{a}	98.37^{a}
T$_4$- SP with ABS ZnNPs	38.30^{a}	78.07^{b}	88.57^{b}	90.53^{b}
T$_5$- FS with ABS ZnNPs	33.13^{b}	74.43^{b}	90.14^{b}	92.67^{b}
T$_6$- SP + FS with ABS ZnNPs	39.47^{a}	83.40^{a}	96.87^{a}	98.43^{a}
T$_7$- SP with Comm. ZnNPs	38.07^{a}	76.87^{b}	85.95^{b}	89.10^{b}
T$_8$- FS with Comm. ZnNPs	32.73^{b}	73.60^{b}	87.10^{b}	90.27^{b}
T$_9$- SP + FS with Comm. ZnNPs	38.13^{a}	82.13^{a}	94.83^{a}	97.70^{a}
T$_{10}$- FS with ZnSO$_4$ @ 0.5%	34.23^{b}	69.50^{c}	81.20^{c}	83.63^{c}
T$_{11}$- RDF	32.80^{b}	65.13^{d}	76.13^{d}	78.27^{d}
T$_{12}$- Control	27.67^{c}	59.67^{e}	71.20^{e}	73.43^{e}
S.Em.±	**1.14**	**1.37**	**1.58**	**1.63**

SP-Seed priming; FS-Foliar spraying; BS-Bacterial (Pseudomonas) synthesized; ABS-Actinobacterial synthesized; Comm. ZnNPs - Commercial zinc nanoparticles; Seed priming @ 500 ppm and foliar spraying @ 500 ppm are common for all nano treated treatments; RDF (100-75-50, N-P- K kg ha^{-1}) common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.05)

Table 3. Number of tillers per meter row length of wheat at different growth stages as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	Number of tillers per meter row length			
	30 DAS	60 DAS	90 DAS	At harvest
T$_1$- SP with BS ZnNPs	80.33^{a}	169.33^{b}	162.67^{b}	159.67^{b}
T$_2$- FS with BS ZnNPs	71.67^{b}	164.67^{b}	158.33^{b}	156.00^{b}
T$_3$- SP + FS with BS ZnNPs	81.00^{a}	184.00^{a}	177.67^{a}	174.33^{a}
T$_4$- SP with ABS ZnNPs	82.67^{a}	170.00^{b}	164.00^{b}	161.00^{b}
T$_5$- FS with ABS ZnNPs	72.00^{b}	167.67^{b}	161.33^{b}	158.67^{b}
T$_6$- SP + FS with ABS ZnNPs	84.00^{a}	186.33^{a}	179.00^{a}	176.33^{a}
T$_7$- SP with Comm. ZnNPs	80.00^{a}	167.67^{b}	159.67^{b}	157.00^{b}
T$_8$- FS with Comm. ZnNPs	72.33^{b}	162.67^{b}	156.67^{b}	153.67^{b}
T$_9$- SP + FS with Comm. ZnNPs	80.67^{a}	182.33^{a}	175.33^{a}	171.33^{a}
T$_{10}$- FS with ZnSO$_4$ @ 0.5%	70.00^{b}	150.00^{c}	144.67^{c}	142.33^{c}
T$_{11}$- RDF	72.33^{b}	137.67^{d}	132.00^{d}	129.67^{d}
T$_{12}$- Control	34.67^{c}	105.33^{e}	98.67^{e}	95.33^{e}
S.Em.±	**2.53**	**4.19**	**3.84**	**3.52**

SP-Seed priming; FS-Foliar spraying; BS-Bacterial (Pseudomonas) synthesized; ABS-Actinobacterial synthesized; Comm. ZnNPs - Commercial zinc nanoparticles; Seed priming @ 500 ppm and foliar spraying @ 500 ppm are common for all nano treated treatments; RDF (100-75-50, N-P- K kg ha^{-1}) common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.05)

Table 4. Leaf area at different growth stages of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	**Leaf area (dm^2 m row length^{-1})**		
	30 DAS	**60 DAS**	**90 DAS**
T_{1}- SP with BS ZnNPs	25.45^{a}	64.75^{b}	67.35^{b}
T_{2}- FS with BS ZnNPs	22.16^{b}	62.89^{b}	68.87^{b}
T_{3}- SP + FS with BS ZnNPs	24.79^{a}	69.64^{a}	73.94^{a}
T_{4}- SP with ABS ZnNPs	25.82^{a}	65.64^{b}	68.24^{b}
T_{5}- FS with ABS ZnNPs	21.65^{b}	63.95^{b}	69.17^{b}
T_{6}- SP + FS with ABS ZnNPs	25.87^{a}	69.83^{a}	74.65^{a}
T_{7}- SP with Comm. ZnNPs	24.92^{a}	64.56^{b}	66.97^{b}
T_{8}- FS with Comm. ZnNPs	21.58^{b}	62.72^{b}	67.56^{b}
T_{9}- SP + FS with Comm. ZnNPs	24.83^{a}	68.96^{a}	72.92^{a}
T_{10}- FS with $ZnSO_4$ @ 0.5%	22.17^{b}	59.43^{c}	63.12^{c}
T_{11}- RDF	21.46^{b}	56.14^{d}	59.32^{d}
T_{12}- Control	18.32^{c}	38.62^{e}	41.34^{e}
S.Em.±	**0.88**	**1.11**	**1.05**

SP-Seed priming; FS-Foliar spraying; BS-Bacterial (Pseudomonas) synthesized; ABS-Actinobacterial synthesized; Comm. ZnNPs - Commercial zinc nanoparticles; Seed priming @ 500 ppm and foliar spraying @ 500 ppm are common for all nano treated treatments; RDF (100-75-50, N-P- K kg ha^{-1}) common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.05)

Table 5. Leaf area index at different growth stages of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	**Leaf area index**		
	30 DAS	**60 DAS**	**90 DAS**
T_{1}- SP with BS ZnNPs	1.13^{a}	2.88^{b}	2.99^{b}
T_{2}- FS with BS ZnNPs	0.98^{b}	2.80^{b}	3.06^{b}
T_{3}- SP + FS with BS ZnNPs	1.10^{a}	3.10^{a}	3.29^{a}
T_{4}- SP with ABS ZnNPs	1.15^{a}	2.92^{b}	3.03^{b}
T_{5}- FS with ABS ZnNPs	0.96^{b}	2.84^{b}	3.07^{b}
T_{6}- SP + FS with ABS ZnNPs	1.15^{a}	3.10^{a}	3.32^{a}
T_{7}- SP with Comm. ZnNPs	1.11^{a}	2.87^{b}	2.98^{b}
T_{8}- FS with Comm. ZnNPs	0.96^{b}	2.79^{b}	3.00^{b}
T_{9}- SP + FS with Comm. ZnNPs	1.10^{a}	3.06^{a}	3.24^{a}
T_{10}- FS with $ZnSO_4$ @ 0.5%	0.99^{b}	2.64^{c}	2.81^{c}
T_{11}- RDF	0.95^{b}	2.50^{d}	2.64^{d}
T_{12}- Control	0.81^{c}	1.72^{e}	1.84^{e}
S.Em.±	**0.04**	**0.05**	**0.05**

SP-Seed priming; FS-Foliar spraying; BS-Bacterial (Pseudomonas) synthesized; ABS-Actinobacterial synthesized; Comm. ZnNPs - Commercial zinc nanoparticles; Seed priming @ 500 ppm and foliar spraying @ 500 ppm are common for all nano treated treatments; RDF (100-75-50, N-P- K kg ha^{-1}) common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.05)

Table 6. Total dry matter production of wheat at different growth stages as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	Total dry matter production (g m row length^{-1})			
	30 DAS	**60 DAS**	**90 DAS**	**At harvest**
T$_{1}$- SP with BS ZnNPs	57.23^{a}	163.28^{b}	259.37^{b}	329.38^{b}
T$_{2}$- FS with BS ZnNPs	45.35^{b}	160.52^{b}	262.17^{b}	332.43^{b}
T$_{3}$- SP + FS with BS ZnNPs	58.35^{a}	180.35^{a}	285.58^{a}	368.37^{a}
T$_{4}$- SP with ABS ZnNPs	58.67^{a}	166.46^{b}	260.62^{b}	332.75^{b}
T$_{5}$- FS with ABS ZnNPs	43.83^{b}	163.24^{b}	264.34^{b}	337.43^{b}
T$_{6}$- SP + FS with ABS ZnNPs	59.42^{a}	182.38^{a}	287.83^{a}	372.24^{a}
T$_{7}$- SP with Comm. ZnNPs	54.68^{a}	160.87^{b}	258.93^{b}	327.89^{b}
T$_{8}$- FS with Comm. ZnNPs	46.36^{b}	156.98^{b}	260.15^{b}	330.25^{b}
T$_{9}$- SP + FS with Comm. ZnNPs	56.84^{a}	178.47^{a}	282.78^{a}	365.76^{a}
T$_{10}$- FS with ZnSO$_{4}$ @ 0.5%	43.25^{b}	145.10^{c}	240.26^{c}	300.32^{c}
T$_{11}$- RDF	45.47^{b}	133.21^{d}	221.35^{d}	272.78^{d}
T$_{12}$- Control	25.73^{c}	106.24^{e}	128.76^{e}	176.56^{e}
S.Em.±	**2.82**	**4.04**	**6.27**	**9.35**

SP-Seed priming; FS-Foliar spraying; BS-Bacterial (Pseudomonas) synthesized; ABS-Actinobacterial synthesized; Comm. ZnNPs - Commercial zinc nanoparticles; Seed priming @ 500 ppm and foliar spraying @ 500 ppm are common for all nano treated treatments; RDF (100-75-50, N-P- K kg ha^{-1}) common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.05)

Table 7. Yield attributes of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	Yield attributes			
	Productive spikes (m^{-2})	**Number of grains per spike**	**Grain weight per spike (g)**	**Test weight (g)**
T$_{1}$- SP with BS ZnNPs	240.67^{b}	43.73^{b}	1.63^{bc}	40.63^{b}
T$_{2}$- FS with BS ZnNPs	243.00^{b}	44.60^{bc}	1.68^{bc}	40.75^{b}
T$_{3}$- SP + FS with BS ZnNPs	256.33^{a}	48.23^{a}	1.81^{a}	42.17^{a}
T$_{4}$- SP with ABS ZnNPs	242.67^{b}	43.40^{bc}	1.65^{bc}	40.67^{b}
T$_{5}$- FS with ABS ZnNPs	246.00^{b}	45.37^{b}	1.70^{b}	40.82^{b}
T$_{6}$- SP + FS with ABS ZnNPs	259.33^{a}	48.50^{a}	1.83^{a}	42.35^{a}
T$_{7}$- SP with Comm. ZnNPs	238.67^{b}	42.93^{c}	1.61^{c}	40.54^{b}
T$_{8}$- FS with Comm. ZnNPs	241.33^{b}	43.87^{bc}	1.67^{bc}	40.69^{b}
T$_{9}$- SP + FS with Comm. ZnNPs	254.67^{a}	47.90^{a}	1.80^{a}	41.94^{a}
T$_{10}$- FS with ZnSO$_{4}$ @ 0.5%	230.33^{c}	40.53^{d}	1.51^{d}	39.43^{c}
T$_{11}$- RDF	221.67^{d}	38.10^{e}	1.40^{e}	38.35^{d}
T$_{12}$- Control	197.33^{e}	32.23^{f}	0.78^{f}	35.64^{e}
S.Em.±	**2.81**	**0.81**	**0.03**	**0.36**

SP-Seed priming; FS-Foliar spraying; BS-Bacterial (Pseudomonas) synthesized; ABS-Actinobacterial synthesized; Comm. ZnNPs - Commercial zinc nanoparticles; Seed priming @ 500 ppm and foliar spraying @ 500 ppm are common for all nano treated treatments; RDF (100-75-50, N-P- K kg ha^{-1}) common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.05)

Table 8. Grain yield, straw yield and harvest index of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	**Grain yield (q ha^{-1})**	**Straw yield (q ha^{-1})**	**Harvest index**
T_1- SP with BS ZnNPs	43.06^{de}	60.56^{b}	0.42^{a}
T_2- FS with BS ZnNPs	44.39^{bc}	61.85^{b}	0.42^{a}
T_3- SP + FS with BS ZnNPs	46.23^{a}	64.28^{a}	0.42^{a}
T_4- SP with ABS ZnNPs	43.25^{cde}	61.13^{b}	0.41^{a}
T_5- FS with ABS ZnNPs	44.61^{b}	62.10^{b}	0.42^{a}
T_6- SP + FS with ABS ZnNPs	46.75^{a}	64.71^{a}	0.42^{a}
T_7- SP with Comm. ZnNPs	42.47^{e}	60.29^{b}	0.41^{a}
T_8- FS with Comm. ZnNPs	43.82^{bcd}	61.36^{b}	0.42^{a}
T_9- SP + FS with Comm. ZnNPs	45.95^{a}	63.97^{a}	0.42^{a}
T_{10}- FS with $ZnSO_4$ @ 0.5%	41.13^{f}	58.32^{c}	0.41^{a}
T_{11}- RDF	39.76^{g}	56.47^{d}	0.41^{a}
T_{12}- Control	17.64^{h}	32.37^{e}	0.35^{b}
S.Em.±	**0.45**	**0.62**	**0.01**

SP-Seed priming; FS-Foliar spraying; BS-Bacterial (Pseudomonas) synthesized; ABS-Actinobacterial synthesized; Comm. ZnNPs - Commercial zinc nanoparticles; Seed priming @ 500 ppm and foliar spraying @ 500 ppm are common for all nano treated treatments; RDF (100-75-50, N-P- K kg ha^{-1}) common for all treatments except control

Note: Means followed by the same letter (s) did not differ significantly by DMRT (p= 0.05)

4. CONCLUSION

Biosynthesis of nanoparticles using microorganisms is considered to be an environmentally friendly approach. Farmers can replace the conventional zinc 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 at 500 ppm and foliar spraying at 500 ppm with zinc nanoparticles biosynthesized by actinobacteria recorded significantly higher plant height, number of tillers per meter row length, leaf area, leaf area index, total dry matter production, yield and yield attributing characters and found on par with seed priming at 500 ppm and foliar spraying at 500 ppm with zinc nanoparticles biosynthesized by *Pseudomonas* and commercial zinc nanoparticles. The positive improvement in nano foliar spray might be due to the rapid translocation and assimilation of Zn nanoparticles, which further led to the expression of growth-accelerated enzymatic activity and auxin metabolism in plants. Zinc acts as an enzyme activator in plants and is directly involved in the biosynthesis of auxin.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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