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# **Seed Priming and Foliar Spraying with Biosynthesized Zinc Nanoparticles on Wheat (***Triticum aestivum* **L.) Production**

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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 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 at 500 ppm and foliar spraying at 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through actinobacteria  $(T_{12})$  recorded significantly higher plant height  $(93.73 \text{ cm})$ , number of tillers per plant  $(4.87)$ , total dry matter production  $(45.57 \text{ g}$  pot<sup>-1</sup>), 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<sup>-1</sup>) and straw yield (5.97 g plant<sup>-1</sup>)and found on par with seed priming at 500 ppm and foliar spraying at 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through *Pseudomonas* (T5).

*Keywords: Actinobacteria; biosynthesis; nanoparticles; seed priming; wheat; 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. Zn nanoparticles (Zn NPs) are among the top three most manufactured and used engineered nanoparticles [2]. ZnO nanoparticles, one of the best source for preventing Zn deficiency and enhancing crop quality and productivity [3]. 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 [4].

Nanotechnology may help bring about a new technological revolution in agriculture. Several problems with conventional biofortification could potentially resolved by nanotechnology [5]. 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 [6]. In comparison to conventional fertilisers, long-term delivery of

plants via nano fertilizers enables enhanced crop growth. As nano fertilizers are added in small amounts, these also prevent soil from becoming burdened with the by-products of chemical fertilisers and reduce the environmental hazards [7].

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 [8]. According to recent studies, seed nano-priming can activate several genes during germination, particularly those involved in plant stress resistance [9]. Using nanotechnology for seed priming is a relatively new field of study; it can be used to target seed biofortification to reduce malnutrition [10].

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 [11]. 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 [12]. Keeping the above facts in view, the present investigation is planned to study the influence of biosynthesized zinc nanoparticles on growth and yield in the wheat crop.

## **2. MATERIALS 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-0.27dS m-1 ; organic carbon- 0.49 %; available nitrogen- 251.37kg ha<sup>-1</sup>; phosphorus- 31.45kg ha<sup>-1</sup>; potassium -363.24kg ha<sup>-1</sup>; zinc- 0.53 ppm and iron –6.78 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 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  $T_1$ - seed priming with BS

(Bacterial (*Pseudomonas*) synthesized) ZnNPs at 500 ppm; T2- foliar spraying with BS ZnNPs at 500 ppm; T3- foliar spraying with BS ZnNPs at 1000 ppm; T4-foliar spraying with BS ZnNPs at 1500 ppm; T<sub>5</sub>-seed priming at 500 ppm  $+$  foliar spraying at 500 ppm with BS ZnNPs;  $T_6$ -seed priming at 500 ppm + foliar spraying at 1000 ppm with BS ZnNPs;  $T_7$ - seed priming at 500 ppm  $+$ foliar spraying at 1500 ppm with BS ZnNPs;T8 seed priming with ABS (Actinobacteria synthesized) ZnNPs at 500 ppm; T<sub>9</sub>- foliar spraying with ABS ZnNPs at 500 ppm;  $T_{10}$ - foliar spraying with ABS ZnNPs at 1000 ppm;  $T_{11}$ -foliar spraying with ABS ZnNPs at 1500 ppm; T<sub>12</sub>-seed priming at 500 ppm + foliar spraying at 500 ppm with ABS ZnNPs;  $T_{13}$ -seed priming at 500 ppm + foliar spraying at 1000 ppm with ABS ZnNPs;  $T_{14}$ - seed priming at 500 ppm + foliar spraying at 1500 ppm with ABS ZnNPs;  $T_{15}$ - RDF (recommended dose of fertilizers-100:75:50,  $N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O$  kg ha<sup>-1</sup>, respectively) and  $T<sub>16</sub>$ -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

	<b>Properties</b>	Value	<b>Methods employed</b>
	<b>Physical properties</b>		
	Particle size analysis		
a.	Coarse sand (%)	6.51	
b.	Fine sand (%)	13.64	
c.	Silt (%)	26.45	
d.	Clay $(\%)$	52.37	International pipette method [13]
е.	Textural class	Clayey	
П.	<b>Chemical properties</b>		
a.	Soil pH (1:2.5 soil: water)	7.76	Potentiometric method [13]
b.	Electrical conductivity (dS $m^{-1}$ )	0.27	Conductivity bridge [13]
C.	Organic carbon (%)	0.49	Walkely and Blacks wet oxidation method [14]
d.	Available nitrogen (kg ha -1)	251.37	Alkaline permanganate method [15]
е.	Available $P_2O_5$ (kg ha $-1$ )	31.45	Olsen's method [14]
f.	Available K <sub>2</sub> O (kg ha <sup>-1</sup> )	363.24	Flame photometer method [14]
g.	DTPA extractable micronutrients (ppm)		
	Zinc (ppm)	0.53	DTPA extractant method [16]
	Iron (ppm)	6.78	

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

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 [17].

## **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 [18]. 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 at 500 ppm and foliar spraying at 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<sup>-1</sup>, respectively) at harvest and found on par with seed priming at 500 ppm and foliar spraying at 1000 ppm with ZnNPs biosynthesized from actinobacteria and *Pseudomonas* (Tables 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 [19]. 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 [20]. The size and shape of the nanomaterial play a critical role in determining easyway passing through the leaves [21]. 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 [22,23]. 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 [24]. 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 [25]. 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 [26].





*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 foliarspraying with biosynthesized zinc nanoparticles**



*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**

*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 attributesof wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles**



*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 indexof wheat as influenced by seedpriming and foliar spraying with biosynthesized zinc nanoparticles**

*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)*

## **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 ZnN Psbio synthesized 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 at 500 ppm and foliar spraying at 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 [27]. 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 [28]. ZnONPs application improved the number of tillers, thousand grain weight, panicle length, and number of fertile spikelets [29-32].

# **4. CONCLUSION**

Seed priming and foliar spraying at panicle initiation stage with zinc nanoparticles biosynthesized through actinobacteria recorded significantly higher plant height, number of tillersper 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.

#### **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 this manuscript.

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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