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Biofortification of Wheat Using Biologically Synthesized Zinc Nanoparticles

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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), Energy-Dispersive X-ray Spectroscopy (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 enrich the zinc content in wheat using biosynthesized zinc nanoparticles.In wheatseed priming @ 500 ppm and foliar spraying @ 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through actinobacteria(T_{12}) recorded significantly higher number of grains per spike (46.73), 1000 grain weight (42.26 g), grain yield (3.95g plant^{-1}), straw yield (5.97g plant^{-1}), zinc content (grain 55.87µg g^{-1} and straw 66.27µg g^{-1}) and zincuptake (grain 220.69 µg plant^{-1}, straw 395.63µg plant^{-1} and total zinc uptake 616.32µg plant^{-1}), which was on par with seed priming @ 500 ppm and foliar spraying @ 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through *Pseudomonas* (T_5).

Keywords: Biosynthesis; nanoparticles; PGPR; seed priming; zinc.

1. INTRODUCTION

Zn 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, Zn plays important role in biosynthesis of proteins, carbohydrates, lipids, and nucleic acids in plants [1]. 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]. Zn NPs 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]. Zn can serve as a cofactor for P-solubilizing enzymes like phosphatase and phytase, and nano-ZnO boosted their activity in the soil [5].

Nanotechnology may help bring about a new technological revolution in agriculture. Several problems (Less application efficiency, soil and environmental pollution and micro nutrient deficiencies in soil, plant and humans) with conventional biofortification could potentially resolved by nanotechnology [6]. 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 [7]. 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 [8]. 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 [9]. 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 [10]. Additionally, nanofertilizers, as opposed to chemical fertilisers, allow for high mineral bioavailability to plants due to their smaller size, greater reactivity, and higher surface area [11].

Wheat is a major food crop cultivated globally, providing food for 35 per cent of the world's population [12]. 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. The most of the wheat types grown today are nutrient poor, especially in Fe and Zn. The most of these minerals are lost during the milling process, which makes them scarce in the human diet and causes malnutrition. Nearly 2 billion people worldwide, especially those in Asia and Africa, suffering from hidden hunger due to their reliance on cereal crops, particularly wheat, for their daily diet [13]. These deficits are particularly widespread among the growing children, pregnant and lactating women and manual labours in highly developed countries. Zn and Fe are the micronutrients that are most commonly linked to micronutrient deficiency globally. Low availability of microelements in soil may be a contributing factor to the decreased level of essential nutrients in crops, due to sub optimal abiotic circumstances, such as abnormally high or low temperature, pH, a lack of water, anaerobic conditions, and the presence of other components [14]. The prevalence of micronutrient deficiencies is higher in humid temperate and tropical regions, where there is extensive leaching caused by excessive precipitation. Another factor is the usage of plant types which have a poor capacity to store adequate amounts of microelements in their edible parts [15].

Increased micronutrient levels in crops can be achieved through biofortification [16]. Biofortified crops have been demonstrated to increase the consumption of micronutrients and significantly improve human health [17]. The three main strategies for biofortification are agronomic, conventional plant breeding, and plant breeding utilising genetic engineering. Agronomic biofortification is considered to be the simplest way to increase the levels of microelements in crops because it focuses on supplying micronutrients that can be directly absorbed by the plant through application with mineral or foliar fertilisers or the improvement of the solubilization and mobilisation of mineral elements in the soil. One of the most affordable methods to lessen dietary mineral deficit in humans is agronomic biofortification [18].

2. MATERIALS AND METHODS

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. 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^{-1} ; phosphorus- 31.45kg ha^{-1}; potassium - 363.24kg ha^{-1}; zinc- 0.53 ppm and iron -6.78 ppm.

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. 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@ 500 ppm; T_2- foliar spraying with BS ZnNPs@ 500 ppm; T_3- foliar spraying with BS ZnNPs@ 1000 ppm; T_4-foliar spraying with BS ZnNPs@ 1500 ppm; T_5-seed priming @ 500 ppm + foliar spraying @ 500 ppm with BS ZnNPs; T_6-seed priming @ 500 ppm + foliar spraying @ 1000 ppm with BS ZnNPs; T_7- seed priming @ 500 ppm + foliar spraying @ 1500 ppm with BS ZnNPs;T_8- seed priming with ABS (Actinobacteriasynthesized) ZnNPs@ 500 ppm; T_9- foliar spraying with ABS ZnNPs@ 500 ppm; T_{10}- foliar spraying with ABS ZnNPs@ 1000 ppm; T_{11}-foliar spraying with ABS ZnNPs@ 1500 ppm; T_{12}-seed priming @ 500 ppm + foliar spraying @ 500 ppm with ABS ZnNPs; T_{13}-seed priming @ 500 ppm + foliar spraying @ 1000 ppm with ABS ZnNPs; T_{14}-seed priming @ 500 ppm + foliar spraying @ 1500 ppm with ABS ZnNPs; T_{15}- RDF (recommended dose of fertilizers-100:75:50, $N:P_2O_5:K_2O$ kg ha^{-1}, respectively) and T_{16}-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.

2.1 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. 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 [19].

2.2 Nutrient Content and Uptake

The wheat grains were crushed with a grinder. Initially, the samples were digested using HNO_3 and $HClO_4$ After digestion with HNO_3 and $HClO_4$, the samples were analyzed for nutrient concentrations using an atomic absorption spectrometer as given by Lindsay and Norvell in [20]. The nutrient uptake was calculated based on the plants' nutrient content and dry matter production, using the following formula.

$$\text{Nutrient uptake (g plant}^{-1}\text{)}$$

$$= \frac{\text{Nutrient concentration (\%)}}{100} \times \text{dry matter (g plant}^{-1}\text{)}$$

2.3 Statistical Analysis

The data collected from the experiment at various growth stages were subjected statistical analysis following the method given by Gomez and Gomez [21]. 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 Yield and Yield Attributes in Wheat

Seed priming (500 ppm) and foliar spraying at panicle initiation stage (500 ppm) with ZnNP sbiosynthesized from actinobacteria (T_6) recorded significantly higher number of grains per spike (46.73) and test weight (42.26 g) which was on par with seed priming @ 500 ppm and foliar spraying @ 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through *Pseudomonas*(T_5) (Table 1). Seed priming @ 500 ppm and foliar spraying@ 500 ppm at panicle initiation stage with ZnNP sbiosynthesized from actinobacteria and *Pseudomonas* increased the wheat yield per plant by 44.16 and 41.97 per cent, respectively than control (Table 2). A sufficient Zn supply boosted the absorption of N at the stage of grain formation, which eventually improved yield [16]. Zinc promotes the synthesis of auxin, which improves the absorption of nitrogen and eventually results in enhanced yield and yield characteristics. The synthesis of carbonic anhydrase enzyme is increased by Zn treatment, which maximises nutrient intake and grain yield by increasing photosynthetic activity in leaves. Zn involvement in the biosynthesis of indole acetic acid, particularly in the initiation of primordial reproductive parts and the allocation of photosynthates towards them, might have an impact on the improved yield and yield components [22]. Adhikari *et al.* [23] revealed that 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.

3.2 Effect of Biosynthesized ZnNPs on Nutrient Content and Nutrient Uptake in Wheat

In wheat seed priming @ 500 ppm and foliar spraying @ 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through actinobacteria(T_{12})recorded significantly highest zinc content (grain 55.87µg g^{-1} and straw 66.27µg g^{-1}) and zincuptake (grain 220.69 µg $plant^{-1}$, straw 395.63µg $plant^{-1}$ and total zinc uptake 616.32µg $plant^{-1}$), which was on par with seed priming @ 500 ppm and foliar spraying @ 500 ppm at panicle initiation stage with zinc nanoparticles biosynthesized through *Pseudomonas* (T_5) (Table 3 and 4).Zn foliar spraying near the heading stage resulted in increasing the absorption and translocation towards the grain for grain development. Foliar application of Zn nanoparticles was most effective at enhancing physiological parameters like chlorophyll content, total soluble sugar, carbonic anhydrase, and grain phytase activity that resulted in the highest grain Zn concentration [24]. As compared to ordinary zinc sulphate, foliar spraying with ZnNPs particles might be more effective due to their larger surface area, which led to increased activity, ion adsorption and faster chemical reaction [25]. Concentration of Zn in the wheat grain recorded higher with foliar application of nano zinc due to increased translocation of Zn in grains as compared to traditional zinc sulphate [26, 27]. Because of their smaller size and greater surface area, which allowed for increase in the zinc absorption and finally it increases the zinc concentration in grain [28]. The higher Zn content in grains was noted with foliar sprayed zinc nanoparticles as compared to conventional foliar treatments. This may be due to greater uptake and absorption from leaf surfaces as well as greater translocation from the place of application to other plant portions which are deficient in zinc [29]. Poornima and Koti [30] reported that compared to bulk zinc, seed primed with nano zinc increased the grain zinc concentration, might be due to nanoscale zinc oxide has a higher uptake and translocation efficiency than bulk zinc form, also reported that zinc nanoparticles with high specific surface and surface reactivity not only easily adsorbed on physical surfaces but also reacted with biological proteins and resulted in their uptake and subsequent quick and efficient translocation to the sink.

Table 1. Yield attributesof wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	**Yield attributes**	
	No of grains per spike	**Test weight (g)**
T$_1$- SP with BS ZnNPs @ 500 ppm	41.57^{b}	40.25^{b}
T$_2$- FS with BS ZnNPs @ 500 ppm	43.13^{b}	40.62^{b}
T$_3$- FS with BS ZnNPs @ 1000 ppm	42.40^{b}	40.43^{b}
T$_4$- FS with BS ZnNPs @ 1500 ppm	38.43^{c}	38.76^{c}
T$_5$- SP + FS with BS ZnNPs @ 500 ppm	45.37^{a}	42.19^{a}
T$_6$- SP + FS with BS ZnNPs @ 1000 ppm	45.10^{a}	41.87^{a}
T$_7$- SP + FS with BS ZnNPs @ 1500 ppm	39.53^{c}	39.12^{c}
T$_8$- SP with ABS ZnNPs @ 500 ppm	41.93^{b}	40.32^{b}
T$_9$- FS with ABS ZnNPs @ 500 ppm	43.20^{b}	40.79^{b}
T$_{10}$- FS with ABS ZnNPs @ 1000 ppm	42.70^{b}	40.57^{b}
T$_{11}$- FS with ABS ZnNPs @ 1500 ppm	38.57^{c}	38.94^{c}
T$_{12}$- SP + FS with ABS ZnNPs @ 500 ppm	46.73^{a}	42.26^{a}
T$_{13}$- SP + FS with ABS ZnNPs @ 1000 ppm	46.27^{a}	42.15^{a}
T$_{14}$- SP + FS with ABS ZnNPs @ 1500 ppm	39.70^{c}	39.17^{c}
T$_{15}$- RDF (100:75:50,N:P$_2$O$_5$: K$_2$O kgha^{-1})	37.93^{c}	38.52^{c}
T$_{16}$- Control	32.50^{d}	35.46^{d}
S.Em.±	**0.47**	**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 treatmentsexcept control

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

Table 2. 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 kgha^{-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 treatmentsexcept control

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

Table 3. Zinc concentration in wheat as influenced by seed priming and foliarspraying with biosynthesized zinc nanoparticles

Treatment details	Zinc concentration (µg g^{-1})	
	Grain	**Straw**
T_1- SP with BS ZnNPs @ 500 ppm	47.59^c	62.13^b
T_2- FS with BS ZnNPs @ 500 ppm	51.62^b	61.46^b
T_3- FS with BS ZnNPs @ 1000 ppm	50.57^b	60.57^b
T_4- FS with BS ZnNPs @ 1500 ppm	43.35^d	56.78^c
T_5- SP + FS with BS ZnNPs @ 500 ppm	54.96^a	65.26^a
T_6- SP + FS with BS ZnNPs @ 1000 ppm	54.83^a	64.73^a
T_7- SP + FS with BS ZnNPs @ 1500 ppm	44.92^d	57.62^c
T_8- SP with ABS ZnNPs @ 500 ppm	48.57^c	62.35^b
T_9- FS with ABS ZnNPs @ 500 ppm	52.21^b	61.87^b
T_{10}- FS with ABS ZnNPs @ 1000 ppm	51.73^b	60.95^b
T_{11}- FS with ABS ZnNPs @ 1500 ppm	43.69^d	57.34^c
T_{12}- SP + FS with ABS ZnNPs @ 500 ppm	55.87^a	66.27^a
T_{13}- SP + FS with ABS ZnNPs @ 1000 ppm	54.92^a	65.59^a
T_{14}- SP + FS with ABS ZnNPs @ 1500 ppm	45.00^d	58.23^c
T_{15}- RDF (100:75:50,N:P_2O_5: K_2O kgha^{-1})	43.10^d	55.97^c
T_{16}- Control	40.65^e	52.16^d
S.Em.±	**0.50**	**0.59**

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

Table 4. Zinc uptake of wheat as influenced by seed priming and foliar spraying with biosynthesized zinc nanoparticles

Treatment details	Zinc uptake (µg plant^{-1})		
	Grain	**Straw**	**Total**
T_1- SP with BS ZnNPs @ 500 ppm	152.76^c	318.73^b	471.49^b
T_2- FS with BS ZnNPs @ 500 ppm	172.41^b	326.97^b	499.38^b
T_3- FS with BS ZnNPs @ 1000 ppm	165.87^b	317.39^b	483.26^b
T_4- FS with BS ZnNPs @ 1500 ppm	119.21^d	262.32^c	381.54^c
T_5- SP + FS with BS ZnNPs @ 500 ppm	213.79^a	386.99^a	600.79^a
T_6- SP + FS with BS ZnNPs @ 1000 ppm	208.35^a	376.08^a	584.44^a
T_7- SP + FS with BS ZnNPs @ 1500 ppm	125.78^d	269.09^c	394.86^c
T_8- SP with ABS ZnNPs @ 500 ppm	158.34^c	322.35^b	480.69^b
T_9- FS with ABS ZnNPs @ 500 ppm	177.51^b	332.86^b	510.37^b
T_{10}- FS with ABS ZnNPs @ 1000 ppm	173.30^b	320.60^b	493.89^b
T_{11}- FS with ABS ZnNPs @ 1500 ppm	121.46^c	266.06^c	387.52^c
T_{12}- SP + FS with ABS ZnNPs @ 500 ppm	220.69^a	395.63^a	616.32^a
T_{13}- SP + FS with ABS ZnNPs @ 1000 ppm	212.54^a	383.70^a	596.24^a
T_{14}- SP + FS with ABS ZnNPs @ 1500 ppm	126.90^d	273.68^c	400.58^c
T_{15}- RDF (100:75:50,N:P_2O_5: K_2O kgha^{-1})	118.09^d	258.02^c	376.12^c
T_{16}- Control	50.81^e	118.92^d	169.74^d
S.Em.±	**3.24**	**7.11**	**11.82**

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

4. CONCLUSIONS

Seed priming and foliar spraying with biosynthesized zinc nanoparticles at panicle initiation stage recorded significantly higher yield and yield attributes, nutrient content. Nanomaterials, particularly in the form of nanofertilizers, have the ability to reduce various stress situations and increase the crop yields by an increase in photosynthesis, nitrogen metabolism, seedling development, carbohydrate and protein synthesis, and the transport of nutrients from roots to leaves. Nanoparticles are commonly synthesized using physical and chemical processes. However, the chemicals used in synthesis are dangerous and have a negative impact on the environment, where as physical techniques needs lot of energy, which is less efficient. Therefore, biological synthesis of nanomaterials is an ideal option which is economically profitable and eco friendly in nature. Seed priming followed by foliar spraying with biosynthesized zinc nanoparticles using actinobacteria recorded significantly higher zinc content and zinc uptake.

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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