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Green synthesis, characterization and field evaluation of zinc, copper and silver nanoparticles for improving growth, yield and seed quality of bread wheat (*Triticum aestivum* L.) under heat stress

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Abstract

Heat stress during flowering and grain filling severely limits the growth, physiological efficiency and yield of bread wheat (*Triticum aestivum* L.). Nanotechnology-based seed priming has recently emerged as a sustainable strategy for improving crop stress tolerance. In the present study, zinc (ZnNPs), copper (CuNPs) and silver (AgNPs) nanoparticles were green synthesized using aqueous leaf extract of *Azadirachta indica* as a reducing and stabilizing agent and were characterized using UV-Visible spectrophotometry, particle size analysis, scanning electron microscopy and X-ray diffraction. The synthesized ZnNPs, CuNPs and AgNPs showed characteristic absorption peaks at 287, 284 and 374 nm, mean particle diameters of 96.56, 122.3 and 74.97 nm and predominantly spherical, flower-like/irregular and spherical/quasi-spherical morphologies, respectively, with crystalline hexagonal and cubic structures. A field experiment was conducted in a randomized complete block design with seven seed priming treatments and three replications, using two bread wheat varieties, HD-3090 and UAS-304, under heat stress conditions. Seed priming with green synthesized nanoparticles significantly improved plant height, productive tillers, chlorophyll content, normalized difference vegetation index, proline content, membrane stability index, spike length, number of seeds per spike, thousand grain weight, seed yield, germination percentage, seedling growth and vigour indices, compared with the untreated control, while also advancing flowering and maturity. Seed priming with zinc nanoparticles at 300 parts per million for 4 hours recorded the highest seed yield (3668.40 and 3722.22 kilograms per hectare in HD-3090 and UAS-304, respectively) along with superior seedling vigour, while silver and copper nanoparticle treatments were most effective for membrane stability, proline accumulation and other stress-physiological traits. The results indicated that *Azadirachta indica* mediated green synthesized nanoparticles, particularly zinc nanoparticles applied through seed priming, are an eco-friendly and effective approach for improving wheat productivity under heat stress.

Keywords: Green synthesis, nanoparticles, *Azadirachta indica*, seed priming, heat stress, wheat

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops and serves as a staple food for a large proportion of the world's population, but its productivity and quality are seriously affected by abiotic stresses, particularly heat stress during flowering and grain filling, which adversely influences seed germination, plant growth and grain development, resulting in reduced yield and seed quality [1]. Conventional approaches to mitigate heat stress, including breeding for heat-tolerant cultivars and improved agronomic practices, are often time-consuming and of limited effectiveness, prompting interest in nanotechnology-based interventions [2].

Nanoparticles possess unique physicochemical properties, including a high surface-to-volume ratio, enhanced reactivity and improved efficiency compared with their bulk counterparts, which make them attractive for improving nutrient delivery, enhancing plant tolerance to abiotic stresses and increasing crop productivity [3]. Although nanoparticles can be synthesized through physical and chemical routes, these often require hazardous chemicals and high energy inputs. Green synthesis, in which bioactive compounds present in plant extracts act as reducing and stabilizing agents, offers an eco-friendly, economical and sustainable alternative [4].

Azadirachta indica (neem) leaves are rich in flavonoids, phenolics, alkaloids, tannins and terpenoids, which facilitate the bioreduction of metal ions into nanoparticles and their subsequent stabilization^[5].

Zinc plays a vital role in auxin biosynthesis, enzyme activation and antioxidant defence, and zinc nanoparticles have been shown to enhance seed germination and seedling vigour^[6]. Copper nanoparticles are of interest for their high reactivity and potential in improving stress tolerance^[7], while silver nanoparticles have demonstrated beneficial effects on germination, physiological performance and drought or heat tolerance in wheat^[8]. In view of this, the present investigation was undertaken with the specific objectives of green synthesis and characterization of zinc, copper and silver nanoparticles using *Azadirachta indica* leaf extract, and evaluation of the effect of the selected green synthesized nanoparticles, applied through seed priming, on growth, yield and seed quality parameters of bread wheat under field heat-stress conditions.

Materials and Methods

Green synthesis and characterization of nanoparticles were carried out during rabi 2025-26 at the Green Nanotechnology Laboratory and the Department of Seed Science and Technology, University of Agricultural Sciences (UAS), Dharwad, Karnataka, India. The field experiment was conducted during rabi 2025-26 at the All India Coordinated Bread Wheat and Barley Improvement Project, Main Agricultural Research Station (MARS), UAS Dharwad (15°04' N, 75°00' E; 751 m above mean sea level), situated in the Northern Transitional Zone of Karnataka, India.

Fresh leaves of *Azadirachta indica* were collected from the university campus, washed thoroughly with distilled water and 20 grams of leaf pieces were extracted in 200 millilitres of distilled water at 80 °C for 15 minutes. The extract was cooled, filtered through Whatman No. 1 filter paper and stored at 4 °C. Stock solutions of zinc acetate, copper acetate and silver nitrate (300 parts per million each) were prepared in distilled water, and the respective precursor solution and leaf extract were combined in a 4:1 ratio for nanoparticle synthesis. Zinc nanoparticles were synthesized on a magnetic stirrer at 70 °C with continuous stirring at 500 revolutions per minute for 60 minutes, while copper and silver nanoparticles were synthesized by reduction in brown amber bottles for 24 hours. The synthesized nanoparticles were characterized using a UV-Visible spectrophotometer over a wavelength range of 200-700 nanometres, a Particle Size Analyzer based on dynamic light scattering, Scanning Electron Microscopy on gold-coated samples and an X-ray diffractometer with copper radiation over an angular range of 5° to 80°.

A field experiment was conducted in a Randomized Complete Block Design with seven treatments and three replications, using two bread wheat varieties, HD-3090 and UAS-304, sown at a spacing of 20 × 10 centimetres with a net plot size of 3.0 × 1.2 metres. The treatment details were T₁-ZnNPs at 200 ppm for 6 hours, T₂-ZnNPs at 300 ppm for 4 hours, T₃-CuNPs at 100 ppm for 2 hours, T₄-CuNPs at 100 ppm for 4 hours, T₅-AgNPs at 75 ppm for 4 hours, T₆-AgNPs at 100 ppm for 4 hours and T₇-untreated control. Seeds were treated with the respective nanoparticle suspension, dispersed by ultrasonication, shade-dried to optimum moisture and sown. Plant height, days to 50

percent flowering, days to maturity and number of productive tillers per metre were recorded on five randomly selected plants per plot. Chlorophyll content, normalized difference vegetation index, proline content, membrane stability index and total dehydrogenase activity were recorded at anthesis and grain filling stages. Spike length, number of seeds per spike, thousand grain weight and seed yield were recorded at harvest and germination percentage, shoot and root length, seedling dry weight and seedling vigour indices were recorded following the standard germination test. The data generated were statistically analysed following the method described by Panse and Sukhatme^[9].

Results and Discussion

Green synthesis of zinc, copper and silver nanoparticles using *Azadirachta indica* leaf extract was confirmed by characteristic colour changes of the reaction mixtures, from light brown to dark brown for zinc nanoparticles, sky blue to dark brown for copper nanoparticles and light brown to brick red for silver nanoparticles, indicating reduction of the respective metal ions. UV-Visible spectroscopic analysis showed characteristic absorption peaks at 287, 284 and 374 nanometres for zinc, copper and silver nanoparticles, respectively, attributable to surface plasmon resonance, corroborating Elumalai and Velmurugan^[5] for zinc oxide nanoparticles synthesized using *Azadirachta indica* leaf extract. Particle size analysis revealed mean particle diameters of 96.56, 122.3 and 74.97 nanometres for zinc, copper and silver nanoparticles, respectively, all within the nanoscale range, while scanning electron microscopy revealed predominantly spherical morphology for zinc and silver nanoparticles and flower-like, irregular morphology for copper nanoparticles. X-ray diffraction confirmed a crystalline hexagonal structure for zinc nanoparticles and crystalline cubic structures for copper and silver nanoparticles, with the absence of impurity peaks indicating high phase purity in all three cases, in agreement with Matinise *et al.*^[10] and Dulta *et al.*^[11] (Table 1).

Plant height, days to 50 percent flowering, days to maturity and number of productive tillers per metre were significantly influenced by seed priming with green synthesized nanoparticles (Fig. 1). Significantly higher plant height was recorded with ZnNPs at 300 ppm for 4 hours (T₂-80.95 centimetres in HD-3090) and ZnNPs at 200 ppm for 6 hours (T₁-84.44 centimetres in UAS-304), while the untreated control (T₇) recorded the lowest plant height (65.24 and 60.12 centimetres, respectively). The minimum days to 50 percent flowering was recorded with CuNPs at 100 ppm for 4 hours (T₄) and AgNPs at 75 ppm for 4 hours (T₅) in HD-3090 (60.72 days each) and with T₂ in UAS-304 (54.83 days), against the maximum with the untreated control (75.00 and 69.36 days, respectively). Timely completion of flowering and maturity under nanoparticle priming may be attributed to enhanced nutrient utilization and physiological efficiency that promote earlier reproductive development, corroborating Munir *et al.*^[12] and Sturikova *et al.*^[13] in wheat.

Chlorophyll content, proline content and membrane stability index were significantly enhanced by nanoparticle priming compared with the untreated control. The highest chlorophyll content at grain filling was recorded with AgNPs at 100 ppm for 4 hours (T₆) in HD-3090 (50.21) and CuNPs at 100 ppm for 4 hours (T₄) in UAS-304 (50.95),

while the untreated control (T₇) recorded the lowest values (37.89 and 36.85, respectively). Proline content and membrane stability index were highest with AgNPs treatments (T₅ and T₆), reflecting improved stress-adaptive response and reduced oxidative damage under heat stress, corroborating Ahmed *et al.* [14] and Shukla *et al.* [15] in wheat.

Number of seeds per spike, thousand grain weight and seed yield differed significantly due to nanoparticle priming treatments (Table 2, Fig. 1). Significantly higher seed yield was recorded with ZnNPs at 300 ppm for 4 hours (T₂) (3668.40 and 3722.22 kilograms per hectare in HD-3090 and UAS-304, respectively), followed by ZnNPs at 200 ppm for 6 hours (T₁), while the untreated control (T₇) recorded the lowest seed yield (2868.45 and 2872.26 kilograms per hectare, respectively). The higher seed yield under zinc nanoparticle priming may be attributed to improved nutrient uptake, enzyme activation and efficient translocation of

assimilates towards developing grains, corroborating Singh and Kumar [16] in sunflower and Niyigaba *et al.* [17] in wheat. Germination percentage, root length, shoot length and seedling vigour indices were significantly higher under nanoparticle priming compared with the untreated control (Table 3, Fig. 2). The highest germination percentage was recorded with CuNPs at 100 ppm for 4 hours (T₄-92.00 and 92.67 percent in HD-3090 and UAS-304, respectively), on par with ZnNPs at 300 ppm for 4 hours (T₂), while the untreated control (T₇) recorded the lowest germination (82.33 and 85.67 percent, respectively). Enhanced germination and seedling vigour under nanoparticle priming may be attributed to improved water uptake, activation of metabolic processes and efficient mobilization of stored food reserves during germination, corroborating Rawat *et al.* [18] and Kausar *et al.* [19] in wheat and Tamindžić *et al.* [20] in field pea.

Table 1: Characterization of green synthesized zinc, copper and silver nanoparticles using *Azadirachta indica* leaf extract

Characterization techniques	Zinc nanoparticles	Copper nanoparticles	Silver nanoparticles
UV-Visible spectrophotometer	Absorbance peak at 287 nm	Absorbance peak at 284 nm	Absorbance peak at 374 nm
Particle Size Analyzer	Mean diameter 96.56 nm	Mean diameter 122.3 nm	Mean diameter 74.97 nm
Scanning Electron Microscopy	Spherical	Flower-like, irregular	Spherical, quasi-spherical
X-ray diffractometer	Crystalline hexagonal	Crystalline cubic	Crystalline cubic

Table 2: Effect of green synthesized zinc, copper and silver nanoparticles on number of seeds per spike, thousand grain weight and seed yield in bread wheat

Treatments	Seeds per spike		Thousand grain weight (g)		Seed yield (kg/ha)	
	HD-3090	UAS-304	HD-3090	UAS-304	HD-3090	UAS-304
T ₁ : ZnNPs @ 200 ppm 6 h	45.45	41.52	40.74	41.85	3550.78	3444.44
T ₂ : ZnNPs @ 300 ppm 4 h	55.55	45.93	44.10	43.80	3668.40	3722.22
T ₃ : CuNPs @ 100 ppm 2 h	46.95	44.66	41.87	40.79	3277.37	3083.33
T ₄ : CuNPs @ 100 ppm 4 h	54.34	40.94	41.60	41.75	2970.68	2988.06
T ₅ : AgNPs @ 75 ppm 4 h	49.01	43.75	42.16	42.58	3350.00	3111.11
T ₆ : AgNPs @ 100 ppm 4 h	50.40	42.34	43.62	41.90	3070.78	3055.55
T ₇ : Control (untreated)	38.95	35.38	35.89	35.37	2868.45	2872.26
Mean	48.66	42.08	41.43	41.15	3250.92	3182.42
S.Em.±	2.15	1.82	1.47	1.53	142.19	133.68
C.D. at 5%	6.62	5.61	4.54	4.72	438.14	411.92

Table 3: Effect of green synthesized zinc, copper and silver nanoparticles on germination, root length and shoot length in bread wheat

Treatments	Germination (%)		Root length (cm)		Shoot length (cm)	
	HD-3090	UAS-304	HD-3090	UAS-304	HD-3090	UAS-304
T ₁ : ZnNPs @ 200 ppm 6 h	87.67	91.67	16.98	15.60	11.68	14.75
T ₂ : ZnNPs @ 300 ppm 4 h	89.00	91.33	17.33	16.32	11.85	16.27
T ₃ : CuNPs @ 100 ppm 2 h	86.67	93.33	16.88	15.45	11.25	14.92
T ₄ : CuNPs @ 100 ppm 4 h	92.00	92.67	17.18	15.60	11.58	15.67
T ₅ : AgNPs @ 75 ppm 4 h	86.33	89.67	16.60	14.92	11.23	14.63
T ₆ : AgNPs @ 100 ppm 4 h	87.67	87.33	16.93	14.97	11.55	13.90
T ₇ : Control (untreated)	82.33	85.67	15.78	12.62	10.28	11.50
Mean	87.38	90.24	16.81	15.07	11.34	14.52
S.Em.±	1.28	1.15	0.21	0.30	0.22	0.34
C.D. at 1%	5.53	4.99	0.92	1.29	0.96	1.49

NS: Non-significant

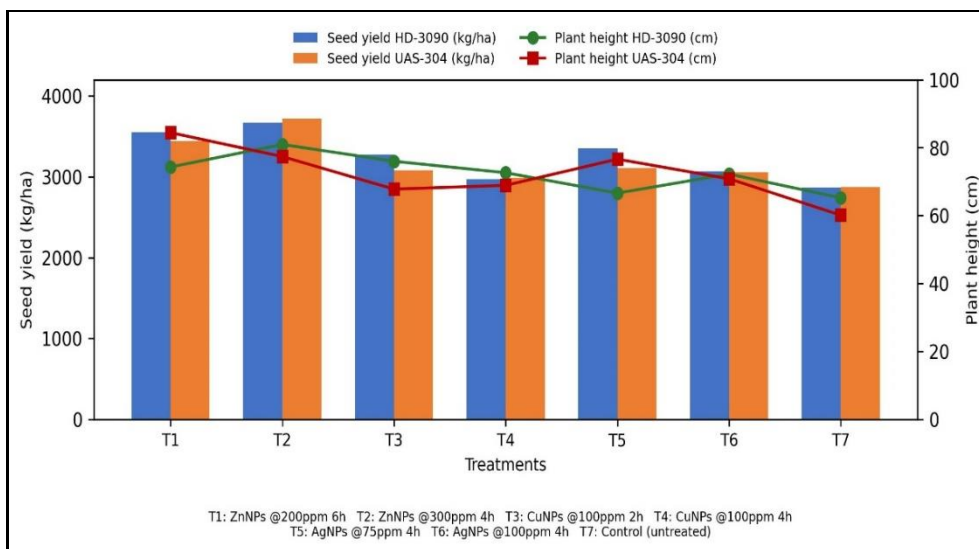


Fig 1: Effect of green synthesized zinc, copper and silver nanoparticles on seed yield (kg/ha) and plant height (cm) of bread wheat cv. HD-3090 and UAS-304

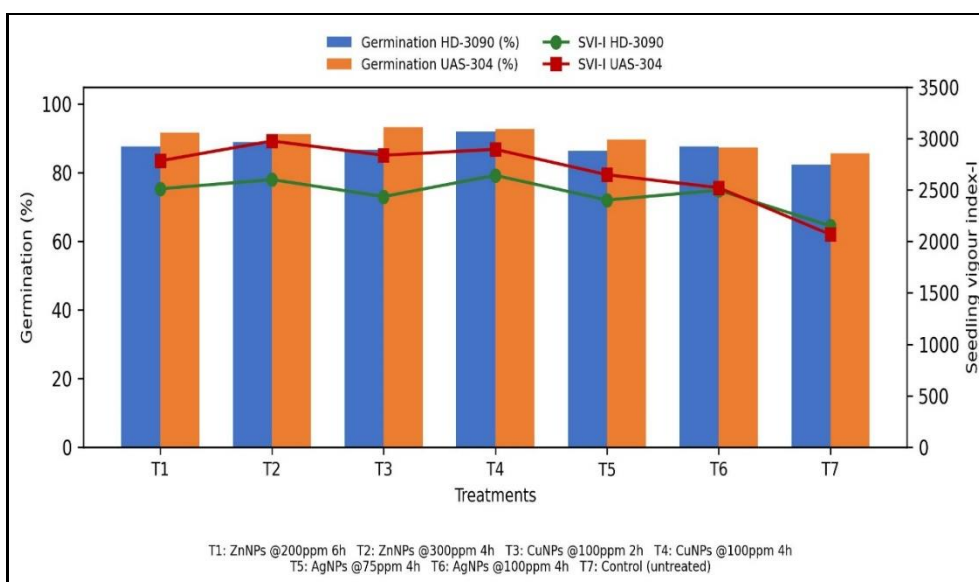


Fig 2: Effect of green synthesized zinc, copper and silver nanoparticles on germination (%) and seedling vigour index-I of bread wheat cv. HD-3090 and UAS-304

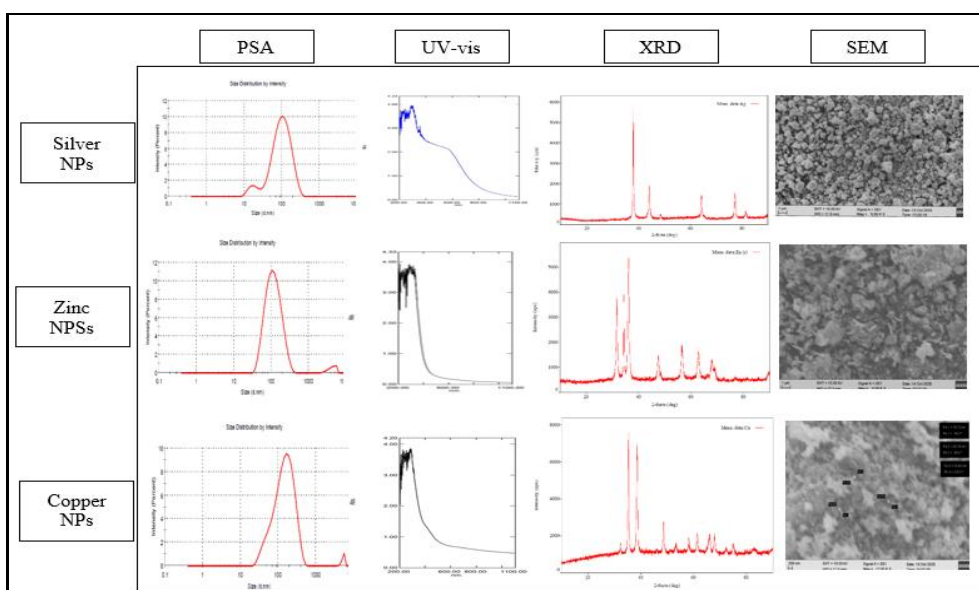


Plate 1: Characterization of nanoparticles through Particle size analyser, UV-visible spectroscopy, X-Ray Diffraction and Scanning electron microscope.

Conclusion

Zinc, copper and silver nanoparticles were successfully green synthesized using *Azadirachta indica* leaf extract, as confirmed by UV-Visible spectroscopy, particle size analysis, scanning electron microscopy and X-ray diffraction. Seed priming of bread wheat with the selected green synthesized nanoparticles significantly improved growth, physiological, yield and seed quality parameters under heat stress conditions, with zinc nanoparticles at 300 parts per million for 4 hours recording the highest seed yield and seedling vigour, while silver and copper nanoparticles were particularly effective for physiological stress-tolerance traits. Hence, seed priming with *Azadirachta indica*-mediated green synthesized zinc nanoparticles can be recommended as a simple, eco-friendly and sustainable approach for improving bread wheat productivity under heat stress, subject to further validation through multi-location field trials.

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