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Effect of Green Synthesized Silver and Zinc Nanoparticles on Seed Quality OF Maize

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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

Nanotechnology is an emerging science and has wide applications in agriculture. Green synthesized nanoparticles are safer, cost effective and eco-friendly over chemical and physical synthesis. The laboratory experiment was conducted to investigate the effectiveness of green-

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synthesized silver and zinc nanoparticles on seed quality of maize hybrid (GH-150125) during 2023-24 at the Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad. Green synthesis of silver and zinc nanoparticles were mediated through neem (*Azadirachta indica*) leaf extract is as reducing and capping agent. The standardization for effective concentration and priming duration for silver and zinc nanoparticles were standardized from different concentrations of (250 ppm, 500 ppm, 750 ppm and 1000 ppm). For priming effective duration were standardized from six, eight and twelve hours inclusive of hydropriming and control (untreated). The effective concentrations of 250-750 ppm and duration of 8 h and 6 h from AgNPs and ZnNPs recorded higher respectively as compared to control and hydropriming. In laboratory, the seed treated with zinc nanoparticles at 750 ppm for 6 h recorded significantly higher germination percentage of (97 %), shoot length (21.53 cm), root length (20.17cm) and seedling vigour index I (4045) compared to control. Hence, the results conclude that ZnNPs at 750 ppm for 6 hours was superior as seed treatment in maize to enhance seed germination and seedling growth than other treatments.

Keywords: Maize; neem; priming; seed quality; silver nanoparticle; zinc nanoparticle.

1. INTRODUCTION

Maize (*Zea mays* L.), also known as corn, is a versatile cereal grain that serves as a staple food for millions of people worldwide. Originated in southern Mexico around 10,000 years ago, maize is the most widely cultivated crop globally as it is not only a primary source of nutrition for humans and livestock but also a significant raw material for various industrial applications [1]. Despite its higher adaptability and productivity, maize faces numerous biotic and abiotic stresses, such as pathogen attacks, pest infestations, drought and extreme temperatures, which can adversely affect seed quality and germination rate, ultimately impacting overall crop yield and productivity. These challenges necessitate innovative strategies to improve seed health, germination, and stress tolerance.

Nanotechnology has emerged as a powerful tool in agricultural science, offering potential solutions for enhancing crop growth, productivity, and sustainability [2]. Recent advancements in green synthesis methods, which utilize plant extracts or biological agents and have provided an eco-friendly as well as cost-effective approach to nanoparticle production, reducing the reliance on toxic chemicals and environmental pollutants.

Neem is native to India and has a remarkable medicinal properties and traditional usage across the world. Neem (*Azadirachta indica*), belongs to the Meliaceae family, contains phytochemicals such as terpenoids and flavonoids which play critical roles as stabilizers, reducing agents and capping agents in nanoparticle synthesis [3]. Among various nanomaterials, silver (Ag) and zinc (Zn) nanoparticles have garnered significant attention due to their unique physicochemical properties, including their antimicrobial, antioxidant, and growth-promoting capabilities [4].

Zinc is an essential micronutrient for humans, animals and plants. It acts as a key component in several enzymes that drive various metabolic processes in plants. Furthermore, zinc plays a significant role in enhancing plant resistance against diseases, supporting photosynthesis, maintaining cell membrane integrity, promoting protein production, aiding pollen development, and boosting the levels of antioxidant enzymes and chlorophyll in plant tissues [5].

The use of silver (Ag) and zinc (Zn) nanoparticles has been shown to enhance seed quality through nano-priming, as evidenced by numerous studies. Groundnut seeds treated with Zn and Ag nanoparticles demonstrated significantly better performance compared to the control group in terms of germination rates, shoot length, root length, and vigor index [6]. Specifically, the application of green-synthesized silver and zinc nanoparticles has emerged as an innovative approach to improving seed quality, promoting germination, and boosting stress resilience in maize. These nanoparticles, synthesized using environmentally friendly methods, can potentially interact with seeds at a cellular level, enhancing seed vigor and providing protection against pathogens. Additionally, they can induce physiological and biochemical changes that improve nutrient uptake and boost the plant’s natural defense mechanisms.

This investigation is on the effect of green-synthesized silver and zinc nanoparticles on the seed quality in maize. By analyzing key seed quality parameters such as germination rate, seedling length and seedling vigor. This research aims to provide insights into how nanotechnology can be harnessed to boost maize crop productivity in a sustainable manner.

2. MATERIALS AND METHODS

A laboratory experiment was carried out during 2023-24 at Green Nanotechnology Laboratory, Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad. This experiment was carried out to investigate the effect of green-synthesized silver (Ag) and zinc (Zn) nanoparticles on the seed quality of maize. The green synthesis of silver (Ag) and zinc (Zn) nanoparticles was mediated by neem (*Azadirachta indica*) leaf extract, which was initially prepared by boiling neem leaf powder in distilled water and then filtered through Whatman No. 1 paper. This extract served as the reducing and stabilizing agent for the synthesis of the nanoparticles. To synthesize silver nanoparticles, silver nitrate stock solution was gradually added to the leaf extract. The mixture was then placed on a magnetic stirrer at 70°C and stirred continuously for 30 minutes, followed by ultrasonication. This process resulted in a color change from light brown to dark brown, indicating the reduction of silver ions in the solution. For the synthesis of zinc nanoparticles, zinc sulfate solution was added dropwise to the neem leaf extract. The mixture was then heated to 70°C and stirred continuously with a magnetic stirrer for 60 minutes, followed by ultrasonication. The green-synthesized nanoparticles were characterized by using UV-Visible spectrophotometer (UV-Vis), Particles Size Analyzer (PSA), Scanning Electron Microscope (SEM) to determine their shape, size and other relevant parameters.

The experiment was arranged in a completely randomized design (CRD) with three replications and nine treatments to assess different concentrations and exposure times of nanoparticles during seed priming. For T_1, T_2 and T_3- seeds were primed with silver nanoparticles (AgNPs) at the concentration of 250, 500 and 750 ppm respectively for duration of 12 h (T_1) and 8 h (T_2 and T_3) and Seeds were primed with silver nanoparticles (AgNPs) at concentrations of 250, 500, and 750 ppm, respectively, for durations of 6 h (T_4 and T_6) and 8 h (T_5) and T_7: Seed primed with 1mM $AgNO_3$ and T_8: Seeds subjected to hydropriming for 16 h. The seeds were primed with nanoparticle solutions at a seed-to-solution ratio of 1:2, ensuring proper exposure to the nanoparticles. After priming, the seeds were shade-dried under ambient conditions until they regained their original moisture content. Observations on key seed quality parameters, including germination percentage, seedling vigor index, root and shoot length and electrical conductivity were recorded to evaluate the effect of the nanoparticle treatments. The results were analyzed statistically to determine the significance of differences between treatments.

The germination test was conducted with three replications, each consisting of 50 seeds, using the between paper towel method as outlined by ISTA procedures [7]. Germination percentage was recorded on the 10th day, based on the number of normal seedlings and expressed in percentage. From each replication, ten normal seedlings were randomly selected on the 10th day of the standard germination test to record shoot and root length, expressed in centimeters using a metric scale. These seedlings were then placed in butter paper packets and dried in a hot air oven at 80 ± 2°C for 24 h. After drying, the seedlings were cooled in desiccator for 30 min and their dry weight was measured by electronic balance, expressed in grams to calculate seedling dry weight. The vigor index was calculated from the formula provided by Abdul-Baki and Anderson [8] and expressed as a numerical value. For electrical conductivity measurement, 5 grams of seeds from each replication were weighed and soaked in 25 ml of distilled water at a temperature of 25 ± 1°C. After 24 h of soaking, the solution was decanted and the volume was adjusted to 25 ml by adding distilled water. The Electrical conductivity of the seed leachate was measured by a digital conductivity bridge (ELICO) with a cell constant of 1.0, and the mean values were reported in deci Siemens per meter (dS/m) [9]. This experimental setup was designed to assess the potential of green-synthesized silver and zinc nanoparticles to enhance seed quality in maize, providing insights into their role in improving germination, seedling vigor, and overall seed health.

3. RESULTS AND DISCUSSION

Among the treatments, the higher germination percentage was recorded in T_6 (ZnNPs 750 ppm) primed seeds (97.00%), which was on par with T_3 (AgNPs 750 ppm) primed seeds (96.66%), which is also on par with T_2 (AgNPs 500 ppm) primed seeds (95.67%) compared to control (T_9-92.33%) (Table 1 and Fig. 1 & 3). Significant variations for abnormal seedling percentage were recorded among the treatments. The lower abnormal seedlings percentage was recorded in T_6 (ZnNPs at 750 ppm for 6 h) (2.67 %), while the higher abnormal seedlings percentage was recorded in T_9 (control) (7.00 %) (Table 2 & Fig. 3). The nanoscale size of particles enables them to easily penetrate the seed coat, facilitating enhanced absorption and utilization by the seeds. The positive impact of these nanoparticles (NPs) on germination can also be attributed to the increased precursor activity of nanoscale zinc in producing essential biomolecules, which are vital nutrients for plant growth. Additionally, NPs triggered oxidation-reduction reactions through superoxide ions during germination, leading to the neutralization of free radicals in the germinating seeds [10]. This effect is linked to the up-regulation of aquaporin genes in the germinating seeds triggered by nanopriming, as well as increased reactive oxygen species (ROS) production compared to untreated seeds and other priming methods. Nanopriming created nanopores that improved water uptake, reset ROS/antioxidant balance in the seeds, facilitated the production of hydroxyl radicals for cell wall loosening, and served as a nanocatalyst to speed up starch breakdown [11-13].

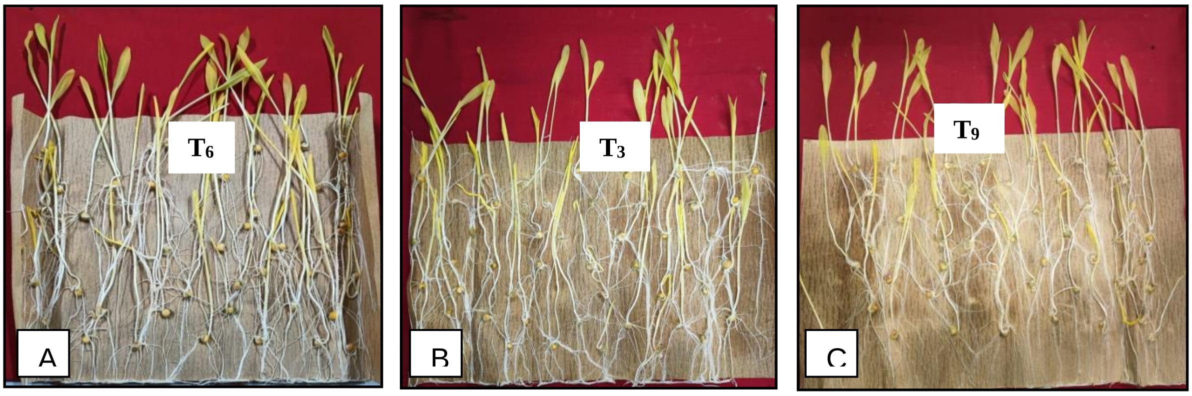
Significantly higher shoot length (cm) was observed in T_6 (ZnNPs at 750 ppm) treated seeds (21.53 cm), on par with T_3 (AgNPs at 750 ppm) as a length of 21.20 cm compared to control (T_9-18.07 cm). The longer root length was recorded in T_6 (ZnNPs at 750 ppm for 6 h) treated seeds (20.17 cm), on par with T_3 (AgNPs at 750 ppm for 8 h) (19.77 cm), T_2 (AgNPs at 500 ppm for 8 h) (19.63 cm) as compared to seeds primed with Silver nitrate (1 mM) (T_7-17.93 cm) the control (T_9 - 17.10 cm), with the shorter root length (Table 1 and Fig. 2 & 4). Nanoparticles

Table 1. Effect of seed priming with Ag and Zn nanoparticles on germination percentage, shoot length, root length and seedling vigour index I in Maize

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	SVI I
T_1-SP with AgNPs @ 250 ppm for 12 h	95.00 (77.08)*	18.60	19.57	3626
T_2-SP with AgNPs @ 500 ppm for 8 h	95.67 (77.99)	19.90	19.63	3820
T_3-SP with AgNPs @ 750 ppm for 8 h	96.66 (79.48)	21.20	19.77	3919
T_4-SP with ZnNPs @ 250 ppm for 6 h	94.00 (75.82)	18.27	18.03	3412
T_5-SP with ZnNPs @ 500 ppm for 8 h	94.67 (76.65)	19.20	19.23	3639
T_6-SP with ZnNPs @ 750 ppm for 6 h	97.00 (80.03)	21.53	20.17	4045
T_7-SP with 1mM $AgNO_3$	93.00 (74.66)	18.60	17.93	3360
T_8 - Hydropriming for 16 h	93.33 (75.04)	19.00	18.73	3509
T_9 –Control (Untreated)	92.33 (73.93)	18.07	17.10	3248
S.Em (±)	**0.79**	**0.39**	**0.23**	**54.17**
C.D (1%)	**3.2**	**1.60**	**0.95**	**220.5**

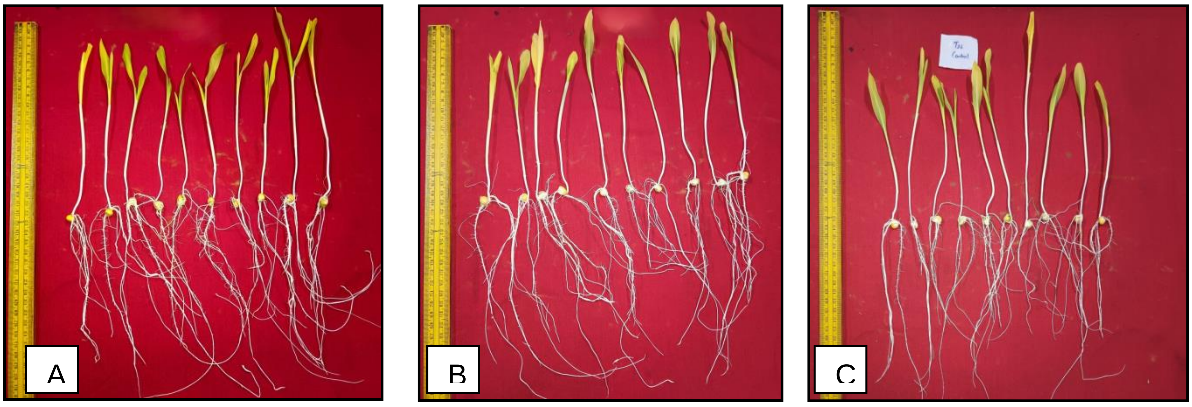
Table 2. Effect of seed priming with Ag and Zn nanoparticles on seedling dry weight, seedling vigour index II , percent abnormal seedlings and Electrical conductivity in Maize

Treatments	Seedling dry weight (g/10 seedling)	SVI II	Abnormal seedlings (%)	Electrical conductivity (dS/m)
T_1-SP with AgNPs @ 250 ppm for 12 h	1.61	152.79	4.67	0.00497
T_2-SP with AgNPs @ 500ppm for 8 h	1.65	159.07	3.67	0.00527
T_3-SP with AgNPs @ 750 ppm for 8 h	1.76	168.61	3.33	0.00676
T_4-SP with ZnNPs @ 250ppm for 6 h	1.61	151.07	3.67	0.00476
T_5-SP with ZnNPs @ 500 ppm for 8 h	1.69	159.93	4.00	0.00539
T_6-SP with ZnNPs @ 750 ppm for 6 h	1.92	186.38	2.67	0.00569
T_7-SP with 1mM $AgNO_3$	1.51	140.31	5.67	0.00633
T_8 - Hydropriming for 16 h	1.53	142.40	5.33	0.00649
T_9 –Control (Untreated)	1.36	125.50	7.00	0.00793
S.Em (±)	**0.03**	**3.50**	**0.29**	**0.00015**
C.D (1%)	**0.14**	**14.3**	**1.20**	**0.00062**



A) ZnNps @ 750 ppm for 6h **B) AgNps @ 750 ppm for 8h** **C) Control**

Fig. 1. Effect of seed priming with Ag and ZnNPs on germination (%) of maize



A) ZnNps @ 750 ppm for 6h **B) AgNps @ 750 ppm for 8h** **C) Control**

Fig. 2. Effect of seed priming with Ag and ZnNPs on seedling length (cm) of maize

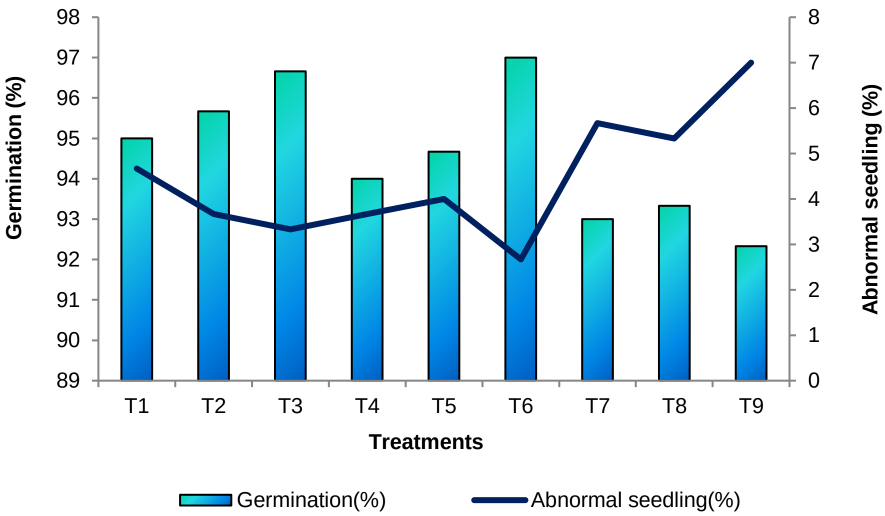


Fig. 3. Effect of seed priming with silver and zinc nanoparticles on percent germination and abnormal seedlings percentage in maize

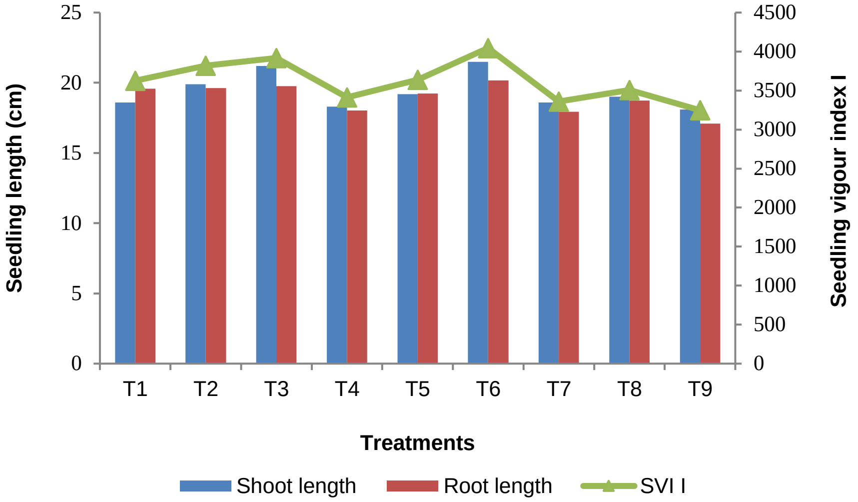


Fig. 4. Effect of seed priming with Silver and Zinc nanoparticles on seedling length and Seedling vigour index I in Maize

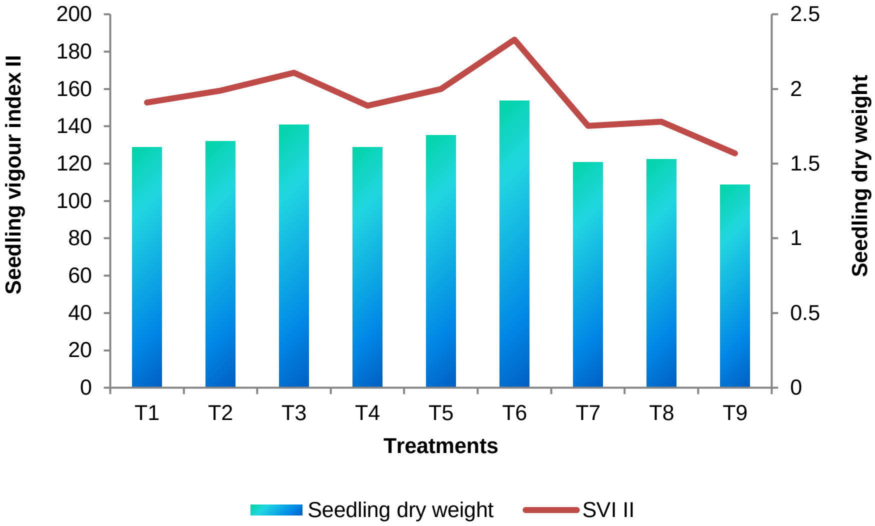


Fig. 5. Effect of seed priming with silver and zinc nanoparticles on seedling dry weight and seedling vigour index II in maize

have a positive impact on the activity of phytohormones, particularly Indole Acetic Acid (IAA), which plays a key role in promoting plant growth. Zinc-rich ZnO nanoparticles can increase IAA concentrations in roots, leading to improved seedling development [14]. These results are in consistent with findings of Anandaraj and Natarajan [15].

Treatment of ZnNPs at 750 ppm (T_6- 1.92 g) primed for 6 h showed higher seedling dry weight, followed by AgNPs at 750 ppm (T_3-1.76 g) primed for 8 h as compared to hydropriming and the control. While, significantly lower seedling dry weight was recorded in control (T_9 - 1.36 g) (Table 2 & Fig. 5). This effect is due to increased synthesis and activity of hydrolytic enzymes during the initial stages of germination, coupled with efficient mobilization of food reserves in the seeds, which promotes early seedling emergence and growth [16]. As seedling growth accelerated, dry matter production also was increased. These observations are in line with the findings of Pandey et al. [17], who reported that Zn nanoparticles enhanced IAA levels in the roots of

Cicer arietinum, consequently increasing the seedling growth rate.

Significantly higher seedling vigor index-I was recorded in T_6 (ZnNPs at 750 ppm for 6 h) primed seeds (4045) as compared to control (T_9 - 3248)(Table 1 & Fig. 4). The higher Vigor Index-II was recorded in T_6 (ZnNPs at 750 ppm for 6 h, 186.38), followed by T_3 (AgNPs at 750 ppm for 8 h, 168.61). The lower seedling vigor index-II was recorded in the control (T_9 – 125.50) (Table 2 & Fig. 5). The enhanced vigor can be attributed to the overall improvement in germination and seedling development traits. A possible reason for this effect is that the oxygen released during this process facilitates respiration and promotes better germination and seedling vitality [18]. Significantly lower electrical conductivity was recorded in T_4 (ZnNPs – 0.00476), while the higher Electrical Conductivity was observed in T_9 (control – 0.00793) (Table 2). According to Sahebi et al. [19], nanoparticles improve plasma membrane stability by providing the cell membrane with more robust lipids. Additionally, studies indicate that lower concentrations of nanoparticles help maintain seed coat integrity, thereby minimizing solute leakage and decreasing electrical conductivity [20]. Seed priming with Zn nanoparticles not only enhances seed quality but also plays a role in defense. Seed priming with ZnONPs enhanced the activity of antioxidant enzymes and increased the levels of organic solutes and ions (Na+, K+, Ca^{2+} and $Mg^{2+)}$, indicating a stimulated defensive response to salinity stress [21].

4. CONCLUSION

Green synthesis of AgNPs and ZnNPs were successfully carried out through neem (*Azadirachta indica*) leaf extract as reducing and capping agents. . The priming of maize seeds with ZnNPs and AgNPs at a concentration of 750 ppm recorded significant improvements in seed quality parameters compared to the control group under laboratory conditions. These findings affirm the efficacy of ZnNPs and AgNPs as priming agents to enhance seed quality of maize, while also highlighting the benefits of eco-friendly nanoparticle synthesis methods. The positive outcomes suggest a promising application of these nanoparticles in agricultural practices, potentially leading to improved crop yields. Future investigations should focus on understanding the mechanisms behind these enhancements and assessing their effectiveness in field conditions to further validate their agricultural applications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. Author(s) hereby declare that generative AI technologies such as ChatGPT, version-ChatGPT-4, Model-GPT-4, Source-OpenAI have been used during editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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