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Unlocking onion potential: Effect of sulphur and zinc oxide nanoparticles on seed germination and vigour under controlled environmental conditions

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Abstract

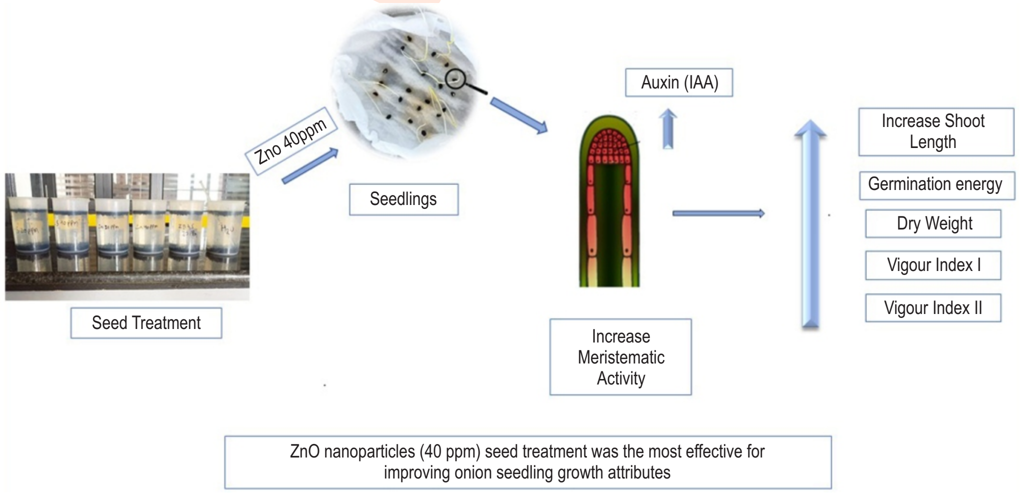
Aim: The present study aimed to evaluate the effects of sulphur and zinc oxide nanoparticles seed priming on the growth and quality attributes of onion seedlings during the year 2023-24 at Lovely Professional University, Phagwara, Punjab.

Methodology: The experiment comprised a total of six treatments, viz., Control, SNPs 20 ppm, SNPs 40 ppm, ZnO NPs 20 ppm, ZnO NPs 40 ppm and ZnO+ SNPs 20 ppm each. The experimental layout was completely randomized design with three replications. Seed priming involved soaking onion seeds for six hours in dark conditions at different concentrations of SNPs and ZnO NPs, dissolved in deionized water.

Results: The application of sulphur and zinc oxide nanoparticles showed a positively significant effect on various seedling-related attributes of onions. Seed priming with Zinc oxide particles at 40 ppm had showed a significant improvement in germination energy, shoot length, root length, seedling dry weight, vigour index I and vigour index II. However, the maximum germination percentage and germination rate index were recorded with the treatment combination of ZnO+SNPs 20 ppm.

Interpretation: Developing seed priming protocols using nanoparticles, especially ZnO nanoparticles may revolutionise the nursery industry. However, deeper research is required to understand how these nanoparticles interact with biomolecules and influence various traits in plants.

Key words: Germination, Onion, Seed priming, Sulphur nanoparticles, Zinc



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Introduction

Allium cepa L., commonly known as onion, is known to be the most versatile crop among the bulb crops. It belongs to the family Alliaceae, sharing diploid chromosome number (n=16) (Ochar and Kim, 2023). It is referred to as the "queen of the kitchen" and is the oldest and most widely consumed bulb crop in the world. While it is thought to have originated in Central Asia, it is now an important and highly regarded vegetable crop grown in India (Ijeoma *et al.,* 2023). Because of its distinctive flavour and aroma, onion is served as both a vegetable and a spice. Being a vegetable crop, it also has unique nutritive and medicinal properties, such as vitamins, iron and calcium. It also contains a fair amount of sulphur compound, namely allyl propyl disulphide and quercetin, that acts as an antioxidant and anticancer (Al-Amri and Abdaly, 2021). Being one of the most commercially grown and exported vegetable, onion cultivars face of challenges such as low viable seed, high seed rate, nutritional requirement, biotic and abiotic stresses, pos-tharvest loses, etc. Nursery raising of onion requires around 8-10 kg of seeds per hectare, however the requirement of healthy seedlings is around 6.5 lakh seedlings for 1 hectare based on spacing (15×10 cm).

Seed priming with nanoparticles has shown increased seed germination, seedling vigour, enzyme activities, micronutrients absorption, etc. in different crops (Panghal *et al.,* 2023; Arun *et al.,* 2017; Laware and Raskar, 2014; Farooq *et al.,* 2012). The term "nanomaterial" typically refers to materials with dimensions between 1 and 100 nanometres. Metal nanoparticles, are engineered materials that deliver essential nutrients in a controlled and efficient manner to crops, enhancing nutrient uptake and reducing environmental nutrient losses (Dastjerdi *et al.,* 2015). Nanoparticles can directly influence plant seed metabolism and disrupt hormonal production, making plants more resilient to environmental stresses (Dilnawaz *et al.,* 2023). They enhance the production of reactive oxygen species (ROS), which play a role in various metabolic pathways and increase the mobilization of storage proteins and the levels of phytohormones (Manzoor *et al.,* 2024). Nanoparticles shows significant biocidal characteristics, containing antibacterial and antifungal properties, which might be applied to develop resistant against pathogens damage crops. Zinc oxide (ZnO) and Sulphur (S) Nanoparticles (NPs) enhance seed vigour, germination and plant growth.

Zinc and sulphur play vital roles in various biological processes and their appropriate use can mitigate the effects of deficiencies, ensuring healthier and more robust plant development (Chandrasekaran *et al.,* 2020). Several studies have confirmed that seed priming with Zn particles has shown a significant and promising impact on crop improvement-related attributes. One of the case study has confirmed that the application of ZnO NPs at 20 $\mu g\ ml^{-1}$ had a significant effect on seed and seedlings-related attributes of onion such as seed germination percentage, radical length and seedlings length has been recorded with same treatment (Laware and Raskar, 2014). Sulphur plays an important role in the formation of amino acids, enzymes, proteins reducing oxidative stress, aiding in chlorophyll formation (Al-Bakry *et al.*, 2024). It is also important for synthesizing oils, amino acids, oligopeptides, vitamins, other cofactors and different secondary metabolites in the *Allium* species (Meher *et al.,* 2016). These sulphur-containing secondary metabolites play a vital role in defence against various pathogens and acts as a signalling molecule for crucial cellular processes (Al-Khayri *et al.*, 2023). Most of the studies have been generalized across various crops or carried out under controlled laboratory conditions with less focus on *Allium* species. Thus, there remains a significant gap in understanding the exact mechanistic impact and a proper concentration of S and ZnO nanoparticles, especially in relation to seed priming method to increase germination for onion. Therefore, this study was conducted to focused to evaluate the effects of zinc oxide and sulphur nanoparticles at different concentration on seed germination and seedling vigour in onion.

Materials and Methods

Experimental site and condition: This experiment was conducted at the Laboratory of the Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab. For the experiment, the laboratory conditions were maintained at 25°C along with the moisture content of 44-95%.

Treatment details and priming of seeds: High-yielding onion variety, namely "PRO-7", released by PAU, Ludhiana, was used for the study. This experiment consisted of six treatments with different nanoparticles and their concentrations. The control (T_0) was treated with distilled water, sulphur nanoparticles (SNPs) were applied at two concentrations: 20 ppm (T_1) and 40 ppm (T_2). Zinc oxide nanoparticles (ZnO NPs) were also evaluated at two levels: 20 ppm (T_3) and 40 ppm (T_4). A combined treatment of zinc oxide and sulphur nanoparticles (ZnO + SNPs) at 20 ppm each (T_5). APS <100 nm sulphur and zinc oxide nanoparticles powder were obtained from Vedayukt India Private Limited. Primarily, 20 and 40 mg of SNPs and ZnO NPs were dissolved in 1 litre of deionized water and magnetically stirred for 45 min. Control onion seeds were soaked in distilled water, while the primed seeds were treated with SNPs and ZnO NPs at their respective concentrations for 6 hrs in dark (Zaim *et al.*, 2023). To ensure safety while working with nanoparticles, necessary precautions were followed such as wearing protective gear, working in well-ventilated areas and the guidelines set by the institution and environmental authorities.

Seed germination and seedling-related attributes of onion

Seed germination-related attributes of onion: Germination tests were conducted following the ISTA (1999) guidelines. Twenty onion seeds were sown in three replicates on sterilized petri dishes, each lined with two autoclaved sheets of Whatman filter paper. The petri dishes were placed in a seed germinator (manufactured by Narang Scientific Works Pvt. Ltd.) and maintained under dark conditions. Various seed and seedling-related characteristics for onion were examined: The Germination percentage and germination energy (%) was calculated using the formula given in ISTA (1999). The germination rate index is the sum of the ratios of seeds germinated each day (G_n) to the corresponding day number (D_n), providing an estimate of germination speed and calculated using the formula given in AOSA (1983). Mean germination time was calculated using formula given by Ellis and Roberts (1981). Seedling vigour index I and II was calculated following protocol developed by Hozayn *et al.* (2015):

Seedling-related attributes of onion: The shoot length of Seedlings was measured on the 12th day of germination in ten randomly selected normal seedlings. The total length was measured from the tip of the primary leaf to the base of the seed using a scale and the mean value was then expressed in centimetres. The average root length of seedling was also measured on the 12th day of germination. Ten randomly selected normal seedlings were used to determine the seedling root length. The total root length was measured from the tip of the root to the base of the seed using a measuring scale. The mean value was then expressed in centimetres. Apart from this, to determine the dry weight of seedling, ten seedlings were selected and kept in an oven at 60°C for 48 hrs. After this period, the weight of the seedlings was measured and the average value was recorded in milligrams.

Statistical analyses: The observed data was statistically analysed by following ANOVA applying suitable for Complete randomized design using SPSS software. In addition, the Duncan Multiple Range Test was applied to group the means at 5% probability level.

Results and Discussion

ANOVA (mean sum of square) of nine onion seedling characters are depicted in Table 1. The perusal of data revealed that the germination percentage was significantly influenced by the application of ZnO and SNPs (Fig. 1a). Results showed that among all treatments, priming with T_5 was the most effective in boosting germination percentage, followed by T_4, whereas the T_0 treatment yielded the lowest germination percentage. However, there was no statistically significant difference between the T_1 and T_2. Zinc plays a vital role in different physiological processes during seed germination, including enzyme activation (Nissa *et al.,* 2024). When zinc is used to prime tomato seeds, it likely promotes the release of enzymes that break down complex molecules into simpler forms, making them more readily available for the developing seedling (Hamsaveni *et al.,* 2003). Seed priming in maize with $ZnSO_4$ for 24 hr increased stand establishment by 29% compared to untreated seeds (Foti *et al.,* 2008). Sulphur is an essential macronutrient for plants and its metabolism is crucial during seed germination (Chen *et al.,* 2022). Sulphur is involved in the synthesis of sulphur-containing amino acids like cysteine and methionine, which are vital for protein synthesis and enzyme function (Mondal *et al.,* 2022). ZnO and SNPs contribute to the breakdown of complex molecules by promoting the release of enzymes which may be the reason for increased germination percentage in this experiment.

Germination energy, which is used to determine the speed of germination, showed significant differences among different treatments (Fig. 1b). Treatment T_4 resulted in the highest seed germination energy, followed by T_3, T_0 and T_5 treatments, which were statistically non-significant with each other. The lowest germination energy was observed with T_1 treatment, which was statistically similar to T_2. In *Capsicum annuum* L., seed priming with 750 ppm ZnO-NPs was found to improve germination energy (Afrayeem *et al.,* 2016). Zinc plays an important role in promoting early seedling growth by increasing the formation of phytohormone auxin, specifically indole acetic acid (Pandey *et al.,* 2010). The recorded improvement in germination energy of onion seeds in this experiment suggests that zinc ions released by ZnO NPs stimulated auxin biosynthesis, potentially leading to better germination energy outcomes. The germination rate index was significantly affected

Table 1: Analysis of variance (mean sum of squares) for nine characters in onion seedling

Characters	Source of variation (Mean sum of squares)	
	Treatment	Error
d.f.	5	12
Germination percentage	38.299*	2.910
Germination energy	93.339*	2.518
Germination rate index	1.749*	0.314
Mean germination time	0.006NS	0.027
Vigour index-I	42739.171*	798.669
Seedling shoot length	1.450*	0.030
Seedling root length	0.775*	0.013
Seedling Dry weight	2.940*	0.126
Vigour index-II	24731.480*	236.145

Significant at 5 % probability level

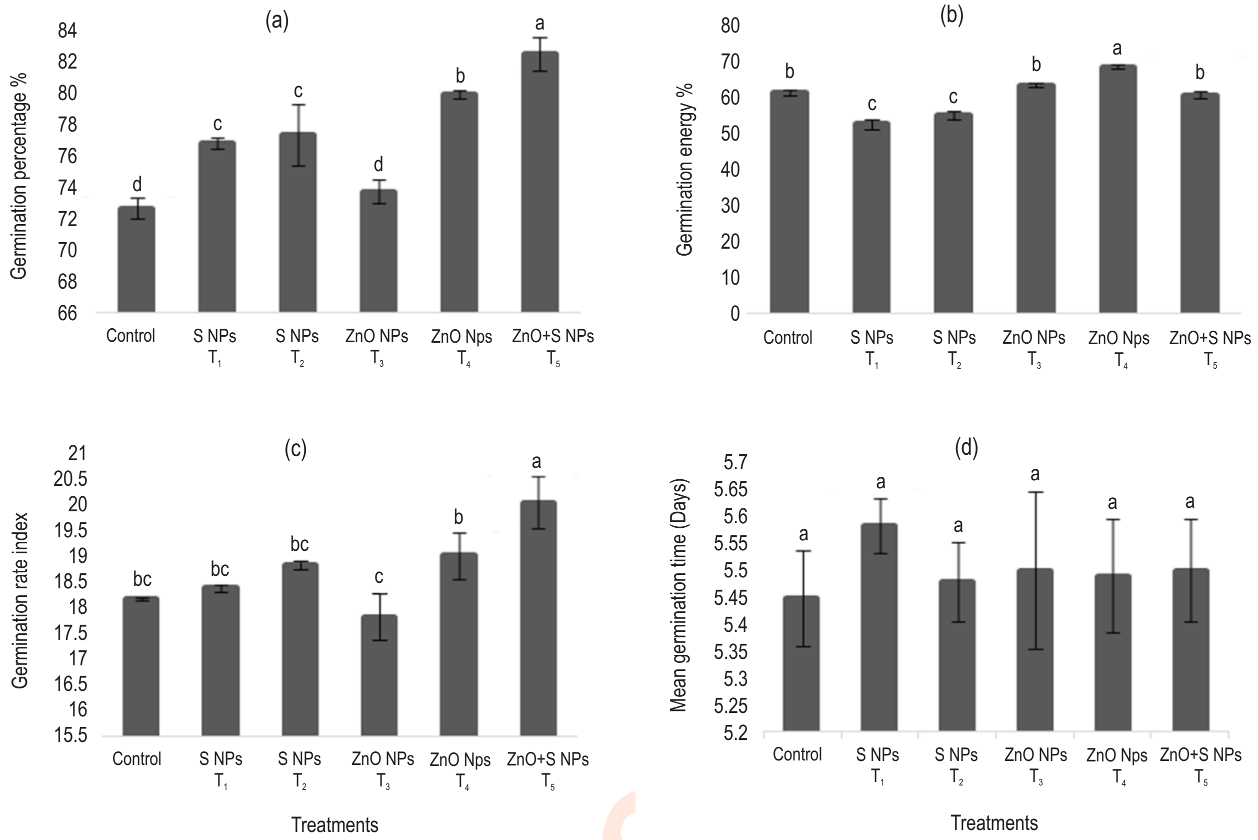

Fig. 1: Effect of ZnO NPs and SNPs on (a) Germination percentage (%), (b) Germination energy (%), (c) Germination rate index, (d) Mean germination time (Days). Means with same letter(s) are not significantly different at 5 % probability level.

by seed treatment with SNPs and ZnO NPs (Fig. 1c). The seed priming with T_5 provided the maximum germination rate index, followed by T_4 treatment, whereas, T_3 treatment showed the lowest value. The increase in germination rate index may be attributed to SNPs and ZnO NPs promoting elongation of the meristematic area and synthesis of sulphur-containing amino acids in plants. Seed priming with ZnO NPs enhance seed germination and plant development, thereby, improving the plant growth and yield in different crops (Pathak *et al.,* 2023; Ishfaq *et al.,* 2025). Sulphur is also vital for balanced nutrition, nutrient absorption and various physiological processes such as formation of sulphur-containing amino acids, which promotes germination (Dudhat *et al.,* 2012; Yadav *et al.,* 2021).

The increase in germination rate index, as observed in the experiment, is positively influenced due to the synergistic effect of zinc and sulphur nanoparticles. The mean germination time was not significantly influenced by the application of zinc and sulphur nanoparticles. However, application of T_1 resulted in the highest mean germination time, which was statistically equal to T_0, T_2, T_3, T_4 and T_5. The T_0 had the least mean germination time (Fig. 1d). These findings align with the previous reports indicating that seed priming did not significantly influence the mean germination time (Jagosz, 2015; Wajid et al., 2018). The results showed significant variations in seedling shoot length (Fig. 2a). The longest seedling length was recorded in seeds primed with T_4, whereas the shortest was in T_0. These results are aligned with previous reports indicating the increased seedling length in zinc primed seeds (Tuiwong *et al.*, 2022; Donia and Carbone, *2023*). Zinc acts as a cofactor for over 300 enzymes and is crucial for the synthesis of tryptophan, a precursor of auxin (IAA), which promotes rapid cell division and elongation, which may lead to increased seedling length (Hamsaveni *et al.,* 2003; Castillo *et al.,* 2018).

Significant differences were also noted in seedling root length among different treatments (Fig. 2b), T_4 treatment resulted in the highest root length, followed by T_3 whereas the lowest root length was observed in control plants. Previous studies have showed that zinc accumulation in young radicles supports protein synthesis, cell elongation, membrane integrity and abiotic stress tolerance during early seedling development (Cakmak, 2000; Mazhar *et al.,* 2023; Ozturk *et al.,* 2006). Seedling dry biomass was significantly affected by ZnO NPs and SNPs (Fig. 2c). The

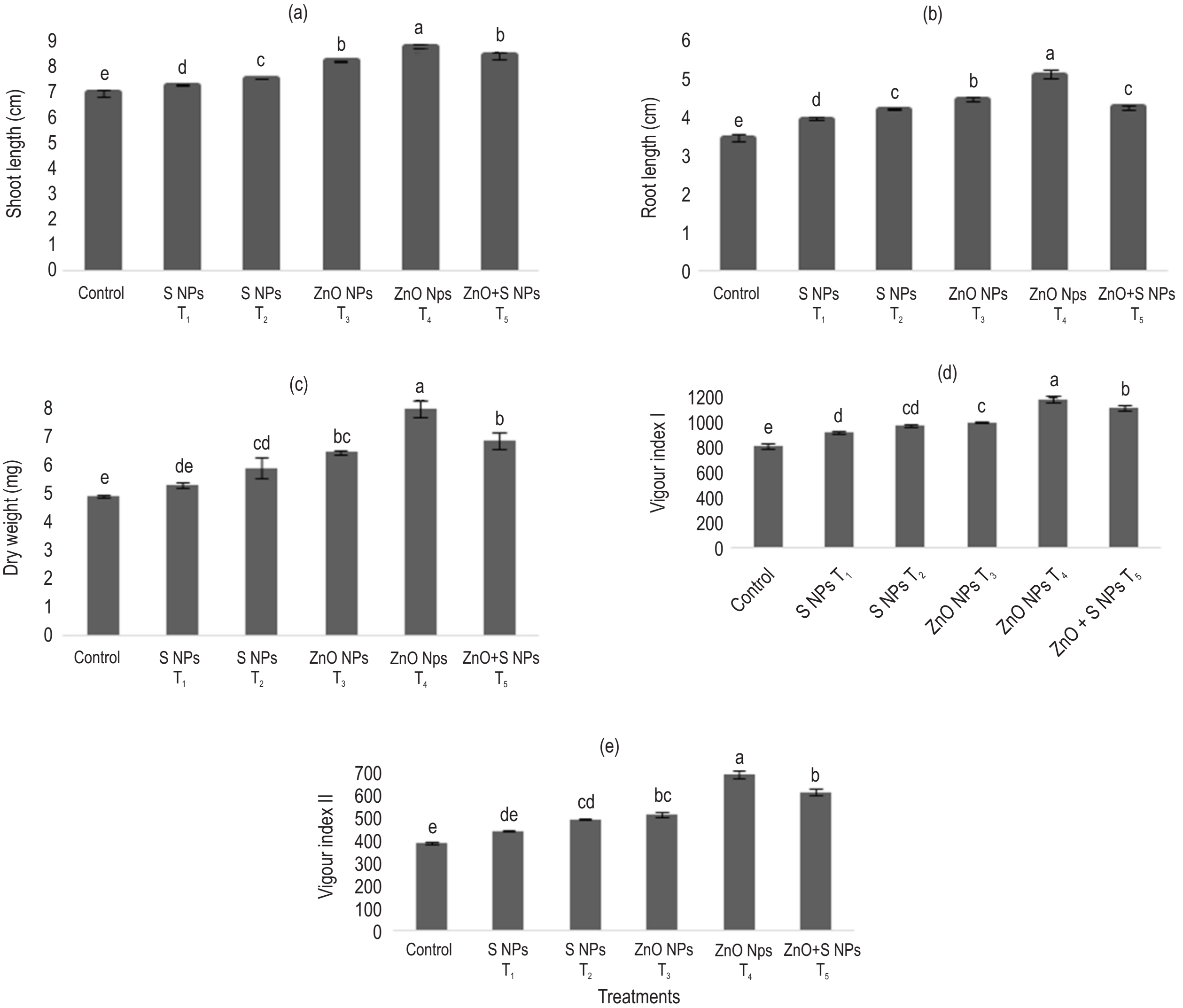

Fig. 2: Effect of ZnO NPs and SNPs on (a) Seedling shoot length (cm), (b) Seedling root length (cm), (c) Dry weight, (d) Seedling vigour index I, (e) Seedling vigour index II. Means with same letter(s) are not significantly different at 5 % probability level.

highest dry biomass was recorded in T_4 treated plants, whereas T_0 treatment showed lowest dry biomass. No significant differences were found between T_1 and T_2 treatments. Increased biomass using zinc treatment is attributed to stimulated metabolic activity which enhance root and shoot growth (Ariraman *et al.,* 2022; Ishfaq *et al.,* 2025). The highest vigour index I was recorded in treatment treated plants T_4, followed by T_5, with the lowest in vigour index I recorded in control plants. Whereas treatments T_1 and T_2 showed statistically similar values (Fig. 2d). Zinc and sulphur seed priming improves seedling vigouras compared to untreated seeds by initiating pre-germination metabolic processes and increasing cell division leads to increase shoot and root length (Rehman *et al.,* 2018; Reis *et al.,* 2018).

Similarly, vigour index II showed positive effects of different priming treatments (Fig. 2e), with T_4 treatment exhibiting the highest effect, followed by T_5 and the lowest in control. Zinc enhances plant growth by stimulating metabolic pathways that promote root and shoot development, resulting in increased biomass and dry weight (Dastjerdi *et al.,* 2015). The increased vigour index I and vigour index II are likely due to the zinc-induced rise in seedling root shoot length and dry weight.

The study concluded that ZnO and S nanoparticle priming significantly enhanced onion seed germination and seedling growth *in vitro.* Especially 40 ppm of ZnO NPs exhibited the highest values for germination energy, shoot length, root length, seedling dry weight, vigour index I and vigour index II, while combination of 20 ppm ZnO NPs and 20 ppm SNPs resulted in the highest germination percentage and germination rate index.

These findings may assist manufacturers in the development of nano-fertilizers, particularly suitable for seed priming and seedling/nursery industries. Further crop-based and biosafety studies are necessary before recommendation forlarge-scale application in different crops.

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Data availability: The data used to support the findings of the study are included in the article.

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