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Enhancing Mustard Seed Germination and Growth through Seed Priming with Green-Synthesized Zinc Oxide Nanoparticles

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ABSTRACT

This study investigates the effect of seed priming with green-synthesized zinc oxide nanoparticles (ZnO-NPs) on germination and growth in two mustard varieties (PDZM31 and PM28). ZnO-NPs were synthesized using *Coriandrum sativum* leaves extract through a green synthesis approach. Seeds were primed with varying concentrations (20 ppm and 40 ppm) of ZnO-NPs before sowing. Results showed a positive correlation between ZnO-NP concentration and seedling growth up to a specific threshold. In PDZM31, both 20 ppm and 40 ppm concentrations exhibited favorable effects on seedling growth and germination. Similarly, in PM28, 20 ppm and 40 ppm concentrations promoted seedling growth and germination. These findings suggest that seed priming with green-synthesized ZnO-NPs can be a potential strategy to enhance germination and growth in mustard crops.

Keywords Green synthesis, Nanoparticles, Seed priming, Seedlings, Germination, Zinc oxide.

INTRODUCTION

Nanoparticles are extraordinarily small particles, typically ranging from 1 to 100 nanometers in size (1 nm = 10^{-9} meters) (Chandran *et al.* 2006). Nanotechnology occupied a prominent position among the most recent technological advancements in agriculture. It possessed significant potential to transform agricultural systems and augment food production to meet demands efficiently and economically (War *et al.* 2020). Nanobiotechnology introduces an innovative toolkit for manipulating and improving crop production through the application of nanoparticles, nanofibers, nanoemulsions and nanocapsules (Chandran *et al.* 2006). NPs come into contact with plant cells, where they were taken up through plasmodesmatal or endocytic pathways and transported via Apo plastic and/or symplastic routes. Once beneficial NPs reached different parts of plants, they boosted photosynthetic rate, biomass measure, chlorophyll content, sugar level, and build-up of osmolytes and

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antioxidants (Singh *et al.* 2021). With the emergence of nanotechnology, more efficient and less polluting nano formulations were continuously developed for sustainable agriculture. These cutting-edge materials possessed the potential to modify plant physiology upon entering the complex plant-soil system, offering opportunities to study their subsequent effects (Mittal *et al.* 2020). Biological methods, such as using plant extracts, are particularly attractive due to their environment friendly nature and potential scalability (Tymoszuk and Wojnarowicz 2020). Plant crude extracts contain unique secondary metabolites such as phenolic acids, flavonoids, alkaloids and terpenoids, which play a pivotal role in reducing ionic components into bulk metallic nanoparticles (Aromal and Philip 2012). These primary and secondary metabolites actively engage in redox reactions to facilitate the eco-friendly synthesis of nano size particles (Kim *et al.* 2007). Zinc is indeed essential for plants as a micronutrient, meaning it is required in small quantities but plays crucial roles in plant growth and development. These nanoparticles have gained attention in agriculture due to their potential applications in enhancing plant growth and stress tolerance (Nam and Luong 2019). Zinc oxide nanoparticles possess remarkable optical, physical, and antimicrobial properties, making them highly promising for enhancing agricultural practices. Recognized as a "generally recognized as safe (GRAS)" material by the Food and Drug Administration (Huang *et al.* 2001). ZnO is an n-type semiconducting metal oxide which has drawn interest in past two–three years due to its wide range of applicability in the field of electronics, optics and biomedical systems (Agarwal *et al*. 2017). Green synthesis is an eco-friendly approach to producing nanoparticles by utilizing biological entities like plants, bacteria, fungi or algae. This sustainable process not only minimizes environmental impact but also often yields nanoparticles with unique properties and enhanced biocompatibility (Abuzeid *et al.* 2023). Coriander, commonly known as Dhania in India, is the herb *Coriandrum sativum*, which is used in the current study. The significant role of natural plant biomolecules, such as proteins and enzymes, is crucial in the bioreduction of metal salts during nanoparticle synthesis (Agarwal *et al.* 2017). *Brassicas*, particularly mustard, hold historical significance as one of the earliest crops cultivated by humans, with mentions in ancient scriptures dating back to around 5000 BC (ICAR Bharatpur 2018). Mustard used in food often consists of a blend of seeds from various *Brassicaceae* species, such as *Sinapis alba* L. (white or yellow mustard), *Brassica nigra* (black mustard), and *Brassica juncea* L. (brown or oriental mustard). Mustards are functional foods with advantageous physiological impacts on humans. *Sinapis alba* can serve as a reservoir for a diverse array of active constituents, including isothiocyanates, phenolics, dithiolthiones and dietary fiber (Abul-Fad *et al*. 2011). Mustard seeds, on the other hand, encompass protein, carbohydrates, dietary fiber, and fat. Additionally, the seeds contain a spectrum of vitamins, including vitamin C and K, along with diverse trace minerals like calcium, iron, zinc, selenium, copper, manganese, and magnesium, and electrolytes such as sodium and potassium (Tian and Deng 2020). Mustard cultivation is primarily suited to temperate climates, but it can also thrive in specific tropical and subtropical regions as a cold-weather crop (Shekhawat *et al.* 2012). India ranks as the third-largest producer of rapeseed-mustard globally, contributing over 13% of total world output during the period 2020-21 to 2024-25. However, its average yield for 2024-25 is 1,461 kg ha^{-1}, which is only about 60.7% of the global average of 2,070 kg ha^{-1}, indicating a notable yield gap (Kumar *et al.* 2025). Indian mustard (*Brassica juncea* L.) is the most important oilseed crop because it account for 21% of the total oilseeds is area and 23% of the total oilseeds production in the country. The differential trends in seed yield of Indian mustard under a particular agro climatic condition have been noticed due to varying moisture and nutrient status of the soil. It is responsive to plant nutrient, especially nitrogen and sulfur. Available soil moisture and nutrient management are important factor for improving crop productivity (Kumar *et al.* 2011).

MATERIALS AND METHODS

Plant samples (coriander leaves) for nanoparticles synthesis were collected from the local vegetable market of Balawala, Dehradun. Reagents and chemicals were purchased from Sigma Aldrich.

Seed samples PDZM31 and PM28 were collected from the "BIHAR STATE SEED AND OR-

GANIC CERTIFICATION AGENCY (BSSOCA)", Patna (Bihar).

Preparation of plant extract

The coriander leaves were washed with distilled water to remove soil debris and then shade dried for 15–20 days. The dried sample was then crushed into fine powder by electric blender and stored.10 g of dried coriander leaves were weighed and soaked in 250 milliliters of distilled water, and the mixture was boiled on a hot plate at 90˚C. The leaf extract was then filtered through Whatman filter paper (no 42) and stored at 4˚C for further use.

Zinc oxide nanoparticles synthesis

A 50 mL solution of 0.02 M zinc acetate was first prepared. Then, 2 M NaOH solution was added dropwise while stirring the mixture to maintain a pH of 12. Subsequently, 25 mL of the previously prepared aqueous coriander leaf extract was incorporated into the zinc acetate solution under continuous stirring. A color change to pale white was observed in the mixture, suggesting the formation of nanoparticles. The mixture was then centrifuged at 10,000 rpm for fifteen minutes, and the resulting pellets were collected. To remove impurities, pellets were washed sequentially with distilled water and ethanol. Finally, the obtained pellets were dried in an oven at 50°C for 30 minutes (Fig. 1).

Characterization of zinc oxide nanoparticles

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was done to characterize the morphology of synthesized zinc oxide nanoparticles. The SEM images revealed the presence of both individual ZnO nanoparticles and aggregates.

Fourier-transform infrared spectroscopy (FTIR)

FTIR analysis was done to analyze the presence of synthesized nanoparticles and functional group (present in the phytochemicals) responsible for the synthesis of nanoparticles as reducing and stabilizing agents. The wave lengths in the IR spectrum are measured in wave numbers (cm^{-1}). The intensity of the absorption is measured in absorbance. The high intensity peaks in the spectrum correspond to the functional groups in the molecule that are absorbing light. By looking up the wave numbers of different functional groups in a table, you can identify the functional groups that are present in the molecule. In the spectrum, the peak at 1343 cm^{-1} is due to O-H bending vibrations. The peak at 456 cm^{-1} is due to Zn-O stretching vibrations. IR spectroscopy is a useful tool for identifying the functional groups in molecules and for studying the

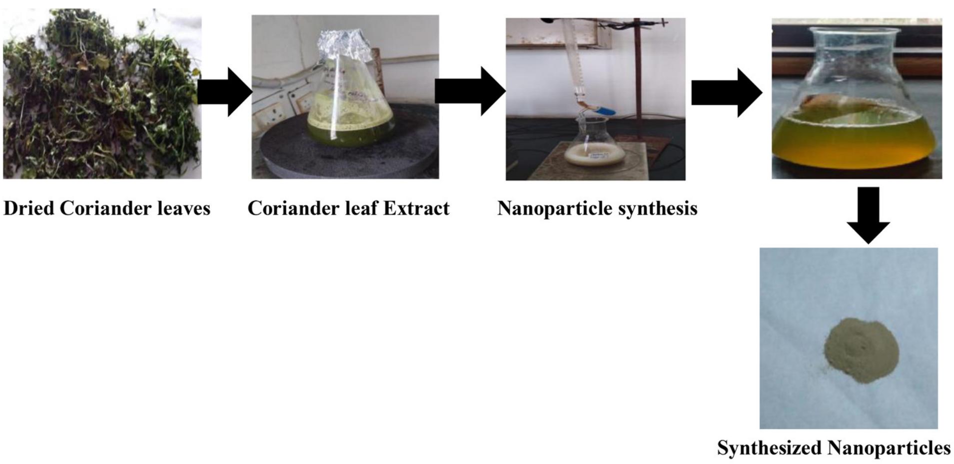


Fig.1. Green Synthesis of Zinc Oxide Nanoparticles.

structure of molecules. It is also used to study how molecules interact with each other.

Germination of seed

The study conducted on the effects of zinc oxide nanoparticles (ZnO NPs) on mustard seeds germination and seedling growth.

Seed preparation**:** Healthy and uniform mustard seeds were selected. The seeds were sterilized using a 0.1% Mercuric Chloride solution for 1 minute. After sterilization, the seeds were rinsed thoroughly with sterilized distilled water.

Treatment of seeds**:** Six different treatments were applied, each with a varying concentration of ZnO nanoparticles (10, 20, 30, 40 and 50 ppm). A control group was also included, where seeds were treated with sterilized water only. Ten seeds were used for each concentration of ZnO NPs. The seeds were exposed to the ZnO NP treatments for 48 hrs.

Seed sowing and evaluation**:** Following the treatment period, the seeds were sown in the field for further observation. The effects of the treatments were evaluated using a randomized complete with two replicates. The following characteristics in the 20-day-old seedlings were observed.

- o Germination percentage
- o Root length
- o Shoot length
- o Root fresh weight
- o Shoot fresh weight
- o Root dry weight
- o Shoot dry weight

RESULTS AND DISCUSSION

Synthesis of Nanoparticles:

Characterization of nanoparticles

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was utilized to characterize the morphology of the zinc oxide nanoparticles synthesized using coriander leaf extract. The SEM images revealed the presence of both individual ZnO nanoparticles and aggregates. As depicted in Fig. 2 the SEM analysis confirmed the nanoscale size of the particles ranges from 70.90 nm 141.8 nm embedded within the sample treated with coriander leaf extract. Notably, the SEM results did not indicate any significant flexing behavior at different magnifications.

Fourier-transform infrared spectroscopy (FTIR)

An infrared (IR) spectrum of zinc oxide nanoparticles. An IR spectrum is a graph that shows how much light a sample absorbs at different wave lengths. The wave lengths in the IR spectrum are measured in wave numbers (cm^{-1}). The intensity of the absorption is measured in absorbance. The high intensity peaks in the spectrum correspond to the functional groups in the molecule that are absorbing light. By looking up

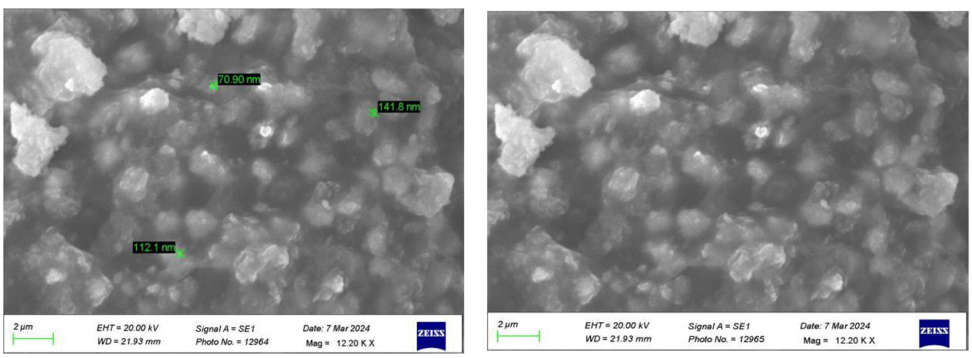


Fig.2. SEM images of synthesized ZnO Nanoparticles size ranges from 70.90nm-141.8nm

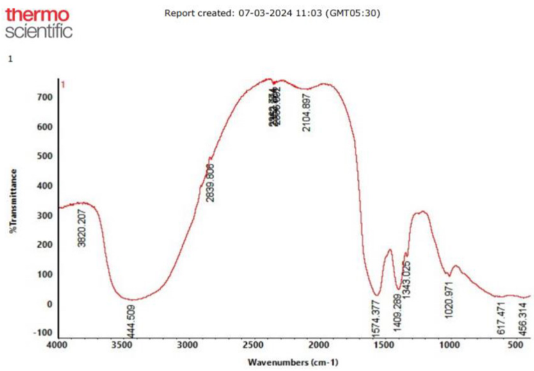


Fig.3. FTIR Plot for Synthesized ZnO Nanoparticles.

the wave numbers of different functional groups in a table, you can identify the functional groups that are present in the molecule. In the spectrum, the peak at 1343 cm^{-1} is due to O-H bending vibrations. The peak at 456 cm^{-1} is due to Zn-O stretching vibrations (Fig. 3).

Effect of zinc oxide nanoparticles on seed germination and seedling growth

All five concentrations of zinc oxide nanoparticles exhibited a positive influence on germination and seedling growth in PDZM31, and PM28 mustard seed varieties compared to the control group after 20 days (Fig. 4). Significant differences were observed across all measured characteristics in all four varieties treated with the six different zinc oxide nanoparticle concentrations. Notably, all concentrations of nanoparticles led to enhanced germination

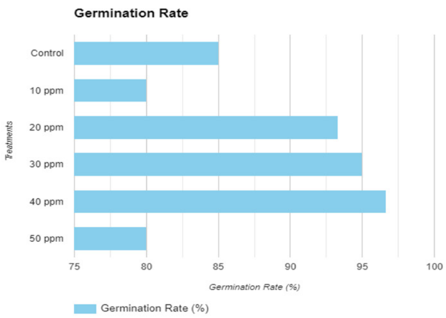
[A]

[B]

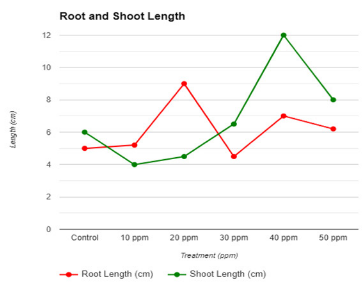
Fig.4. Image showing effect of different concentrations of synthesized nanoparticles on 20 days old mustard seedlings, **[A]** PDZM31 **[B]** PM28

percentages in all mustard seed varieties compared to the control.

All five concentrations of zinc oxide nanoparticles positively impacted germination and seedling growth across all mustard seed varieties (as shown



A



B

Fig.5. Graph Depicting Effect of Nanoparticles on Germination **[A]** Root and Shoot length **[B]** (PDZM31).

Table 1. Effect of zinc oxide nanoparticles on seedling characters in PDZM31.

Treatment (ppm)	Germination (%)	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Control	85.000	5.000	6.000	0.040	0.900	0.030	0.210
10 ppm	80.000	5.200	4.000	0.030	0.300	0.020	0.290
20 ppm	93.333	9.000	4.500	0.090	0.590	0.030	0.450
30 ppm	95.000	4.500	6.500	0.040	1.500	0.030	0.300
40 ppm	96.667	7.000	12.000	0.100	2.100	0.030	0.370
50 ppm	80.000	6.200	8.000	0.040	1.940	0.020	0.800
GM	89.333	6.117	6.833	0.057	1.122	0.027	0.403
SEm	2.943	0.667	1.229	0.011	0.245	0.002	0.083
SEd	4.163	0.943	1.739	0.016	0.346	0.003	0.117
CV (%)	5.71	18.91	31.17	33.33	37.83	12.96	35.73
CD (%)	9.144	2.072	3.819	0.035	0.761	0.006	0.258

Table 2. Effect of zinc oxide nanoparticles on seedling characters in PM28.

Treatment (ppm)	Germination (%)	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Control	85.000	5.000	5.600	0.040	0.900	0.030	0.003
10 ppm	80.000	3.000	5.000	0.050	0.700	0.040	0.008
20 ppm	95.000	7.500	4.000	0.080	0.500	0.020	0.008
30 ppm	90.000	4.000	6.000	0.040	1.000	0.060	0.200
40 ppm	96.667	7.000	8.000	0.090	1.540	0.040	0.230
50 ppm	80.000	4.200	3.500	0.060	1.230	0.050	0.270
GM	87.778	5.117	5.350	0.060	0.978	0.038	0.120
SEm	2.943	0.707	0.645	0.008	0.143	0.007	0.043
SEd	4.163	1.000	0.912	0.012	0.202	0.010	0.061
CV (%)	5.81	23.92	20.87	23.09	25.33	31.62	62.39
CD (%)	9.144	2.197	2.003	0.026	0.444	0.022	0.134

in Tables 1–2). Significant differences were observed among the six treatments for all characteristics in mustard seeds. Germination percentages were higher for all concentrations of ZnO-NPs compared to the control group in mustard seeds. The highest germination percentage was achieved at 40 ppm (96.67%) followed by 30 ppm (95.0%) and 20 ppm (93.33%) in PDZM31 (Fig. 5A). While in PM28, the highest germination was at 40 ppm (96%) followed by 20 ppm (95%) (Fig. 6A). Previously reported that carbon nanotubes dramatically accelerated germination and growth rates in tomato (Priya *et al.* 2015).

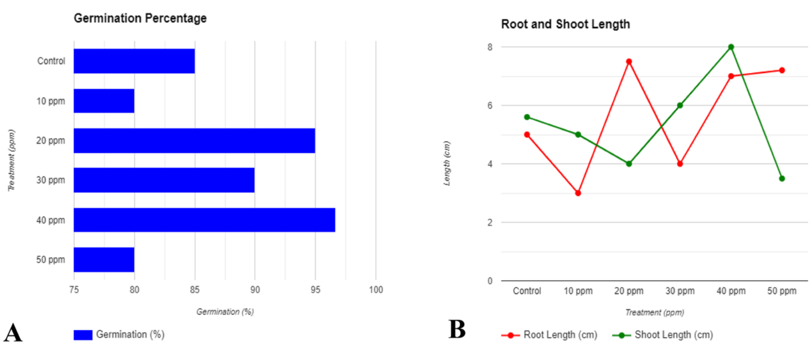


Fig.6. Graph Depicting Effect of Nanoparticles on Germination **[A]** Root and Shoot length **[B]** (PM28).

The most favorable impact on root and shoot length was observed at a concentration of 40 ppm across all mustard varieties. However, in the case of PDZM31, root length reached a maximum of 9.000 cm at 20 ppm (Fig. 5), although the overall best results for both root and shoot length were still obtained at 40 ppm.

The maximum fresh weight of both root and shoot was recorded at 40 ppm for PDZM31 (0.100 g and 2.100 g, respectively) (Table 1). Similarly, the highest fresh weight for root and shoot was also observed at 40 ppm in PM28 (Table 2). Consequently, the overall optimal results for fresh weight were achieved at the 40 ppm concentration reported that seedlings exhibited enhanced growth compared to controls when exposed to specific optimal concentrations of nanoparticles. However, growth inhibition was noted at concentrations exceeding this optimum (Mahajan *et al*. 2011).

Both 40 ppm and 20 ppm concentrations exhibited comparable positive effects on PDZM31 germination and seedling growth (Fig. 5). Also the growth rate and seedling growth of PM28 were enhanced at 20 ppm and 40 ppm (Fig. 6). Importantly, no detrimental effects were observed on seed germination when applying the standardized 40 ppm concentration of zinc oxide nanoparticles.

CONCLUSION

Zinc oxide nanoparticles were synthesized through a biological method utilizing *Coriandrum sativum* leaves extract. This green synthesis approach was considered environmentally friendly compared to traditional physical and chemical methods. The synthesis of ZnO nanoparticles using coriander leaves provided an alternative to chemical synthesis and held potential as bio-nano-fertilizers. In this present study, the effect of zinc oxide nanoparticles on seed germination and plant growth was investigated using different varieties of mustard. A positive correlation was observed between ZnO-NP concentration and seedling germination and growth up to a specific threshold. Beyond this point, a decline in shoot and root length was evident, suggesting a potential phytotoxic effect at higher nanoparticle concentrations. In PDZM31, both 40 ppm and 20 ppm concentrations exhibited favorable effects on seedling growth and germination. In case of PM28, both 20 ppm and 40 ppm concentrations promoted seedling growth and germination.

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