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Zinc Oxide Nano Priming: A Cutting-edge Strategy for Mung bean (*Vigna radiata* L.) Seed Bio-stimulation

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ABSTRACT

Background: The research outlines a study utilizing zinc oxide (ZnO) as a nano-priming agent synthesized through combustion. ZnO nanoparticles are of significant attraction because of their distinct properties and combustion synthesis proves to be a versatile and effective method for their preparation. The exothermic nature of a combustion reaction offers a rapid and energy-efficient route to obtain nanoparticles with specific properties. Mung beans are rich in nutrients, easy to digest, with low glycemic index and show antioxidant and versatility as sprouted form, soup, dal, dessert and processed products like starch for noodles and flours. Mung beans are a globally traded commodity and may generate an income source for farmers.

Methods: In this work, at 500°C, ZnO nanoparticles were prepared using zinc nitrate and ascorbic acid. Characterization techniques, including UV-vis spectroscopy, XRD, RAMAN and FE SEM, confirmed ZnO nanoparticles. The experiment involved seed priming with varying concentrations of the ZnO nanoparticle (from 1 ppm to 5 ppm) suspension, with a control group that was left untreated.

Result: The results revealed that treating mung bean (Vigna radiata) seeds with a concentration of 5 ppm ZnO nanoparticles positively influenced seed germination, improved shoot and root lengths and overall biomass, compared to unprimed seeds and suggested ZnO nanoparticles as an effective nano-priming agent for promoting seedling growth and development.

Key words: Combustion synthesis, Germination, Priming, *Vigna radiata*, Zinc oxide nanoparticles.

INTRODUCTION

The global food supply faces numerous challenges due to the increasing global population. It is predicted that the human population will hit 9.7 billion by 2050. Due to this population increase, the demand for food will increase between 59% to 98% (Haris *et al*., 2023). Another challenge is providing nutritious food. An important legume, mung bean, is recognized for its medicinal and nutritional values (Liang *et al.,* 2022). It is rich in protein, fiber, vitamins and minerals and free from gluten and cholesterol.

Mung bean (*Vigna radiata* L., Family: Fabaceae), popularly called green gram or moong bean, has been consumed for about 3,500 years (Junjariya *et al*., 2024). It has been cultivated mainly in India and introduced worldwide (Choudhary *et al*., 2025). This Mung bean sprouted food is gaining demand as a therapeutic food (Geng *et al*., 2022). Because of its dual benefits in medicine and food, it is accepted as a Medicine Food Homology (MFH). In a few restaurants, sprouted Mung bean is sometimes served as a main or side dish (Salvador *et al*., 2021). It is also produced as value-added products (Naik *et al*., 2020). Mung bean cultivation faces enormous challenges such as poor seed quality, weeds and pests, degradation of soil, inadequate farming practices, quality issues and postharvest wastage (Dikr *et al*., 2023).

Nanomaterials can be used as fertilizer that prevents nutrients from prematurely converting into gaseous or chemical form (Verma *et al*., 2023). The commonly utilized NPs were zinc (Zn), titanium (Ti), iron (Fe), copper (Cu), manganese (Mn) and aluminum (Al) (Maity *et al*., 2022). Reports indicate that ZnO NPs priming in Maize seeds increases nutrient, antioxidant enzymes, biomass yield, growth rate and photosynthetic performance under adverse environments and reduces Malondialdehyde (MDA) and Shoot Reactive Oxygen Species (ROS) accumulation (Salam *et al*., 2022). The use of ZnO NPs Priming in cucumber plants improved physical parameters such as germination rate, the height of the plant, leaf number and area, flower yield and its fruit length, weight, diameter, color, firmness and chemical parameters such as total soluble solids, ascorbic acid, titratable acidity (Nisar *et al*., 2022). ZnO NPs priming in mung bean seeds under salinity conditions enhanced germination percent, physical parameters, also increased antioxidant enzyme activity, proline content, specifically superoxide dismutase (SOD) and peroxidase (POX) (Sherpa *et al*., 2024). The ZnO NPs

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priming in seeds of tomatoes under a stressful environment increases germination, chlorophyll, enzyme-mediated and non-enzyme mediated antioxidant protective mechanism and adjusts osmotic conditions (Ghosh *et al*., 2024). The ZnO seed priming stimulated germination and increased fruit number, density, diameter and phytochemicals were noticed in Bitter gourd seeds (Mazhar *et al*., 2024). Germination and total phenol content were improved by ZnO NPs priming in sunflower seeds (Al-Sudani *et al*., 2024). The ZnO NPs priming in rapeseed boosts germination and vigor index (VI), increases the physical parameters, absorption of nutrients and antioxidants and adjusts osmotic protection leading to proline, soluble protein and sugar increase. Reduces ROS, degradation of chlorophyll and biosynthesis pigment under salinity (El-Badri *et al*., 2021).

In the present work, mung bean seeds were selected due to the enormous benefits mentioned. ZnO NPs were used to prime the seeds due to their popularity, easy availability and low price. The ZnO NPs are synthesized through a simple combustion method. Various techniques like UV visible spectroscopy, XRD, RAMAN and FE SEM were used to characterize the synthesized ZnO NPs. Priming the seed is a low-cost, feasible technique and can advance germination in the early growth stage of plants in adverse environments. Mung bean seeds were primed at different ZnO NPs concentrations. After seed germination, the physical and phytochemical parameters of the sprouts were examined and a comparison was carried out between the control group seeds and primed seeds.

MATERIALS AND METHODS

Chemicals

All Chemicals used were analytical-grade and were not further modified, with ultra-pure water used in all procedures.

Synthesis of ZnO NPs

The self-propagating high-temperature synthesis (SHS) technique was employed to prepare ZnO NPs at the Nanomaterials Synthesis Laboratory, Noorul Islam Centre for Higher Education, Kumaracoil. In a typical experiment, zinc nitrate hexahydrate (0.2 g) and ascorbic acid (0.1 g) were mixed in an agate mortar. The blend was placed into an alumina crucible and heated in a muffle furnace at 500°C in stagnant air for 3 hours to yield the ZnO NPs as shown in (Fig 1) (Kumar *et al*., 2011).

Characterization of nanoparticles

The ZnO NPs were characterized for their optical properties, crystalline nature, vibrational modes and morphology. The optical properties were analyzed using a Systronic M2202 double-beam UV-visible spectrophotometer. ZnO NPs crystalline structure was studied through X-ray diffraction utilizing the BRUKER D2 Phaser instrument. Vibrational modes of the ZnO NPs were obtained using a RAMAN spectrophotometer (EZRaman, N785, Enwave Optronics, USA). Additionally, the ZnO NPs morphology was investigated with FESEM-SUPRA55, manufactured by Carl Zeiss, Germany.

Seed priming experiment in mung bean (Vigna radiata) seeds

The mung bean seeds were washed in distilled water to remove surface dust and treated with 70 % ethanol for 2 minutes to remove surface microbes. Afterward, a 15-minute rinsing with 1% sodium hypochlorite was conducted to eradicate any bacteria or fungi contamination on its surface and subjected to 5 consecutive washes with ultrapure water (Tadayon *et al*., 2023). Then, priming with ZnO NPs of various concentrations and the control group were treated with distilled water in a closed environment at room temperature for 24 hours. After that, seeds from each treatment were placed separately in Petri dishes above sterile tissue paper and 5 mL of distilled water was added (Masilamani *et al*., 2025). These seeds were kept undisturbed for a week to grow within the Petri dish. The entire experiment was carried out in triplicate at the Nano Applications and Research Laboratory, Noorul Islam Centre for Higher Education, Kumaracoil, from January 2024 to June 2024, under undisturbed, sterile conditions to avoid contamination.

Seedling growth parameters

After a week of germination, the shoot and root lengths and the dry and wet weights of the germinated mung bean seeds were measured.

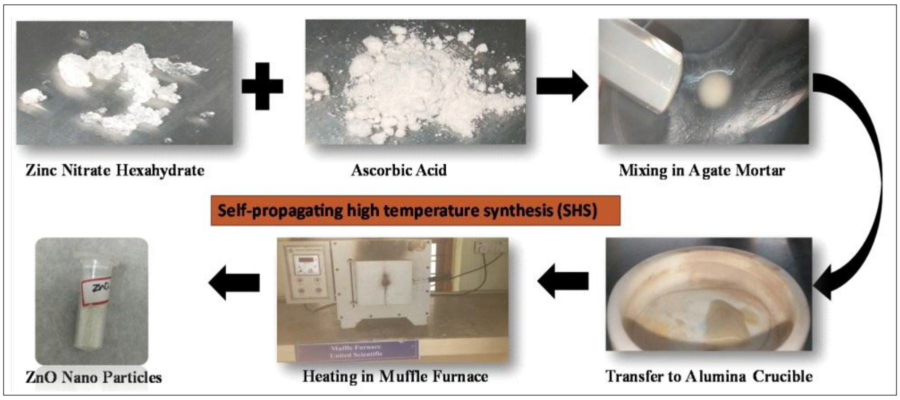


Fig 1: Self-propagating high-temperature synthesized (SHS) ZnO NPs.

Dry weight

The seeds were left to dry overnight inside an oven at 40°C for 3 days (Anaemene *et al*., 2022). After complete drying, the dry biomass (DWs) was measured.

Plant extract preparation

The dried plant (0.5 g) was extracted in a reflux condenser using ultra-pure water of 50 mL for 2 hours at 100°C. The extracts were collected and stored at 4°C (Bitwell *et al*., 2023).

Carbohydrate content

The total carbohydrate was estimated using the Anthrone method. Briefly, 2 % Anthrone reagent 4 mL was added to the extract of 0.1 mL and the solution was incubated for 15 min at 90°C. This was cooled immediately after heating. The blank was prepared only with the Anthrone reagent and without the extract. The greenish-blue solution absorbance was recorded in a UV-vis spectrophotometer at 620 nm. The carbohydrate content was calculated using a calibration graph prepared using glucose as the stock solution (Plummer 1990).

Protein content

The total protein present was estimated using Lowry's method with Bovine Serum Albumin (BSA) serving as the standard. This method employs two types of reagents, Reagent 1 contains 0.4% NaOH and 2% Na_2CO_3 of 48 ml, 1% NaK tartrate and 0.5% $CuSO_4.5H_2O$. Reagent 2 contains 10% of the Folin-Ciocalteu reagent. To the 0.1 mL extract, reagent 1 of 4.5 mL was mixed and 10 min incubated. Subsequently, 0.5 mL of reagent 2 was added and incubated for 30 min. A blank was prepared with reagents 1 and 2 alone. The absorbance of the blue-colored solution was measured at 660 nm (Redmile-Gordon *et al*., 2013).

Total phenol content

The total phenolic content (TPC) present in the extract was estimated by using the Folin-Ciocalteu method, with gallic acid serving as the standard. In this procedure, 0.1 mL extract was added to Folin-Ciocalteu's phenol reagent of 1.5 mL and left at room temperature for around 5 minutes. Subsequently, 20% sodium carbonate of 4 mL is added and incubated at room temperature for 30 minutes. Using a calibration graph TPC was estimated. The blue-colored solution absorbance was recorded at 765 nm (Isaac *et al*., 2012).

RESULTS AND DISCUSSION

UV-vis spectroscopy result

The UV-visible wavelength range shown in (Fig 2 A) is between 355-400 nm. A strong absorption was measured at 367 nm, which is in accordance with previous research work done by a solvothermal approach and confirms the ZnO NPs (Singh *et al*., 2012).

The energy bandgap of the material was measured from Tauc's plot at 3.1 eV, as shown in (Fig 2 B) calculated using equation (1).

$$(\alpha h\nu)^{\gamma} = A\,(h\nu - E_g) \qquad \text{.... (1)}$$

XRD result

X-ray Diffraction (XRD) was used to characterize the ZnO NPs as shown in (Fig 3). The phase is found to be Hexagonal with JCPDS Card No. 36-1451, conformed XRD patterns of 500°C calcinated ZnO NPs. The reflections observed at 31.8° , 34.4° , 36.3° , 47.5° , 56.6° , 62.8°, 66.4°, 67.9°, 69.1°, 72.5° and 76.9°, were indexed to planes as [100], [002], [101], [102], [110], [103], [200], [112], [201], [004], [202]. It states that ZnO NPs have no additional peaks and are highly pure. The average crystallite size (D) was calculated as 10 nm by using the Scherrer equation given below in Equation (2) (Jayswal and Moirangthem, 2018).

$$D = \frac{0.9\lambda}{\beta\cos\theta} \qquad \text{.... (2)}$$

RAMAN result

The ZnO NPs Raman spectrum is shown in (Fig 4) measured under 785 nm red LASER excitation. The observed signal at 245 cm^{-1}, was due to interstitial zinc (Zni) affection (Song *et al*., 2019). The signal found at 403 cm^{-1} resulting from A1 (TO) phonons oxygen-dominated polar mode (Abdelouhab *et al*., 2020). The remaining signals at 523 cm^{-1}, 549 cm^{-1},

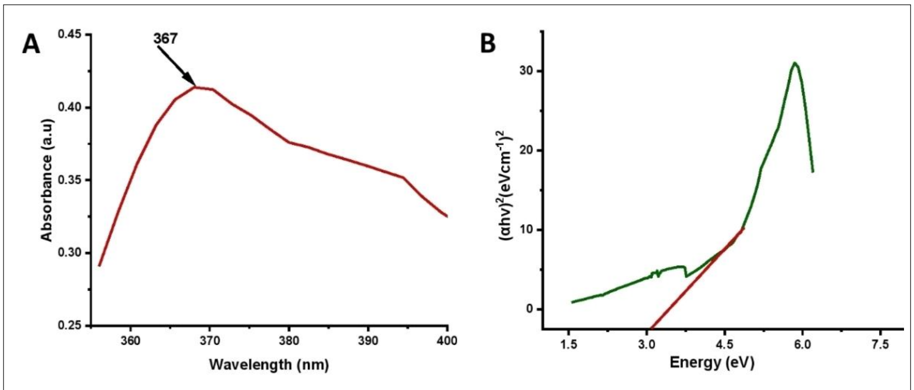


Fig 2: (A) UV-visible spectrum of ZnO NPs, (B) Tauc plots drawn (αhv)2 versus photon energy (hv) of ZnO NPs.

635 cm^{-1}, 688 cm^{-1} were due to oxygen vacancy defect (Vo) (Song *et al*., 2019), longitudinal optical (LO) phonon modes of A1 and E1 modes (Khan *et al*., 2015), Second-order Raman scattering by LA(X) or LA(L) phonons and the combinational mode [LO (Γ) + TO(X)] (Milekhin *et al*., 2012), E1 (TO) mode (Lo *et al*., 2010).

FESEM image

The morphology of the ZnO NPs was examined using Scanning electron microscopy (SEM) illustrated below (Fig 5). SEM analysis further revealed a diverse range of particle sizes, spanning from 20 to 40 nanometers. This extensive size distribution shows the polydispersity inherent in the synthesized ZnO NPs.

Germination rate and seed vigor index

Germination was confirmed upon the emergence of a 2 mm radicle from the seed. After 1 week, all the germinated seedlings from each of the 3 replications, 5 were chosen and measured for both root and shoot lengths (centimeters) and the wet and dry weights (grams). The germination of mung bean seeds at different concentrations is illustrated in (Fig 6).

Various growth and development parameters were assessed, such as germination percentage (GP) Equation (3), seedling vegetation index (VI) Equation (4) and mean emergence time (MET) Equation (5). The results of these analyses are presented in Table 1 (Abdul Baki *et al*., 1973).

$$GP = \frac{n}{N} \times 100\% \quad \text{.... (3)}$$

$$VI = (\text{Mean root length} + \text{Mean shoot length}) \times \text{Germination percentage (\%)} \quad \text{.... (4)}$$

$$MET = \frac{\Sigma\, Dn}{\Sigma\, N} \quad \text{.... (5)}$$

The observed increase in the germination rate can be linked to the entry of NPs through seed coat absorption, the nature of the increased surface area to small size subsequently traversing by parenchymatous tissues, spacing enter cells easier. The positive germination percent indicates that accelerated amylase activity may improve simple sugar stock and lead to embryo viability. Additionally, the involvement of ROS during seed germination plays a crucial role in these processes (Setty *et al*., 2023).

In basic, the quality of seeds was determined through 2 major parameters, GP and VI (Hussain *et al*., 2021). The simple calculation for determining VI is the percentage of germination multiplied by the shoot and root length. The results revealed that (3 ppm) showed a higher VI value of (3098.00) as compared with other treatments. A higher VI suggests greater vitality and potential for robust seedling growth (Roy *et al*., 2024). For higher VI, priming for 24 hours is found to be best (Muttaqin *et al*., 2019). Mung bean seed germination with ZnO NPs priming was dosage dependent and it inhibited faster growth and seedling traits (Sherpa *et al*., 2024).

Seedling growth assay

After growing the seeds for 7 days, measurements were taken for Wet weight (WW) and dry weight (DW), shoot length and root length. The results (Fig 7) showed that a higher material mass is yielded in terms of WW and DW when the ZnO concentration is at 5 ppm, compared with other treatments. The pivotal function in the pre-growth phase is supported by well-developed roots, which leads to the subsequent expansion of leaves by efficient nutrient and mineral absorption (Nakasha *et al*., 2014).

Carbohydrate content

The total carbohydrate levels in the control and NP-treated seedlings are shown in (Fig 8A). Zn is a crucial component in carbohydrate metabolism, contributing to improved and uniform seed germination. A decreased level of carbohydrate content at (5 ppm) than control was observed. The physiological discrepancy between ZnO NPs and

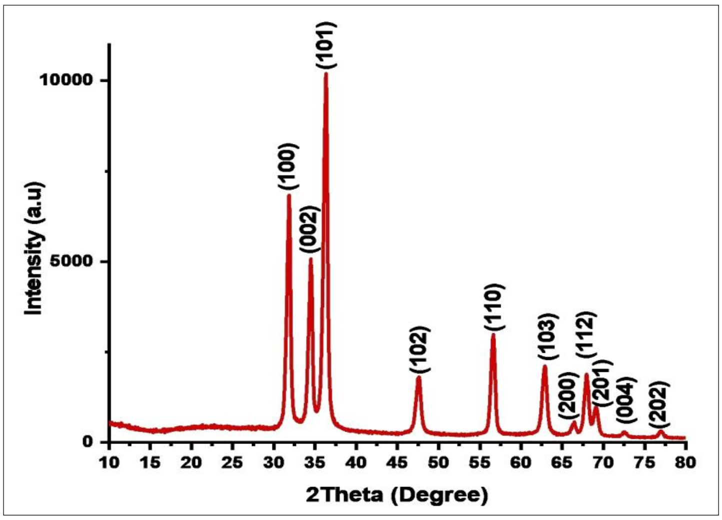


Fig 3: XRD diffraction pattern of ZnO NPs.

control was because Zn^{2+} is slowly released from ZnO NPs. In lettuce seeds ZnO NP treatment, decreased total carbohydrate content, because NPs trigger the early breakdown of carbohydrates and reduce overall carbohydrates (Rawashdeh *et al*., 2020). These findings are consistent with our study, as ZnO NPs promote faster growth through the rapid utilization of carbohydrates.

Protein content

The germinated seeds' protein content was measured after 7 days and shown in (Fig 8B). Numerous studies have found that NPs mimic the antioxidant enzyme's role and act as catalase (Wu *et al*., 2019). NP's role in promoting the antioxidant defense mechanisms of plants, contributing to the resistance to survive in challenging environmental

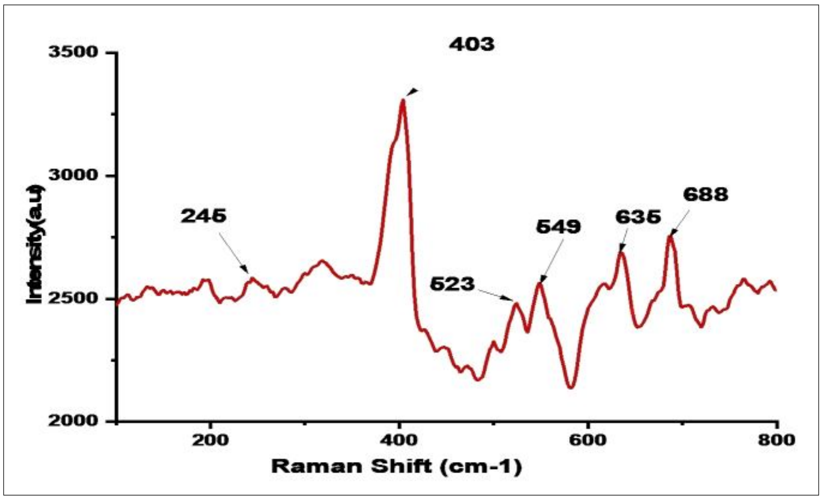
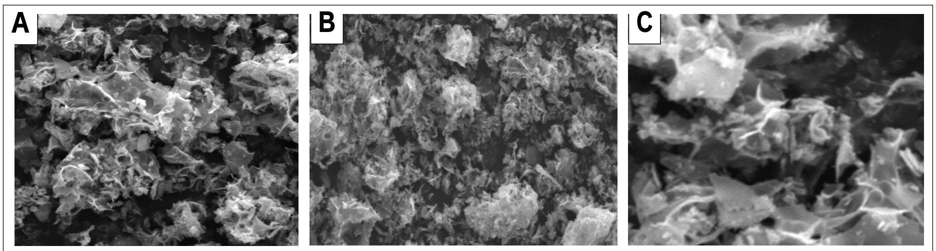
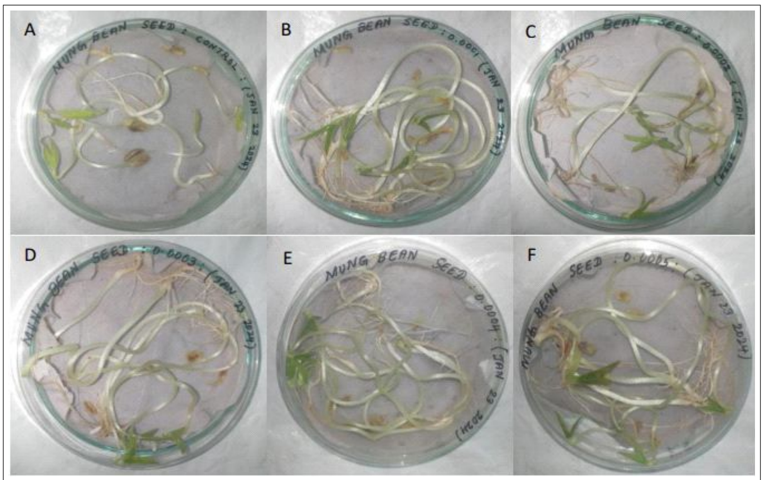


Fig 4: RAMAN spectra of ZnO NPs.



(A) 50.00 KX, (B) 20.00 KX and (C) 2.50 KX.

Fig 5: FESEM Images of synthesized ZnO NPs at different magnifications.



Control (A); 1 ppm (B); 2 ppm (C); 3 ppm (D); 4 ppm (E); 5 ppm (F)

Fig 6: The Petri plates containing seedlings treated with different concentrations of ZnO NPs.

conditions (Rawashdeh *et al*., 2020). The total protein content result shows that the controlled seeds exhibit higher protein stability due to no externally induced stress.

Table 1: S1 (Control); S2 (1 ppm ZnO); S3 (2 ppm ZnO); S4 (3 ppm ZnO); S5 (4 ppm ZnO); S6 (5 ppm ZnO); GP = germination percentage; MET = mean emergence time; VI = seedling vigour index.

Treatment	GP	VI	MET
S1	100	2381.99	1
S2	100	3056.66	1
S3	100	2842.66	1
S4	100	3098.00	1
S5	100	2818.66	1
S6	100	2912.66	1

NPs-treated seeds experience a decline in protein levels due to mild stress but are still found non-toxic even at 5 ppm.

Phenol content

The NP-treated seedling's Total Phenolic Content (TPC) is shown in (Fig 8C). The phenolic levels show an increasing trend in primed seeds compared with unprimed ones. Phenolic acids act as secondary metabolites of plants and are connected with biotic and abiotic plant responses to stress and dissolve in estimating the total activity of antioxidants. This improvement in TPC was particularly notable in treatment with 5 ppm ZnO NPs compared with other treatments. The increased phenolic contents may be indicative of a defense mechanism in overcoming potential NP-mediated phytotoxicity (Sorahinobar *et al*., 2023). The increase in phenolic production was found to be 1.3-fold compared to the control.

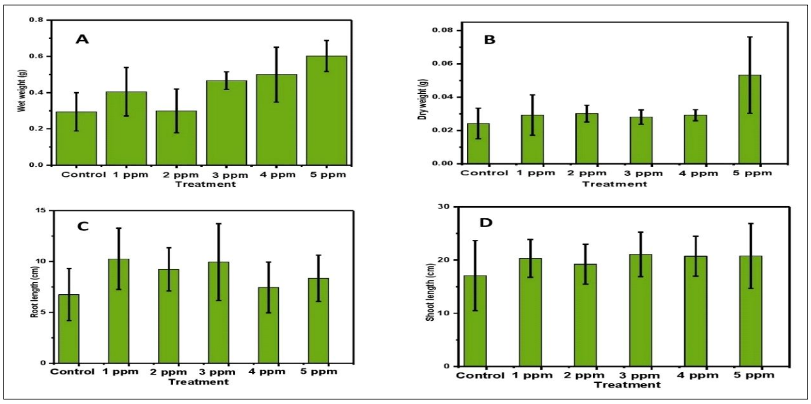


Fig 7: (A) Wet Weight of germinated mung bean seeds measured after 7 days, (B) Dry Weight of germinated mung bean seeds measured after 7 days, (C) Shoot length of germinated mung bean seeds measured after 7 days, (D) Root length of germinated mung bean seeds measured after 7 days.

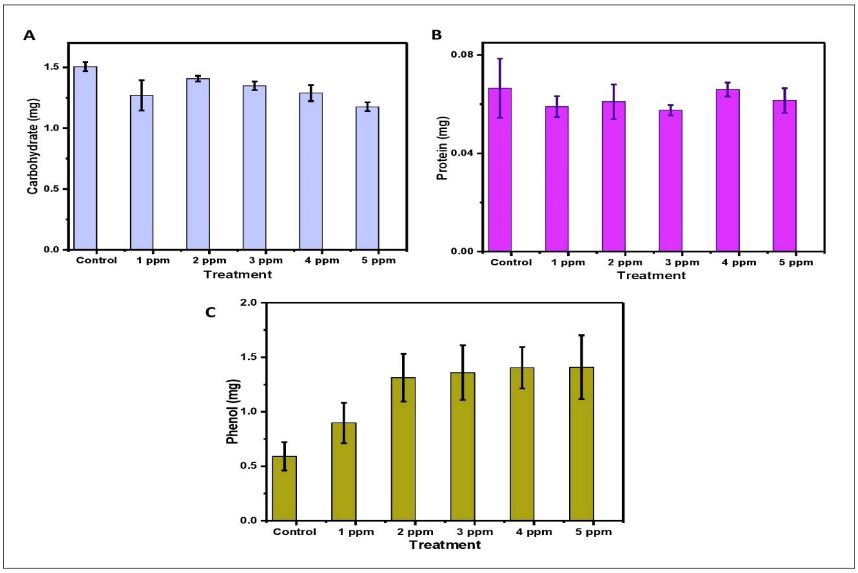


Fig 8: (A) Carbohydrate content in the germinated Mung bean after 7 days, (B) Protein content in germinated Mung bean after 7 days, (C) Phenol content in germinated Mung bean after 7 days.

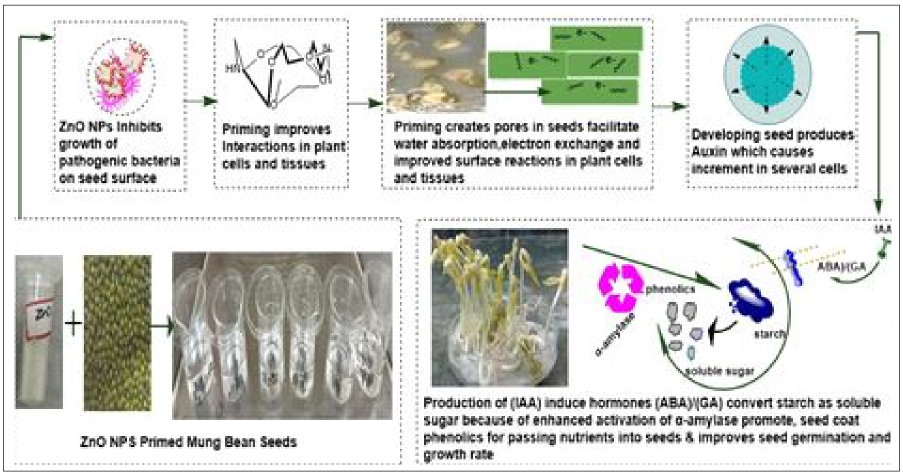


Fig 9: Mechanism of ZnO Nano priming.

Mechanism of action

The mechanism behind ZnO NPs priming is illustrated in (Fig 9). Nanoscale materials possess diverse physicochemical properties, allowing them readily absorbed by seeds than bulk materials. Modifying surface chemical characteristics of nanomaterials may improve their molecular interaction with seedlings and reduce the waste of priming agents. Multiple studies have shown their capacity to hinder the development of both pathogenic and non-pathogenic bacteria and fungi. NPs' notable properties allow for electron exchange and improve surface reactions in plant cells and tissues. Nano-priming induces nanopores, facilitating the absorption of water (Donia *et al*., 2023). The Zinc causes an increment in several cells and a larger size by producing auxin for the development of plants (Hussain *et al*., 2021). Nano-primed seeds, uptake water so that Indole-3-acetic acid (IAA) induces the hormones Abscisic acid (ABA)/Gibberellic acid (GA) that may convert starch into soluble sugar because of enhanced activation of α-amylase. This enabled the seed's respiration response. Also, the hormones promote seed coat phenolics for passing nutrients into seeds and improve the seed germination and growth rate. But still, there was no clear internal mechanism reported on seed growth (Nile *et al*., 2022).

CONCLUSION

In agricultural practice, priming the seed is an alternative and easiest method for growing crops in stressful and normal conditions. The optimization of the seed priming technique improved crop performance, enhancing the productivity of plant crops and offering sustainable solutions for promoting overall agricultural productivity. Zn, a crucial micronutrient for plants, plays a vital role in various physiological functions. An adequate Zn supply in plants supports optimal growth and overall productivity in agricultural systems. Among NPs, ZnO NPs are the most extensively utilized, with several reports highlighting their beneficial effects on the ecosystem. This present study demonstrated the efficacy of ZnO NPs priming in improving biomass production in Mung bean seedlings compared to untreated seeds. Moreover, seed-priming with ZnO NPs enhances shoot height, root length and shoot biomass. The current findings indicate the effectiveness of ZnO NPs in improving seedling growth and development. Therefore, greater plant growth with NPs may result from better nutrient mobilization and stimulated microbial activity in the rhizosphere. Hence, nanotechnology is used to enhance germination and yield. In the future, research needs to be focused on practical field trial experiments.

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Authors' contributions

Jeyani Shalu; Carried out the experiments. Jeyani Shalu and Rimal Isaac; Co planned the experiments. Jeyani Shalu, Rimal Isaac and R. Revathi; Contributed to the interpretation of results and wrote the manuscripts.

Ethical approval

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest related to this article.

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