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Green Synthesis of ZnO Nanoparticles from *Caesalpinia bonducella* Seed and Application in Nano-priming-Assisted *Vigna mungo* Seed Germination

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Abstract

The green synthesis of zinc oxide nanoparticles employing *Caesalpinia bonducella* as a bio-reductant explores their potential applications in plant growth promotion through nano-priming and environmental safety. Conventional seed treatments often lead to risks to the environment and toxicological concerns. There is an urgent need for safe and sustainable solutions to improve seed germination and plant growth. This study aims to evaluate the efficacy of ZnO nanoparticles as a nano-priming agent on *Vigna mungo* seed germination and root, shoot development at varying concentrations. Employing XRD, SEM, UV-vis Spectroscopy, FTIR, and Zeta potential measurements, the synthesized ZnO nanoparticles were comprehensively characterized, confirming their successful production. Following characterization, the study delves into the pioneering exploration of nano-priming, evaluating the impact of ZnO nanoparticles on *Vigna mungo* germination and plant growth parameters (root and shoot lengths) at defined concentrations. Furthermore, the same concentrations were utilized to assess nanoparticle toxicity through mortality tests on *Artemia* larvae. This investigation underscores the potential of biogenic ZnO nanoparticles as potent nano-priming agents and unveils *Caesalpinia bonducella* as a valuable source for nanoparticle synthesis. Compared to the control group, the study demonstrates significantly positive effects in the germination index and overall plant growth in seeds treated with ZnO nanoparticles. Therefore, the ZnO nanoparticles improve seed germination and growth in *Vigna mungo* with minimum toxicity, ensuring a safe and efficient agricultural application.

Keywords*: Caesalpinia bonducella, Nano priming, ZnO nanoparticles, Vigna mungo.*

I. INTRODUCTION

The ongoing field of green nanotechnology focuses on the eco-friendly synthesis of nanoparticles using natural resources. This approach holds immense promise for mitigating environmental concerns associated with traditional chemical methods.[1] The natural resource offers a diverse array of biomolecules, such as flavonoids, terpenoids, and alkaloids that can act as both reducing and stabilizing agents during nanoparticle synthesis, these biomolecules promote nanoparticle formation through various mechanisms based on their nature and source. Specifically, flavonoids act as an antioxidant that reduces metal ions and stabilize nanoparticles, terpenoids facilitate metal ion reduction while alkaloids reduce metal salt and provide stability to nanoparticles. [2][3,4] *Caesalpinia bonducella* seed powder was chosen as a base material due to its inherent profile of bioactive compounds and its diverse phytochemical composition, which includes flavonoids, alkaloids, and phenolic chemicals that act as natural reducing and stabilizing agents. This environmentally friendly approach is consistent with green synthesis principles, providing a sustainable, cost-effective alternative to traditional chemical methods while harnessing the plant's bioactive qualities to improve the efficacy and stability of the nanoparticles. [5,6][7,8] These compounds not only facilitate the reduction of metal ions but also possess potential applications in agriculture and medicine.[9,10] Moreover, employing plant-derived precursors aligns

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perfectly with the increasing demand for sustainable and environmentally benign nanoparticle synthesis methods.

Zinc is vital for plant growth and development, involving enzyme activity, protein synthesis, and hormone regulation. Its deficiency hinders chlorophyll production, reduces photosynthetic efficiency, and results in stunted development, interveinal chlorosis, and malformations such as smaller leaves or necrotic regions. Zinc deficiency in legume grains has a negative impact on reproductive structures, resulting in poor pod development, smaller grain size, and decreased nitrogen fixation due to defective nodule growth. This leads to decreased yields and poor grain quality. Sustainable approaches, such as soil amendments, foliar sprays, and nano-enabled treatments, are crucial for combating zinc deficiency and enhancing crop yield.[11,12]

In particular, ZnO nanoparticles have garnered significant interest across diverse fields due to their unique chemical and physical properties.[13–15] Zinc nanoparticles (Zn NPs) have significant advantages over traditional zinc fertilizers, such as improved nutrient uptake, slow and controlled release, and lower application rates. They improve seed germination, plant development, yield, and stress tolerance while reducing nutrient loss, leaching, and environmental contamination.Zn NPs, which act as both nutrients and antimicrobial agents, offer a long-term and efficient solution for increasing crop yield and environmental safety.[16]

Seed germination is a pivotal stage in the plant life cycle, and its enhancement can significantly improve agricultural productivity by increasing crop yield and ensuring more uniform and timely growth. *Vigna mungo* seeds were chosen because their cultivation involves concerns such as poor germination, low yield, and vulnerability to environmental stressors, all of which have a substantial impact on farmer output. These concerns highlight the need for novel approaches to boost growth and resilience, making this crop suited for testing sustainable interventions such as nanoparticle-based treatments.[17]

Recent studies have highlighted the ZnO nanoparticle's ability to improve seed germination and seedling growth by acting on improved water uptake, boosting nutrient availability, and enhancing stress tolerance in seeds with the help of nano priming. Nano-priming, a technique that involves treating seed directly with the nanoparticle, has emerged as a promising strategy to improve germination rates, seedling vigor, and overall crop yield. [18,19] By integrating the green synthesis of ZnO nanoparticles with nano-priming techniques, this research aspires to contribute to sustainable agricultural practices that promote seed germination and minimize the environmental footprint associated with nanoparticle production.

This research aims to develop a cost-effective and eco-friendly route for ZnO nanoparticle production, eliminating the use of hazardous chemicals and energy-intensive processes.[20] We explore the potential of *Caesalpinia bonducella* seed powder as a green precursor for ZnO nanoparticle synthesis and delve into their application in nano-priming-assisted seed germination of *Vigna mungo*. This research aims to offer valuable insights into the potential of green nanotechnology for sustainable agriculture through a comprehensive analysis of the synthesized ZnO nanoparticles and their influence on *Vigna mungo* seed germination. The findings could pave the way for developing eco-friendly seed priming techniques, further propelling sustainable agricultural practices.

II. MATERIALS AND METHODS

➢ *Aqueous seed Extract Preparation*

Commercially acquired *Caesalpinia bonducella* seeds were purchased from an Ayurvedic shop and ground into a fine powder. The seed powder was mixed with $D.H_2O$ in a 1:10 ratio respectively and heated to 60°C for 120 mins.[21] The extract was then filtered using Whatman no. 42 filter paper and stored in a sealed container at 4°C for further use.

➢ *Nanoparticle Synthesis*

Zinc acetate dihydrate served as the precursor for the ZnO nanoparticle synthesis. A total of 160mL of 2mM zinc acetate solution was mixed with 40mL of the prepared *Caesalpinia bonducella* extract under constant stirring. The mixture was then titrated dropwise with 2M NaOH solution until it reached pH 12. After continuous agitation with a magnetic stirrer for 1h 30 mins, the reaction mixture was centrifuged to isolate the precipitate. The pellet was re-dispersed in $D.H_2O$, sonicated, and centrifuged three times for thorough washing. The final product was then re-suspended in 70% ethanol, centrifuged, and dried. Finally, the ZnO nanoparticles were calcined at 300-400°C for 3h in a muffle furnace followed by drying overnight in an 80°C hot air oven.[22]

➢ *Characterization*

The synthesized ZnO nanoparticles were characterized using various techniques:

- *Fourier Transform Infrared (FTIR) Spectroscopy:*

Analysis was performed using a Thermo Nicolet iS50 equipped with a KBr mode spectrophotometer (Shimadzu Corporation, Japan). Spectra were recorded in the wavenumber range of 4000 - 400 cm^{-1}.

- *X-ray Diffraction (XRD):*

Crystallinity and phase identification were determined using a Bruker D8 Advance X-ray diffractometer (Germany) equipped with a high-power Cu X-ray source (2.2 kW anode) generating Cu Kα radiation (λ = 0.154 Å).

- *Scanning Electron Microscopy (SEM):*

The morphology of the nanoparticles was analyzed using a Thermo Fisher FEI QUANTA 250 FEG field emission scanning electron microscope (running at 5-30

kV). At 30 kV, the microscope provided high-resolution imaging with a resolution of 1.2 nm.

- *UV-Visible (UV-Vis) Spectroscopy:*

The optical properties of the nanoparticles were characterized using a JASCO V-670 PC twin-beam spectrophotometer (Shimadzu Corporation, Japan).

➢ *Toxicity Evaluation in Artemia salina*

- *Hatching and Preparation:*

Artemia salina eggs were obtained from an aquaculture lab at Vellore Institute of Technology, Vellore, Tamil Nadu, India. For hatching, the eggs were placed in a container with artificial seawater prepared according to standard protocols from Claus et al. 1981.[23] The container was maintained at a slightly elevated temperature (28-30°C) under continuous aeration and a light source for 24h - 48h to promote hatching. Healthy nauplii were easily identified by their continuous swimming activity.

- *Toxicity Assay:*

The toxicity of ZnO nanoparticles towards *Artemia salina* was evaluated using different nanoparticle concentrations: 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL. The ZnO nanoparticles were first suspended in D.H_2O using sonication for 30 mins to ensure proper dispersion. Serial dilutions were then performed to obtain the desired concentrations for the assay.[24] A six-well plate was used for the toxicity assay. Each well-contained 10mL nanoparticle solution contains different concentrations of ZnO nanoparticles. Well-1 served as the control and contained only D.H_2O. The remaining wells were filled with seawater containing different ZnO nanoparticle concentrations as follows: Well-2 (500 µg/mL), Well-3 (250 µg/mL), Well-4 (125 µg/mL), and Well-5 (62.5 µg/mL). Ten healthy and actively swimming nauplii were carefully transferred to each well using a dropper. The plates were then incubated for 48h at room temperature under static conditions. The number of surviving nauplii in each well was counted after 24h and 48h of exposure to assess the toxicity of the ZnO nanoparticles.[25]

➢ *Nano-Priming Application:*

Vigna mungo seeds underwent standard surface sterilization to proceed with nanoparticle treatment. Different concentrations of ZnO nanoparticle solutions were prepared with D.H_2O: control (untreated), 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL. The *Vigna mungo* seeds were then treated with the nanoparticle suspensions for 6h. Following the treatment, seeds were placed on a moistened absorbent paper placed in a petri dish. The Petri dishes were maintained at a constant room temperature of 25°C.[26][27] Seed germination was monitored daily for 7 days, recording the number of germinated seeds at each time point.

➢ *Germination and Physiological Measurements:*

A seed was considered germinated when the radicle emerged from the seed coat. The radicle length of each germinated seed was measured daily. Ten seedlings were randomly selected from each concentration with at least two replicates per concentration (a total of 20 seedlings per concentration that includes 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL).[28,29] These seedlings were used for root and shoot length measurements.[30] To investigate the following parameters after seven days: germination percentage (GP), germination energy (GE), germination value (GV), mean daily germination (MDG), germination rate (GR), and peak value (PV), the following formulas were used to generate germinating seed indexes from the germinated counts.

i. $$Germination\ Percentage = \frac{Germinated\ seeds}{Total\ no\ of\ seeds} \times 100$$

ii. $$Germination\ Energy = \frac{Number\ of\ seeds\ germinated\ on\ the\ 7th\ day}{Total\ no\ of\ seeds}$$

iii. $$Mean\ Daily\ Germination = \frac{Germination\%}{Total\ experiment\ days}$$

iv. $$Peak\ \text{Value} = \frac{Maximum\ number\ of\ germinated\ seeds\ at\ one\ day}{Total\ no\ of\ days}$$

v. $$Germination\ Value = PV \times MDG$$

vi. $$Seed\ Vigor\ Index = (Shoot\ lenght + Root\ lenght) \times Germination\ Percentage$$

Where, PV: Peak Value; MDG: Mean Daily Germination

III. RESULTS

➢ *ZnO Nanoparticle and Characterization*

- *UV-Vis Spectroscopy and Band Gap Analysis:*

A UV-Vis spectrophotometer was used to record the absorption spectrum of the synthesized ZnO nanoparticles. The spectrum (Fig.1) shows a peak within the range of 200nm - 300nm. This peak is attributed to the intrinsic band gap absorption of ZnO nanoparticles, not surface plasmon resonance (SPR). SPR typically occurs in metal nanoparticles, not metal oxides like ZnO.[31]

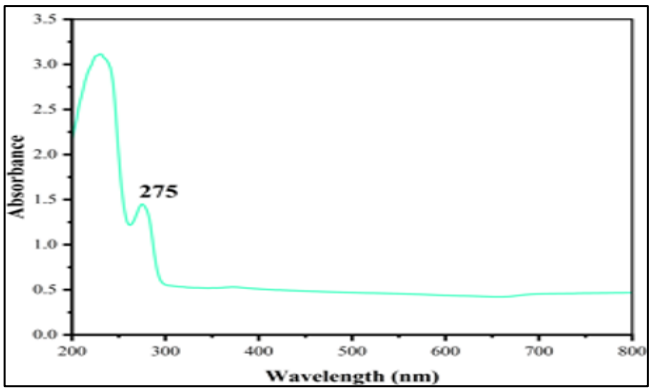


Fig 1 UV Spectroscopy

- *Fourier Transform Infrared (FTIR) Spectroscopy:*

The FTIR analysis of the ZnO nanoparticles synthesized using *Caesalpinia bonducella* seed extract was performed to identify the functional groups present on the nanoparticle surface and their corresponding transmittance peaks.[32] The FTIR spectrum (Fig.2) revealed three distinct transmittance maxima at 1400.69 cm^{-1}, 1032.53 cm^{-1}, and 866.99 cm^{-1}. These peaks can be attributed to the following functional groups: The **peak at 1400.69 cm^{-1}:** This peak likely corresponds to amide-I stretching vibrations, indicating the presence of organic molecules (possibly from the seed extract) adsorbed onto the ZnO nanoparticle surface. Amide-I stretches involve the C=O bond and the C-N bond in organic molecules. **The peak at 1032.53 cm^{-1}** could be assigned to C-O stretching vibrations of secondary alcohols or carbohydrates potentially originating from the seed extract. **The peak at 866.99 cm^{-1}:** The broad peak at 866.99 cm^{-1} might be due to overlapping contributions from in-plane bending vibrations of aromatic C-H bonds or alkene (C=C) stretching.

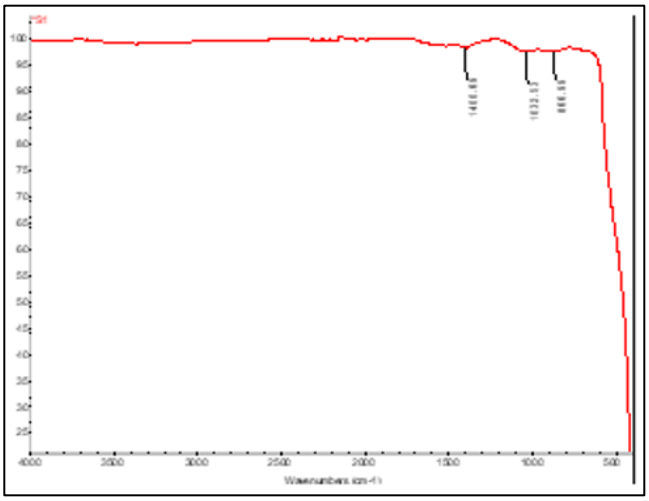


Fig 2 FTIR

- *X-ray Diffraction (XRD) Analysis:*

The crystal structure and phase identification of the synthesized ZnO nanoparticles were determined using X-ray diffraction analysis.[33] The obtained XRD pattern (Fig.3) was compared with the reference pattern for ZnO (JCPDS – 01-070-8072 card number). The XRD pattern exhibited eleven distinct peaks at 2θ values of [list the observed 2θ values for each peak, e.g., 31.8°, 34.4°, etc.]. These peaks can be indexed to the following Miller indices planes in the hexagonal wurtzite structure of ZnO: (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202). The good agreement between the observed peaks and the standard JCPDS data confirms the successful synthesis of crystalline ZnO nanoparticles.

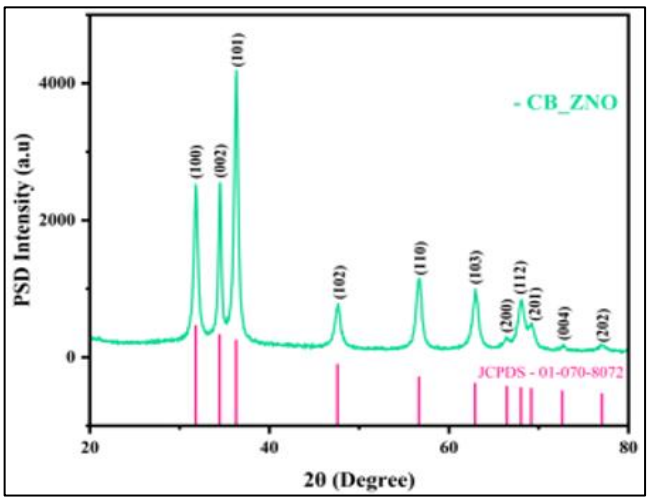


Fig 3 XRD

- *Zeta Potential Analysis:*

The surface charge of the ZnO nanoparticles was determined using zeta potential analysis. As shown in Fig.4, the nanoparticles exhibited a negative surface charge with a maximum value of approximately -91.4 mV. This negative charge arises from the presence of capping molecules adsorbed onto the nanoparticle surface. The electrostatic repulsion between the negatively charged particles contributes to enhanced stability in suspension by preventing aggregation.[34] The observed variability in particle size might be attributed to other factors during the synthesis process.

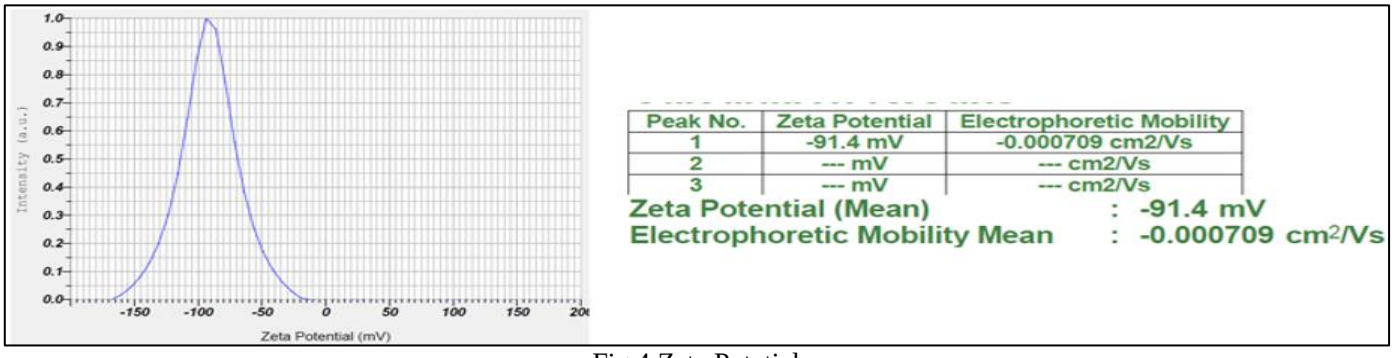


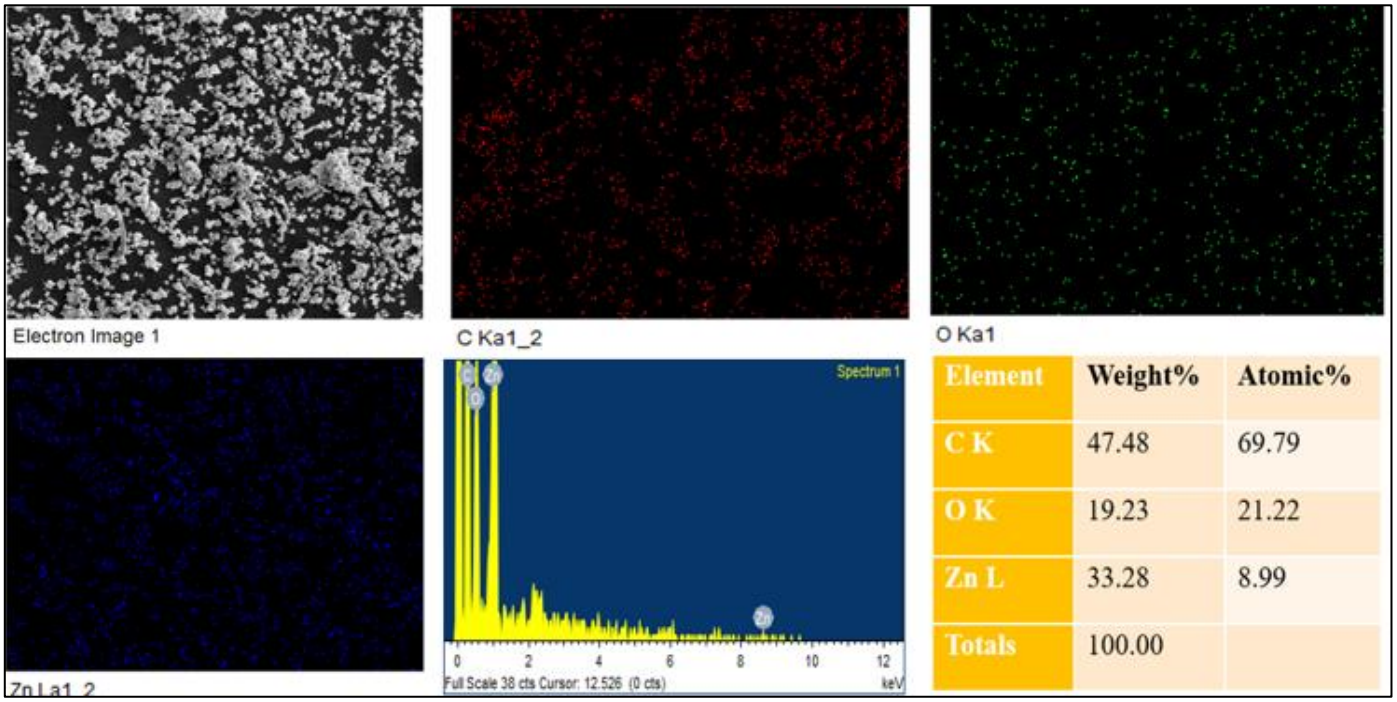
Fig 4 Zeta Potetial

- *Scanning Electron Microscopy (SEM) Analysis:*

The morphology and size of the synthesized ZnO nanoparticles were investigated using scanning electron microscopy. Fig.5 shows the SEM image, revealing that the ZnO nanoparticles are primarily spherical. While the text mentioned a high aspect ratio for some ZnO nanoparticles, the image in this case suggests a more equidimensional (spherical) morphology. The observed tendency for aggregation after annealing at 400°C can be attributed to strong inter-particle forces like van der Waals forces and electromagnetic interactions between the ZnO nanoparticles.[35]

- *Energy-dispersive X-ray spectroscopy (EDX) analysis:*

EDX analysis was performed (Fig.5) to identify the elemental composition of the ZnO nanoparticles. The EDX spectrum confirms the presence of zinc and oxygen, the primary elements for ZnO.[36] The presence of a small carbon peak might be due to residual organic compounds from the synthesis process or surface contamination. Overall, the EDX analysis supports the formation of ZnO nanoparticles.



Element	Weight%	Atomic%
C K	47.48	69.79
O K	19.23	21.22
Zn L	33.28	8.99
Totals	100.00	

Fig 5 Characterization of ZnO NP bySEM

➢ *Toxicity Evaluation by Artemia Mortality Test for ZnO Nanoparticles*

In all treatment groups (including the control), a significant number of nauplii (>50%) survived after both 24h and 48h of exposure. This suggests that the green-synthesized ZnO nanoparticles exhibit low toxicity toward *Artemia salina* at the tested concentrations as shown in Fig. 6.

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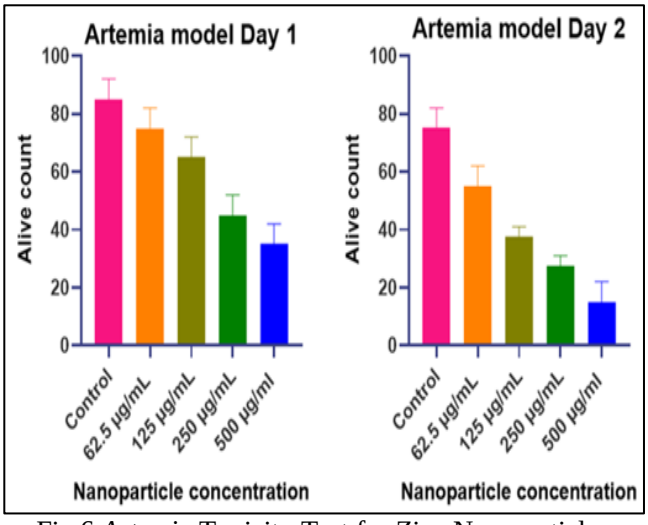

Fig 6 *Artemia* Toxicity Test for Zinc Nanoparticles

➢ *Nano Priming – Vigna mungo Seed*

Nano priming was used to assess ZnO nanoparticle's effect on seed germination by supplying essential nutrients and growth factors. Over a week, germinated seeds were counted, and root and shoot lengths were measured on the final day. The results revealed that ZnO nanoparticle priming significantly enhanced seed germination and vigor index when compared to the control. The highest germination percentage (63.3%) and vigor index (810.24) were observed at 65.5 µg/mL, demonstrating the favorable influence of lower doses. Additionally, germination energy, mean daily germination, peak value, and germination value all peaked at this concentration, showcasing the efficiency of ZnO nanoparticles in boosting seedling growth (Fig. 7). These findings underscore the potential of ZnO nanoparticle priming to enhance seed performance and sustainably improve agricultural yield.

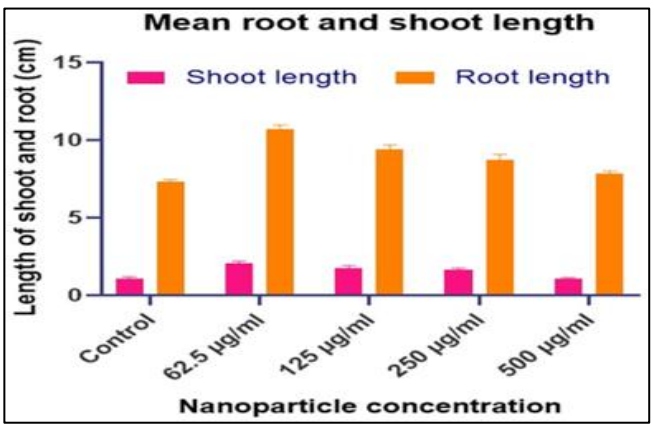

Fig 7 Nano Priming *Vigna mungo Seed*

Table 1 Physiological Parameters of *Vigna mungo* Seed

Parameters	Control	65.5µg/ml	125µg/ml	250µg/ml	500µg/ml
Germination Percentage	36.6	63.3	56.6	50	43.3
Germination Energy	0.36	0.63	0.56	0.50	0.43
Mean Daily Germination	5.22	9.04	8.08	7.14	6.1
Peak Value	0.57	1.42	1.14	0.85	0.71
Germination Value	2.97	12.83	9.21	6.06	4.33
SVI	308.53	810.24	633.92	518.5	384.93

In the *Artemia* toxicity test for zinc nanoparticles, lower concentrations (62.5 and 125 µg/mL) resulted in greater survival rates. It indicates that the produced nanoparticles have minimal harmful effects on *Artemia* at these dosages.

Nano priming *Vigna mungo* seeds with zinc nanoparticles at 62.5 µg/mL resulted in significantly longer shoot and root lengths than other doses, indicating optimal seedling growth.

IV. DISCUSSION

Caesalpinia bonducella extract was used to synthesize ZnO nanoparticles, which naturally stabilizes the nanoparticle production.[37] The extract's bioactive components possess antioxidant, anti-inflammatory, antibacterial, and antidiabetic effects. This plant has traditionally been used in herbal therapy to treat fevers, asthma, and gastrointestinal disorders. [38] This study used *Caesalpinia bonducella* seed extract to reduce metal ions and produce nanoparticles. ZnO nanoparticles made from *Grewia flavescen* have strong antibacterial action, and the synthesis of nanoparticles using leaf extract has already been reported.[39] Aqueous Leaf Extract of *Aquilegia pubiflora* is utilized for the synthesis of ZnO nanoparticles.[40] *Eucalyptus globules* Leaf is used to synthesize ZnO nanoparticles with significant antifungal activity.[41] The synthesized nanoparticles were characterized with various techniques, primarily UV spectroscopy, which evaluates the interaction between nanoparticles and light to disclose features like band gap energies and optical behavior.[42] In the case of synthesized ZnO nanoparticles, the spectrum shows a peak between 200 nm and 300 nm, indicating intrinsic band gap absorption. In comparison, the studies have shown absorption peaks in the 300-600 nm range, including a significant peak at 340 nm, which confirms the synthesis of ZnO nanoparticles.[43] In contrast, the ZnO nanoparticle's absorption peak at 370 nm was reported in a study.[44] This distinction reflects the optical characteristics of ZnO nanostructures. With the presence of nanomaterial characterized by UV spectroscopy, the functional group identification was carried out by FTIR spectroscopy analysis, which measures the infrared radiation absorption by the sample, and plotted against the wavelength.[45] Correlating the absorption band or vibration band with the chemical compounds of the sample, it is possible to identify the biomolecules found in the *Caesalpinia bonducella* seed extract which are involved in the reduction phase of ZnO nanoparticles formation and confirm the functional group.[46] This study the resultant spectrum with different absorption peaks with insights that include 1400.69 cm^{-1}(C=O stretching and C-N bond), 1032.53 cm^{-1}(amino acid C-O stretching), and 866.99 cm^{-1} (C-H bonds). These inferences were aligned very similarly with the various reported studies, such as FT-IR spectra of other findings which have reported the C-N stretch at 1396 cm^{-1}, C-O stretching at 1037$cm^{-1,}$ and C-H bending at 819 cm^{-1} with no significant difference in peaks.[46] Similar absorbance peaks from different studies occur to show a consistent pattern, which validates the presence of ZnO as a functional group.[47] [48]

X-ray diffraction data shows ZnO nanoparticles have a crystalline structure with discrete lattice plane peaks. The crystalline character of the synthesized ZnO nanoparticles is confirmed by the analysis, which shows that they align well with the conventional lattice planes. The (100), (002), and (101) planes specifically match the measured peaks, showing the wurtzite hexagonal phase of ZnO. The uniformity of the diffraction pattern confirms the ZnO nanoparticles and validates the lattice constants by confirming the crystalline structure from different research highlighted the structural integrity of ZnO nanoparticles.[49] According to a subsequent study, characterization by zeta potential is essential for comprehending and managing the activities of nanoparticles.[49] ZnO nanoparticles' substantial negative surface charge of -91.4 mV was found by measuring their zeta potential, suggesting that they had strong electrostatic stability in suspension. The significant negative charge ensures the nanoparticle's stability in solution by preventing aggregation and encouraging dispersion. Adsorbed molecules significantly improve ZnO nanoparticles' electrostatic stability and performance in a variety of applications.[50] The zeta potential analysis highlights the stability and practical efficacy of ZnO nanoparticles by showcasing their significant negative surface charge.

The ZnO nanoparticles in this study were spherical, with some aggregation resulting from strong inter-particle interactions, according to the SEM analysis. Furthermore, the composition of the synthesized ZnO nanoparticles was validated by EDX analysis, which also showed the presence of oxygen and zinc. However, ZnO nanoparticles with hexagonal shapes and diameters ranging from 11 to 25 nm were reported. [51], indicating that distinct production techniques affect ZnO nanoparticle sizes and morphologies. Similarly, studies have observed that the synthesis of ZnO nanoparticles was supported by the presence of zinc and oxygen in their sample as revealed by EDX elemental mapping.[52] The synthesis method's efficacy observed the spherical morphology and confirmed the elemental composition. Following the confirmation of ZnO nanoparticles, the

study proceeds with a lethality assay to support nano priming. Several toxicity assays for nanoparticles were developed in recent years with different model organisms such as bacteria, zebrafish embryos, daphnia, and rainbow trout. [53] [54] [55] [56] *Artemia salina* was chosen as the test species for the current study due to its standard test principles, rapidity, and convenience. Also, in recent times different studies on metal and metal oxide nanoparticles have undergone toxicity testing with *Artemia salina.*[57]

Different concentrations were tested on the target species to determine the possible toxicity of ZnO nanoparticles. The findings revealed that at lower concentrations, the species survived under standard environments, with negligible toxicity compared to conventional metal and metal oxide nanoparticles. At the lowest concentration of 62.5 µg/mL, *Artemia* showed higher survival rates than other concentrations.

A similar ecotoxicology study has reported an LC50 of 24 µg/mL, which is much less than the value found in this research.[58] LC50 values of 100 µg/mL, 89 µg/mL, and 86 µg/mL [59] [60] [61] have also been reported which are consistently lower than this study. Variations in the size and shape of nanoparticles can be related to these differences in LC50 values, as these factors have a substantial impact on the levels of toxicity. According to this study, green synthesized ZnO nanoparticles are less harmful than those found in earlier research, probably because of variations in the nanoparticle's properties.

This finding demonstrates the potential of ZnO nanoparticles as a viable and efficient priming agent for improving *Vigna mungo* seed germination and seedling vigor. ZnO nanoparticle treatment shows significant growth in the length of the root and stem. Various nanoparticles derived from different green synthesis methods have a great influence on plant growth. Different green synthesis methods yield different nanoparticles, each of which has a significant impact on plant growth. Zinc is a crucial element essential for several physiological and biochemical processes necessary for seed development and early plant growth. Root length, shoot length, and total yield are among the important plant growth characteristics that zinc nanoparticles have been reported to significantly improve.[62] The cluster bean (*Cyamopsis tetragonoloba*) showed a notable increase in shoot and root growth, indicating that the biologically produced zinc nanoparticles proved to be very beneficial for plant nutrition.[63] Furthermore, as shown by the increased yields and grain quality seen in field crops like wheat, numerous nanoparticles have proven their capacity to improve nutritional value through nanoparticle priming.[64]

After imbibition, zinc ions in water diffuse passively through the micropyle, allowing them to enter the seed coat and the interior seed tissues. These ions are essential for activating zinc-dependent enzymes such as amylases and proteases. Amylase activity catalyzes the hydrolysis of stored starch into glucose, generating metabolic energy.[65] In contrast, proteases break down reserve proteins into amino acids, which are required for protein synthesis and cellular growth. This coordinated enzymatic activity accounts for the increased germination efficiency reported in *Vigna mungo* seeds.[66] Zinc ions thus play an important role in modifying enzymatic activity, acting as a cofactor to improve the catalytic effectiveness of amylases and proteases, ultimately driving the biochemical processes required for optimal germination in *Vigna mungo* seeds.

Zinc nanoparticles play an important function in seed germination because their nanoscale size increases bioavailability and allows for efficient penetration through the micropyle and seed coat. Their large surface area allows for controlled zinc ion release, resulting in sustained activation of zinc-dependent enzymes including amylases and proteases. This focused contact enhances nutrient mobilization and biochemical activities required for germination. Zinc absorption and seedling growth can both be significantly improved by ZnO nanoparticles.[67] ZnO synthesized from *Aloe barbadensis miller* leaf extract promotes the growth of wheat, rice, chili, mung bean, and red gram.[68] The *Ocimum* leaf extract was used to create silver and copper nanoparticles, which helps green gram seed germination.[69] An onion extract was utilized to create silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs), which are used to prime-aged onion seeds.[70]

Zinc oxide nanoparticles boost zinc uptake while also increasing the shoot and root length, plant biomass, germination, antioxidant enzyme activity[71], and photosynthetic mechanism, which also improves the ultracellular structure of cell organelles and the stability of the photosynthetic mechanism.[72] These results demonstrate the potential of ZnO nanoparticles as a viable and efficient priming agent for improving *Vigna mungo* seed germination and seedling vigor. At lower concentrations, both seedling vigor index and germination rates improved significantly, demonstrating that ZnO nanoparticles can play an important role in boosting agricultural production. As a result, the study reveals that lower concentrations produce favourable results, proving the safety and potential of using nanomaterials in agricultural techniques.

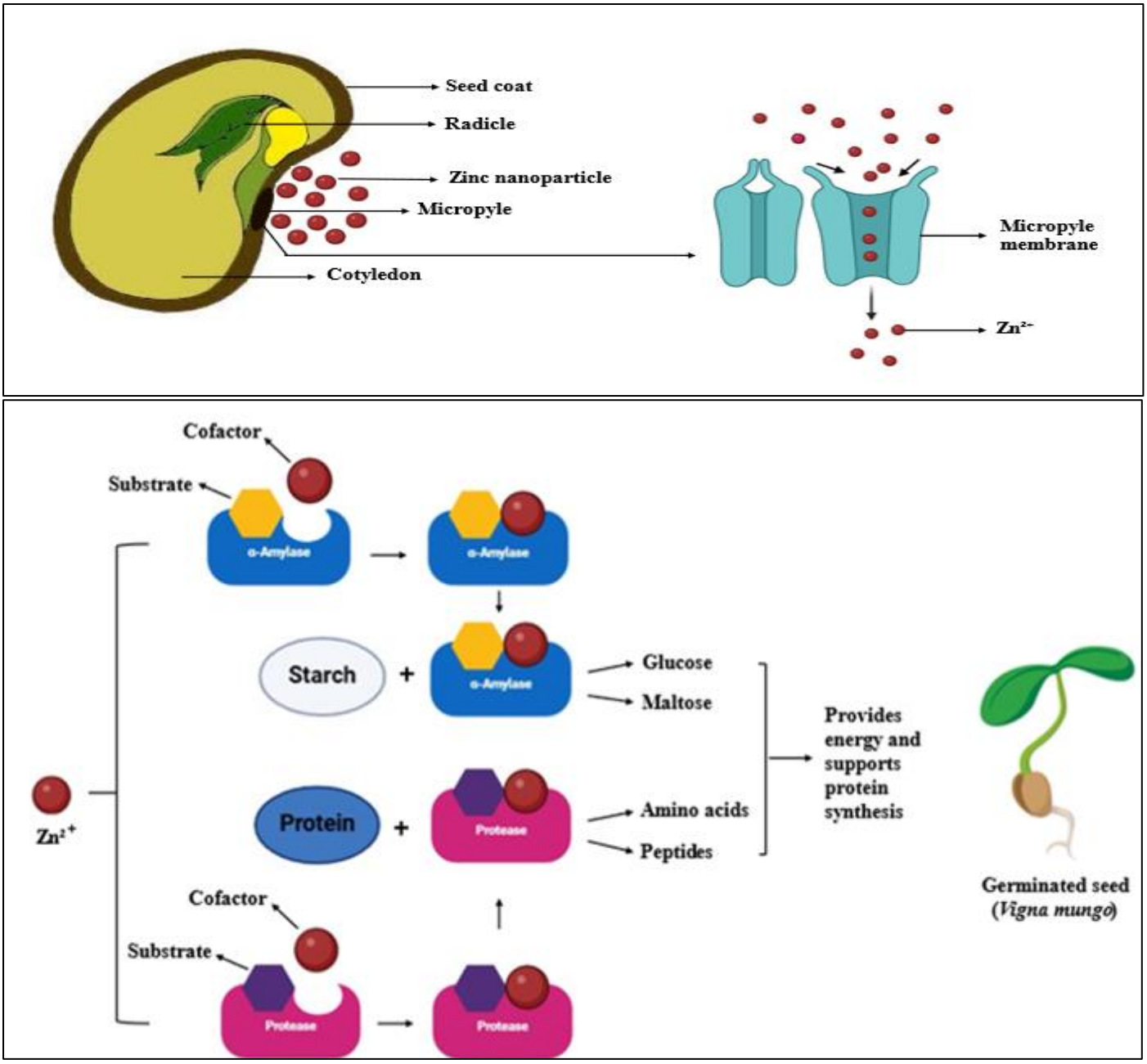

Fig 8 Diagrammatic View of Seed Germination Utilizing ZnO NP

Zinc nanoparticles (ZnO NPs) are taken into the seed via the micropyle during imbibition. The micropyle acts as a passageway for nanoparticles, allowing them to enter the seed coat. A closer cellular view reveals the passage of zinc ions (Zn^{2+}) through micropyle membrane channels, allowing their internalization into seed structure. Zinc ions activate essential hydrolytic enzymes such as α-amylase and protease. α-Amylase converts starch to glucose and maltose, while protease degrades proteins into amino acids and peptides. These products provide energy and critical building blocks, hence promoting protein synthesis and overall seed metabolism. The coordinated biochemical actions improve seed germination, vigor, and early seedling growth in *Vigna mungo*.

V. CONCLUSION

The green facile synthesis method has been used to produce Zinc oxide nanoparticles from *Caesalpinia bonducella* seed extract which was then characterized with different techniques and methods to analyze the structural and optical integrity. After the confirmation, ZnO nanoparticles underwent a toxicity assay with *Artemia salina* as the test species, the assay concluded that ZnO nanoparticles have an acceptable low toxicity. Further, the nano priming study indicates the ZnO nanoparticle capability on significant promotion of seed germination and seedling growth vigor on *Vigna mungo* seeds. This study provides a comprehensive view of the phenotypic alteration of seeds by ZnO nanoparticles, and more research and understanding of physical, metabolic, and genomic level alteration mechanisms on seeds may reveal the potentiality of ZnO nanoparticles as a "Sustainable tool in modern agriculture".

- *Credit Authorship Contribution Statement*

Study conception and design - Doveit Antony Charles, Ramkumar Katturajan; material preparation, data collection, analysis, and writing the first draft of the manuscript - Gokul Kumar, Suriya Velu, Nafisa

Thabassum Nisar. Approval of the final manuscript - Sabina Evan Prince.

- *Declaration of Competing Interest*

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- *Data Availability*

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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