Green-Synthesized Silver Nanoparticles as a Sustainable Seed Priming Agent for Improved Rice Cultivation

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ABSTRACT

This research investigates the effects of silver nanoparticle (AgNP) priming, achieved through both green and chemical synthesis, on the germination and early growth of rice seeds in Bangladesh. Green AgNPs, synthesized using neem extract, showed smaller particle sizes (21.19 nm) and better performance than chemically synthesized AgNPs (27.238 nm). Seeds primed with AgNPs (1–200 mg/L) exhibited enhanced root and shoot growth, with optimal results at 70–160 mg/L. Germination remained high (95–100%) in both treatments, indicating low toxicity. The improved efficacy of green AgNPs is attributed to the presence of neem's bioactive phytochemicals, which also contribute to their environmentally benign synthesis, avoiding hazardous chemicals and minimizing ecological impact. Findings suggest nanopriming as a cost-effective, scalable technique for improving rice productivity. Further field validation and safety assessments are recommended to support their integration into eco-friendly agricultural practices.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a vital food crop for over half the global population and remains the cornerstone of food and economic security in Bangladesh. However, productivity continues to be challenged by multiple abiotic and biotic stresses, including drought, poor seed vigor, and climate variability. Early seedling establishment, particularly germination success, is crucial for achieving optimal plant density and yield potential in the field.

Seed priming is a proven pre-sowing strategy to improve germination and seedling vigor by modulating metabolic and physiological processes. Nanopriming using nanoparticles (NPs) in priming solutions has emerged as a powerful enhancement of traditional priming, improving water absorption, enzymatic activation, and redox balance [1], while reducing microbial contamination risks [2], [3], [4]. Among various NPs, silver nanoparticles (AgNPs) are particularly promising due to their unique physicochemical and antimicrobial properties, which positively influence germination and early growth [5], [6].

Chemically synthesized AgNPs, such as those produced via sodium borohydride reduction, are effective but may raise cytotoxicity and environmental safety concerns [7], [8]. In contrast, green synthesis using plant extracts offers an eco-friendly, biocompatible approach, leveraging natural reducing and stabilizing agents such as polyphenols and flavonoids [9], [10]. *Azadirachta indica* (neem) is frequently used in green synthesis due to its phytochemical richness and widespread availability [10].

Although increasing interest in AgNPs for agricultural use, comparative studies on green versus chemically synthesized AgNPs in rice seed priming especially for high-yielding Bangladeshi cultivars are still scarce. This study addresses that gap by assessing the effects of both synthesis methods on rice seed germination and early seedling growth, promoting a safer and more sustainable nanotechnology-based approach to rice production in Bangladesh.

2. METHODOLOGY

2.1 Synthesis of Silver Nanoparticles (AgNPs)

AgNPs were synthesized via two different methods: a plant-mediated green approach and a conventional chemical route.

2.1.1 Green Synthesis of AgNPs

Azadirachta indica (neem) was selected for the preparation of green-synthesized AgNPs, due to its high phytochemical content of flavonoids, terpenoids, and alkaloids, which act as both reducing and stabilizing agents. These compounds not only reduce silver ions to form nanoparticles but also provide a dual stabilization mechanism, both electrostatic

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and steric, that helps keep the nanoparticles dispersed and prevents their agglomeration.

The green synthesis process is illustrated in Fig. 1, where fresh leaves of neem were rinsed thoroughly with distilled water and air-dried. A total of 25 grams of leaves were boiled in 100 mL of deionized water for 15 minutes to extract bioactive compounds. After filtration, this extract served as a natural reducing and capping agent. It was added dropwise to a 1 mM aqueous solution of silver nitrate ($AgNO_3$) under continuous stirring at ambient temperature. The progression of the reaction was visually confirmed by a color transition from pale yellow to brown, indicating AgNP formation.

(a) (b) (c) (d)

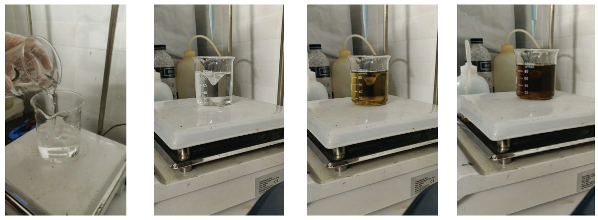
Fig. 1: (a) $AgNO_3$ Solution (b) Adding Neem Extract to the $AgNO_3$ Solution Drop Wise. (c) AgNP Solution after 1 Hour of Stirring (d) AgNP Solution after 24 Hours of Cold Storage

2.1.2 Chemical Synthesis of AgNPs

In chemical synthesis, Trisodium citrate was initially tested as a reducing agent but failed to produce stable AgNPs, causing aggregation. Sodium borohydride ($NaBH_4$) was then chosen for its high reduction efficiency, producing small, uniform, and stable nanoparticles.

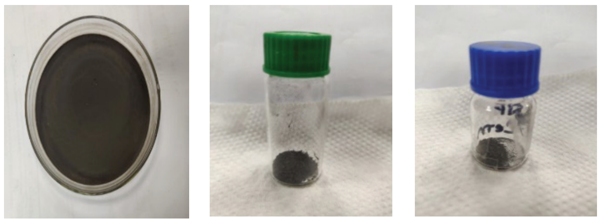
The chemical synthesis process is illustrated in Fig. 2, where a 1 mM $AgNO_3$ solution was prepared and trisodium citrate ($Na_3C_6H_5O_7$) solution was added to the silver nitrate ($AgNO_3$) solution. The $NaBH_4$ solution was then slowly added dropwise to the $AgNO_3$ solution, where the solution was initially clear. After 1 mL of $NaBH_4$ was added, the solution was observed to turn pale yellow, indicating the formation of AgNPs. Finally, after 3 mL of $NaBH_4$ was added, the solution turned dark brown, confirming the complete reduction of silver ions to metallic silver and the formation of stable nanoparticles. Trisodium citrate was used as a stabilizing agent in the chemical route to prevent particle agglomeration [8].

After the synthesis procedure, centrifugation was performed to separate nanoparticles from the liquid phase shown Fig. 1(d) & Fig. 2(d). The nanoparticles were then transferred to a petri dish displayed Fig. 3 (a) and air-dried. Once dried, AgNPs from both green and chemical synthesis were obtained in powder form, as shown in Fig. 3. The green-synthesized AgNPs of Fig. 3(b) are finer and more uniform due to neem-based stabilization, while the chemically synthesized AgNPs of Fig. 3(c) show more aggregation, highlighting the superior stability of the green-synthesized nanoparticles. Due to the unclear nature of these figures, higher-magnification FE-SEM images are provided later for a clearer illustration of the physical properties and differences between the two types of nanoparticles.



(a) (b) (c) (d)

Fig. 2: (a) Adding $Na_3C_6H_5O_7$ Solution to $AgNO_3$ Solution (b) Before Adding $NaBH_4$ (c) Forming AgNP as Turned Pale Yellow after adding 1ml of $NaBH_4$ (d) AgNP Solution Turned into Dark Brown after Adding 3ml of $NaBH_4$



(a) (b) (c)

Fig. 3: (a) AgNPs after Drying (b) Green Synthesized AgNP Powder form (c) Chemical Synthesized AgNP Powder form

2.2 Materials and Reagents

In this study, a variety of chemical reagents, biological materials, and seeds were employed to synthesize and evaluate AgNPs and their effects on rice seed priming. Table 1 provides a comprehensive overview of the raw materials used, including their chemical names.

2.3 Characterization of AgNPs

Optical characteristics and the presence of surface plasmon resonance were analyzed through UV–Visible spectrophotometry. Additionally, Field Emission Scanning Electron Microscopy (FE-SEM) was utilized to evaluate the morphology and estimate particle size distribution. Fourier Transform Infrared Spectroscopy (FTIR) analysis was used to identify the functional groups involved in the reduction and stabilization of AgNPs, confirming the presence of biomolecules or capping agents on their surface.

Table I: Materials and Reagents

Material	CAS Number	Supplier	Country
Azadirachta indica (neem) leaves	-	Local Source	Bangladesh
Silver Nitrate ($AgNO_3$)	7761-88-8	Sigma-Aldrich	USA
Sodium Borohydride ($NaBH_4$)	16940-66-2	Sigma-Aldrich	USA
Trisodium Citrate ($Na_3C_6H_5O_7$)	6132-04-3	Sigma-Aldrich	USA
Deionized Water (DI Water)	-	Labtex	Bangladesh
BRRI-88 Rice Seeds	-	BRRI	Bangladesh

2.4 Seed Priming Procedure

BRRI-88 rice seeds (certified) were used for the study. Various concentrations of AgNPs, ranging from 1 to 200 mg/L, were prepared by diluting the synthesized particles in deionized water. Every 30 rice seeds were soaked in these AgNP solutions for 12 hours, as shown in Fig. 4, at ambient temperature with mild agitation to ensure even treatment. The control treatment consisted of water-only hydropriming, where an equivalent number of seeds were soaked in deionized water for the same duration. After soaking, the seeds were dried under aseptic conditions on Whatman No. 1 filter paper, which has a pore size of 11 µm, and stored in paper bags at room temperature until further use.

Fig. 4: Rice Seed Priming using Different Concentrations of AgNPs and Control Treatments

2.5 Germination Bioassay

After soaking the AgNP-primed seeds, they were transferred into sterile Petri dishes lined with two layers of Whatman No. 1 filter paper (pore size 11 µm), which were moistened with approximately 15–20 mL of normal water so that the seeds were submerged under the water to maintain proper moisture for seed germination. Each dish received thirty seeds, and all treatments were replicated three times. The dishes were incubated in a controlled growth chamber at 25 ± 1°C under a 16-hour light and 8-hour dark photoperiod. Germination was recorded daily for a period of seven days, with seeds considered germinated when the radicle extended at least 2 mm, is illustrated in Fig. 5.

On the final day, both root and shoot lengths were measured from scanned image of Fig. 5(b) captured by microscope using ImageJ software which is shown in Fig. 5(d). To measure the root and shoot length, a scale reference is required, which was also captured at the same height as the seed images using a microscope as shown in Fig. 5(c). Germination rates were calculated, and seedling vigor indices were computed using standard equations based on shoot and root measurements. Statistical analysis was performed to identify significant treatment effects.

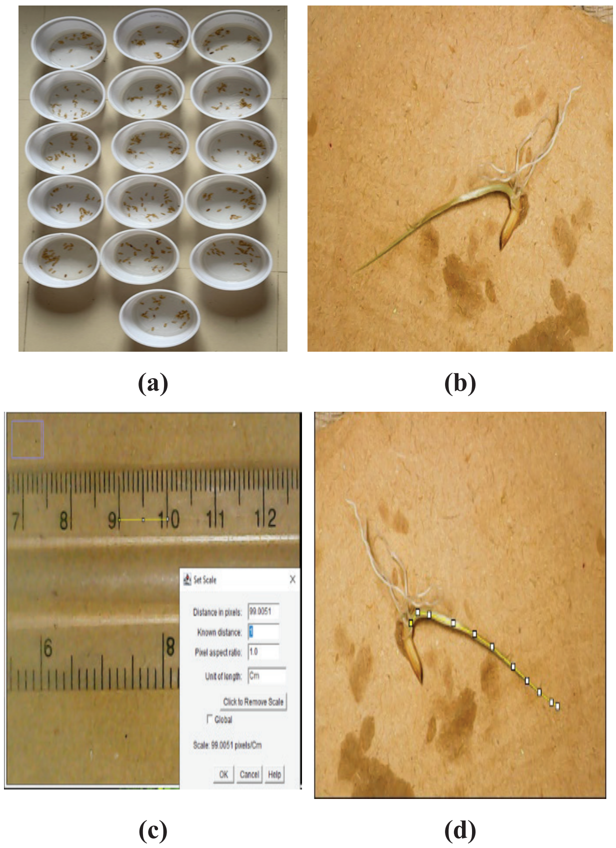


Fig. 5: (a) Performing Germination in Normal Water; (b) Images Captured by a Microscope for Measuring Root and Shoot Lengths ; (c) Scale set-up in Image J (d) Root-Shoot Length Measuring using Image J Software

3. LITERATURE SURVEY

3.1 Rice Production and Climate Stress in Bangladesh

Rice cultivation is the backbone of Bangladesh's agriculture. The widespread adoption of high-yielding modern rice varieties has contributed significantly to national food security [1]. However, the sector faces increasing threats from climate change, including rising temperatures, water scarcity, and erratic weather patterns [3]. These factors, especially during the dry Boro season, result in elevated irrigation demands and reduced productivity [3].

3.2 Nanoparticle Synthesis Approaches

Nanoparticles can be synthesized via top-down or bottom-up methods. The top-down approach (e.g., milling, etching, ablation) breaks bulk materials into nanoparticles but often involves high energy use and toxic byproducts. In contrast, bottom-up methods (e.g., precipitation, sol-gel, pyrolysis) assemble particles atom-by-atom, offering better control over size and morphology, though some processes still involve hazardous chemicals.

The green synthesis approach used in this research follows the bottom-up method shown in Fig. 6. Specifically, it employs biological agents like plant extracts (in this case, *Azadirachta indica*, or neem) to reduce metal ions and form nanoparticles. This method is eco-friendly, non-toxic, and cost-effective, making it highly suitable for sustainable agricultural practices, such as seed priming[11].

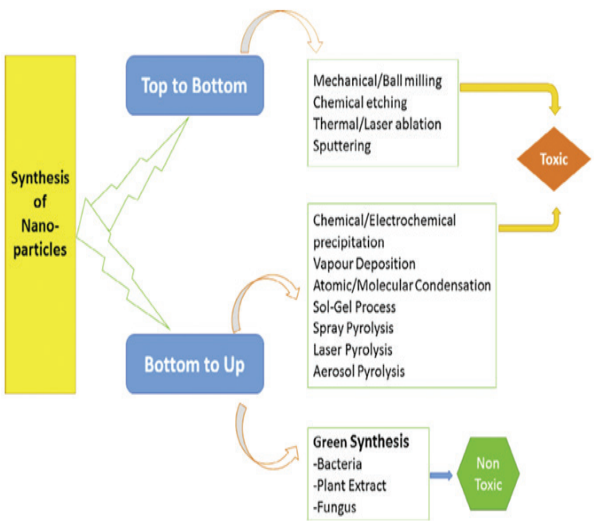


Fig. 6: Different Approaches to the Synthesis of Silver Nanoparticles [11]

3.3 Effect of AgNP Priming on Germination and Root Shoot Growth

Seed priming is a pre-sowing method that activates metabolic processes through controlled hydration, improving seed vigor and stress tolerance. Hydropriming and osmopriming are common in rice and enhance germination, seedling growth, and biochemical activity [1], [12]. However, their effectiveness can be inconsistent under stress. Nano-priming, using nanoparticles like AgNPs and ZnO, offers a more efficient alternative by improving water uptake, enzyme activation, and antioxidant responses [1], [3], [5], [13]. This technique shows great potential for enhancing rice performance under challenging environmental conditions.

Many studies on seed priming, such as those by W. Mahakham [14], have recorded germination over a period of six days and it is shown in Fig. 7. In contrast, the present study monitored germination daily for seven days, as this duration provided sufficient time for seedling development and vigor assessment. While previous studies have attempted seed priming in rice, our research distinguishes itself by comparing green-synthesized AgNPs with chemically synthesized AgNPs in rice seed priming. This comparison, particularly using *Azadirachta indica* (neem) as the source for green synthesis, has not been fully explored in agricultural practices for rice. Our research identifies a specific AgNP concentration range, that optimally promotes root and shoot length in rice seedlings.

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3.4 Prospects and Challenges

Green synthesis methods, such as using *Azadirachta indica*, provide safer, eco-friendly alternatives to chemical routes [9], [10]. However, challenges persist, including nanoparticle toxicity, inconsistent results due to variable NP size and concentration, and limited understanding of long-term environmental effects [2], [7], [8]. In addition, future studies could explore multiple rice varieties to compare the effects of AgNP priming across different cultivars.

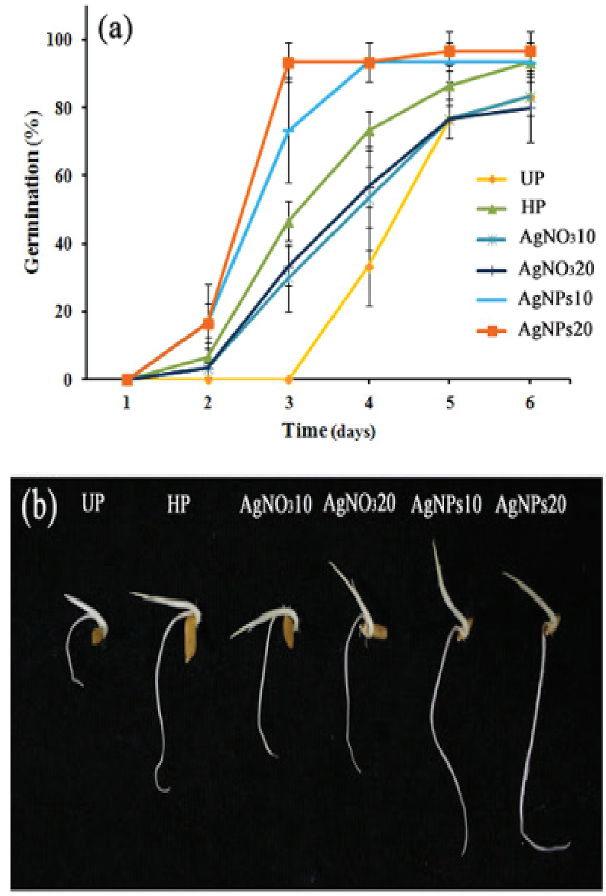


Fig. 7: (a) Germination Rate of Rice Seeds after Priming with Different Priming Agents and Phenotype of Rice Seedlings after 6-Day Germination (B) Data are Presented as Means of three Replicates Containing 10 Seeds Each ± Standard Error of Means [13]

4. RESULTS AND DISCUSSIONS

4.1 Functional Group Analysis Using FTIR

FTIR analysis revealed distinct functional groups responsible for nanoparticle synthesis and stabilization. The FTIR spectrum of green-synthesized AgNPs shows a strong peak between 3430–3600 cm^{-1} for O–H stretching (alcohols and phenols), a weaker peak at 1595 cm^{-1} for N–H stretching (aromatic secondary amines), and a faint band near 1450 cm^{-1} for C=O stretching (likely from flavonoids, aiding stabilization) shown in Fig. 8.

In contrast, the FTIR spectrum of chemically synthesized AgNPs shows a sharp band at 1585 cm^{-1}, indicating C–C aromatic stretching and N–H bending (primary amines) [15]. This suggests less effective interaction between sodium citrate and Ag^+ ions, possibly due to changes in citrate's structure during or after nanoparticle formation [8].

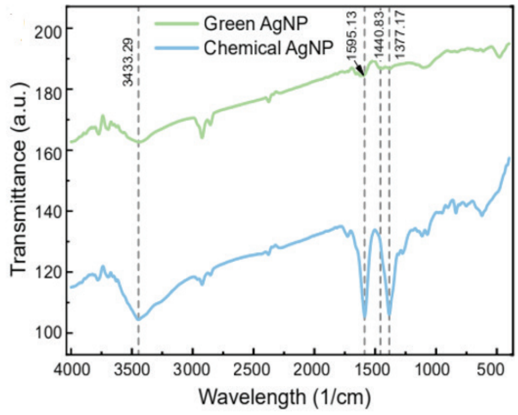


Fig. 8: FT-IR Transmittance Spectra of Green Synthesized AgNP & Chemically Synthesized AgNP

4.2 Optical Properties of AgNPs

UV-Visible spectroscopy was employed to investigate the optical behavior of AgNPs.

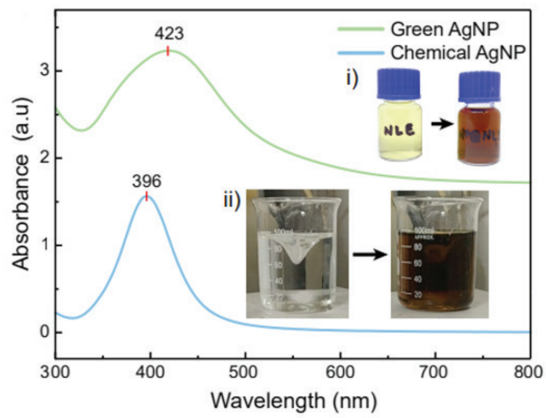
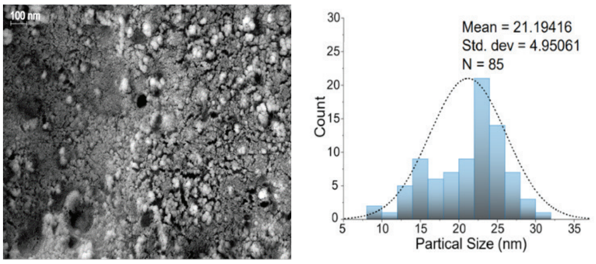


Fig. 9: UV-Vis Absorption Spectra of Green Synthesized AgNP & Chemically Synthesized AgNP

AgNPs were synthesized using both green (neem extract) and chemical (silver nitrate and sodium citrate) methods. The resulting solution underwent a color change from clear to dark brown, indicating successful nanoparticle formation Tests using UV-Vis spectroscopy confirmed that nanoparticles were successfully created in both methods. The green method showed a peak at 423 nm, and the chemical method showed a peak at 396 nm both peaks are signs that the AgNPs formed correctly shown in Fig. 9. Since there were no extra peaks, it also means there were no unwanted substances in the mixtures. However, the peak for the green method was broader, suggesting that the particles were of different sizes. This is likely because the Neem extract has various natural chemicals and the reaction conditions weren't as controlled.

4.3 Particle Morphology (FE-SEM)

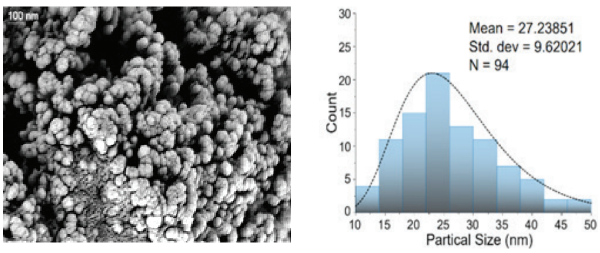
Field Emission Scanning Electron Microscopy (FE-SEM) highlighted clear morphological variations between the two synthesis approaches.



(a) (b)

Fig. 10: (a) FE-SEM Image of Green Synthesized AgNPs and (b) Particle Size Distribution for Green Synthesized AgNPs

Fig. 10 represents the Field Emission Scanning Electron Microscopy (FE-SEM) images of AgNPs synthesized through a green method. The analysis reveals that the particles display a diverse array of shapes and are irregularly distributed on the substrate. Measurements taken from 85 individual nanoparticles indicate an average size of 21.19 nm, with a standard deviation of 4.95 nm, emphasizing the structural heterogeneity among the particles.



(a) (b)

Fig. 11: (a) FE-SEM Image of Chemically Synthesized AgNPs and (b) Particle Size Distribution for Chemically Synthesized AgNPs

In contrast, Fig. 11 shows the FE-SEM images of AgNPs produced via chemical synthesis. This sample comprises a mix of both small and large particles, with an average size of 27.238 nm and a standard deviation of 9.62 nm, based on measurements of 94 nanoparticles. The chemically synthesized particles exhibit a tendency to form agglomerates, resulting in clusters that incorporate a range of particle sizes.

4.4 Effect of AgNPs on Seed Germination Rate

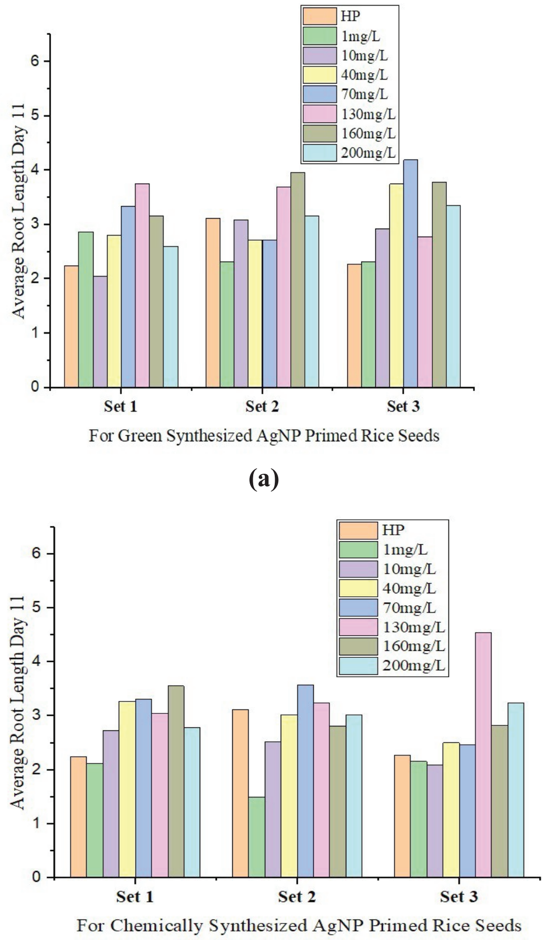
Although the germination percentage was measured for both AgNPs-primed and hydroprimed seeds, no significant difference was observed between the two treatments. Both AgNPs priming and hydropriming resulted in nearly identical germination rates of 95-100%, indicating that AgNPs priming does not negatively affect seed viability or germination.

4.5 Root and Shoot Growth Enhancement

Notable improvement in seedling root and shoot development was recorded following AgNP priming.

4.5.1 Effect of AgNPs on Root Length

The root length response to green-synthesized AgNPs showed variability across three experimental sets that represents in Fig. 12(a), with optimal growth at 130 mg/L in Set 1, 160 mg/L in Set 2, and 70 mg/L in Set 3. Despite these inconsistencies, the concentration range of 70–160 mg/L generally promoted better root growth, suggesting its potential for effective priming.



(b)

Fig. 12: (a) Impact of Green Synthesized AgNPs and (b) Chemically Synthesized AgNPs on the Root Length (in cm) of Rice Seeds

The results in Fig. 12(b) show that the effect of chemically synthesized AgNPs on root length varies across experimental sets, with Set 1 showing optimal growth at 160 mg/L, Set 2 at 70 mg/L, and Set 3 at 130 mg/L. This inconsistency suggests that the root length response to AgNPs is not dose-dependent or linear. Despite this, the concentration range of 70–160 mg/L appears most effective for promoting root growth. Based on the average results in Fig. 13, chemically synthesized AgNPs showed the highest root length at 130 mg/L, while green synthesized AgNPs reached optimal growth at 70 mg/L. Despite the differences in peak concentrations, both types of AgNPs were most effective within the 70–160 mg/L range, compared to other concentrations. The shoot length response to green synthesized AgNPs in Fig. 14(a) showed no consistent pattern across sets, with optimal concentrations of 130 mg/L, 160 mg/L, and 70 mg/L in Set 1, Set 2, and Set 3, respectively.

4.5.2 Effect of AgNPs on Shoot Length

Despite these variations, the 70–160 mg/L range generally yielded the best results, suggesting it is effective for enhancing shoot growth, though the optimal concentration varies between sets. The shoot length response to chemically synthesized AgNPs in Fig. 14(b) showed no consistent pattern across sets, with peak growth at 130 mg/L in Set 1 and Set 3, and at 200 mg/L in Set 2. However, the optimal shoot length generally fell within the 130–200 mg/L range, indicating this concentration range is most effective for promoting shoot growth.

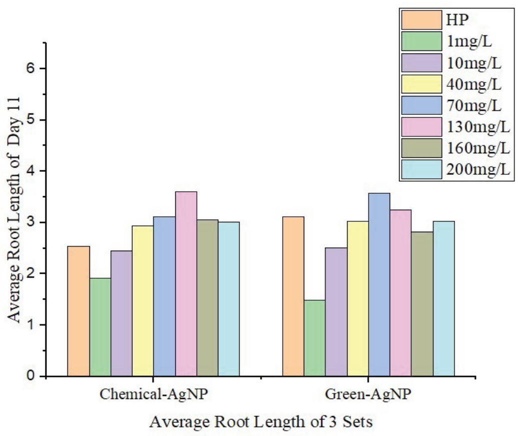


Fig. 13: Impact of Green-synthesized and Chemical- Synthesized AgNPs on the Average Root Length (in cm) of Rice Seeds

In Fig. 15, both chemically synthesized and green synthesized s AgNPs promoted optimal shoot elongation within the 70–200 mg/L range. Chemically synthesized AgNPs showed peak growth at 130 mg/L (130–200 mg/L range), while green synthesized AgNPs were most effective at 160 mg/L (70–160 mg/L range). These results suggest that the 70–200 mg/L range is optimal for enhancing shoot length while minimizing toxicity.

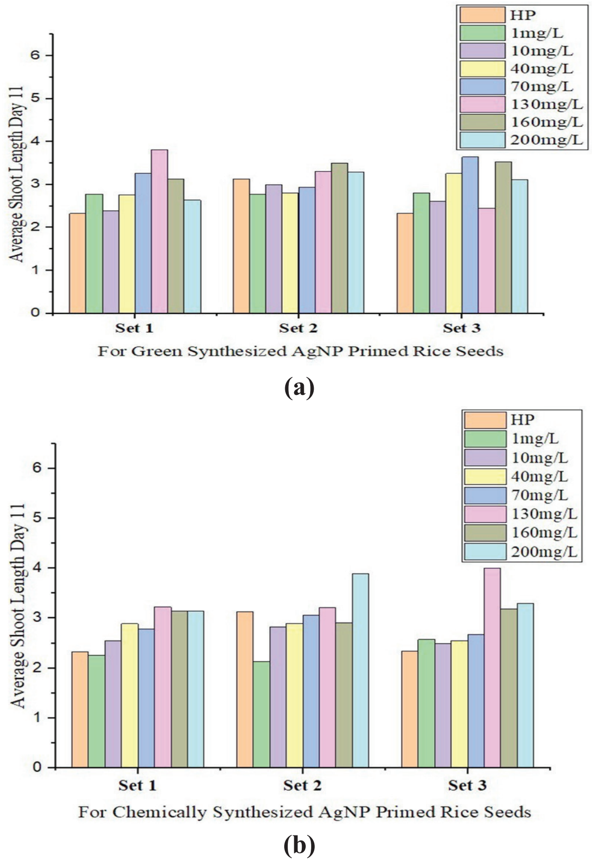


Fig. 14: (a) Impact of Green Synthesized AgNPs and (b) Chemically Synthesized AgNPs on the Shoot Length (in cm) of Rice Seeds

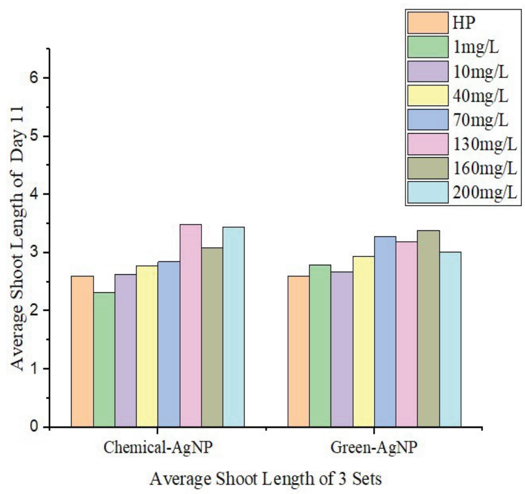


Fig.15: Impact of Green-Synthesized and Chemical-Synthesized AgNPs on the Average Shoot Length (in cm) of Rice Seeds

However, significant differences in the enhancement of root and shoot length were observed between hydropriming and AgNP priming, indicating that AgNP priming had a noticeable impact on seedling growth during the early stages.

5. CONCLUSION

This study demonstrates that both hydropriming and silver nanoparticle (AgNP) treatments significantly enhance rice paddy early seedling development under controlled conditions. Both hydroprimed and AgNP-primed seeds exhibited germination rates of 95-100%, confirming that AgNP priming does not compromise seed viability. AgNP treatments, whether chemically or green synthesized notably improved root and shoot growth. Although the response to AgNP concentration varied across experimental sets, both types showed optimal performance within specific ranges. For root development, the effective concentration window was 70–160 mg/L, while shoot elongation was maximized within 70–200 mg/L. Therefore, based on the range observed for both root and shoot growth, the optimal concentration of AgNPs for maximizing overall seedling growth performance is within the 70–160 mg/L range. These findings indicate that AgNPs, when applied within the appropriate dosage range, can act as potent biostimulants to enhance seedling vigor. However, due to the inconsistent trends across sets, further investigation is needed to elucidate the mechanisms of action and to standardize application protocols for agricultural use.

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