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BIOSYNTHESIS OF GREEN NANOPARTICLES FROM *AZADIRACHTA INDICA* (L.) AND EVALUATION OF PLANT GROWTH PROMOTING ABILITY

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ABSTRACT
Azadirachta indica (L.) is extensively utilized for pest management in sustainable agriculture. This study presents the green production of silver nanoparticles utilizing *A. indica* (L.) leaf extract and evaluates their ability to promote plant growth in wheat (*Triticum aestivum* L.). The leaf extract of A. indica reduces $AgNO_3$ and stabilizes the AgNPs. Nanoparticles were synthesized, and their formation was confirmed by a color change from yellow to dark brown, further validated by UV-Vis spectrophotometry. UV-Vis spectrophotometry revealed a pronounced surface plasmon resonance (SPR) peak at 445 nm, signifying successful nanoparticle formation. The AgNPs produced were purified by centrifugation and subsequent drying, and then evaluated for their growth-promoting ability. Wheat seedlings were subjected to different concentrations of green AgNPs (25, 50, 75, and 100 mg L^{-1}) along with a control under controlled conditions. Growth parameters such as root length, shoot height, and fresh weight for both root and shoot were measured at seven days of treatment. The findings showed significant improvement in seedling growth at lower AgNPs concentrations (25 and 50 mg L^{-1}), while higher concentrations (75 and 100 mg L^{-1}) led to growth suppression, indicating a concentration-dependent effect. The findings of the study emphasize the potential of A. indica-mediated AgNPs as a non-hazardous plant growth enhancer with prospective uses in sustainable agriculture, subject to careful maintenance of an appropriate dose to prevent phytotoxic effects.

KEYWORDS: *Azadirachta indica (L.);* Plant extract; Green nanoparticles; Wheat; Sustainable agriculture

1 INTRODUCTION
Nanotechnology is reforming crop management, offering tools that directly interact with plant systems where biological processes originate (Virk et al. 2024). Among synthesized nanoparticles, green-synthesized silver nanoparticles (AgNPs) have proved their significance. AgNPs exhibit unique physicochemical properties, environmental compatibility, and diverse biological activities (Sati et al. 2025). Green technology for the synthesis of nanoparticles employs plant extracts and their reducing and stabilizing properties, thereby avoiding hazardous chemicals that may harm the environment (Khan et al. 2022). Plant phytochemicals such as flavonoids, terpenoids, and polyphenols readily reduce metal ions and prevent their aggregation (Singh et al. 2018).

In sustainable agriculture, neem is conventionally utilized for crop protection and productivity enhancement owing to its richness in bioactive compounds, which makes it suitable for nanoparticle biosynthesis (Dhaware et al. 2025). Its leaves contain limonoids, azadirachtin, and other secondary metabolites that can reduce metal ions and stabilize nanoparticles (Islas et al. 2020). The green approach to nanoparticle synthesis is eco-friendly and biocompatible, reducing environmental risk (Abuzeid et al. 2023).

Growing interest exists in the role of green nanoparticles in plant growth and physiology. Studies report that low doses of plant-derived AgNPs can promote seed germination, root and shoot elongation, and biomass accumulation in various crops (Yang et al. 2025). Wheat seeds primed with Citrus hystrix D.C.-derived AgNPs at 20 mg L^{-1} achieved 96% germination while un-primed seed germination was 83% (Mahakham et al. 2017). Green-AgNPs synthesized using Coriandrum sativum leaf extract at high concentration reduced root and shoot elongation and fresh weight in Lupinus termis L., while at low dose they improved root-shoot elongation and fresh weight (Al-Huqail et al. 2018). AgNPs synthesized using neem leaf extract enhanced root and shoot growth in tomato at 5 and 10 ppm, while at higher doses (25 and 50 ppm) root and shoot lengths were 20% shorter than the control (Ansari et al. 2023). Green-AgNPs synthesized using Cotula cinerea extract enhanced nutrient uptake in wheat (Kesbi et al. 2025), stimulated cellular metabolism in tobacco plants (Huang et al. 2026), and modulated phytohormone signalling in T. aestivum by enhancing ABA content (Wahid et al. 2020). However, at high concentrations, nanoparticles showed negative effects on plants.

Wheat is a staple cereal of global significance, and evaluation of the growth-promoting potential of nanoparticles is therefore of great importance. Early seedling vigour, especially root development, is an indicator of nutrient uptake and ultimately crop yield (Wang et al. 2016). Understanding how biosynthesized green nanoparticles influence this early growth stage is essential for assessment of agronomic utility and safety.

This study reports the biosynthesis of AgNPs using A. indica (L.), their characterization, and a systematic evaluation of their effects on wheat seedling development, bridging gaps in our understanding of green nanoparticle-plant interactions and assessing the potential of A. indica (L.)-derived nanoparticles as growth-promoting agents.

2. METHODOLOGY

2.1 Green-AgNPs Synthesis

Green silver nanoparticles (AgNPs) were produced via a green synthesis reaction mediated by Azadirachta indica, following the protocol previously described by Saini et al. (2024) with minor modifications. Plant material was thoroughly washed under running tap water and then with distilled water to remove surface contaminants, and air-dried in shade for 7-14 days. Dried material was ground to fine powder using a mechanical grinder. Approximately 10 g of powder was soaked in distilled water (1:10 ratio) for 24 hours at room temperature and filtered through Whatman No. 1 filter paper. The filtrate was stored at 4°C until use. A 1 mM $AgNO_3$ solution was prepared by dissolving 169.87 mg $AgNO_3$ in 1 L distilled water. Plant extract was added to $AgNO_3$ solution at a 1:2 ratio (extract: $AgNO_3$) under dark conditions to avoid photo-reduction. The mixture was incubated until a color change from yellow to dark brown was observed. The mixture was centrifuged at 12,000 rpm for 20 minutes; the pellet was washed three times with distilled water and dried at 37°C.

2.2 Characterization of Nanoparticles

The morphology, size distribution, shape, and stability of synthesized AgNPs were evaluated using various spectrophotometric and microscopic techniques.

2.2.1 UV-Vis Spectrophotometry

The reduction of $AgNO_3$ by plant extract in the reaction mixture, resulting in colour change, was monitored by UV-Vis spectrophotometry. A. indica leaf extract was used to reduce Ag^+ from 1 mM $AgNO_3$, producing silver nanoparticles that turned the solution from yellow to dark brown. The scanning was performed over the wavelength range of 300-700 nm with a resolution of 1 nm. A distinct surface plasmon resonance (SPR) peak at approximately 445 nm confirmed nanoparticle formation (Figure 1).

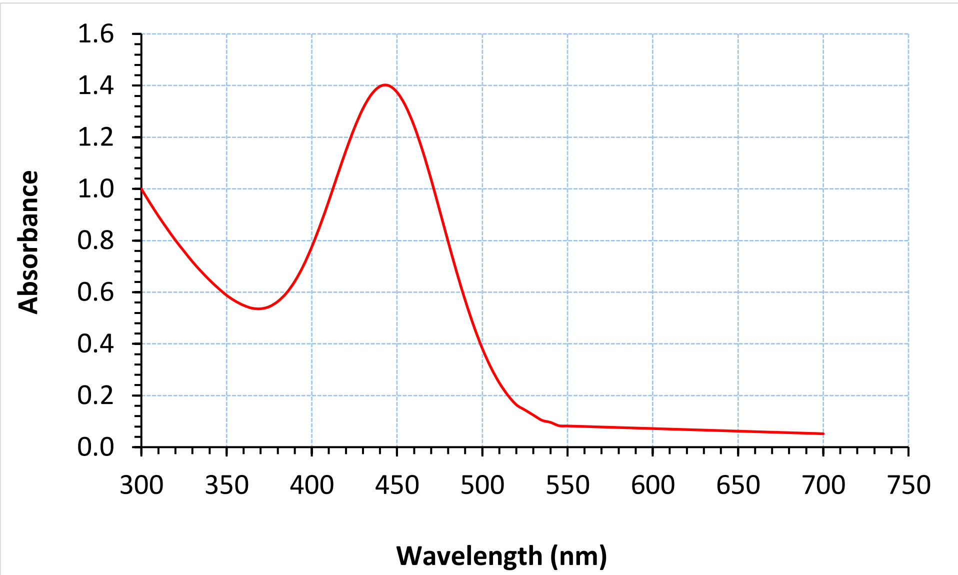

Fig. 1 UV-Vis spectra of biosynthesized AgNPs using *A. indica*.

2.2.2 X-Ray Diffraction (XRD)

The crystalline structure of the synthesized green AgNPs was characterized using X-ray diffraction (XRD). The XRD pattern of neem-synthesized AgNPs displays characteristic peaks of metallic silver in a face-centered cubic (FCC) structure. Prominent sharp peaks around 38° 2θ (111 plane), 44° (200 plane), 64° (220), and 77-80° (311) match JCPDS 04-0783 for pure Ag, confirming high crystallinity (Figure 2). The strong intensity at around 38° indicates preferred orientation along (111), typical for bio-reduced AgNPs stabilized by neem phytochemicals such as terpenoids and flavonoids.

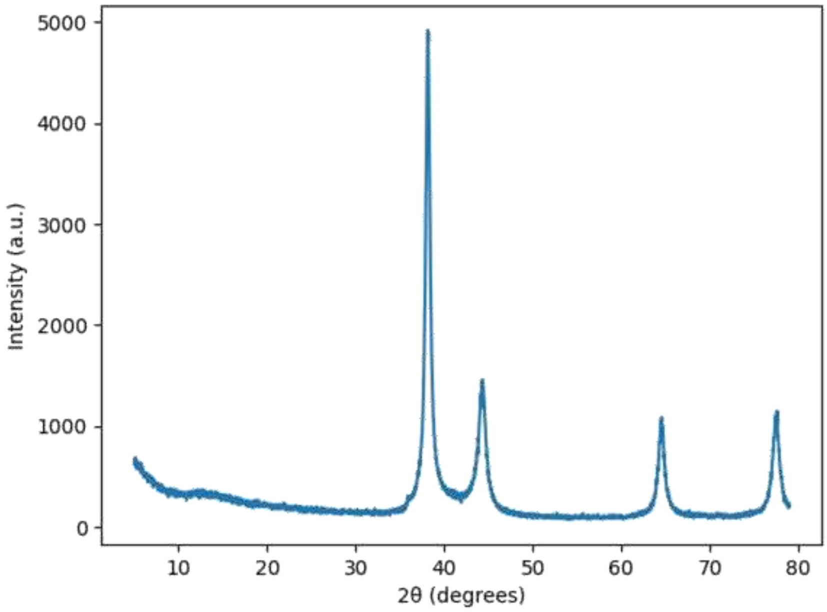

Fig. 2. XRD pattern of neem-mediated silver nanoparticles.

2.2.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was carried out to investigate the possible functional groups present in the green-synthesized Ag-NPs and involved in the reduction and stabilization of the metal nanoparticles. The spectrum exhibits prominent absorption bands in the region of 1700-1500 cm^{-1}, corresponding to carbonyl (C=O) stretching and amide I and II vibrations, indicating the presence of proteinaceous or polyphenolic compounds derived from neem leaf extract (Figure 3). Additional bands observed around 1650 cm^{-1} further support the presence of amide linkages. Weak absorption bands at approximately 2049 and 1979 cm^{-1} may be associated with surface-bound species or metal-ligand interactions, indicating coordination between functional groups of biomolecules and the silver nanoparticle surface. The peak near 2344 cm^{-1} is attributed to atmospheric CO_2 and is not considered intrinsic to the sample. The band at approximately 662 cm^{-1} is assigned to C-halogen stretching or Ag-O vibrations, supporting interaction between silver and oxygen-containing functional groups.

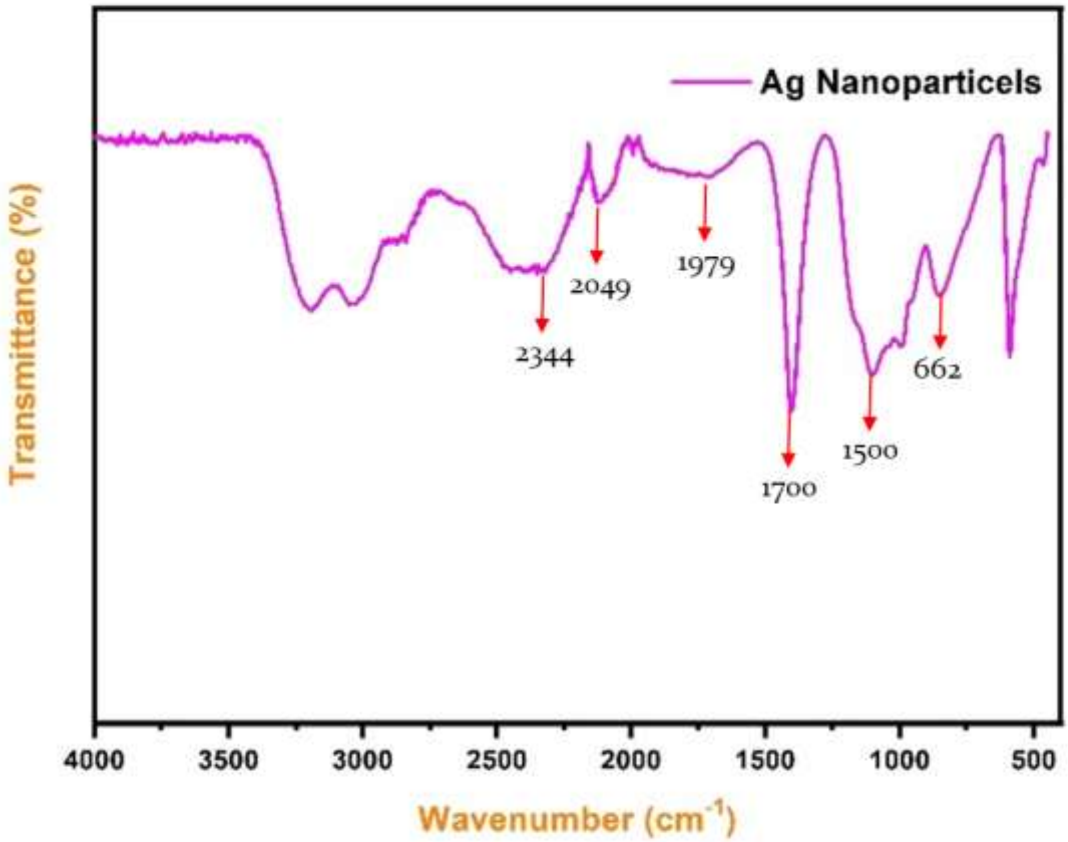

Fig. 3. FTIR analysis of green silver nanoparticles synthesized using neem leaf extract.

2.2.4 Scanning Electron Microscopy (SEM)

The particle size distribution and surface morphology of the green-synthesized Ag-NPs were examined using SEM. The micrograph reveals the formation of densely agglomerated nanostructures with a cauliflower-like morphology (Figure 4). The particles appear as clusters composed of smaller, quasi-spherical primary units. Based on the scale bar (100 nm), the primary particle size is estimated to be in the range of 20-40 nm, while agglomerated structures extend beyond 100 nm. The rough surface texture and clustering behaviour indicate strong interparticle interactions. The significant difference between SEM-derived particle size and XRD-derived crystallite size confirms that the nanoparticles are polycrystalline in nature, with each particle observed in SEM consisting of multiple smaller crystallites aggregated together.

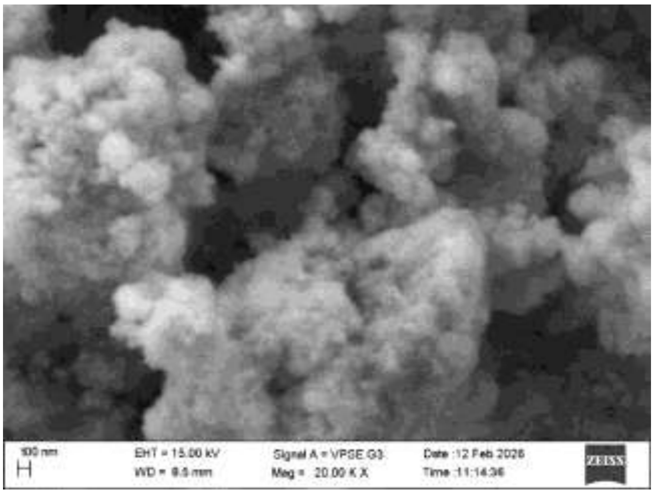

Fig. 4 SEM analysis of green silver nanoparticles synthesized using neem leaf extract.

2.2.5 Energy Dispersive X-ray Spectroscopy (EDS)

To determine the elemental composition of synthesized green AgNPs EDS analysis was performed (Figure 5). The spectrum shows strong typical silver peaks, with weaker AgM and Lβ/Lγ emissions and a prominent Ag Lα peak near 3.0 keV. Also the quantitative analysis confirming synthesis of AgNPs from the pure metallic silver sample as it exhibited no any impurities in their analysis. No additional elemental peaks were observed which also indicated the purity of the synthesized AgNPs.

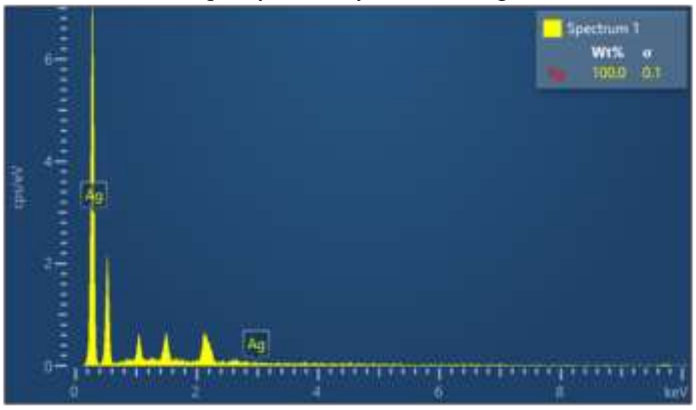

Fig. 5. EDS spectra of green silver nanoparticles synthesized using neem leaf extract.

2.2.6 Electrochemical Performance of Ag nanoparticles

The electrochemical behavior of the synthesized Ag nanoparticles was investigated by cyclic voltammetry (CV) (Figure 6).

The electrochemical behavior of the AgNPs were analyses using cyclic voltammeter (CV) (Figure 6). A prominent anodic peak near -0.7 V corresponding the oxidation of metallic silver to surface silver oxide (Ag_2O/Ag^+), and a cathodic peak near -0.25V endorsed the reduction of the Ag. The reversible electrochemical behavior of the AgNPs demonstrated by the redox peak. Additionally, the broad Voltametric profile and high current response indicated the electrochemically active surface and efficient electron transfer. These characteristics attributed to the nanosize AgNPs and high conductivity.

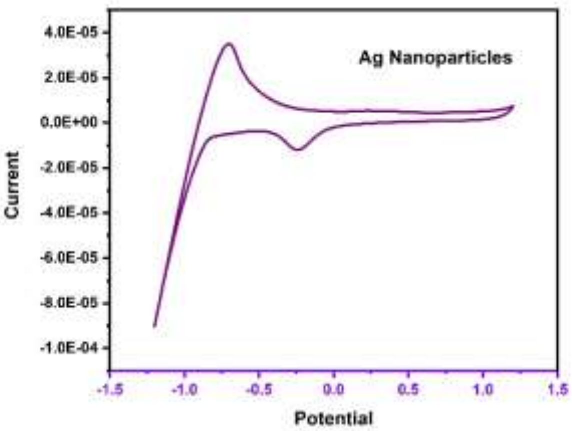

Fig. 6. CV profile of green silver nanoparticles synthesized using neem leaf extract.

2.2.7 Zeta Potential Analysis of Ag Nanoparticles

To evaluate surface charge and colloidal stability, zeta potential analysis were done (Figure 7). The zeta potential value approximately -20 mV revealing the synthesized AgNPs. This negative surface potential originates from the adsorption of stabilizing functional groups, which mitigates particles aggregation via interparticle electrostatic repulsion. While this value does not reach the standard benchmark of ± 30 mV indicative of highly stable colloids, it nonetheless denotes adequate stability of functional applications. Consequently, this surface charge configuration is projected to facilitate uniform dispersion and sustain an elevated active surface area. This ultimately optimizing the performance of the AgNPs across catalytic, sensing, and biomedical applications.

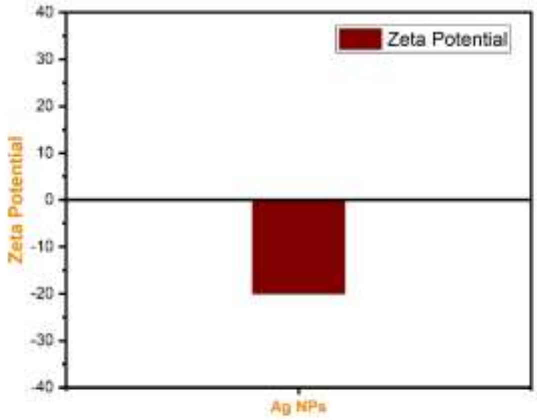

Fig. 7. Charge distribution of green silver nanoparticles synthesized using neem leaf extract.

3 RESULTS AND DISCUSSION

3.1 Evaluation of the Effect of Neem-AgNPs on Wheat Seedling

Wheat seeds were procured from a certified supplier. Healthy, uniform-sized seeds were surface-sterilized with 0.1% mercuric chloride ($HgCl_2$) solution, soaked in double-distilled water (ddH_2O) for 12 hours, and kept in the dark for germination. Germinated seeds were transferred to plastic pots filled with sterilized sand and supplied with 50 mL half-strength Hoagland solution per pot. After 7 days, plants were subjected to different concentrations of neem-AgNPs solution (25, 50, 75, and 100 mg L^{-1}) along with a control. Shoot height and root length were measured on the 7th day of treatment. The fresh and dry weights of seedlings were also measured and recorded.

Growth was assessed seven days after treatment. At the two lower concentrations, seedlings displayed significant stimulation: root length increased from 12.2 cm (control) to 14.5 cm (25 mg L^{-1}) and 16.4 cm (50 mg L^{-1}) (Figure 8a), while shoot length rose from 13.1 cm to 15.3 cm and 16.8 cm, respectively (Figure 8b). Fresh weight measurements followed the same pattern, with root fresh weight rising from 78.6 mg (control) to 99.1 mg at 25 mg L^{-1} and 115.9 mg at 50 mg L^{-1} (Figure 8c). Shoot fresh weight increased from 117.7 mg to 129.4 mg and 142.8 mg at 25 and 50 mg L^{-1}, respectively (Figure 8d).

In contrast, higher doses suppressed growth: at 75 mg L^{-1}, root length fell to 10.3 cm and shoot length to 11.6 cm; at 100 mg L^{-1} they dropped further to 7.5 cm and 8.7 cm, respectively (Figure 5). Marked reductions in fresh weight of root and shoot were also observed at higher doses. At 75 mg L^{-1}, root fresh weight decreased to 64.4 mg and shoot fresh weight to 96.4 mg; at 100 mg L^{-1}, fresh weight of root and shoot further decreased to 51.3 mg and 58.9 mg, respectively.

Compared to the control, lower doses (25 and 50 mg L^{-1}) of nanoparticles increased root length by 18-34% and shoot length by 17-28%. Root and shoot fresh weights increased by 26-48% and 10-21%, respectively. At higher doses (75 and 100 mg L^{-1}), root lengths were 16-39% shorter than control and shoot lengths were 12-34% shorter. Root and shoot fresh weights were reduced by 18-35% and 18-50%, respectively, compared to control.

Table 1 Growth parameters of wheat seedlings under different treatments of AI-AgNPs (mean ± SD).

Treatment → **Parameter ↓**	**Control**	**25 mg/L**	**50 mg/L**	**75 mg/L**	**100 mg/L**
Root Length (cm)	12.2 ± 1.0	14.5 ± 1.1	16.4 ± 1.0	10.3 ± 1.5	7.5 ± 1.6
Shoot Height (cm)	13.1 ± 1.1	15.3 ± 1.0	16.8 ± 1.2	11.6 ± 1.7	8.7 ± 1.3
Root FW (mg)	78.6 ± 6.5	99.1 ± 4.9	115.9 ± 5.3	64.4 ± 8.4	51.3 ± 7.0
Shoot FW (mg)	117.7 ± 3.8	129.4 ± 4.3	142.8 ± 7.5	96.4 ± 11.9	58.9 ± 10.1

FW = Fresh Weight; values represent mean ± SD (n = 5).

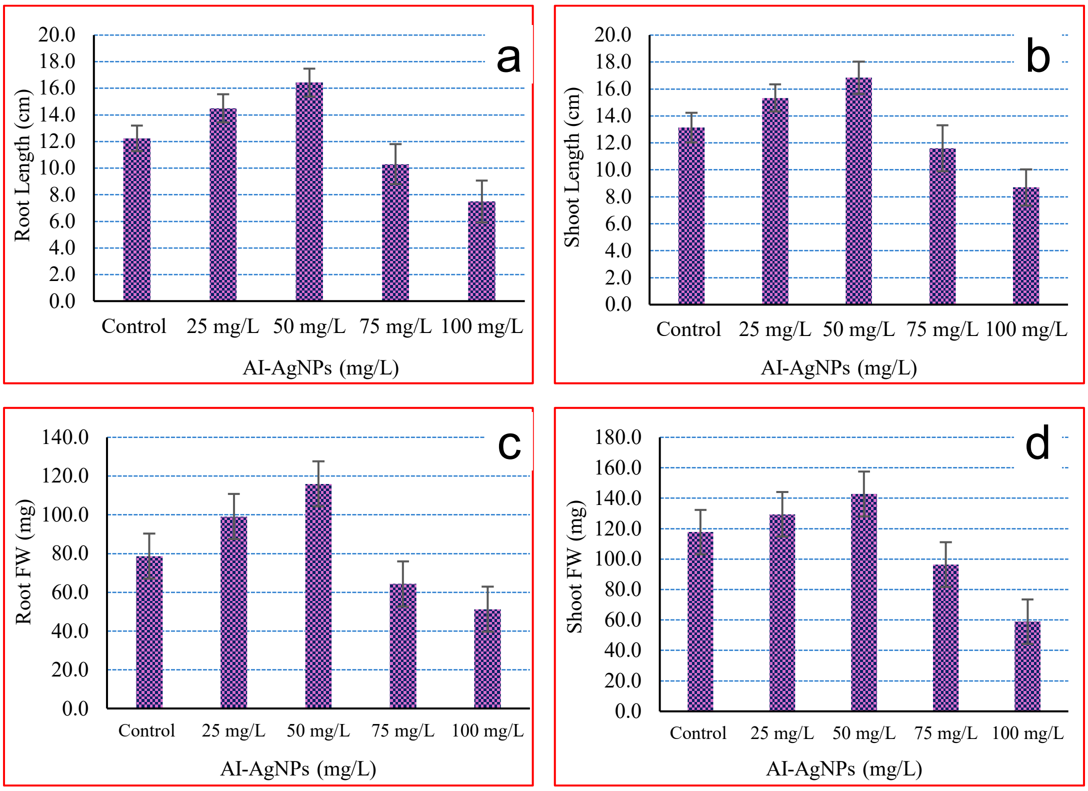

Fig. 8 Impact of different concentrations of Green-AgNPs on (a) root length, (b) shoot height, (c) root fresh weight, and (d) shoot fresh weight. Data are mean ± SD.

The present study demonstrates that A. indica-mediated AgNPs exert a clear concentration-dependent influence on early wheat seedling development. The biphasic response-growth stimulation at low dose and inhibition at higher dose-is not incidental, but rather reflects a central biological signal consistent with how nanoparticles interact with plant physiology.

The formation of AgNPs was reflected by the characteristic color transition and a distinct surface plasmon resonance peak at 445 nm, consistent with biologically synthesized AgNPs reported previously (Bhuyar et al. 2020; Abada et al. 2025). The ability of neem leaf extract to act simultaneously as a reducing and stabilizing agent can be attributed to its rich pool of secondary metabolites, including flavonoids, terpenoids, and limonoids (Uthaya Kumar et al. 2020; Sutrisno et al. 2024). These compounds not only facilitate nanoparticle synthesis but may also influence their biological behaviour. Green-synthesized AgNPs have been shown to exhibit superiority over chemically produced nanoparticles upon application to plants (Lawal et al. 2025).

At 25 and 50 mg L^{-1}, AgNPs treatment significantly enhanced root and shoot elongation as well as fresh biomass accumulation. The stronger stimulation of root growth compared to shoots is particularly important from an agronomic perspective, as early root development directly determines nutrient and water uptake efficiency (Wang et al. 2016). This pattern suggests that low concentrations of green AgNPs may enhance cellular division and elongation in meristematic tissues, possibly by improving nutrient availability, modulating hormone balance, or mildly stimulating metabolic activity. Similar growth-promoting responses at low AgNP doses have been reported in wheat, tomato, and other crops, reinforcing the idea that nanoparticles can function as nano-elicitors within an optimal concentration window (Iqbal et al. 2019; Ansari et al. 2023; Masood et al. 2025; Kesbi et al. 2025).

However, the response curve sharply reversed at 75 and 100 mg L^{-1}, where pronounced reductions in root length, shoot length, and fresh weight were observed. This inhibitory effect reflects the onset of phytotoxicity, a well-documented risk associated with excessive nanoparticle exposure (Vishwakarma et al. 2017; Yan and Chen 2019). At higher concentrations, AgNPs may disrupt membrane integrity, interfere with cellular redox balance, and impair water and nutrient transport, ultimately suppressing growth (Das and Paul 2025). The stronger decline in root parameters compared to shoots further suggests that roots, as the primary site of nanoparticle contact and accumulation, are more vulnerable to silver nanoparticle-induced stress (Pan et al. 2024).

This study found that A. indica-mediated AgNPs can act as a green growth enhancer for wheat when applied at ≤ 50 mg L^{-1}, but become phytotoxic at higher concentrations. Silver nanoparticles show promise as plant growth enhancers, but only within a narrow and controlled concentration range. Their effectiveness at 50 mg L^{-1} highlights promise for sustainable agriculture, while the inhibitory effect at higher doses serves as a clear caution against indiscriminate use. Future work should focus on mechanistic studies involving oxidative stress markers, nutrient uptake dynamics, and phytohormone regulation to fully define the safe and effective application of green-AgNPs.

4 CONCLUSIONS

Green silver nanoparticles were successfully synthesized using Azadirachta indica (L.) leaf extract and confirmed by UV-Vis spectrophotometry (SPR peak at 445 nm), XRD (FCC crystalline structure, JCPDS 04-0783), FTIR (functional group identification), and SEM (particle size 20-40 nm). Application of neem-AgNPs to wheat (Triticum aestivum L.) seedlings revealed a clear concentration-dependent biphasic response: growth promotion at ≤ 50 mg L^{-1} and phytotoxicity at ≥ 75 mg L^{-1}. These findings demonstrate the potential of A. indica-mediated AgNPs as eco-friendly plant growth promoters in sustainable agriculture, contingent on careful dose management.

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