JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(4), 136–147
https://doi.org/10.12911/22998993/199819
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Received: 2024.12.11
Accepted: 2025.01.31
Published: 2025.02.17

Impact of seed treatment with *Azadirachta indica* based silver nanoparticles on the early growth and resistance of soybean plant under drought stress

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ABSTRACT

Nanoparticles enhance plant growth and resilience to diverse environmental stressors but can also adversely affect plants. This study investigated the impact of neem-mediated silver nanoparticles (AgNPs) in seed treatment on soybean plants' early growth and resistance under drought stress. AgNPs were synthesized using a neem leaf extract reductant. Characterization of AgNPs used dynamic light scattering and SEM with EDX spectroscopy. The experiment was arranged in randomized complete block design with four replicates. The main plot was field water capacity (FWC) at 100%, 80%, 60%, and 40%, representing the level of drought stress. The subplots represented seed treatments (control, AgNP priming, and AgNP coating). Results showed that AgNPs had a z-average of 55 nm, PDI of 0.449, and zeta potential of -28.8 mV. We detected Ag, C, O, and N in the spherical AgNPs. Seed priming and coating with AgNPs reduced plant height, shoot dry weight, root dry weight, and root volume. As the level of drought stress increased, seed priming increased malondialdehyde content, while the effect of seed coating was more moderate than that of seed priming. In this study, seed treatment with AgNPs enhanced the proline content and activity of peroxidase to mitigate the adverse effects of drought stress.

Keywords: antioxidant enzyme, malondialdehyde, peroxidase, proline, seed coating, seed priming.

INTRODUCTION

Soybeans are among the most important food commodities worldwide. In the cultivation of soybean plants, some constraints result in the disruption of growth and development, leading to suboptimal production. One of these constraints is the drought stress caused by climate change.

Drought describes the availability of less soil water, which is below the critical limit for plant growth and development (Fathi and Tari, 2016). This water shortage causes physiological changes, such as disruption of nutrient availability and transportation, and various metabolic activities, resulting in decreased plant growth and yield (Hasan et al., 2021). Soybeans are sensitive to water shortages (Bortoluzzi et al., 2020). In response to water shortages, plants adapt by activating several osmolyte compounds and antioxidant enzymes to control excess levels of reactive oxygen species (ROS) in the cells formed due to oxidative damage (Zhou et al., 2021). In addition, there are changes in plant morphology, such as reductions in plant height and leaf area (Dong et al., 2019).

Seed treatment before planting has been reported to form an early plant memory that is resistant to various biotic and abiotic stresses in the field (Mahboob et al., 2019; Saha et al., 2022; Sharma et al., 2015). Seed treatments include seed priming, seed conditioning, and seed coating. This technology has the advantages of easy application and cost-effectiveness (Mahboob et al., 2019). Seed treatment uses various materials that can promote seed growth, such as osmolytes, hormones, and nutrients. The latest technologies used as seed treatment materials are nanomaterials, such as nanoparticles (Espirito Santo Pereira et al., 2021; Nile et al., 2022).

Nanoparticles have been reported to stimulate faster embryo growth and modulate the osmotic system and plant resistance making them more resistant to stress (Acharya et al., 2020; González-García et al., 2022; Mazhar et al., 2023). Nanoparticles can enter plants through roots and leaves and accumulate in various plant tissues, causing physiochemical and metabolic changes. The impact of these changes depends on factors such as size, shape, concentration, surface charge, nanoparticle composition, and specific plant species (Kumari et al., 2024).

Silver nanoparticles are metal nanoparticles that are widely used in various fields such as medicine, food, textiles, and agriculture (Zhang et al., 2016). Silver nanoparticles have attracted much attention because of their good conductivity, chemical stability, and unique optical and electrical properties; therefore, they are used as catalysts, sensors, and antibacterial, antiviral, and antifungal agents (Khodashenas and Ghorbani, 2019). Silver nanoparticles have also been reported to increase plant growth and yield under abiotic stress conditions such as salinity, heat, flooding, and drought (Alabdallah et al., 2021; Bsoul et al., 2023; Mustafa et al., 2015). Silver nanoparticles induce the formation of hydroxyl radicals and increase aquaporin gene expression, thus accelerating water absorption and initiating enzymatic processes such as carbohydrate degradation (Mahakham et al., 2017).

However, there are still very few studies and literature that discuss the role of silver nanoparticles on plant growth and resistance, especially under drought-stress conditions (Abasi et al., 2022). In addition, nanoparticles are also reported to have negative impacts on plants and the environment (Cox et al., 2016; Nie et al., 2023; Tripathi et al., 2017). Impacts that occur such as decreased plant growth, DNA damage, and others (Khan et al., 2023; K. Kumari et al., 2024). Therefore, this study aimed to investigate the impact of seed treatment using silver nanoparticles synthesized with neem leaf extract reductant on the early growth and resistance of soybean plants under drought stress.

MATERIAL AND METHODS

Synthesis and characterization of silver nanoparticles

Neem leaf extract was prepared by boiling fresh neem leaves with aquabidest in an Erlenmeyer flask. The ratio of neem leaves to aquabidest was 1:10 (w/v) (Ahmed et al., 2016). Boiled water was allowed to reach room temperature and filtered using Whatman No.1 paper. A solution of 1 mM $AgNO_3$ was prepared by dissolving 0.17 g of $AgNO_3$ in 1000 mL of distilled water and stored at 4–8°C. Analytical grade silver nitrate ($AgNO_3$) was obtained from Merck (Germany). Silver nanoparticles were synthesized by mixing 1 mM $AgNO_3$ solution with neem leaf extract at 9:1 (v/v). The solution mixture was stirred using a hotplate magnetic stirrer at 60 °C for 20 min until the solution turned reddish-brown.

Characterization was conducted at the Research Center for Nanoscience and Nanotechnology, Institut Teknologi Bandung, and Integrated Laboratory and Research Center, Universitas Indonesia. The particle size and zeta potential of the AgNPs were measured using a particle size analyzer (Horiba SZ-100). The surface of the AgNPs was imaged using a scanning electron microscope (Hitachi SU3500) with energy-dispersive X-ray (EDX) spectroscopy. Fourier transform infrared spectroscopy (FTIR) profile of AgNPs was analyzed using Thermo Fisher Scientific Nicolet iS50.

Seed priming and coating treatment

The soybean cv. Grobogan seeds were used in this experiment. Initial seed germination percentage and vigor index were 80.5% and 65.5%, respectively. Seed priming was performed by soaking the seeds for 6 h at 25 °C in a 10% AgNPs solution, then air-dried to reach the initial moisture content. The selection of a 10% AgNP concentration was based on the previous study (Arridho et al., 2024), which resulted in the highest increase in soybean seed vigor (radicle emergence) compared to 2.5–7.5% AgNPs.

Sodium alginate (NaAlg) adhesive (Sigma-Aldrich) was used in seed coating. A 1% NaAlg suspension was prepared by dissolving 0.5 g of sodium alginate powder in 50 ml of distilled water. The mixture was magnetically stirred for 30 min until it became homogeneous. Soybean seeds were coated with a 1% NaAlg–10% AgNPs suspension manually using a jar. Stirring was performed by shaking the jar for 10 min until the suspension was evenly distributed over the entire seed surface and aerated before planting. The ratio of soybean seeds, 10% AgNPs, and 1% NaAlg was 10:1:1 (w/v/v) (Manggung et al., 2014).

Experimental procedures

The experiment used a completely randomized split-plot design, with four replicates. The main plot had a field water capacity of 100%, 80%, 60%, and 40%, representing the level of drought stress. The subplots show seed treatment methods of control, AgNP priming, and AgNP coating. The concentration of AgNPs used was 10% of the synthesized stock solution.

Soybean seed planting was conducted in a greenhouse of Bogor Agricultural University, Bogor, Indonesia. The max/min temperature was 39.1/ 23.5 °C with an average temperature in the greenhouse during the experiment of 29 °C. Primed and coated seeds were planted at four seeds per plastic pot. The planting medium consisted of soil and compost at a ratio of 2:1. NPK fertilizer was applied at 125 kg/ha (Hapsoh et al., 2019), one week after planting and three weeks after planting. The field water capacity of the planting media was determined based on the gravimetric method of Haridjaja et al. (2013). Drought stress treatment began 14 d after planting and continued until 35 d after planting (during the vegetative phase).

Plant growth parameters

The observed growth variables were field seedling emergence, plant height, leaf area, shoot dry weight, root dry weight, and root volume. Plant height was measured every week after the seeds were planted, and other variables were measured during the last observation, 35 days after planting.

Biochemical parameters

Biochemical plant resistance variables were measured using leaf samples on the 10th day after the plants were subjected to drought stress. Plant biochemical resistance variables included malondialdehyde (MDA), proline, peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT). Malondialdehyde levels were measured using the method as described by Wang et al. (2013). The proline content was measured using the method described by Bates et al. (1973). Peroxidase enzymes were measured based on Kar and Mishra (1976) and Mishra (1976). superoxide dismutase enzyme levels were measured according to Wu et al. (2014). The measurement of catalase activity was based on the method described by Bertholdo-Vargas et al. (2009). Protein content was measured following Lowry (1951) and was used as a dividing factor in measuring antioxidant enzyme activity.

Statistical analysis

The data were processed and analyzed using Microsoft Excel 2021 and R-Studio software. Analysis of variance (ANOVA) was used, followed by Duncan's multiple range test (DMRT) at the 5% level.

RESULTS AND DISCUSSION

Characterization of silver nanoparticles

Size distribution analysis of silver nanoparticles (AgNPs) was performed by dynamic light scattering (DLS). The results revealed that the synthesized AgNPs exhibited a mean particle diameter of 74.9 nm with a z-average of 55 nm (Fig. 1). The particle size distribution was larger than that reported by S. A. Kumari et al. (2022) and Ahmed et al. (2016), which produced an average diameter of 38.5 nm and 34 nm, respectively. The accumulated size of the metal core, capping agent, and electrical double layer between the particles determine the size of the nanoparticles (Hemlata et al., 2020). The polydispersity index (PDI) was 0.449, indicating moderate size distribution uniformity.

Zeta potential measurement was conducted to evaluate the surface charge and colloidal stability of the synthesized AgNPs. Figure 2 displays the zeta potential distribution curve of the AgNPs, which exhibits a single dominant peak centered at -28.8 mV with a relatively narrow distribution. The negative surface charge can be attributed to the presence of stabilizing agents adsorbed onto the nanoparticle surface (Kumari et al., 2024). The uniformity of the surface charge is crucial for preventing agglomeration through electrostatic repulsion mechanisms (Duval et al., 2018).

Microscopic analysis using a scanning electron microscope (SEM) showed that the formed AgNPs were spherical (Fig. 3a). Based on the EDX

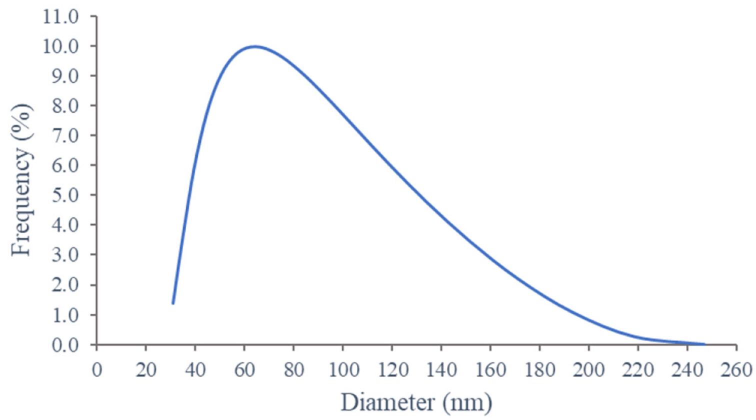

Figure 1. Particle size distribution of AgNPs

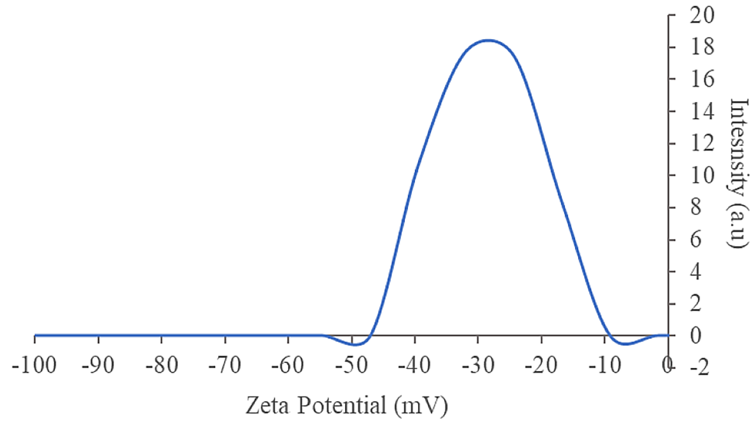

Figure 2. Zeta potential of AgNPs

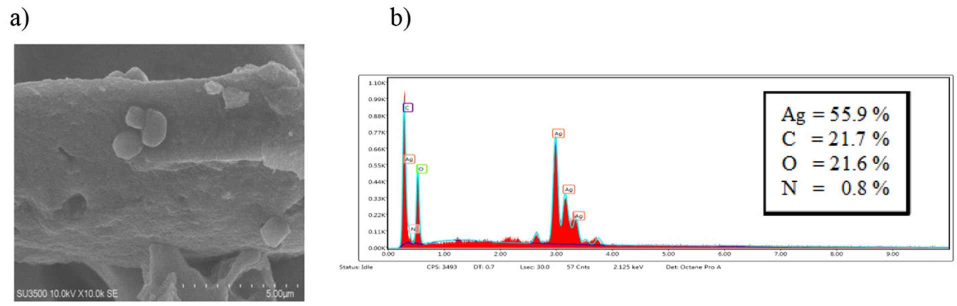

Figure 3. Electron microscopy study: (a) SEM analysis and (b) Energy-dispersive x-ray (EDX) Spectra of AgNPs

spectroscopy analysis, the successful synthesis of AgNPs using *Azadirachta indica* leaf extract as a reducing agent was confirmed (Fig. 3b). The EDX spectrum exhibited distinctive peaks corresponding to the various elements present in the synthesized AgNPs. The predominant peak observed at approximately 3 keV is characteristic of Ag, confirming the formation of AgNPs in the sample. The spectrum also reveals the presence of additional elements, indicated by lower-intensity peaks in the 0–1 keV range, which can be attributed to carbon (C), oxygen (O), and nitrogen (N). These elements are likely derived from the organic compounds present in the neem leaf extract, which serve as both reducing and capping agents during the synthesis process (Hemlata et al., 2020).

Plant growth parameters

Seed treatment significantly influenced the field emergence of soybean seeds (Table 1). Soybean seeds in the AgNP priming treatment showed

Table 1. Result of ANOVA of plant growth parameters

Parameters	Field water capacity	Seed treatment	Interaction	CV (a)	CV (b)
Field emergence (%)	–	0.040*	–	35.1%	–
Shoot dry weight (g)	0.004*	0.000*	0.001*	34.4%	17.8%
Leaf area (cm^2)	0.038*	0.093ns	0.542ns	26.4%	18.8%
Root dry weight (g)	0.000*	0.000*	0.001*	21.2%	16.7%
Root volume (cm^3)	0.000*	0.000*	0.000*	28.7%	15.6%
Plant height W1 (cm)	0.315ns	0.245ns	0.925ns	9.1%	14.2%
Plant height W2 (cm)	0.621ns	0.077ns	0.852ns	11.2%	12.4%
Plant height W3 (cm)	0.265ns	0.005*	0.165ns	12.4%	9.8%
Plant height W4 (cm)	0.009*	0.015*	0.525ns	12.6%	10.7%

Notes: Symbol (*) represents statistical significance and (ns) indicates non-significant. CV is the coefficient of variance. W (1–4) represents the first week until the fourth week after planting.

the highest field emergence value among all treatments but were not different from the control. AgNP coating reduced the field emergence of soybean seeds compared to the control, but the difference was insignificant (Table 2). This was thought to be due to the adhesive that slightly slowed the rate of water imbibition into soybean seeds.

Seed treatment with AgNPs significantly affected the early growth of soybean plants i.e. shoot dry weight, root dry weight, root volume, and plant height. The interaction of seed treatment and field water capacity significantly affected shoot dry weight, root dry weight, and root volume. Seed treatment only and its interaction with field water capacity did not affect soybean leaf area. The leaf area was affected only by the field water capacity treatment (Table 1).

AgNP priming reduced shoot dry weight by 32.4%, and AgNP coating reduced shoot dry weight by 27.8% compared with the control. AgNP priming significantly reduced shoot dry weight under FWC 100% and FWC 80%, whereas shoot dry weight at FWC 60% and FWC 40% was not significantly different from the control. AgNP coating treatment only reduced considerably shoot dry weight at FWC 100%, while the lower field water capacity showed approximately the same value as the control (Fig. 4).

AgNP priming reduced the root dry weight and volume by 28.6% and 38.03%, respectively. AgNP coating reduced root dry weight and volume by 28.6% and 40.1%, respectively (Table 2). A significant reduction in root dry weight occurred after AgNP priming and coating treatment at FWC 100% and 80%. Seed priming and coating with AgNPs tended to maintain root dry weight at FWC 60% and 40% (Fig. 5). AgNP priming had a significant effect on reducing root volume at FWC 100% and 80%, but not at FWC 60% and 40%. Meanwhile, AgNP coating only significantly reduced the root volume at 100% FWC. Generally, seed priming

Table 2. Effect of seed treatment on plant growth parameters

Parameters	Control	Seed treatment			
		Priming	Deviation (%)	Coating	Deviation (%)
Field emergence (%)*	68.75ab	73.44a	6.8	53.12b	-22.7
Shoot dry weight (g)*	0.71b	0.48a	-32.4	0.51a	-27.8
Leaf area (cm^2)	10.51a	8.80a	-16.3	9.47a	-9.9
Root dry weight (g)*	0.14b	0.10a	-28.6	0.10a	-28.6
Root volume (cm^3)*	1.42b	0.88a	-38.0	0.85a	-40.1
Plant height W1 (cm)	20.40a	18.60a	-8.8	18.90a	-7.3
Plant height W2 (cm)	39.90a	35.50a	-11.0	36.50a	-8.5
Plant height W3 (cm)*	61.30b	52.80a	-13.9	55.50a	-9.5
Plant height W4 (cm)*	76.90b	67.50a	-12.2	68.70a	-10.7

Notes: Mean ± (SD) values followed by the same letters within each row are non-significantly different based on DMRT ($p < 0.05$), and W (1–4) represents the first week until the fourth week after planting.

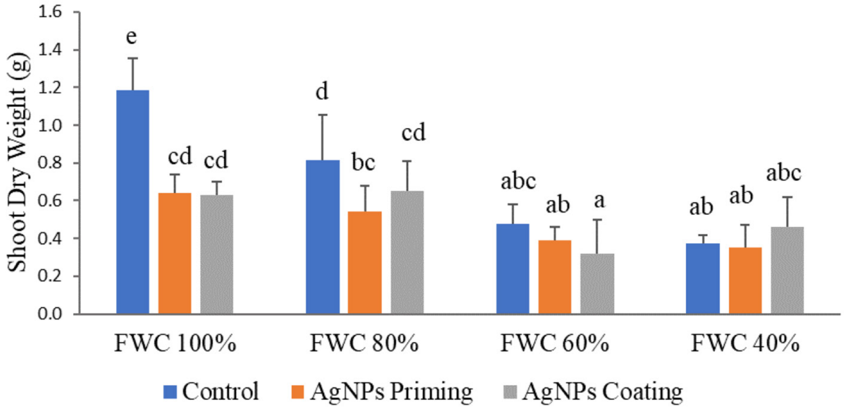

Figure 4. Effect of interaction between seed treatment and field water capacity on shoot dry weight

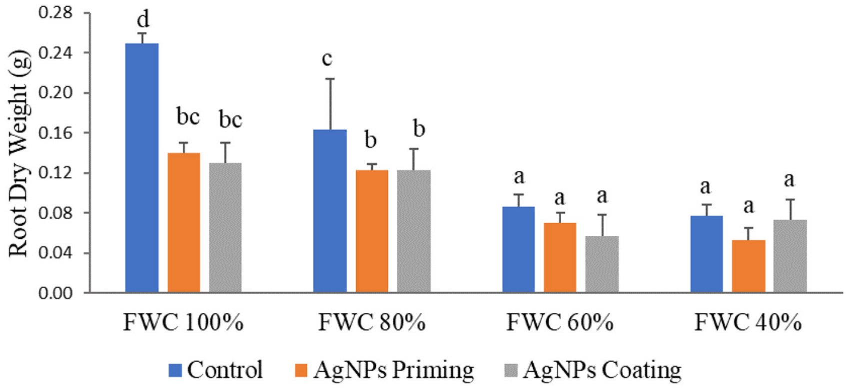

Figure 5. Effect of interaction between seed treatment and field water capacity on root dry weight

and coating with AgNPs maintained the root volume at FWC 60% and 40% (Fig. 6).

This decrease in soybean biomass was also reported by Quintela et al. (2024) when administered to soybean plants through the roots with an average AgNP size of 60 nm, however, the damaging effect was less than that of the $AgNO_3$ treatment. Li et al. (2017) also reported a decrease in soybean plant biomass after exposure to AgNPs through roots and leaves, but the impact was greater when administered through the roots.

In addition, plant height responded significantly to seed treatment and field water capacity in the third week (W3) and fourth week (W4) (Table 1). AgNP priming reduced plant height by 12.2–13.9%, whereas AgNP coating reduced plant height by 9.5–10.7% compared to the control (Table 2). Quintela et al. (2024) reported that

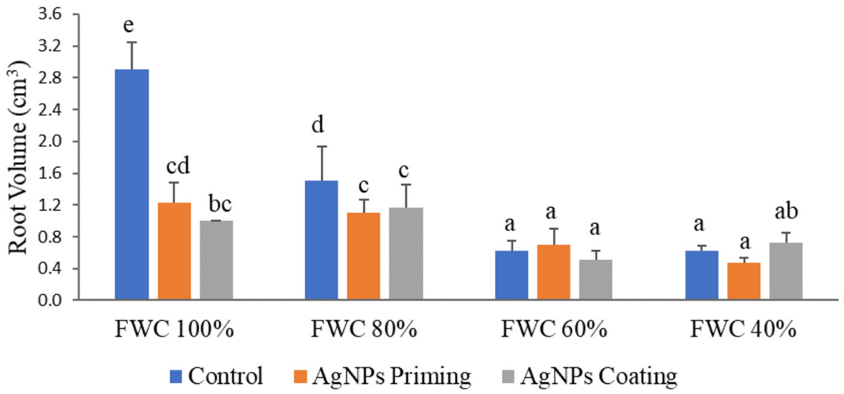

Figure 6. Effect of interaction between seed treatment and field water capacity on root volume

AgNP application through irrigation reduced soybean plant height by 12.6% compared with the control. This decrease might be related to MDA levels, which increased with increasing levels of drought stress and exposure to AgNPs (Fig. 7). Excessive MDA levels can cause lipid peroxidation, DNA damage, and protein degradation. Further impact is a decrease in the photosynthesis rate due to the reduced chlorophyll in the leaves. Decreased cell membrane fluidity and permeability results in reduced water and nutrient uptake. In addition, AgNPs reduce auxin accumulation and increase ethylene signaling resulting in impaired plant elongation (Yan and Chen, 2019).

Plant resistance parameters

Resistance in plants is significantly related to antioxidant enzymes, also known as defense enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Alici and Arabaci, 2016). Seed treatment and field water capacity significantly affected proline levels, whereas the interaction of the two treatments did not affect changes in proline levels. Seed treatment, field water capacity, and their interactions significantly affected changes in MDA levels. Field water capacity treatment significantly affected CAT enzyme activity, but seed treatment and the interaction had no significant effect. None of the treatments had a significant effect on SOD activity (Table 3).

Proline is an osmolyte compound that regulates the osmosis of plant cells to maintain their turgor when experiencing abiotic stress (Dai et al., 2017). Seed treatment with AgNPs significantly increased the proline levels. AgNP priming increased proline content by 15.9%, and AgNP coating increased proline content by 32.8% (Table 4). Similar results were also reported by Nair and Chung (2015), who reported increasing proline levels in mung bean plants after treatment with AgNPs in the growing medium. This increase indicated that AgNP priming and coating modulate plants to increase the production of proline compounds, thereby increasing soybean plants' adaptation and tolerance to drought stress.

Malondialdehyde (MDA) is a compound formed due to lipid peroxidation, commonly used to represent reactive oxygen species (ROS) to describe the level of oxidative damage experienced by plant cells. The higher the MDA level, the higher the level of damage. In this experiment, AgNP priming increased MDA levels by 80% and AgNP

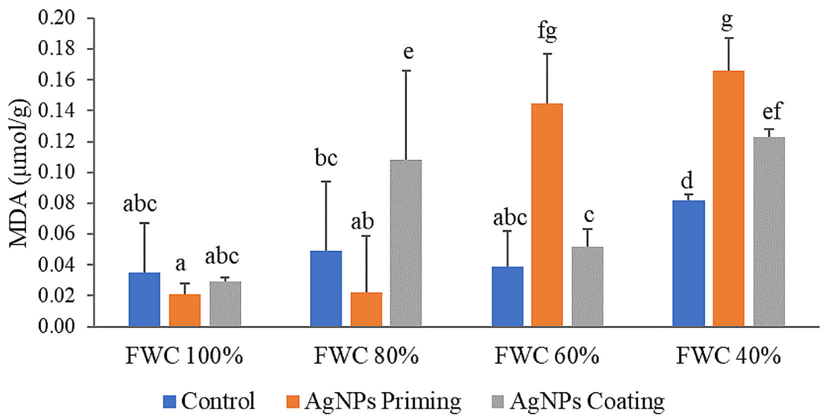

Figure 7. Effect of interaction between seed treatment and field water capacity on MDA content

Table 3. Result of ANOVA of plant resistance parameters

Parameters	Field water capacity	Seed treatment	Interaction	CV (a)	CV (b)
Proline (µmol/g)	0.020*	0.001*	0.141ns	17.6%	15.3%
MDA (µmol/g)	0.011*	0.006*	0.001*	59.8%	33.1%
CAT (µmol/g)	0.005*	0.313ns	0.231ns	39.1%	34.6%
POD (U/mg)	0.260ns	0.096ns	0.011*	41.7%	28.1%
SOD (U/mg)	0.158ns	0.223ns	0.098ns	83.4%	99.2%

Notes: Symbol (*) represents statistical significance and (ns) indicates non-significant. CV: coefficient of variance, MDA: malondialdehyde, CAT: catalase enzyme, POD: peroxidase enzyme, and SOD: superoxide dismutase enzyme.

Table 4. Effect of seed treatment on plant resistance parameters

Parameters	Control	Seed treatment			
		Priming	Deviation (%)	Coating	Deviation (%)
Proline (μmol/g)*	30.18ᵃ	34.99ᵇ	15.9	40.08ᶜ	32.8
MDA (μmol/g)*	0.05ᵃ	0.09ᵇ	80.0	0.08ᵇ	60.0
CAT (μmol/g)	0.03ᵃ	0.03ᵃ	0.0	0.02ᵃ	-33.3
POD (10^{-3} U/mg)	0.45ᵃ	0.58ᵃ	28.9	0.56ᵃ	24.4
SOD (10^{-6} U/mg)	1.06ᵃ	0.60ᵃ	-43.4	0.57ᵃ	-46.2

Notes: Mean ± (SD) values followed by the same letters within each column are non-significantly different based on DMRT ($p < 0.05$). MDA: malondialdehyde, CAT: catalase enzyme, POD: peroxidase enzyme, and SOD: superoxide dismutase enzyme.

coating increased MDA levels by 60% (Table 4). Seed priming and coating with AgNPs significantly increased MDA levels at FWC 60% and 40%. AgNP coating significantly increased MDA levels at 80% and 40% FWC compared with the control (Fig. 7). These results are in line with those of Nair and Chung (2015), who reported an increase in MDA and H_2O_2 levels in the roots of mung bean plants after exposure to silver nanoparticles (20–50 mg/L) through the growing medium.

Table 4 also shows that seed priming and coating with AgNPs increased the activity of POD enzymes, meanwhile, the antioxidant enzymes SOD and CAT tended to decrease their activity, but all were not significantly different from the control. Although the field water capacity treatment and seed treatment had no significant effect, their interaction significantly affected POD enzyme activity (Table 3).

Seed priming and coating with AgNPs increased POD enzyme activity at FWC 100%, but there was a decrease in POD enzyme activity at FWC 80% after AgNP coating treatment. Priming and coating with AgNPs did not significantly affect the changes in POD enzyme at FWC 60% and 40% even though the activity increased compared to the control (Fig. 8). This indicates that the increase in POD enzyme activity at FWC 100% was due to the increase in MDA levels modulated by seed priming and coating with AgNPs. However, its effectiveness was reduced at lower field water capacity due to increasing MDA levels as the stress levels increased (Fig. 7). Jumrani and Bhatia (2019) stated that POD enzyme activity in soybean plants can decrease because of drought stress. In this study, it was found that POD enzymes responded more to seed treatment with AgNPs than SOD and CAT enzymes.

SOD enzyme plays a role in the release of excess superoxide (O_2^-) compounds into oxygen (O_2) and hydrogen peroxide (H_2O_2) during stress. CAT enzyme promotes the decomposition of H_2O_2 into water, thereby preventing oxidative damage caused by ROS in plant cells. The POD enzyme induces the synthesis of salicylic acid and elicits an immune response in plants (Alici and Arabaci, 2016; Jiang et al., 2013).

The reduction in plant biomass observed in this experiment suggests that AgNPs may have

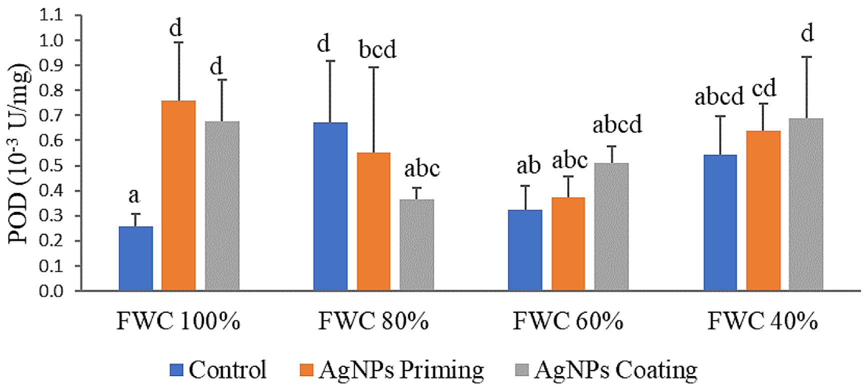

Figure 8. Effect of interaction between seed treatment and field water capacity on POD enzyme activity

toxic effects on plant growth. This negative effect may be due to oxidative damage, as indicated by the increase in MDA levels after seed treatment and drought stress (Fig. 7). K. Kumari et al. (2024) stated that AgNPs can release Ag^+ ions that interfere with the photosynthesis process by replacing Cu^+ in plastocyanin. Ag^+ ions can also bind to DNA, disrupting the respiration process and leading to the formation of reactive oxygen species (ROS).

The effect of AgNPs in this experiment may not be the main cause of the excessive increase in MDA in soybean plants. This increase was probably also influenced by the high temperature in the greenhouse during the experiment, which was 29 °C, with maximum and minimum temperatures of 39.1/23.5 °C. Soybean plants are sensitive to temperature increases and water shortages. Jumrani and Bhatia (2019) reported that MDA levels increased in dry stress media by 5%, 27%, and 60% when the temperature increased from 30/22 °C to 34/24 °C, 38/26 °C, and 42/28 °C, respectively.

In some studies, AgNP treatment on plants has produced two opposite effects. The positive impact of AgNPs can increase plant germination and growth, both morphologically, physiologically, and molecularly, resulting in optimal crop yields (Nile et al., 2022). The negative impact of AgNPs causes toxicity in plant growth, which leads to reduced harvest quantity and quality of the seeds produced (Cox et al., 2016). Several factors, including the size, shape, and concentration of AgNPs, plant species, plant development stage, method, and duration of exposure influence this difference (Yan and Chen, 2019).

The mechanism by which nanoparticles promote plant growth is the maintenance of ROS homeostasis. ROS production is useful in cell division and elongation during plant organ development (Choudhary et al., 2020). Nanoparticles modulate ROS production while maintaining excessive amounts of ROS by stimulating the formation of antioxidant enzymes. If AgNPs are applied to plants at high concentrations, excessive ROS can be produced. If it cannot be controlled by antioxidant enzymes, it causes oxidative stress in the plant cells and tissues. Ansari et al. (2023) reported that foliar application of 10 ppm AgNPs increased the growth and yield of tomato fruit. Conversely, applying AgNPs at a higher concentration (50 ppm) decreased the plant biomass and tomato fruit weight.

CONCLUSIONS

Biosynthesis using the neem leaf extract reductant produced a uniform particle size distribution with a z-average of 55 nm and a polydispersity index of 0.449. The zeta potential of AgNPs of -28.8 mV indicates moderate solution stability, thus they can be stored longer. AgNPs were spherical and contained Ag, C, O, and N elements that formed the structure of the plant-extract-based AgNPs. Seed treatment with AgNPs reduced the shoot dry weight, root dry weight, root volume, and plant height. The decrease in the growth performance of soybean plants during the vegetative phase was due to an increase in MDA levels after treatment. AgNP priming increased MDA levels as drought stress levels increased, while the effect of AgNP coating was more moderate than that of AgNP priming. However, seed treatment with AgNPs enhanced the proline content and activity of POD enzymes to mitigate the adverse effects of drought stress. Based on this study, AgNPs have the potential to be used as a seed treatment to increase plant tolerance to drought stress. In the future, it is recommended that the effects of drought stress and seed treatment with AgNPs be tested on the growth and yield of soybean plants under optimal temperature conditions.

Acknowledgement

The authors express appreciation to the Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research and Technology, Postgraduate Research Scheme for Doctoral Dissertation Research (PDD) on behalf of Prof. Dr. Satriyas Ilyas (Contract No. 18860/IT3.D10/PT.01.03/P/B/2023).

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