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Biosynthesis of silver nanoparticles and effectiveness of seed nanopriming in improving vigor and health of soybean seeds

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Abstract

Soybean seeds deteriorate rapidly and are infected by many seed-borne fungi, which reduce seed quality. Biosynthesized nanoparticles are believed to be an environmentally safe approach for increasing seed quality. This study aimed to develop a biosynthetic formulation of silver nanoparticles (AgNPs) and test its effectiveness in improving the physiological and health qualities of soybean seeds. AgNPs were synthesized by mixing 1 mM AgNO3 solution and neem leaf extract (9:1 v/v). AgNPs characterization was performed using UV-vis spectrophotometry, dynamic light scattering, and transmission electron microscopy. Seed priming treatments were tested for evaluation of seed viability and vigor: control, hydropriming, and AgNPs priming (4, 8, 12, and 15 ppm). The treatments applied for the evaluation of seed health were control, AgNO3 priming (4, 9, 13, and 17 ppm), and AgNPs priming (4, 8, 12, and 15 ppm). The experiments used a completely randomized design. The results showed that the absorbance peak of AgNPs was at λ=414 nm, with a z-average of 43.9 nm and a PDI of 0.322, and the particles were spherical. Seed nanopriming with 15 ppm AgNPs is recommended to improve soybean seed viability and vigor.

Keywords: AgNO3, AgNPs, Azadirachta indica, neem leaf extract, seed-borne fungi.
Abbreviations: GP_germination percentage; MGP_maximum growth potential; DW_dry weight of normal seedlings; RE_radicle emergence; VI_vigor index; SG_speed of germination; T50_median germination time; CV_coefficient of variation; PDI_ polydispersity index; SPR_ surface plasmon resonance; TEM_transmission electron microscopy; NUV_near ultra violet; DMRT_ Duncan Multiple Range Test; ROS_ reactive oxygen species; NaOCl_sodium hypochlorite; DAS_day after sowing; CRD_ completely randomized design.

Introduction

Soybeans are a strategic food commodity with excellent market potential in Indonesia. The need for soybean availability is increasing annually, but productivity still needs to be improved. Production is predicted to decline by 3 percent per year, reaching 0.56 million tons/ha in 2024 (Hulu, 2023). One of the causes of low productivity in soybean cultivation is the use of low-quality seeds.

Farmers prefer to use soybean seeds from non-formal seed production systems that lack clear seed quality.

Low-quality soybean seeds are characterized by low viability and vigor and are heavily contaminated by seed-borne pathogens. The causes of low seed viability and vigor include untimely seed harvesting, physical damage to seeds during processing, and storage conditions that shorten seed life (Manggung et al., 2014; Rao et al., 2023). Seed-borne pathogens can damage and accelerate seed deterioration and are very dangerous if transmitted when planting seeds in the next growing season. Pathogens that interfere with the growth of soybean plants include fungi (Hapsari, 2021), bacteria (Sotelo et al., 2021), and viruses (Purnamawati et al., 2019). Fungi are the dominant seed-borne pathogens that infect soybean seeds (Soesanto et al., 2020).

Seed priming is a pre-planting treatment that enables seeds to undergo physiological changes, allowing for faster and more uniform germination (Devika et al., 2021). There are various types of seed priming, including hydropriming, halopriming, osmopriming, solid matrix priming, thermopriming, and biopriming (Ilyas et al., 2015; Paparella et al., 2015; Nurkartika et al., 2018). Nanopriming is a seed-priming technique that is gaining attention for improving vigor and seed health (Pereira et al., 2021; Kandhol et al., 2022).

Nanopriming is more promising than conventional priming techniques for improving germination, growth, and crop yield (Khalaki et al., 2016; Mahakham et al., 2017; Younis et al., 2019). The advantage of nanoparticles in seed priming is their ability to optimize electron exchange and particle surface reactions associated with various components of plant cells and tissues (Nile et al., 2022). This ability makes nanoparticles capable of modulating plant metabolic systems while eliminating pathogens that infect seeds and plants (Pereira et al., 2021; Kutawa et al., 2021).

Silver nanoparticles (AgNPs) can be synthesized using physical, chemical, and biological methods (Kutawa et al., 2021). Biological synthesis methods can use microorganisms and plant extracts as reducers (Bansal et al., 2015) and are believed to be more environmentally friendly. The advantages of nanoparticle synthesis using biological methods with plant extracts are that they are relatively straightforward to work with, and materials are easily obtained. The basic principle is that plant extracts act as reducers, converting Ag+ ions into uncharged and stable Ag0 (Keat et al., 2015). Secondary metabolites in plants, including phenolic compounds and terpenoids, play an active role in reducing Ag^+ ions (Mahakham et al., 2017). Bio-synthesized AgNPs are enveloped by secondary metabolites from plant extracts, making the nano-particles more stable (Song and He, 2021) and safer for seeds by slowly producing reactive oxygen species (ROS) (Mahakham et al., 2017).

The neem plant (Azadirachta indica) contains abundant secondary metabolites such as azadirachtin and nimbin (Saleem et al., 2018). Almost every part of neem (e.g., the stem, bark, roots, leaves, gum, seeds, fruits, and flowers) can be used as a medicinal and vegetable pesticide (Wylie and Merrell, 2022). During AgNP synthesis, neem extract functions as both a reducing and capping agent, so it needs only small amounts. The capping process contributes to nanoparticle stability, minimizes their toxicity, and strengthens their antifungal activity (El-Kadi et al., 2018; Dutt et al., 2022; Guilger-Casagrande et al., 2022).

The use of plant extracts as a reducer in the synthesis of AgNPs and the priming effect on seeds have been investigated by Mahakham et al. (2017) for rice seeds and by Acharya et al. (2019) for onion seeds. The application of biosynthesized silver nanoparticles using neem extract has been carried out by several researchers (Lalitha et al., 2013; Ahmed et al., 2016; Chinnasamy et al., 2021; Dutt et al., 2022), but no one has tried to use it for nanopriming of soybean seeds. Thus, this study aimed to develop AgNPs using neem leaf extract as a reducing and stabilizing agent and to test their effectiveness through seed nanopriming to increase seed vigor and reduce the number of soybean seeds infected with seed-borne fungi.

Results

Biosynthesis and characterization of silver nanoparticles

The reduction process changed the color of the solution from pale yellow to reddish-brown (Figure 1). The localized surface plasmon resonance (LSPR) pattern forms an absorbance peak, which is an important parameter for successful nanoparticle synthesis. The absorbance peaks of AgNO3, neem leaf extract, and AgNPs were at wavelengths of 300 nm, 330 nm, and 414 nm, respectively (Figure 2).

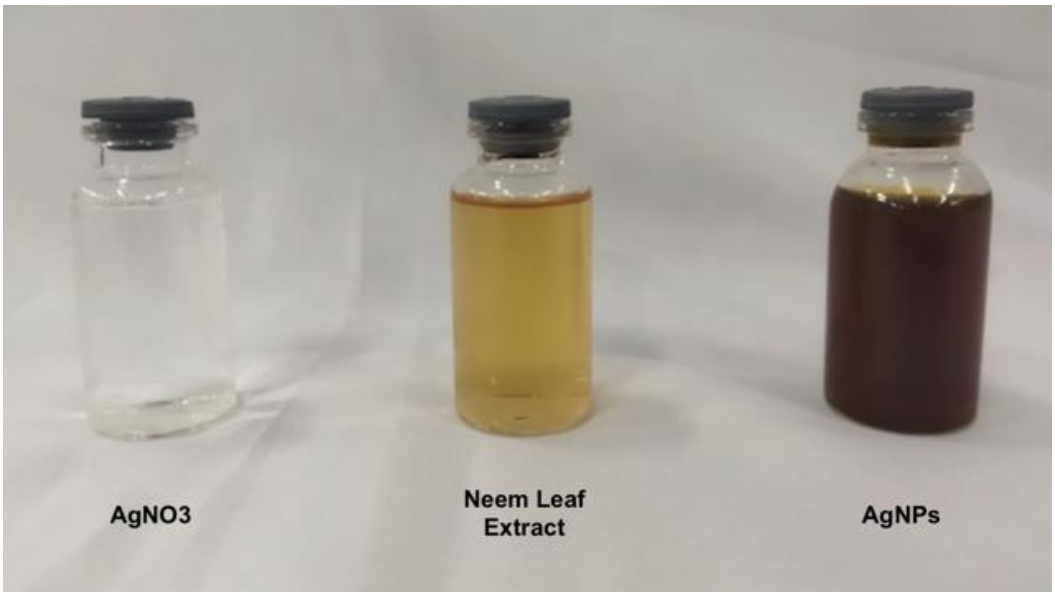

Fig. 1. The color difference of precursor (AgNO3), reducer (neem leaf extract), and synthesized solution (AgNPs).

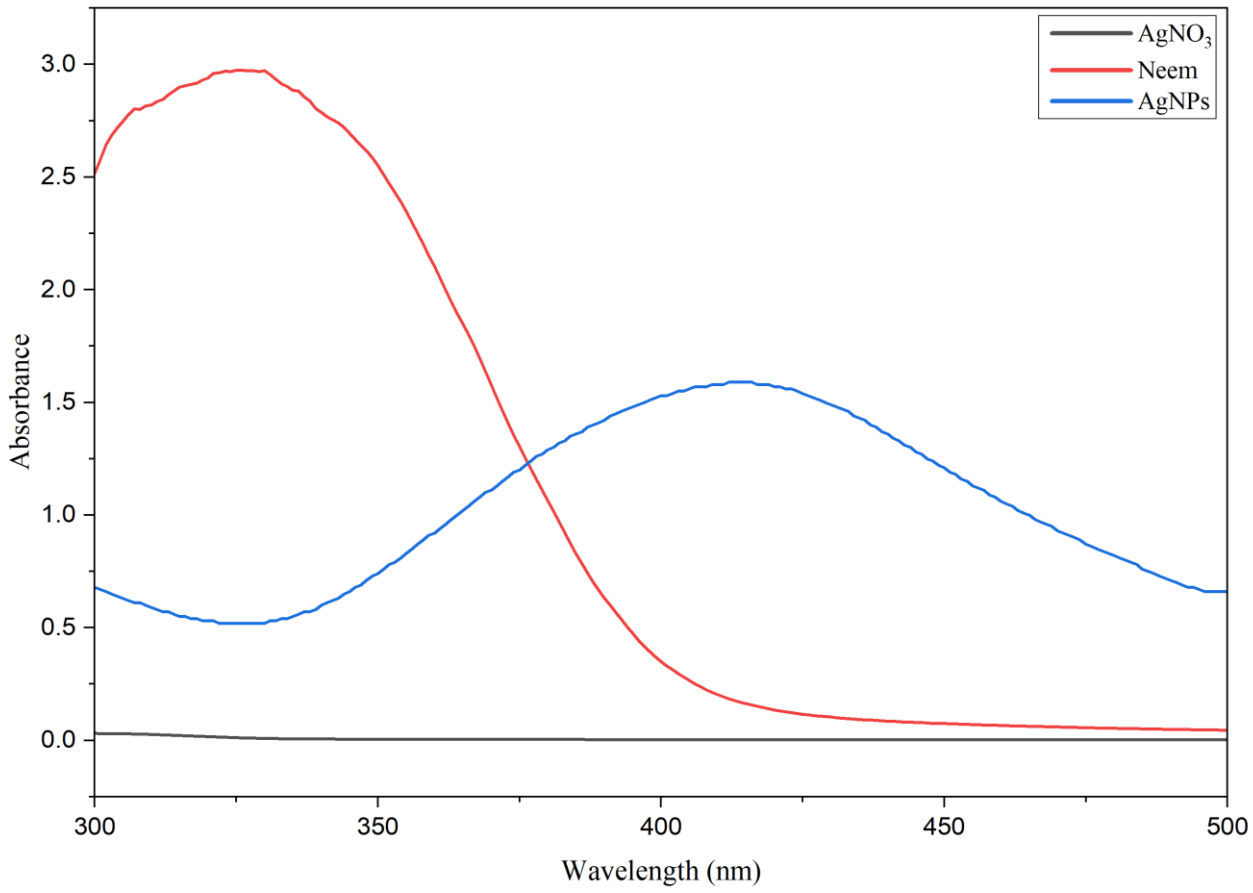

Fig. 2. UV-Vis spectra of AgNO3 solution, neem leaf extract, and AgNPs.

The size distribution of AgNPs showed a diameter of 27.4–118.7 nm with a z-average of 43.9 nm and a polydispersity index of 0.322 (Figure 3), indicating that the AgNPs formed were relatively uniformly dispersed.

Transmission electron microscopy (TEM) was used to identify the size, shape, and crystalline morphology of AgNPs. The analysis showed that the AgNPs were well dispersed and mostly spherical, with a diameter of 9–23 nm (Figure 4).

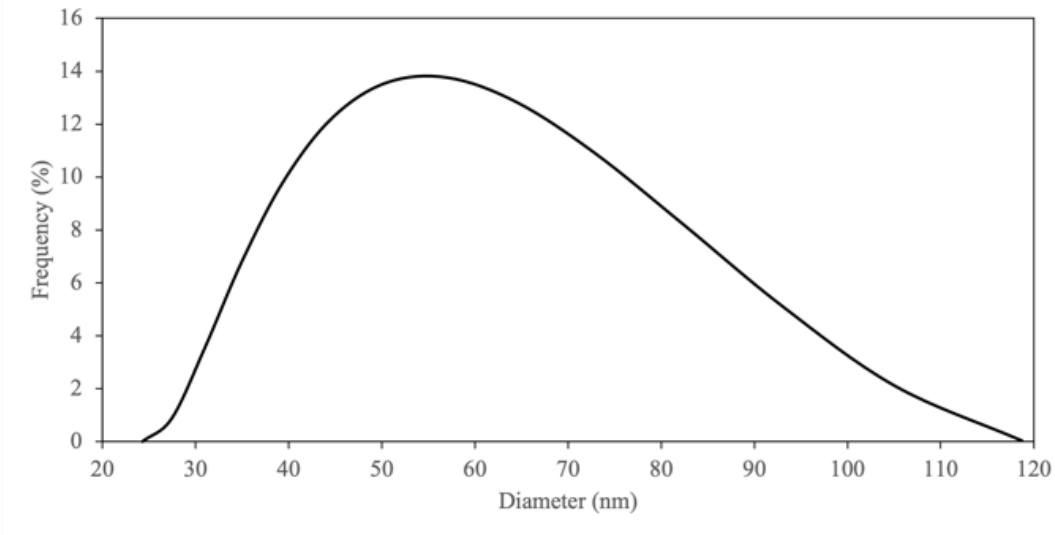

Fig. 3. Size distribution of AgNPs measured by particle size analyzer (PSA).

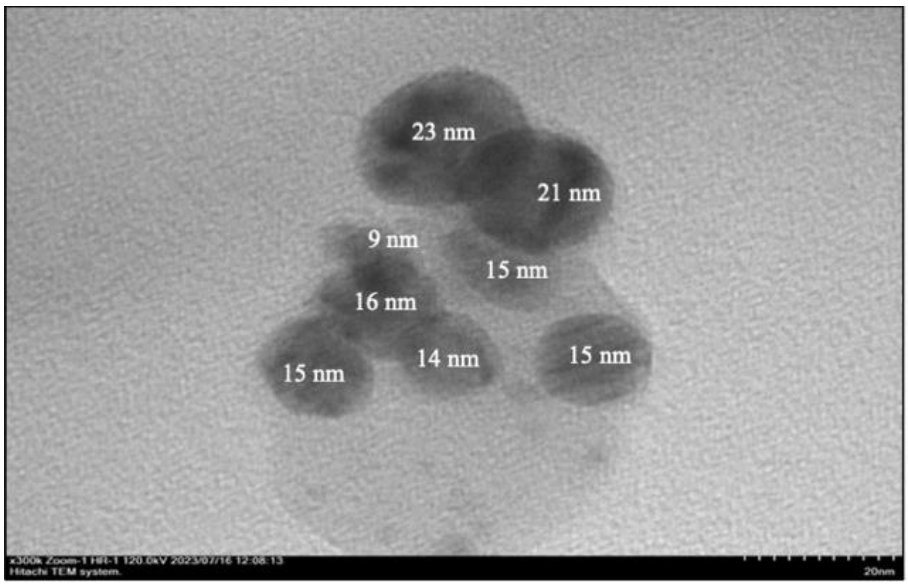

Fig. 4. Morphology of AgNPs using TEM at 300,000x magnification.

Effect of nanopriming on seed viability and vigor

AgNPs priming did not have a significant effect on germination percentage (GP), dry weight of normal seedlings (DW), vigor index (VI), speed of germination (SG), and median germination time (T50). However, priming did not decrease soybean seed viability and vigor. AgNPs priming significantly increased the maximum growth potential (MGP) at 15 ppm concentration (Table 1) and radicle emergence (RE) at 8–15 ppm concentration (Table 2).

Table 1. Effect of seed nanopriming on the viability of soybean seeds.

Seed Priming	Seed Viability	Seed Viability	Seed Viability
Seed Priming	**GP (%)**	**MGP (%)**	**DW (g)**
Control	78.5 ± 3.0	69.5 ± 3.4 c	1.51 ± 0.15
Hydropriming	72.5 ± 5.7	81.0 ± 6.6 ab	1.32 ± 0.14
4 ppm AgNP	74.0 ± 9.1	78.0 ± 7.3 abc	1.27 ± 0.18
8 ppm AgNP	81.0 ± 2.6	74.5 ± 4.4 bc	1.24 ± 0.11
12 ppm AgNP	77.5 ± 11.5	78.0 ± 6.5 abc	1.34 ± 0.23
15 ppm AgNP	75.5 ± 6.0	86.5 ± 3.4 a	1.50 ± 0.13
Significance	ns	*	ns
CV (%)	9.2	7.1	11.8

Means ± SD sharing the same letter in a column do not significantly differ (P < 0.05) by DMRT. Symbol (*) represents statistical significance, and (ns) indicates non-significance. GP: germination percentage, MGP: maximum growth potential, DW: dry weight of normal seedlings, and CV: coefficient of variation

Table 2. Effect of seed nanopriming on the vigor of soybean seeds.

Seed Priming	Seed Vigor	Seed Vigor	Seed Vigor	Seed Vigor
Seed Priming	**RE (%)**	**VI (%)**	**SG (% etmal-1)**	**T50 (day)**
Control	18.5 ± 6.0 b	60.0 ± 5.9	19.0 ± 1.2	4.1 ± 0.4
Hydropriming	20.5 ± 11.1 b	62.5 ± 3.0	17.9 ± 1.5	3.6 ± 0.3
4 ppm AgNP	23.0 ± 15.4 b	61.5 ± 7.0	19.0 ± 0.5	3.6 ± 0.4
8 ppm AgNP	51.0 ± 4.2 a	65.0 ± 4.8	18.3 ± 0.7	3.8 ± 0.4
12 ppm AgNP	44.0 ± 14.3 a	62.0 ± 8.5	18.6 ± 1.0	3.8 ± 0.5
15 ppm AgNP	54.5 ± 10.0 a	65.0 ± 3.5	18.5 ± 2.5	3.7 ± 0.5
Significance	*	ns	ns	ns
CV (%)	20.5	9.2	7.5	11.5

Means ± SD in a column sharing the same letter in a column do not significantly differ (P < 0.05) by DMRT. Symbol (*) represents statistical significance, and (ns) indicates non-significance. RE: radicle emergence, VI: vigor index, SG: speed of germination, T50: median germination time, and CV: coefficient of variation

Compared to the control, hydropriming and 15 ppm AgNPs priming increased the MGP value by 11.5% and 17%, respectively (Table 1). Priming with 4–12 ppm AgNPs did not significantly increase MGP. In other words, 15 ppm AgNPs enhanced the quantity of germinated seeds under optimal field conditions. The RE value was highest in the 15 ppm AgNPs treatment and lowest in the control treatment. Priming with 8–15 ppm AgNPs increased RE value by 25.5–36% compared to the control (Table 2). In this study, AgNPs priming increased the RE of soybean seeds as the AgNPs concentration increased. A high percentage of RE indicated high seed vigor.

Effect of nanopriming on the incidence of seed-borne fungi

Priming treatments significantly affected the incidence of seed infection and contamination by seed-borne fungi. The lowest incidence of fungal infection occurred in the control, whereas the highest incidence occurred in the 8 ppm AgNP priming treatment. The AgNO3 and AgNPs priming treatments significantly increased the incidence of fungal infection compared to the control, but there was no difference between the two treatments at all concentrations. The dominant seed-borne fungi infecting soybean seeds were from the genus Fusarium sp., followed by Mucor sp., Aspergillus sp., Penicillium sp., and Trichoderma sp. (Table 3).

Table 3. Incidence of seeds infected with seed-borne fungi at 7 days after sowing (DAS).

Seed Priming	Fungal Incidence (%) 1)	Seed-Borne Fungi (%)	Seed-Borne Fungi (%)	Seed-Borne Fungi (%)	Seed-Borne Fungi (%)	Seed-Borne Fungi (%)
Seed Priming	**Fungal Incidence (%) 1)**	**Asp**	**Fus**	**Tric**	**Pen**	**Muc**
Control	11.0 ± 21.9 (1.8) c	5	6	0	0	0
4 ppm AgNO3	50.0 ± 0.0 (7.1) ab	4	37	0	1	8
9 ppm AgNO3	43.0 ± 9.7 (7.3) ab	10	31	0	2	0
13 ppm AgNO3	37.0 ± 18.6 (6.0) ab	10	21	0	3	3
17 ppm AgNO3	42.0 ± 33.7 (6.1) ab	3	24	0	0	15
4 ppm AgNP	64.0 ± 18.8 (7.9) ab	2	48	3	0	11
8 ppm AgNP	69.0 ± 31.7 (8.1) a	16	17	2	1	33
12 ppm AgNP	56.0 ± 28.2 (7.3) ab	23	18	0	2	13
15 ppm AgNP	30.0 ± 20.9 (5.1) b	6	23	0	0	1
Mean		8.8	25.0	0.6	1.0	9.3
Significance	*					
CV (%)	30,7					

1) Means ± SD sharing the same letter in a column do not significantly differ (P < 0.05) by DMRT. The numbers in parentheses are the results of square root transformations. Symbol (*) represents statistical significance. Asp: Aspergillus sp., Fus: Fusarium sp., Tric: Trichoderma sp., Pen: Penicillium sp., Muc: Mucor sp., and CV: coefficient of variation

Discussion

The synthesis of nanoparticles causes a color change in the resulting solution. The color change during synthesis occurs because of the LSPR excitation of the AgNPs (Mahakham et al., 2017). AgNPs solutions have characteristic LSPR patterns depending on the particle characteristics, such as the size, shape, and dielectric properties of the synthesized materials (Hemlata et al., 2020). The resonance pattern forms an absorbance peak, which is an important parameter for successful nanoparticle synthesis. Fajri et al. (2022) reported that AgNPs have an absorbance peak in the 400–450 nm wavelength range. In this study, biosynthesis produced AgNPs with an absorbance peak at 414 nm. In contrast, Ahmed et al. (2016) revealed that the absorbance peak of AgNPs synthesized with a neem extract reductant was approximately 436–446 nm. Differences in absorbance peaks can occur depending on the concentration of the

AgNO3 precursor, the amount of plant extract, and incubation time. The wavelength increased with increasing leaf extract concentration. The peak absorption intensity increased with increasing silver nitrate salt concentration and incubation time (Ahmed et al., 2016; Hemlata et al., 2020). The LSPR pattern was also related to the spherical shape of the AgNPs (Figure 4) (Jyoti et al., 2016).

The size distribution (z-average) of the AgNPs was 43.4 nm, which is similar to that reported by Kumari et al. (2022), who produced AgNPs using neem leaf extract with an average size of 38.5 nm. AgNPs synthesized using mimba extract produced a relatively small average size. Hemlata et al. (2020) produced AgNPs using Cucumis prophetarum leaf extract with an average size of 90 nm, and Arulnangai et al. (2025) produced AgNPs using Centella asiatica leaf extract with an average size of 132.8 nm. Phytochemicals in the neem extract contribute to the hydrodynamic size of AgNPs. The hydrodynamic size is determined by the accumulated size of the metal core, capping agent, and electrical double layer between particles. Different phytochemical compounds present in plant extracts are responsible for the formation of particles of various sizes (Hemlata et al., 2020). A polydispersity index (PDI) of 0.338 indicated that the particle size distribution was relatively uniform. PDI values ranged from 0 to 1, indicating the diversity of the particle sizes formed. An immense PDI value has a diverse particle size distribution, and the particles are easily aggregated and sedimented. A small PDI value indicates well-dispersed particles.

AgNPs were tested on soybean seeds using seed priming techniques to determine their effects on seed viability and vigor. This test was conducted to predict the ability of soybean seeds to grow normally when planted in the field. The germination percentage (GP), a routine test indicator of seed viability, was not improved by AgNPs priming. However, priming with 15 ppm AgNPs increased the maximum growth potential (MGP) of soybean seeds. MGP was defined as the percentage of all seeds that germinated until the last observation in a seed germination test (Haq et al., 2023). A higher MGP than the control confirmed that the AgNP treatment optimized energy use for the germination process. Song and He (2021) reported that nanoparticles can protect chloroplasts from deterioration and extend chloroplast photosynthesis time by increasing antioxidant enzyme activity. Reinforced by Mahakham et al. (2017), dehydrogenase activity increased in seeds treated with AgNPs priming compared to those without AgNPs priming.

Seed vigor is a concept that describes several characteristics that determine seed quality and the potential for plant uniformity in a field with a wide range of environmental variables (Finch-Savage and Bassel, 2016). Priming with 8–15 ppm AgNPs increased soybean seed vigor due to radicle emergence. This test was conducted to predict soybean seed vigor by observing radicle emergence of at least 2 mm during early seedling growth (Astuti et al., 2020); thus, seed vigor could be predicted quickly. This increase in radicle emergence might be caused by faster water absorption. Feizi et al. (2013) reported that AgNP priming for a particular duration produces hydroxyl radicals (OH-) that loosen the seed coat cells and induce seed germination. Subsequently, Mahakham et al. (2017) reported that AgNPs induced the formation of hydroxyl radicals and increased aquaporin gene expression, thus accelerating water absorption and initiating enzymatic processes, such as carbohydrate degradation.

The effect of AgNPs on the sporulation of soybean seed-borne fungi was also tested using priming techniques. In general, fungal sporulation continued in seeds incubated for seven days, regardless of whether the seeds were primed with AgNO3 or AgNPs. Although priming with AgNPs did not suppress the incidence of fungus compared to the control, the incidence of fungus at 15 ppm AgNP was lower than that at other AgNP concentrations. Seed-borne fungi can have a negative impact on seed quality, reduce germination percentage, and contribute to seed deterioration. The germination percentage of soybean seeds decreased by 6–48% during storage due to infection by seed-borne fungi (Olszak-Przyby´s et al., 2024).

Fungal sporulation was higher in seeds primed with AgNO3 and AgNPs than in the control, presumably because of the compounds released by the seeds during priming that are beneficial for fungal growth. Ajmal et al. (2022) reported that nutrients, such as microelements, carbon, and nitrogen, induce fungal sporulation. The effect of AgNO3 and AgNP treatments, which were not significantly different, may be due to the wide distribution of AgNP particle size, making them less effective in controlling fungal growth. Akpinar et al. (2021) suggested that the growth of Fusarium oxysporum mycelium was most effectively suppressed by AgNPs measuring 3 nm. Similarly, Azzaz et al. (2017) reported that AgNPs with a diameter of 13.5 ± 2.6 nm are effective against Candidus utilis.

Materials and Methods

Plant materials

Grobogan variety of soybean seeds was used in this trial, one of the Indonesian varieties of soybean, with a moisture content of 9.2% and a germination rate of 89% on the seed quality test label.

Biosynthesis and characterization of silver nanoparticles

The neem leaves were washed with water until clean, and then cut into small pieces. The leaf pieces were boiled for 30 min with aquabidest in an Erlenmeyer flask (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 AgNO3 was prepared by dissolving 0.17 g of AgNO3 in 1000 mL of distilled water. Analytical-grade silver nitrate (AgNO3) was obtained from Merck (Germany).

Silver nanoparticles (AgNPs) were synthesized by mixing 450 mL of 1 mM AgNO3 solution with 50 mL of neem leaf extract at a 9:1 ratio (Hemlata et al., 2020). The solution mixture was stirred using a hotplate magnetic stirrer at 60°C (Ansari et al. 2023) for 20 min (Oraibi et al. 2022) until the solution turned reddish-brown.

AgNPs characterization was conducted at the Center for Nanoscience and Nanotechnology, Institut Teknologi Bandung. The absorbance spectrum of the AgNPs was measured using a UV-Vis spectrophotometer (Thermo Scientific Evolution 220). The particle size distribution of the AgNPs was measured using a particle size analyzer (Horiba SZ-100). The crystalline structure of the AgNPs was analyzed using a transmission electron microscope (TEM) (Hitachi HT7700). AgNPs diameter size based on TEM was measured using ImageJ.

Effect of nanopriming on seed viability and vigor

Experimental design

The experiment was conducted using a completely randomized design (CRD) with four replicates. The priming treatments included an untreated control, hydropriming, and AgNP priming. Hydropriming used distilled water, and AgNPs were used at concentrations of 4, 8, 12, and 15 ppm. The concentration used is based on Ansari et al. (2023), who reported that AgNP priming concentrations below 20 ppm are safe for seed viability and vigor.

Treatments and observation

The seeds were sterilized with 1% NaOCl for 1 min and then washed three times with distilled water. The seeds were soaked in the AgNP solution for 6 h at 25°C and air-dried to the initial moisture content level for 48 h at 20°C. The ratio of seeds to AgNP solution was 3:10 (w/v). Germination was tested using the between-paper test method, incubated in an ecogerminator (IPB 72-1 type) at 25 ± 2°C, with 50 seeds used for each replicate. Seed viability and vigor were evaluated based on germination percentage (GP), maximum growth potential (MGP), speed of germination (SG), vigor index (VI), median germination time (T50), and dry weight of normal seedlings (DW).

GP was calculated based on the percentage of normal seedlings on the fifth and eighth days. DW was calculated as the dry weight of all normal seedlings at the final count. MGP was calculated based on the percentage of normal and abnormal seedlings until the final count (eighth day). The VI was calculated based on the percentage of normal seedlings in the first count (fifth day). SG was determined based on the number of normal seedlings that could germinate every 24 h (etmal) during the standard seed germination test (eight days). SG was calculated using the following formula (Haq et al., 2023):

$$SG = \sum_0^t (\%NS / etmal) \quad (1)$$

where SG is the speed of germination, t is the observation time, %NS is the percentage of normal seedlings at each observation time, and etmal is converted from observation time every 24 h.

T50 is the time required for 50% of the total seed population to germinate. The value was determined using the formula described by Farooq et al. (2005):

$$T_{50} = t_i + [(N/2 - n_i)(t_j - t_i)] / (n_j - n_i) \quad (2)$$

where N is the final number of germinating seeds, and nj and ni are the cumulative number of seeds germinated by adjacent counts at times tj and ti, respectively, when $n_i < N/2 < n_j$.

The RE test was performed using the top-on-paper method. The seeds were sown on three layers of moist paper, covered with two layers of moist paper, placed in a plastic box, and germinated at 25 ± 2°C. Each replicate contained 50 seeds. The RE value was the number of seeds with radicles ≥ 2 mm long (ISTA, 2018), and observations were made 42 h ± 15 min after sowing (Astuti et al., 2020).

Effect of nanopriming on seed health

Experimental design

The experiment was conducted using a CRD with five replicates. The priming treatments included untreated control, AgNO3 priming (4, 9, 13, and 17 ppm), and AgNP priming (4, 8, 12, and 15 ppm).

Treatments and observation

The seeds were placed on three layers of moist pμμaper in petri dishes, each containing 20 seeds. Seed health tests were performed using the deep-freezing blotter method. The seeds were incubated at 25°C for 24 h under alternating cycles of 12 h NUV light and darkness, then at -20°C for 24 h, and returned to alternating cycles of 12 h NUV light and darkness for five days. The incidence of seed-borne fungi was observed.

Statistical analysis

Data were analyzed using Microsoft Excel 2021 and IBM SPSS Statistics 22.0. Analysis of variance was employed to analyze experimental data, and significant treatment

means were examined using Duncan Multiple Range Test (DMRT) at a probability level of 0.05.

Conclusion

AgNPs were successfully synthesized using neem leaf extract, with an absorbance peak of λ=414 nm, z-average of 43.9 nm, PDI of 0.322, and spherical particles. Seed priming with 15 ppm AgNPs increased soybean seed viability due to the maximum growth potential and seed vigor due to radicle emergence. Therefore, seed priming with 15 ppm AgNPs is recommended for improving the viability and vigor of soybean seeds.

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Statements of Contributions

Conceptualization: SA, SI, TAD, and AM; Data curation: SA; Funding acquisition; SI, TAD, EW, and SA; Investigation: SA; Project administration: SA, SI and TAD; Supervision: SI, TAD, EW, AQ and AM; Validation: SI, TAD and AM; Visualization: SA; Writing - original draft: SA; Writing - review and editing: SA, SI and TAD. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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