



Biogenic Silver Nanoparticles Synthesized Using *Rivina humilis* Leaf Extract Suppress *Ganoderma boninense* in Oil Palm Seedlings

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Abstract

Basal stem rot (BSR) caused by *Ganoderma boninense* severely constrains global oil palm productivity, while conventional chemical fungicides often lack efficacy and cause negative ecological impacts, necessitating sustainable alternative control strategies. Therefore, this study aimed to biogenically synthesize silver nanoparticles (AgNPs) using *Rivina humilis* leaf extract (AgNPs-Rh) and evaluate their antifungal activity. AgNPs-Rh were characterized using UV-Vis spectroscopy, a particle size analyzer (PSA), gas chromatography–mass spectrometry (GC–MS), and Fourier-transform infrared spectroscopy (FTIR). *In vitro* antifungal activity was assessed via the poisoned plate method. *In vivo* efficacy was evaluated on oil palm seedlings inoculated with *G. boninense* at 4 months of age and subsequently treated with AgNPs-Rh at initial formulation concentrations of 250, 500, and 1,000 ppm. Results confirmed the successful biogenic synthesis, showing a surface plasmon resonance peak at 415 nm and an average hydrodynamic size of 77.3 nm with a polydispersity index of 0.308. FTIR and GC–MS analyses identified secondary metabolites, including phytol, as key stabilizing agents. *In vitro* assays demonstrated that 100 ppm AgNPs-Rh inhibited radial growth by 58.07%. *In vivo*, the 250 ppm application optimally suppressed BSR, reducing disease incidence and severity to 0% while significantly enhancing stem diameter and restoring total leaf chlorophyll content. In conclusion, AgNPs-Rh exhibit significant antifungal efficacy against *G. boninense*. This stable, biogenic formulation demonstrates strong potential as a highly promising, eco-friendly biocontrol alternative to synthetic fungicides for BSR management in oil palm nurseries.

Keywords: antifungal activity; basal stem rot; green synthesis; nursery disease management; phytopathogen control

Cite this as: Saragih, W. S., Dewi, R. R., & Wagino. (2026). Biogenic Silver Nanoparticles Synthesized Using *Rivina humilis* Leaf Extract Suppress *Ganoderma boninense* in Oil Palm Seedlings. *Caraka Tani: Journal of Sustainable Agriculture*, 41(4), 605-620. doi: <http://dx.doi.org/10.20961/carakatani.v41i4.123069>

INTRODUCTION

The palm oil industry is an essential part of the agricultural sector in Indonesia, making a significant contribution to the national economy (Purnomo et al., 2020). From upstream to downstream, this industry plays an important role in creating jobs, driving economic growth, contributing to non-oil and gas export earnings,

and supplying biofuels and biodiesel in Indonesia (Kutawa et al., 2021). Despite its economic importance, the sector is increasingly threatened by outbreaks of the soil-borne pathogen *Ganoderma boninense*, the causal agent of widespread basal stem rot (BSR; Rebitanim et al., 2020). Initially, infected palms may continue to

* Received for publication July 3, 2026

Accepted after corrections September 25, 2026

produce fruit, but they eventually wither and die (Jazuli et al., 2022), leading to a drastic decline in productivity and threatening the sustainability of production (Khoo and Chong, 2023).

In Bilah Hulu Sub-district, BSR infection across 4,901 ha of smallholder plantations led to a 26.75% decline in annual production (Harefa et al., 2023). As the greatest constraint for oil palm crops in Southeast Asia, BSR can damage approximately 80% of plants during the nursery and production stages (Flood et al., 2022), with incidence rates in Indonesia projected to rise significantly by 2050 if inadequately managed (Paterson, 2023; 2025). The pathogen's facultative saprophytic characteristics allow it to persist for long periods in plant debris, making effective control challenging (Aziz et al., 2021).

Current strategies for managing *G. boninense*, including physical, mechanical, chemical, and biological methods, have not yielded significant long-term results (Tan et al., 2021). Furthermore, the extensive use of chemical fungicides is often ineffective and poses several negative impacts on the environment and human health (Siddiqui et al., 2021). This necessitates the development of innovative, environmentally friendly, and sustainable approaches (Khan et al., 2023; Karunarathna et al., 2024). One highly promising approach is the application of nanotechnology, specifically the green synthesis of biogenic silver nanoparticles (AgNPs) using plant extracts as a delivery system.

The rationale for selecting AgNPs over other types of metallic nanoparticles, such as gold (AuNPs), copper (CuNPs), or zinc (ZnNPs), is driven by both scientific efficacy and economic viability. While gold nanoparticles exhibit excellent biocompatibility, they lack the strong intrinsic antifungal properties required to combat aggressive agricultural pathogens. Conversely, AgNPs release Ag^+ ions that effectively disrupt fungal cell membranes and respiratory enzymes. Although silver precursors are relatively more expensive than those of other metals, the overall application is highly cost-effective. AgNPs possess a multi-targeted antifungal mechanism, allowing them to be highly efficacious even at extremely low doses (micro-quantities). Furthermore, green synthesis significantly cuts production costs by eliminating the need for expensive chemical stabilizers. Compared with the massive economic losses caused by BSR, this minimal-dose intervention is highly advantageous.

According to Sameeh (2023), green-synthesized nanoparticles ranging from 1 to 100 nm in size are environmentally friendly and capable of improving the physical and chemical properties of soil, thereby significantly enhancing plant growth. The extremely small size of bionanofungicides facilitates their effective penetration into and interaction with targeted pathogens, thereby improving inhibitory efficiency. Riya et al. (2023) reported that *Rivina humilis* contains high levels of phytoconstituents and bioactive compounds with strong antioxidant activity. AgNPs from *R. humilis* have shown antibacterial activity against *Brucella* species (*B. abortus*, *B. melitensis*, *B. suis*) at a concentration of $800 \mu\text{g ml}^{-1}$.

While previous studies have demonstrated the antibacterial efficacy of *R. humilis*-derived silver nanoparticles (AgNPs-Rh), such as against *Brucella* species (Raghava et al., 2021), there are currently no reports evaluating their specific antifungal potential against *G. boninense*, particularly for applications in oil palm seedlings. Biological methods have gained significant attention due to their synthesis efficiency, environmental compatibility, and the good performance of nanoparticles against phytopathogens (Dávila Costa and Romero, 2025).

Another study by Khatun et al. (2023) reported advancements in nanotechnology, exploring various nanoparticles, including plant-derived nano-sized biofungicides. Previous studies have broadly demonstrated the general antimicrobial capabilities of plant-mediated AgNPs, including significant antibacterial activity (Tessema et al., 2023). Building on this fundamental premise, recent scientific interest has increasingly focused on their antifungal potential, which is highly critical for managing aggressive agricultural phytopathogens such as *G. boninense*. The use of AgNPs synthesized using *Azadirachta indica* leaf extract inhibited the fungi *Sclerotinia sclerotiorum*, *Colletotrichum falcatum*, *Colletotrichum gloeosporioides*, and *Rhizoctonia solani* by 68 to 80% (Singh et al., 2024). Furthermore, bionanofungicides have been applied to infected plants to suppress infection, induce the plant defense system, improve delivery system efficiency, control the release of active ingredients, and prevent environmental damage (Kutawa et al., 2021).

The direct use of crude secondary metabolites from *R. humilis* has shown limited effectiveness, largely due to their relatively larger molecular and

structural forms. Moreover, plant extract-based bionanofungicides remain underutilized for the *in vivo* control of *G. boninense* in oil palm, despite the higher surface-to-volume ratio of AgNPs (1 to 100 nm), which enhances reactivity and antimicrobial efficiency (Laib et al., 2025). Currently, there are no reports evaluating their specific antifungal potential against *G. boninense*, particularly for *in vivo* application in oil palm seedlings. Therefore, this study aims to evaluate the suppressive effect of biogenic AgNPs-Rh synthesized from *R. humilis* leaf extract against the pathogenic activity of *G. boninense* in oil palm seedlings, offering a sustainable bionanofungicide alternative for BSR management.

MATERIALS AND METHOD

Experimental location and materials

This study was conducted from June to December 2025 at the experimental plots of the Medan City Department of Food Security, Agriculture, and Fisheries and the Biology Laboratory of Universitas Tjut Nyak Dhien, North Sumatra, Indonesia. Fresh *R. humilis* leaves (5,000 g) were collected from the PT Socfindo Indonesia oil palm plantations. The leaves were thoroughly washed and dried in an oven at 40 °C for 144 hours, reaching a final moisture content of 7.74%. The dried leaves were then pulverized into a crude powder, with an average bulk density of 0.4 g ml⁻¹ and a final moisture content of 6.01%.

For the extraction process, 200 g of the crude powder was macerated in 2,000 ml of 70% ethanol at a 1:10 (w/v) ratio in each of 6 separate glass bottles for 72 hours. The macerated mixtures were filtered through Whatman No. 1 filter paper, and the combined filtrates were concentrated using a rotary evaporator at 40 °C and 130 mbar, followed by further concentration in a water bath at 50 °C under atmospheric pressure. The extraction process yielded a concentrated extract of 14.75%, based on the total initial weight of the crude powder used.

The concentrated extract was subsequently used for the synthesis of AgNPs (Raghava et al., 2021). Aqueous *R. humilis* filtrates at concentrations of 250, 500, and 1,000 ppm were prepared by dissolving 250, 500, and 1,000 mg of the concentrated extract, respectively, in 1 l of double-distilled water, followed by magnetic homogenization and vacuum filtration. The chemical reagents used for nanoparticle synthesis, including silver nitrate (1 mM AgNO₃) as the Ag⁺ ion precursor for the formation of AgNPs,

sodium hydroxide (0.1 M NaOH) for pH adjustment, trisodium citrate dihydrate (10 mM TSC·2H₂O) to facilitate nanoparticle formation and stabilization, and polyvinylpyrrolidone (10% PVP K30) as a steric stabilizer, were of analytical grade (Merck).

The *G. boninense* isolate was obtained from the Plant Pathology Laboratory collection at the Faculty of Agriculture, Universitas Sumatera Utara. To prepare the inoculum, rubberwood blocks (RWB) measuring 6 cm × 6 cm × 6 cm were sterilized by autoclaving at 121 °C for 20 minutes. The sterile blocks were then aseptically inoculated with active *G. boninense* cultures on potato dextrose agar (PDA) media. The blocks were sealed in sterile containers and incubated for 4 weeks until the entire wood surface was visibly covered with white mycelium, serving as the criterion for full colonization.

Oil palm seedlings were transplanted from the nursery into 40 cm × 50 cm polybags in the main nursery at 3.5 months of age. After an adaptation period, *G. boninense* inoculation was conducted when the seedlings were 4 months old using a direct root-contact technique. Specifically, a fully colonized RWB was placed 10 cm above the base of the polybag, and the seedling roots were positioned directly on top of the RWB to ensure physical contact before being covered with soil.

The first AgNPs-Rh treatment was administered one day after inoculation, when the seedlings were 4 months old. Subsequent applications were conducted at 2-week intervals for 3 months, with the final application performed at week 12. The final assessment of seedling growth, disease incidence, and disease severity was conducted 3 months after the first AgNPs-Rh application, when the seedlings were approximately 7 months old.

Biogenic synthesis of silver nanoparticles

The biogenic synthesis of silver nanoparticles (AgNPs-Rh) was conducted using *R. humilis* filtrates at 3 initial concentrations (250, 500, and 1,000 ppm). For each formulation, 200 ml of the respective filtrate was adjusted to pH 6.0 by adding a few drops of 0.1 M NaOH, then mixed with a 1 mM AgNO₃ solution at a 1:4 (v/v) filtrate-to-AgNO₃ ratio. Subsequently, 30 ml of 10 mM TSC·2H₂O was added gradually, and the mixture was continuously stirred at 300 to 400 rpm and 40 °C. As the solution developed a brown color, 10 ml of 10% PVP K30 was gradually added as a steric stabilizer. Stirring was continued

until the characteristic final color of each formulation was obtained. The final color became progressively darker with increasing initial filtrate concentration, appearing light golden-brown in AgNPs-Rh 250, golden-brown in AgNPs-Rh 500, and dark golden-brown in AgNPs-Rh 1000. The resulting formulations were designated as AgNPs-Rh 250, AgNPs-Rh 500, and AgNPs-Rh 1000, according to the initial concentration of the *R. humilis* filtrate. The suspensions were stored as prepared and used directly for subsequent characterization and biological assays without purification.

Characterization of AgNPs

Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was performed on the post-synthesis supernatant following centrifugation to detect residual bioactive metabolites potentially involved in the reduction and stabilization of AgNPs. Rather than definitively confirming the capping mechanism, this analysis demonstrates which compounds remain detectable in solution and potentially contribute to stabilization. The separation was conducted using a Shimadzu GC–MS–QP2010 equipped with a DB-5ms capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed from 60 to 280 °C at a rate of 10 °C minute⁻¹. A 1 µl sample was injected in splitless mode, using helium (He) as the carrier gas at a constant flow rate of 1.0 ml minute⁻¹. Mass spectra were acquired under electron ionization at 70 eV across a mass range of *m/z* 40 to 500. Compounds were identified based on their ion fragmentation patterns and relative molecular masses by comparison with the NIST 14 database (matching threshold > 80%), and their relative abundances were calculated from peak areas (Fu et al., 2021).

Fourier-transform infrared spectroscopy (FTIR)

FTIR analysis of the nanoparticle solution was performed using the KBr pellet method. The spectrum was recorded at a resolution of 4 cm⁻¹, and the scanning range was set from 400 to 4,500 cm⁻¹ (Çolak, 2025). A dry sample of 0.002 g was ground with 0.198 g of KBr and pressed into a thin disc. Subsequently, the disc was placed in an FTIR sample holder and analyzed using

a Shimadzu IR Prestige-21 instrument (Japan). The spectrum obtained was compared with the standard FTIR table. Functional group identification for AgNPs-Rh was performed with reference to the Sigma-Aldrich IR spectrum table.

Particle size analyzer (PSA)

The particle size, distribution, and polydispersity index (PDI) of the synthesized nanoparticles were evaluated using a Horiba SZ-100-Z particle size analyzer (PSA, Japan) through the dynamic light scattering (DLS) method. A 1.5 ml aliquot of the AgNPs-Rh solution from *R. humilis* was transferred into a cuvette and analyzed at a scattering angle of 90°, a temperature of 25 °C, and a viscosity of 0.895 cP (Serunting et al., 2024).

Antifungal *in vitro* assay against *G. boninense*

The experiment was conducted using a completely randomized design with the poisoned plate method (Erhonyota et al., 2023) on PDA medium. The treatment concentrations used were 0 (control), 20, 40, 60, 80, and 100 ppm, with 5 replicates per treatment. Fungal mycelium of *G. boninense* was collected using a 0.5-cm-diameter cork borer and placed at the center of a 90-mm petri dish containing PDA medium. The plates were incubated at 30 °C. Colony diameter was measured on day 3 (H3; 72 hours), day 6 (H6; 144 hours), and day 9 (H9; 216 hours), with the final observation conducted when the colonies in the control treatment reached the edge of the petri dish. Each independently inoculated petri dish was considered one biological replicate and served as the experimental unit. No separate technical replicates were performed; therefore, the 5 petri dishes per treatment represented 5 biological replicates rather than 5 technical measurements from a single experimental unit. Subsequently, the diameter of the colonies was measured every 2 days and stopped once the pathogen colonies in the control filled the petri dish. The percentage inhibition of radial growth (PIRG) was calculated using Equation 1 (Darlis et al., 2023).

In vivo evaluation of oil palm seedlings

The main nursery trial was divided into 2 blocks. These included the AgNPs-Rh block, comprising NC (Negative Control: plants without inoculation), PC (Positive Control),

$$\text{PIRG (\%)} = \frac{r_1 - r_2}{r_1} \times 100\% \quad (1)$$

Where PIRG is the inhibition percentage, *r*₁ is the fungal colony growth in the control, and *r*₂ is the fungal colony growth in AgNPs-Rh treatment.

$$DI (\%) = \frac{\text{Number of seedlings identified as diseased}}{\text{Total number of seedlings per treatment}} \times 100\% \quad (2)$$

$$DSI (\%) = \frac{\sum(A \times B)}{\sum n \times 5} \times 100\% \quad (3)$$

Where A is the disease scale (score 0-5), B is the number of seedlings showing the disease scale per treatment, n is the total number of plants observed, and 5 is the constant representing the highest score.

Table 1. Pathogen infection scores in oil palm seedlings, adapted from Rakib et al. (2015)

Score	Symptoms on oil palm seedling
0	Healthy plants or normal leaves
1	One to three leaves exhibiting chlorosis
2	Appearance of fungal mass, leaves may show chlorosis or early necrosis
3	> 3 chlorotic or necrotic leaves, with or without visible fungal growth
4	Severe chlorosis or necrosis on at least 50% of the leaves
5	Dead plants with or without fungal mass

R1 (250 ppm), R2 (500 ppm), and R3 (1,000 ppm). Meanwhile, the synthetic fungicide block served as a comparative treatment using Anvil 50 SC (Syngenta; active ingredient: hexaconazole 50 g l⁻¹, formulation: suspension concentrate). This block consisted of NC, PC, F1 (0.375 ml l⁻¹), F2 (0.5 ml l⁻¹), and F3 (0.75 ml l⁻¹). These concentrations were selected based on the manufacturer's label recommendations (0.5 to 1.0 ml l⁻¹ for horticultural crops) and on reports of the efficacy of hexaconazole against *G. boninense* in mature oil palm via trunk injection and soil drench methods. Application to the plants was carried out by watering 100 ml per plant around the base of the stem at 2-week intervals. There was no fertilizer treatment. Each treatment consisted of 5 replicate seedlings (n = 5). During the observation period, one replicate in the PC of the fungicide block died due to severe *Ganoderma* infection (score 5) before the final observations, resulting in n = 4 for the final growth and physiological assessments in that specific group. Disease incidence (DI) and disease severity index (DSI) were assessed 3 months after the first AgNPs-Rh application (3 MAA) and calculated according to Rakib et al. (2015) using Equation 2.

The DSI was assessed based on visually observable symptoms (Table 1) using the numerical values of the disease class (Rakib et al., 2015), as shown in Equation 3. The growth parameters for oil palm seedlings were plant height, number of leaves, stem diameter, and total chlorophyll content.

Histopathological analysis

The basal stem condition of the selected seedlings was documented macroscopically. Root

samples from the corresponding seedlings were fixed in FAA solution for 18 to 24 hours. The root samples were subsequently washed with 70% ethanol for 15 minutes and rinsed under running water for another 15 minutes. They were embedded in an appropriate medium and sectioned using a cryostat at a thickness of 6 to 8 µm. Afterward, the sections were stained with a single safranin dye for approximately 16 hours, washed with 70% ethanol for 30 minutes, and rinsed in water for 30 minutes. Finally, the stained sections were mounted on glass slides, sealed with coverslips, and examined under a microscope using 4×, 10×, and 40× objective lenses.

Statistical analysis

Polynomial regression was conducted using Minitab® statistical Software (2026) to evaluate the relationship between AgNPs-Rh concentration and PIRG (%) in the *in vitro* assay. Linear, quadratic, and cubic models were compared using replicate-level observations. *In vivo* plant growth parameters were analyzed using analysis of variance (ANOVA), and significant differences among treatment means (*p* < 0.05) were further analyzed using Tukey's HSD test.

RESULTS AND DISCUSSION

AgNPs were synthesized by introducing *R. humilis* extract into an AgNO₃ solution as an additional reactant. The reduction process was driven by continuous stirring. Nanoparticle formation was initiated as the solution progressively transitioned from yellow to light brown and reddish-brown within 30 minutes,

showing the successful synthesis of AgNPs-Rh. Similar observations have been reported in previous studies using various plant extracts, including *Citrus aurantifolia* fruit (Mustapha et al., 2023), *Acacia cyanophylla* leaves (Jalab et al., 2021), and *Aloe vera* leaves (Liknaw et al., 2025).

Characterization of green-synthesized AgNPs-Rh

GC-MS

As shown in Figure 1, GC-MS analysis of the post-synthesis supernatant of AgNPs-Rh detected 2 diterpenoid-related compounds, namely phytol (retention time: 31.318 minutes) and neophytadiene (retention time: 29.316 minutes). The detection of these compounds in the post-synthesis supernatant indicates that they remained in the soluble fraction after nanoparticle separation. GC-MS profiling of biological extracts has similarly reported phytol and neophytadiene as detectable constituents (Hussein et al., 2020). However, because the present study analyzed only the post-synthesis supernatant, the results demonstrate the persistence of these metabolites in the soluble fraction but do not establish their adsorption onto, or functional role as capping agents on, the AgNP surface. The detection of phytol is particularly relevant to the antifungal objective of this study, as phytol-containing extracts and purified phytol have previously shown inhibitory activity against *G. boninense* (Abdul Aziz et al., 2019).

UV-Vis spectroscopy

The formation of AgNPs-Rh was initially confirmed by UV-Vis spectroscopy, which showed a sharp surface plasmon resonance (SPR) peak at a wavelength of 415 nm. This specific absorption in the 375 to 450 nm range indicates that the nanoparticles tend to be spherical in shape with a narrow and consistent size distribution,

as supported by previous TEM/DLS studies (Kaur et al., 2021; Hang et al., 2024). Furthermore, FTIR analysis showed the inclusion of phenolic and carbonyl groups in the *R. humilis* extract, indicating their roles as both reducing and capping agents. This explains the observed colloidal stability in DLS measurements, with a hydrodynamic diameter of 77.3 nm and PDI of 0.308.

Table 2 shows that as the size of AgNPs-Rh increases, the SPR absorption peak shifts toward longer wavelengths, or the red end of the spectrum (redshift). The smallest particles in the 10 to 20 nm range have an SPR peak at 400 to 410 nm, while an increase in size to over 80 nm or the occurrence of aggregation shifts the peak beyond 450 to > 500 nm (Di Fraia et al., 2025).

As shown in Figure 2, the sharp, symmetrical SPR peak at a wavelength of 415 nm confirmed the successful biosynthesis of monodisperse AgNPs through secondary metabolites such as flavonoids, polyphenols, and alkaloids in the filtrate of *R. humilis* as an effective bioreductant and capping agent (Mandal et al., 2024). Similarly, Kaur et al. (2021) stated that a wavelength (λ_{max}) of 415 nm confirmed the formation of AgNPs, while the absence of a second peak in the region > 500 nm. This suggests that the synthesized nanoparticles are stable and resistant to aggregation (de Oliveira et al., 2025).

Table 2. Correlation between AgNPs particle size and maximum wavelength, adapted from Agnihotri et al. (2014) and Paramelle et al. (2014)

Size of AgNPs (nm)	SPR Peak (λ_{max} /nm)
10–20	400–410
20–40	410–420
40–80	420–450
> 80	450–500+

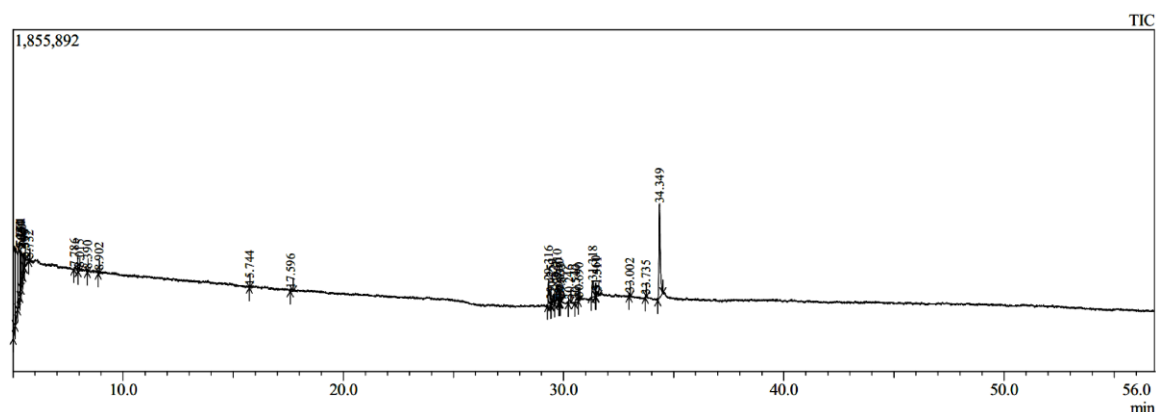


Figure 1. The GC-MS chromatogram profile of the supernatant

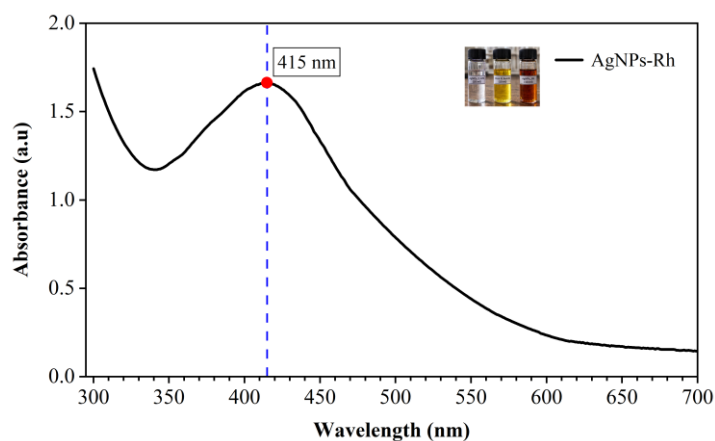


Figure 2. UV-Vis spectrum synthesized AgNPs-Rh

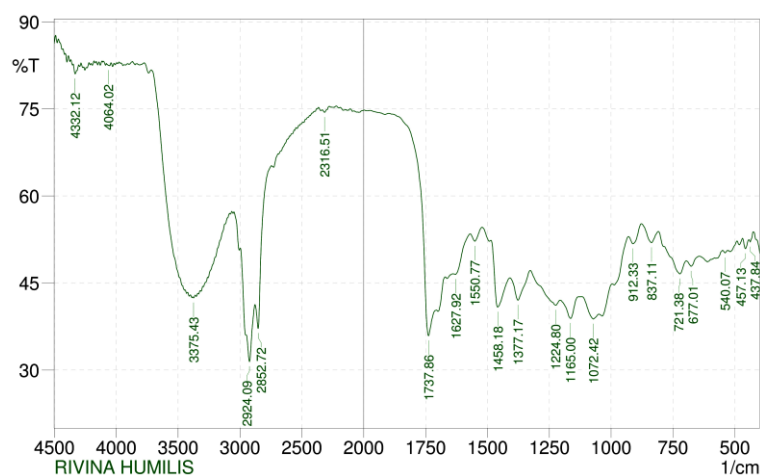


Figure 3. FTIR spectrum of synthesized AgNPs-Rh

FTIR

FTIR analysis showed the presence of secondary metabolite functional groups in the *R. humilis* filtrate during the bioreduction and stabilization of AgNPs-Rh. In the FTIR spectrum (Figure 3), the presence of a sharp and intense peak at a wavenumber of $1,737.86\text{ cm}^{-1}$ shows the stretching vibration of the carbonyl (C=O) group in the aldehyde compound. This group, along with hydroxyl, acts as the primary reducing agent in converting silver ions into the nanoform. Furthermore, the efficiency of the capping process and particle stabilization is enhanced by the presence of strong absorption bands in the fingerprint region, namely at $1,224.80\text{ cm}^{-1}$ (vinyl ether C–O vibration) and $1,165.00\text{ cm}^{-1}$ (tertiary alcohol C–O vibration), as well as a significant peak at $1,072.42\text{ cm}^{-1}$ representing the sulfoxide group (S=O). These interactions are supported by signals in the halogen region at wavenumbers of 677.01 cm^{-1} and 540.07 cm^{-1} (C–Br), which help prevent particle aggregation (Yadav et al., 2025). These results show that *R. humilis* filtrate

functions as a bioreductant and stabilizer of bionanofungicides with antifungal activity against *G. boninense*.

PSA

The reduction in particle size increases the specific surface area and the stability of the formulation, thereby optimizing the bioavailability of the active flavonoid compounds penetrating plant tissues and fungal cell walls (Lestari et al., 2025). Figure 4 illustrates that AgNPs-Rh synthesized using *R. humilis* extract had an average particle size (Z-Average) of 77.3 nm and a PDI of 0.308. The particle size distributions had median values of 75.5 and 79.0 nm, with sizes below 100 nm showing successful formation of stable nano-structures. These physicochemical characteristics are crucial for ensuring effective penetration of antifungal compounds into plant tissues and inhibiting the growth of *G. boninense* mycelium. Therefore, optimizing particle size through PSA produces precise molecular penetration and stronger inhibitory activity compared to conventional

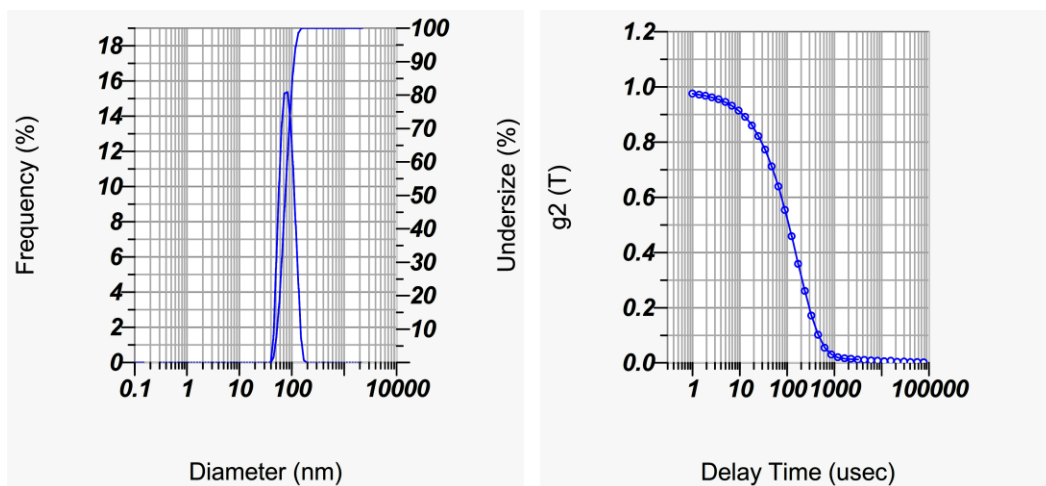


Figure 4. DLS particle size distribution and autocorrelation function of synthesized AgNPs-Rh

extracts, providing more stable and biologically efficient seedling protection (Madaniyah et al., 2025).

The *in vitro* antifungal activity of AgNPs-Rh against *G. boninense*

AgNPs-Rh were evaluated in the laboratory to test their antifungal activity in inhibiting the growth of *G. boninense*. The results showed that all concentration treatments inhibited the growth of *G. boninense* compared to the control (Table 3). Tukey's HSD test ($p < 0.05$) showed PIRG values increased significantly across all treatment levels, ranging from 11.43% (20 ppm) to a peak of 58.07% (100 ppm). In comparison, the control (T0) showed no growth inhibition (0%).

Polynomial regression analysis using 30 individual PIRG observations showed a strong concentration-response relationship (Figure 5). Among the models evaluated, the quadratic model was selected as the more parsimonious empirical model. The quadratic regression equation was $\text{PIRG (\%)} = 0.825 + 0.4027X + 0.001643X^2$, with $R^2 = 99.30\%$, $R^2 \text{ adjusted} = 99.24\%$, and

$R^2 \text{ predicted} = 99.15\%$. Both the linear (X) and quadratic (X^2) terms were significant ($p < 0.001$). The 95% confidence intervals were -0.609 to 2.259 for the intercept, 0.3352 to 0.4701 for X , and 0.000996 to 0.002290 for X^2 . The addition of the cubic term was not significant ($p = 0.058$); therefore, the quadratic model was retained. These results indicate that increasing AgNPs-Rh concentrations was associated with increased inhibition of *G. boninense* mycelial growth *in vitro*.

The efficacy of AgNPs-Rh against *G. boninense* *in vivo*

The results of the *in vivo* assessment conducted 3 months after the first AgNPs-Rh application, when the seedlings were approximately 7 months old, showed that AgNPs-Rh and fungicide treatments suppressed disease incidence and severity in the test plants (Figure 6). In Figure 6, NC plants were free of the pathogen (0%), while the PC (which received no antifungal treatment) showed the highest susceptibility, with infection rates ranging from 60 to 80%

Table 3. Colony diameter of *G. boninense* and percentage inhibition of radial growth (PIRG) at different AgNPs-Rh concentrations

Concentrations (ppm)	Colony diameter (mm)			PIRG (%)
	H3 (72 hours)	H6 (144 hours)	H9 (216 hours)	H9 (216 hours)
0	35.90±1.07 ^a	67.94±0.93 ^a	88.70±0.63 ^a	0.00±0.00 ^f
20	33.44±1.28 ^b	62.74±0.57 ^b	78.56±0.74 ^b	11.43±0.95 ^e
40	30.22±1.02 ^c	56.00±0.47 ^c	72.48±1.44 ^c	18.29±1.51 ^d
60	25.72±0.91 ^d	49.74±0.92 ^d	60.66±1.04 ^d	31.61±1.16 ^c
80	21.40±1.83 ^e	41.14±0.69 ^e	51.00±1.53 ^e	42.51±1.53 ^b
100	17.68±0.97 ^f	33.10±1.04 ^f	37.20±1.79 ^f	58.07±1.86 ^a

Note: Values are presented as mean±SD (n = 5). H3, H6, and H9 indicate observations at 72, 144, and 216 hours, respectively. Different lowercase letters within each column indicate significant differences among concentrations according to Tukey's HSD test at $p < 0.05$

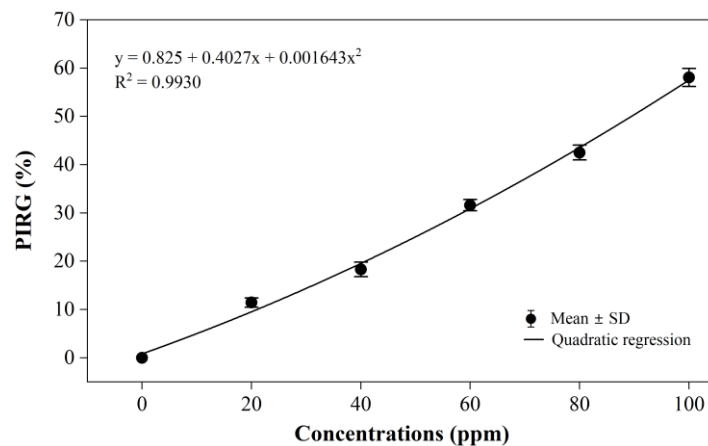


Figure 5. Effect of AgNPs-Rh concentration on the percentage inhibition of radial growth (PIRG) of *G. boninense* in vitro

Note: Data points represent mean±SD (n = 5). The line represents the quadratic regression fitted to the 30 individual PIRG observations

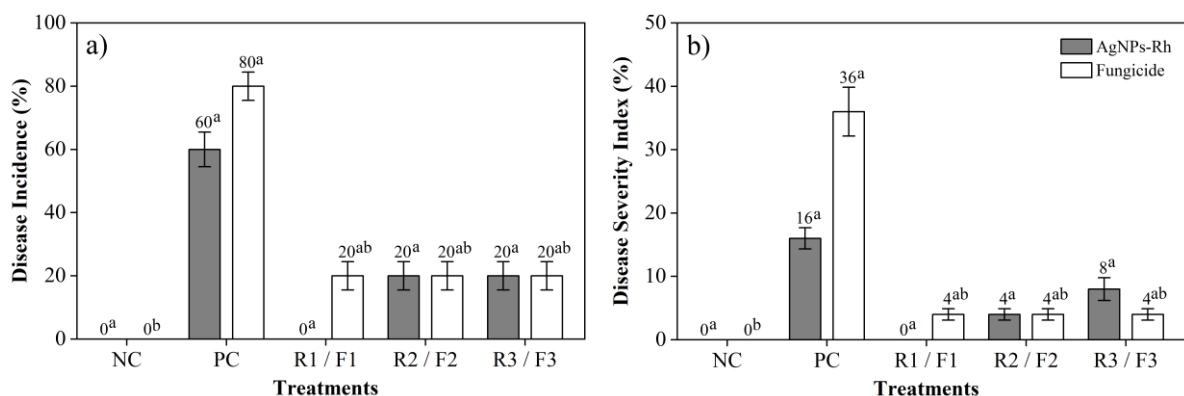


Figure 6. Effect of AgNPs-Rh and fungicide treatments on disease incidence (a) and disease severity index (b) of oil palm seedlings at 3 months after the first AgNPs-Rh application

Note: The legend indicates the experimental block (AgNPs-Rh or fungicide trial block). NC and PC plants within both blocks did not receive any antifungal treatments. Different lowercase letters above bars show a significant difference according to Tukey's HSD test at $p < 0.05$

and severity ranging from 16 to 36%. In the AgNPs-Rh treatment group, the application of a 250-ppm concentration (R1) achieved optimal effectiveness by reducing disease incidence and severity to 0%. All treated plants showed a significant decrease in disease incidence and severity compared to the PC. The PC group exhibited the highest disease susceptibility, reaching an incidence of 80%, which was significantly different from both the AgNPs-Rh and fungicide treatments. This shows that AgNPs-Rh are effective at a concentration of 250 ppm, serving as a potential competitive, efficient, and sustainable nanotechnology-based biocontrol agent for oil palm plantations.

Previous studies have shown that *G. boninense* is a soil-borne fungus capable of causing BSR, a serious threat to oil palm plantations, with potential economic losses reaching 43%.

Furthermore, Suwandi et al. (2024) evaluated the effectiveness of a mixed planting system including oil palm seedlings and taro plants (Japanese, Bogor, and Indralaya varieties) in suppressing *G. boninense*. The results showed that the presence of taro plants significantly reduced oil palm root necrosis (by 82 to 96%), lowered the disease index (by 65 to 71%), and accelerated the decomposition of inoculum sources.

According to Zakaria (2023), BSR infection and spread occur through root-to-root contact, and basidiospores that colonize roots also play a role. In the early stages of infection, the pathogen behaves as both a biotroph and a necrotroph, releasing cell wall-degrading enzymes that trigger the defense response of the host. Furthermore, Bharudin et al. (2022) identified key metabolic pathways involved in oil palm defense mechanisms against *G. boninense*, showing their

potential application as biomarkers for early disease detection. To control BSR, integrated disease management strategies are commonly used, including cultural practices, chemical control, and the application of biocontrol agents or fertilizers (Zakaria, 2023).

The physiological response and growth parameters of oil palm seedlings

Based on the results in Figure 7, oil palm seedlings inoculated with *G. boninense* exhibited growth inhibition, as indicated by significantly smaller plant height and stem diameter in the PC treatment compared to the NC. AgNPs-Rh treatment yielded a positive response at a concentration of 250 ppm, significantly increasing stem growth compared to the PC treatment. The synthetic fungicide at a concentration of 0.750 ml l⁻¹ (F3) effectively restored the physiological health of the plants, bringing the leaf chlorophyll content back to levels comparable to the healthy

control group. However, the number of leaves did not show a significant difference across all treatments. Similar observations were reported by Rahmadhani et al. (2020), who identified BSR caused by *G. boninense* as a major oil palm disease that is difficult to control.

The chlorophyll content of infected plants (PC) treated with AgNPs-Rh or fungicides showed a decrease (50.05 and 54.61 SPAD). At a concentration of 250 ppm, AgNPs-Rh were able to increase the SPAD value to 57.60±5.57. This shows that AgNPs-Rh can mitigate chloroplast damage caused by pathogens or excessive reactive oxygen species (ROS) responses, as chlorophyll degradation can be minimized, consistent with Jian et al. (2022). Similarly, as conceptualized by Ong et al. (2021), AgNPs-Rh in our system may have restored oil palm seedlings through an elicitation mechanism, simultaneously triggering a systemic defense

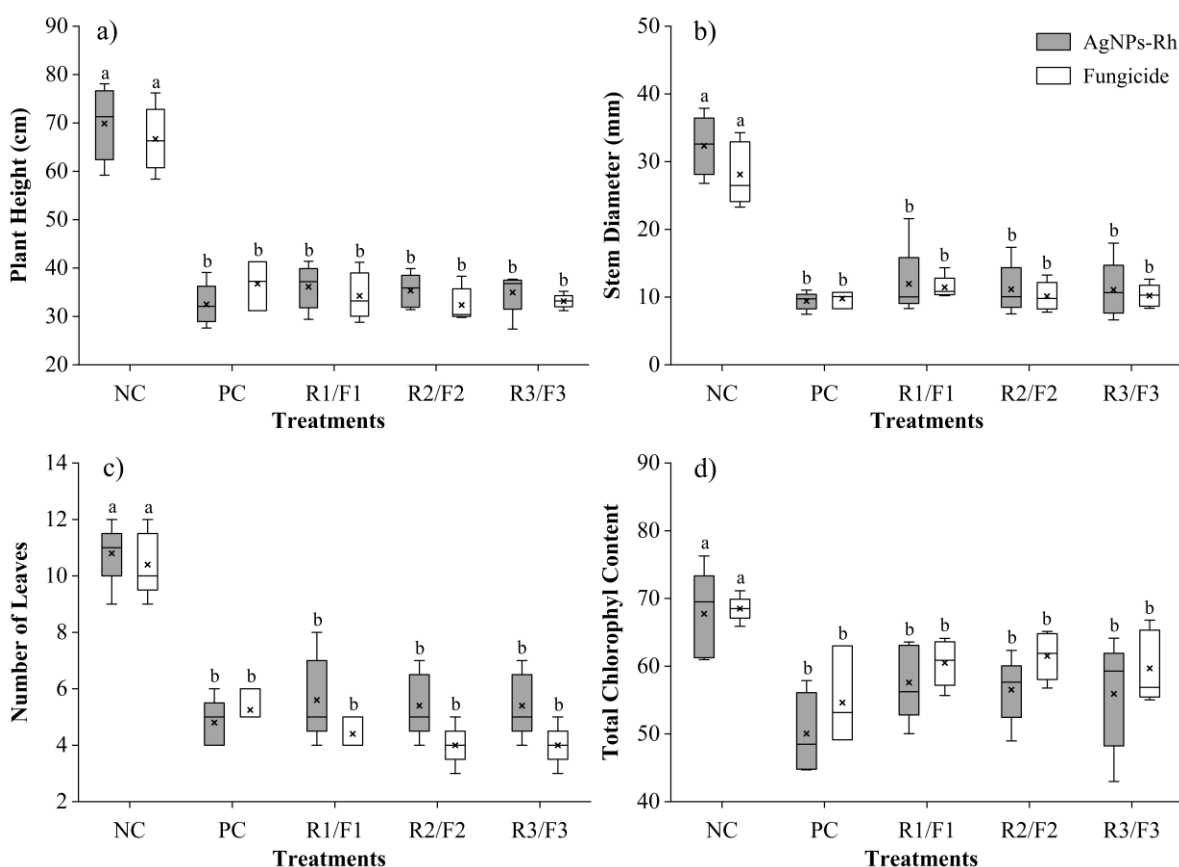


Figure 7. Physiological responses of oil palm seedlings treated with AgNPs-Rh and synthetic fungicides against *G. boninense*: (a) plant height, (b) stem diameter, (c) number of leaves, and (d) total chlorophyll content

Note: Biological replicates were used (n = 5; for the PC in the fungicide blocks: n = 4 due to plant mortality caused by *Ganoderma* infections). Different letters above the boxplots show significant differences according to Tukey's test at $p < 0.05$. NC (negative control); PC (positive control); AgNPs-Rh: R1 (250 ppm), R2 (500 ppm), R3 (1,000 ppm); Fungicide: F1 (0.375 ml l⁻¹), F2 (0.500 ml l⁻¹), F3 (0.750 ml l⁻¹)

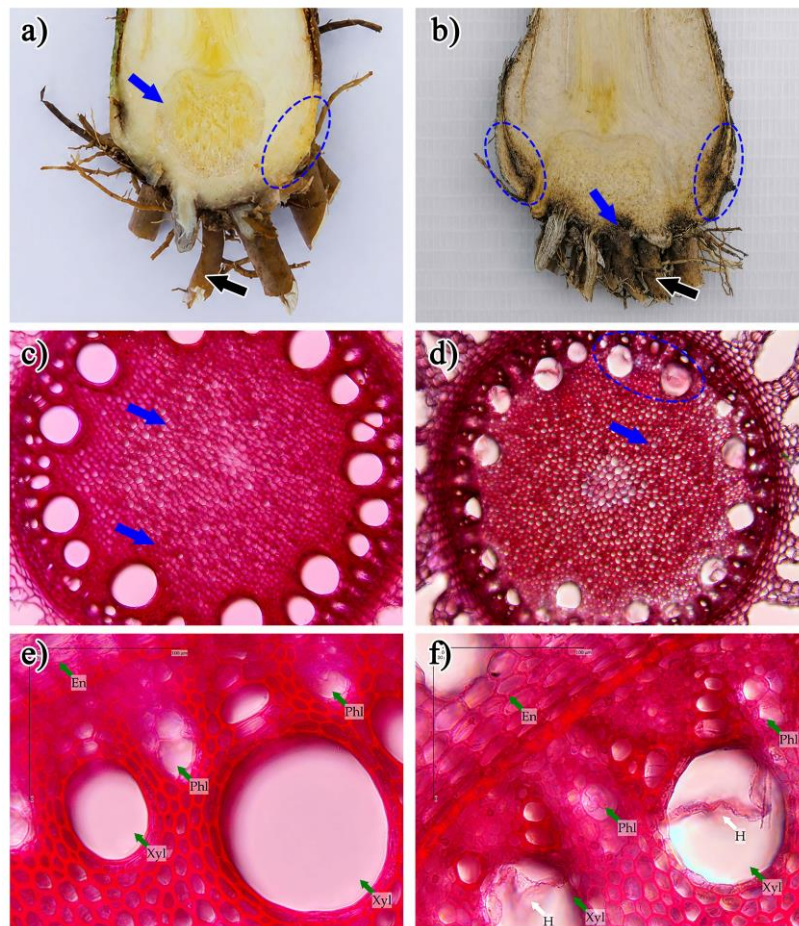


Figure 8. Macroscopic and histopathological observations of basal stem and root tissues of oil palm seedlings infected with *G. boninense*. (a, b) Macroscopic appearance of the basal stem of seedlings treated with AgNPs-Rh R1 (250 ppm) and the PC, respectively; (c, d) Cross-sections of root samples obtained from the corresponding seedlings and observed at 10× objective magnification; (e, f) Higher-magnification views of the corresponding root tissues observed at 40× objective magnification, showing the xylem and phloem structures

Note: En = endodermis; Phl = phloem; Xyl = xylem; H = fungal hyphae. Black arrows with white outlines indicate the root tissues sampled for microscopic examination. Blue arrows and ellipses indicate the areas of interest, while green arrows indicate the xylem and phloem structures

response. This process includes the induction of H_2O_2 accumulation as a signaling molecule for the systemic acquired resistance (SAR) pathway, alongside enhanced biosynthesis of the hormones salicylic acid (SA) and jasmonic acid (JA), which suppress pathogen colonization. Additionally, elicitation increases the activity of the enzyme phenylalanine ammonia-lyase (PAL), accelerating lignin biosynthesis. This process thickens the oil palm cell walls, making them more resistant to degradation by the pathogen's hydrolytic enzymes.

Histopathological assessment of oil palm root tissues

Ganoderma boninense infection in oil palm causes necrotic symptoms at the basal stem,

which are characterized by a change in tissue color from yellowish-white to dark brown. This change is associated with tissue degradation during disease development. As the infection progresses, *G. boninense* mycelia colonize root tissues, including the cortex and xylem vessels, leading to host tissue deterioration and loss of tissue integrity (Khoo and Chong, 2023).

The histopathological observation was designed as a representative qualitative assessment to illustrate differences in tissue structure between the untreated PC and the 250 ppm AgNPs-Rh treatment. Figure 8a shows the basal stem tissue from the AgNPs-Rh R1 treatment, which appears healthy and yellowish-white, without visible signs of necrosis. The

vascular tissues (xylem and phloem) appear intact and organized, while the cortex remains relatively compact (Figure 8c). No fungal hyphae are visibly observed in the xylem in the corresponding higher-magnification image (Figure 8e). In contrast, infection by *G. boninense* causes necrosis at the basal stem, characterized by a dark brown discoloration (Figure 8b). The corresponding root cross-section shows structural disruption of the vascular tissues (Figure 8d), while fungal hyphae are visible in association with the disrupted tissue in the higher-magnification image (Figure 8f).

Potential application of AgNPs-Rh for BSR management

The development of AgNPs-Rh shows significant antifungal efficacy against *G. boninense* mycelium *in vitro* and under the tested nursery conditions. The utilization of *R. humilis* phytochemicals as reducing and stabilizing agents offers an alternative approach for the green synthesis of these nanoparticles. The application of AgNPs-Rh showed an ability to maintain leaf numbers across treatments, thereby preserving agronomic performance under pathogen-induced stress during the nursery stage. The antifungal effectiveness of these nanoparticles, which matched that of synthetic fungicides on certain growth parameters in our controlled setting, indicates their potential as a candidate for managing BSR.

While this study provides foundational data on the efficacy of AgNPs-Rh in inhibiting *G. boninense* growth, their direct application in oil palm plantation management requires extensive further evaluation. Because the current data only support antifungal efficacy under nursery conditions, the sustainability and environmental safety of this bionanofungicide remain critical questions for future research. Future studies must rigorously evaluate the environmental fate of these nanoparticles, including measurements of silver residues, soil accumulation, leaching, plant uptake, and potential ecotoxicity to non-target organisms and microbial communities, before any large-scale field applications can be recommended. Additionally, further research is needed to understand the biological mechanisms underlying their activity in controlling *G. boninense* infections in oil palm plantations.

CONCLUSIONS

AgNPs synthesized from *R. humilis* extract were successfully characterized through UV-Vis analysis, exhibiting an SPR peak at 415 nm and an average particle size of 77.3 nm by PSA. GC-MS and FTIR analyses confirmed that phytols and oxygen-containing functional groups serve as natural capping agents. Furthermore, applying a 250-ppm concentration effectively suppressed disease symptoms, stimulated physiological growth in oil palm seedlings, and increased stem diameter. Consequently, green-synthesized AgNPs-Rh function as a sustainable bionanofungicide, offering an eco-friendly alternative to synthetic fungicides for routine main nursery applications while enhancing systemic plant defense against *G. boninense*.

ACKNOWLEDGEMENT

This study was funded by the Directorate General of Research and Development, Ministry of Higher Education, Science, and Technology (MOHEST) of the Republic of Indonesia, under the Applied Research - Model Output scheme (Grant No. 122/C3/DT.05.00/PL/2025 and Derived No. 19/SPK/LL1/AL.04.03/PL/2025). The authors are grateful to the Institute for Research and Community Service (LPPM) at Universitas Tjut Nyak Dhien for their support under Contract No. 010/UTND.10/PL/VI/2025. Furthermore, the authors would like to thank the reviewers and editors for their valuable comments and suggestions.

DECLARATION OF GENERATIVE AI USE

During the preparation and revision of this manuscript, the authors used Grammarly for grammar correction, spelling checks, and language refinement to improve the readability and clarity of the text. Additionally, Consensus AI was used solely to assist in identifying potentially relevant scholarly literature and sources. The authors independently verified, read, and cited the original publications before inclusion. All scientific content, experimental data, interpretations, citations, and references were critically reviewed, evaluated, and finalized by the authors. The authors retain full responsibility for the accuracy, validity, originality, and integrity of the manuscript.

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