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Mycogenic Silver Nanoparticles Synthesized by *Trichoderma reesei*: Physicochemical Characterization and Antibacterial Activity Against *Ralstonia solanacearum*

Sazia Siddiqui¹, Ayesha Khatoon¹, Nida Sultan¹, Badruddeen²,
Juber Akhtar², Aisha Kamal^{2*}

¹Department of Biosciences, Integral University, Lucknow, 226026, India

²Department of Pharmacy, Integral University, Lucknow, 226026, India

²Department of Bioengineering, Integral University, Lucknow, 226026, India

*Corresponding author: Dr. Aisha Kamal,

e-mail: aishakamal04@gmail.com

ORCID-0000 0003 2303 0344

ABSTRACT:

Background: Bacterial wilt (Bw) is one of the most important constraints in tomato production and is caused by the bacterium *Ralstonia solanacearum*. Green synthesis of silver nanoparticles (AgNPs) using fungal metabolites is an eco-friendly approach to alternative methods of chemical synthesis.

Objectives: Objective of the study was synthesis and characterization of AgNPs by cell-free filtrate of *Trichoderma reesei* and the evaluation of the antibacterial activity against the bacterium *R. solanacearum*.

Methods: Morphological and ITS rDNA were used to identify *Trichoderma reesei* (PX585131), isolated from the tomato rhizosphere soil. Its cell-free filtrate was used for the synthesis of AgNPs which was characterized by UV–Vis, FTIR, TEM, DLS and zeta potential analysis. The antibacterial activity was assessed by disc diffusion and broth inhibition method.

Results: The presence of AgNPs was confirmed based on the colour change and SPR band. TEM showed predominately spherical shaped nanoparticles with diameter range of 8–50 nm, whereas DLS micrograph showed hydrodynamic diameter of 191.9 nm (PDI 0.307) and zeta potential of –15.8 mV. FTIR suggested that proteins and polysaccharides are responsible for the capping of nanoparticles. The synthesized AgNPs were found to possess antibacterial activity against *R. solanacearum*.

Conclusion: The AgNPs were synthesized using green synthesis using cell-free filtrate of *Trichoderma reesei* with the desired physicochemical properties and antibacterial activity against *R. solanacearum*.

INTRODUCTION

Nanotechnology has become a transformative multidisciplinary field bridging physics, chemistry, biology, and materials science (Das et al. 2024). Of the numerous nanomaterials studied, silver nanoparticles (AgNPs) have gained remarkable interest owing to their unique physicochemical properties and potent antimicrobial activity against numerous pathogens (Bamal et al. 2021; Khan et al. 2018). AgNPs are increasingly integrated into medical coatings, wound dressings, agricultural disease-management

tools, environmental remediation systems, and food-safety technologies (Harish et al. 2022). Their widespread application has intensified the demand for cleaner, safer, and more sustainable synthesis approaches.

Conventional physical and chemical routes for AgNP production rely on high energy inputs and toxic reducing or stabilising agents, such as hydrazine and hazardous organic solvents, leaving environmentally harmful residues (Abbas et al. 2024; Ahmad et al. 2024; Dasauni et al. 2025). These drawbacks highlight the urgency of developing eco-friendly synthesis methods that minimize chemical burden while retaining high nanoparticle quality. Biological synthesis using plants, bacteria, fungi, and enzymes has emerged as a sustainable alternative because it employs naturally occurring biomolecules which reduces energy requirements, and eliminates toxic by-products (El-Sayyad et al. 2024; Gunjyal et al. 2023).

Among biological systems, fungi offer significant advantages for nanoparticle production. Their ability to generate large biomass, secrete abundant extracellular metabolites, and tolerate high metal-ion concentrations makes them superior biofactories for nanomaterial synthesis (Oke et al. 2023; Wadhwa et al. 2024). *Trichoderma* species are especially potent candidates because of their fast growth, rich metabolic repertoire, and well-established roles as biocontrol agents and plant growth promoters in agriculture. *Trichoderma* species are particularly promising due to their rapid growth, metabolic diversity, and established roles in agriculture as enhancing plant health and protecting crops from pathogens (Altaf et al. 2024; Madbouly 2021). *Trichoderma* species produce a rich repertoire of hydrolytic enzymes, phenolics, terpenoids, peptides, and extracellular proteins that can act simultaneously as reducing and capping agents for metal nanoparticles (Alghuthaymi et al. 2022; Keswani et al. 2019; Khan et al. 2025).

Although many studies have explored fungal-mediated nanoparticle synthesis, comparative analyses of different *Trichoderma* species and their distinct nanomaterial-forming capacities remain limited. Moreover, mechanistic insights into how species-specific metabolite profiles influence nanoparticle morphology and antimicrobial potency are insufficiently addressed in existing literature.

The present study provides a systematic, side-by-side evaluation of *Trichoderma* species viz. *T. reesei* isolated from agricultural soils in Uttar Pradesh, India, for their ability to biosynthesize AgNPs through a clean, chemical-free process. Both morphological and ITS-based molecular identification confirmed strain identity before use. Cell-free fungal filtrates were employed as dual reducing and stabilizing agents, and the resulting AgNPs were rigorously characterized using UV–vis spectroscopy, FTIR, TEM, DLS, and zeta-potential analysis to elucidate structural properties of biogenic silver nanoparticles. In this study we evaluated the in-vitro antimicrobial activity of TR-AgNPS in suppressing *R. solanacearum*, which is a plant pathogen. This research can be beneficial for sustainable agriculture.

2. MATERIALS AND METHODS

2.1 Isolation of *Trichoderma* strains and Species Determination

Samples of Soil were collected from the rhizospheric soil of tomato fields in Uttar Pradesh, India during the autumn and winter cropping seasons. Samples were transferred into sterile, clearly labelled polybags and stored at 4 °C until subsequent processing (Mistry and Bariya, 2022).

Fungal isolation was done using the serial dilution plating approach as outlined by Bissett et al. (2015). Briefly, 1 g of soil was dispersed in 9 mL of sterile distilled water, followed by serial dilution to 10^{-7} . From each dilution, 0.1 mL aliquots were plated on PDA and incubated at 28 °C for 7 days. Colonies exhibiting morphological characteristics typical of *Trichoderma* were transferred to fresh PDA plates to obtain pure cultures.

Preliminary identification was based on microscopic examination of lactophenol cotton blue-stained mounts. A small mycelial fragment was placed in 3% KOH on a clean slide, and observation of diagnostic structures including branched conidiophores, lageniform phialides, and characteristic conidia were done microscopically (Atzori et al. 2023).

For molecular verification, genomic DNA was extracted from actively growing cultures using a modified CTAB protocol. The Internal Transcribed Spacer (ITS) region of rDNA was amplified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). PCR parameters were refined to obtain clear, specific amplicons. Products were resolved on a 1.5% agarose gel, and target bands (~500–600 bp) were excised, purified, and processed for sequencing. Confirmed *Trichoderma* isolates were assigned laboratory accession numbers and stored at 4 °C for nanoparticle synthesis.

2.2 Collection of Cell-Free Fungal Filtrate

T. reesei was first grown on PDA plates for 7 days. From these cultures, 5-mm mycelial discs were transferred into 100 mL of sterile PDB aseptically. The flasks were incubated at 28 ± 2 °C at 150 rpm on a rotary shaker for 7 days to promote vigorous mycelial development and secretion of extracellular metabolites (Gemishev et al. 2022).

The cultures were filtered through Whatman No. 1 paper to harvest the mycelial biomass and rinsed with sterile distilled water to remove residual medium components. Then the cleaned biomass was resuspended in 100 mL of sterile distilled water and maintained at 28 °C without agitation for 24 h to enable the secretion extracellular proteins and metabolites into the aqueous phase (Manasa 2023). The mixture was subsequently filtered to obtain a clear, cell-free fungal filtrate, which was utilized immediately for the synthesis of silver nanoparticle (Alves and Murray, 2022).

2.3 Fungal-Mediated Synthesis of Silver Nanoparticles

Silver nanoparticles were biosynthesized using cell-free filtrates of *T. reesei*. Equal volumes of AgNO_3 and fungal filtrate were mixed in sterile 250 mL flasks and incubated at 25 ± 2 °C in the dark to avoid photoreduction. Gradual shift from pale yellow to brown within 24–96 h confirmed Ag^+ reduction and AgNP formation (Durán et al. 2023; Gemishev et al. 2022; Subhash, 2017). Two controls positive (AgNO_3 without fungal filtrate to confirm absence of abiotic reduction) and negative (Fungal filtrate without AgNO_3 to exclude pigment interference) were maintained. After the color stabilized, mixtures were centrifuged at 15,000 rpm for 15 min. Pellets were rinsed with sterile distilled water, resuspended in deionized water, and kept at 4 °C for further analysis.

2.4 Physicochemical Characterization of synthesized AgNPs

Characterization of synthesized AgNPs were done by comprehensively using spectroscopic and microscopic techniques (EL-Moslami et al. 2017; Ogunleye et al. 2023; Ramírez-Valdespino and Orrantía-Borunda 2021). UV–Visible spectroscopy was first employed to record absorbance spectra between 300–700 nm, enabling the detection of SPR peaks that confirm AgNP formation. FTIR spectra ($4000\text{--}400\text{ cm}^{-1}$) were recorded to elucidate the functional groups associated in the reduction and stabilization of Ag^+ (Pasiczna-Patkowska et al. 2025). TEM was used to determine the size distribution, morphology and potential aggregation of nanoparticles, while DLS analysis of hydrodynamic diameter and polydispersity index (PDI) provided additional information on the colloidal stability and uniformity (Filippov et al. 2023). Zeta potential analysis was performed to assess the surface charge and stability of the nanoparticle suspension, where higher absolute values reflect stronger electrostatic repulsion and improved dispersion (Konappa et al. 2021).

2.5 Antimicrobial Assay

The antimicrobial potential of AgNPs synthesized from *Trichoderma* species was evaluated against Gram-negative bacteria (*Ralstonia solanacearum*). All assays were performed under aseptic conditions using sterile techniques and following standard microbiological protocols. All experiments were carried out with three independent replicates.

2.5.1. Assessment of Antibacterial Potential of Synthesized Nanoparticles

The antibacterial potential of AgNPs was determined by disc diffusion method, following CLSI guidelines with slight modifications (Kakian et al. 2024). Overnight bacterial cultures in nutrient broth were adjusted to 0.5 McFarland ($\sim 10^8$ CFU/mL) and uniformly spread onto Mueller–Hinton Agar (MHA; Himedia, India) plates with sterile swabs. Disc measuring 6 mm in diameter were aseptically loaded with 50 μL of AgNP suspensions at 25, 50, 75, and 100 $\mu\text{g/mL}$. Gentamicin (10 $\mu\text{g/mL}$) taken as the positive control, while DW (at the concentration present in AgNP stocks) and sterile distilled water were served as negative controls.

Plates were maintained at 28 °C for 24–48 h in upright position. Zones of inhibition (ZI) were measured using a digital Vernier caliper, taking the mean of two perpendicular diameters for improved accuracy. All measurements were performed blinded to treatment to minimize bias.

2.5.2. Determination of MIC

MIC values were examined using the broth microdilution method following CLSI M07-A9. Sterile 96-well microplates were used to prepare two-fold serial dilutions of AgNPs from 50 to 200 $\mu\text{g/mL}$ in Mueller–Hinton Broth. 100 μL of bacterial

suspension were inoculated in each well, adjusted to $\sim 10^5$ CFU/mL and incubated at 28°C for 48 h. MIC was determined as the lowest concentration of AgNP that completely inhibited visible growth. To minimize turbidity-related interference from AgNPs, OD₆₀₀ measurements were corrected using nanoparticle-only controls (Singh and Mishra 2022).

2.6 Statistical Analysis

All experiments were performed in triplicate, and results are reported as mean $\pm$ SD. Antimicrobial and nanoparticle characterization data were analysed using one-way ANOVA followed by Tukey's post hoc test for pairwise comparisons. Statistical significance was defined as $p < 0.05$. All analyses were carried out using MS Excel.

3. RESULTS

3.1 *Trichoderma* Isolation and Species Determination

A total of nine fungal isolates exhibiting colony characteristics typical of the genus *Trichoderma* were obtained from rhizosphere soils collected from tomato fields in Uttar Pradesh, India. On Potato Dextrose Agar (PDA), the isolates initially produced fast-growing, compact, cottony white mycelia that gradually transitioned to light green and later dark green upon sporulation, an established diagnostic trait of *Trichoderma spp.* Colonies developed a slightly granular texture at maturity, consistent with conidial formation patterns reported for agriculturally important *Trichoderma* taxa.

Microscopic examination further confirmed these preliminary morphological observations. All isolates displayed branched, pyramidal conidiophores bearing typical flask-shaped phialides arranged in clusters, along with smooth-walled, ellipsoidal to nearly spherical conidia (Asis et al. 2021). These characters strongly suggested the presence of *Trichoderma reesei* frequently reported from crop-associated soils and recognized for robust sporulation and distinct conidial morphology (Chan et al. 2023). Representative morphological and microscopic images are presented in Figure 1.

To confirm species identity beyond morphological inference, molecular characterization of the isolates was performed by amplifying the Internal Transcribed Spacer (ITS) region using universal primers ITS1 and ITS4. PCR amplification produced single, sharp bands of approximately 500–600 bp, corresponding to expected ITS fragment lengths for *Trichoderma species*. Sequencing of the amplicons followed by BLASTn analysis against the NCBI GenBank database revealed greater than 99% similarity with reference sequences of *T. reesei* (Hui 2013; Laidani, 2019). These high-percentage matches provided definitive species-level identification and validated earlier morphological classification.

Phylogenetic analysis using the neighbour-joining method further supported this identification. The isolates formed well-resolved clades clustering with authenticated *T. reesei* sequences, supported by strong bootstrap values ($>90\%$), demonstrating a close evolutionary relationship with their respective reference strains (Figure 2), consistent with other ITS-based *Trichoderma* phylogenies (Calumby et al. 2025; Thepbandit et al. 2024).

Together, the integrated morphological, microscopic, and molecular analyses verified the identity of the selected isolates as *Trichoderma reesei*. The verified strain was submitted to the NCBI and assigned a unique accession number, *Trichoderma reesei* (PX585131), and preserved for use in downstream biosynthesis experiments. Notably, the comparative assessment of both isolates suggested that although they shared typical *Trichoderma* morphological features.

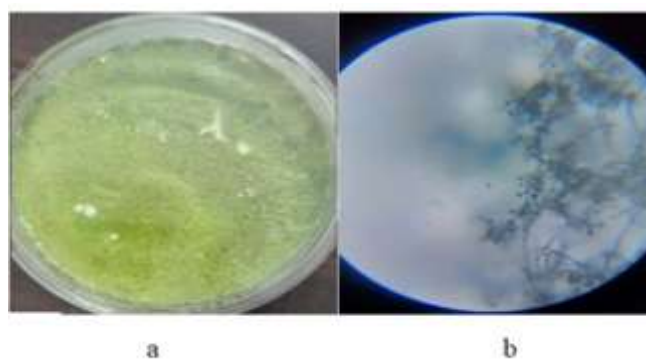


Figure 1. Morphological features supporting identification of *Trichoderma* isolates.

T. reesei showing characteristic PDA colony morphology with green sporulation and lactophenol cotton blue micrographs depicting diagnostic structures viz. branched conidiophores, lageniform phialides, and smooth, ellipsoidal conidia.

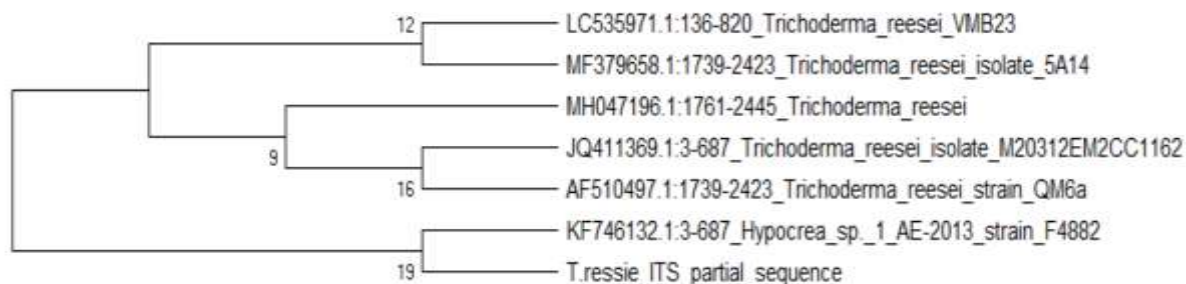


Figure 2. Phylogenetic analysis inferred from ITS sequences confirming the identity of the isolates as *T. reesei*. Strong bootstrap values (1,000 replicates) support their placement within well-defined clades alongside corresponding GenBank reference sequences.

3.2 Visual Confirmation of Silver Nanoparticle Biosynthesis

When fungal filtrates were incubated with 1 mM AgNO_3 , a progressive change in colour from pale yellow to deep brown was occurred over 48–72 h. This transformation, absent in both controls (AgNO_3 alone and filtrate alone), indicates Ag^+ ions reduction and the formation of AgNPs driven by fungal metabolites. The brown chromaticity corresponds to surface plasmon resonance (SPR) and provided an initial qualitative confirmation of nanoparticle formation (Shahzadi et al. 2025). The darkening intensity was in *T. reesei* (TR) -mediated suggesting efficient ion reduction (Figure 3).



Figure 3. Visual confirmation of silver nanoparticle synthesis

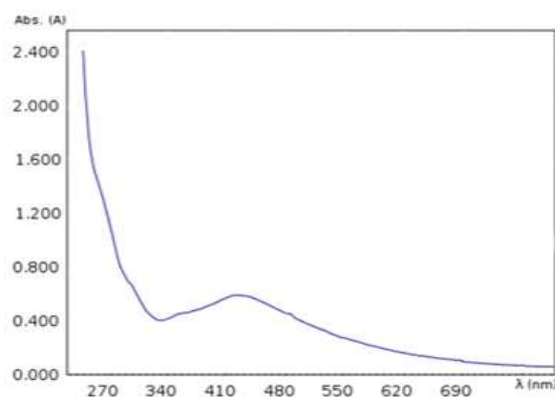
3.3 Structural and Functional Characterization of AgNPs

The biosynthesized silver nanoparticles (AgNPs) obtained from *Trichoderma reesei* (TR) was comprehensively characterized through various standard techniques to confirm their formation, stability, and structural properties.

3.3.1 UV–Visible Spectroscopy

TR- AgNPs reaction mixtures exhibited a characteristic SPR band between 420–440 nm (Figure 4), a spectral signature confirming the reduction of Ag^+ to metallic Ag^0 nanoparticles (Heikal et al. 2024; Thepbandit et al. 2024). The absence of secondary shoulders or broadened peaks indicates that nanoparticle preparations were largely monodisperse and well-stabilized (Lasmi et al. 2025).

Notably, TR-AgNPs displayed a sharper and more intense SPR peak suggesting narrower size distribution and more efficient stabilization by extracellular metabolites.

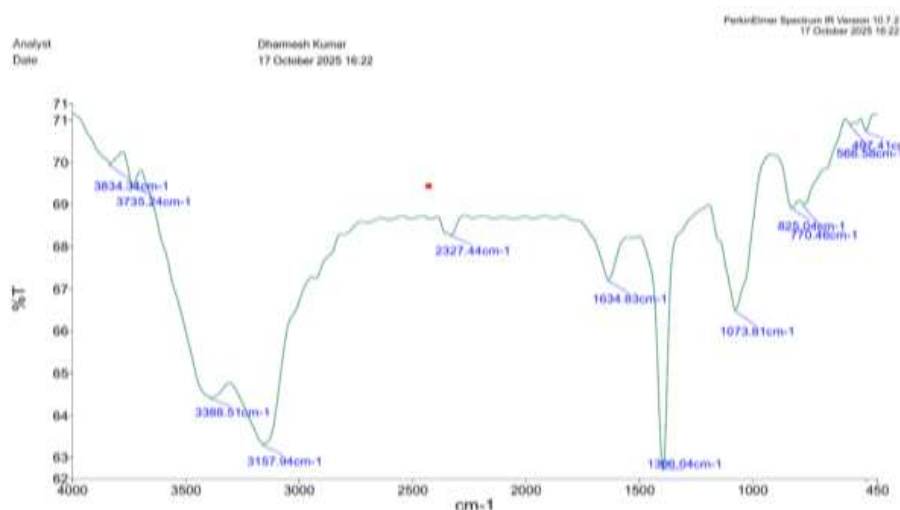


a

Figure 4. UV–Visible spectra of AgNPs synthesized by *T. reesei* (TR). Nanoparticle suspensions exhibit characteristic SPR peaks at 420–440 nm.

3.3.2 FTIR Analysis

FTIR results verified the role of fungal biomolecules in reducing and stabilizing the AgNPs (Figure 5). The spectrum of TR–AgNPs exhibited prominent absorption bands at 3834, 3388, 3157, 1634, 1396, 1073, and 497 cm^{-1} . The broad region spanning 3800–3100 cm^{-1} attributed to O–H and N–H stretching vibrations, suggesting the participation of hydroxyl- and amine-containing metabolites such as phenolic compounds and proteins in silver ion reduction. A strong peak at 1634 cm^{-1} assigned to amide (C=O) stretching, indicating proteinaceous capping on the nanoparticle surface. Additional bands at 1396 and 1073 cm^{-1} associated with C–N and C–O stretching, respectively, pointing to the involvement of polysaccharides. The weak band observed around 497 cm^{-1} is characteristic of Ag–O bonds, confirming the formation of silver nanoparticles (Pasiczna-Patkowska et al. 2025).



a

Figure 5. FTIR spectra of synthesized silver nanoparticles using *Trichoderma reesei*

3.3.3 TEM Analysis of Synthesized Nanoparticles

TEM micrographs revealed predominantly spherical nanoparticles with minimal aggregation, measuring approximately 8–50 nm for TR–AgNPs (Figure 6). No major aggregation was observed, indicating effective capping by fungal biomolecules (Hodoroaba et al. 2016).

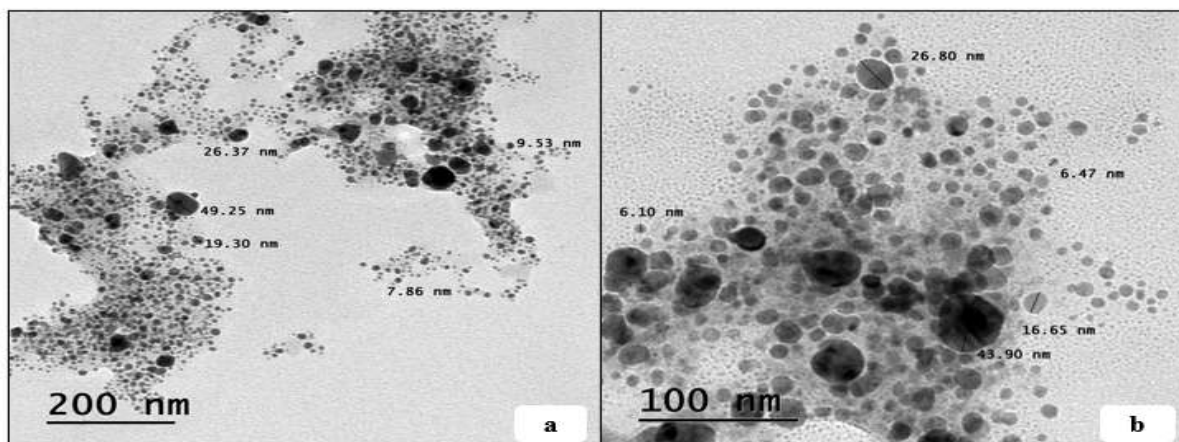


Figure 6. TEM images of biosynthesized silver nanoparticles (AgNPs) produced by *T. reesei* (a–b) showing predominantly spherical, well-dispersed nanoparticles with minimal aggregation.

3.3.4 Hydrodynamic Size and PDI Measurement

The hydrodynamic diameters determined by DLS (Figure 7) were larger than those observed in TEM ranging between 60–85 nm for both nanoparticle types reflecting the hydration layer and biomolecular corona surrounding the particles (Filippov et al. 2023; Shukla et al. 2023).

Polydispersity index (PDI) values of 0.2–0.3 for TR-AgNPs indicated a narrow size distribution and confirmed the colloidal uniformity of the biosynthesized nanoparticles.

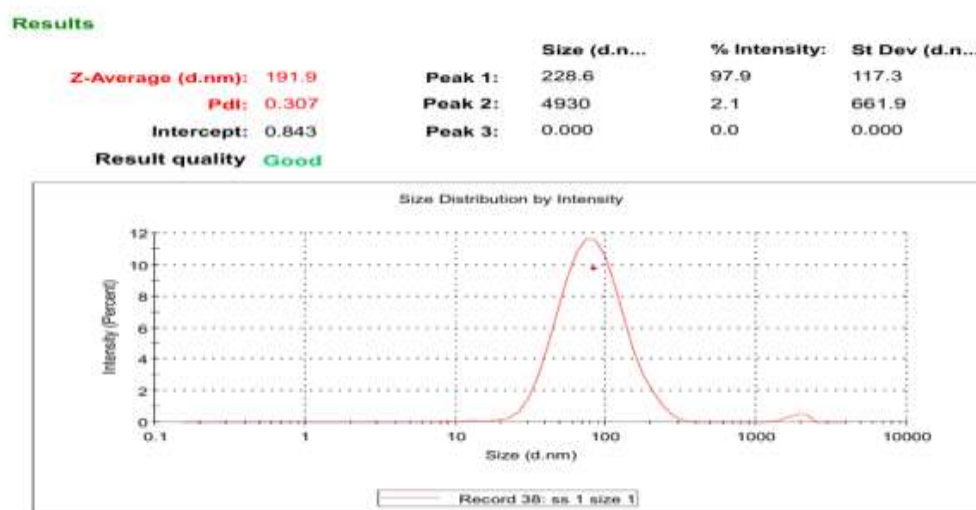


Figure 7. DLS size distribution profiles of silver nanoparticles synthesized by *T. reesei* showing hydrodynamic diameters and colloidal uniformity.

3.3.5 Zeta Potential–Based Stability Analysis

Zeta potential values -15.8 mV (TR-AgNPs) (Figure 8) demonstrated moderate but sufficient negative surface charge to prevent aggregation through electrostatic repulsion. These values fall within the typical stability range for biosynthesized silver nanoparticles, supporting the role of extracellular fungal metabolites in imparting electrostatic stability (Bélteky et al. 2019).

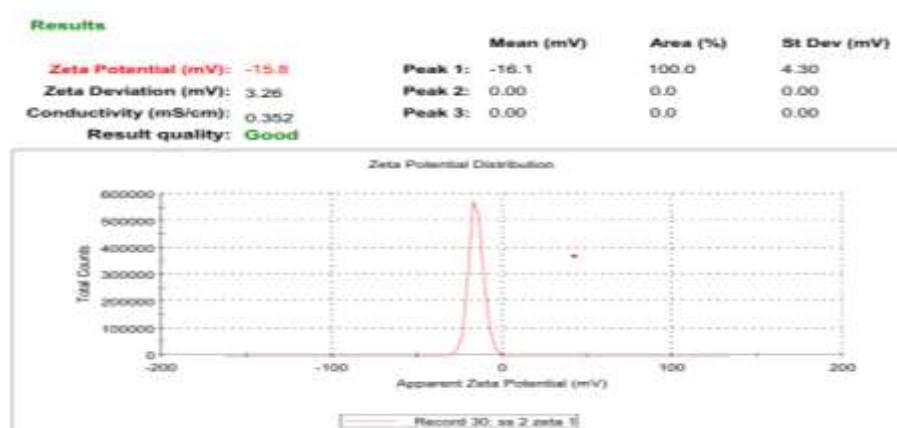


Figure 8. Zeta potential analysis of silver nanoparticles synthesized by *T. reesei* (TR).

Collectively, the spectroscopic and microscopic analyses confirm that both *Trichoderma* species successfully mediated the biosynthesis of spherical, stable, and moderately monodisperse silver nanoparticles.

3.4 Antimicrobial Efficacy of Biosynthesized Silver Nanoparticles

3.4.1 Disc Diffusion assay

TR–AgNPs exhibited clear, dose-dependent antibacterial activity, with inhibition zones increasing from 25 to 100 $\mu\text{g mL}^{-1}$. Gentamicin consistently produced the largest zones, whereas DW showed no inhibition (Figure 9). TR–AgNPs at all concentrations, producing zones of inhibition (e.g., *R. solanacearum* (plant pathogen): 12.00 ± 0.45 mm (Table 1). Gram-negative strains (*R. solanacearum*), likely due to the absence of an outer membrane, which facilitates nanoparticle contact with the cell surface. The enhanced activity of TR–AgNPs corresponds with their smaller size, higher surface area, slightly more negative zeta potential, and stable biomolecular capping, as indicated by TEM, DLS, and FTIR analyses.

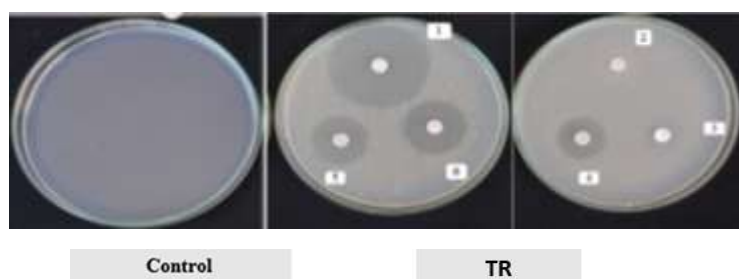


Figure 9. Dose-dependent antibacterial activity of *Trichoderma*-mediated AgNPs (25-100 μL) against four bacterial pathogens. Panels show inhibition zones for *R. solanacearum*: 1 = Gentamicin; 2 = Distilled water; 3–6 = AgNPs at 25, 50, 75, and 100 μL respectively.

MIC supported the disc diffusion results, with TR–AgNPs showing inhibitory effect. The close proximity of MIC and disc diffusion (ZOI) values suggests bactericidal action. Collectively, these results demonstrate that *T. reesei*–derived AgNPs possess favourable physicochemical features that translate into superior antimicrobial performance (ZOI)(Figure 10).

Table 1. Antimicrobial activity of AgNP produced TR to counter Plant pathogen (*Ralstonia solanacearum*) gm negative bacteria.

Bacterium	AgNP Concentration (μL)	TR–AgNPs (mm)Mean $\pm$ SD	Gentamicin (+ve control) (mm)	Distilled water (–ve control) (mm)
<i>Ralstonia solanacearum</i>	25	0.4124 ± 0.314	28.1 ± 0.623	-
	50	5 ± 0.452		-
	75	11.856 ± 0.312		-
	100	12 ± 0.451		-

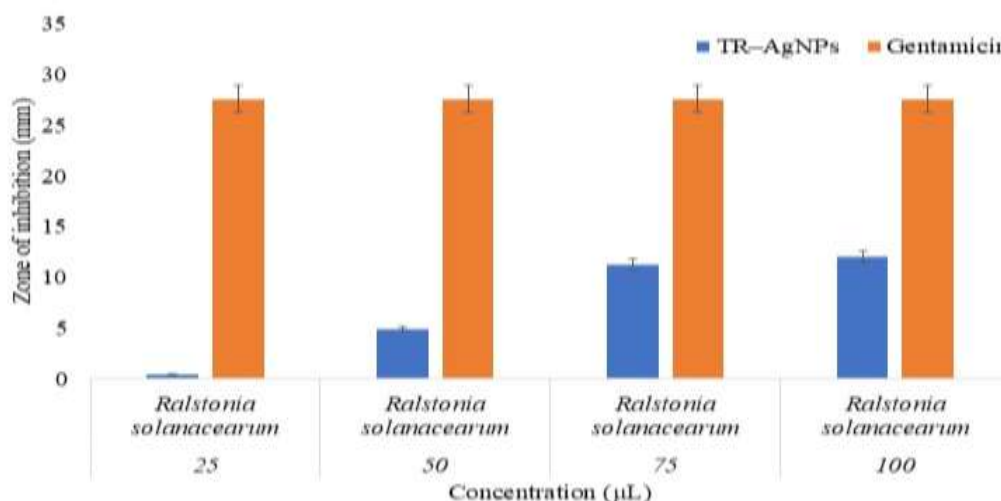


Figure 10. Concentration-dependent antibacterial activity of *Trichoderma*-derived silver nanoparticles (AgNPs). Bar graph showing mean $\pm$ SD ($n = 3$) inhibition zone diameters for TR-AgNPs (25–100 μ L) against *R. solanacearum*. Gentamicin served as the positive control. According to one-way ANOVA ($F(8,18) = 142.61$, $p < 0.001$) and Tukey's HSD test ($p < 0.05$) and result is statistically significant.

3.3.4.2 MIC Results

The broth microdilution method (BMM) was used to determine the minimum inhibitory concentration (MIC) of TR-AgNPs against *R. solanacearum*. The minimum inhibitory concentration (MIC) of TR-AgNPs was found to be 50 ppm after 48 hours of incubation at 28°C, from visual observation of bacterial growth (Kulkarni et al. 2025). To quantitatively determine the growth of bacteria, its OD₆₀₀ value was measured at each concentration (Figure 11). Based on these results, it can be concluded that TR-AgNPs showed antibacterial activity under the experimental conditions against *R. solanacearum*. The results prove that *Trichoderma* mediated AgNPs gives a significant antimicrobial effect in lower concentration. Overall, the results showed that the *Trichoderma* derived AgNPs have antibacterial activity which is concentration dependent. Some concentrations were able to produce significant growth inhibition under the in vitro conditions tested and the growth inhibition of *R. solanacearum* was evaluated with OD₆₀₀ measurements.

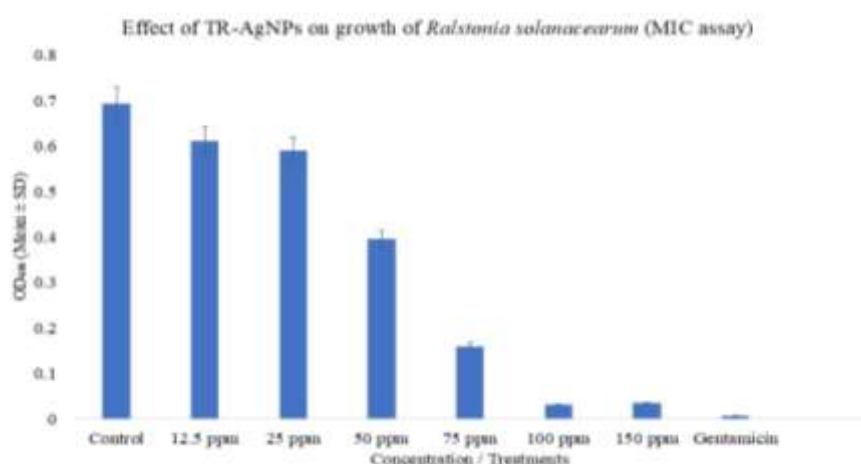


Figure 11. Effect of *Trichoderma reesei*-derived silver nanoparticles (TR-AgNPs) on the growth of *Ralstonia solanacearum* (MIC assay). The bacterial growth was determined by measuring the optical density (OD₆₀₀) after 48 hours of incubation. Data are means $\pm$ SD ($n = 3$, independent biological replicates). Statistically significant differences according to one-way ANOVA ($F(8,18) = 146.52$, $p < 0.001$) and Tukey's HSD test ($p < 0.05$).

4. DISCUSSION

The findings presented here demonstrate that cell-free filtrates of agriculturally relevant *T. reesei* (TR), provide an effective, environmentally benign platform for the synthesis of stable silver nanoparticles (AgNPs) with pronounced antimicrobial activity, and highlight how species-specific metabolite profiles shape nanoparticle physiochemistry and bioactivity.

Visual and spectroscopic evidence (brown colour change and a clear SPR band at ~420–440 nm) confirm rapid bioreduction of Ag^+ to Ag^0 , while FTIR signatures implicate proteins, polysaccharides and phenolic moieties as the principal reducing and capping agents, consistent with fungal-mediated mechanisms reported elsewhere (Anjum et al. 2023; Gemishev et al. 2022; Pasieczna-Patkowska et al. 2025). The sharp and narrow nature of these peaks suggested that the particles were uniformly distributed and that aggregation was minimal (Duman et al. 2024; Zaheer et al. 2023). TEM and DLS together reveal that TR-derived AgNPs are smaller, uniform and the slightly negative zeta potential that accounts for their enhanced colloidal stability. The higher zeta potential (-15.8 mV) and well-defined spectral peaks of TR-derived nanoparticles further support the idea that particle structure and biological performance are closely related (Zuhrotun et al. 2023). These difference attributable to species-specific variations in extracellular metabolites, including proteins, enzymes, phenolics, and polysaccharides, that govern nanoparticle nucleation and capping (Bélteky et al. 2019; Filippov et al. 2023; Sidhu et al. 2022). These biochemical distinctions highlight the importance of fungal strain selection in optimizing green nanomaterial synthesis. A correlation between the synthesized AgNPs concentration and the *R. solanacearum* inhibition was established in the antibacterial assays. The anti-bacterial effect of the fungal mediated AgNPs against the phytopathogenic bacteria was found in both the disc diffusion and broth inhibition method, which was in line with the previously reported study (Dilbar et al., 2023; Soulaïmani et al. 2025; Tomah et al. 2023). The antibacterial activity of AgNPs could be attributed to a few possible complementary interaction mechanisms. AgNPs can interact with the cell envelope of bacteria, and the Ag^+ ions released by AgNPs can react with thiol-containing proteins and enzymes of bacteria, and impact bacteria respiration and ATP production.

While the study establishes a strong foundation, further work would strengthen the translational potential. Future investigations should focus on identifying the key metabolites responsible for reduction and stabilization, assessing nanoparticle behaviour under real field conditions, and evaluating potential ecological impacts on non-target organisms. Large-scale production strategies and formulation development for agricultural applications also warrant attention.

Overall, the study highlights the significant potential of *Trichoderma*-mediated silver nanoparticles as sustainable, broad-spectrum antimicrobial agents and underscores the critical influence of fungal species on nanoparticle characteristics and biological performance. In addition, AgNPs can induce the production of ROS that can cause oxidative stress and damage to cellular components like proteins and DNA. Gram negative bacteria have thin peptidoglycan layers along with outer membrane containing lipopolysaccharides which may enable a contact between the nanoparticles and the bacteria and release silver ions from the nanoparticles (Ayaz et al. 2023). Additional development work is needed before adopting the technology for field-scale use, however, especially in terms of formulation stability, dose optimization, field-scale validation, phytotoxicity, environmental persistence, and long-term safety.

5. CONCLUSION

This study demonstrates that *Trichoderma reesei* is effective and sustainable biological systems for the green synthesis. Comprehensive characterization confirmed that species successfully mediated the reduction of Ag^+ ions and produced spherical, moderately monodisperse nanoparticles, more uniform, and more electrostatically moderate stable AgNPs. These findings highlight the importance of fungal strain selection in optimizing the physicochemical properties and bioactivity of biogenic nanoparticles. This study also presents extensive information using molecular identification of *T. reesei*, physicochemical characterization of the synthesized nanoparticles, and in-vitro antibacterial assessment. Importantly, the eco-friendly synthesis route employed in this study eliminates the use of hazardous chemicals, reduces environmental burden, and supports the global shift toward sustainable nanotechnology.

Overall, the study provides strong evidence that TR-AgNPS can be used for further research, particularly hold considerable promise as environmentally compatible antimicrobial agents for agricultural disease management and potentially broader biomedical and biotechnological applications.

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Statements & Declarations

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Author Contributions

All authors made substantial contributions agreed to submit to the current journal, gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

Data Availability:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval:

This study does not involve human participants, animal subjects, or any sensitive personal data. Therefore, no ethical approval is required for the conduct of this research.

Consent for publication Not applicable.

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