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Biosynthesis of *Trichoderma harzianum* mediated silver nanoparticles (AgNPs), their characterization and *in vitro* antimicrobial potential against *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii*

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Abstract

Silver nanoparticles (AgNPs) were efficiently produced using the extracellular culture filtrate of fungal biocontrol agent *Trichoderma harzianum*. UV-Visible spectroscopy analysis of *T. harzianum*-mediated AgNPs exhibited a sharp and well-defined absorption peak at 428.87 nm with a greatest absorbance of 2.143, indicating high NPs concentration. Transmission Electron Microscopy (TEM) analysis revealed that *T. harzianum*-derived AgNPs were predominantly spherical, well dispersed, and varied in dimensions from 13.28 to 15.12 nm. The antimicrobial capability of *T. harzianum*-mediated AgNPs was evaluated against test soil-borne fungal pathogens, namely *F. oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii*, using the poisoned food technique under *in vitro* conditions. Results revealed that in case of *F. oxysporum* f. sp. *lycopersici*, *Trichoderma harzianum*-derived AgNPs significantly restricted fungal growth, with inhibition increasing progressively from 28.61 % at 10 ppm to a maximum of 81.11 % at 100 ppm and an analogous trend of inhibition was noted against *Sclerotium rolfsii*, with growth inhibition increasing from 15.55 % at 10 ppm to 82.22 % at 100 ppm.

Keywords: Silver nanoparticles (AgNPs), *Trichoderma harzianum*, Silver nitrate (AgNO₃), UV-Visible spectroscopy, Transmission Electron Microscopy (TEM), Poisoned food technique, *F. oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii*

1. Introduction

Synthesis of nanoparticles represents a cornerstone of nanotechnological advancement. Silver nanoparticles (AgNPs) have been thoroughly investigated in recent decades, and their application in agriculture offers an alternative protective measure against diseases and pests, which could lessen our reliance on environmentally harmful fungicides and pesticides. Species belonging to genus *Trichoderma* are commonly acknowledged for their role in biological control of plant pathogens. These filamentous fungi exhibit mycoparasitic behavior through mechanisms such as hyphal coiling and secretion of cell wall-degrading enzymes (Zin and Badaluddin, 2020) [16]. Additionally, *Trichoderma* species possess NADH-dependent enzymes, incorporating nitrate reductase that hold significant importance in nanoparticle synthesis and stabilization (Muhammad *et al.* 2021) [11]. Prior investigations have illustrated that AgNPs produced through *Trichoderma* spp. exhibit strong inhibitory effects against a range of phytopathogenic fungi and bacteria (Elgorban *et al.* 2016; Chandrasekhar *et al.* 2016) [5, 2]. Biosynthesis AgNPs was carried out by using the extracellular culture filtrate of fungal biocontrol agent *Trichoderma harzianum* and their characterization was done by using UV-Visible spectroscopy and Transmission Electron Microscopy (TEM) available at Eco-friendly Disease Management and Beneficial Microbes Research Laboratory, MPKV, Rahuri (M.S) India.

2. Materials and Methods

2.1 Chemical

Silver nitrate (AgNO₃) used in the present investigation was procured from Hi-Media Laboratories Pvt. Ltd. The molecular weight of AgNO₃ is 169.87 g mol⁻¹.

2.2 Synthesis of silver nanoparticles from culture filtrate of fungal culture

90 mL of aqueous solution of 5mM Silver nitrate (AgNO_3) (equivalent to 170 ppm) was mixed with 10 mL of *T. harzianum*, culture filtrate for the synthesis of silver nanoparticles in a 250 mL conical flask. The conical flask was covered with the aluminum foil and the whole mixture was incubated at room temperature for 24 hrs. The color change of silver nitrate from colorless to brown color was

considered as indicator of formation of silver nanoparticles through reduction of silver ions from Ag^+ to Ag^0 .

The silver nanoparticles solution was centrifuged at 10000 rpm for 20 minutes to separate the AgNPs. The resultant pellet was kept in hot air oven overnight at 60 °C to make a fine powder of nanoparticles. The stepwise protocol employed for the biosynthesis of silver nanoparticles using *Trichoderma harzianum* culture filtrate is illustrated in Plate 1.



Plate 1: Photographs showing biosynthesis of silver nanoparticles from *Trichoderma harzianum* culture filtrate.

2.3 Characterization of Biosynthesized Silver Nanoparticles

Silver nanoparticles synthesized by *T. harzianum* culture filtrate was analyzed using visual observation in color change and techniques such as UV-visible spectroscopy and Transmission Electron Microscopy (TEM).

Visual observation in color change

Visual observation in color change and color intensity during the incubation period of 24 hrs. was recorded.

UV-visible spectroscopy

UV-Visible spectroscopy analysis was done by using Evolution 201 UV-Visible Spectrophotometer available at

Department of Soil science, NRC on Grape, Pune. The reduction of silver ions were monitored after 24 hrs. by recording the UV-Vis spectrum of the reaction medium after diluting a small aliquot of the sample into deionized water. The spectra of the surface plasmon resonance of AgNPs in the reaction mixture was recorded using UV-Vis spectrophotometer at wavelengths between 200 to 800 nm.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was utilized for the analysis of shape and size and used to image the synthesized AgNPs. TEM analysis of the sample was carried out at Eco-friendly Disease Management and Beneficial Microbes Research Laboratory, MPKV, Rahuri.

2.4 Culture Collection

The pure cultures of test fungal pathogens included *Fusarium oxysporum* f. sp. *lycopersici*, the causal organism of *Fusarium* wilt of tomato, and *Sclerotium rolfsii* responsible for stem rot disease of groundnut were obtained from Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra and the fungal pathogens were maintained on Potato Dextrose Agar (PDA) medium.

2.5 *In vitro* antimicrobial potential of *Trichoderma harzianum* mediated biosynthesized silver nanoparticles (AgNPs) against test fungal plant pathogens

Silver nanoparticles synthesized by *T. harzianum* culture was evaluated under *in vitro* conditions against the test fungal plant pathogens by poisoned food technique, using PDA as basal medium to observe the inhibitory effect of these AgNPs on the mycelial growth of test fungal plant pathogens.

Double strength PDA medium (Potatoes-400 g, Dextrose-40 g, Agar 40 g, Distilled Water-1000 mL) was prepared by doubling the amount of constituents except distilled water and the medium was sterilized at 15-20 psi pressure for 20 minutes. Simultaneously, different concentrations (@ 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm) of silver nanoparticles were prepared in sterilized distilled water so as to get desired concentration of AgNPs after mixing the AgNPs solutions in the double strength media in the 250 ml flask. The PDA added with the AgNPs was shaken well to mix thoroughly and then poured into the Petri plates and allowed to solidify. A small culture bit of 5 mm size of seven days old culture of the test pathogen was cut with a sterile cork borer and picked up with the help of sterilized inoculation needle and then inoculated in the centre of each Petri plate under aseptic conditions in the laminar air flow and incubated at 27 ± 1 °C for a period of 7 days. The Petri plates poured with double strength PDA medium without amending AgNPs was served as untreated control.

Observations on radial mycelial growth/colony diameter

(mm) of the test pathogens was recorded after 7 days. Percent inhibition of mycelial growth of the test pathogens over untreated control was calculated.

3 Results and Discussion

3.1 Characterization of Biosynthesized AgNPs from *Trichoderma harzianum* Culture Filtrate

Visual Observation (Color Change)

The reaction mixture comprising *Trichoderma harzianum* culture filtrate and silver nitrate solution exhibited a gradual but pronounced color change from colorless/light yellow to dark brown during the incubation period of 24 hrs. The highest dark color intensity (+++) was recorded for *T. harzianum*-mediated synthesis confirming efficient bioreduction. The color change observed in *Trichoderma*-mediated synthesis is a well-recognized qualitative indicator of AgNPs formation. These results validate previous results by Singh and Raja, 2011^[14]; Elamawi *et al.* 2018^[3]; El-Wakil, 2020^[6]; El-Ashmony *et al.* 2022^[4] where brown coloration was considered a primary indicator of AgNP formation using *Trichoderma* spp. culture filtrates.

UV-Visible Spectroscopy

UV-Visible spectrophotometric analysis of *Trichoderma harzianum* mediated silver nanoparticles revealed a distinct, sharp and well-defined absorption peak at 428.87 nm, with a maximum absorbance value of 2.143 confirming the formation of nanosized silver particles as graphically showed in graph Figure 1. Peak was narrow and symmetrical, indicating homogeneous particle size distribution and minimal aggregation.

The observed SPR peak lies within the characteristic wavelength range reported for biologically synthesized AgNPs and indicates uniform particle size distribution. Comparable absorption peaks were observed earlier for *Trichoderma*-mediated AgNP synthesis, ranging from 385 to 435 nm, validating the present findings by Elamawi *et al.* 2018^[3]; Kaman and Dutta, 2019^[9]; El-Wakil, 2020^[6]; El-Ashmony *et al.* 2022^[4]; Narware *et al.* 2023^[12].

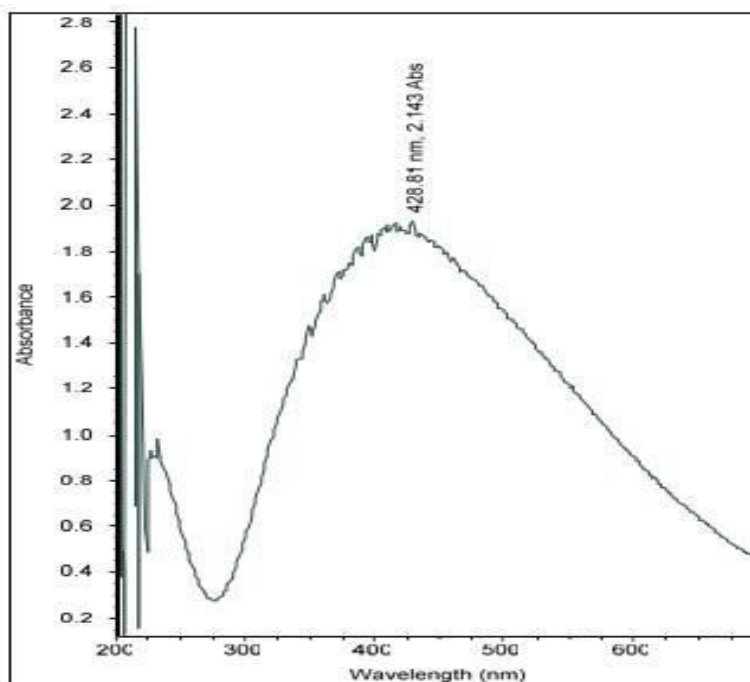


Fig 1: Graphical presentation of UV-visible spectroscopy characterization of biosynthesized silver nanoparticles from *Trichoderma harzianum* culture filtrate.

Transmission Electron Microscopy (TEM).

TEM analysis revealed that AgNPs synthesized utilizing *T. harzianum* culture filtrate were predominantly spherical NPs with smooth edges, well dispersed, and non-agglomerated. Particle size ranged from 13.28 nm to 15.12 nm, indicating narrow size distribution and uniform morphology as shown in photograph of Plate 2. Individual nanoparticles were clearly distinguishable, with minimal clustering or agglomeration. The consistent morphology suggested effective stabilization by fungal biomolecules. The observed nanoscale dimensions confirmed successful synthesis of true nanoparticles rather than bulk silver particles.

TEM provides direct evidence of nanoparticle morphology and size distribution, which are critical parameters influencing biological activity. The spherical shape observed in *Trichoderma*-derived nanoparticles is commonly associated with enhanced antimicrobial efficacy due to increased surface area. The narrow size distribution and lack of aggregation indicate efficient capping by extracellular fungal proteins, which limit particle growth and prevent coalescence. Such controlled morphology enhances nanoparticle stability and reactivity. These findings demonstrate the suitability of *Trichoderma harzianum* as a robust biological agent for producing uniformly shaped and biologically active silver nanoparticles.

Similar spherical morphology and size ranges have been reported for *Trichoderma*-derived silver nanoparticles in earlier studies by Elamawi *et al.* 2018 ^[3]; Kaman and Dutta, 2019 ^[9]; El-Wakil, 2020 ^[6]; El-Ashmony *et al.* 2022 ^[4], confirming the reliability of the biosynthesis protocol employed in the present investigation.

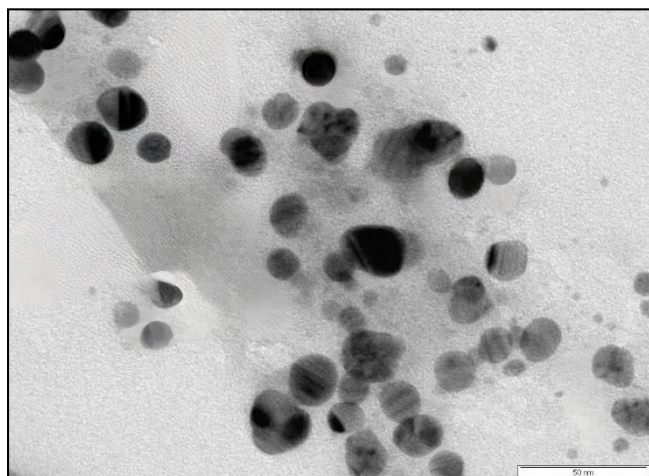


Plate 2: TEM Micrograph showing the relatively spherical shape silver nanoparticles with a size range of 13.28 to 15.12 nm, synthesized by using *Trichoderma harzianum* culture filtrate.

3.2 *in vitro* Evaluation of Antifungal Potential of *Trichoderma harzianum* Mediated Biosynthesized AgNPs Against *Fusarium oxysporum* f. sp. *lycopersici*.

The results revealed a clear concentration-dependent suppression of mycelial growth of the test pathogen. At the lowest concentration (10 ppm), the mycelial growth measured 64.25 mm, corresponding to 28.61 % inhibition, indicating limited antifungal activity. Increasing the concentration to 25 ppm resulted in moderate suppression, with radial growth reduced to 53.00 mm and inhibition enhanced to 41.11 %. A substantial reduction in fungal growth was observed at 50 ppm, where the mycelial

diameter declined to 41.50 mm, recording 53.88 % inhibition.

Marked inhibition was evident at higher concentrations. At 75 ppm, mycelial growth was restricted to 26.25 mm, resulting in 70.83 % inhibition, which was significantly superior to lower concentrations. Maximum antifungal effect was seen at 100 ppm, where radial growth was reduced to 17.00 mm, corresponding to 81.11 % inhibition, and this treatment was significantly leading over all other concentrations. In contrast, the untreated control exhibited full mycelial growth (90.00 mm) with no inhibition. The morphological effects of nanoparticle treatments are depicted in photographs of Plate 3, while quantitative information is depicted in Table 1.

Table 1: *in vitro* Evaluation of antifungal potential of *T. harzianum* mediated biosynthesized silver nanoparticles (AgNPs) against *F. oxysporum* f. sp. *lycopersici*.

Tr. No.	Treatment details	Radial Growth of pathogen	% Inhibition
T ₁	AgNPs @ 10 ppm	64.25	28.61 (32.34)
T ₂	AgNPs @ 25 ppm	53	41.11 (39.88)
T ₃	AgNPs @ 50 ppm	41.50	53.88 (47.23)
T ₄	AgNPs @ 75 ppm	26.25	70.83 (57.31)
T ₅	AgNPs @ 100 ppm	17	81.11 (64.24)
T ₆	Control	90	00.00
	SE±	1.12	
	CD at 1 %	4.56	

*Figures in parentheses are arc sine transformed values

The present findings clearly demonstrate the strong antifungal potential of *Trichoderma harzianum* mediated AgNPs against *F. oxysporum* f. sp. *lycopersici*. The progressive increase in growth inhibition with increasing AgNPs concentration indicates a direct relationship between nanoparticle dose and antifungal efficacy. At higher concentrations, AgNPs likely interact more effectively with fungal cell walls and membranes, leading to structural damage, disruption of membrane permeability, and interference with metabolic processes, ultimately suppressing hyphal extension.

The pronounced inhibition observed at 75 and 100 ppm suggests that higher nanoparticle density enhances contact with fungal hyphae, resulting in increased cellular stress and growth arrest. The superior efficacy of *Trichoderma*-derived AgNPs may also be attributed to the presence of fungal metabolites acting as capping agents, which could synergistically enhance nanoparticle toxicity.

The results of the present investigation are in close agreement with earlier reports. Al-Abboud (2018) ^[1] documented complete growth inhibition of *F. oxysporum* at 80 ppm utilizing AgNPs biosynthesized from *T. harzianum*. Similarly, Elamawi *et al.* (2018) ^[3] reported high inhibition percentages of AgNPs prepared by *Trichoderma longibrachiatum* against various fungal pathogens, involving *F. oxysporum*. El-Wakil (2020) ^[6] demonstrated significant antifungal activity of AgNPs synthesized from various *Trichoderma* species against *Fusarium* spp., with *T. hamatum* showing the highest inhibitory response.

Recent findings by Thepbandit *et al.* (2024) ^[15] further support the present results, reporting 83.66 % inhibition of *Fusarium solani* by *T. harzianum*-derived AgNPs. Additionally, the antifungal effectiveness observed in this study is inline with previous investigations conducted by Jo *et al.* (2009) ^[8], Kim *et al.* (2012) ^[10], Jolanta *et al.* (2013) ^[7]

and Ouda (2014) [13], who collectively demonstrated the broad-spectrum antifungal action of AgNPs against plant pathogenic fungi.

Overall, the results confirm that AgNPs derived from

Trichoderma harzianum demonstrate strong antifungal action against *F. oxysporum* f. sp. *lycopersici*, with higher concentrations showing significantly enhanced inhibitory effects.

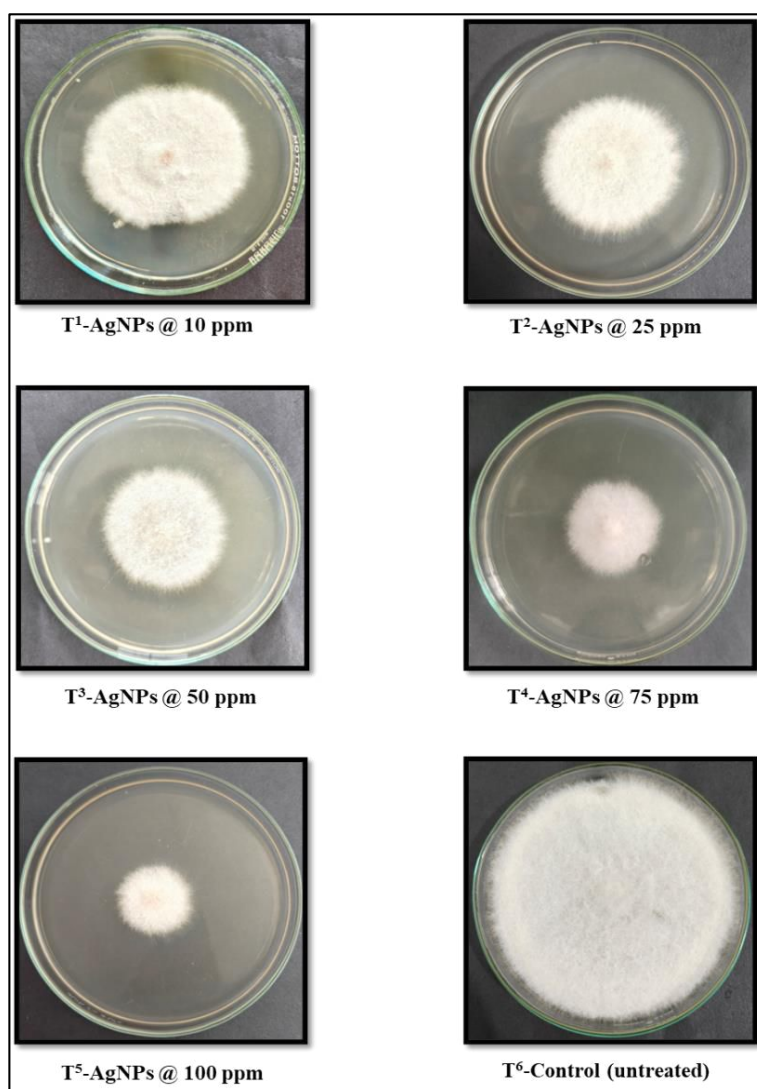


Plate 3: Photograph showing *in vitro* evaluation of antifungal potential of *Trichoderma harzianum* mediated biosynthesized silver nanoparticles (AgNPs) against *Fusarium oxysporum* f. sp. *lycopersici*.

3.3 *In vitro* Evaluation of Antifungal Potential of *Trichoderma harzianum* Mediated Biosynthesized Silver Nanoparticles (AgNPs) Against *Sclerotium rolfii*.

In case of *Sclerotium rolfii*, at the lowest concentration (10 ppm), limited inhibition (15.55 %) was observed, with radial growth measuring 76.00 mm. Increasing the concentration to 25 ppm caused a little raise in inhibition to 23.33 %, with mycelial growth of 69.00 mm. A pronounced reduction was observed at 50 ppm, where mycelial growth declined to 45.50 mm, corresponding to 49.44 % inhibition.

At higher concentrations, antifungal activity increased sharply. At 75 ppm, radial growth was restricted to 25.00 mm, registering 72.22 % inhibition, which was statistically significant. The highest inhibition (82.22 %) was recorded at 100 ppm, with minimal mycelial growth of 16.00 mm. The control showed complete growth (90.00 mm). The outcomes are depicted in Table 2 and photographs of Plate 4.

The suppression of *Sclerotium rolfii* observed in this investigation highlights the strong antifungal capability of AgNPs prepared utilizing *Trichoderma harzianum*. The marked reduction in mycelial growth at higher nanoparticle

concentrations suggests that *S. rolfii*, despite its aggressive growth habit and sclerotial nature, is highly susceptible to biologically synthesized AgNPs.

Table 2: *In vitro* evaluation of antifungal potential of *Trichoderma harzianum* mediated biosynthesized silver nanoparticles (AgNPs) against *Sclerotium rolfii*.

Tr. No.	Treatment details	Radial Growth of the pathogen	% Inhibition
T ₁	AgNPs @ 10 ppm	76	15.55 (23.22)
T ₂	AgNPs @ 25 ppm	69	23.33 (28.88)
T ₃	AgNPs @ 50 ppm	44.5	49.44 (44.68)
T ₄	AgNPs @ 75 ppm	25	72.22 (58.19)
T ₅	AgNPs @ 100 ppm	16	82.22 (65.06)
T ₆	Control	90	00.00
	SE _±	0.8250	
	CD at 1 %	3.3582	

*Figures in parentheses are arc sine transformed values

Previous research provides corroborative evidence for the present observations. Kaman and Dutta (2019) [9]

documented substantial growth inhibition of *S. rolfii* when silver nanoparticles derived from *Trichoderma asperellum* were applied, with inhibition exceeding 70 per cent at 100 ppm concentration. Their findings demonstrated that sclerotial fungi are particularly sensitive to nanosilver, a trend that was clearly reflected in the present study.

Investigations conducted by El-Ashmony *et al.* (2022) [4] further emphasized the antifungal potential of *Trichoderma*-based AgNPs, where nanoparticles synthesized from *T. harzianum* caused substantial suppression of *Sclerotinia sclerotiorum* and *S. rolfii*. The observed reduction in linear mycelial growth across varying concentrations indicated that the antifungal action of AgNPs is not limited to a single pathogen but extends to multiple soil-borne fungi with similar infection strategies.

The heightened antifungal response against *S. rolfii* might be because of dual action of AgNPs and antifungal metabolites produced by *Trichoderma* species. These metabolites, including hydrolytic enzymes and secondary antifungal compounds, may become associated with the nanoparticle surface, thereby enhancing penetration and disrupting fungal cellular integrity. Consequently, the present findings reaffirm the suitability of *Trichoderma harzianum*-mediated silver nanoparticles as an effective biological alternative for controlling *Sclerotium rolfii*.

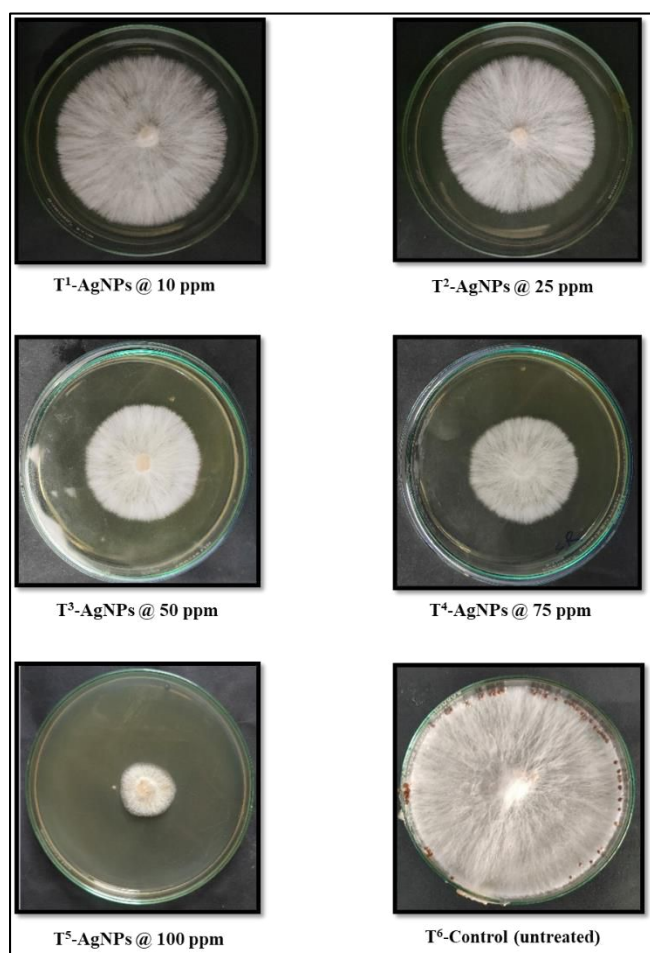


Plate 4: Photograph showing *in vitro* evaluation of antifungal potential of *Trichoderma harzianum* mediated biosynthesized silver nanoparticles (AgNPs) against *Sclerotium rolfii*.

Conclusion

Successful biosynthesis of AgNPs was attained utilizing *Trichoderma harzianum* culture filtrate. *Trichoderma*-

mediated biosynthesized AgNPs demonstrated significant antifungal efficacy against *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotium rolfii*. The significant *in vitro* inhibition of test pathogens underscores the potential of this biosynthesized AgNPs as a sustainable, eco-friendly component of integrated disease management strategies, offering an alternative to reduce reliance on synthetic chemical pesticides.

References

1. Al Abboud MA. Fungal biosynthesis of silver nanoparticle and their role in control of fusarium wilt of sweet pepper and soil-borne fungi *in vitro*. Int J Pharmacol. 2018;14(6):773-780.
2. Chandrasekhar Y, Ahammed SK, Prasad TNVK, Devi RSJ. Evaluation of fungicidal activity of mycogenic silver nanoparticles against *Sclerotium rolfii* inciting stem rot disease in groundnut (*Arachis hypogaea* L.). Indian Phytopathol. 2016;69(4):609-613.
3. Elamawi RM, Al-Harbi RE, Hendi AA. Biosynthesis and characterization of silver nanoparticles using *Trichoderma longibrachiatum* and their effect on phytopathogenic fungi. Egypt J Biol Pest Control. 2018;28:28-28.
4. El-Ashmony RMS, Zaghloul NSS, Milošević M, Mohany M, Al-Rejaie SS, Abdallah Y, Galal AA. The biogenically efficient synthesis of silver nanoparticles using the fungus *Trichoderma harzianum* and their antifungal efficacy against *Sclerotinia sclerotiorum* and *Sclerotium rolfii*. J Fungi (Basel). 2022;8(6):597-597. doi:10.3390/jof8060597.
5. Elgorban AM, Aref SM, Seham SM, Elhindi KM, Bahkali AH, Sayed SR, Manal MA. Extracellular synthesis of silver nanoparticles using *Aspergillus versicolor* and evaluation of their activity on plant pathogenic fungi. Mycosphere. 2016;7:844-852.
6. El-Wakil DA. Antifungal activity of silver nanoparticles by *Trichoderma* species: synthesis, characterization and biological evaluation. Egypt J Phytopathol. 2020;48(1):71-80.
7. Jolanta P, Marcin B, Renata S, Mirosław B. Nanosilver against fungi: silver nanoparticles as an effective biocidal factor. Acta Biochim Pol. 2013;60(4):795-798.
8. Jo YK, Kim BH, Jung G. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Dis. 2009;93:1037-1043.
9. Kaman PK, Dutta P. Synthesis, characterization and antifungal activity of biosynthesized silver nanoparticle. Indian Phytopathol. 2019;72:79-88.
10. Kim SW, Jung JH, Lamsal K, Kim YS, Min JS, Lee YS. Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. Mycobiology. 2012;40(1):53-58.
11. Muhammad T, Tahir F, Ahmad SA, Muhammad DH, Amin UR. Role of *Trichoderma* as a biocontrol agent (BCA) of phytoparasitic nematodes and plant growth inducer. J Invertebr Pathol. 2021;183:107626-107626.
12. Narware J, Singh SP, Manzar N, Kashyap AS. Biogenic synthesis, characterization, and evaluation of synthesized nanoparticles against the pathogenic fungus *Alternaria solani*. Front Microbiol. 2023;14:1159251-1159251. doi:10.3389/fmicb.2023.1159251.
13. Ouda SM. Antifungal activity of silver and copper nanoparticles on two plant pathogens, *Alternaria*

- alternata* and *Botrytis cinerea*. Res J Microbiol. 2014;9(1):34-42.
14. Singh P, Raja RB. Biological synthesis and characterization of silver nanoparticles using the fungus *Trichoderma harzianum*. Asian J Exp Biol Sci. 2011;2(4):600-605.
15. Thepbandit W, Papathoti NK, Hoang NH, Siriwong S, Sangpueak R, Saengchan C, Laemchiab K, Kiddeejing D, Tonpho D, Buensanteai K. Bio-synthesis and characterization of silver nanoparticles from *Trichoderma* species against cassava root rot disease. Sci Rep. 2024;14:12535-12535.
16. Zin NA, Badaluddin NA. Biological functions of *Trichoderma* spp. for agriculture applications. Ann Agric Sci. 2020;65:168-178.