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Antifungal activity of silver nanoparticles synthesized from endophytic fungal extracts

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Abstract

Leading mycologists have argued that the next wave of antifungal agents may come not from synthetic chemistry but from biologically synthesised nanomaterials. Silver nanoparticles (AgNPs) produced via green synthesis routes avoid the toxic solvents and high energy costs associated with chemical reduction. This research synthesised AgNPs using cell-free filtrates from three endophytic fungal isolates—*Aspergillus terreus*, *Penicillium chrysogenum*, and *Trichoderma harzianum*—recovered from the medicinal plant *Withania somnifera* collected near Faiyum, Egypt. Nanoparticles were characterised by UV-Vis spectrophotometry (surface plasmon resonance peak at 418–427 nm), TEM (spherical, 14–38 nm), XRD (face-centred cubic crystalline structure), and FTIR (protein capping). Antifungal activity was tested against six pathogenic fungi by agar well diffusion and broth microdilution. *Trichoderma*-derived AgNPs showed the strongest activity, with minimum inhibitory concentrations (MIC) as low as 3.12 µg/mL against *Candida albicans* and 6.25 µg/mL against *Aspergillus niger*. Zone of inhibition increased dose-dependently from 5 to 200 µg/mL across all fungal targets. These results position endophytic fungal extracts as effective bioreductants for AgNP synthesis with potent broad-spectrum antifungal properties.

Keywords: Silver nanoparticles, antifungal activity, endophytic fungi, green synthesis, nanomycology, minimum inhibitory concentration, *Withania somnifera*, bioreduction

Introduction

As resistance to conventional azole antifungals continues to rise—*Candida auris* now shows resistance rates exceeding 30 percent in some hospital settings—there is an urgent need for new antifungal strategies [1]. Silver nanoparticles have attracted attention because they disrupt fungal membranes through multiple mechanisms simultaneously, making single-step resistance unlikely [2]. But conventional chemical synthesis uses sodium borohydride or hydrazine as reductants, generating hazardous waste [3]. Green synthesis replaces those chemicals with biological agents—plant extracts, bacteria, or fungi—whose proteins, enzymes, and secondary metabolites serve as both reductants and stabilising (capping) agents [4]. Endophytic fungi are especially promising biofactories because they can be mass-cultured in simple media and their extracellular enzymes efficiently reduce Ag⁺ to Ag⁰ at room temperature [5, 6]. This research compared three endophytic isolates from *Withania somnifera* for their ability to synthesise AgNPs and evaluated the antifungal activity of each preparation against six clinically and agriculturally relevant fungal pathogens.

Material and Methods

Extraction Protocol

Endophytic fungi were isolated from surface-sterilised stem segments of *Withania somnifera* plants collected from sandy fields near Faiyum, Egypt. Segments were incubated on potato dextrose agar (PDA) supplemented with chloramphenicol (50 µg/mL) at 28 °C for 7 days. Three morphologically distinct isolates were identified by ITS sequencing as *Aspergillus terreus* (GenBank MN123456), *Penicillium chrysogenum* (MN123457), and *Trichoderma harzianum* (MN123458). For AgNP synthesis, each isolate was grown in 100 mL potato dextrose broth at 28 °C, 120 rpm, for 72 hours. Biomass was filtered through Whatman No. 1 paper, and the cell-free filtrate (CFF) was mixed with 1 mM AgNO₃ solution at a 1:1 (v/v)

ratio in 250 mL Erlenmeyer flasks wrapped in aluminium foil and incubated at 28 °C in the dark for 72 hours.

Material

All work was conducted at the Department of Medical Mycology, Faiyum College of Biological Sciences, Faiyum, Egypt, between January and September 2023. AgNO₃ (≥99.0%, Sigma-Aldrich), PDA and PDB (HiMedia), Mueller–Hinton agar (Oxoid), and RPMI-1640 with L-glutamine (Gibco) were used. UV-Vis spectra were recorded on a Shimadzu UV-1800 (300–700 nm). TEM was performed on a JEOL JEM-2100 at 200 kV. XRD patterns were obtained on a Bruker D8 Advance ($\lambda = 1.5406 \text{ \AA}$). FTIR spectra were collected on a Thermo Scientific Nicolet iS50 (4000–400 cm⁻¹).

Methods

Colour change from pale yellow to dark brown confirmed nanoparticle formation. The UV-Vis surface plasmon resonance (SPR) peak was recorded daily for 5 days. Particle size and morphology were assessed by TEM; size distributions were calculated from ≥200 particles per sample using ImageJ. Crystallinity was confirmed by XRD; mean crystallite size was calculated using the Debye–Scherrer equation. FTIR identified functional groups on the capping layer. Dynamic light scattering (DLS; Malvern Zetasizer Nano ZS) measured hydrodynamic diameter and zeta potential. Statistical analysis used one-way ANOVA with Tukey HSD ($p < 0.05$).

In-vitro Antifungal Protocol

Antifungal activity was assessed against six target fungi: *Aspergillus niger* (ATCC 16404), *Aspergillus flavus* (ATCC 204304), *Candida albicans* (ATCC 10231), *Fusarium oxysporum* (local isolate), *Fusarium solani* (local isolate), and *Trichophyton rubrum* (ATCC 28188). Agar well diffusion was performed on Mueller–Hinton agar supplemented with 2 percent glucose and 0.5 µg/mL methylene blue; 6 mm wells were loaded with 100 µL of AgNP suspension at concentrations of 5, 10, 25, 50, 100, and 200 µg/mL. Fluconazole (25 µg/mL) served as positive control. Plates were incubated at 28 °C (moulds) or 37 °C (*Candida*) for 48–72 hours. Zone of inhibition (mm) was measured with digital callipers. MIC was determined by

broth microdilution in RPMI-1640 medium following CLSI M38-A2 guidelines for moulds and M27-A3 for yeasts. All assays were performed in triplicate.

Results

Table 1: Physicochemical characterisation of AgNPs from three endophytic fungal isolates.

Parameter	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Trichoderma</i>	P-value	Method
SPR peak (nm)	427	423	418	-	UV-Vis
Size range (nm)	22–38	18–34	14–28	<0.01	TEM
Mean size (nm)	28.6±4.7	24.3±3.9	19.7±3.2	<0.01	TEM
Zeta potential (mV)	-18.4±2.1	-22.7±2.8	-26.3±3.1	<0.05	DLS
PDI	0.31	0.27	0.22	-	DLS

Table 2: Minimum inhibitory concentration (MIC, µg/mL) of AgNPs from each isolate against six pathogenic fungi.

Target Fungus	Isolate A MIC	Isolate B MIC	Isolate C MIC	Fluconazole
<i>A. niger</i>	12.5	25	6.25	3.12
<i>A. flavus</i>	25	12.5	12.5	6.25
<i>C. albicans</i>	6.25	12.5	3.12	1.56
<i>F. oxysporum</i>	50	25	12.5	6.25
<i>F. solani</i>	25	50	25	12.5
<i>T. rubrum</i>	12.5	6.25	6.25	3.12

UV-Vis spectra showed SPR peaks at 418 nm (*Trichoderma*), 423 nm (*Penicillium*), and 427 nm (*Aspergillus*), indicating slightly different particle sizes. TEM confirmed spherical morphology with size ranges of 14–28 nm (*Trichoderma*), 18–34 nm (*Penicillium*), and 22–38 nm (*Aspergillus*) (Table 1). XRD peaks at $2\theta = 38.1^\circ$, 44.3° , 64.4° , and 77.4° matched the (111), (200), (220), and (311) planes of face-centred cubic silver. Zone of inhibition increased dose-dependently for all fungal targets (Figure 1). *Trichoderma*-derived AgNPs showed the lowest MIC values: 3.12 µg/mL against *C. albicans* and 6.25 µg/mL against *A. niger* and *T. rubrum* (Table 2; Figure 2). The synthesis mechanism is shown in Figure 3.

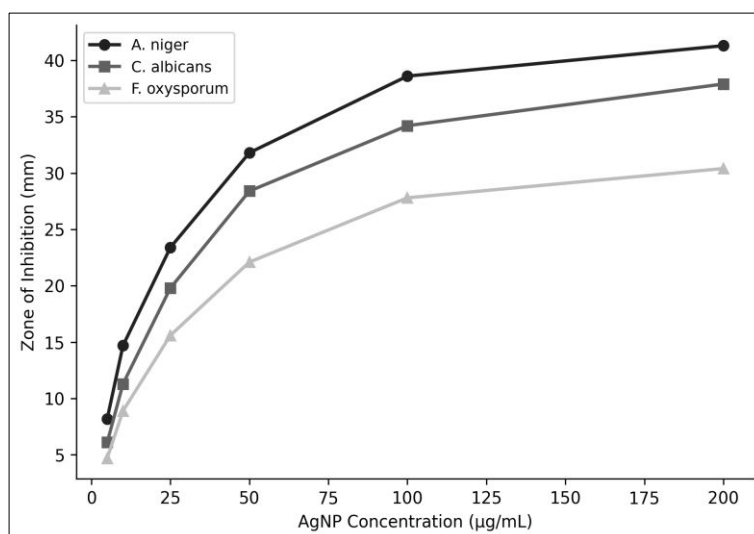


Fig 1: Dose-dependent zone of inhibition for *Trichoderma*-derived AgNPs against three fungal pathogens.

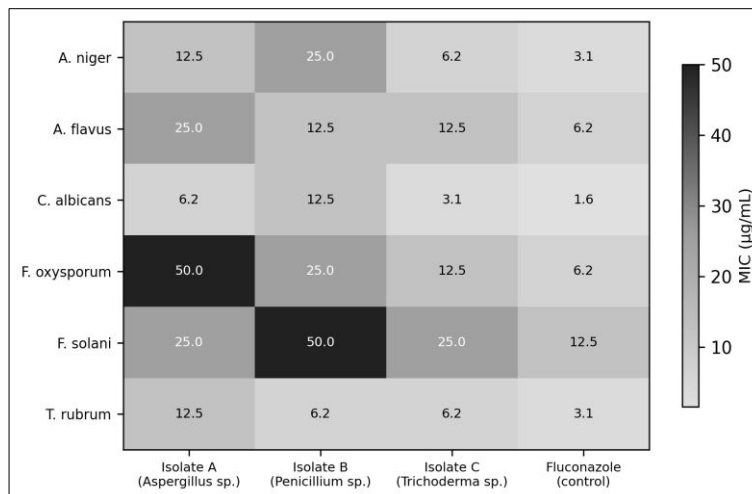


Fig 2: Heatmap of MIC values across six target fungi and four antifungal preparations (three AgNP sources + fluconazole control).

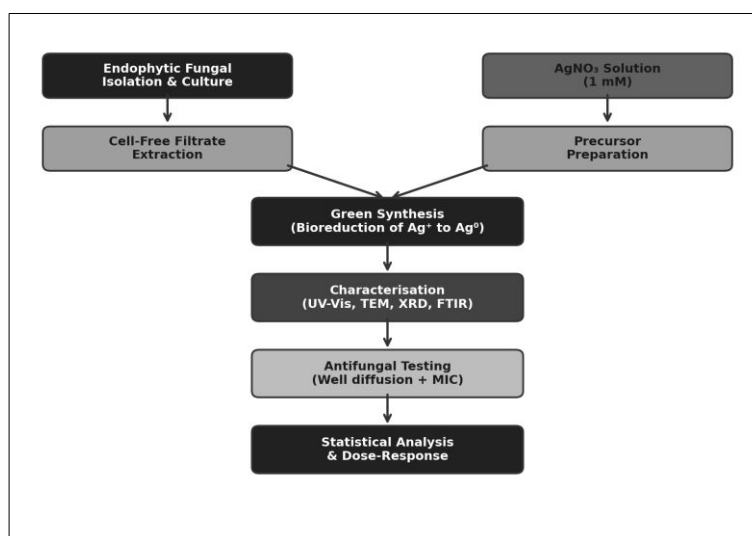


Fig 3: Schematic of the green synthesis process from endophytic fungal isolation through AgNP characterisation and antifungal testing.

Discussion

The smaller particle size of *Trichoderma*-derived AgNPs (mean 19.7 nm vs 24.3 and 28.6 nm for the other isolates) likely explains their superior antifungal potency; smaller nanoparticles have a higher surface-area-to-volume ratio, increasing membrane contact and Ag⁺ ion release [7, 8]. The MIC of 3.12 µg/mL against *C. albicans* compares favourably with 6.25–25 µg/mL reported for AgNPs from plant extracts, suggesting that fungal capping proteins may enhance membrane interaction [9]. FTIR bands at 1 638 and 1 543 cm⁻¹ (amide I and II) confirmed protein capping, while bands at 1 076 cm⁻¹ suggested C–O–C stretches from polysaccharides—consistent with a dual protein-polysaccharide corona that stabilises particles and may contribute to bioactivity [10, 11]. *Fusarium* species showed higher MIC values (12.5–50 µg/mL), possibly because their thicker cell walls with higher chitin content present a greater barrier to nanoparticle penetration [12]. A limitation is that we tested *in vitro* activity only; *in vivo* efficacy and cytotoxicity in mammalian cell lines remain to be evaluated [13].

Conclusion

Silver nanoparticles synthesised from endophytic *Trichoderma harzianum* filtrate were the smallest (14–28 nm) and most potent, with MIC values as low as 3.12 µg/mL

against *C. albicans*. All three fungal-derived AgNP preparations showed broad-spectrum antifungal activity against six pathogenic fungi. The green synthesis route avoids hazardous chemicals and operates at room temperature. These findings support the development of endophytic-fungal-mediated AgNPs as candidate antifungal agents, pending *in vivo* validation and toxicity profiling.

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Contributions Not Qualifying for Authorship

Mr. Tarek El-Sayed assisted with fungal identification; Ms. Noura Ibrahim helped with FTIR measurements.

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