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Mycogenic Silver Nanoparticles From Endophytic *Trichoderma atroviride* with Antimicrobial Activity

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Abstract: There is an increasing interest in developing nanoparticles with diverse biologic activities. To this end, we prepared 10 to 15 nm silver nanoparticles (AgNP) from native isolates of *Trichoderma atroviride*. Within this study, endophytic fungi hosted four medicinal plants in Saint Katherine Protectorate, South Sinai, Egypt have been isolated by surface sterilization technique on four isolation media. Ten species, based on their frequency of occurrence, out of twenty recovered taxa were tested for their capability to synthesize extracellular AgNPs. *Trichoderma atroviride* hosted *Chiliadenus montanus* was found to be the best candidate for the production of mycogenic AgNPs among all examined species. The mycosynthesized AgNPs were compared with chemically synthesized and characterized using Ultraviolet-visible (UV-vis) spectroscopy, Raman spectroscopy, X-ray diffraction (XRD) and high-resolution transmission electron microscopy (HRTEM) techniques. The HRTEM result showed the distribution of spherical AgNPs ranging from 10 to 15 nm. *Trichoderma atroviride* isolate was subjected to sequencing for confirmation of phenotypic identification. The internal transcribed spacer (ITS) 1–5.8 s – ITS2 rDNA sequences obtained were compared with those deposited in the GenBank Database and registered with accession number MH283876 in the NCBI Database. Antibacterial, anticandidal and antifungal effects of chemically and mycosynthesized AgNPs were examined at various concentrations in vitro against six pathogenic bacteria and 4 pathogenic fungi by agar well diffusion technique. Standard antibiotics; Gentamicin, Amoxicillin, Clotrimazole, and Nystatin at 5 μg/disk were taken as positive controls, while 5% DMSO was used as the negative control. Our data revealed that the application of mycogenic AgNPs at a concentration of 100 ppm resulted in maximum inhibition of pathogenic bacteria and fungi. These data suggest that AgNPs from native isolates of *Trichoderma atroviride* (MH283876) offer a source of rapid synthesis of eco-friendly, economical biomaterials that show antimicrobial activities.

Keywords: Antimicrobial; endobionts; mycogenic AgNPS; Saint Katherine Protectorate

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1 Introduction

Based on their unique features, nanoparticles (NPs) are characterized by small with a large surface area and application in diverse fields, the fabrication of matter at the nanoscale (1-100 nm) has been improving rapidly in the last decade [1-3]. Besides chemical or physical methods or nano-synthesis of NPs by microorganisms and higher plants, fungi (myconanotechnology) came recently to attract the scientist's attention for the production of NPs [4-13]. Besides having a rich repertoire of enzymes, the use of fungi offers distinct advantages including ease of maintenance and scaling [14-16]. Due to the changes placed upon the ecosystems the number of plant species that are becoming extinct is increasing rapidly [17]. It has been shown that endophytic fungi can help in the conservation of plants as a sustainable source of biological resources [18]. Among NPs, the AgNPs have been produced that offer potent antimicrobial activity against an array of plant and animal pathogens and their inclusion in wound dressing has enhanced wound healing [19, 20]. Biogenic AgNPs have been widely applied in control of infection, in food and water preservation and in various cosmetic, pharmaceutical and medical products [21-23].

Endobiontic fungi are microfungi that host plant tissue without any apparent pathological symptoms intercellularly and/or intracellularly [18, 24, 25]. To be able to sustain steady symbiosis, endophytes develop chemical substances that enhance the development of plants and benefit them to acclimatise better to the harsh environment [26]. As a treasure mine of bioactive metabolites, endophytic fungi considered a sustainable source of various natural products *viz:* quinones, saponins, alkaloids, steroids, phenolic acids, terpenoids, and tannins that exhibit antimicrobial and anticancer properties [27]. Medicinal plants and their endophytes constitute a significant supply of over 80% of the natural drugs, bioactive compounds & secondary metabolites, worldwide [18, 28].

Recently a “bioprospecting” term was applied to refer an old practice for searching useful bioactive compounds and other potentially valuable biochemical products from nature [28, 29]. Recently, fungi included in the interest of biotechnology as organisms important not only for the crucial roles they undertake in nature as recyclers but because of their immense importance to the ecosystem components especially humans [30]. The scientific community worldwide in the last two decades concentrated on the fungal endophytes as a sustainable and eco-friendly source for the production of bioactive metabolites that may be effective candidates for the first-class drugs for the treatment of human diseases. It was recently indicated that endophytic fungi produce about 51% of new bioactive substances in comparison with 38% came from terricolous fungi. During the selection of the hosting plants of endophytic fungi, unique environments, diversity, ethnobotanical history and endemicity should be considered during selection and sampling processes [28, 29]. The mountainous region of southern Sinai exhibits greater biodiversity than the rest of Egypt, and 4350 km^2 of this area was declared a Protectorate in 1996 [31]. In South Sinai, Bedouin’s traditional folk medicine depends upon approximately 170 plant species that inhabit the area [32]. The associated endophytic fungi of medicinal and/ or endemic plants are ideal for the synthesis of nanoparticles due to the ease of large-scale production in bioreactors compared to plants that require more sophisticated preparation [33] and because downstream processing is more manageable [19].

Endophytes have been most widely studied for their ability to develop antibacterial, antioxidant, antifungal, and antidiabetic compounds and their ability to synthesis nanoparticles has also been described to some extent [34-36]. The development of an eco-friendly and reliable process for the green synthesis of nanoparticles is a huge step in the application of nanotechnology. One approach with immense potential is based on the mycosynthesis of nanoparticles by plant hosting microfungi (endophytes).

During our continuous bioprospecting of endophytic fungi in Saint Katherine Protectorate, fungal endobionts hosted four wild medicinal plants were isolated and surveyed for their capability to green synthesize AgNPs. *Trichoderma atroviride* hosted in *Chiliadenus montanus*, the most potent taxon for production was selected. The mycosynthesized AgNPs were characterized using UV-vis (UV-vis), Raman

spectroscopy, X-ray diffraction (XRD) and high-resolution transmission electron microscopy (HRTEM). The antibacterial and antifungal efficacy of the mycosynthesized AgNPs was studied against phyto and human pathogenic bacteria (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Shigella flexneri*, and *Salmonella typhimurium*), yeast (*Candida albicans*) and filamentous fungi (*Aspergillus brasiliensis, A. niger* and *Fusarium oxysporum*).

2 Materials and Methods

2.1 Plant Sampling and Isolation of Endophytic Fungi

One hundred samples representing the four-plant species under investigation were collected from Wadi Itlah, Wadi Tala and Wadi El-Arbaein respectively. Aerial parts (25 samples/ plant) from *Chiliadenus montanus* (Vahl) Brullo; *Origanum syriacum* L.; *Verbascum sinaiticum* Benth and *Artemisia herba-alba* Asso were sampled according to Abdel-Azeem et al. [18]. For isolation of endophytic microfungi, small parts of the aerial parts of each plant were surface-sterilized according to Abdel-Azeem et al. [28]. Sterilized pieces were plated out on 400 petri dishes with 4 different isolation media supplemented with a bacteriostatic and a bactericidal to suppress bacterial growth and incubated at 28°C [28].

2.2 Phenotypic Identification

Relevant identification keys were used for phenotypic identification of the recovered microfungi e.g., for *Penicillium* [37], *Aspergillus* [38], dematiaceous hyphomycetes [39, 40], *Fusarium* [41], different taxa [42], ascomycetes of soil [43], *Chaetomium* [44] and *Alternaria* [45]. Taxonomic position, assignments and name corrections of all recovered taxa were compared with the Index Fungorum Partnership [46].

2.3 AgNPs Synthesis

2.3.1 Synthesis of Mycogenic AgNPs

Capabilities of ten taxa with a high frequency of occurrence, among all recovered taxa, for the biosynthesis of Ag-NPs have been surveyed. Taxa were *Alternaria alternata*; *A. atra*; *Aspergillus flavus*; *A. niger*; *Curvularia lunata*; *Fusarium oxysporum*; *Nigrospora oryzae*; *Trichoderma atroviride*; *Chaetomium globosum* and *Penicillium chrysogenum*. The selected species were grown on slants of potato dextrose agar at room temperature for two weeks and then maintained at 4°C until required. The fungi were subsequently cultured in 250-mL Erlenmeyer flasks containing 100 mL potato dextrose broth (PDB) at 25°C on a shaker at a speed of 120 rpm for 5 days. The biomass was harvested by separation through a plastic sieve. To remove any medium components, the mycelium of each taxon was washed with sterilized distilled water three times. Subsequently, in 500 ml Erlenmeyer flasks, 20 g of biomass was mixed with 200 ml of sterilized deionized water and agitated under the same conditions for 72 h at 25°C. The flask content was filtered through sterilized Whatman® qualitative filter paper, grade 1 and the filtrate was used for the synthesis of myconanoparticles. 50 ml of 1 mM $AgNO_3$ solution was mixed and agitated in the dark at 25°C with 50 ml of cell filtrate in a 250 ml Erlenmeyer flask for the production of AgNPs. For comparison, control consists of biomass filtrate only without the silver ions was included in the experiment [47].

2.3.2 Synthesis of Chemical AgNPs

In order to compare mycogenic synthesized AgNPs with chemically synthesized AgNPs, the latter AgNPs were prepared according to Fang et al. [48].

2.4 Characterization of AgNPs

Visual observation of the colour change of the cell filtrate was used as a preliminary detection of AgNPs. Positive samples were subjected to optical measurements by using a Perkin Elmer UV-Vis Spectrophotometer (Jasco V-570) and scanning the spectra between 300 and 700 nm at a resolution of

1 nm. The morphology of the samples was studied via high-resolution transmission electron microscopy (HRTEM) on JEOL JEM 2100 (JEOL, Japan) at an accelerating voltage of 200 kV. Selected area electron diffraction (SAED) was performed using HRTEM. The obtained nanomaterials were exanimated by X-ray diffraction, (XRD, Shimadzu XD-1 diffractometer) with Cu Kα radiation (λ = 0.15406 nm). Raman spectra were measured by the dispersive Raman microscope (Model Sentera, Bruker, Germany), the laser wavelength was 532 nm (doubled Nd: YAG laser (neodymium-doped yttrium aluminum garnet) at 10 mW power.

2.5 Molecular Identification

As a potent taxon for the production of AgNPs, *Trichoderma atroviride* was molecularly identified. The CTAB extraction procedure [49] was applied to extract rDNA. Amplification of the internal transcribed spacer (ITS) region of rDNA was applied by PCR [50] using the primer combination of ITS1F/ITS2 according to Abdel-Azeem et al. [51]. By using Geneious 9.0 [52] software a consensus sequence was assembled and compared to those deposited in GenBank using BLASTn for identification.

2.6 Antimicrobial Activity of AgNPs

The potentiality of chemically and mycogenic AgNPs against bacteria were determined against six pathogenic bacteria (*S. aureus* ATCC 29213, *B. cereus* ATCC 6629, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *Sh. flexneri* ATCC 12022, and *S. Typhimurium* ATCC 14028) by agar well diffusion technique [53]. For determination of the antibacterial activity mycosynthetic AgNPs, the diameter of the inhibition zone after 24 h of incubation at 37°C was measured. The anticandidal and antifungal activities were determined against four fungal taxa (*Candida albicans* ATCC10231, *Aspergillus brasiliensis* ATCC 16404, *Aspergillus niger* ATCC 16404, and *Fusarium oxysporum* f. sp. *lycopersici* SCFUN1965). Taxa of pathogenic bacteria and fungi were obtained from the international microbial centers (American Type Culture Collection (ATCC) and Suez Canal University Fungarium (SCUF)). For the preparation of the AgNPs' colloidal solution, AgNPs dissolved in 5% dimethyl sulfoxide (DMSO, 100 ppm/mL) and samples were sonicated for 15 min at 28°C. Four standard antibiotics (Gentamicin, Amoxicillin, Clotrimazole, and Nystatin) with a concentration of 5 μg/disk were taken as positive controls and negative control was represented by 5% DMSO and incubated at 37°C. Inhibition zones were recorded after 24 h for *C. albicans* and 72 h for filamentous fungi. Triplicates of each sample were used and by measuring the inhibition zone, both antibacterial and antifungal activities were determined.

3 Results and Discussion

During the whole study 20 species with a total number of 636 fungi and 1 yeast colony-forming units (CFU) were recovered and deposited in the Fungarium of Suez Canal University (SCUF). Recovered taxa belonged to three phyla, five classes, eight orders, nine families and *incerta sedis* were recorded among higher taxonomic levels (orders and families). The order Eurotiales was most represented by accommodating the highest range of species (7 species). Pleosporales came second including 4 species followed by Hypocreales (3 species). The lowest range of species was recorded in the remaining orders by one or two species. Family Aspergillaceae had the highest contribution to the recovered taxa (7/20) followed by Pleosporaceae (4 species). The rest of the families were represented only by one taxon.

Anamorphic Ascomycota recorded 15 species which represented 75% of the total taxa followed by teleomorphic Ascomycota (3 species, 15%), Basidiomycota (1 species, 5%) and one species of Zygomycota, namely, *Syncephalastrum racemosum* representing 5% of the 20 species recovered. Taxonomically recovered taxa belonged to 17 genera where *Aspergillus* and *Penicillium* were dominated over the isolated genera. *Aspergillus* came first (4 species including the sexual stage of *Emericella*; 36.66% of the total isolates) followed by *Penicillium* (3 species including the asexual stage of *Talaromyces*; 15%) and the rest of taxa were showed only one or two species each. Concerning the association between endophytic fungi and medicinal host plants (Tab. 1), *Artemisia herba-alba* and

Table 1: Total count, number of cases of isolation out of 400 plates and percentage of frequency of isolated taxa

Species	Source				TC	NCI	%F
	A	C	O	V			
Alternaria alternata (Fr.) Keissl.	41	22	10	37	110	42	10.5
Aspergillus niger Tiegh.	6	18	30	24	78	40	10
Nigrospora oryzae (Berk. & Broome) Petch	9	15	17	22	63	33	8.25
Aspergillus flavus Link	9	18	19	11	57	29	7.25
Chaetomium globosum Kunze	8	27	8	11	54	27	6.75
Fusarium oxysporum Schltdl.	17	9	14	9	49	26	6.5
Penicillium chrysogenum Thom	25	8	9	7	49	24	6
Trichoderma atroviride P. Karst.	11	22	6	8	47	22	5.5
Alternaria atra (Preuss) Woudenb. & Crous	6	7	14	11	38	20	5
Curvularia lunata (Wakker) Boedijn	7	9	11	2	29	13	3.25
Cladosporium cladosporioides (Fresen.) DeVries	5	0	12	0	17	9	2.25
Aspergillus nidulans (Eidam) G. Winter	0	0	10	0	10	6	1.5
Penicillium brevicompactum Dierckx	7	0	2	0	9	5	1.25
Talaromyces stipitatus C.R. Benj.	0	0	0	10	**10**	7	1.75
Sarocladium strictum (W.Gams) Summerb.	2	0	0	2	4	2	0.5
Syncephalastrum racemosum Cohn ex J. Schröt.	0	2	0	2	4	2	0.5
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	1	0	2	0	3	2	0.5
Aspergillus terreus Thom	2	0	0	0	2	2	0.5
Exserohilum monoceras (Drechsler) K.J. Leonard & Suggs	0	0	2	0	2	1	0.25
Phanerodontia chrysosporium (Burds.) Hjortstam & Ryvarden	1	0	0	0	1	1	0.25
Yeast	0	0	1	0	1	1	0.25
Total	157	157	167	156	637		
Fungal species richness per plant	16	11	16	13	**20 species**		

Note: where, TC = Total Count, NCI = Number of cases of isolation, %F = Percentage of frequency, A = *Artemisia herba-alba*, C = *Chiliadenus montanus*, O = *Origanum syriacum*, and V = *Verbascum sinaiticum*.

Origanum syriacum hosted the highest fungal richness represented by 16 species each followed by *Verbascum sinaiticum* (13 species) and *Chiliadenus montanus* (11 species). Based on the total CFU, *Origanum syriacum* came first among all studied plants by recording (167 CFU), while *Verbascum sinaiticum* hosted the lowest count (156 CFU).

The sequence obtained from the *T. atroviride* isolate was 575 bp in length (Fig. 1). The *T. atroviride* sequence was checked against the NCBI database using the BLAST homology search, and ITS data of the *T. atroviride* isolate was 100% identical to GenBank data. Native *T. atroviride* was deposited in the GenBank database with an accession number of MH283876 (Fig. 2). Our work revealed that the counts of endophytic fungal populations recovered from endemic plants in Saint Katherine Protectorate were relatively moderate. This result is in agreement with various studies viz. Selim et al. [54, 55], Abdel-Azeem et al. [18, 56]. Some taxa like *Chaetomium globosum*, *Alternaria alternata*, and *Nigrospora oryzae* were recorded as common species hosted in the plants under investigation. These associations

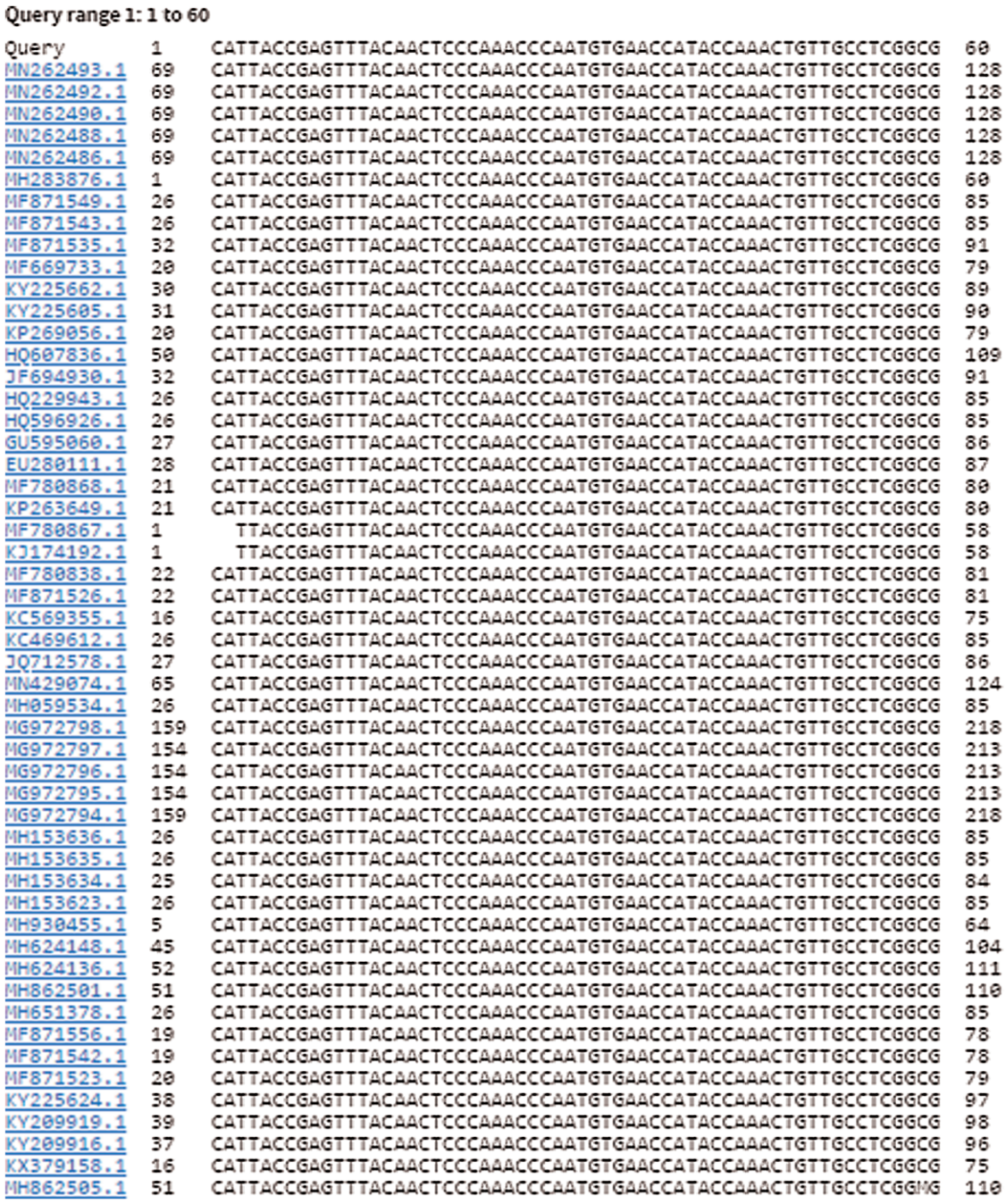


Figure 1: ITS sequence of *T. atroviride* (SCUF-1511- MH283876) isolate and the alignments among 60 similar *Trichoderma* species

may be referred to as the chemical compositions of the host plants [30]. Some plants in Saint Katherine Protectorate tolerated adverse environmental conditions, e.g., water stress, altitude, and evaporation which reflected on the production of various chemical compounds by their hosted endophytic fungi [18, 29, 56].

A comprehensive study on the biosynthesis of silver nanoparticles by natural mycogenic extracts from *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum,*

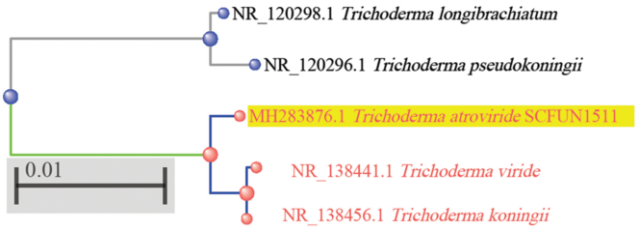


Figure 2: ITS sequence tree of *T. atroviride* (SCUF-1511- MH283876) made though NCBI blast based on neighbor-joining method with max sequence difference of 0.75

Nigrospora oryzae, Trichoderma atroviride, Chaetomium globosum, and *Penicillium chrysogenum* are described in this work. The aqueous silver ions were reduced to silver nanoparticles when added to the mycogenic extract of the former fungal species. It was observed that the colour of the solution turned from yellow to bright yellow and then to dark brown, which indicated the formation of silver nanoparticles.

Due to their ability to secret reducing and stabilizing components, fungi are one of the most efficient and promising producers of AgNPs [57]. As an effective technique, UV–Vis spectroscopy considered a well prominent to monitor the development and stability of AgNPs. The limiting factors of the λmax of silver nanoparticles colloidal in the visible region are the size and the shape of these particles. The absorption peak was observed at approximately 416.6 nm due to surface plasmon resonance (SPR) for AgNPs (Fig. 3). The intensity, shape, and position of the SPR band are highly affected by the dielectric constant of the medium of silver nanoparticles (the size, shape, and dispersity) as mentioned in Mie's theory [58, 59]. The intensity of SPR shown in Fig. 1 indicates that the formation of the AgNPs was more intense in the mycogenic extract than in the chemically synthesised sample. The absorption peak of the Ag-mycogenic sample shifted to smaller wavelengths, indicating more homogeneous particles than the chemical sample [60].

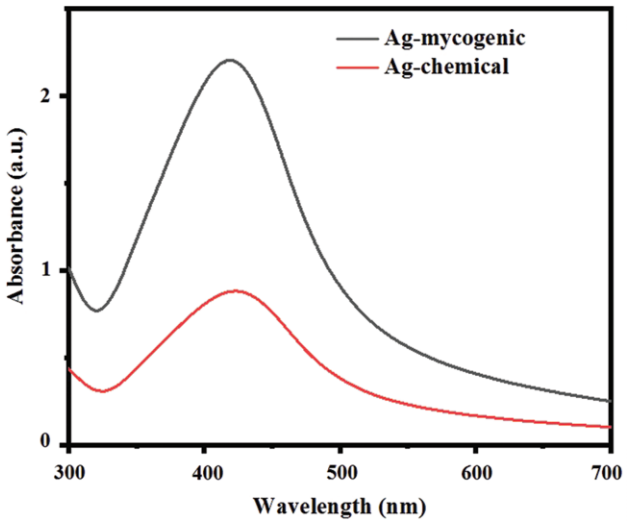


Figure 3: UV-visible spectra of Ag-mycogenic and Ag-chemical

The HRTEM images of the mycogenic AgNPs are presented in Fig. 4a. The phenology of silver nanoparticles was found to be spherical in shape and ranged between 10 to 15 nm. The nanoparticles were not in direct contact and were surrounded by a thin layer of organic material, indicating mycogenic stabilization of silver nanoparticles. Previous studies carried by various investigators showed that spherical nanoparticles are highly stable thermodynamically due to preservation by a sufficient amount of

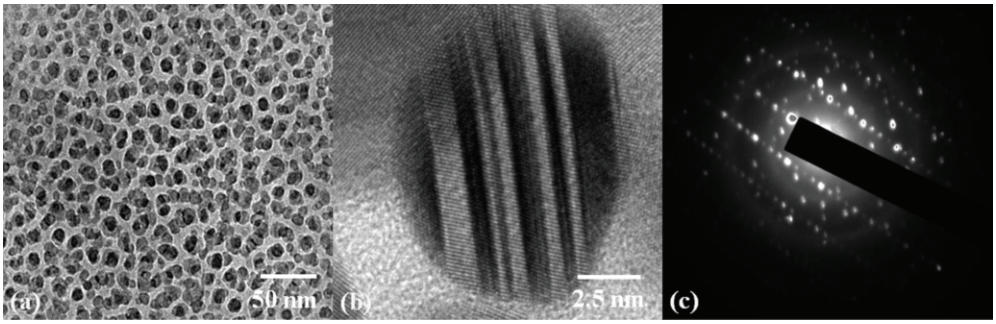


Figure 4: Ag-mycogenic TEM (a), HRTEM (b) and SAED (c)

biomolecules [61]. After HRTEM the spherical silver nanoparticles showed lattice spacing of 0.238 nm associated with (111) plane (Fig. 4b). The selected area electron diffraction pattern (SAED) in Fig. 4d exhibited the high crystallinity of silver nanoparticles approved by following XRD diffraction patterns.

Figure 5a shows the XRD patterns of mycogenic AgNPs. It can be seen that the silver phase (JCPDS no. 00-004-0783) with lattice planes structure (111), (200), (220), (311) correspond to 2θ values of 38.12°, 44.31°, 64.44° and 77.39°, respectively. The mean size of silver nanoparticles was calculated to be 28.7 nm by Scherrer's equation [62, 63], which was compatible with the TEM images. Also, the d-spacing of (111) plane is 2.36 A°, which complemented the value observed by HRTEM.

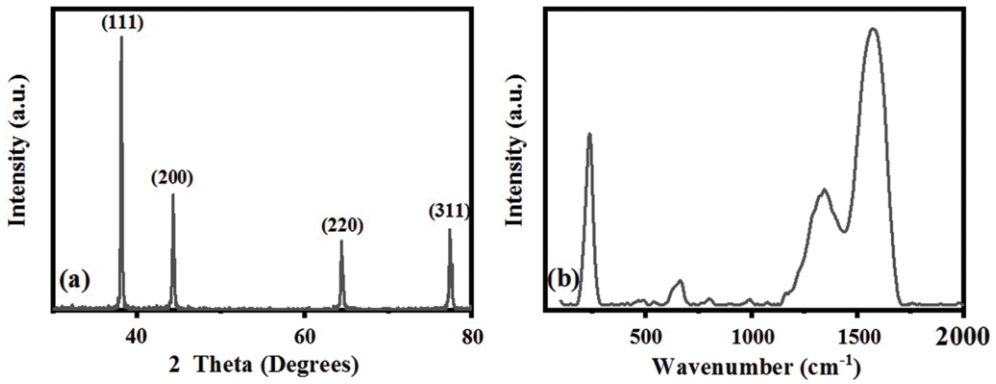


Figure 5: XRD of Ag- mycogenic (a) and Raman of Ag- mycogenic (b)

Various studies confirmed that Raman spectroscopy is a useful and important technique for the characterization of the molecules present on the surface of AgNPs. Lin-Vien [64] mentioned that vibrational bands of the Raman spectra are of the great significance of the determination of the secondary structure of the biomolecules [64]. The results obtained by Raman scattering (Fig. 5b), shows that C=O, which is adsorbed on the surface of silver nanoparticles, was confirmed by the presence of the peak at 1345 cm^{-1}. A peak at 1570.5 cm^{-1} was also observed. This refers to the presence of essential amino acids with aromatic side chains [65, 66]. The band at 236 cm^{-1} refers to Ag-OCO- [67]. These peaks clearly present the molecules on the mycogenic AgNPs surface.

The antimicrobial activity of both AgNPs (chemically and mycogenic synthesized by *T. atroviride*) was examined against various pathogenic organisms as mentioned in materials and methods by using the good diffusion method. The diameter of the inhibition zones (mm) around each well-containing silver nanoparticles solution is represented in Fig. 6.

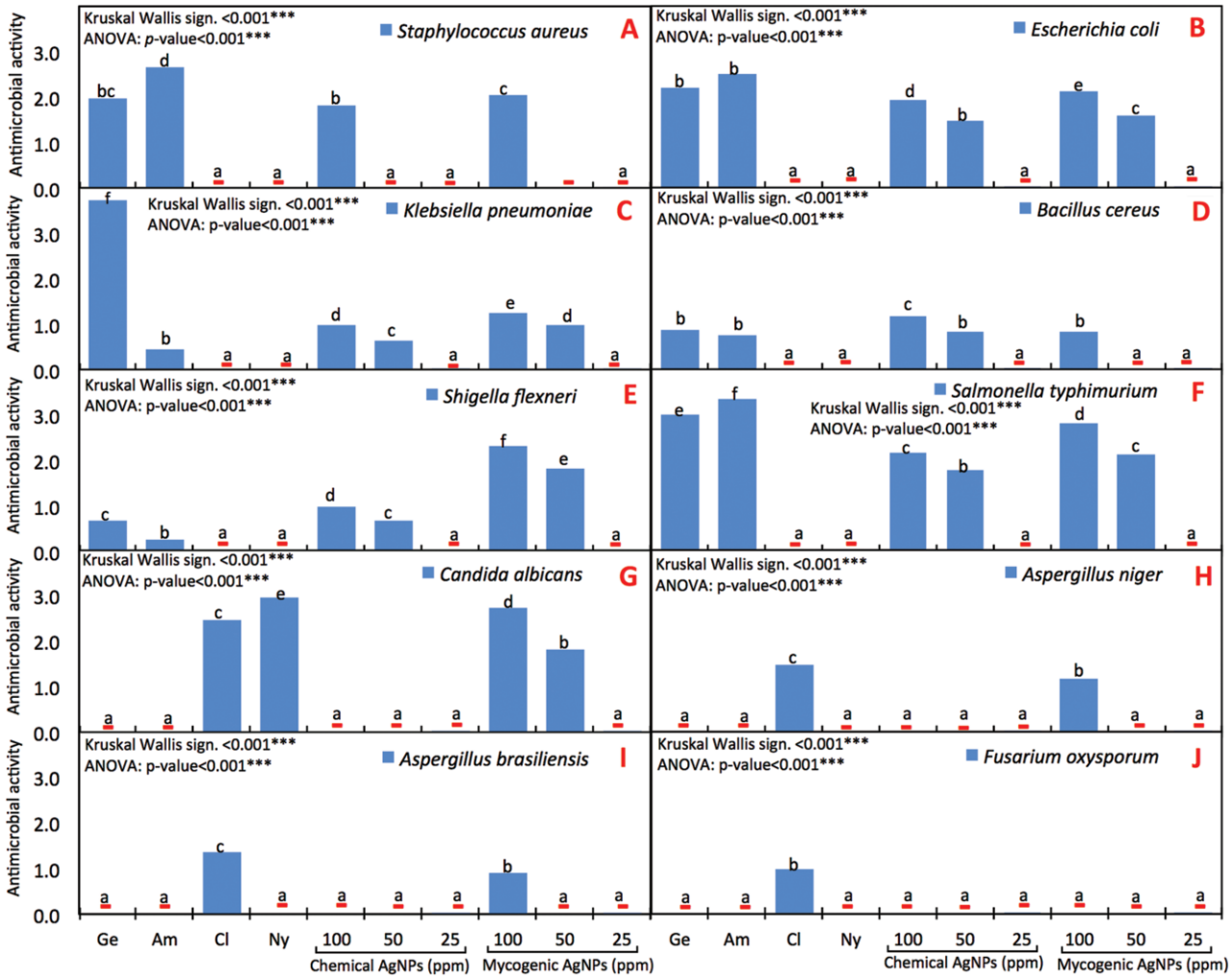


Figure 6: Illustrates the comparative results of antibacterial activities of mycogenic and chemical AgNPs (25 ppm, 50 ppm, 100 ppm) by agar well diffusion technique. Clotrimazole (Cl), Nystatin (Ny), Gentamicin (Ge), and Amoxicillin (Am) were used as positive controls for the Gram-positive bacteria, Gram-negative bacteria, yeasts, and fungi, respectively. Data represented are the mean of triplicates. Means with different letters are significantly different according to Smarts at $P < 0.05$

The silver nanoparticles showed potent antibacterial activity and to a lesser extinct antifungal activity. The mycogenic silver nanoparticle showed significant ($P < 0.05$) antifungal activity against *Candida albicans*, and *Aspergillus* sp. especially in 100 ppm concentration. While the *Fusarium oxysporum* f. sp. *lycopersici* showed a complete resistance towards both the mycogenic and chemical AgNPs (Fig. 6j). In regard to antibacterial activity, the mycogenic AgNPs effectively inhibited most of the bacterial growth at high and intermediate concentrations; 100 and 50 ppm respectively (Figs. 6a–6f). The most prominent inhibition zones were achieved with *Klebsiella pneumoniae* (Fig. 6c), *Shigella flexneri* (Fig. 6e), *Salmonella typhimurium* (Fig. 6f), and *Escherichia coli* (Fig. 6b). With these bacterial strains, the mycogenic AgNPs had a more significant antibacterial effect than the chemical AgNPs of the same concentration. It was also noticed that the mycogenic AgNPs can only stop the growth of *Staphylococcus aureus*, and *Bacillus cereus* (Figs. 6a and 6d) at high concentrations (100 ppm). Finally, the mycogenic AgNPs showed antimicrobial activities with efficiencies comparable to the positive controls, especially toward Gentamicin and Amoxicillin for pathogenic bacteria and Clotrimazole and Nystatin for pathogenic fungi.

Many reports discuss the promising approach of AgNPs for the development of novel antimicrobial compounds [68-72]. The findings from our antimicrobial studies, which are in accordance with the findings of many authors, confirm the possibility of achieving a higher inhibition rate toward a broad range of pathogenic microbes, even with a lower concentration [73-75]. Several mechanisms are combined in describing the antibacterial effect of AgNPs against many types of pathogenic bacteria. Dorau and his co-authors [76] found that AgNPs are responsible for bacterial cell lysis by inhibiting sulfhydryl group formation in the cell wall and disrupting membrane-bound enzymes. It has also been reported that such progression is associated with interrupting the respiratory chain and cell division or raising the rate of ROS formation such as hydroxyl radicals and superoxides [77, 78]. In fact, many reports proved that the activity of AgNPs is size-dependent [79, 80]. A direct correlation has been recorded between AgNP concentration and the antibacterial effect and was found to be bacterial class-dependent [80, 81]. Feng et al. [82] and Fayaz et al. [83] found that the susceptibility of AgNPs against Gram-negative and Gram-positive bacteria is probably due to the variation in the chemical structure of the bacterial cell walls.

The mycogenic AgNPs showed significant antifungal activity, and our data in matching with those obtained from Kim et al. [84], Lamsal et al. [85], Panáček et al. [86] and Elgorban et al. [87] who proved that AgNPs had a significant effect on the plant pathogenic fungi; *Raffaelea* and *Colletotrichum* and *Candida* species. This is possibly due to the high affinity of AgNPs to attack and adhere to fungal mycelium, significantly destructing the membrane integrity, and thus controlling the plant pathogen [88]. However, the mechanism of antifungal activity of AgNPs still needs further investigation.

4 Conclusion

The point that microbial resistance to elemental silver is rare [88] draws attention to the raised desire for applying AgNPs as a powerful antimicrobial agent in biomedical applications. The increase in the literature, including our own study, on this issue, will help drive future research and development of cost-effective metallic nanoparticle synthesis, with required therapeutic effects. The mycogenic AgNPs synthesized by *Trichoderma atroviride* were characterized and showed potential antifungal and antibacterial activity, noted through growth inhibition of most fungal and bacterial species (Fig. 7). Moreover, we conclude a direct connection regarding the concentration of AgNPs and its antimicrobial potential.

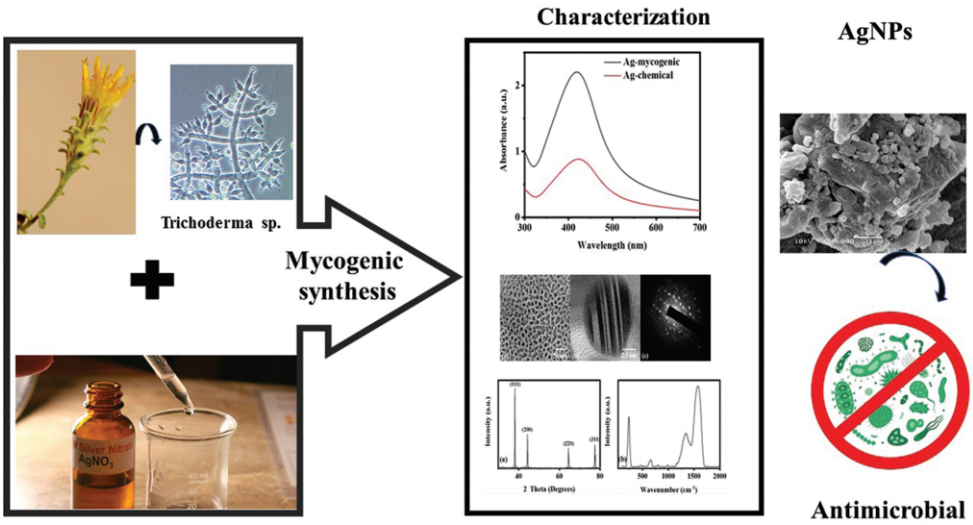


Figure 7: Schematic on green synthesis of AgNPs by *Trichoderma atroviride* hosted *Chiliadenus montanus*

Our study shows the necessity of exploring more environments and examination of its microfungal community to find novel or safe biological products with therapeutic applications. This research is a necessity to impact the global threat of microbial resistance to the majority of the recognized commercially accessible medicines.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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