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Mycogenical Synthesising AgNPs Using Two Native Egyptian Endophytic Fungi Isolated from Poisonous Plants

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THE current study biosynthesized silver nanoparticles (AgNPs) using two fungal endophytes isolated from two Egyptian dangerous plants, *Amaranthus viridis* (Amaranthaceae) and *Lotus corniculatus* (Fabaceae). The fungal isolates were identified using traditional methods, and the 18S rRNA gene sequence was used to confirm the identification. With average particle diameters of 15–27nm for *Alternaria alternata* and 11–21nm for *Aspergillus niger*, respectively, fungal strains produced spherical AgNPs. In the UV-Vis spectra, the absorption peaks of AgNPs ranged from 459–462nm. FT-IR spectra proved the existence of proteins as capping agents in AgNPs. Antimicrobial experiments revealed that AgNPs inhibited the growth of strains of fungi (*Candida albicans and Aspergillus niger*) and strains of harmful bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*). Cell wall lysis, distortion, and the distinction between the plasma membrane and the cell wall were all visible in transmission electron microscopy (TEM) micrographs of AgNPs-treated bacterial strains, as was complete cell lysis. In addition, AgNPs-treated fungal strains had a large gap between the plasma membrane and the cell wall, as well as lysed cell walls and severe plasmolysis in the protoplasm of the fungal cell. The findings suggest that in the future, bio-produced AgNPs might be used as efficient antibacterial and antifungal agents.

Keywords: Ag-nanoparticles, Antimicrobial activity, Endophytic fungi, Green synthesis, Poisonous plants.

Introduction

One of the most recent nanotechnology research methodologies is the synthesis of silver nanoparticles (AgNPs). There are many different fields in which it might be used. AgNPs efficiently inhibit a wide range of microorganisms (Youssef et al., 2019; Ismail et al., 2022; Khan et al., 2022). Nanoparticles (NPs) with a diameter of less than 100nm were biosynthesized for a specific purpose. These particles are non-toxic, inexpensive, environmentally safe, time-saving, and long-lasting. The generated AgNPs were found to have antimicrobial action (El-Dein et al., 2021; Mostafa et al., 2021; Sharma et al., 2022). The biosynthesis of AgNPs employing biological tools, including plant metabolites and microorganisms, has many benefits over chemical and physical techniques because of its single-step synthesis to reduce and stabilise silver (bulk) into AgNPs, low cost, and potent biological activities (El-Zahed et al., 2021). Antimicrobials such as silver and silver nitrate ($AgNO_3$) are efficient against harmful bacteria and fungi (Abdelsalam et al., 2018). Endophytes are microscopic creatures that live within plant tissues and do not harm them. These microorganisms are made up of the bacterial and fungal populations that colonise inside the host's tissues and reside there for most of their lives without producing visible symptoms of plant disease (Tadych & White, 2019). Endophytes generated from medicinal plants are gaining popularity due to their capacity for the production of novel secondary metabolites with potential pharmacological uses

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(Tripathi & Joshi, 2019). Endophytes are an appealing target for typical host-derived bioactive chemicals due to an increase in demand for natural goods from medicinal plants (Mostafa et al., 2021). Microbiologists were interested in looking for potential medicines in endophytes from medicinal plants (Saengsawang et al., 2012; Bhuyar et al., 2021). Despite chemical antibacterial compound synthesis's undeniable achievements and success, nature remains a precious treasure and a permanent, renewable, and highly attractive supply of a variety of safe natural products (Dzoyem et al., 2017). Exploring endophytic fungi or bioactive compounds is becoming increasingly important, as endophytic fungi represent a promising alternative source. As a result, secondary metabolites are extremely important (Darwish et al., 2020). Some of these needs may be met by non-chemical approaches and non-selective fungicide applications. Therapeutic plant extracts and secondary metabolites produced by endophytic fungi were previously discovered as antimicrobial bioagents (Al-Maqtari & Al-Doaiss, 2020; Tariq et al., 2020). In *Fusarium solani*, an antifungal compound called camptothecin was found (Rai et al., 2022). Terpenoids have also been shown to have antibacterial effects. The antibacterial properties of terpenoids have also been discovered. *Penicillium janthinellum*, an endophytic fungus, produces the polyketide citrinin, which possesses antibacterial properties against *Leishmania* sp. (Preethi et al., 2021). The antibacterial and antifungal compounds were discovered in endophytes isolated from *Tectona grandis* and *Samanea samane* (Mathur et al., 2021). In the Botanical Garden of New Damietta, both the genera *Amaranthus* and *Lotus* are flourishing as weeds (Serag et al., 2020). *Amaranthus viridis* is a poisonous annual herb that grows to a height of 60-80cm and has a pale green stem. The oval leaves are 2-4cm wide and 3-6cm long, and they have a 5cm long petiole. The oval leaves measure 2-4cm wide and 3-6cm long and possess long (5cm) petioles, with several branches emerging from the base. The Lotus genus (Fabaceae family) contains over 100 species that are found all over the world, primarily in the Mediterranean region. It is distinguished from other members of the pea family by its five leaves and head-like umbels of vivid yellow blossoms (Taeckholm, 1974; Boulous et al., 2009). In the past, plants from the genus *Lotus* were employed as antimicrobials in medicine, contraception, prophylactics, and treatments for sexually transmitted diseases. *Amaranthus* spp. is a flowering plant that has traditionally been used for food (a good source of protein) and medicine (Bokaeian et al., 2013). Utilizing plants in NPs synthesis is a novel first step toward truly green chemistry, owing to the fact that can be used widely without using harmful chemicals, and because it is more affordable, high carrier capacity, high stability, and environmentally safe than chemical and physical processes (Chaudhari et al., 2007). Currently, bacteria and fungi are used to make NPs, but leaf extracts are being employed to save money and eliminate the requirement for specific culture preparation and isolation procedures (Koyyati et al., 2014).

Although several earlier studies have looked at the antibacterial efficacy of chemically manufactured AgNPs (Schacht et al., 2013), we are looking at biologically synthesised AgNPs. The presented work investigated the antibacterial and antifungal potential of AgNPs made using two fungal endophyte extracts isolated from two toxic Egyptian weeds: *A. viridis* and *L. corniculatus*, taken from the Botanical Gardens of New Damietta, Egypt.

Materials and Methods

Collection of plant samples

Samples of plants were collected at the Botanical Garden in New Damietta, Egypt (31° 25' 3.1440" N and 31° 48' 51.9984" E). The two poisonous plants (Fig. 1) were *Amaranthus viridis* (Amaranthaceae) and *Lotus corniculatus* (Fabaceae). Pure populations, healthy individuals, and minimal human interference were used as selection criteria.

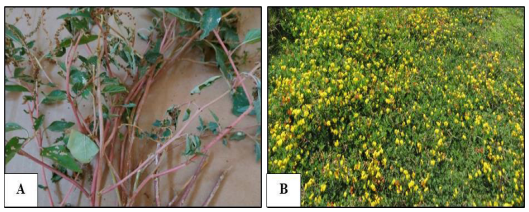

Fig. 1. (A) *Amaranthus viridis* (Amaranthaceae), (B) *Lotus corniculatus* (Fabaceae)

Chemicals

Chemicals $AgNO_3$, fluconazole (Diflucan), and penicillin G potassium (buffered Pfizerpen) were obtained from Spain (Panreac Quimica) and Pfizer Inc. (New York, NY), respectively. The chemicals and culture media were acquired from India (Sigma Aldrich Chemical Pvt. Ltd.).

Microbial strains

Strains of bacteria, including *Pseudomonas aeruginosa, Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*, and some fungus strains (*Aspergillus niger* and *Candida albicans*), were kindly provided by Botany and Microbiology Department, Faculty of Science, Damietta University.

Isolation and identification of endophytic fungi

The plants fresh aerial components (leaf and stem) and healthy root parts were disassembled and inserted in sterile glass bags. All tissues were sterile in the lab using the method described by Schulz et al. (1993). The plant tissues were washed with care under running water. The surface was sterilised by dipping it for 60 seconds in 70% ethanol, 3min in NaClO (5% accessible chlorine), and 30sec in a 75% ethanol solution. The leaves were sterilised using a method of sterilisation (Suryanarayan & Thennarasan, 2004). The plant roots, stems, as well as leaves, placed in Petri dishes after being thinly sliced, contain Potato Dextrose Agar (CMO-139, PDA, Oxoid, UK). The drug streptomycin (100mg/L), provided by Himedia Company, India, was additionally administered to the PDA to stop the development of endophytic bacteria. At 28±2°C, Petri dishes were incubated. Endophytic fungal growth was monitored on a regular basis in these Petri plates. Endophytic fungus isolated in its purest form was established following many transfers of hyphal tips onto brand-new PDA dishes. Endophytic fungi that were isolated were morphologically recognised at Damietta University, Faculty of Science laboratory. Eight of the nine endophytic fungi (*A. nidulans*, *P. viridicatum*, *A. flavus*, *A. niger*, etc.) are members of the Aspergillaceae family, while one (*Alternaria alternata*) is a member of the Pleosporaceae family. PCR with ITS1 and ITS4 primer pairs was utilised to identify isolated fungi at the molecular level. The identification was performed at Applied Co. Ltd. in Egypt, and the manufacturer's procedure was used to extract DNA from the cultures (White et al., 1990). For rRNA gene sequencing and PCR (polymerase chain reaction), the ITS4 (reverse) and ITS1 (forward) primers have been used to perform PCR in the reaction mixture. ITS4 (5'- TCC GCT TGA TAT GC -3') and ITS1 (5'- TCC GTA GGT GAA CCT GCG G -3') are identical primers. After a first denaturation at 96°C (4min.), 36 cycles of primer annealing at 58°C (30sec.), elongation at 72°C (90sec), and denaturation at 94°C were performed for one minute. A 5min final elongation step at 72°C was permitted. Gel electrophoresis (2% agarose in Tris-acetate ethylenediaminetetraacetic acid) and a low-DNA-mass ladder were used to quantify DNA. The sequencing and gel purification of the products of the amplified PCR were assigned to Solgent Co. Ltd. (South Korea). BLAST (the website of the National Center of Biotechnology Information) was used to search the sequences. To do sequence phylogenetic analysis, Meg Align (DNA Star) software version 5.05 was used. Gen Bank was consulted for the sequences and given accession numbers. The antifungal and antibacterial activity of the isolated endophytic fungi was assessed. This led to its selection for the synthesis of AgNPs.

Endophytic fungus extract preparation

Alternaria alternata (AC: OK483494) and *Aspergillus niger* (AC: OK483678) were used for the biosynthesis of AgNPs**.** Both *A. alternata* and *A. niger* were cultivated for 6 days on an incubator shaker at 28°C and 150rpm in potato dextrose broth. The fungus's progress was tracked on a regular basis. The fungus biomass had been filtered using filter paper (Whatman No. 1). To remove the media, the filtered bulk was rinsed three times with distilled H_2O_2. After weighing (20g) of the extracted fungal mass, it was mixed with 100mL of distilled H_2O_2 in an Erlenmeyer flask to make endophytic fungal extracts. For the extraction process, these flasks were shaken at 180rpm while being incubated at 28°C for 24h. Following this process, the cell-free filtrate was filtered using Whatman No. 1.

Biosynthesis and characterization of AgNPs

The synthesis of silver nanoparticles was done according to the method of Sandhu et al. (2017). At room temperature, 20mL of the fungal extract were added to an 80mL (2mM) aqueous solution of silver nitrate. To prevent photo-oxidation, aluminium foil was used to keep the flasks covered. The reaction mixture had to be standardised before proceeding to the next step of AgNPs synthesis. The time constraints for colour change and AgNP creation were finalised in accordance with the standardization. After 1, 12, 24, 36, 48, 72, and 96h, respectively, aliquots of samples were removed to observe the colour shift from transparent to dark brown. A Shimadzu UV-Vis Spectrophotometer was used to determine the success of AgNPs synthesis by

evaluation of the solution's absorbance between 300–700 nm at various time intervals (1, 12, 24, 48, 72, and 96h) to ascertain the success of AgNPs production.

Antimicrobial potential

The antimicrobial activity of AgNPs was tested; gram-positive bacteria are combated utilising the agar- well diffusion method, including *S. aureus* and *B. cereus* (gram-positive bacteria), *P. aeruginosa* and *E. coli* (gram-negative bacteria), and *A. niger* and *A. alternata* (fungi), in accordance with the recommendations of the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards, 2006). Mueller-Hinton agar and Dox agar media were ready, autoclaved, and inoculated with a 100-liter culture of 0.6 McFarland standard bacteria or fungus (1-2109CFU/mL). 100mL of 150g/mL AgNPs, fluconazole (antifungal), and penicillin G (antibacterial) were produced separately and applied to small (5mm) wells punched in agar plates. Plates for both fungal and bacterial cultures were incubated for 48 hours at 37°C and 48 hours at 30°C, respectively. Zones of inhibition (ZOI) have recently been quantified in millimetres (mm).

Ultrastructure studies

The ultrastructure of AgNPs-treated microbial strains (150g/mL) was examined using TEM in comparison to the ultrastructure of control bacteria (200KV, Japan's JEOL JEM-2100, Electron Microscope Unit, Mansoura University).

Fourier transform infrared analysis (FT-IR)

Nicolet iS10, purchased from Thermo Fisher Scientific, Waltham, MA 02451, United States, was used for FT-IR analysis. The spectra were recorded in the absorption frequency range 400–4000cm^{-1}.

Dynamic light scattering (DLS)

Particle size can be determined by the DLS technique using an ALV/DLS-5000 instrument (ALV GmbH).

Statistical analysis

The data were statistically analysed by using SPSS software (version 25). The data was expressed using the mean and standard deviation. In each test, $P<0.05$ was regarded as significant (O'Connor, 2000).

Results

From *A. viridis* and *L. corniculatus*, two fungal endophytes were isolated. Using classical identification, the isolates of fungal organisms were identified as *Alternaria alternata* and *Aspergillus niger*. The internal transcribed spacer (ITS) was used to validate the traditional identification (Figs. 2-5).

Molecular identification of the isolated endophytic fungi

The species of PCR amplicon isolated with forward ITS (550) primers was subjected to DNA sequence analysis and nucleotide sequencing. To create the phylogenetic tree (García-Varela et al., 2000), the collected 620 nucleotides from the initial fungus strain were compared to the equivalent 18S ribosomal RNA gene that existed in the international gene bank (Figs, 2-5).

Characterization of the biosynthesized AgNPs

Alternaria alternata (AC: OK483494) and *Aspergillus niger* (AC: OK483678) were used for the biosynthesis of AgNPs. In the presence of sunlight and at room temperature, *A. niger* and *A. alternata* produced AgNPs in a matter of minutes. The peak in the UV-Vis spectra between 459–462nm of *A. niger* and *A. alternata*, respectively, confirmed the synthesis of AgNPs (Fig. 6A). The negative charge of the produced AgNPs was confirmed using the Zeta potential (-19.2 and -12.7mV for *A. niger* and *A. alternata*, respectively) (Fig. 6B and C). The FT-IR spectra indicated the existence of proteins connected to the fungal AgNPs (Fig. 6D and E) that were generated. The O–H bond is represented by a band at 3413.39 and 3419.17 cm^{-1} *for A. niger* and *A. alternata,* respectively. The bands at 2931.27 (C–H bond), 1628.59 (Amid), and 1073.19 (C–O–C) cm^{-1} for *A. niger* and 2928.38, 1633.41, and 1071.26cm^{-1} for *A. alternata* indicate that, phenolic hydroxyl groups were utilised as a reducing factor during the production process. The vibrations in the proteins are shown by the band at 609.39cm^{-1} caused by the C–CL bond (Song et al., 2009). TEM images of biosynthesized AgNPs (Fig. 6F and G) revealed spherically shaped and well-dispersed NPs with mean sizes of 11– 21nm for *A. niger*, and 15– 27nm for *A. alternata*, respectively.

CAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTG
TAATTGGAATGAGTACAATTTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGT
GCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAA
GCTCGTAGTTGAAACTTGGGCCTGGCTGGCGGGTCCGCCTCACCGCGTGCACTCGTCCGG
CCGGGCCTTCCTTCTGAAGAACCTCATGCCCTTCACTGGGGTGCTGGGGAATCAGGACTT
TTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACGTTAGCATGGAA
TAATAAAATAGGGCGTGCGTTTCTATTTTGTTGGTTTCTAGAGACGCCGCAATGATTAAC
AGGAACAGTCGGGGGCATCAGTATTCAGTTGTCAGAGGTGAAATTCTTGGATTTACTGAA
GACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAGTGAACGAAAGTTA
GGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCGTAAACTATGCCGACTAGGGA
TCGGGCGATGTTCTTTTTC

Fig. 2. Nucleotide sequence of *A. alternata* (Accession number (AC): OK483494)

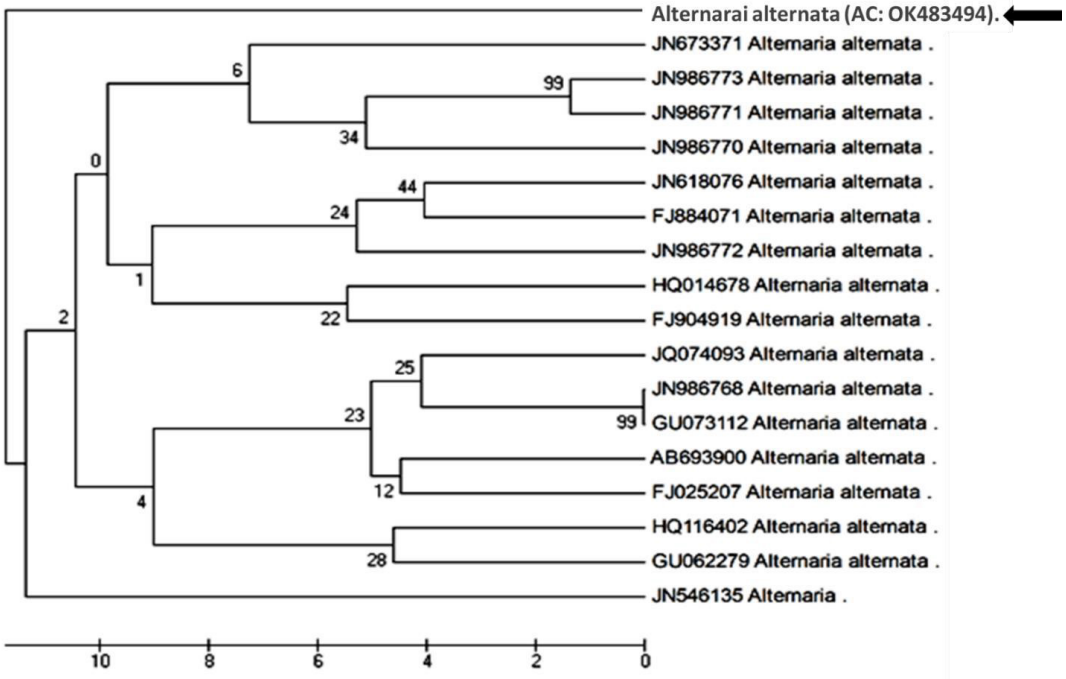

Fig. 3. The sequence of the rDNA internal transcribed spacer (ITS) region and phylogenetic tree of *A. alternata* (AC: OK483494). Numbers on the tree branches indicate the relatedness of species on a scale from 0 to 100 [The higher the number, the more closely related the species are when compared to the matching 18s ribosomal RNA gene that existed in the international gene bank to obtain the phylogenetic tree (Garca-Varela et al., 2000). The second fungus strain analysis comprised 400 nucleotides]

GCGAGAAGGCTTGAAATAATGTCTCCGCGGGGGTCTTTGTCTAATGCGGATGTAGCATCGG
CGGGCCGTCCGAGTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCG
GAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGT
TAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGA
TAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCC
CTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGT
GTTGGGTCGCCGTCCCCCTCTCCGGGTTTATTTT

Fig. 4. Nucleotide sequence of *A. niger* (AC: OK483678)

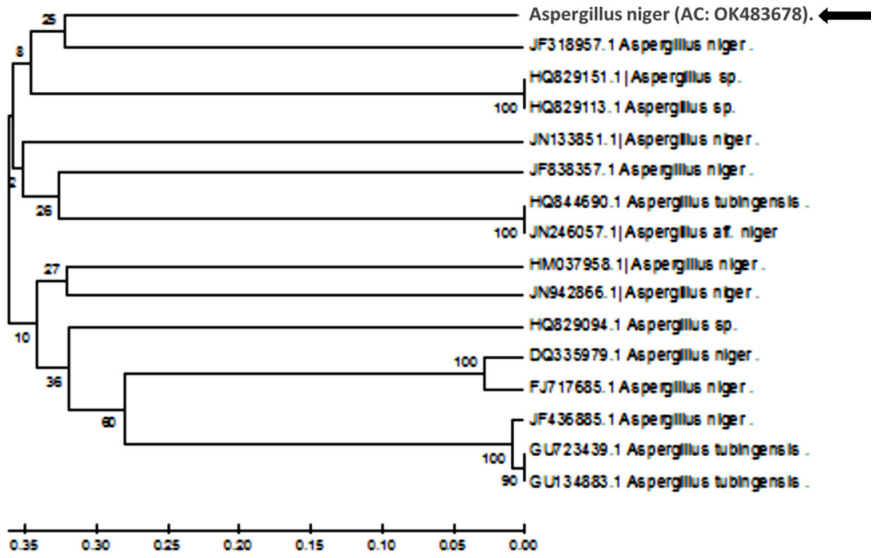

Fig. 5. Sequence of the rDNA internal transcribed spacer region and phylogenetic tree of *A. niger* (AC: OK483678). Numbers on the tree branches indicate the relatedness of species on a scale from 0 to 100 [The higher the number, the closer is the relatedness of species]

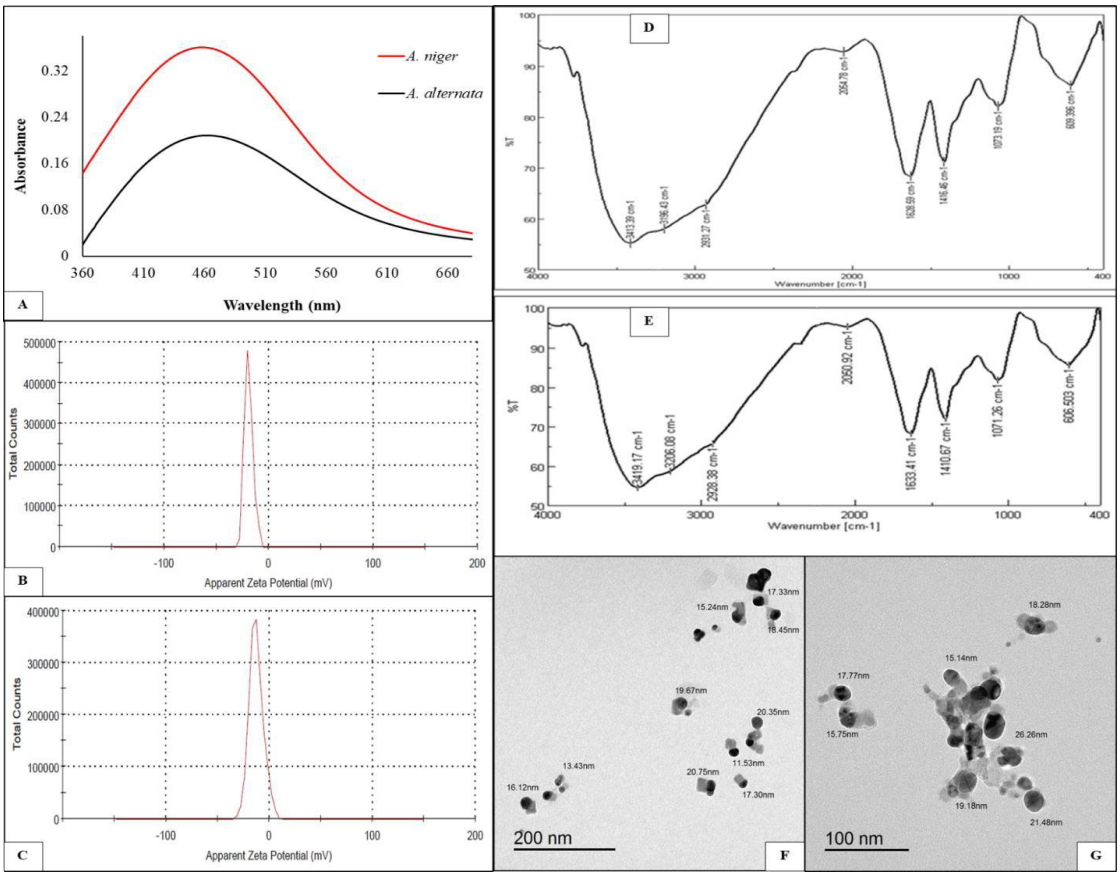

Fig. 6. Characterization of AgNPs. The UV-Vis spectra of AgNPs using *A. niger* and *A. alternata* (A). Zeta potential measurements analysis of AgNPs using *A. niger* (B), and *A. alternata* (C). FT-IR spectra of biosynthesized AgNPs by *A. niger* (D), and *A. alternata* (E). TEM of the produced AgNPs using *A. niger* (F), and *A. alternata* (G). scale bar = 200 and 100nm, respectively

Antimicrobial activity of biosynthesized AgNPs

Most of the bacteria and fungi were found susceptible to the prepared AgNPs that showed antimicrobial potential against the tested microbes (Figs. 7, 8). Between samples treated with and without AgNPs, there are considerable differences in the antibacterial effects in comparison with penicillin G and fluconazole as standard drugs. Between the microbial strains, there were highly significant ($P<0.05$) differences in the diameter of the inhibition zone *(B. cereus*, *C. albicans, P. aeruginosa*, *E. coli*, *A. niger*, and *S. aureus*). The three separate common harmful microorganisms, *P. aeruginosa*, *E. coli*, and *S. aureus*, with diameters of 12, 13, and 16mm, respectively, could all be inhibited by the generated AgNPs. In addition, the AgNPs generated by *A. niger* showed strong antifungal activity against *C. albicans* (16mm).

The ultrastructural alterations of the treated and control bacteria with green-produced AgNPs are shown in Fig. 9. As illustrated in Fig. 9A, untreated *B. cereus* was rod-shaped and had complete cell walls. The cell walls of *B. cereus* (Fig. 9B) became wrinkled and deteriorated after exposure to AgNPs, causing the bacterial cell membrane to rupture. AgNPs had a positive effect on the cell walls and cell membranes of the treated bacteria. The produced AgNPs have been proven to have bactericidal properties, killing microorganisms. The morphological alterations in the treated *S. aureus* cells, as well as the inhibition of cell expansion, were visible in TEM micrographs. A small quantity of DNA is also present; however, the alterations included the suppression of *S. aureus* multiplication.

A. niger hyphal cells with a normal cell membrane, compact cytoplasm, and cell wall were observed in untreated hyphal cells (Fig. 10A). Besides the accumulation in the cell nucleus, AgNPs accumulation in the cytoplasm and cytoplasmic membrane may be the key reason for the important morphological alteration. The treated *A. niger* TEM micrographs (Fig. 10B) revealed numerous alterations, including reduced cell size and significant cytoplasmic leakage.

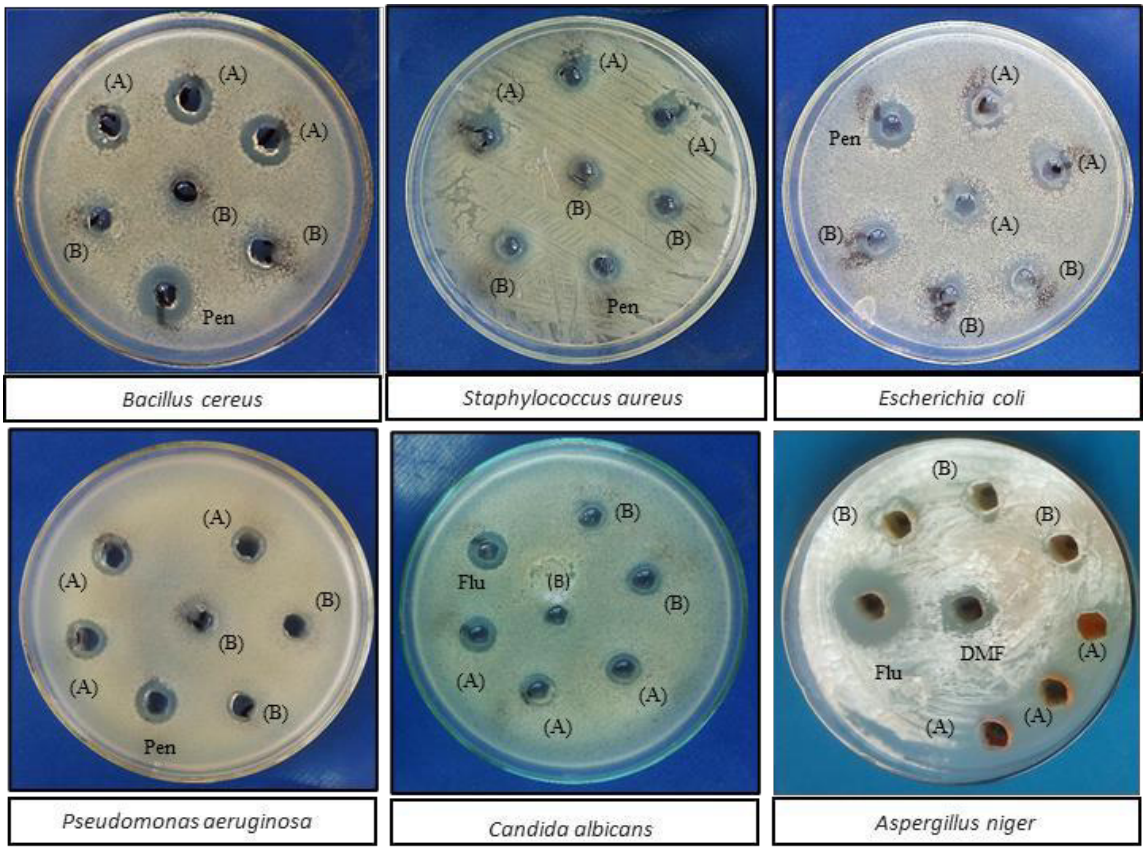

Fig. 7. Antimicrobial activities of AgNPs of *A. niger* (A), and AgNPs of *A. alternata* (B) using agar well diffusion method where DMF (dimethylformamide), Pen (penicillin G), and Flu (fluconazole)

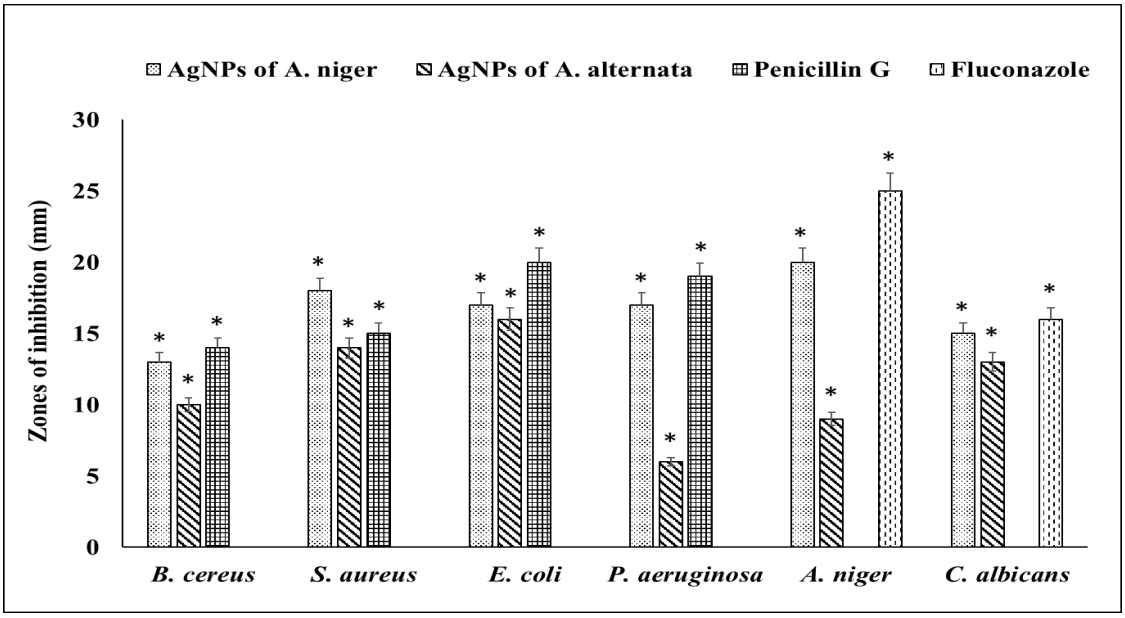

Fig. 8. Antimicrobial activities of the prepared AgNPs in comparison with penicillin G and fluconazole as standard drugs (highly significant=*P<0.05, n= 3) [Values are mean± SD]

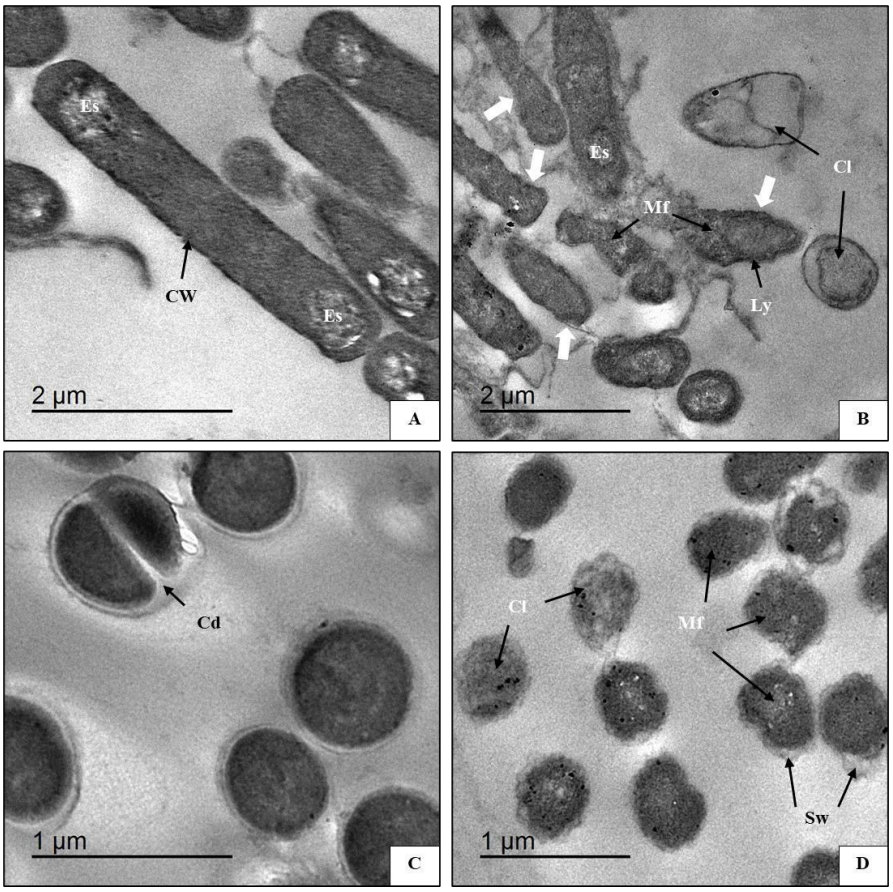

Fig. 9. The bactericidal effect of AgNPs on the ultrastructure of *B. cereus* and *S. aureus*. A negative control (without AgNPs treatment; A &C). In the treated samples (at 150µg/mL; B), there are irregular rods (white arrows) with lysed cell walls (Ly), malformed cells (Mf), the separation between the cell wall and plasma membrane (Sw) and complete cell lysis (Cl) [Note the endospore formation (Es)]

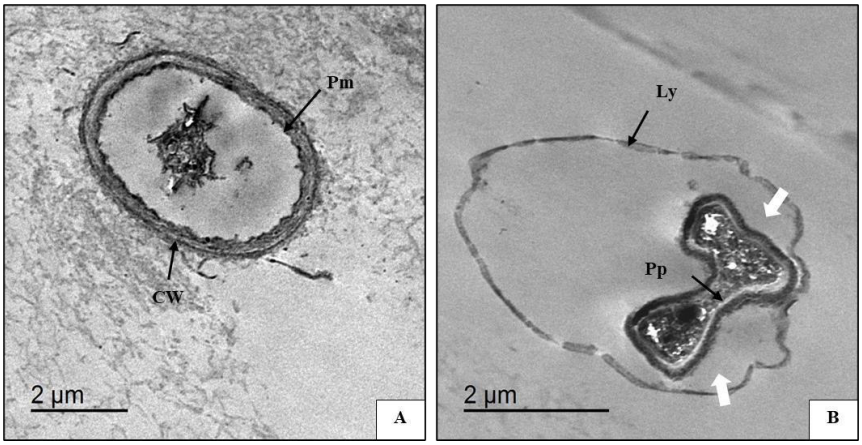

Fig. 10. The antifungal effect of AgNPs on the ultrastructure of *A. niger*. A negative control (without AgNPs treatment; A). In the treated sample (at 150µg/mL; B), there was a great separation between the cell wall, and plasma membrane 688 (white arrows) with lysed cell walls (Ly) and a severe plasmolysis action in 689 the protoplasm of fungal cells

Discussion

AgNPs have been a popular research topic in microbiology, agriculture, medicine, optics, and other fields. As a result, new techniques for more effective AgNPs with antibacterial activity had to be developed. The capacity of various isolated fungal endophytes isolated from poisonous plants to produce AgNPs was investigated in this work. Endophytic fungi are a type of fungal community that lives in healthy plant tissues and has yet to be discovered. Endophytic fungal strains have been shown to produce bioactive substances have numerous uses in agriculture, medicine, and industry (Seetharaman et al., 2017). As a result, endophytic fungi provide a long-term source of NP synthesis. As opposed to plants and other microbes, fungi are good nano-factories for the production of metal nanoparticles due to their capacities for bioaccumulation of metals, secretion of extracellular enzymes, higher tolerance, easy biomass production, rapid mycelial growth, and a cost-effective and affordable time growing process (Ottoni et al., 2017). Fungi are able to manufacture nanometals because of their potent enzyme system and high metal tolerance (Osorio-Echavarría et al., 2021). Endophytic fungal strains have been reported to synthesise AgNPs, such as *Phomopsis liquidambaris*, *A. niger*, *A. flavus*, *A. alternata*, *Cladosporium cladosporioides*, *F. oxysporum*, *F. solani*, and *Trichoderma* sp. (Seetharaman et al., 2017; Madakka et al., 2018). *A. alternata* extract is used in the biosynthesis of AgNPs (Baharvandi et al., 2015). AgNPs are widely recognised for their brown colour in aqueous solutions and for possessing a prominent absorbance band at 400–460nm, which is brought on by vibrations in metal generated by excitation of surface plasmon NPs (Durán et al., 2007). AgNPs absorption peaks at 430nm, are indicating that fungal metabolites play an essential function in the reduction of metal ions (Al-Zubaidi et al., 2019). The UV-Vis spectra of the *A. niger* cell filtrate with $AgNO_3$ revealed a prominent broad absorption peak at 440nm (Sagar & Ashok, 2012). The extracellular production of AgNPs by *A. niger* was reported by an absorbance peak at 420nm (Gade et al., 2008), and the *A. alternata* cell filtrate generated an absorbance band at 420nm (Gajbhiye et al., 2009). A UV-Vis spectroscopic investigation of AgNPs production employing *A. alternata* revealed a prominent broad peak at 430nm (Ibrahim & Hassan, 2016). Ghazwani (2015) used three fungi to study the biosynthesis of AgNPs: *A. niger, F. oxysporum,* and *A. solani,* and found that the absorption peak at 435–445nm is caused by tyrosine and tryptophan residues in the protein being electronically excited. Nanomaterial stability and aggregation are regarded as universal challenges that limit their medical and commercial applications. The exterior capping agents are critical in determining the form, size, and stability of NPs by preventing aggregation (Duan et al., 2015). The nanometals negative charge may increase the repulsion force between particles, and reducing accumulation (Siddique et al., 2013). *Streptomyces noursei* H1-1, *B. cereus* A15, and *R. stolonifer* A6-2 strains were employed for the biosynthesis of AgNPs with great stability (Alsharif et al., 2020), which was proven by strongly negatively charged surfaces

of -18.5, -19.9, and -16.6mV, respectively, for the used strains. *Colletotrichum* sp. culture filtrates make AgNPs varying in diameter from 5–60nm, and the secreted fungal biomolecules may have worked as stabilising agents to prevent aggregation (Azmath et al., 2016). Additionally, it was found in the current study that the produced AgNPs contained proteins. Proteins may be involved in the production as stabilising agents (Gopinath et al., 2013). Also, proteins associated with fungal AgNPs may operate as stabilising and capping agents (Ballottin et al., 2016). AgNPs size and morphology have a strong impact on their value and applications. As previously stated, spherical AgNPs are the smallest and have the maximum antibacterial action (Hillaireau & Couvreur, 2009; Syu et al., 2014). The particle size was discovered to be 35nm (Ibrahim & Hassan, 2016). Farrag et al. (2020), who used *A. niger* to make spherically shaped AgNPs with a diameter of 25 nm, while the fungal strain *A. alternata* produces well-dispersed, spherically shaped AgNPs that range in size from 20–45nm (Sarkar et al., 2011). All pathogens tested demonstrated that the biosynthesized compound had a good antibacterial effect (Kaidi et al., 2021). According to the current findings, the AgNPs have a maximum inhibitory area against gram-positive bacteria and a minimal inhibitory area against gram-negative bacteria. Biosynthesized AgNPs from *A. niger*, which have antibacterial action against *B. subtilis*, *P. aeruginosa*, *E. coli,* and *S. aureus* (Kalaiselvan & Rajasekaran, 2009). With *S. aureus*, the largest zone of inhibition had a diameter of 15mm. Zones of inhibition in *B. subtilis, P. aeruginosa*, and *E. coli* cultures were around 10, 11, and 14mm in diameter, respectively. The AgNPs produced by *A. niger* ATCC 16404 demonstrated good antibacterial efficacy against all pathogens examined, but there were discrepancies in that gram-negative bacteria have thinner cell walls than gram-positive bacteria (Mohammed, 2015). This could lead to a rise in antibiotic resistance among gram-positive bacteria. The *A. niger* fungal isolate used to biosynthesize AgNPs had significant antifungal efficacy against pathogenic plant fungi such as *F. oxysporum, A. flavus,* and *P. digitatum* (Al-Zubaidi et al., 2019). The *A. alternata* extract used to make AgNPs inhibits *B. subtilis, E. coli*, *B. cereus, P. aeruginosa, Micrococcus luteus,* and *Proteus vulgaris* growth (Sarkar et al., 2011a). The green-produced AgNPs demonstrated effective antibacterial and antifungal activity against *B. cereus, Klebsiella pneumoniae, M. phaseolina*, *E. aerogenes,* and *A. alternata* (Bernardo-Mazariegos et al., 2019). The inhibition and mechanism of action of AgNPs may be accounted for by the large contact areas of the tiny AgNPs generated (Baker et al., 2005). Furthermore, the cell walls of these bacteria have different designs and compositions (Yu et al., 2014; Guzman et al., 2012). Only macromolecules can enter gram-positive bacteria with a strong peptidoglycan coating. In gram-negative bacteria, on the other hand, the thinner peptidoglycan covering and the abundance of existing cell wall pores allow molecules to pass through easily. Using AgNPs as an antibacterial result in membrane breakdown, cytoplasmic component loss, and eventually cell death (Wang et al., 2017). Additionally, gram-positive bacteria have highly negative charges on their cell walls, which may aid them in their ability to fight bacteria by attracting neutrophil attracting protein A (Morones et al., 2005). The form and cell membrane function may be influenced by AgNPs. Also, bacterial permeability and respiration are disrupted (Kvítek et al., 2005). Interaction with phosphorus-containing compounds prevents protein synthesis and DNA replication (Morones et al., 2005), also by deactivating biological enzymes, and increases reactive oxygen species production (Sahayaraj et al., 2012). Furthermore, the accumulation of AgNPs in the cell nucleus, cytoplasmic membrane, and cytoplasm induced a shrinkage of the treated cells size as well as cytoplasmic content leakage, which confirmed the results of this investigation (Abdel-Hafez et al., 2016; Sarkar et al., 2011b).

Conclusion

The current research demonstrated a green, cheap, and simple biosynthetic method for AgNPs using the fungal extracts of two endophytes isolated from two poisonous plants. The spherical nanoparticles produced through biosynthesis were called AgNPs, with a well-diffused size range of 11–28 nm. The findings suggest that biosynthesized AgNPs could be used in the future as effective antibacterial and antifungal agents.

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Conflict of interest: The authors declare that they have no conflict of interest.

Authors' contributions: MSS and MAE collected and identified the plant samples. MME and MIA provided the microbial strains for the antimicrobial tests. HRM, MSS, MTM, and MAE isolated and identified the endophytic fungal strains. MME conducted experiments on the biosynthesis and antimicrobial activities of AgNPs. MAE and HRM cover the costs of molecular identification of fungal isolates, characterization of AgNPs, and ultrastructural studies of treated microbes. HRM, MSS, MTM, MAE, and MME wrote the draft of the manuscript. All authors read and approved the manuscript.

*Availability of data and materials:*The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval: Not applicable.

Abbreviations
AgNPs: Silver nanoparticles
PNC: Polymer-based nanocomposites
UV-vis: Ultraviolet-visible
FT-IR: Fourier transform infrared spectroscopy
TEM: Transmission electron microscope
PDA: Potato dextrose agar medium
PDB: Potato Dextrose Broth
MHA: Mueller-Hinton agar
ZOI: Zones of inhibition
ROS: Reactive oxygen species

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التخليق الفطرى لجسيمات الفضة النانوية باستخدام نوعين من الفطريات المصرية المحلية المعزولة من النباتات السامة

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يتبين من هذه الدراسة التخليق الحيوي لجسيمات الفضة النانوية (AgNPs) من نباتين سامين مصريين، وهما *Amaranthus viridis* (Amaranthaceae) و *Lotus corniculatus,* (Fabaceae) تم التعرف على العزلات الفطرية باستخدام الطرق التقليدية وتم استخدام تسلسل الجين 18S rRNA للتحقق من صحة التعرف. تم تحديد *Alternaria alternata* و*Aspergillus niger* حيث تم تخليق جزيئات AgNPs بشكل كروي بواسطة سلالات فطرية، بمتوسط أحجام للجسيمات تتراوح من 11-21 نانومتر لـ *A. niger* و15-27 نانومتر لـ *A. alternata*، على التوالي. في أطياف UV-Vis لفطر *A. niger* و *A. alternata*، تراوحت قمم امتصاص AgNPs من 459 إلى 462 نانومتر. تم تأكيد وجود البروتينات كعوامل تغطية مع AgNPs بواسطة أطياف FT-IR أظهرت اختبارات مضادات الحساسية للميكروبات لـ AgNPs أنها تثبط السلالات البكتيرية الممرضة *Bacillus cereus* و *Staphylococcus aureus* و *Escherichia coli* و *Pseudomonas aeruginosa* والسلالات الفطرية *A. niger* و *Candida albicans*. كان تحلل جدار الخلية والتشوه والفصل بين جدار الخلية وغشاء البلازما مرئيًا في الصور المجهرية TEM للسلالات البكتيرية المعالجة بـ AgNPs وتحلل الخلية الكامل. بالإضافة إلى ذلك، فإن السلالات الفطرية المعالجة بـ AgNPs لها فصل كبير بين جدار الخلية وغشاء البلازما وجدران الخلايا المتحللة وانحلال البلازما الحاد في بروتوبلازم الخلية الفطرية. تعتبر النتائج التي تم الحصول عليها نتائج واعدة لاستخدام AgNPs المركب حيوياً كعوامل قوية مضادة للجراثيم ومضادة للفطريات.