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Original article

Biosynthesis of silver nanoparticles by endophytic fungus *Fusarium proliferatum* isolated from Allium sativum and its antimicrobial effect against multidrug resistant bacteria

Mohamed M. Gharieb [1], Mai S. Khatab [1], Azza M. Soliman[1], Marwa S. Taha [2*]

1- Department of Botany & microbiology, Faculty of Science, Menoufia University, Shebeen El-Koom, Menoufia, Egypt.

2- Department of Medical Microbiology and Immunology, Faculty of Medicine, Tanta University, Egypt

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ABSTRACT

Background: Biosynthesis of silver nanoparticles (AgNPs) by endophytic fungi is a potential biological nanomanufacturing technique. Endophytic fungus provides a cost-effective and environmentally friendly method for producing nanoparticles. **Methods**: In the current investigation, endophytic fungal isolates were identified as *Fusarum proliferetum* visually and genetically. The purified silver particles' crystalline structure, particle size distribution, and morphology were analyzed using UV-Vis, X-ray diffraction, and high-resolution transmission electron microscopy. **Results**: Fourier transform infrared spectroscopy (FT-IR) of the particles indicated the presence of many functional groups involved in the synthesis process, such as bio reducers and capping agents. Optimization of physio-chemical parameters for biosynthesis of silver nanoparticles was at pH 7, substate concentration 1mM, salinity value 0.1 NaCl and incubation period 72 hrs. silver nanoparticles were shown to have an antibacterial impact on multidrug resistant bacteria, as evidenced by an inhibitory zone that increased with nanoparticle concentration. Furthermore, the effect of silver nanoparticles on Gram negative bacteria was better than Gram positive bacteria as evidence by MIC results. Scanning electron microscopy analysis revealed significant distortions caused by green produced silver nanoparticles on bacterial cell morphology. **Conclusion**: The research findings suggest that GS-AgNPs have significant efficacy as a safe and potent antibacterial agent against bacteria that are resistant to many antibiotics.

Introduction

One significant risk to the public's wellness is the worldwide expansion of multidrug resistant (MDR) microorganisms [1]. The inability to discover more antimicrobials to avoid and treat bacterial diseases, combined with antibiotic overuse are the main causes of antibiotic resistance and the end of the antibiotic golden era [2].

Undoubtfully, MDR strains harmed patients' health and finances and posed a threat to numerous lives [3]. Numerous significant organizations, such as the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), have declared antibiotic resistance to be a "global public health concern". These worries raise the need for developing new therapeutic treatments [4]. Nanotechnology is one of these innovative

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* *Corresponding author:* Marwa S. Taha
E-mail address: *marwa.taha@med.tanta.edu.eg*

beneficial techniques that investigators highlight for combating antibiotic resistance by leveraging the benefits of nanomaterials [5].

Substances at the nanoscale possess distinct physical, chemical, and electromagnetic characteristics. These unique characteristics enable them to communicate with various pathogens. Metallic and non-metallic nanoparticles (NPs) are being employed in antibacterial applications. The utilization of metallic silver nanoparticles (AgNPs) as antimicrobial, sensor and anticancer agents is on the rise. Biologically manufactured silver nanoparticles are very stable, can dissolve easily, give off a lot of particles, and kill microbes. The process for generating AgNPs is also safe and doesn't cost much [6]. Currently, the synthesis of AgNPs is a highly important research focus within the realm of nanotechnology [7].

The usage of conventional physical and chemical methods for creating nanoparticles is restricted because they involve hazardous chemicals, consume plenty much energy, and require expensive downstream processing [8]. Several biological techniques, such as the use of microbes, enzymes, and plant extracts, have been proposed as potential environmentally benign, less harmful, high output, fast synthesis, and less expensive energy downstream processes for the green synthesis of nanoparticles [9].

Numerous microbes, including bacteria, yeast, and fungi, can create AgNPs via processes that occur inside or outside of cells [10]. Fungi produces massive protein quantities that contribute to the throughput of AgNPs. Extracellular mycosynthesis of AgNPs has been documented employing various molds, as *Aspergillus niger* and *Penicillium* species [11]. There is insufficient research on the mycosynthesis of AgNPs by endophytic fungi, which inhabit the interior tissues of plants in a mutually beneficial relationship and are regarded as a promising reservoir of bioactive substances. Endophytes involve both fungi and bacteria,

Endophytic fungi have been the subject of the most research [12].

Certain fungi create secondary metabolites, which are chemical compounds with numerous applications, including antioxidants, anti-cancer, antibacterial, and anti-fungal [13]. Although earth has about 300,000 plant species, many of which include endophytic fungi. Several studies have shown beneficial substances in endophytic fungal flora [14]. Nevertheless, the utilization of endophytic fungus for nanoparticle production has only been adopted by a limited group of researchers [15-17].

The goal of this research is to isolate some endophytic fungi from medicinal plants, identify fungal isolates, and test them for synthesis of AgNPs. Then, assess the activity of these AgNPs against some MDR organisms.

Material and methods

This cross-sectional study was conducted at the department of Botany & Microbiology, Faculty of Science, Menoufia University and department of Medical Microbiology and Immunology, Faculty of Medicine, Tanta University, Egypt.

Isolation of endophytic fungi from medicinal plants

Randomly selected samples including leaves, roots and stem of several medicinal plants were obtained from different environmental habitats. The samples underwent surface sterilization using a standard technique with specific modifications [18]. Plant samples were washed with tap water to remove dust and soil particles and then washed with sterile distilled water. Small pieces were immersed in 70% ethanol for 60 seconds and then washed with 4% sodium hypochlorite solution for one minute. They were rinsed in sterile distilled water several times and dried between autoclaved blotting paper times.

Samples were chopped into small pieces and placed on Petri plates with 100 g/ml streptomycin-containing potato dextrose agar (PDA) (house-made, containing (g/L): Agar power 16, potato 200 {peel potatoes, cut into 1 cm pieces, boiled for 20 min, filtered to obtain filtrate}, and dextrose 20, pH 6.0). Petri plates were coated with Parafilm and kept at 26±2°C in a moist incubator for 20 days, monitoring fungal emergence daily. Endophytes from explants with morphological variability (color, growth pattern) were sub-cultured and maintained as axenic cultures on freshly prepared PDA plates.

Medical plant endophytic fungi were identified according to a previously reported method [19]. Endophytic fungi from plant segment cuts were continuously sub cultured on antibiotic-free sterile PDA plates until pure cultures were obtained. Endophytic fungi were identified through

morphological analysis macroscopically and microscopically using a light microscope.

Screening of the active endophytic fungal isolates for production of silver nanoparticles

Purified isolates were tested for silver ion tolerance on Potato Dextrose medium (solid and liquid). Solid media: A disc of fungal mycelium was aseptically inoculated on a PDA plate supplemented with 1mM silver nitrate. The inoculated plates were incubated at 28±2 ºC for at least 7 days. Appearance of brown or dark color under the surface relative to control (metal free inoculated media) was used to determine the capacity of pure isolates to produce silver nanoparticles. The isolates capable of producing silver nanoparticles were chosen for further testing. Liquid media: Pure fungus isolated from screening tests were inoculated onto potato Dextrose liquid medium containing 1mM silver nitrate. The inoculated flasks were incubated in an orbital rotary shaker at 120 rpm and 28±2 ºC for 7 days. Appearance of brown or dark color of the silver-ion-inoculated flasks relative to control (metal free inoculated media) was used to determine the capacity of pure isolates to produce silver nanoparticles.

Identification of the most active endophytic fungus

The selected fungal isolate was identified by morphological, microscopic examination and rRNA gene sequencing.

Morphological characterization

Endophytic fungal strain was identified macroscopically and microscopically using a bright-field and phase contrast microscope on slides generated from cultures stained with lactophenol cotton blue [20]. Similarly, typical taxonomic keys were employed to determine morphological traits [21].

Molecular identification by polymerase chain reaction (PCR) and rRNA sequencing

Identification of selected isolate by PCR was performed at Animal Health Research Center, Dokki, Giza, Egypt. The primers used were Oligonucleotide primers shown in **Table 1 supplementary** [22]. The cycle parameters of the primers during PCR were determined [23], using the Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit. as shown in **Table 2 supplementary**. The same primers were used to sequence purified PCR products using dNTPs in the reaction mixture [24] at Elim biopharmaceuticals, USA by Animal Health Research Center.

Optimization of physio-chemical parameters for biosynthesis of nanoparticles

a- Substrate concentration

For the creation of silver nanoparticles, different concentrations of silver nitrate (0.1, 0.5, 1, 1.5, 2.0 mM) were incubated with 50 ml of cell filtrate and stirred at 28±2 ºC in the dark for 72 hours. 1 ml sample was taken, and the absorbance was measured with a UV-Visible spectrophotometer at a resolution of 1 nm.

b- pH value

Numerous PH values (4, 5, 6, 7, and 8) were prepared. 1 ml sample was taken, and the absorbance was measured with a UV- Visible spectrophotometer with a resolution of 1 nm.

c- Salinity

In this work (0.1,0.2,0.3) and 0.4% (w/v) sodium chloride (NaCl) were utilized, and the biosynthesis of silver nanoparticles analysis was held out.

d- Incubation period

Silver nanoparticle biosynthesis was studied at varied incubation times (24,48,72,120 and 168 hrs.).

Characterization of Green Synthesized AgNPs

Ultraviolet Visible (UV–Vis) Spectral Analysis

The UV-visible absorption spectra of the AgNPs suspension were measured using a UV-vis-NIR spectrometer (JASCOV-570, China) in a quartz cuvette. The wavelengths ranged from 300 to 800nm [25].

X-ray Diffraction (XRD) Analysis

An X-ray (D2 Phaser 2nd Gen 209450) was used to study the crystal structure of Ag-NPs. X-rays were used to penetrate the powdered Ag-NPs and scan them over the area of 2 θ from 0° to 80°.

Fourier Transform Infrared Spectroscopy Analysis (FTIR)

FTIR analysis was used to identify the secondary metabolites contained in the plant extract as well as the functional groups [26] on the AgNPs (FTIR-Bruker ALPHA II, Germany). The various functional groups present in the sample are in charge of the stabilization and reduction processes for AgNPs production. Capping agents stabilize and regulate the excessive development of nanoparticles; therefore, it is critical to select the right capping agent [27].

Antimicrobial Activity Assay

Isolation of pathogenic microorganisms

Antibacterial activities were tested against five MDR clinical pathogens (*Acinetobacter baumannii, klebsiella pneumonia, Escherichia coli (E.Coli), Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa*) Obtained from diverse clinical specimens from Tanta University Hospitals patients. Isolates were identified using standard microbiological techniques. The VITEK2 System (Biomerieux, Marcy-l'Etoile, France) was used to confirm the identification of the species.

Multidrug-resistant isolates were pathogenic bacterial strains that were resistant to at least three kinds of antimicrobials: penicillin and cephalosporins (including inhibitor combinations), fluroquinolones, and aminoglycosides [28].

Antibacterial assay of the green synthesized AgNPs

The inhibitory effects of green produced AgNPs were tested on five MDR clinical pathogens. Mueller–Hinton agar (Oxoid, UK) was used for this triplicate experiment. 100 µL of 0.5 McFarland of standardized inoculum was evenly distributed on the plate surface. Following that, sterile discs of filter paper (6 mm diameter) loaded with various concentrations of green produced silver nanoparticle solution (10µl, 20µl, 50µl, 70µl, 100µl) were transferred to seeded agar plates. Sterile filter paper discs saturated with 20 µL of distilled water served as a negative control. The plates were incubated for 24 hours at 37 °C. The zone of inhibition was calculated and compared to the control [29].

Minimum inhibitory concentration (MIC) and Minimal Bactericidal Concentration (MBC)

According to CLSI guidelines [30], broth microdilution procedures employing 96-well microtiter plates assessed AgNPs MICs. Twofold dilutions of Green synthesized silver nanoparticles suspensions were made to make final concentrations ranging from 100mg/mL to 0.195mg/mL after adding 100 µl to each well, which was earlier seeded by 100 µl Mueller Hinton broth media (Oxoid, UK). Then, 100 µL of (10^8 CFU/ml, 0.5 McFarland's standard) bacterial suspension was inoculated into every well. The positive control only had broth that had been inoculated and negative control contained the same serial dilution without inoculated broth. After incubation at 37 °C for 24 h. The MIC was determined by visually checking the wells for turbidity and recording the lowest concentration at which no bacteria grew. Furthermore, all plates were measured on ELISA (MR2MH005, London, England) to determine MIC. Three duplicates of each experiment were performed, and mean values were noted.

To calculate MBCs, 30 µL was transferred from each well to Mueller Hinton agar medium and incubated at 37 °C for 24 hours. When the lowest antibiotic concentration kills 99.9% of bacteria, the MBC endpoint is attained.

Scanning Electron Microscopy (SEM) investigation of bacterial cells

A bacterial stock culture with about 10^8 CFU/mL was added to 10 µL of sterilized nutrient broth containing the MIC of AgNPs. The mixture was then kept at 37°C for 18 hours as a test. After incubation, cultures were centrifuged for 5 minutes at 3000 rpm. The supernatant was discarded, and the pellet rinsed three times with distilled water after centrifugation. Following the washing process, the pellets were reconstituted in a fixation solution for subsequent analysis. To evaluate the influence of produced AgNPs on bacterial cell morphology, bacterial cells were generated, coated with gold using an ion sputter evaporator (JFC-1100E-JEOL), and studied using a scanning electron microscope (JEOL JSM-IT200, Japan).

Statistical analysis

Three replications of the experimental findings were made and average results were represented as mean ± standard deviations. Tukey's test was used in R to compute adjusted p values, and significance was recognized when the adjusted p value was equal to or less than 0.05 between the comparable treatments. This study utilized Microsoft Excel 2010 for experimental design and statistical analysis to demonstrate the relationship between response and variable effects.

Results

Isolation, purification and Screening of the most active fungal isolates in respect to production of silver nanoparticles (Ag-NP)

This study isolated 12 fungal colonies from 12 medicinal plants (Zingiber officinale, Allium sativum, Psidium guajava, Allium cepa, Mentha, Cactaceae, Eruca, Salvia officinalis, Euphorbia milii, Ocimum basilicum, Capsicum annuum, Salix) on PDA medium. Subsequently, the strains were subjected to growth analysis on PD agar and broth with 1mM silver nitrate to identify the best effective

strains in tolerating silver nitrate. After incubation period, three isolates (Zingiber officinale, Allium sativum, Psidium guajava) change their color to dark brown color compared to control. Next, the endophytic fungus isolated from Allium sativum with the highest potency has been chosen for further investigation, focusing on its ability to produce an intense color and distinct peak **(Figure 1 supplementary)**.

Identification of the most potent AgNPs producer

Morphologic identification

The most potent AgNPs endophytic fungus isolated from Allium sativum appeared macroscopically as irregularly white and cottony colonies changing color over time. Large amounts of dense mycelia are produced when colonies grow on PDA medium. By light microscopic (LM) examinations, microconidia among hyaline hyphae appeared **(Figure 1**).

Molecular identification

Most effective endophytic fungus for AgNPs synthesis was isolated from Allium sativum, and identified by PCR as *Fusarium*, Then the PCR products of *Fusarium* was submitted to be identified using ribosomal RNA (rRNA), The result is molecularly identified as *Fusarium proliferatum* and recorded in gene bank under the name of *Fusarium* and with accession number of OP024233. Phylogenetic tree analysis **(Figure 2)**.

Optimization for biosynthesis of AgNPs by *Fusarium*

Regarding the effect of different substrate concentration, the maximum production of silver nanoparticles was observed at 1mM concentration as shown in **Figure 3A**. Concerning the effect of different PH, From the UV-Visible absorption spectra readings, maximum production of silver nanoparticles was observed when shows sharp peak at PH 8 compared to other PH values (4,5,6,7) that was tested as shown in **Figure 3B**. Considering the effect of Salinity , production of silver nanoparticles was studied at different salinity value (0.1, .02, 0.3 and 0.4 % NaCl). Our findings indicated that maximum production of silver nanoparticles was at salt concentration 0.1%. Furthermore, salt concentration 0.3 and 0.4 % are not favorable to produce silver nanoparticles. **Figure 3C**. In view of effect of different incubation period, the production of silver nanoparticles was tested by treated fungal cell filtrate with 1mM silver nitrate at different incubation period (24,32,48,72 and 120 hrs.) the Maximum production of silver nanoparticles was at incubation period 72hrs as shown in **Figure 3D**.

Characterization of Green Synthesized AgNPs

To confirm the green synthesis of AgNPs, the sample of *Fusarium proliferatum* underwent characterization using multiple techniques. UV–Vis spectroscopy showed that the fungal cell filtrate treated with silver nitrate had a strong peak at around 420 nm after 72 hours as shown in **Figure 4A**. The XRD pattern of the prepared silver nanoparticles revealed a cubic crystal structure **(Figure 4B)**. The primary strong characteristic peaks of silver nanoparticles at 2θ are 32º, 37.9º, 54.6º, and 67.2º which correspond to the crystal faces of 111,200, 220, and 311 silver nanoparticles, respectively. TEM Microscopy demonstrated that the green synthesized silver nanoparticles had a normal spherical form with smooth surfaces and sizes ranging from 6 to 19 nm **(Figure 4C)**. In FTIR spectrum of AgNPs, bands observed were from 3439 to 617 cm−1, indicating the presence of active functional groups. FTIR analysis of synthesized silver nanoparticles suggests that biological molecules such as alkaloids, phenols, flavonoids, amino acids, glycosides, and tannins play a role in the biotransformation of silver ions to nanoparticles and their stability in aqueous medium **(Figure 4D)**.

Antibacterial activity of Green Synthesized AgNPs

It was noted that all five tested microbes show inhibition zones at low concentration of silver nanoparticles and the inhibition zones increased with increasing concentration of silver nanoparticles. However, no inhibition zone was noted in the control (cell free water extract alone). This is the order in which the greatest zone of inhibition was seen: *Pseudomonas aeruginosa* > *Acinetobacter baumannii* > *MRSA* > *E.Coli* > *klebsiella pneumonia.*

Minimum inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC)

In this study MIC and MBC of silver nanoparticles were tested against two MDR bacteria *Acinetobacter baumannii* (Gram negative bacteria) and *MRSA* (Gram positive bacteria). Our findings revealed that the MIC of *Acinetobacter baumannii* and *MRSA* was 0.195µg/ml and 6.25µg/ml respectively. The antimicrobial activity of silver nanoparticles was more effective against Gram-negative bacteria compared to Gram-positive bacteria **(Figure 6)**. Besides, the results of MBC

assay indicated that MBC of green synthesized AgNPs against *Acinetobacter baumannii* and *MRSA* was 0.195µg/ml and 6.25µg/ml respectively.

The influence of the green synthesized silver nanoparticles on the bacterial cells

SEM revealed significant external ultrastructural changes in *Acinetobacter baumannii* and *MRSA* cells treated with biosynthesized AgNPs **(Figure 7)**.

The untreated cells of *Acinetobacter baumannii* exhibited a typical rod-shaped morphology with a uniform surface, as depicted in **Figure 7A**. While, a biosynthesized AgNP-treated cell had an uneven, wrinkled, twisted and damaged outer surface and collapsed cell membranes **(Figure 7B)**. Typical *MRSA* cells were sphere-shaped with smooth, undamaged cell surfaces **(Figure 7C)**. After treatment with AgNPs, the spherical-shaped *MRSA* cell appeared distorted and destroyed with a hole in the center and looked deformed **(Figure 7D)**.

Figure 1. Fungal isolate grown on potato Dextrose Agar media for 72hrs (left) and under microscope in bright – field (right)

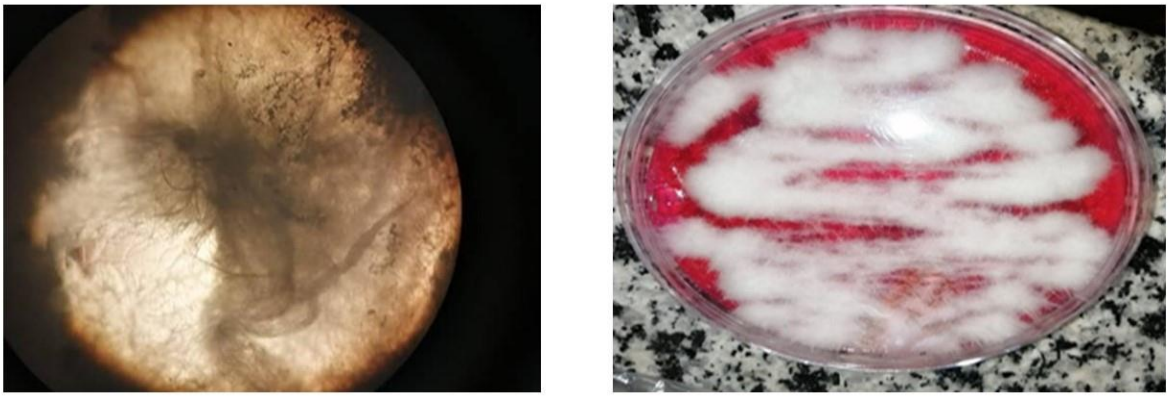


Figure 2. Maximum phylogenetic relatedness of detected *Fusarium* isolates, as evidenced by an unrooted tree grouping the tested strain with *Fusarium proliferatum*.

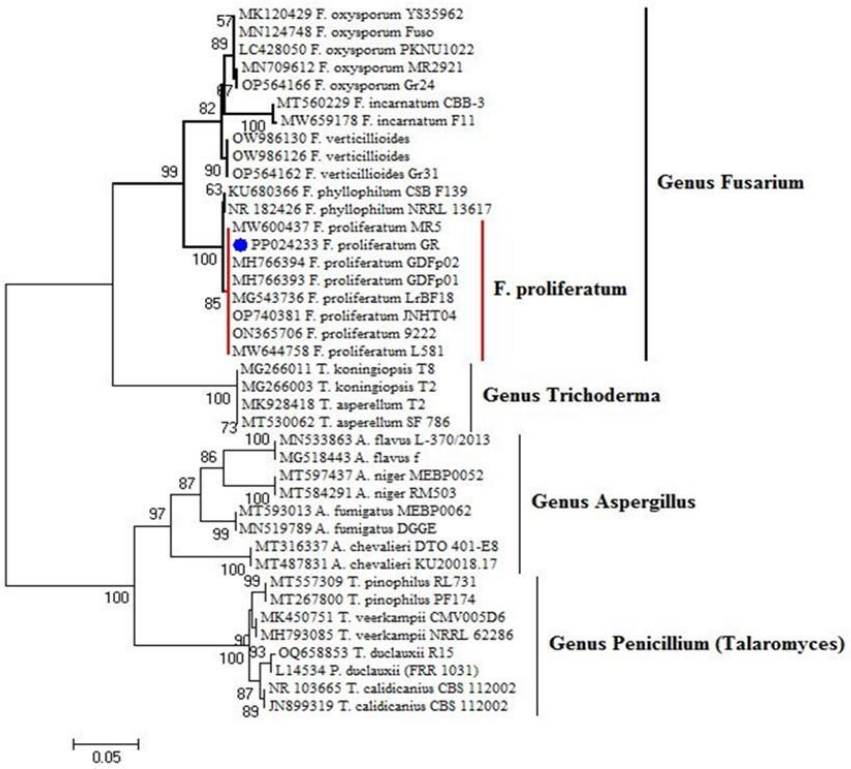


Figure 3. Effect of different parameters on the production of silver nanoparticles on UV- Visible spectra after 72hrs of incubation. A: Effect of Substrate concentration; A=0.1mM, B=0.5mM, C=1.5mM, D=1mM, E=Control, F=2.0mM, B: PH value; A=PH 4, B=PH 5,C=PH 8,D=PH 6 and E=PH 7. C: Salinity value; A=0.4 %, B= 0.2%, C=0.1%, D=Control, E=0.3%. D: Incubation period; A=72hrs, B= 48hrs, C= 120hrs, D= 24 hrs, E=32 hrs.

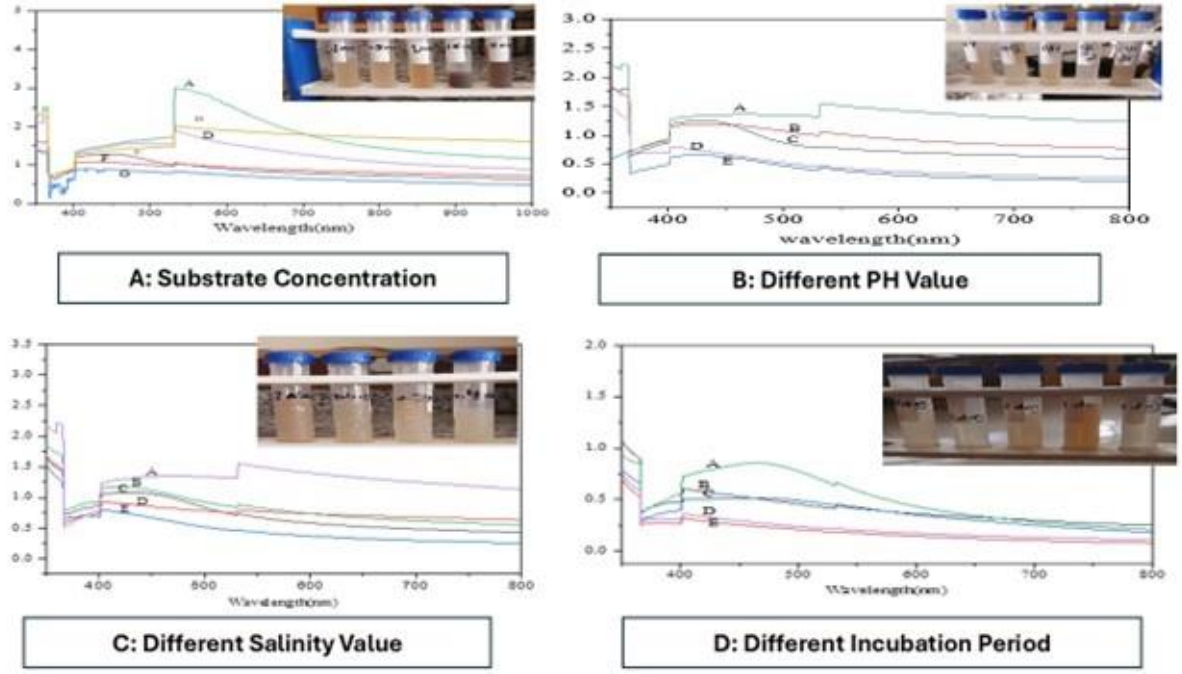


Figure 4. Characterization of Green Synthesized AgNPs produced by *Fusarium proliferatum*. A: UV-Visible spectra of AgNo3. B: X- RAY diffraction analysis (XRD). C: Electron Micrograph of TME for silver nanoparticle D: FTIR Spectroscopic micrograph of synthesized silver nanoparticles

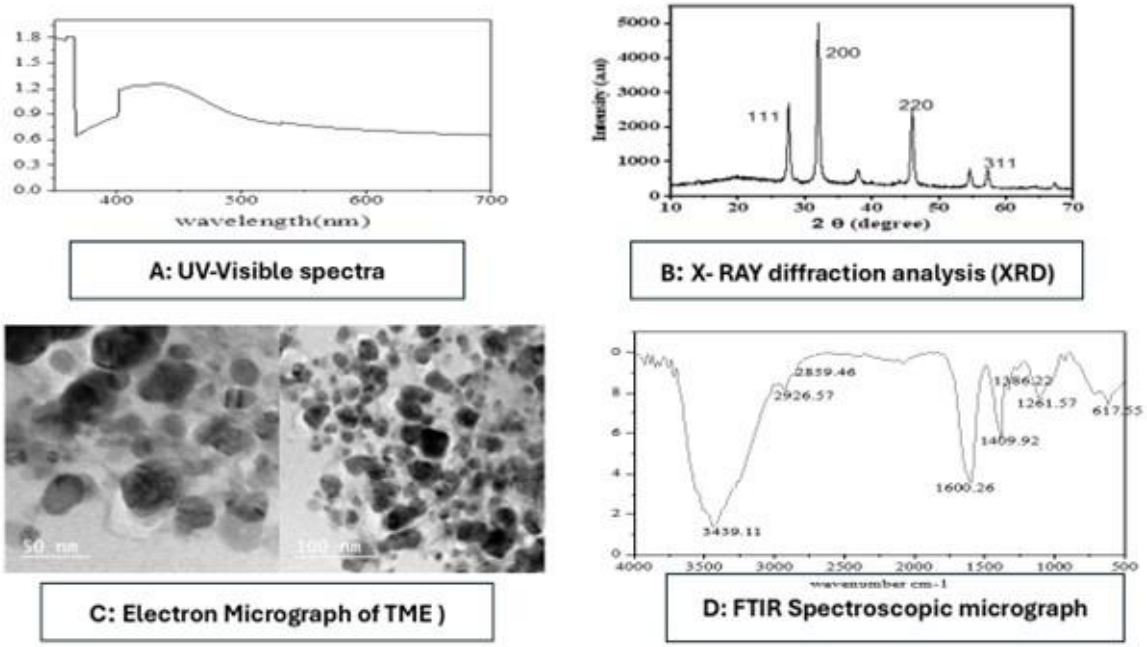


Figure 5. Inhibition zones of silver nanoparticles against five different MDR bacterial strains (A: *Acinetobacter baumannii*, B: *Pseudomonas aeruginosa*, C: *E.Coli*, D: *MRSA*, E: *Klebsiella pneumonia*). Inhibition zones increased with increasing concentration of silver nanoparticles.

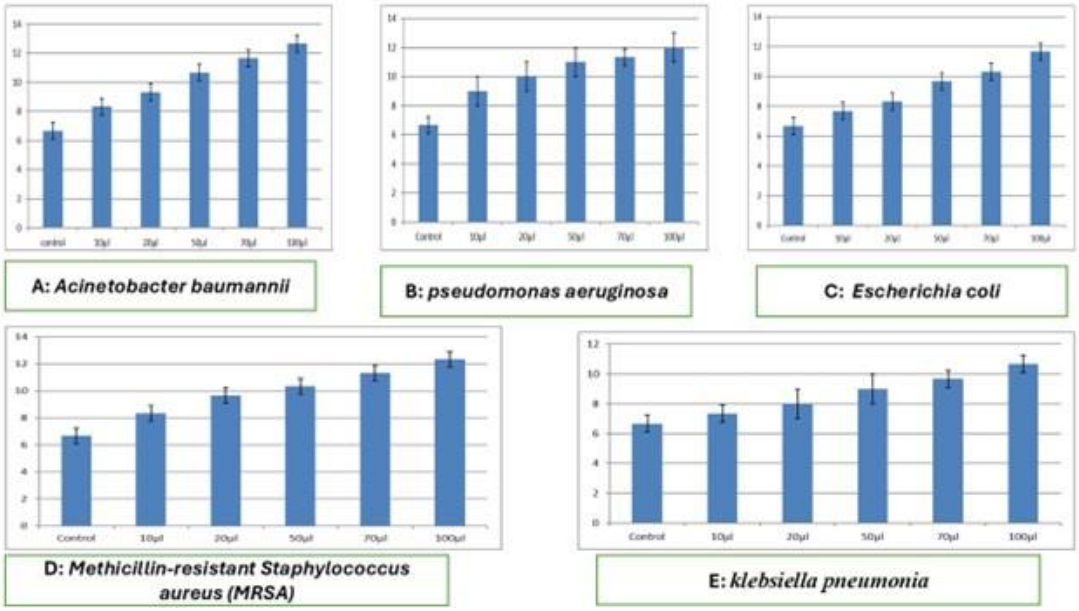


Figure 6. Minimum inhibitory concentration of silver nanoparticles against *Acinetobacter baumannii* and Methicillin-resistant *Staphylococcus aureus* (*MRSA*).

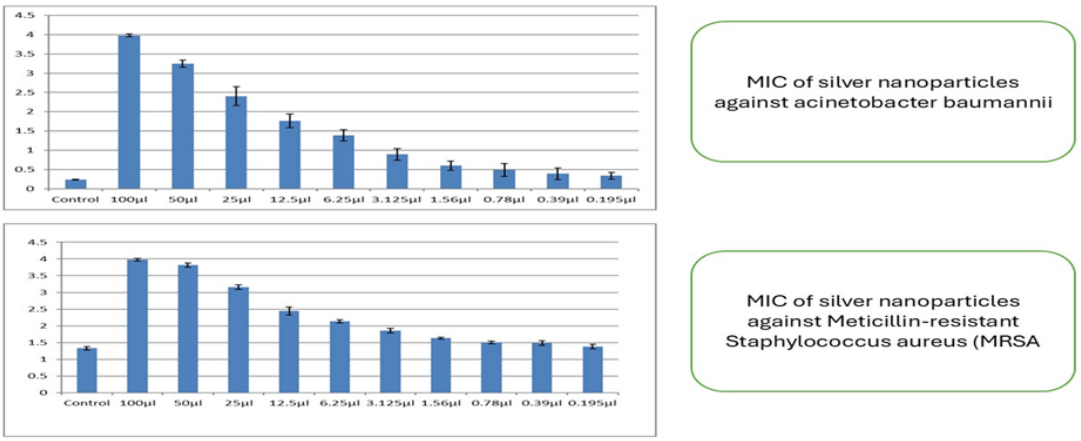
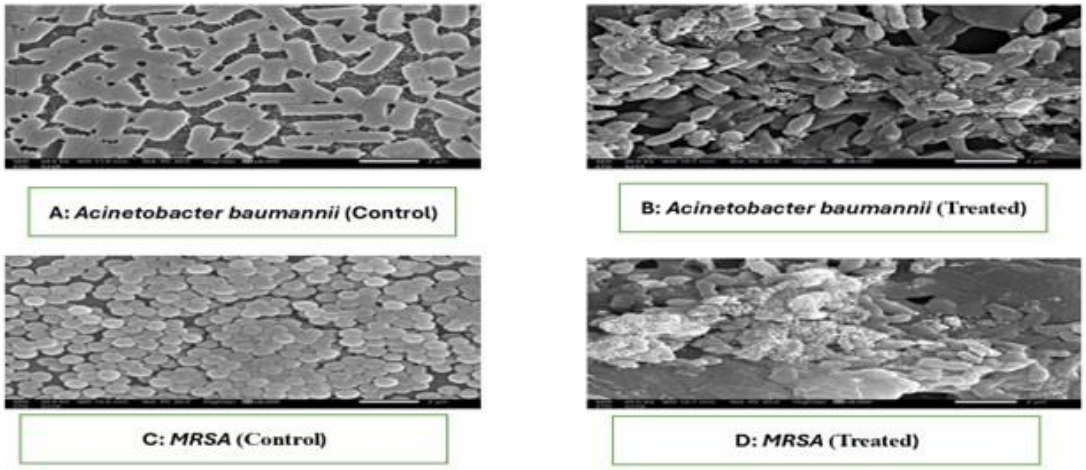


Figure 7. Scanning electron microscopy for A-*Acinetobacter baumannii* (control); B- *Acinetobacter baumannii* (treated by minimum inhibitory concentration of AgNo3); C- Methicillin-resistant *Staphylococcus aureus* (control); D- Methicillin-resistant *Staphylococcus aureus* (treated with minimum inhibitory concentration of AgNo3).



Discussion

The invention of innovative nano biomedicine has altered the treatment of a variety of infectious and non-infectious disorders [31]. Silver nanoparticles are the most effective way to reduce resistance to antibiotics in pathogens [14].

Color change observation is a typical method for assessing microbiological isolates for producing silver nanoparticles [32]. Our investigation found that Allium sativum produced the greatest Myco-SNPs and turned dark brown compared to controls. Similar findings were observed by Abd Elnaby et al. [33].

The endophytic fungus that had the most effectiveness in producing AgNPs was molecularly identified as *Fusarium proliferatum* and preserved in GenBank under the Accession Number. OP024233. Nevertheless, there has been no investigation carried out on the mycosynthesis of AgNPs from the endophytic *Fusarium proliferatum*. Hence, this study is the initial exploration of the biological properties of Myco-SNPs derived from endophytic *Fusarium proliferatum*, with the intention of utilizing them in future medical and pharmaceutical endeavors.

A greater rate of growth can be promoted, and the output of products can be increased by optimizing physiochemical characteristics. Regarding the effect of different substrate concentrations, this study revealed that the maximum production of silver nanoparticles was observed at 1mM concentration. The results of our study were consistent with the findings of Banu et al. [34], who employed a concentration of 1 mM AgNO3 to synthesize SNPs using a fungal extract derived from *Rhizopus stolonifer*. Concerning the effect of different PH, our findings revealed that maximum production of silver nanoparticles shows sharp peak at PH 8. these findings lined up with EL-Zawawy et al. [12] who reported that pH 7 was the optimal point for Myco-SNPs formation. Furthermore, previous studies found similar findings [34-36]. In view of effect of different incubation period**,** our outcomes indicated the maximum production of silver nanoparticles was at incubation period 72 hrs. These results disagreed with those of Elamawi et al. [37], who found that the maximum SNP production occurred in a similar manner between 4 and 5 days of incubation. Conversely, some studies have reported varying SNPs biosynthetic incubation periods, including three days [38].

To confirm the green synthesis of AgNPs, the sample of *Fusarium proliferatum* underwent characterization using multiple techniques. Result of UV–Vis spectroscopy, showed that the fungal cell filtrate treated with silver nitrate had a strong peak at around 420 nm after 72 hours. EL-Zawawy et al. [12] also reported similar results, stating that the sample of *A. flavipes* AUMC 15772, when examined using UV-Vis spectroscopy, exhibited a single and intense peak at 420 nm for the Myco-SNPs. The nanoparticles produced in this study were analyzed using powder XRD to validate their composition as silver and obtain structural information. The results showed that the prepared silver nanoparticles revealed a cubic crystal structure. These findings were near to Shaaban, et al. [39]. Additionally, the green synthesized silver nanoparticles had a normal spherical form with smooth surfaces and sizes ranging from 6 to 19 nm using TEM Microscopy. This was in accordance with Gupta et al. [40].

FTIR analyses of AgNPs indicated**,** bands observed were from 3439 to 617 cm−1, indicating the presence of active functional groups. In harmony with our results Gupta et al. [40] posted that the bands are noticed at 3295.71, 1635.67, 1541.60, 1043.84, and 516.36cm−1. Furthermore, previous articles offer significant evidence to corroborate the current conclusions.

Regarding the effectiveness of green synthetized AgNPs as a bacterial growth inhibitor, our data showed that all five microorganisms exhibited inhibitory zones at low silver nanoparticle concentrations and increased with increasing concentrations. The highest zone of inhibition was observed in the following order: *Pseudomonas aeruginosa* > *Acinetobacter baumannii* > *MRSA* > *E.Coli* > *Klebsiella pneumonia.* This was in accordance with Seetharaman et al. [41]. This can be attributed to the presence of a dense peptidoglycan layer, which allows AgNPs to adhere efficiently to the bacterial cell wall, hence impeding their antibacterial function. Similarly, EL-Zawawy et al. [12] reported that Myco-SNPs shown remarkable efficacy against specific multidrug-resistant wound infections, albeit with modest variations. *Pseudomonas aeruginosa* was the most susceptible pathogen, followed by *Klebsiella pneumoniae* and *Staphylococcus aureus*, in that order. Nevertheless, Gupta et al. [40] demonstrated that the produced

POAgNPs effectively suppressed the growth of both bacteria in a manner that depended on the concentration. The highest level of antibacterial activity was observed against *S. aureus*.

Regarding MIC results, our findings revealed that the MIC of *Acinetobacter baumannii* and *MRSA* was 0.195µg/ml and 6.25µg/ml respectively. However, the effect of silver nanoparticles on Gram negative bacteria was better than Gram positive bacteria**.** This outcome was consistent with the research conducted by Shaaban, et al. [39] who observed that MIC and MBC of 1.25 mg/mL demonstrate that AgNPs manufactured using green methods exhibit a bactericidal effect against *S. aureus* and *P. aeruginosa*. Furthermore, EL-Zawawy et al. [12] reported that the strains: *Pseudomonas aeruginosa* and *Klebsiella pneumonia* exhibited the highest sensitivity, as evidenced by their lowest MIC of 8 µg/mL. *Staph. aureus* had the lowest sensitivity among the strains tested, with the highest recorded MIC of 32 µg/mL. Unlike previous research, MIC of *E. coli, P. aeruginosa, and B. subtilis* was found to be 84.28, 74.26, and 94.43 µg/mL, respectively [42]. Similarly, the MICs for *K. pneumonia* and *S. aureus* [42] were found to be 60 and 500 µg/mL, respectively. The variations in MIC values can be ascribed to the colloidal condition, concentration, and size of Myco-SNP. Nanoparticles of smaller sizes exhibit greater toxicity towards pathogens compared to larger ones, mostly due to their enhanced diffusion capabilities. The most optimal effectiveness was observed for nanoparticles with sizes below 50 nm [43].

The impact of AgNPs on bacterial cells was examined using scanning electron microscopy. The results show deformation effects of green-synthesized AgNPs on cell morphology, indicating the effectiveness of the green synthesis process. The morphological alterations in the cell wall demonstrated that the newly generated AgNPs may interfere with the operation of regular cells. Our findings demonstrated that *Acinetobacter baumannii* were more sensitive than *MRSA*, this may be due to Gram-negative and Gram-positive bacteria's cell wall structure and foundation. The recent findings were supported by earlier research. [44]. Nevertheless, several research refuted this assertion [45, 46].

There have been several postulated mechanisms for AgNPs' antibacterial action. Among these, the widely accepted process is that positively charged silver ions in AgNPs interact with negatively charged sulfur or phosphorus-containing bio-macromolecules such nucleic acids and proteins, altering bacteria's structure. The structural alterations caused by the deformation of the cell wall and cell membrane may compromise the metabolic process and eventually lead to cell death [47]. Furthermore, it has been proposed that nanoparticles create many free radicals, which cause cell membrane damage through increased membrane permeability and ultimately cell death [48].

Conclusion

The study discovered a new type of fungus called *Fusarium proliferatum* that was found inside Allium sativum. This fungus was found to have the capability to manufacture Myco-SNPs, which are nanoparticles with a distinct peak at 420 nm. The effectiveness of Myco-SNPs against MDR microorganisms was demonstrated by their antibacterial activity, as indicated by the MIC and MBC. The data acquired in this study demonstrates, for the first time, that endophytic *Fusarium proliferatum* has the capacity to create Myco-SNPs with potent antimicrobial activity against MDR pathogens under optimal conditions. The Myco-SNPs produced in this study have significant potential for application in the pharmaceutical industry as a potential treatment for infections.

Ethical consideration

The study protocol was approved by Tanta University's Faculty of Medicine Research Ethics Committee (36264PR811/8/24).

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Author contribution

All authors participated in this research's conception, design, material preparation, data collecting, and analysis, or paper draft. Final manuscript read and approved by all authors.

Data availability

Contact the author for data.

Acknowledgments

None

Conflicts of interest

The authors declare no conflict of interest.

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