Fusarium mangiferae as New Cell Factories for Producing Silver Nanoparticles

Haider M. Hamzah^{1*}, Reyam F. Salah², and Mohammed N. Maroof³

¹Department of Biology, College of Science, University of Sulaimani, Slemani, Kurdistan Region, Iraq

²Department of Biology, College of Science, Tikrit University, Iraq

³Department of Biology, College of Education for Pure Sciences, Tikrit University, Iraq

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*Corresponding author
Phone: +9647708912248;
E-mail: haider.hamzah@univsul.edu.iq

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Finding a safe and broad-spectrum medication is a goal of scientists, pharmacists, and physicians, but developing and fabricating the right medicine can be challenging. The current study describes the formation of silver nanoparticles (AgNPs) by *Fusarium mangiferae*. It involves the antibiofilm activity of the nanoparticles against *Staphylococcus aureus*. It also involves cytotoxic effect against mammalian cell lines. Well-dispersed nanoparticles are formed by *F. mangiferae*. The sizes of the nanoparticles were found to range from 25 to 52 nm, and UV-Vis scan showed absorption around 416–420 nm. SEM, TEM, and AFM results displayed spherical and oval shapes. Furthermore, the FTIR histogram detected amide I and amide II compounds responsible for the stability of AgNPs in an aqueous solution. AgNPs were observed to decrease the formation of biofilm at 75% (v/v). DNA reducing, smearing, and perhaps fragmentation were noticed after treating the bacterial cells with 50% (v/v). Additionally, cell lysis was detected releasing proteins in the supernatant. It was also observed that the AgNPs have the ability to cause 59% cervical cancer cell line (HeLa) deaths at 25% (v/v), however, they showed about 31% toxicity against rat embryo fibroblast transformed cell lines (REF). The results of this study prove the efficiency of AgNPs as an antibiofilm against *S. aureus*, suggesting that AgNPs could be an alternative to antibiotics. It must also be emphasized that AgNPs displayed cytotoxic behavior against mammalian cell lines. Further studies are needed for assessing risk in relation to the possible benefit of prescribing AgNPs.

Keywords: *Fusarium mangiferae*, silver nanoparticles, *Staphylococcus aureus*, antibiofilm, mammalian cell lines

Introduction

Advances in nanotechnology have allowed the synthesis of nanoparticles which are considered a feasible alternative to antibiotics and seem to be the solution to the problem of microbial multidrug resistance. Increasingly, multidrug-resistant (MDR) microbes are a growing dilemma and the cause of a public health crisis, particularly in the treatment of infectious diseases [1]. On the other hand, several pathogens can form biofilms composed of extracellular polymeric substances (EPS) that are responsible not only for a communication phenomenon but display resistance to

antibiotics and host defense systems [2, 3, 4]. Besides that, biofilm attaches to many medical devices and foreign body materials (e.g., catheters, contact lenses, and artificial heart valves) causing chronic and hard-to-treat infections, particularly in immunocompromised patients [5, 6]. Thus, experts are facing a lot of challenges to find robust drugs or antimicrobial agents that are effective against biofilm-forming microbes such as *Staphylococcus aureus*.

S. aureus is an opportunistic pathogen; some strains are resistant to a wide range of antibiotics and are capable of evading the host immune system [7]. There are no complete therapies for treating and preventing chronic

biofilm-mediated infections; however, several available alternative choices such as silver nanoparticles (AgNPs) are in use and are being tested [8, 9]. Several publications have hypothesized that AgNPs are involved in disruption of the membrane with the generation of reactive oxygen species (ROS) and ultimately lead to the death of pathogens [6, 10, 11]. One possible mechanism is that AgNPs have the ability to adhere to the bacterial cell wall and produce cracks and pits, through which the internal cell contents are released [12]. Although AgNO₃ has potential toxicity to humans, its nanoparticles are medically promising for being highly effective as antimicrobial agents [1] and the least toxic [13]. However, the toxicity of AgNPs needs to be studied extensively to determine whether the benefits of taking nanoparticles outweigh potential risks. At the same time, silver nanoparticles have potential therapeutic properties in treating a variety of diseases including cancers. For instance, AgNPs induced cytotoxic effects in Dalton's lymphoma ascites (DLA) tumor system in vitro, inhibiting tumor progression and thereby effectively controlling disease development without toxicity to normal cells [14]. Furthermore, Arora *et al.* [15] have suggested a promising role for AgNPs as novel chemo-preventive agents against UVB-irradiation-induced skin carcinogenesis. Other studies have illustrated the in vitro inhibitory effects of AgNPs on human lung epithelial cells and rat cells [16, 17]. Generally, silver ions disrupt the mitochondrial permeability transition pore in mitochondrial membranes, increasing mitochondrial swelling, and eventually resulting in cellular apoptosis or necrosis as a result of ROS production [18, 19].

In recent years, there has been an increasing interest in mycosynthesis of AgNPs due to its efficacy [1, 20, 21]. In this context, filamentous fungi have become important for AgNP makers, where producing unique nanoparticles plays a key supporting role in the growing industry of synthetic biology. The filamentous fungi *Fusarium* species that have previously been used in reducing aqueous silver ions extracellularly to generate silver NPs, are *F. oxysporum* [22], *F. acuminatum* [23], *F. semitectum* [24], *F. solani* [25], *F. culmorum* [26], *F. graminearum*, *F. scirpi*, *F. tricinctum*, *F. proliferatum*, *F. equiseti*, and *F. moniliforme* [27]. To the best of our knowledge, there is no report of *F. mangiferae* as being a new AgNP factory. *F. mangiferae* has been associated with mango malformation [28]. However, mango leaves inoculated with *F. mangiferae* isolate did not produce any disease symptoms and remained healthy [29]. In this study, we synthesized AgNPs using the filamentous fungus *F. mangiferae* and investigated its antibiofilm activity against biofilm forming *S. aureus*. Additionally, we conducted experiments

to detect the cytotoxicity behavior of AgNPs against two mammalian cell lines.

Materials and Methods

Synthesis of AgNPs

F. mangiferae (FQ) was used for the synthesis of AgNPs. Colonies of *F. mangiferae* were grown on Potato Dextrose Agar (PDA) plates for 6 days at 28°C and were used to inoculate 250 ml MYPG medium (consisting of 3 g malt extract, 3 g yeast extract, 2 g peptone, and 10 g glucose per liter) in Erlenmeyer flasks. The flasks were incubated at 28°C for 7 days with shaking at 150 rpm using shaking incubator (GFL, Germany). Mycelial pads were harvested aseptically and washed three times with distilled water. The biomass of fungi was weighed and then used for the synthesis of nanoparticles according to Ahmed *et al.* [1].

Characterization of AgNPs

The synthesized NPs were characterized based on specific surface plasmon resonance peak, shape, size and interaction between protein and NPs. To confirm the synthesis of AgNPs, 3 ml of sample was used for UV-Vis scan at wavelengths ranging from 300–600 nm using a Genesys 6 spectrophotometer (Thermo Electron Corporation, USA). The nanoparticles were subjected to microscopic characterization, *i.e.* scanning electron microscope (SEM), transmission electron microscope (TEM), and atomic force microscope (AFM) [1]. To identify the presence of possible biomolecules, Fourier transmission infrared spectroscopy (FTIR, WQF-520, Biotect, England) was employed [1].

Antigrowth and Antibiofilm Activities of AgNPs

An isolate of *S. aureus* obtained from Sulaimani Teaching Hospital in Kurdistan Region, Iraq, was multidrug resistant. The activity of AgNPs was tested against *S. aureus* as inhibitors using a microtiter plate. Fresh overnight culture of pathogenic bacterial isolate was adjusted to be 10⁸ cells per ml. A bacterial culture of 120 µl was placed in a 96-well microtiter plate and then 80 µl of an appropriate dilution of AgNPs (25%, 50%, 75%, and 100% (v/v)) was added. Nutrient broth (200 µl) was used as a negative control and 120 µl of bacterial culture mixed with 80 µl of fungal extract was also employed as a negative control. The microtiter plates were incubated at 37°C for 18 h [1]. The optical density (OD) at 600 nm was measured using Microplate Spectrophotometer (Biotect µQuant, USA). Growth percentage was calculated based on the average and standard deviation of triplicate results. Besides the growth inhibition assay, spot inoculation was also evaluated. Five µl was taken from each well and spotted on nutrient agar and Congo red agar (CRA) plates. Then all plates were incubated at 37°C for 18 h. CRA was used to detect, qualitatively, biofilm production. CRA was prepared by mixing 3.7% (w/v) Brain Heart Infusion broth (Difco) with 0.08% (w/v) Congo red (Sigma-Aldrich, Germany) supplemented with 5% (w/v) sucrose and 1.5% (w/v) agar [30].

Biofilm formation was inspected according to Mathur *et al.* [31] with a few modifications. Nutrient broth (NB) was inoculated with bacterial cells and incubated under shaking for 24 h at 37°C. Cells were adjusted to be 10^8 cell/ml, then a Tissue Culture Plate (TCP) approach was used. About 200 µl cultures were placed in a 96-well microtiter plate. The plate was then incubated for 24 h at 37°C under a gentle shaking in microplate incubator-shaker PST-60 HL Plus (BOECO, Germany). Biofilm was measured by discarding the medium and rinsing the wells three times with 200 µl phosphate buffer saline (PBS, pH 7.2). After drying, the attached cells were stained with crystal violet (0.1%) for 30 min. After staining, the liquid was discarded and the wells were rinsed three times with distilled water. The plate was then allowed to dry at room temperature, after which 200 µl ethanol (95%) was added to the wells to solubilize the stain. The absorbance at 595 nm was measured via Microplate Spectrophotometer (Biotech µQuant, USA). OD readings were used as an indicator of the bacterial biofilm formation, and then converted to percent of inhibition (%). To confirm that the AgNPs had anti-biofilm activity, different concentrations (0%, 25%, 50%, 75%, and 100% (v/v)) of AgNPs were used. Biofilm formation was determined as mentioned above. This experiment was performed three times and then averages and standard deviations (STDEV) were calculated using Microsoft Excel.

Outer Membrane Disturbing Test

In order to elucidate the antibacterial mode of AgNPs on cell membranes, scanning electron microscopy (SEM) technique was used. One hundred microliters of broth culture of *S. aureus* incubated with AgNPs (50% (v/v)) for 24 h were centrifuged at $3,000 \times g$ for 10 min using Centrifuge 5702 R (Eppendorf, Germany). The cells were then washed with PBS and fixed in 2.5% glutaraldehyde for 24 h at room temperature. Thereafter, cells were dehydrated through a graded ethanol series (70%, 85%, 90%, and 100%). Then, the sample from each group was sputter coated with carbon. The same procedure was followed without AgNPs and served as a negative control [32].

Protein Leakage Assay

The effect of silver nanoparticles on membrane damage was further studied by quantifying the leaked cytoplasmic proteins. Protein leakage from bacterial cells was determined using Biuret method (CHRONOLAB SYSTEMS, Spain). Briefly, bacterial cell suspension with an absorbance of 0.2 at 600 nm ($\sim 10^8$ cells per ml) was prepared in NB and treated with AgNPs at 0%, 25%, 50%, 75%, and 100% (v/v) for about 12 h. Samples were centrifuged at $3,000 \times g$ for 20 min using Centrifuge 5702 R (Eppendorf, Germany), and supernatants were subjected for protein quantification.

DNA Profile Analysis

To assess DNA reduction, genomic DNA from *S. aureus* (before and after treatment with 50% AgNPs) was extracted using the protocol described by Thottappilly *et al.* [33] and Onasanya *et al.*

[34] with some modifications. Briefly, about 0.3 g of washed bacterial cells were suspended in 200 µl of $2 \times$ CTAB buffer (50 mM Tris, pH 8.0; 0.7 mM NaCl; 10 mM EDTA; 2% hexadecyltrimethylammonium bromide; 0.1% 2-mercaptoethanol), followed by the addition of 100 µl of 20% sodium dodecyl sulfate and incubated at 65°C for 20 min. DNA was purified by three extractions with phenol:chloroform:isoamyl alcohol (24:25:1) and then cold 99% ethanol was added to the aqueous layer and kept at -20°C for 20 min. After this stage, the solution was centrifuged at $10,000 \times g$ for 20 min using Mikro 200R (Hettich, UK). After washing with 70% ethanol, the DNA was dried and resuspended in 50 µl of sterile distilled water. The DNA profile was checked by electrophoresis on a 1% agarose gel in 1xTAE (45 mM Tris-acetate, 1 mM EDTA, pH 8.0).

Cytotoxicity Assay

Rat embryo fibroblast transformed cell lines (REF cells) and cervical cancer cell line (HeLa cells) were used in this study. The cell lines were obtained from the Iraqi Center for Genetics and Cancer Research-Baghdad, Iraq, and maintained in RPMI 1640 (Sigma-Aldrich, Germany) supplemented with 5% calf bovine serum (ICCMGR), 100 units/ml penicillin, and 100 µg/ml streptomycin. Cell proliferation was evaluated according to Betancur-Galvis *et al.* [35] with modifications. In brief, mammalian cell lines ($120 \mu\text{l}$ of 10^8 cell/ml) was seeded in a 96-well tissue culture plate. Then, 80 µl of different concentrations of AgNPs (0%, 25%, 50%, 75%, and 100% (v/v)) were added. Cells were cultured overnight at 37°C and were then washed with PBS, followed by 50 µl of crystal violet solution (crystal violet solution was prepared according to Mather and Roberts (1998) [36]). Afterwards, the plate was measured at 492 nm using a microtiter plate reader (Organon Teknika Reader 230S, Austria). The data were analyzed using Microsoft Excel and depicted as a graphic representation. The assay was performed in triplicate and cytotoxicity was expressed as the percentage of viable cells at different concentrations of samples. The percentage of cytotoxicity (Inhibition %) was calculated as: $(A - B)/A \times 100$, where A is the mean optical density of untreated wells and B is the optical density of treated wells. IC_{50} , the value that kills 50% of cultured cells, was calculated from the dose-response curve.

Results

Characterization of AgNPs

F. mangiferae (FQ) was able to convert AgNO_3 into AgNPs after 190 h of incubation as noticed visually by color change from purple to dark brown, whereas the control vessels developed no change in their original color (Fig. 1; inset). The wavelength scan of UV-Vis spectra revealed a broad peak between 416 to 419 nm indicating the surface plasmon resonance (SPR) nature of the AgNPs that is present in the dark brown supernatant (Fig. 1).

Fig. 1. UV-Visible absorption spectrum of AgNPs.

(a) AgNO_3 solution (1 mM) control. (b) Biomass of FQ test sample before incubation. (c) Supernatant of FQ; control. (d) Biomass of FQ; test sample. **Inset:** Color change of filtrate from purple (b) to dark brown (treated) after synthesis of AgNPs (d).

SEM was used to determine the presence and size of AgNPs. After drying, the thin layer of AgNPs was analyzed. The scanning electron micrograph shows a presence of high-density AgNPs with mainly spherical shape (Fig. 2A; white arrows). The data obtained from TEM micrographs show that the synthesized AgNPs have a spherical shape and the size ranged from 25–52 nm uniformly distributed

and also showed a few aggregations (Fig. 2B; white arrows). A two-dimensional horizontal cross section of the AgNPs by AFM indicated that the surface topography of the synthesized silver nanoparticles was almost spherical in shape (Fig. 2C). A histogram analysis revealed that the mean particle size was approximately 48.5 nm.

The absorption peaks of AgNPs (Fig. 3) are located in the region of $523\text{--}3,388\text{ cm}^{-1}$ in FT-IR. The band at $3,388\text{ cm}^{-1}$ is assigned to the stretching vibrations of -NH identified as the amide I band, whereas the bands at $1,626\text{ cm}^{-1}$ represent -NH_2 . At $1,523\text{ cm}^{-1}$ shows the stretching vibration of -C=O (carbonyl). On the other hand, the bands at $1,404\text{ cm}^{-1}$ and $2,939\text{ cm}^{-1}$ represent C-H and C-H_2 , respectively.

Antigrowth and Antibiofilm Activities of AgNPs

The action of AgNPs on multidrug-resistant clinical isolate *S. aureus* was carried out by comparing growth and biofilm rate under control and test conditions (Fig. 4). AgNPs show antimicrobial activity in which 75% (v/v) of AgNPs inhibited 59% growth of *S. aureus*. Additionally, AgNPs reduced the biofilm formation in *S. aureus* significantly. Notably, changes in colony colors on CRA in the presence of nanoparticles indicate that AgNPs have significant roles as antibiofilm activity against *S. aureus* (Fig. 4; inset). The biofilm formation is diminished to about 50% when 50% (v/v) of AgNPs was used (Fig. 4).

Fig. 2. Micrograph of AgNPs synthesized by FQ.

(A) SEM image. (B) TEM image. (C) AFM images (Left upper picture depicts the 3D image of the AgNPs; whereas left lower picture represents the 2D image of the AgNPs horizontal cross section. Right image indicates the histogram analysis showing the average particle size 48.5 nm).

Fig. 3. FT-IR spectrum of silver nanoparticles synthesized by FQ. Green boxes depict C=O, -NH, -NH₂, C-H and C-H₂ bands.

Outer Membrane Disruption and DNA Profile of *S. aureus* by the Action of AgNPs

In the present study, the preliminary mechanistic actions of AgNPs on *S. aureus* have been investigated. The outer membrane of *S. aureus* has been inspected by examining the cells by SEM and determining protein leakage (Figs. 5A and 5B). *S. aureus* cells were treated with various concentrations (0%, 25%, 50%, 75%, and 100% (v/v)) of AgNPs, then protein concentration was estimated in the supernatants (Fig. 5B). Notably, AgNPs have defected and possibly ruptured the outer membrane of *S. aureus* cells in comparison with untreated cells. To confirm whether AgNPs were able to increase the decomposition of genomic DNA, agarose gel electrophoresis was performed. Gel electrophoresis image (Fig. 5C) revealed that the bands of DNA of the treated cells have diminished and maybe fragmented as noticed with the plasmids DNA.

Cytotoxicity Assay of AgNPs on Mammalian Cell Lines

The cytotoxicity of AgNPs was assessed in vitro against two different cell lines; cervical cancer cell line (HeLa cells) and rat embryo fibroblast transformed cell line (REF cells) at different concentrations (0%, 25%, 50%, 75%, and 100% (v/v)). The data elucidate a direct dose-response relationship; cytotoxicity increased at higher concentrations (Fig. 6). A significant inhibition of HeLa cells was noticed; 25% (v/v) of AgNPs generates ~59% cell death in comparison with the REF cells since 25% (v/v) resulted in ~31% cell death.

Discussion

To our knowledge, this is the first study investigating the use of *Fusarium mangiferae* in synthesizing AgNPs. Ultimately, these findings support the hypothesis that *F. mangiferae* has

Fig. 4. Growth and biofilm inhibition of *S. aureus* in the presence of AgNPs using microtiter plate technique.

Bacterial cells adjusted to 1×10^8 per ml; 120 μ l broth culture were mixed with 80 μ l of different concentrations of AgNPs in each microtiter plate well. The plate was incubated for 18–24 h at 37°C. Optical density was measured at 600 nm and 595 nm for growth and biofilm inhibition, respectively. Then readings were converted to growth (%) and biofilm inhibition (%). **Inset:** Five μ l was taken from each well of growth inhibition assay plate and spotted onto the nutrient agar plate (A) and Congo red agar plates; pictures were taken from separate CRA plates (B).

Fig. 5. Outer membrane disruption and DNA profile of *S. aureus* by the action of AgNPs. (A) SEM images (Left picture depicts the untreated cells; whereas right picture represents the treated cells with 50% AgNPs) (B) Protein leakage. (C) Genomic DNAs profile, black arrows represent plasmids profile. One percent agarose in TAE buffer was used and gel run at 80 Volts for an hour. Ladder used was 1 kb.

the ability to drive AgNP formation, with equal or greater efficacy as compared to other *Fusarium* species. Furthermore, as a primary focus of this work, we observed successful antigrowth and antibiofilm activities of AgNPs by evaluating it against *Staphylococcus aureus*. Although this has not been

Fig. 6. Cytotoxic effect of AgNPs against HeLa and REF cell lines.

HeLa and REF cell lines were treated with different concentrations of AgNPs and incubated at 37°C for 24 h. Cell death values were measured and converted to percentages of inhibition.

designed to assess the mode of action against human cell lines, these results are promising and warrant further investigation. Therefore, future studies aimed at assessing the AgNPs' mode of action following HeLa cell line application, and producing ample and clinically feasible sources of AgNPs for testing in larger rodent and small animal studies are necessary to translate these findings to clinical use.

In this study, *F. mangiferae* showed resistance to relatively high concentrations of copper and silver metals (data not shown). FQ might develop a new survival strategy as an adaptive response to unfavorable environmental conditions, therefore this study offers some important insights into a plethora of unanswered questions concerning the mechanisms involved in metal resistance of fungal strains. FQ was able to reduce silver ions to silver NPs since it is well known that AgNPs in aqueous solutions show a yellowish brown color [1, 22]. The reduction took place through catalysis by the reducing agent secreted from the culture of FQ into the solution. *Fusarium* species have been used previously for NP production [1, 22, 37]; however, previous studies did not investigate *F. mangiferae* for the synthesis of AgNPs. The formation of AgNPs took about a week's incubation to develop the brown color. This slow

silver reduction could be the result of undesirable growth surroundings, due to the presence of relatively high concentrations of silver nitrate. Mycosynthesis of silver NPs might occur by the activity of secreted reductase enzymes during the phases of fungal growth [37]. However, the limited fungal growth under such growth conditions may perhaps lead to the delayed excretion of the enzymes, which leads to the slow reduction of silver nitrate. Notably, the solution developed a dark brown color; therefore it was subjected and analyzed spectrophotometrically using UV-Vis in the scan range of 300–600 nm. Interestingly, AgNPs absorbed light at different wavelengths and were excited due to charge density at the interface between conductor and insulator to give a respective peak on UV-Vis spectrophotometry [1, 37]. This finding is in agreement with several other studies [38, 39].

The SEM image of silver NPs shows aggregated particles, most likely due to the presence of the fungal metabolites that are acting as capping agents. Besides, high-density AgNPs with spherical shape were recognized by SEM. Similarly, a previous study has shown that the shapes of AgNPs synthesized by *F. oxysporum* were spherical to oval with little aggregation [1, 40]. TEM and AFM are two of the most widely applied techniques to analyze the structure and size of NPs. Together these analyses provide considerable insight into the morphology and particle size distribution profile of the AgNPs synthesized by FQ. AFM three-dimensional analysis showed the average roughness and inhomogeneity of the cluster formation of AgNPs. Various studies have reported that mycosynthesized AgNPs have spherical shapes and are uniformly distributed without significant agglomeration [1, 22, 41, 42]. Moreover, Gaikwad *et al.* [27] have compared the average size of AgNPs from six *Fusarium* species by TEM analysis, which confirmed the spherical shape of synthesized silver nanoparticles within the range of 2–68 nm. Further, Ingle *et al.* [23] showed that AgNPs from the fungus *F. acuminatum* were spherical with a broad size distribution in the range of 5–40 nm.

Fungal extracellular metabolites, mainly proteins, are the putative biomolecules responsible for the capping and stabilizing of the nanoparticles detected by FTIR, which renders the formed AgNPs' potential for various real-life applications. In this study, the FTIR results corroborate the findings of previous work in this field [1]. The FTIR results also suggest that there is an association between the biopolymer protein and stability of AgNPs. The biopolymer forms a ligand-shell surrounding AgNPs at the core, forming a silver polymer core-shell structure. Consequently, the polymeric shell decreases the surface potential that is

responsible for the accumulation of the silver nanoparticles to larger aggregates [41].

S. aureus is considered life-threatening due to its biofilm formation on surgical equipment, medical implants, and other devices, leading to diminished results of antibiotic treatment. Therefore, finding and perhaps fabricating a pervasive and environmentally friendly material to impede biofilm-forming bacteria is challenging. Antibacterial and antibiofilm activities of AgNPs have been appraised against multidrug-resistant gram-positive *S. aureus*. *S. aureus* shows resistance to a relatively high concentration of AgNPs, and this tolerance might be attributed to the presence of a self-produced extracellular matrix in the isolate and its roles in the defense mechanisms [43, 44]. Colonies of the slime-producing strains manifest characteristic blackening on CRA. Accordingly, biofilm-forming clinical isolates of staphylococci show either black or dark-red colonies with accompanying dry crystalline consistency, whereas non-biofilm producers might appear as dark colonies without crystalline formation [31, 45, 46]. The change in colony appearance might be attributed to side reaction between the slime production and Congo red, which ultimately yielded a distinctive coloration.

Although the inhibition of bacterial growth and biofilm development indicates that AgNPs possess a biocidal activity, the mechanisms that contribute to cell death is poorly understood. As anticipated, the cell membrane of *S. aureus* was deteriorated by the action of AgNPs, and it was clearly supported by the resulted protein content in the supernatant. The protein concentration was considerably high with the cells treated with AgNPs. It has previously been reported that AgNPs were able to adhere to the bacterial cell wall and produce cracks and pits, through which the internal cell contents are released [12]. Moreover, Prabhu and Poulouse, 2012, have stated that AgNPs are involved in the disruption of the membrane with a generation of reactive oxygen species (ROS), which ultimately lead to the death of pathogens [10].

Gel electrophoresis was performed to evaluate the action of AgNPs on the genome of *S. aureus*. Noticeably, AgNPs reacted with DNA of *S. aureus* since both chromosomal and plasmids DNA seem to be reduced or degraded enormously in the case of treated cells. Additionally, the RNA smearing was immensely decreased. AgNPs possibly prevented DNA replication via binding, and eventually, causing cell death. Chen *et al.* [44] have reported that silver nanoparticles not only condense DNA, but also combine and coagulate with the cytoplasm of damaged bacteria, which results in the leakage of the cytoplasmic component. AgNPs could cause

severe oxidative stress in *S. aureus* and might produce ROS leading to alteration of proteins, inhibition of enzymes, and nucleic acids reduction [9].

Based on the results obtained from the preliminary endeavor, and as noticed with the eukaryotic system, AgNPs exhibited cytotoxic effect against both HeLa and REF cell lines; however, HeLa cells were found to be more sensitive than REF cells. Based upon the results of the cytotoxicity assay, dose-response curves were extrapolated, and IC₅₀ was found to be around 20% v/v for HeLa cell lines. HeLa cells and other human cancer-derived cell lines are commonly employed in medical studies for assessing the efficacy of chemotherapies, anticancer agents, drug quality control and testing the expression of recombinant proteins [47, 48]. Kaba and Egorova [49] have indicated the significant toxicity of AgNPs toward the HeLa and U937 tumor cells. Consequently, AgNPs may prove to be a possible candidate for the creation of corresponding antitumor drugs. AgNPs caused mitochondrial dysfunction as well as induction of ROS thereby causing changes in membrane structure and inducing DNA damage, chromosomal aberrations, and enzyme inactivation, which eventually leads to cell death [50]. Moreover, Salaheldin *et al.* [13] have reported that human breast carcinoma cell line MCF7 cells featured remarkable vacuolation, thus indicating a potent cytotoxic activity by the action of AgNPs synthesized by *F. oxysporum*. Sambale *et al.* [50] have found that silver nanoparticles induced apoptosis, whereas silver ions stimulated necrosis in four different mammalian cell lines.

Our work has led us to conclude that this study is likely to be of great interest to the vision of researchers who are interested in nanomedicine disciplines. This study has demonstrated, for the first time, that *Fusarium mangiferae* is harnessed to synthesize nanoparticles. The study here provides an exciting opportunity for the mode of action of AgNPs to advance the knowledge of using AgNPs as an alternative to antibiotics. This paper's findings also shed light on the toxicity of AgNPs on cervical cancer (HeLa) and non-cancerous rat embryo fibroblast transformed cell lines which were found to be dose-dependent. These findings offer a foundation for future animal studies to confirm our results and assess whether *F. mangiferae*-AgNPs can generate cell death at rates similar to anticancer medications with or without side effects.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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