ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Bactericidal Activity of Silver Nanoparticles Produced by *Fusarium solani* against the Multidrug-Resistant Bacteria.

Basma H. Amin[1], and Reyad Mohamed El-Sharkawy[2]*.

[1]The Regional Centre for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.
[2]Botany and Microbiology Department, Faculty of Science, Benha University, Egypt.

ABSTRACT

The recent emergence of novel therapeutic agents replacing traditional antibiotics has received tremendous attention due to their unique efficiency against the quick developed multidrug-resistance pathogenic bacteria. In the present study, the biosynthesis of silver nanoparticles (AgNPs) by *Fusarium solani* and their antibacterial efficiency against different multidrug-resistant bacteria was investigated. The reducing capability of silver ions to AgNPs by *F. solani* was confirmed by UV–visible spectroscopy showing a surface plasmon resonance of 420 nm and Energy dispersive X-ray analysis. The biosynthetic nanosilver were monodispersed, spherical to oval shape, irregular in shape with an average molecular size of 6–15 nm and high negative charge as revealed from Transmission electron microscopy and Zeta analyses. The antibacterial effect of nanosilver was examined against the investigated multidrug resistant bacteria, *Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*. The developed AgNPs had a significant antibacterial activity on all tested bacterial isolates. The bactericidal effect of AgNPs was highly concentration-dependent and the minimum inhibitory concentration of AgNPs against MDR bacteria was 40 ppm. Notably, the antibacterial susceptibility of Gram-positive bacteria was higher when compared with that of Gram-negative bacteria as confirmed by the reduction in bacterial growth and the morphological changes occurred in bacterial cells ultrastructure. Thus, the fungus *F. solani* can be considered a potential bio-factory for the biosynthesis of AgNPs with a remarkable bactericidal activity against the examined multidrug resistance pathogenic bacteria.

Keywords: *Fusarium solani*; Silver nanoparticle; Multidrug-resistant bacteria; Antibacterial activity; Minimum inhibitory concentration; Bacterial cell ultrastructure.

https://doi.org/10.33887/rjpbcs/2019.10.6.25

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Multidrug-resistance (MDR) pathogenic bacteria are one of the worldwide crises in the latest years. They are widely distributed in hospital areas; represent a major health problem as it is associated with less susceptibility and more toxicity of broad-spectrum antibiotics. The rapid evolution of antibiotic resistance by various pathogenic bacteria necessitates development of novel antimicrobial agents with unique mode of action. Nanotechnology offers synthesis of different nanoparticles with a potential inhibitory effect against various microbial cells. Among the biosynthesized nanoparticles, Ag-nanoparticles are characterized by its low toxicity toward human cells, oligodynamic symmetry, exhibiting higher toxicity against viruses, bacteria and other eukaryotic microorganisms [1-5]. In addition, AgNPs have aptly been investigated for their antibacterial property because the silver nanoparticles have high surface area, which will lead to excellent antimicrobial activity as compared with Ag^+ metal [6, 7].

The biosynthetic ability of Ag-nanoparticles by plants, microorganisms (bacteria, fungi, yeast and actinomycetes) and enzymes has been reported as ecofriendly technique, alternative to chemical and physical synthesized AgNPs [8-10]. Fungal synthesis of AgNPs is either intracellularly or extracellularly and is preferable over bacteria due to high production of reducing enzymes, amino acids, peptides, organic acids and the facile recapture of nanoparticles [11-13].

Green synthesis of AgNPs has gained much attention due to its ability to reduce toxicity of AgNPs toward human cells through biomolecular capping of nanoparticle. Microorganisms have been reported to synthesis protein, amino acids, peptides, stabilizing agents, cytochromes and enzymes during the process of different metallic nanoparticles synthesis [12]. Diverse biomolecules obtained from living system perform several functions; capping protein, stabilizing cofactors, natural and reductive agent for Ag-nanoparticle, inhibiting their aggregation. Interestingly, a previous report shows that the synthesized AgNPs at low concentration has a significant therapeutic effect on more than 650 diseases causal organisms [14]. The antibacterial activity of biosynthesized nanoscale Ag^+ is significantly correlated to the shape and size of the AgNPs, depending upon the kind of organism and conditions employed in the biosynthetic processes. The nanoscale silver can perform its action through generation of reactive oxygen species, damaging DNA, mitochondrial dysfunction, drastic damage to cell wall, cell membrane and different cellular components. Therefore, AgNPs consider a promising nanoantibiotics since it can perform several medicinal applications [12, 15-16].

As the cytomorphological structure of Gram-positive bacteria and Gram-negative bacteria are much dissimilar, the acting mechanism of AgNPs on bacterial growth curve and cell ultrastructure may be different. Therefore, the present work was conducted to biosynthesize and characterized extracellular Ag^+ nanoparticles from *Fusarium solani*. This study has been extended to evaluate the effectiveness of AgNPs on the growth and ultrastructure of various multidrug-resistant bacteria; *Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*.

MATERIALS AND METHODS

Microorganisms and Chemicals

Fusarium solani and Multi-drug resistance (MDR) pathogenic bacteria were friendly provided from the Culture Collection and Identification Unit at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The fungal pure culture was grown and maintained on potato dextrose agar (PDA) for 5 days at 28 °C. MDR bacteria including *Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* and *Klebsiella pneumonia* were cultured on nutrient agar medium. Silver nitrate was purchased from Sigma-Aldrich.

Extracellular synthesis of Ag-nanoparticles

Fungal spore suspension (10^5 spores/ml) was inoculated into PDA and incubated with shaking (150 rpm) at room temperature for 5 days. The mycelial-free filtrate (MFF) was obtained through filtration via Whatman filter paper No. 1 (Whatman, Piscataway, NJ, USA). $AgNO_3$ solution (1 mM, prepared in deionized water) was added to the obtained MFF and was incubated for 3 days at 28 °C with shaking at 150 rpm in dark

condition. Color changes were used as an indication for the biosynthesized Ag-nanoparticles in the reaction mixtures, comparing to control of $AgNO_3$ [17].

Characterization of biosynthesized Ag-nanoparticles

The developed nanoscale silver synthesized by *F. solani* was assessed by a double beam spectrophotometer (UV/visible, Cary 100, Varian) at 200 to 700 nm, [18]. The presence of Ag^+ in the biosynthesized nanoparticles was affirmed by Energy dispersive X-ray (EDX) analysis. The EDX analysis was performed via X-ray micro-analyzer (Oxford 6587 INCA) which attached to scanning electron microscope (JEOL JSM-5500 LV) at 20 kV. The EDX spectrum was detected from the densely characterized nanosilver region via the spot mode method. Quanta 200 FEG was used for the nanosilver crystallites analysis [19]. The Ag-nanoparticles size and charge were detected by Zetasizer Nano S90 (Malvern Instruments Ltd., U. K.) [20]. The biosynthesized AgNPs size and morphology were determined by Transmission electron microscope (TEM, JEOL GEM-1010 transmission electron microscope at 70 kV) at the Regional Center for Mycology and Biotechnology, Egypt. A drop containing AgNPs was deposited onto carbon-coated copper grids (CCG) and then exposed to the infra light for 30 min. The micrograph was analyzed by JEOL - JEM 1010 - Transmission Electron Microscope at 70 kV in the RCMB, Al-Azhar University.

Antibacterial activity of synthesized Ag-nanoparticles

The antibacterial effect of synthesized Ag-nanoparticles was evaluated against multi-drug resistant bacteria by the disk diffusion method. MDR bacterial suspensions (50 µl, 10^5 cfu/ml) of overnight culture were seeded into Luria-Bertani (LB) medium, shake well and poured into sterilized Petri-dishes. Prior to use, different concentrations of aqueous AgNPs (5, 10, 20, 40, 80, 100 ppm, 20 µl) was loaded on filter paper discs and placed onto LB agar plates surface [21]. The sizes of the inhibition zones were assayed after incubation for 24 h at 37 °C.

In order to observe the influence of Ag-nanoparticles on the bacterial growth of different MDR pathogenic bacteria, 7 ml of LB broth was inoculated with 500 µL of *Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*. Different concentrations of synthesized AgNPs (5-100 ppm, 100 µl) was added to the bacterial culture and then incubated at 37 °C for 24 h. At 600 nm wavelength, optical density (OD) was recorded at regular time intervals (i.e. 2, 4, 6, 8, 10, 12, 24 h postinoculation). Untreated control of the same volume of bacterial broth without AgNPs was used. Broth medium alone or with corresponding nanosilver solutions were used as blank to determine the turbidity appearing as a result of bacterial growth [22].

Effect of Ag-nanoparticles on bacterial cellular ultrastructure

For evaluating the morphological changes occurred by AgNPs, the tested multi-drug resistant bacteria were treated with Ag-nanoparticles and the cellular alterations were observed through TEM. MDR pathogenic bacteria (10^5 $CFUml^{-1}$) were treated or not with synthesized AgNPs at 20 nm (sub-MIC dose). The experiments were conducted at 37 °C for 6 h and 150 rpm. The cultures were centrifuged for 30 min at 300 × g and the pellets were washed with phosphate buffered saline. Samples for TEM were fixed in 2.5 % glutaraldehyde, pH 7.4. The samples were post-fixed for 5 min in potassium permanganate solution at room temperature. Subsequently, the samples were serially dehydrated using ethanol (50 to 100 %) and passed for 10 min in acetone: ethanol (1: 2, 1: 1 and 2: 0, v/v), each at room temperature and embedded in epoxy resin capsule. Ultrathin sections (~70 nm) were stained by uranyl acetate (5%) and lead citrate (0.5%) before observation in JEOL- JEM microscope [23].

Statistical Analysis

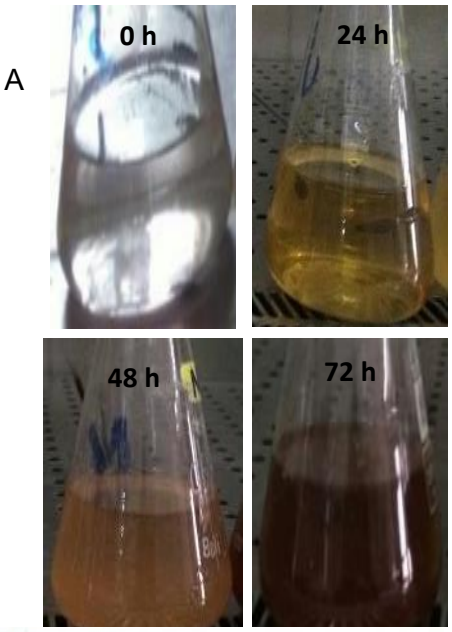
The results were expressed as the mean values of three replicates with the standard deviation (s.d.).

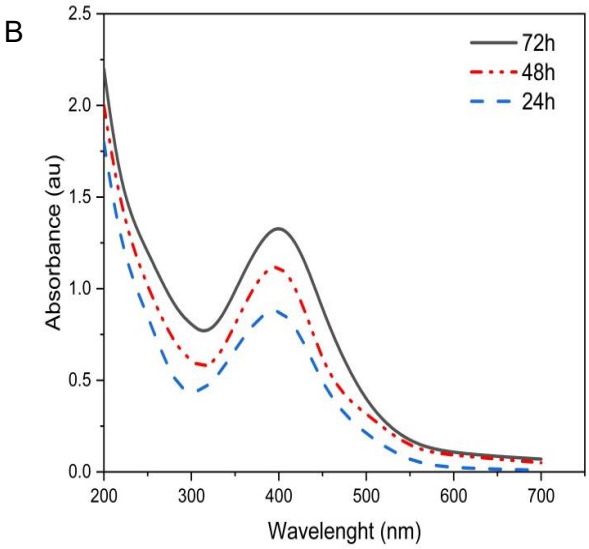
ISSN: 0975-8585

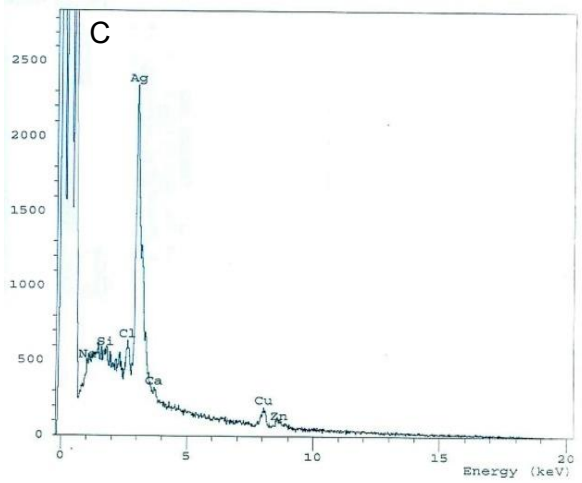
RESULTS AND DISCUSSION

Characterization of Ag-nanoparticles synthesized by *Fusarium solani*

The reaction mixture was gradually changed to brown color during the biosynthetic process of silver nanoparticles by *F. solani* (Figure 1A), indicating the role of fungal filtrate in nanoparticle synthesis. The absence of agglomeration and precipitation affirmed the stability and homogeneity of the synthesized AgNPs, showing the existence of stabilizing agent. The whole reduction of Ag^+ ions to AgNPs was detected after 3 days of incubation (Data not shown). The colloidal Ag-nanoparticles synthesized by *F. solani* were detected by UV-Visible spectroscopy (Figure 1B), showing a characteristic surface plasmon resonance (SPR) band of 420 nm. The biosynthetic nanosilver showed a well characterized absorption peak at 420 nm when the fungal filtrate of *Fusarium solani* was mixed with $AgNO_3$ [24]. The characteristic SPR band of AgNPs may be due to a collective oscillation of free conduction electron [25]. The biosynthetic capability of AgNPs by fungal mycelial extract is attributed to the existence of different enzymes secreted by fungus in the mycelial-free filtrate which delivers a mechanism for Ag^+ ions reduction [26]. In UV–visible spectra, the existence of a single SPR band is a good evidence for production of isotropic nanoparticles [27].







Element	Weight %	Atomic %
Na	16.60	44.00
Si	0.98	2.12
Cl	2.12	3.65
Ca	1.13	1.72
Cu	6.24	5.98
Zn	3.67	3.42
Ag	69.26	39.11
Total	100.00	

ISSN: 0975-8585

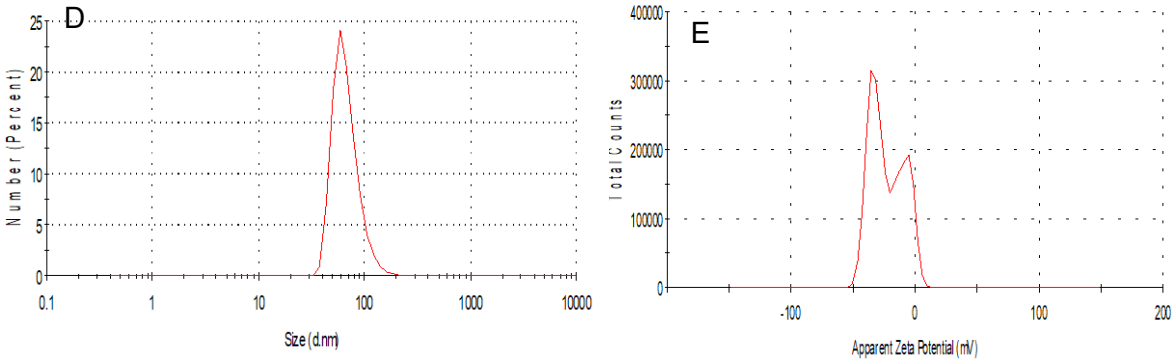


Figure (1): Colloidal Ag-nanoparticles synthesized by *Fusarium solani* were detected by (A) Color change of the fungal filtrate amended with 1 mM $AgNO_3$ solution after 24h, 48h and 72h of incubation, comparing to control of $AgNO_3$ solution, (B) UV-Vis spectrum, (C) EDX spectrum, (D) size distribution and (E) zeta potential.

The reduction of Ag^+ to nanocrystalline silver by fungal filtrate of *F. solani* was affirmed by the presence of a characteristic peak at 3 keV, which is corresponding to the SPR (Figure 1C). The weight percentage of Ag^+ was detected as 69.26%. The formation of nanoscale silver by various fungal isolates was determined by EDX [18, 19]. The nanometer size and zeta potential of bio-AgNPs was detected by Zetasizer Nano S90 (Figure 1D, E). The histograms of Ag-nanoparticles showed two zeta peaks of -32.4 mV and -6.13 mV. The high negative charge of bio-AgNPs confirms the stability and repulsion of biosynthesized nanoscale silver synthesized by *F. solani*. The higher anionic strength of biosynthesized AgNPs is necessary for nanoparticles stability, cellular membranes permeation and different biological application [28, 29].

The size and morphology of the mycosynthesized nanosilver were determined from TEM analysis (Figure 2). The micrograph exhibited a well dispersed, spherical to oval shape without agglomeration. The average molecular size of the biosynthetic nanosilver was in the range from 6 to 15 nm. *Penicillium expansum* has ability to synthesize a well-distributed AgNPs with average diameter of 14-25 nm, while, *Aspergillus terreus* produced AgNPs with average diameter of 10-18 nm [30]. The average diameter of the developed AgNPs obtained from zetasizer was inconsistent with that determined from TEM micrograph. This variation in the average diameter of colloidal AgNPs has been attributed to the existence of hydrated and capping protein surrounding nanosilver crystal surface, facilitating attachment and transfer of Ag^+ ions into bacterial cells [17, 31].

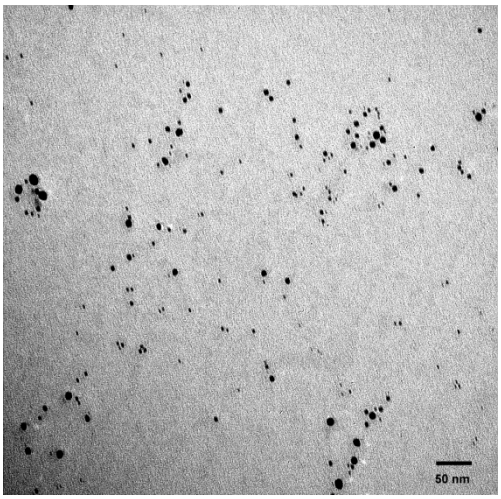


Figure (2): Transmission Electron Microscope (TEM) micrographs of the biosynthesized AgNPs obtained from *F. solani* (scale bar equivalent to 50 nm).

ISSN: 0975-8585

Antibacterial activity of biosynthesized AgNPs on MDR bacteria using the disk diffusion method

The antibacterial effect of biosynthesized AgNPs from *F. solani* was investigated against different MDR pathogenic bacteria using the disk diffusion method. Different concentrations of nanosilver solution were investigated and the diameter of inhibition zones was shown in Figure 3. The synthesized AgNPs was effective against all the tested MDR pathogenic bacterial strains. The relative inhibitory effect of the bioactive AgNPs on the bacterial growth was significantly increased with rising concentration of AgNPs. Gram-positive bacteria reveal more susceptibility toward nanosilver particles than Gram-negative bacteria.

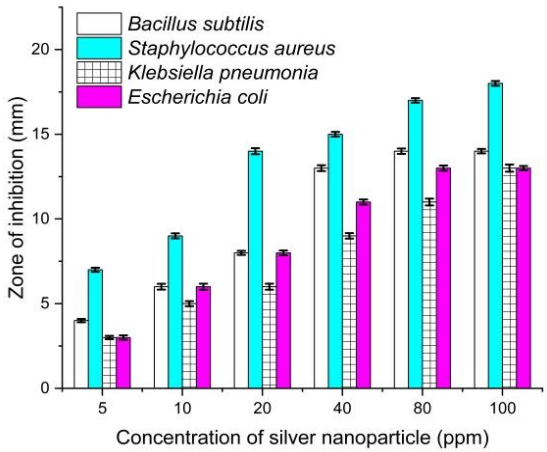


Figure (3): Antibacterial activity of different silver nanoparticles concentrations against various MDR bacterial strains.

Effect of various Ag-nanoparticles concentrations on bacterial growth

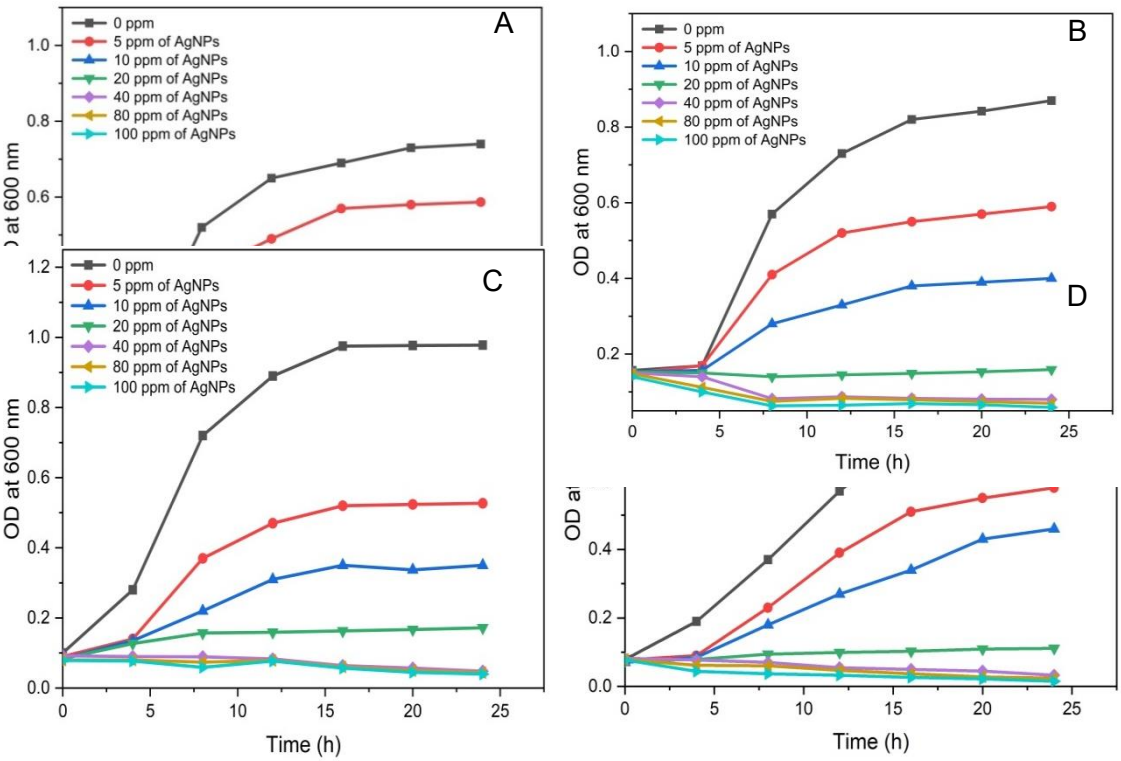


Figure (4): Growth of *B. subtilis* (A), *S. aureus* (B), *K. pneumonia* (C) and *E. coli* (D) cells subjected to various concentrations of nanosilver synthesized by *F. solani*.

The bacterial growth analysis was used to evaluate the antibacterial effect of AgNPs synthesized by mycelial free filtrate of *F. solani*. Bacterial culture of the tested MDR pathogenic bacteria displayed typical

bacterial growth. However, the existence of various concentrations of nanosilver (5-100 ppm) obviously suppresed the growth and reproduction of bacterial cells. The bacterial denisty of different MDR pathogens exhibited a significant reduction corresponding to each concentration of AgNPs as compared to the control growth profile. This may be arising from the antimicrobial activity of nanosilver which causes a significant reduction in the bacterial growth. From the obtained data (Figure 4), *B. subtilis* and *S. aureus* treated with 40-100 ppm AgNPs were almost dead after 8 h, while, *K. pneumonia* and *E. coli* treated with 40-100 ppm AgNPs were almost dead after at 12 h. These results indicates a faster growth inhibition of Gram-positive bacteria over Gram-negative bacteria. The lowest concentration that fully inhibited visible bacterial growth (MIC) was detected according to [32]. Interesingly, the MIC of AgNPs against the investigated multidrug resistance pathogenic bacteria was 40 ppm.

Effect of different Ag-nanoparticles concentrations on the MDR bacterial cellular ultrastructure

The interaction between biosynthesized AgNPs and multi-drug resistance pathogenic bacterial cells was visualized by TEM comparing to control without silver nanoparticle. Depending on the degree of bacterial cell damage, biosynthesized silver nanoparticles are more toxic toward Gram-positive bacteria than Gram-negative bacteria in the following sequence: *Staphylococcus aureus* > *Bacillus subtilis* > *Escherichia coli* > *Klebsiella pneumonia*. The untreated bacterial cells in the supernatant exhibited a well-preserved cellular ultrastructure with intact surface, homogeneous and electron-dense cytoplasm, continuous cytoplasmic membrane, intact cell wall and capsule (Figure 5 A-D). By contrast, the treated MDR bacterial cells with biosynthesized AgNPs exhibited significant changes in bacterial morphology. The most notably deformations was a disrupted cell wall (appears as membrane corrugations with some blisters on the surface), completely damaged cell membrane and deformation of intracellular structure are presented in Figure 5 E-H. The leakage of intracellular contents is attributable to the distortion and dysfunction of the cell membrane. Interestingly, the nucleoside part of the nucleotide was observed as a breaking thread.

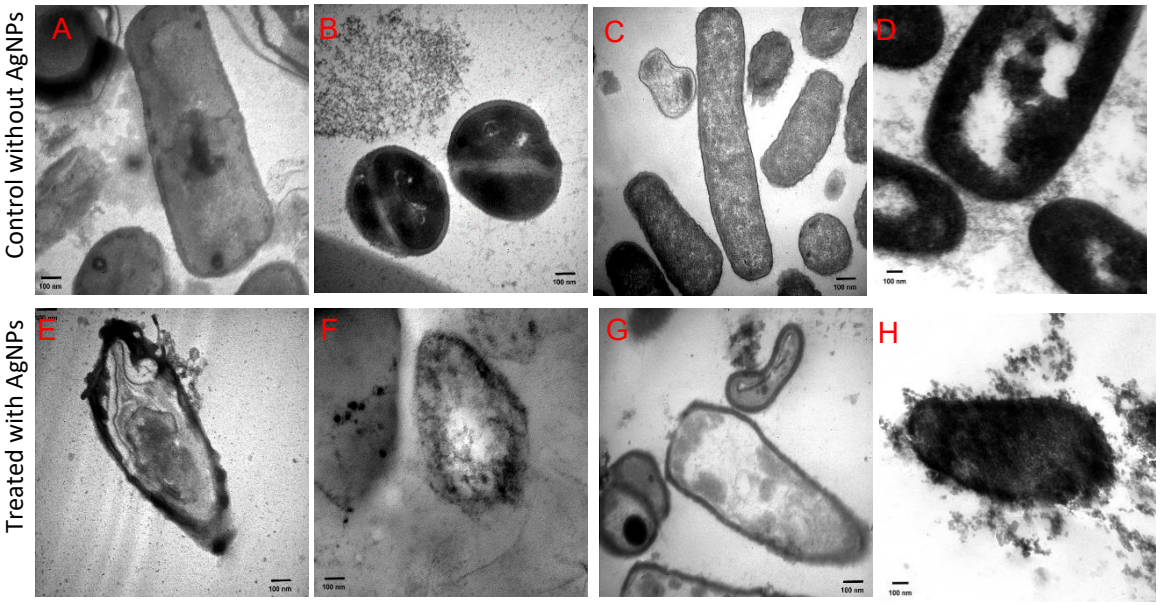


Figure (5): Morphological alterations of *Bacillus subtilis* (A, E), *Staphylococcus aureus* (B, F), *Escherichia coli* (C, G) and *Klebsiella pneumonia* (D, H) treated with silver nanoparticles (20 nm) at 37ºC for 6 h. Untreated bacterial cells (upper panel) showed a well preserved cell wall, cytoplasmic membrane and homogeneous cytoplasm. Treated bacterial cells (lower panel) with biosynthesized AgNPs had severed damaged cytoplasmic membrane and cell wall (B). Scale bar equivalent to 100 nm in all micrographs.

The antibacterial effect of nanosilver on *E. coli* was reported by [33]. The silver nanoparticle showed a significant antibacterial effect against *S. aureus* [34] and *B. subtilis* [22]. The damaged influence of biosynthetic silver ions on different multidrug resistant bacteria [35, 36]. In contrast with our results, [37] mentioned that Gram-positive bacteria are slightly tolerance to nanosilver than Gram-negative bacteria. The increasing antibacterial activity of nanosilver was possibly attributable to the deleterious impact of Ag^{+} ions on cell wall, electron transport chain, DNA, protein and lipids. The elevated generation of intracelluer reactive oxygen

species (ROS) and free radicles mediated by AgNPs resulted in a programming cell death [5, 38]. In addition, Antimicrobial activity of AgNPs may attribute to their physical properties, sizes and crystallographic structure as reported by [39].

CONCLUSIONS

Fusarium solani has ability to synthesis highly stable, monodispersed Ag-nanoparticles with various particle sizes. The biosynthetic AgNPs showed a significant antibacterial activity against various multi-drug resistant bacteria, *B. subtilis*, methicillin-resistant *S. aureus*, *K. pneumonia* and *E. coli*. MDR bacterial cells exposed to AgNPs were substantially damaged as affirmed by examining the bacterial cell ultrastructure. Thus, *F. solani* offers a desired fungal nano factory for sustainable production of silver nanoparticles that can control the investigated multidrug resistance pathogenic bacteria.

REFERENCES

[1] Nasreen IH and Taranath TC. Biosynthesis of nanoparticles using microbes—A review. Colloids and Surfaces B: Biointerfaces 2014, 121: 474–483.

[2] Singh D, Vandana R, Shivaraj N, Jyothi H, Singh K, Mathew J. Optimization and characterization of silver nanoparticle by endophytic fungi *Penicillium* sp. isolated from *Curcuma longa* (Turmeric) and application studies against MDR *E. coli* and *S. aureus*. Bioinorganic Chemistry and Application 2014; Article ID 408021, 8 pages.

[3] Ouay B and Stellacci F. Antibacterial activity of silver nanoparticles: a surface science insight. Nano Today 2015; 10: 339–354.

[4] Patra JK, Baek KH. Antibacterial Activity and Synergistic Antibacterial Potential of Biosynthesized Silver Nanoparticles against Foodborne Pathogenic Bacteria along with its Anticandidal and Antioxidant Effects. Frontiers Microbiology 2017; 8:167.

[5] Roy A, Bulut O, Some S, Mandal AK, Yilmaz MD. Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. RSC Advances 2019; 9: 2673-2702.

[6] Panacek A, Kvítek L, Prucek R, Kolar M, Vecerova R, Pizúrova N. Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. Journal of Physical Chemistry B. 2006; 110:16248-53.

[7] Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances 2009; 27(1):76-83.

[8] Mohanpuria P, Rana N, Yadav S. Biosynthesis of nanoparticles: Technological concepts and future applications. Journal of Nanoparticle Research 2008; 10(3): 507-517.

[9] Verma VC, Kharwar RN, Gange AC. Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. Nanomedicine 2010; 5: 33-40.

[10] Kashyap PL, Kumar S, Srivastava AK, Sharma AK. Myconanotechnology in agriculture: a perspective. W0rld Journal of Microbiology and Biotechnology 2013; 29:191–207.

[11] Musarrat, J., Dwivedi, S., Singh, B.R., Al-Khedhairy, A.A., Azam, A. and Naqvi, A. Production of antimicrobial silver nanoparticles in water extracts of the fungus *Amylomyces rouxii* strain KSU-09. Bioresearch Technology 2010; 101: 8772–8776.

[12] Singh R, Shedbalkar U, Wadhwani, A, Chopade A. Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. Appl. Microbiol. Biotechnol. 2015; 99: 4579-4593.

[13] Barkhade T. Extracellular biosynthesis of silver nanoparticles using *Penicillium* species. Inter. Journal of Research – Granthaalayah 2018; 6(1): 277-283.

[14] Jeong SH, Hwnag YH, Yi SC. Antibacterial properties of padded PP/PE nonwovens incorporating nano-sized silver colloids. Journal of Material Science 2005; 40:5413–5418

[15] Hulkoti NI, Taranath TC. Biosynthesis of nanoparticles using microbes—A review. Colloids and Surfaces B: Biointerfaces 2014;121: 474–483.

[16] Hu X, Saravankumar K, Jin T, Wang M. Mycosynthesis, characterization, anticancer and antibacterial activity of silver nanoparticles from endophytic fungus *Talaromyces purpureogenus*. International Journal of Nanomedicine 2019; 3427-3438

[17] Rodriguez PL, Harada T, Christian DA, Patano DA, Tsai RK, Discher DE. Minimal 'self' peptides that inhibit phagocytic clearance and enhance delivery of nanoparticles. Science 2013; 339:971.

[18] Hamedi S, Shojaosadati S, Shokrollahzadeh S, Hashemi-Najafabadi S. Extracellular biosynthesis of silver nanoparticles using a novel and non-pathogenic fungus, *Neurospora intermedia*: controlled synthesis and antibacterial activity. World Journal of Microbiolgy and Biology 2014; 30: 693–704.

[19] Ghosh S, Patil S, Ahire M, Kitture R, Kale S, Pardesi K, Cameotra S, Belleare J, Dhavale D, Chopade BA. Synthesis of silver nanoparticles using *Dioscorea bulbifera* tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. International Journal Nanomedicine 2012; 7: 483–496.
[20] Kheybari S, Samadi N, Hosseini S, Fazeli A, Fazeli MR. Synthesis and 16 antimicrobial effects of silver nanoparticles produced by chemical reduction method. Daru : Journal of Faculty of Pharmacy, Tehran University of Medical Sciences 2010; 18: 168–72.
[21] Sarkar S, Atish D, Samir K, Golam M. Facile synthesis of silver nano particles with highly efficient anti-microbial property. Polyhedron 2007; 26: 4419–4426.
[22] Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomaterial 2008; 4**:** 707–716.
[23] Amin BH. Isolation and characterization of antiprotozoal and antimicrobial metabolite from *Penicillium roqueforti.* African Journal of Mycology and Biotechnology 2016; 21(3): 13-26.
[24] Vijayan R, Joseph S, Mathew B. Green synthesis of silver nanoparticles using *Nervalia zeylanica* leaf extract and evaluation of their antioxidant, catalytic, and antimicrobial potentials. Particulate Science and Technology, 2018, 1-11.
[25] Shukla MK, Singh RP, Reddy CRK, Jha B. Synthesis and characterization of agar-based silver nanoparticles and nanocomposite film with antibacterial applications. Bioresource Technology 2012; 107: 295–300.
[26] Bhainsa, K.C. and D'souza, S. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Colloids and Surfaces B: Biointerfaces 2006; 47(2): 160-164.
[27] Sadhasivam S, Shanmugam P, Yun K. Biosynthesis of silver nanoparticles by *Streptomyces hygroscopicus* and antimicrobial activity against medically important pathogenic microorganisms. Colloids and Surfaces B: Biointerfaces 2010; 81:358–362.
[28] Gengan RM, Anand K, Phulukdaree A, Chuturgoon A. A549 lung cell line activity of biosynthesized silver nanoparticles using *Albizia adianthifolia* leaf. Colloids and Surfaces B: Biointerfaces 2013; 105: 87–91.
[29] Aiad I, El-Sukkary M, Soliman EA, El-Awady MY, Shaban SM. Evaluation of Some Cationic Surfactants Based on Dimethylaminopropylamine as Corrosion Inhibitors. Journal of Industreial and Engineering Chemistry 2014; 21: 1029-1038.
[30] Ammar HA, El-Desouky TA. Green. synthesis of nanosilver particles by *Aspergillus terreus* HA1N and *Penicillium expansum* HA2N and its antifungal activity against mycotoxigenic fungi. Journal of Applied Microbiology 2016; 121: 89-100.
[31] Hu CMJ, Zhang L, Aryal S, Cheung C, Fang RH, Zhang L. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. *PNAS* 2011; 108:10980–10985.
[32] Maiti S, Krishnan D, Barman G, Ghosh S and Laha J. Antimicrobial activities of silver nanoparticles synthesized from *Lycopersicon esculentum* extract. Journal of analytical science and technology. 2014, 5(40): 1-7.
[33] Husseiny S, Taher AS, Hend AA. Biosynthesis of size controlled silver nanoparticles by *Fusarium oxysporum*, their antibacterial and antitumor activities. beni-suef uni. Journal of basic and applied Science 2015; 4: 225–231.
[34] Li WR, Xie XB, Shi QS, Duan SS, Ouyang YS, Chen YB. Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*. *Biometals* 2011; 24:135–141.
[35] Wigginton N, de Titta A, Piccapietra F, Dobias J, Nesatyy V, Suter M, Bernier-Latmani R. Binding of silver nanoparticles to bacterial proteins depends on surface modifications and inhibits enzymatic activity. Environmental Science and Technology 44(6); 2163-2168.
[36] Roy P, Das B, Mohanty A, Mohapatra S. Green synthesis of silver nanoparticles using Azadirachta indica leaf extract and its antimicrobial study. Applied Nanoscience 2017; 7(8): 843–850.
[37] Beddow J, Stolpe B, Cole P, Lead JR, Sapp M, Lyons BP, Colbeck I, Whitby C. Effects of engineered silver nanoparticles on the growth and activity of ecologically important microbes. Environmental Microbiology Reports 2014; 6(5): 448–45.
[38] Li WR, Xie XB, Shi QS, Zeng HY, Ou-Yang YS, Chen YB. Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. Applied Microbiology and Biotechnology 2010; 85**:**1115–1122.
[39] Singh, T., Kumari, J., Patnaik, A., Singh, A., Chauhan, R. and Chandel, S. Biosynthesis, characterization and antibacterial activity of silver nanoparticles using an endophytic fungal supernatant of *Raphanus sativus*. Journal of Genetic Engineering and Biotechnology 2017; 15: 31–39.