

Study of Biosynthesis silver nanoparticles by *Fusarium graminearum* and test their antimicrobial activity

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ABSTRACT: Silver nanoparticles (Ag-NPs) were extracellularly biosynthesized using the mold *Fusarium graminearum* that isolated from poultry feed, the fungal isolates were inoculated in a broth medium incubated in a shaker incubator at 25 °C for 8 days, metal nanoparticles were synthesized by treating mycelia (1%w/v) with (1mM, 0.5Mm) of metals oxide solution and incubated in a shaker incubator at 25 °C for 3 days. Many techniques have been used for characterize metal oxides nanoparticle, UV–VIS Spectroscopic Analysis, X-ray Diffraction Analysis (XRD), Atomic Force Microscopy (AFM) and Scanning electron microscope (SEM), which were done at Nanotechnology Center in UOT in Iraq. X-ray Diffraction (XRD) was used to identify these NPs. The nanoparticles exhibited maximum absorbance peak at 440 nm in UV–Vis spectroscopy. From the XRD pattern of Ag-NPs exhibited $2\theta \sim 38.2^\circ$ values, corresponding to the silver Ag (111) crystalline phase indexed. The NPs surface morphology revealed from SEM and AFM images shows formation of well-dispersed Ag-NPs with diameter between one to 95.5 nm and the average of the NPs diameter was (45.5 nm), and the presence of silver was confirmed. It has antimicrobial activity with the most effective concentration (50 µg/µl) against *Pseudomonas aeruginosa*, *Salmonella* sp. *Candida albicans* and (40 µg/µl) against *E.coli*.

KEYWORDS: Biosynthesis, silver nanoparticles, *Fusarium graminearum*, antimicrobial activity.

INTRODUCTION

Throughout human history, fungi have been utilized as a source of food and harnessed to ferment and preserve foods and beverages. In the 20th century, humans have learned to harness fungi to protect human health (antibiotics, anti-cholesterol statins, and immunosuppressive agents), while industry has utilized fungi for large scale production of enzymes, acids, and biosurfactants (Jump & Barredo, 2005). With the advent of modern nanotechnology in the 1980s, fungi have remained important by providing a greener alternative to chemically synthesized nanoparticles. A nanoparticle is a particle having one or more dimensions of the order of 100 nm or less (Paull *et al.*, 2003). Current research has shown that microorganisms, plant extracts, and fungi can produce nanoparticles through biological pathways. (Abou El-Nour *et al.*, 2010; Popescu, *et al.*, 2010; Ghorbani *et al.*, 2011). The most common nanoparticles synthesized by fungi are silver and gold, however fungi have been utilized in the synthesis of other types of nanoparticles including zinc oxide, platinum, magnetite, zirconia, silica, titanium, and cadmium sulfide and cadmium selenide quantum dots. In addition, the extracellular biosynthesis using fungi could also make downstream processing much easier than bacteria, interesting example of NPs biosynthesis using fungi was that the cell-associated biosynthesis of silver using *Fusarium oxysporum* was demonstrated by Ahmad *et al.* (2003). There also have been several reports on the biosynthesis of metal nanoparticles using fungi, including *Fusarium acuminatum* (Bharde *et al.*, 2006), *Fusarium semitectum* (Basavaraja *et al.*, 2008) and *Verticillium* spp. (Mukherjee *et al.*, 2001a). The aims of the present study of biosynthesis metal oxide nanoparticles from *Fusarium graminearum* and test the ability of nanoparticles to inhibit some microorganisms.

MATERIAL AND METHODS

BIOSYNTHESIS OF METAL OXIDE NANOPARTICLES

Five fungal isolates of *Fusarium graminearum* were obtained from Mustansiriyah university / College of Science / department of Biology isolated from poultry feed were grown on Potato dextrose Agar medium (PDA) then used in present study to test their ability to biosynthesize of silver nitrate $\text{Ag}(\text{NO}_3)_2$ nanoparticles .

PRODUCTION OF BIOMASS

The preparation of biomass for biosynthesis of silver nitrate $\text{Ag}(\text{NO}_3)_2$ nanoparticles from the fungi involved the aerobic culturing of fungi in a liquid broth composed of KH_2PO_4 (7.0g/l), K_2HPO_4 (2.0g/l) , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1g/l), $(\text{NH}_4)_2\text{SO}_4$ (1.0g/l) yeast extract, (0.6g/l) and glucose (10g/l). The pH of the media was adjusted to 5.6 ± 0.2 . The flasks containing this culture were incubated at 25°C for 8 days (Sangappa and Padma, 2012).

NANOPARTICLE SYNTHESIS

The harvested mycelia and culture broth were separated by centrifugation at 4500 rpm for 15 min. The mycelia pellet was washed thrice with deionized water. The washed mycelia (1%w/v) were treated with (0.5 ,1,1.5 and 2)mM of silver nitrate $\text{Ag}(\text{NO}_3)_2$. then after incubated at 25°C in darkness at 200 rpm for 3 days. A control experiment containing only 1mM of silver nitrate solution was also performed. All experiments were carried out in triplicates and samples were drawn everyday throughout the days of incubation (Chan and Don, 2012). The biosynthesis was confirmed by UV–VIS Spectroscopic Analysis, X-ray Diffraction Analysis (X-RD) , Atomic Force Microscopy (AFM) and Scanning electron microscope (SEM), which were done at Nanotechnology Center in UOT in Iraq with ethical approval from our department in college of science/ Mustansiriyah University.

UV–VIS SPECTROSCOPIC ANALYSIS

In order to study the formation of silver nitrate nanoparticles, the UV–VIS absorption spectra of the prepared colloidal solutions were recorded using a spectrophotometer against deionized water and fungi mycelium without any addition as blank (Dadosh, 2009).The bioreduction of the Ag NPs solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component after 20 times dilution and measuring the UV–VIS spectrum of the solution at 24 hrs . UV–VIS spectra of these aliquots were monitored as a function of time of reaction on a Shimadzu 1601 spectrophotometer in 200–700 nm range operated at a resolution of 1 nm (Jayaseelan *et al.*, 2012) .

X-RAY DIFFRACTION ANALYSIS (X-RD)

The formation of AgNO_3 NPs was checked by XRD technique using an X-ray diffractometer with $\text{Cu K } \alpha$ radiation ($\lambda = 0.1540 \text{ nm}$), employing a scanning rate of $0.02^\circ \text{ s}^{-1}$ and 2θ ranges from 10° to 80° for silver nitrate $\text{Ag}(\text{NO}_3)_2$. The XY (2θ vs. intensity) data obtained from this experiment were plotted with the Win PLOT program and the angular positions of the peaks were obtained with the same program (Senapati *et al.*, 2005) . The dimensions of the unit cell, hkl values and space group of silver nitrate $\text{Ag}(\text{NO}_3)_2$ nanoparticles were obtained using the DICVOL program in the FullProf 2000 software package and then refinement was carried out through the profile matching routine of FullProf. The Bragg peaks were modeled with pseudo-Voigt function and the background was estimated by linear interpolation between selected background points. The crystallite size (D) and the lattice strain of AgNO_3 NPs were estimated by analyzing the broadening of X-ray diffraction peaks, using Williamson-Hall approach (Prasad and Jha, 2009).

ATOMIC FORCE MICROSCOPY (AFM)

A thin film of the sample was prepared on a glass slide by dropping 100 μl of the sample on the slide, and was allowed to dry for 5 min. The slides were then scanned with the AFM apparatus (Naveen *et al.*, 2010).

SCANNING ELECTRON MICROSCOPE (SEM)

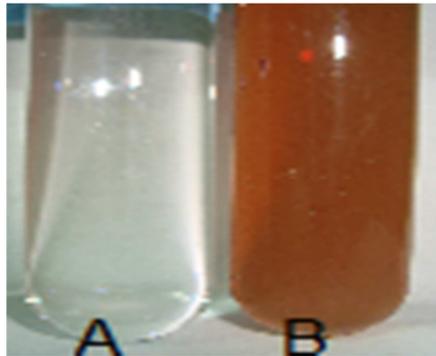
Fungal biomass before and after the formation of AgNO₃ NPs was examined by scanning electron microscope (SEM). Analyzed samples were dried at room conditions for 5 days and small fragments were placed on pin stubs and then coated with carbon under vacuum (Castro-Longoria *et al.*, 2011).

RESULTS AND DISCUSSION

The results of test the ability of five isolates of fungi *Fusarium graminearum* for biosynthesis of Ag- nanoparticles were varied, then chose the most ability of biosynthesizing Ag-NPs in present study, the reason may be due not all fungi isolates secrete the same enzymes that needed for synthesizing NPs even in the same species Ahmad *et al.* (2003).

After 24hrs of the reaction between *Fusarium graminearum* biomass and aqueous solution of Ag(NO₃)₂ led to change the color of the mixture to yellowish brown this is indication of silver nanoparticles formation. The change of the color from pale yellow to yellowish brown due to the excitation of surface Plasmon vibrations in the silver nanoparticles. Control without showed no change in color under the same condition (picture 1).

The fungal cell filtrate after addition of aqueous Ag NO₃(1 mM) was subjected to optical measurements by UV-Vis spectrophotometer analysis showed an absorbance shape peak at 440 nm Figure (1). The fungus was which belongs to the specific for the Ag-NPs. secreted the NADH-dependent nitrate reductase enzyme extracellularly for the reduction Of silver ions in order to synthesise of Ag-NPs (Feng *et al.*, 2000 ; Song *et al.*, 2006).



Picture 1. (A) The crude cell filtrate of *Fusarium graminearum* without Ag(NO₃)₂, (B) with Ag(NO₃)₂ after 24 h

UV-Vis spectroscopy was used to examine size and shape controlled nanoparticles in aqueous suspensions (Yamanaka *et al.*, 2005). The UV-Vis spectra recorded from *Fusarium graminearum* reaction vessel at different reaction times are shown in figure 1. The time at which the aliquots were removed for measurement is indicated next to the respective curves in figure 1. which showed the increase in intensity of silver solution with time indicating the formation of an increased number of silver nanoparticles in the solution. From the figure 1, there was appreciable change in the net magnitude of UV-Vis absorbance of the reaction.

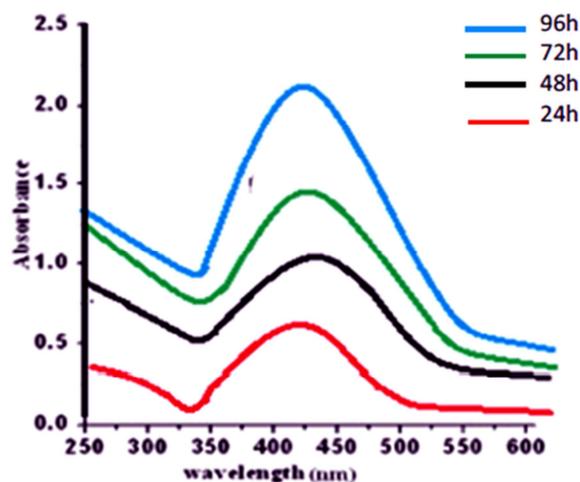


Figure (1): UV-Visible spectroscopy of silver nanoparticles with peak at 440 nm

Under observation The XRD pattern, thus, clearly shows that the Ag-NPs were essentially crystalline. The intensity of the diffraction was much stronger than those of the other diffractions. The XRD diffraction measured in this case resulted in four intense peaks shown in figure 2. Thus, it agrees the Bragg’s reflection of silver nanocrystals . This further confirms that the Ag-NPs formed in the extracellular filtrate are present in the form silver nanocrystals. This further confirms that the Ag-NPs formed in the extracellular filtrate are present in the form silver nanocrystals.

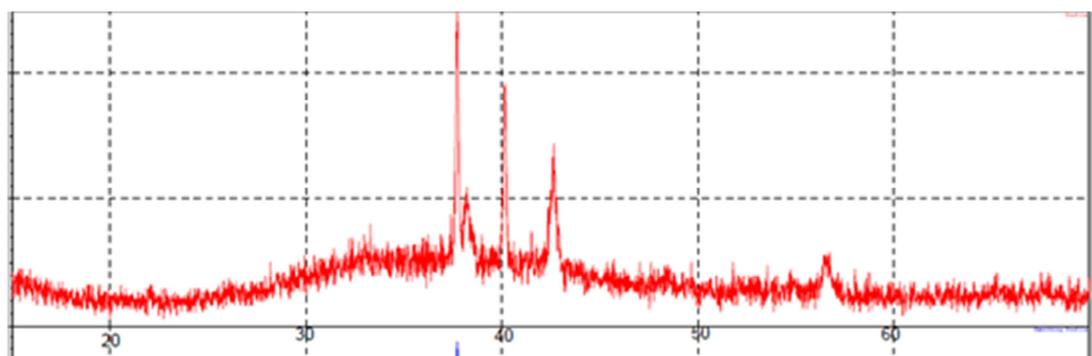


Figure (2): X-ray diffraction patterns of biosynthesized Ag NPs

Atomic force microscopy (AFM) is a very high-resolution type of scanning probe microscopy, with demonstrated resolution on the order of fractions of a nanometer, more than 1000 times better than the optical diffraction limit. The AFM is one of the foremost tools for imaging, measuring, and manipulating matter at the nanoscale (Langfield *et al.*, 2004) .Silver nanoparticles were characterized by AFM for its detail size, morphology and agglomeration of Ag(NO3)2. Figure(3) shows 3D image for Ag NPs and the maximum tip height is (95.5nm).

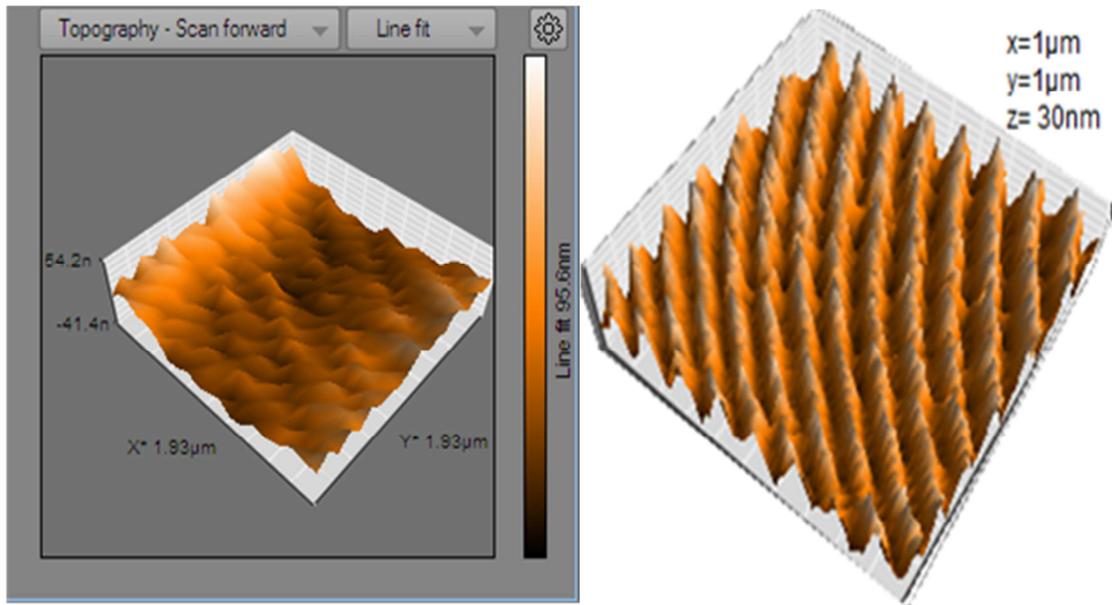


Figure (3): AFM picture shows NPs maximum tip height (95.5 nm)

Ag NPs were biosynthesized in different sizes by three isolates of the size was measured by using AFM the diameter starting from 1 to 95.5 nm and the average of the Nps diameter was (45.5 nm) as it is shown in figure(4).

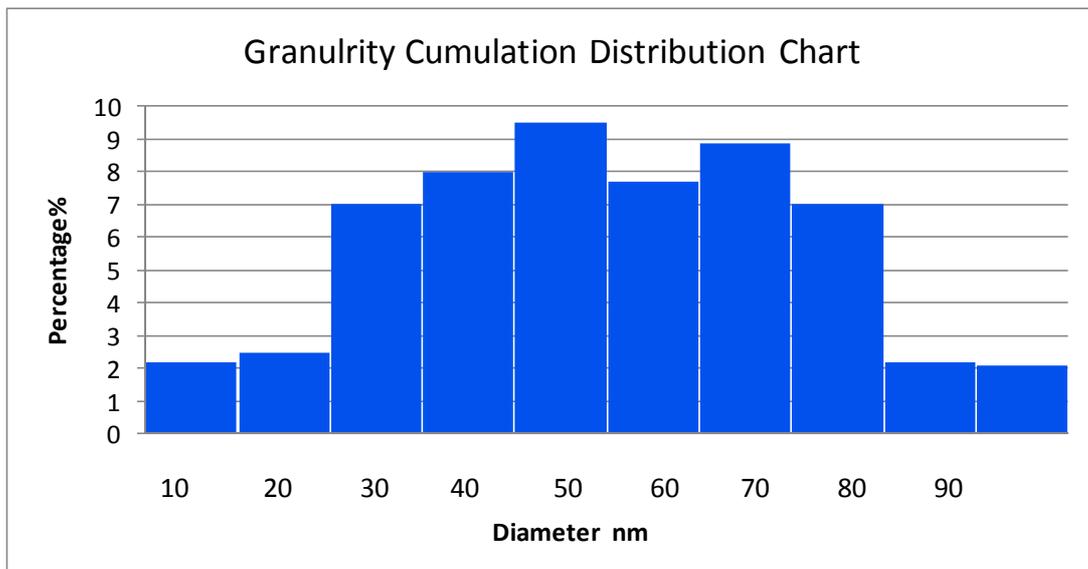
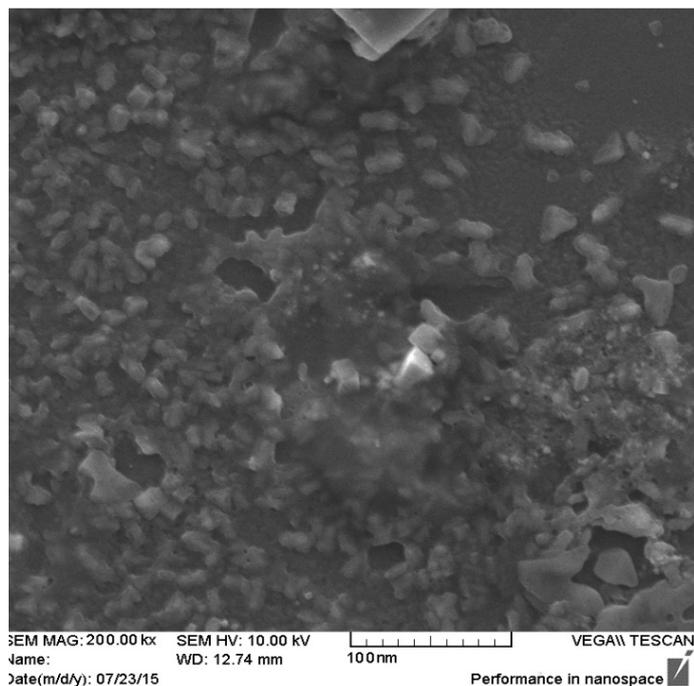


Figure (4): Diameter, volume and cumulation of Ag NPs

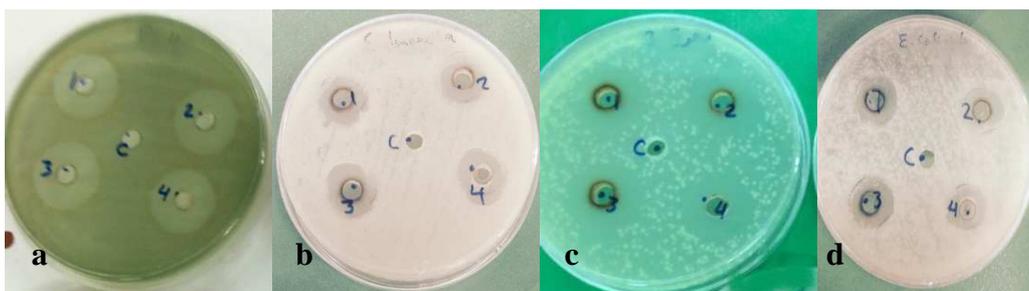
SEM is a kind of electron microscope which images a sample by scanning it using a high-energy electron beam. The electrons then interact with the atoms making up the sample, thus producing signals which reveal information about the sample's composition, surface topography and other properties such as electrical conductivity (Gupta *et al.*, 2006). Scanning Electron Microscope (SEM) surface morphology image showed relatively spherical shape nanoparticles formed with diameter range 40–50 nm in picture 2 which represents the SEM picture of silver nitrate NPs. These pictures confirm the formation of silver nitrate nanoparticles after 24 hrs incubation of aqueous filtrate of *Fusarium graminearum* with 1mM silver nitrate. This picture substantiate the approximate spherical shape to the nanoparticles, and most of the particles exhibit some faceting.



Picture-2-SEM of silver nanoparticle synthesized by treating fungal cell filtrates with 1mM AgNO3 solution

The microbes selected for the present study for the antibacterial activity were *pseudomonas aeruginosa*, *Salmonella* sp., *Candida albicans* and *E. coli*. Inhibition zone was determined by measuring the diameter of bacterial clearance after 24 h. As shown in plate (1), the means diameter of inhibition zone in case of *pseudomonas aeruginosa* were (12.2,13,13.5,14.5) mm and for *Salmonella* sp.(7.3,8.5, 8.8,9.5) mm and in case of *Candida albicans* were (15,16.5,17.5,18.5) and for *E. coli* (7.5,8.1,8.5,8) mm using the concentration (20,30,40,50) µg/µl respectively, this results agreed with (Sadhasivam *et al.*, 2010; Devika *et al.*, 2012).

This study conclude that the nanoparticles synthesized from the fungus open up the exiting possibility of rational strategy of biosynthesis of nanomaterials, and thus, silver nanoparticles has great potential as antimicrobial compound against pathogenic microorganisms studied, and that it can be used in the treatment of infectious diseases caused by bacteria.



Plate(1): Graphs showing the inhibition of silver nanoparticles against some pathogenic bacteria. showing the antimicrobial activity against (a) *pseudomonas aeruginosa* (b) *Salmonella* sp. (c) *Candida albicans* (d) *E. coli*.

- C: control
- 1: 20 µg/µL
- 2: 30 µg/µL
- 3: 40 µg/µL
- 4: 50 µg/µL

CONCLUSIONS

- 1- Silver nanoparticles had been successfully synthesized by the fungus *Fusarium graminearum* and its had highly effective Antimicrobial activity.
- 2- The Nps size from one to 95.5 nm and the average of diameter was (45.5 nm), this diameter is suitable for penetration of bacterial wall.

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