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ECO-FRIENDLY BIOSYNTHESIS OF SILVER NANOPARTICLES BY *ASPERGILLUS PARASITICUS*

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In this study, the synthesis of silver nanoparticles by *Aspergillus parasiticus* were isolated from wheat was identified primarily by changing the color of the extracellular filtrate and confirmed by UV-Vis spectroscopy. The synthesized nano particles have been characterized by the Biophysical techniques like X-ray, Fourier transformation infrared (FTIR), the Transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

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Keywords: silver nanoparticles; biosynthesis; ions; biomass

1. Introduction

The biosynthesis of nanoparticles with the help of biological sources like microorganisms is essential to develop safe a green approach for production materials synthesis without using dangerous substances such as toxic reducing agents and organic solvents [1- 3]. Production of silver nanoparticles (AgNPs) by fungi more suitable for large-scale due to secrete amounts of bioactive substances much higher than bacteria [4]. Many approaches investigated to Biosynthesis of silver nanoparticles using fungi [5-10]. Nanoparticles have higher antimicrobial effect in comparison with the chemical silver nitrate this is related with ratio of surface-to-volume and depend mainly on their size of the AgNPs [11] which ensures direct contact of silver nanoparticles with pathogens [12]. Characterization of these silver nanoparticles can be by many techniques such as Ultraviolet-Visible spectroscopy, fourier transformed-infrared spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). These techniques can show the information about the size and formation, morphology of the particles, capping, and stability.

2. Materials and Method

2.1 Isolation of Aspergillus parasiticus.

The fungus was isolated from wheat from Riyadh, Saudi Arabia, on potato dextrose agar (PDA) and incubated at 25±2 °C. The identification of the fungal isolate was based on morphological and microscopic observations. The identities of the isolates were confirmed by the Regional Center of Fungi and their Applications, Al-Azhar University, Cairo, Egypt.

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2.2 Preparation of cell-free fungal filtrate.

To prepare biomass for biosynthesis studies, the fungus was grown in a liquid medium containing (g/l) KH_2PO_4, 7.0; K_2HPO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.1; $(NH_2)SO_4$, 1.0; yeast extract, 0.6; and glucose, 10.0. The flasks were inoculated and then incubated on an orbital shaker at 27 °C and agitated at 150 rpm. The biomass was harvested after 72 h followed by washing with distilled water to remove any medium component from the biomass. Twenty gram biomass (wet weight) was added to 100 ml of double-distilled water and then incubated for 72 h at 27 °C in an Erlenmeyer flask and agitated under the same conditions. The cell filtrate after the incubation was obtained by passing the sample through Whatman No. 1 filter paper.

2.3 Synthesis of silver nanoparticles using Aspergillus parasiticus.

The filtrate was mixed with 100 ml of carefully weighed 10^{-3} M $AgNO_3$ solution and kept on a shaker at 27 °C. The filtrate alone (without silver nitrate) and pure silver nitrate solution (without cell-free filtrate) were used as positive and negative controls, respectively.

2.4 Characterization of AgNPs

2.4.1. Ultraviolet-Visible Spectrophotometer.

The formation of reduced silver nanoparticles in colloidal solution was monitored using UV-Vis spectral analysis. Color changes in the supernatant were monitored both by visual inspection and absorbance measurements using a Cintra 10e GBC double beam UV–Vis spectrophotometer (Victoria, Australia). It was observed that the suspension had convert from yellow to brown color upon completion of the reaction. The spectra of the surface plasmon resonances of AgNPs in the supernatants were recorded at wavelengths between 200 to 1000 nm. and recorded at different times during biosynthesis. The control (without silver ions) were incubated under the same conditions.

2.5 Fourier transforms infrared.

Fourier transform infrared (FTIR) spectroscopy measurements, the bio-transformed products present in cell-free filtrate after 72 h of incubation were freeze-dried and diluted with potassium bromide in the ratio of 1: 100. FTIR spectrum of samples was recorded on FTIR instrument mode Nicolet 6700 spectrometer of resolution 4 cm-1(Thermo Electron Corporation, USA). All measurements were carried out in the range of 400– 4000 cm-1 at a resolution 4 cm-1.

2.6 X-ray diffraction analysis.

XRD is an important technique to evaluate the formation of silver nanoparticles. The fungal supernatant containing AgNPs was freeze-dried using a Heto Lyophilizer (Heto-Holten, Denmark). Powdered form had used for characterization. The finely powdered sample was analyzed by an X'pert PRO PANalytical diffractometer using CuKα radiation (k = 1.54056 Å) in the range of $20 \leq 2\theta \leq 80 \leq$ at 40 keV.

2.7 Transmission electron microscopy.

The size and morphology of the air-dried silver chloride nanoparticles were characterized by Transmission electron microscopy was performed on a JEOL (JEM-1010) instrument with an acceleration voltage of 80 kV after drying aqueous AgNPs on a carbon-coated copper TEM grid. Samples were dried and kept under vacuum in desiccators before loading them onto the specimen holder. The particle size of the silver nanoparticles was evaluated using Image J 1.45s software 1493.

2.8 Scanning electron microscopy.

The powdered particles were scanned on scanning electron microscope (SEM) using a JEOL (JSM-6380 LA) instrument.

3. Result and Discussion

3.1 Isolation of Aspergillus parasiticus

The identification of the fungal isolate was based on morphological and microscopic observations (Fig. 1)

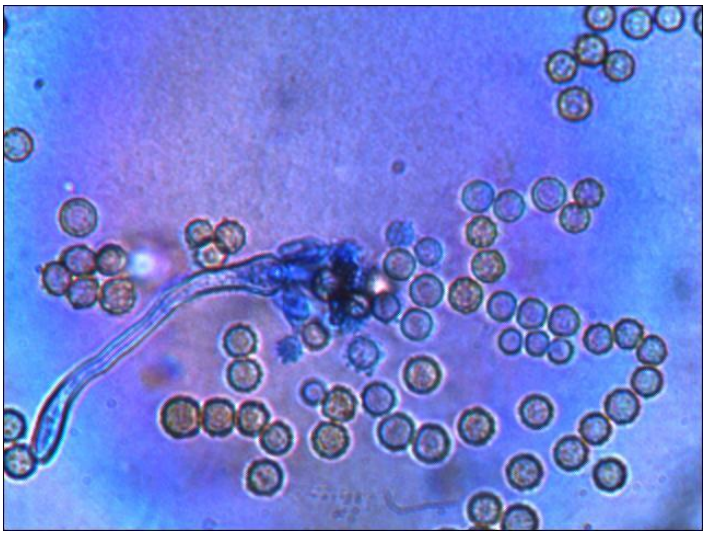

Fig. 1. Morphology of the Aspergillus parasiticus

3.2 Biosynthesis of fungal silver nanoparticles

The synthesis of silver particles using isolates of *Aspergillus parasiticus* was investigated. After addition of $AgNO_3$ into filtered cell-free culture, the samples color of the mixture changed from nearly colorless to brown with intensity increasing during the period of incubation which confirms the formation of nanoparticles [14], [15]. Fig. 2 show that picture of conical flasks containing the filtrate of the *Aspergillus parasiticus* biomass in aqueous solution of 10^{-3}M $AgNO_3$ after 3 days of reaction, control sample (without silver ions) showed no change in color of the cell filtrates when incubated in the same conditions.

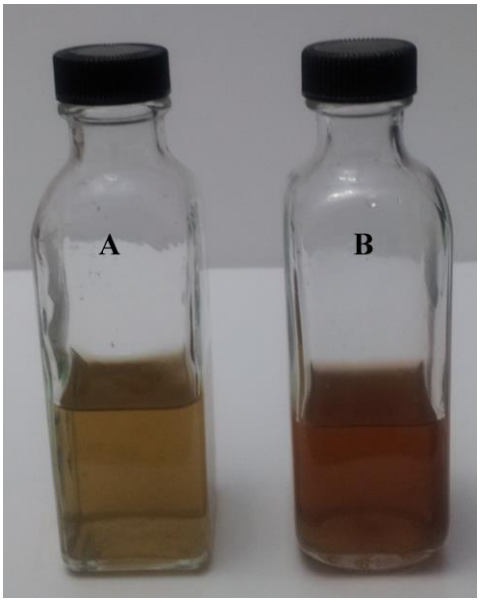

Fig. 2. Picture of two bottles containing the filtrate of the Aspergillus parasiticus biomass in aqueous solution of 10^{-3} M AgNO3 at the beginning of the reaction (A) and after 3 days of reaction (B).

3.3 Characterisation of Synthesized Silver nanoparticles

3.3.1 UV–visible spectroscopy

The formation and stability of the reduced silver nanoparticles was monitored by using by changes of UV-Vis absorption (Fig. 3). The increase in intensity of colour occurred due to gradually increasing the number of silver nanoparticles synthesized from the reduction of silver ions available in the aqueous solution .After 72 h of incubation, no further increase in intensity was recorded indicating complete reduction of precursor silver ions [5].

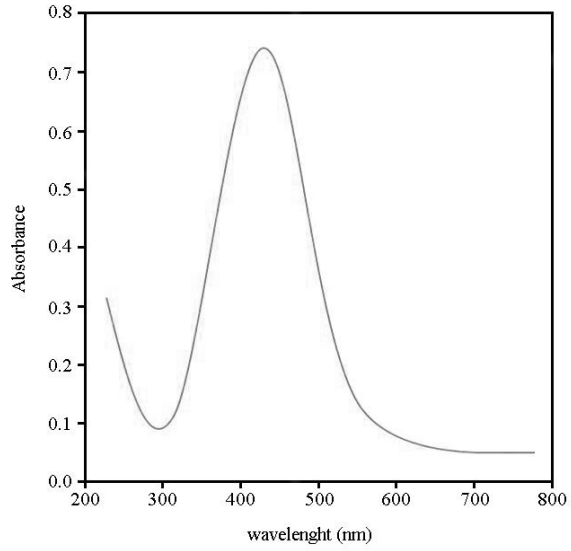

Fig. 3. The UV-Vis spectra recorded for the reaction of fungal cell filtrate with AgNO3 solution

The light absorption pattern of the cell filtrate was continuously monitored after 24 and 72 h in the range of 200-800 nm by using UV-visible spectrophotometer. The average wavelength at which the peak occurred was around 420 nm. after 72 h of starting of the reaction. The size and shape of the synthesized silver nanoparticles reflects the absorbance peak as reported earlier [16]. The changing of the SPR peak to high wavelengths with increase in the size of particle was found to be similar as reported by [17].

3.4 FTIR spectroscopy analysis

FTIR spectroscopy is used to analyze the binding of proteins with silver nanoparticles, and it is possible to characterize the secondary structures involved in the metal nanoparticle-protein interactions which may be responsible for the synthesis and stabilization of silver nanoparticles with the capping agent available in the fungal filtrate. Fig. (4) shows the FTIR spectrum of a freeze-dried powder of silver nanoparticles after 72 h of incubation. The FTIR spectrum contained two bands at 1635.05 cm-1 that correspond to the bending vibrations of the amide II bands of the proteins [18].The presence of the peaks of amino acids supports the presence of proteins in cell-free filtrate. The one band observed at 3295.19 cm^{-1} can be assigned to stretching vibrations of the primary amines [10]. These observations indicate the presence of the proteins binding which can promote the stabilization of the nanoparticles. amino acid and peptides lead to prevent agglomeration by configured a coat which covering the silver nanoparticles as well as may be the possible reason of their stabilization [19] .

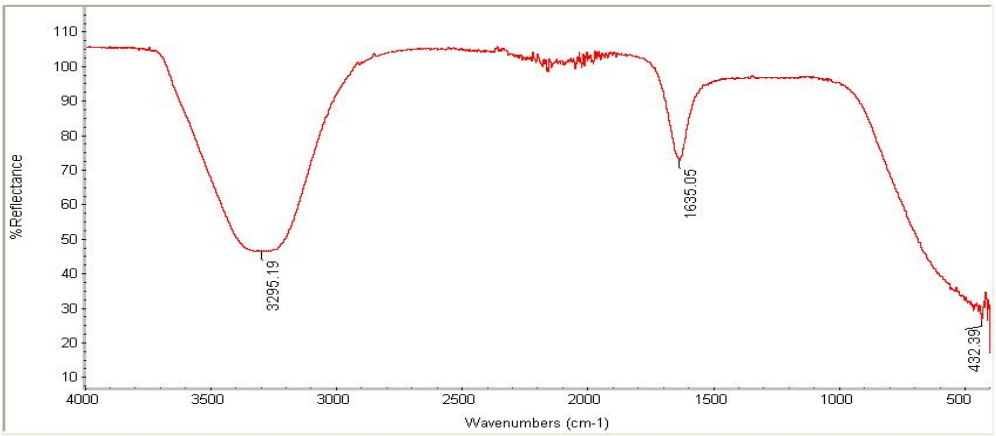

Fig. 4 *Fourier Transform Infrared Spectroscopy (FTIR) spectrum of Ag nanoparticles synthesized by reduction of Ag+ ions by Aspergillus parasiticus*

3.5 X-ray analysis

Reflecting the crystalline of the AgNPs, intense XRD peaks were observed corresponding to the (111), (200), (220) and (311) planes at 2θ angles of 39.49, 44.81°, 68.31°, and 77.41°, respectively (Fig. 5). These results agreement with the unit cell of the face-centered cubic (fcc) structure (JCPDS File No 03-0921) reported by [3].

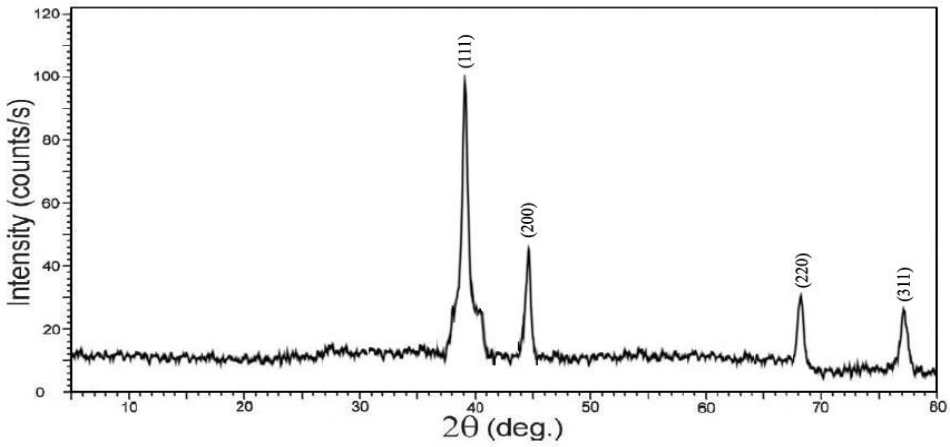

Fig. 5 Representative X-ray diffraction patterns of AgNPs synthesized by Aspergillus parasiticus

3.6 Transmission electron microscopy (TEM)

The transmission electron microscopy (TEM) studies characterized the shape and size of the silver nano particles as shown in fig. 6. The results obtained from the study of TEM indicated that the same nanoparticles are present in spherical shaped without agglomeration. The particle size histogram of silver nanoparticles shown in (Fig. 7). The results obtained that there are variations in the particle size and the average size. The particle size ranges from 5 to 60 nm. The particle distribution presented in the histogram shows that the highest percentage observed of the particles size are in the 20 to 30 nm range. The shape and size of the biosynthesized nanoparticles depend on the temperature and pH of the medium and the microorganisms [10] .size variation of silver nanoparticles which produce by *Aspergillus* species have been reported *such as Aspergillus foetidus* (20-40 nm), *Aspergillus parasiticus* (less than 50 nm), *Aspergillus niger* (5–35 nm) and *Aspergillus terreus* (5–30 nm) [19], [13] and [7] respectively.

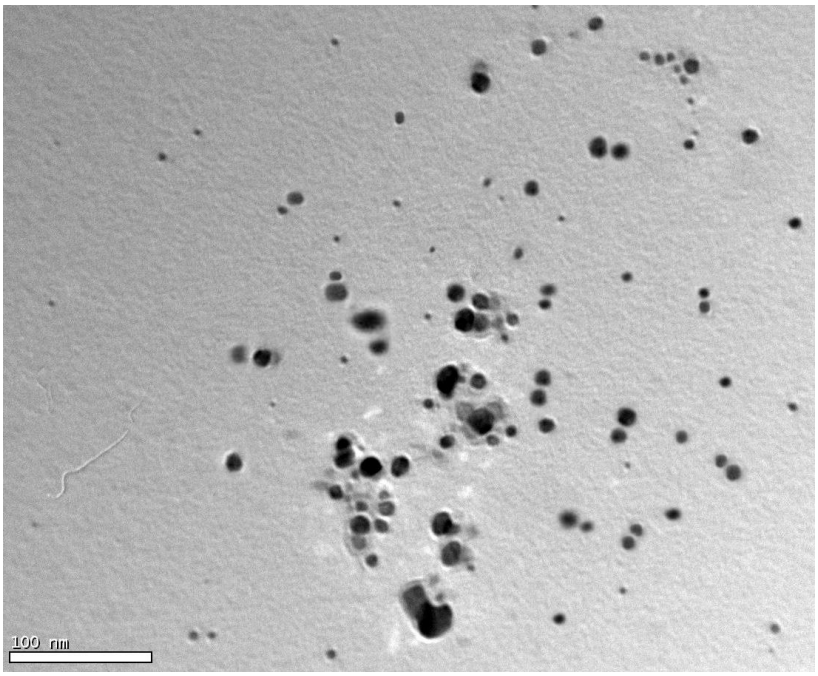

Fig. 6. Transmission Electron Microscopy (TEM) images of synthesized silver nanoparticles by Aspergillus parasiticus

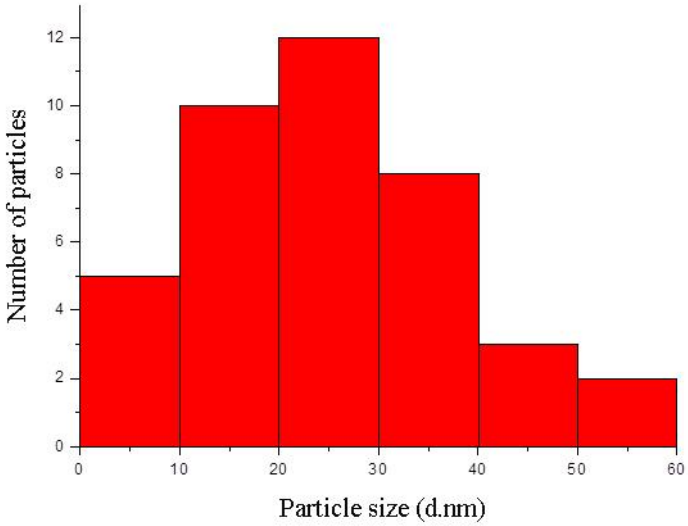

Fig. 7. A particle size distribution histogram of as synthesized silver nanoparticles determined from Transmission Electron Microscopy (TEM) images

3.7 Scanning electron microscopy

Scanning electron micrograph (Fig. 8) it was observed that drying process can affect their size and shape of the nanoparticles may be lead to partially aggregated [20]. SEM micrograph of silver nanoparticles shows, in many cases, the capping agent. Additionally, he particles size measured by SEM can be larger than the size measured by TEM due to aggregated particles [21].

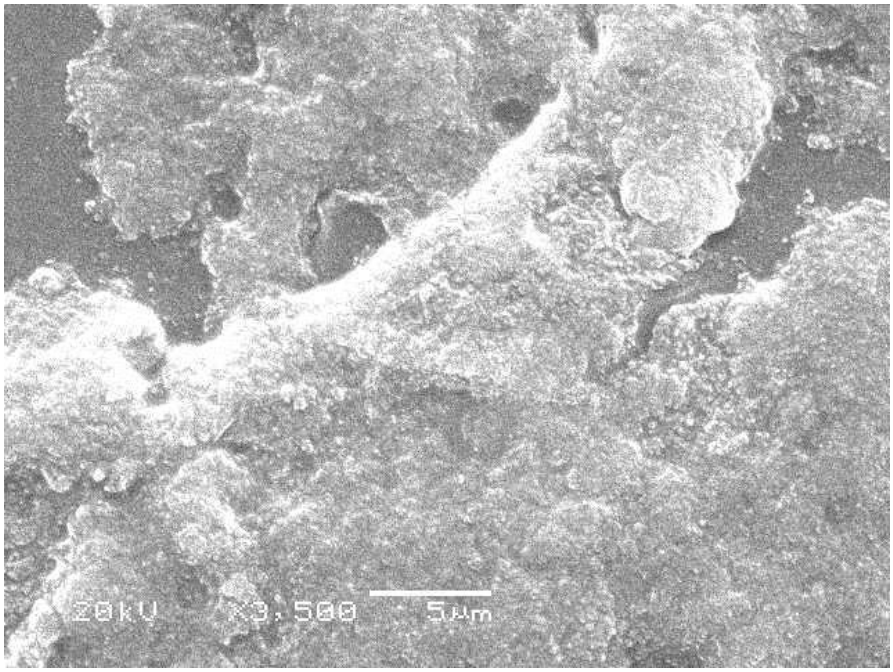

Fig. 8. Transmission Electron Microscopy (TEM) images of synthesized silver nanoparticles by Aspergillus parasiticus

4. Conclusion

In this study, it has been demonstrated that *Aspergillus parasiticu*s is capable of producing silver nanoparticles extracellularly and the silver nanoparticles are quite stable in solution. The characterization of Ag+ ions exposed to this fungus by UV-vis and SDS techniques confirmed the reduction of silver ions to silver nanoparticles. The TEM images suggest that the particles are monodispersed and spherical silver nanoparticles (AgNPs) in the size range of 5-60 nm. The spectroscopic techniques (FTIR and UV-vis) including morphological (TEM), (SEM) andstructural (SDS). SEM suggests that aggregated particles due to the capping agent. Therefore, the particles size measured by SEM can be large than the size measured by TEM.

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