

## **Biosynthesis of Silver Nanoparticles by *Fusarium solani* isolates from Agricultural Soils in Gorgan, Iran**

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**ABSTRACT**

**Background and objectives:** Silver nanoparticles (AgNPs) are major nanomaterials with a variety of applications. The synthesis of nanoparticles by conventional methods is challenging and often requires use of hazardous chemicals. Therefore, there is a growing need for development of environmentally and economically friendly processes for the synthesis of nanoparticles. This study aimed at biosynthesis of AgNPs using a filamentous fungus; *Fusarium solani*.

**Methods:** Twenty-four *Fusarium* isolates were found from several soil samples collected from depth of 1-10 cm. All isolates were identified as *F. solani* based on morphological characteristics. The synthesis of nanoparticles were evaluated after 24, 48, 72 hours of culture. AgNPs were characterized using UV-visible spectroscopy and transmission electron microscopy.

**Results:** The synthesized AgNPs showed maximum absorbance peak at 420 nm after 72 hours. Moreover, most AgNPs were spherical with diameter of between 20 and 40 nm.

**Conclusion:** In this study, we introduced a simple biological process for biosynthesis of AgNPs using *F. solani* isolates from soil samples. The results indicate that fungi may be suitable for safe and cost-effective production of AgNPs.

**Keywords:** Fungi; *Fusarium*; Nanoparticles; Nanotechnology.

## INTRODUCTION

Nanotechnology is an important field of modern science dealing with synthesis and utilization of nanoscaled structures (size range from approximately 1 – 100 nm). Nanomaterials have been used in various area such as biomedical science, cosmetics, food and feed, chemicals, drug delivery, etc. (1). Although various approaches are available for the synthesis of silver nanoparticles (AgNPs) such as reduction in solutions and chemical and photochemical reactions in reverse micelles (2), most of the methods of nanoparticle synthesis are environmentally unfriendly and expensive. Thus, there is a growing need for development of environmentally and economically friendly processes for nanoparticle synthesis, which do not require toxic chemicals (2, 3).

AgNPs are among the most popular nanomaterials used for various biomedical purposes including diagnosis and treatment of infections and tumors. A wide range of bacteria, fungi, algae and plants has been used for the biosynthesis of AgNPs. Fungal systems or myconanofactories have been exploited for the synthesis of metal nanoparticles such as silver, gold, zirconium, silica, titanium, iron and platinum (4, 5). Filamentous fungi such as *Aspergillus*, *Fusarium* and *Penicillium* are advantageous over bacteria and other organisms because of having mycelial mesh that can withstand flow pressure, agitation and other conditions in bioreactors or chambers (3, 6, 7). *Fusarium solani* was used in the present study due to its crucial pathogenic role in several crop diseases. Although a variety of physical and chemical methods have been used for production of metal nanoparticles, these procedures have adverse environmental effects due to hazardous radiation and toxic chemicals (8). In the present study, we used the extracellular protein extract of a white rot fungal strain (*F. solani*) for biosynthesis of AgNps.

## MATERIAL AND METHODS

Soil samples were collected from agricultural lands in Gorgan, Iran. The samples were taken from a depth of 1-10 cm and kept in plastic bags until air-dried at room temperature ( $27\pm 1^\circ\text{C}$ ) for 7 days. After grinding and sieving (with a 0.5 mm sieve) to remove large particles, the soil samples and debris were stored separately in paper bags at  $4^\circ\text{C}$ . One

mL of diluted soil suspension ranging from  $10^2$  to  $10^4$ , was spread on the surface of potato dextrose agar (PDA) medium (Himedia-India). All cultures were incubated at  $25\text{-}30^\circ\text{C}$  for 7–10 days (9). After the incubation period, obtained colonies were sub-cultured separately in Petri dishes containing PDA (in triplicate). Afterward, fungal morphology (color and texture) was observed macroscopically, whereas conidia, conidiophores and arrangement of spores were examined under a compound microscope after lactophenol cotton blue staining. Finally, the fungi were identified with the help of literature (10).

*F. solani* was grown in 100 ml PDA broth at  $28^\circ\text{C}$ . After 5 days, biomass was separated from the medium using Whatman filter paper No.1 and washing with sterile distilled water (three times).

For biosynthesis of AgNPs, 50 ml of cell filtrate were mixed with 10 ml of  $\text{AgNO}_3$  solution (1mM). The reaction mixture without  $\text{AgNO}_3$  was used as negative control. The prepared solutions were incubated at  $30^\circ\text{C}$  with shaking (200 rpm) in the dark for 7 days. Aliquots were prepared from isolates that changed color from yellow to brown (4). The synthesized AgNPs were characterized using a UV-visible spectroscope (Shimadzu UV-2550, US) with a resolution of 1 nm between 300 and 700 nm possessing a scanning speed of 300 nm/min. The reduction of pure  $\text{Ag}^+$  ions was monitored by measuring the UV-visible spectrum of the reaction medium after diluting a small aliquot of the sample into deionized water. One milliliter of the sample was pipetted into a test tube and then diluted with 4 ml of deionized water. All analyses were performed at room temperature. The solution containing nanoparticles showed maximum absorbance at 420 nm.

Size and shape of the biosynthesized AgNPs were studied by transmission electron microscopy (TEM, Zeiss CEM-902A). The samples were prepared by drop-coating the AgNps solution onto the carbon-coated copper grid, and were loaded onto a specimen holder. TEM micrographs and size and shape of the AgNps were recorded (11).

## RESULTS

Overall, 24 *Fusarium* isolates were found in the soil samples, which were later identified as *F. solani* based on their morphological

characteristics (Table 1). The solution changed color to yellowish brown a few hours after exposure to the fungal biomass, which confirms extracellular synthesis of nanoparticles. The control solution without the biomass remained colorless, indicating that the formation of nanoparticles was mediated

by the microbial culture. The extracellular synthesis of AgNPs in AgNO<sub>3</sub> solution was monitored by periodic sampling of the reaction mixture at regular time intervals by using UV-visible spectroscopy. The synthesized AgNPs showed maximum absorbance at 420 nm after 72 hours (Figure 3).

Table 1- Morphological characteristics of *F. solani* isolates from soil

Characteristics	<i>F. solani</i>
Pigmentation	Light orange, yellowish, light brown to dark brown
Microconidia	Abundant in aerial mycelium, 0-2 septate, oval to kidney-shaped
Macroconidia	Sporodochia, 3-7 septate, slightly hook or blunt rounded at the apical, straight, stout
Conidiophore	Long monophialides and branched monophialides
Chlamyospore	Present alone, in pairs or clusters

Figure 1- UV-Vis absorbance spectrum of AgNPs synthesized by *F. solani* after 24 hours

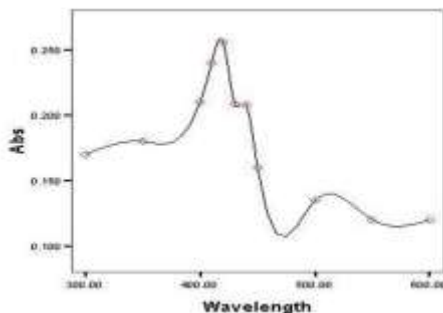


Figure 2- UV-Vis absorbance spectrum of AgNPs synthesized by *F. solani* after 48 hours

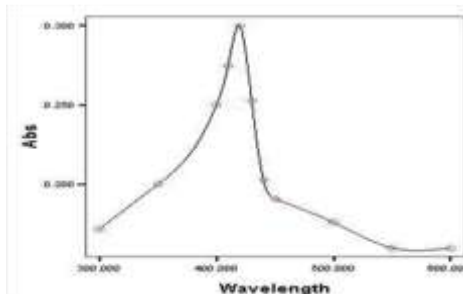
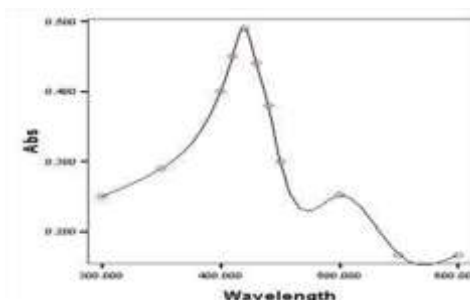
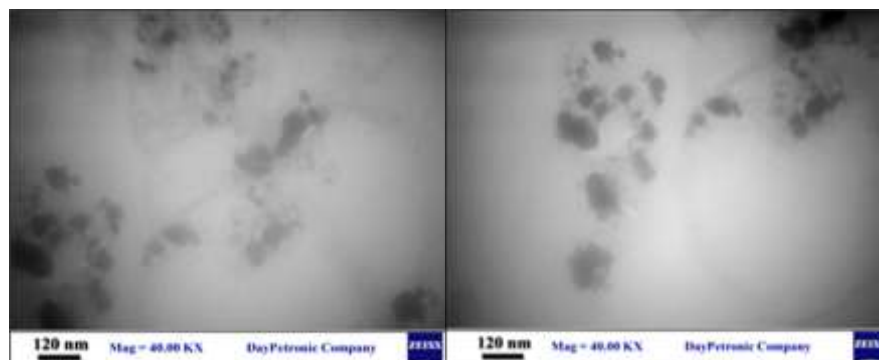


Figure 3- UV-Vis absorbance spectrum of AgNPs synthesized by *F. solani* after 72 hours



The typical bright-field TEM image of the synthesized AgNPs showed that the nanoparticles were isolated and surrounded by a layer of organic matrix at some places, which were presented as mass or bulk in

micrographs (Figure 4). Shape of the nanoparticles was highly variable but mostly spherical. In addition, the nanoparticles were polydispersed with diameter of 20-40 nm.

Figure 4- TEM micrograph of the AgNPs synthesized by *F. solani*

## DISCUSSION

Most methods of nanoparticle synthesis require use of hazardous chemicals. Biosynthesis of nanoparticles with microorganisms is satisfactory, safe and cost-effective. We aimed at the extracellular synthesis of AgNPs using *F. solani* isolates from soil samples. The results indicated the successful synthesis of nanosilver suspensions by the fungi. Production of AgNPs was confirmed by observing color change in the solution from yellowish to brown, which is mainly due to excitation of surface plasmon resonance of the AgNPs (7). UV-visible spectroscopy is a commonly used technique for analysis of AgNPs because metals with free electrons possess plasmon resonance in the visible spectra (12). The UV-visible absorbance spectrum of the biosynthesized AgNPs showed peaks at around 420 nm, which is in line with findings of some previous

studies (4, 13). The TEM micrograph showed presence of spherical-shaped structures with size ranging between 20 and 40 nm, and no agglomeration. In line with our findings, Ingle et al. demonstrated extracellular synthesis of spherical AgNPs with diameter of 16.23 nm using *F. solani* strains (8). In 2008, Basavaraja et al. reported production of spherical AgNPs sized 10-60 nm using *F. semitectum*. The mentioned studies indicate that synthesis of metal nanoparticles via biological processes is rapidly evolving into a crucial branch of nanobiotechnology (14).

## CONCLUSION

In this study, we introduced a simple biological process for biosynthesis of AgNPs using *F. solani* isolates from soil samples. The results indicate that fungi may be suitable for safe and cost-effective production of AgNPs.

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