# MYCOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES MEDIATED BY *ASPERGILLUS TERREUS*

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**Abstract:**

In the present study, silver nanoparticles (AgNPs) were synthesized using Aspergillus terreus. The synthesized AgNPs were characterized by UV-Vis spectroscopy, Fourier transforms infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), Scanning electron microscopy (SEM) and X-ray diffraction spectroscopy (XRD). The absorbance maxima of AgNPs were observed at 432 nm in visible region. The nature of co-ordination between bioactivite compounds secreted by fungi and silver ions were analysed through FTIR spectroscopy. TEM analysis revealed the spherical shape with the size ranged between 5 to 50 nm and SEM revealed the varying morphology of the nanoparticles in the size range of 34.4 nm to 57.8 nm. The crystalline nature of AgNPs was observed with XRD. Aspergillus terreus serve as clean, inexpensive, eco-friendly, reliable and safe method for the biosynthesis of silver nanoparticles.

**Keywords: Silver Nanoparticles, Fungus *Aspergillus terreus*, Mycosynthesis, FESEM, TEM, XRD**

## I. Introduction

Synthesis of nano particles (NP's) using micro-organism as nano factories is an eco-friendly method for producing nano sized particles with specific composition, size, shape and unique physical and chemical properties. (Ahmed *et al.,* 2008; Huang *et al.*, 2007). Because of the unique properties, nanoparticles have diverse applications in different areas such as chemical industries, textile industries, nano medicine, clinical diagnostics (nanobots), electronics, tissue engineering, artificial implantations (Santhosh *et al*., 2011), biosensors (Probin *et al.*, 2012) biological imaging (Nagaraj *et al.*, 2012), biological markers and labeling etc., ( Le *et al.*, 2011; Singh *et al.,* 2011). Microorganisms such as bacteria and fungi serve as potential bio factories for the production of variety of nanoparticles, including copper, gold and silver, etc. (Sastry *et al.*, 2003). The synthesis of nanoparticles of specific composition and size is a rapidly growing area of material science research. New methods in preparation of these materials extend the choice of different properties that can be obtained by various physical and chemical methods (Mandal *et al.,* 2005). Most of the techniques are still in

developmental stage and various problems are often experienced with the stability of the synthesized nanoparticles (Vahabi *et al.,* 2011). Bhaskara Rao *et al.,* (2018) Among these silver nanoparticles have received considerable attention due to their physico-chemical properties, Surface Plasmon Resonance (SPR), surface-enhanced Raman scattering (SERS) or biological application including both *in vivo* and *in vitro* biomedical and industrial research. Addition to this the AgNP's act as very good insecticide for the control of mosquito vectors (Patil *et al.*, 2012). Synthesis of NPS with various sizes and shapes is essential due to their wide applicability which can be mediated by bacteria, fungi and other organisms. It was found that fungi are the most ideal biocatalysts for NP's biosynthesis in contrast to bacteria, as fungi are well-known for producing greater amounts of biologically active substances that makes them more appropriate for large-scale production (Li *et al,* 2012). These bio-based approaches are not only eco-friendly but provide a clean and nontoxic way for the fabrication of nano crystals. Therefore, the main aim of this research work was to produce nanoparticles of silver using the fungal strain *Aspergillus terreus* and to optimize the reaction conditions for bio-reduction of silver ions.

## II. Materials and Methods

### 2.1 Isolation and identification of *Aspergillus terreus*

*Aspergillus terreus* was isolated from soil. The soil samples were collected from a sugarcane field at Thanjavur District, Tamil Nadu. After sample collection, serial dilution was performed for isolating microbial growth from the collected sample. The strain was plated on martin rose Bengal agar media and plates were incubated at $28^0$C for 4d. Individual colonies were picked and further purified by using potato dextrose agar (PDA) medium. Morphological and microscopic observation (such as colour, texture of mycelia, spore observation pattern, etc.,) (Aneja 2005).

### 2.2 Biomass preparation:

*Aspergillus terreus* was grown in potato dextrose (PDA) broth. The flask were inoculated with spores and incubated at $28^o$ C on a rotary shaker at 120 rpm for 48 hrs. The biomass was harvested by filter paper (Whatmann filter paper no 1) and the broth (culture medium) was prepared for biosynthesis of AgNP's.

### 2.3 Synthesis of silver nanoparticles:

*A. ferreus* was maintained on PDA slants for $28^0$C with regular subculture of fresh media.The stock culture of *A.terreus* was inoculated in 500ml SDA (Sabourud Dextrose Agar) andIncubated in $28\pm1^0$C for 1 week. Then, the biomass was separated by whatman fitter paper No.1. The reaction mixture for the synthesis of AgNPs was prepared by blending 10ml of the fungal extracts to 90 ml of 1m mol AgNO3 solution in a 250ml conical flask and incubated at $29^0$C for reduction.

## III. Characterization of AgNP's

### 3.1 UV-Visible Spectrophotometer:

Reduction of silver ions by *Aspergillus terreus* broth and the resulting formation of AgNP's were observed by UV-Visible Spectroscopy. The synthesized AgNP's exhibit unique optical properties due to their Surface Plasmon Resonance (SPR) which depends on the shape, size and distribution of nanoparticles (Ingle *et al*., 2008). To determine the excitation of surface plasmonic vibration of the reduced silver ions a small aliquot of the sample was diluted with distilled water and the absorption maxima was scanned by UV-Visible Spectrophotometer in the range 300-800 nm using perkin-Elmer Lamda 2 UV 198 Visible Spectroscopy.

### 3.2 Fourier transforms infrared and X-Ray diffraction Analysis

The synthesized nanosolution was centrifuged at 60,000 g for 40 min and the pellet were dissolved in deionized water and filtered through Millipore filter paper (0.45 mm). Fourier transforms infrared (FTIR) and X-ray diffraction (XRD) analysis were carried out using a small aliquot of the filtrate containing silver nanoparticles.

### 3.3 Fourier transforms infrared Spectroscopy

In order to identify the possible bio-reducing and capping agent involved in the synthesis of nanoparticles, *Aspergillus terrues* extract was subjected to FTIR spectroscopic analysis. The sample after freeze drying was mixed with potassium bromide (KBr) and pelletized. Sample was recorded by measuring the spectra in the diffuse reflectance mode using Perkin- Elmer spectrum RX1 (wavelength range between 4000 $cm^{-1}$ and 400 $cm^{-2}$).

### 3.4 X-ray diffraction analysis

X-ray diffraction was performed to determine the dimension of biologically synthesized AgNPs with h, k, 1value. The XRD diffractogram of synthesized AgNPs was carried out on a film of the solution drop-coated on to glass substrate on a Phillips PW 1830 instrument with operation conditions of voltage 40 KV and a current of 30 mA in Cu, Ka1 radiation. Particles size (L) of the AgNP's was calculated using PAN analytical expert PRO model instrument following Debye Scherer's equation.

$$L = 0.9/\beta \cos \lambda$$

Whereas the wavelength of the X-ray; β is full width and half maximum; and λ is the Bragg's angle.

### 3.5 Scanning Electron Microcopy (SEM) Analysis

The reaction mixture is centrifuged at 600rpm for 10 min and the pellet obtained was suspended in small amount of sterilized double distilled water. A small amount of this suspension was sprayed onto the glass slide to make a thin film and kept in hot oven to dry. The thin film was used for the SEM analysis.

### 3.6 HRTEM analysis

The morphology of the nano particles was analyzed using the images obtained with a JEOL3010 Transmission Electron Microscope. The silver nanoparticles solution was purified by repeated centrifugation and allowed for sonication and a drop of this solution was used to make a thin layer on the copper coated grid and allowed to dry. The morphology, size and diffraction of the silver nanoparticles were measured at different magnification at 100 kev using JEOL TEM 2100 High Resolution Transmission Electron Microscopy.

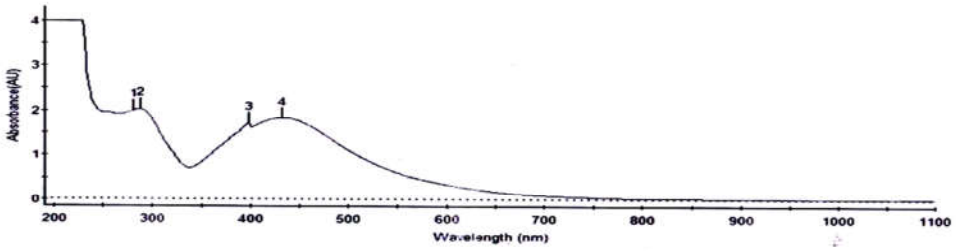
**Figure 1: (A) Silver Nitrate solution (B) Fungal Culture (C) $AgNo_3$ treated Culture medium**

## IV. Results and Discussions:

### 4.1: Biosynthesis of Silver nanoparticles

AgNP's were synthesized using aqueous extract of *Aspergillus terreus*. The $AgNO_3$ solutions turned to dark brown color with the addition of fungal extract indicating the formations of silver nanoparticles within one hour of incubation.The change in colour of the resulting solution was due to excitation of Surface Plasmon Resonance (SPR) and the reduction of Silver ions. After 1 hour of inhibition, further increase in colour intensity was not observed indicating the stability of synthesized nanoparticles. the absorbtion spectra of the resulting mixture was measured with UV-Vis spectrophotometer in the range of 300-800 nm revealed at a peak 432 nm (Fig.2). Anil *et al* (2007) and Duran (2005) also reported the absorption spectra of 432 nm developed during the synthesis of AgNP's from *Aspergillus niger.*

**Figure 2: a UV–Visible spectrum of synthesized AgNP's mediated by *Aspergillus terreus.***




| Name | No. | Peak(nm) | Peak(AU) | No. | Valley(nm) | Valley(AU) |
|---|---|---|---|---|---|---|
| UV-Asper-Gel-Ter | 1 | 279.85 | 2.0036 | | | |
| | 2 | 287.30 | 2.0328 | | | |
| | 3 | 397.55 | 1.7265 | | | |
| | 4 | 432.30 | 1.8331 | | | |

### 4.2: FTIR and XRD analysis:

FTIR Spectroscopy analysis was performed to ascertain the involvement of possible bio compound responsible for reduction of $Ag^{+}$ ions and capping of AgNPs synthesized by using fungal extract (Fig:3) shows the synthesized AgNPs using *Aspergillus terrues* extract and the absorption spectrum manifests prominent transmittance located at five absorption peaks. A broad band at 3447.96 cm-1 is due to O-H stretching of alcohol groups. C-H Stretching of alkene can be assigned the band at 1638.69 cm-1. The band at 1385 cm-1 peak shows the C-H Aldehyde stretching and the peak at 687-98 cm-1 may be due to C-Br stretching of halocompound.

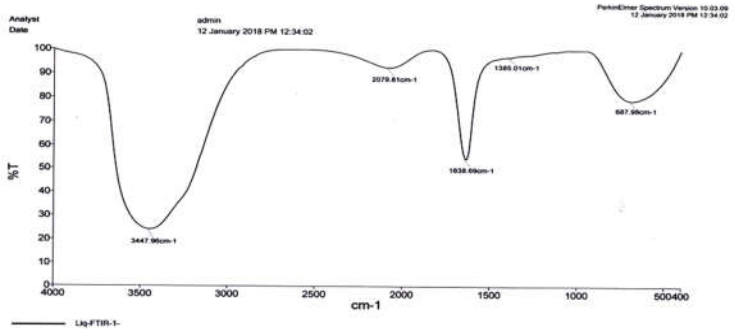
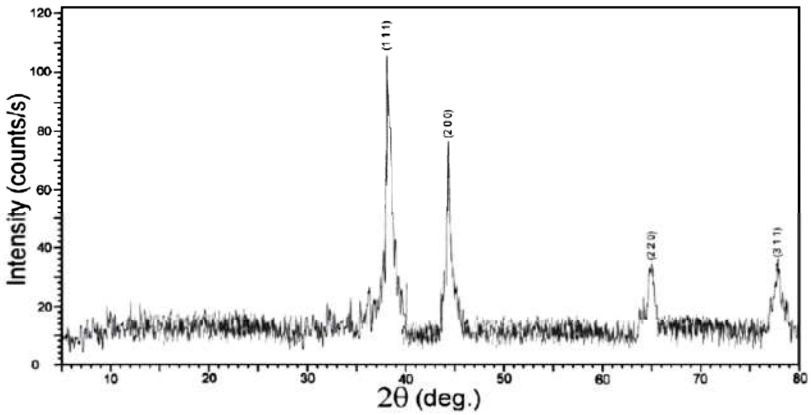



**Figure 3: FTIR Spectrum of *Aspergillus terreus* Silver Nanoparticles.**

XRD- peak corresponding to 2θ angles at 38.25°, 44.48°, 65°, and 77.68°. These peaks corresponds to (111), (200), (220) and (311). Bragg's reflection respectively. The intensity of the (111) diffraction was much stronger than (200). These agreement with the unit cell of the face centered cubic (fcc) structure (JCPDS File No. 04-0783). Mohanasriramula., (2017) reported the synthesis AgNPs from *Aspergillus terrues* and the XRD pattern showed 2θ values at 32.3°, 45.01°, 75.09° assigned to the planes of (111),(200),(311) corresponds to faced cubic structures of AgNPs .

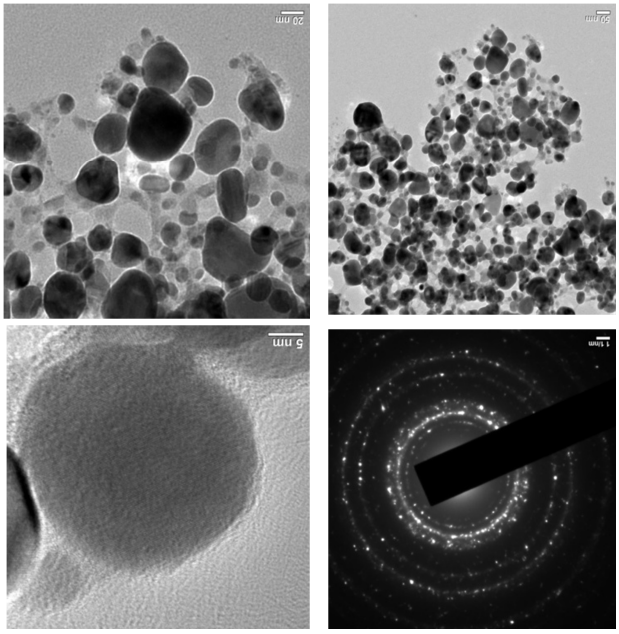
**Fig: 4 XRD Analysis of AgNPs using *Aspergillus terreus***



### 4.3: The High Resolution Transmission Electron Microscopy (HRTEM):

The High Resolution Transmission Electron Microscopy (HRTEM) analysis was done to determine the shape of silver nanoparticles. Majority of synthesized nanoparticles were spherical shape. HRTEM analysis showed particles in the range of 20 nm. Shekhar ,2014 revealed that the shape and size of the green synthesized AgNPs depends on the PH and temperature of the medium as well as the microorganisms. Eepsita *et al.*, (2014) reported the synthesis of AgNP's with a particle size of 10-19 nm from *A.terrues.* Similarly Lanabam & Joshi, 2015 synthesized nanoparticles with a size range of 5-35 nm from *A.niger* an reported nanoparticles of (Lanabam & Joshi 2015) 20-40 nm from *A.foetidus* Roy *et al.,* 2013 reported the synthesis nanoparticles less than 50 nm from *A. paraiticus.*

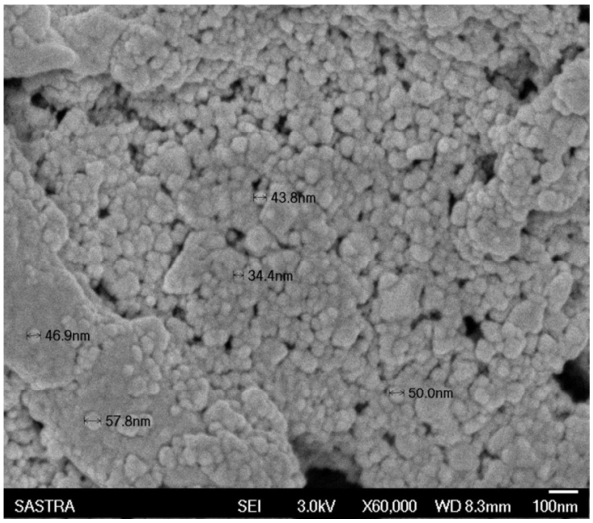
**Fig.5 TEM analysis of AgNPs synthesized from *Aspergillus terreus***



### 4.4: Scanning Electron Microscopy (SEM):

The morphology and the size of the AgNPs were determined using scanning Electron Microscopy (SEM). The Scanning Electron Micrograph reveals that the silver nanoparticles synthesized were slightly aggregated. The synthesized AgNPs were mostly spherical in shape ranging between 34-57 nm (Fig.6). Our present findings were in agreement with Mohana sriramula and Shanmugam sumathi, 2017 who reported the synthesis of spherical shaped nanoparticles with the size ranging between 25-50 nm which was mediated through *Fusarium oxysporum*.

**Figure 6: FESEM Micrograph of AgNP's mediated by *A. terreus*.**




## CONCLUSION

Fungal synthesis of NPs is the cheapest and most environmental of friendly method, which does not require a huge expenditure of energy in comparison of other methods, the silver nanoparticles formation from fungus *Aspergillus terreus* species was confirmed by UV-vis spectroscopy, FTIR (Fourier transform infrared Spectroscopy) and SEM (Scanning electron microscopy), TEM (Transmission electron microscopy) Crystalline nature of AgNPs observed in XRD, *Aspergillus terreus* dominates NPs production because of its wide range of applications, cost effectiveness, simplicity of use and management and superior qualities. The fungus *Aspergillus terreus* used in the present study was found to be efficient for the rapid synthesis of silver nanoparticles.

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