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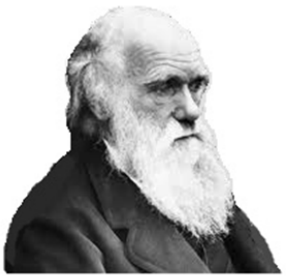

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Biosynthesis of antibacterial silver nano-particles from *Aspergillus terreus*

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ABSTRACT

This paper deals with the bio-synthesis of silver nano-particles using a soil fungi. In this work, FTIR examinations indicated that the encountered soil particles actually contained Silver particles, while XRD analysis confirmed the silver was in the nano-crystalline phase. Moreover, the hurried decline of silver (Ag^+) ions monitored using UV-visible spectrophotometer, demonstrated that these silver nano-particles were formed within 19 minutes. In addition, transmission electron microscopy (TEM) revealed that the synthesized silver nano-particles come in a size ranging between 16-57 nm and have a variety of shapes, including round, rod, and uneven. The present study thus concludes that silver nano-particles were actually produced and that these have admirable antibacterial activity. The current study also indicates that growing concentration increases the rate of reduction and decreases the particle size.

***Keywords*:** Bio-synthesis, Silver nanoparticles, UV, TEM

1. INTRODUCTION

Silver nanoparticles, considered as noble metal, originated to have impending solicitations in numerous arenas, like microelectronics (Y. Li *et al.,* 1999), optical devices (P. V. Kamat *et al.,* 2002), catalysis (Schmid, 1992), drug delivery system (S. Mann, 1996) antibacterial consequence, biological sensors, textile and filters (Elechignerra J., 2005), etc., (Gajendran N., 2007, Kathiresan K. *et al.,* 2009). Synthesis of nano-particles retaining microorganisms has fascinated much due to their customary optical, chemical, photoelectron

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chemical and electronic properties. Numerous biological organisms, such as bacteria, fungi, yeast, and plants, either intra- or extra-cellular (Castro-longoria E., 2010), which are sophisticated production, yield and with low incidentals. Now, a day's mycological synthesis of silver nanoparticles play an important role in medicinal preparations. Fungi are the best candidates in the synthesis of metal nano-particles, because of their ability to secrete large amount of enzyme (Basavaraj S. *et al.*, 2007; Saeed Moharrer, 2012) and easy to isolate from different sources, like soil, air, plants, etc. In the current report, researchers have been reporting the biological methods for the synthesis of silver nanoparticles, using Fungi *Aspergillus terreus* for a potential synthesis of metal nanoparticles.

2. MATERIALS AND METHODS

Sample collection

The soil samples were collected from around the administration building of Palamuru University campus of Mahabubnagar Dist., Telangana State, India, respectively. Samples were transferred into sterile plastic bags and brought to Laboratory, and stored in laboratory conditions for further processing.

Isolation and inoculation

The soil samples were further used for the serial dilution for the soil fungi isolations. The isolated fungi were pure cultured in the repeating of the experiment, repeating number of times, with respectable samples. The isolated fungi were identified as *Aspergillus terreus* using help of Barnet. The fungi were further sub-cultured on PDA plates, and slanted in order to obtain pure culture. Pure isolates were cultured in 250-ml conical flask containing 100 ml liquid media Czepak-dox broth keeping on a rotator orbital shaker for seven days at 120 rpm.

Thereafter, cultured material was sieved by funnel separating media content. Obtained biomass was inoculated in 250-ml conical flask containing 100 ml sterilized distilled water and kept for 3 days on Orbital shaker for agitation at the speed of 150 rpm. After the incubation, the cell filtrate was collected and used for the synthesis of nanoparticles.

Biosynthesis of Silver Nano-particles

10 ml culture filtrate of the fungi was mixed with 50 ml of 1 mM silver nitrate solution in 250-ml conical flask and agitated at room temperature; control (without silver nitrate, only biomass) was also run along with the experimental flask.

After beginning and 24 hours of time interval, the culture filtrate and silver nitrate were turned into orange brown due to reduction of silver nitrate to silver ions, the formation of nanoparticles understood from the UV- Visible spectroscopy and X-Ray diffraction studies.

Characterization of Synthesized Silver Nano-particles

UV- Visible spectroscopy

The reduction of silver ions was confirmed by a qualitative testing of supernatant by UV-Visible spectrophotometer. The UV–Visible spectroscopy measurements were performed on Elico spectrophotometer as a resolution of 1 nm, from 300 to 800 nm.

XRD study

The sample was powdered and prepared for X-Ray diffraction. The target was cuk β (λ = 1.54 Å), the generator was operated as 40 kV and 30 mA current.

The scanning range (20) was selected from 10 to 80 angle, scanning speed of 2.00 deg/min and the chart spread of 20 mm/min were used for a precise determination of lattice parameters. High-purity selection powder was used as an internal standard. The Coherently diffracting Crystallography domain size (dxrd) of the silver nano-particle was calculated from X-Ray diffraction (XRD) line broadening after subtracting the contribution from the cuk β component (Rachignor correction) and correcting the instrumental width. The integral line width was used in the Scherrer formula to calculate dxrd of the (III) plane for silver.

3. RESULTS AND DISCUSSION

In the present work we have reported biological method for the production of silver nanoparticles using selected fungi. The size of the silver nano-particle was found to be 30-55 nm from the Transmission electron microscope observations.

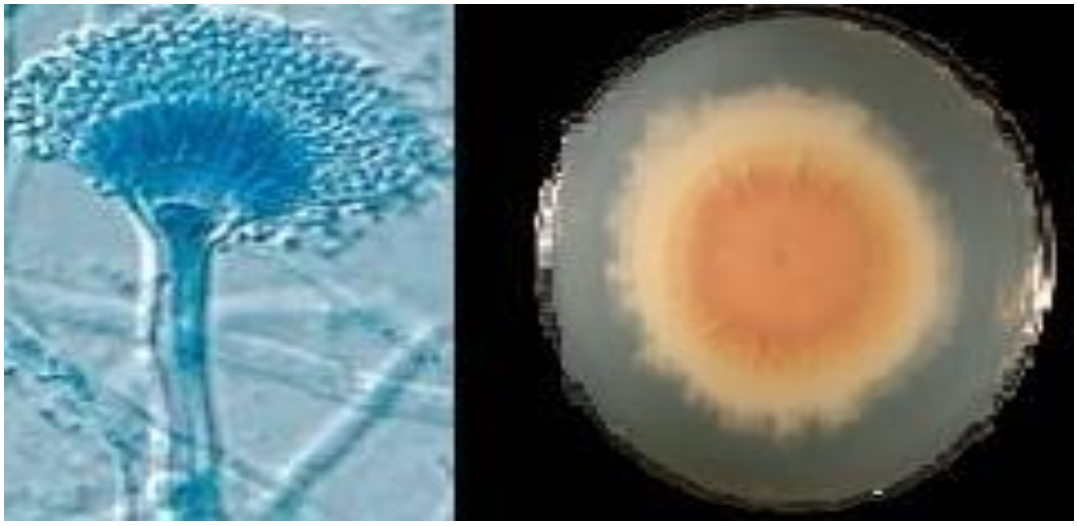

Figure 1. Isolated fungal (*Aspergillus terreus*) mycelium plates and microscopic evidence

Inset of **Fig. 1** shows Fungal Biomass with Silver nitrate ions at the beginning and after 8 and 72 hours of reaction. It was observed that the color of the solution turned from colorless to brown after the 8 hours of reaction, indicating the formation of silver nanoparticles. This arises owing to the surface plasma vibration in the metal nanoparticles (Langford, S.D. and Boor, P.J., 1996; Parikh, C.K., 1989; Schvartsman, S., 1992; Monroy, C.Y Castillo, P., 2000; Martínez, M.E., 2002). This important observation indicates that the reduction of silver nitrate into silver ions went extracellular.

Inset of **Fig. 2** shows distinct and fairly broad absorption band centered at 450 nm. The presence of a broad resonance indicates an aggregated structure of silver nanoparticles in the film.

Figure 2. Mycosynthesis of silver nanoparticles using *Aspergillus terreus* aqueous extract treated with $AgNO_3$ solution at room temperature: (A) Silver nitrate ($AgNO_3$) solution, (B) formation of silver nanoparticles using *Aspergillus terreus* aqueous extract.

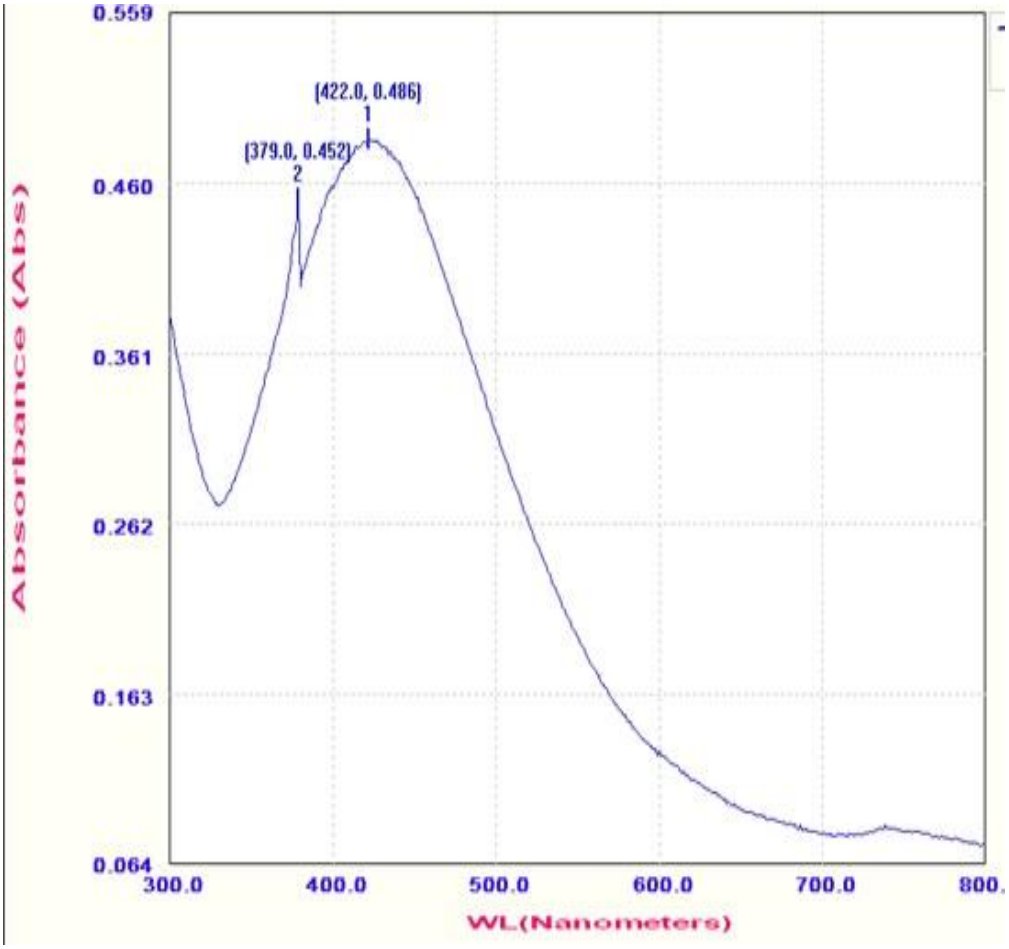

Fig. 3. UV–Vis spectrum of biosynthesized silver nanoparticles, showing surface plasmon peak at 422 nm

UV-Vis spectra recorded from the aqueous silver nitrate solution after 8, 24, 48, and 72 hours of reaction with the biomass are shown as the curve 2, 3, and 4, respectively in **Figure 3**. It is clear that there is a presence of silver particles in solution, thus distinctly pointing to surface reduction of the silver ions as the most probable mechanism for the synthesis of silver nanoparticles by fungus.

A possible mechanism for the presence of silver nanoparticles in the fungal biomass could be the extra-cellular reduction of the silver ions in solution followed by precipitation onto the cells.

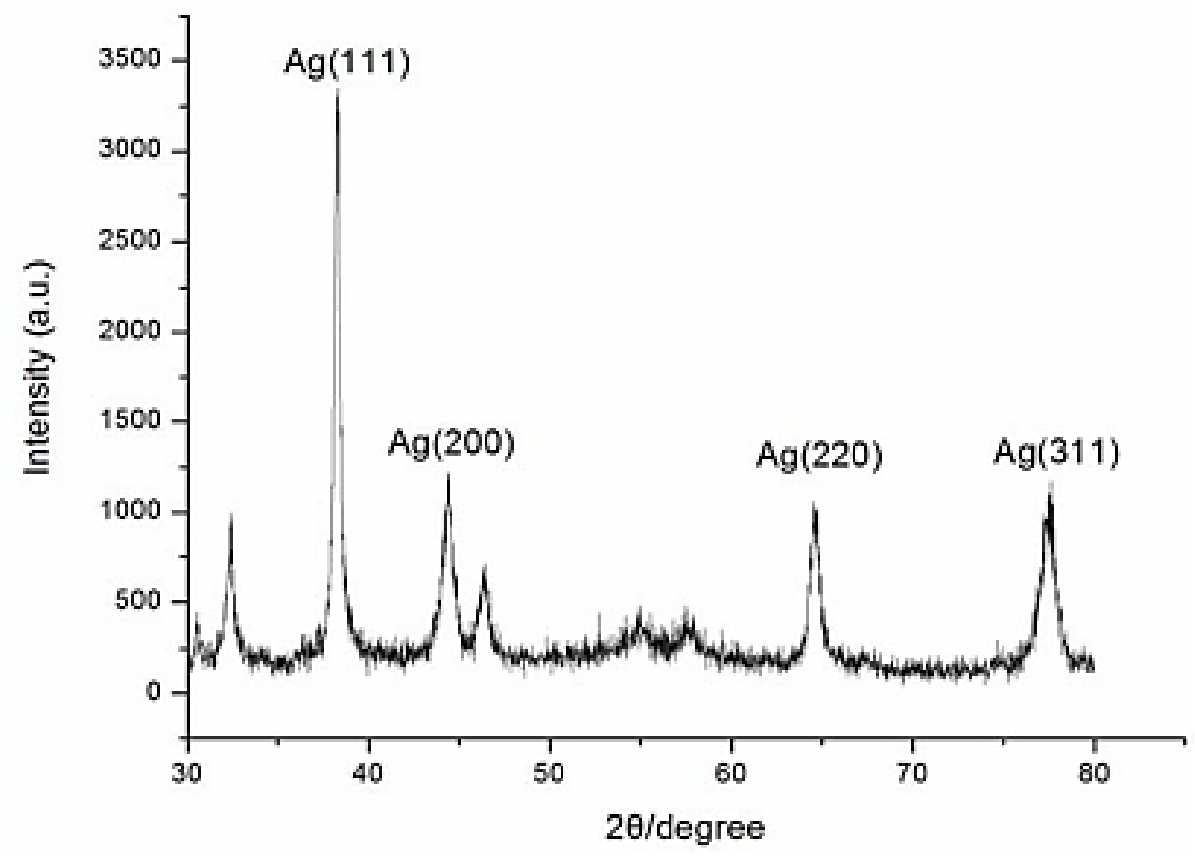

Fig. 4. XRD patterns of Biosynthesized Silvernanoparticles from *Aspergillus fumigatus*

Figure 4 shows XRD analysis, peaks assigned to the corresponding diffraction signals (111), (200), (220), and (311) facets of silver. The mean particle diameter of silver nano-particles was calculated from the XRD pattern according to the line width of the (111) plane, refraction peaks using the Scherrer equation. The calculated average particle size of the silver was found to be 27-55 nm.

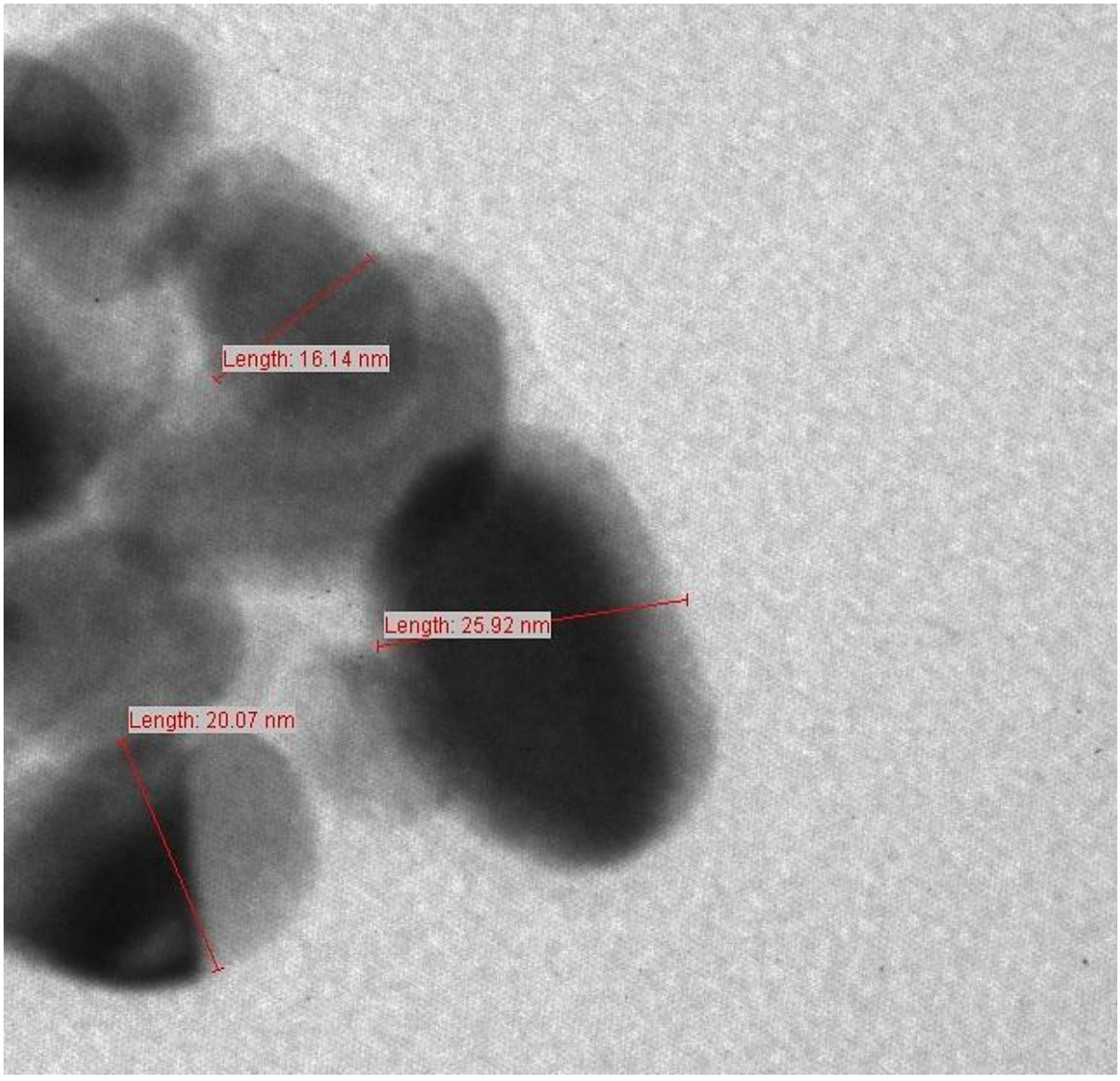

Fig. 5. TEM image of biosynthesized silver nanoparticles from *Aspergillus terreus*

Based on the biosynthesized silver nanoparticles from *Aspergillus terreus* (**Fig. 5**), antibacterial activity at 80 µL/well, with a maximum against *B. Subtilis* followed by *E. coli*, is visible, whereas the mediate antibacterial activity was observed against *C. albicans*, *Staphylococcus*, shown in **Figure 6**.

Two negative controls, i.e., selected fungi aqueous extract and $AgNo_3$ solution did not show any activity. Streptomycin sulfate used as standards alongside test organisms showed the inhibition zones of 21.00 mm, respectively (Fig. 6).

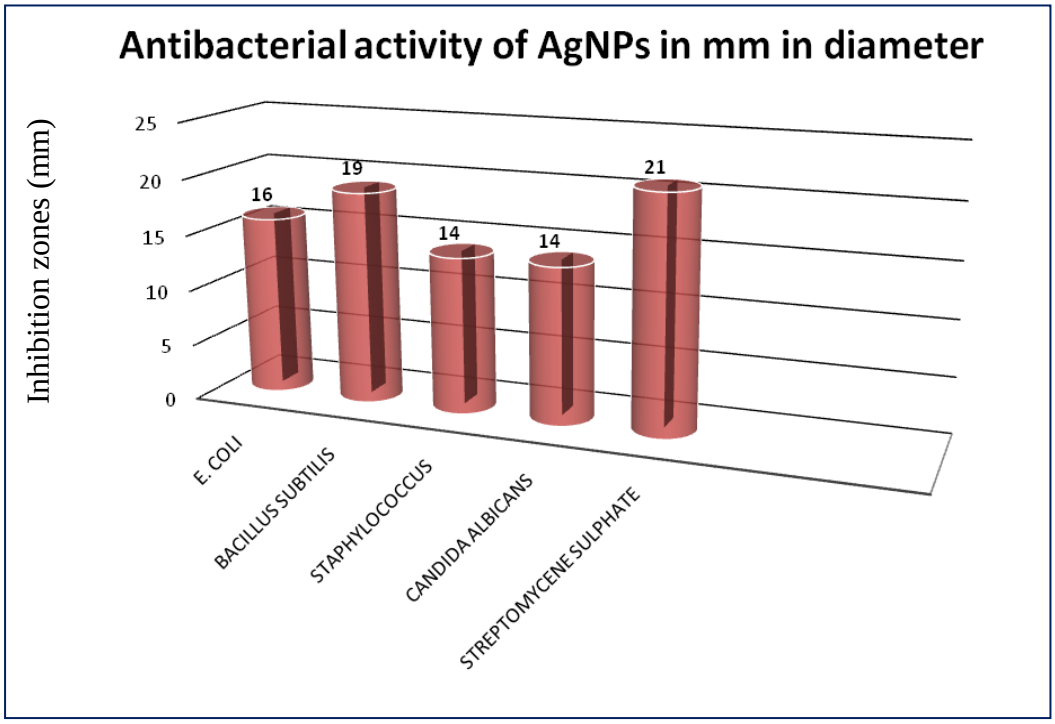

Figure 6. Antibacterial activity of biosynthesized silver nanoparticles from *Aspergillus terreus*

4. CONCLUSION

In the current upshot, the nanoparticles were naturally, eco-friendly synthesized, using selected fungal species biomass isolated from soil of Palamuru University campus soil samples, Mahabubnagar, India. The cubicle scum of fungi was confronted with 1 mm silver nitrate, changing of the mixture from colourless to light orange-brown, and dark-brown.

This indicates the production of silver nanoparticles in the reaction mixture with the size of synthesized nano-particles as measured to be 30-55 nm by XRD analysis and with the TEM observations. The final fallout sand conclusion of the isolated fungi PU-1 (*Aspergillus terreus)* was manufacture of protuberant silver nano-particles.

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