

Characterization and biosynthesis of Silver nanoparticles using a fungus *Aspergillus niger*

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ABSTRACT

An attractive possibility of green nanotechnology is to use microorganisms in the synthesis of silver nanoparticles. Recently, the biosynthesis especially from fungi has emerged as a novel method for the synthesis of silver nanoparticles. Nanoparticles are considered as building blocks of Nanotechnology. In the present work we have screened fungi for the extracellular production of silver nanoparticles. *Aspergillus* sps, *Rhizopus* sps, *Fusarium* sp. and *Penicillium* sp. were the isolates screened and subjected to silver nanoparticles production. Of the tested isolates, the fungus *Aspergillus* sp. showed maximum absorbance at 416 nm which is an indication of Silver nanoparticles production. Further characterization was made by TEM which revealed the shape to be spherical and size ranged between 20-55 nm, EDS showed the presence of elemental silver at 3keV, FTIR spectrum showed the different functional groups, XRD spectrum showed the crystalline nature of the particles and AFM revealed three dimensional structures of the nanoparticles. Of all kinds of nanoparticles silver nanoparticles show great promise in terms of biomedical applications as they exhibit different biomedical activities.

Keywords: *Aspergillus niger*; Silver Nanoparticles; TEM; AFM; FTIR

1. INTRODUCTION

The biosynthesis of nanoparticles has received increasing attention due to the growing need to develop safe, cost effective and environment friendly technologies for nanoparticles synthesis. Out of all kinds of nanoparticles, the metallic nanoparticles, including gold, silver iron, zinc and metal oxide nanoparticles, have shown great promise in terms of biomedical applications, not only due to their large surface area to volume ratio but also because they exhibit different biomedical applications.

With the development of modern antibiotics for the treatment of infectious diseases, the use of silver agents in the clinical setting had been restricted mainly to topical silver sulfadiazine cream in the treatment of burn wounds. Nanotechnology provides a good platform for having promising applications in diagnostics, antimicrobial agents and drug delivery systems (Jaidev and Narasimha 2010). Antimicrobial effects of silver can be

increased by manipulating their size at nano level (Dattu et al 2014). Because of this change in physical chemical properties, silver nanoparticles (AgNPs) have emerged as antimicrobial agents. The development of research for new antimicrobial compounds to improve bactericidal potential is a priority area of research.

An important feature of metal nanoparticles synthesis is their ability to remain dispersed in liquids without agglomeration, this can be achieved by biosynthesis due to proteins in cell filtrate themselves acts as stabilizing and capping agent (Guangquan et al 2012). Microorganisms such as bacteria, actinomyetes and fungi play an important role in remediation of toxic metal through reduction of metal ions and are considered as potential nanofactories. Filamentous fungi are ideal candidates for environmental friendly synthesis of AgNPs (Devi and Joshi 2012).

Biotechnological approach towards the synthesis of nanoparticles has many advantages such as economic viability, possibility of easily covering large surface area by suitable growth of the mycelia, and its green chemistry nature.

In the present study, we have isolated, screened fungal strains for biosynthesis of silver nanoparticles and the synthesized AgNPs were characterized by UV-Vis spectroscopy (T 90+ UV-VIS spectrophotometer), Transmission Electron Microscopy (TEM) (Hitachi H 7500 ID, Japan), Infra Red Spectroscopy (IR) (Perkin Elmer model 783 spectrophotometer), Energy Dispersive Spectroscopy (EDS) (JEOL Model JED - 2300), X-Ray diffractometer (XRD) and Atomic Force Microscopic (AFM) analysis.

2. MATERIALS AND METHODS

2. 1. Isolation of Fungi

Soil samples were collected from Yadgir district, Karnataka for the isolation of fungal strains and cultured on potato dextrose agar (PDA) medium. Isolates were identified based on colony morphology and lacto phenol cotton blue staining. Isolates were sub cultured and preserved for further work.

2. 2. Synthesis of Silver Nanoparticles

The fungus, *A.niger* was grown in 250 ml Erlenmeyer flasks containing 100 ml of MGYB broth (Malt extract 0.3 %, Glucose 1 %, Yeast extract 0.3 % and Peptone 0.5 %) at 29 °C for 72 hr., then mycelia was separated, washed using distilled water and repeated 2-3 times to remove any traces of previous medium content.

Then mycelia was transferred to fresh Erlenmeyer flask containing 100 ml distilled water, kept for incubation at afore said conditions for 48 hr. The suspension was filtered with the help of Whatman's filter paper No. 1, as obtained filtrate was challenged with 1mM AgNO₃ at 29 °C for reduction under dark at 150 rpm.

2. 3. Characterization of Silver Nanoparticles

Synthesized silver nanoparticles were characterized using UV-Vis spectroscopy (T 90+ UV-VIS spectrophotometer) shows specific surface plasmon resonance peak. Transmission Electron Microscopy (TEM - Hitachi H 7500 ID, Japan) reveals the size and shape of nanoparticles. The interaction between protein and AgNPs was analysed by Fourier Transform Infrared Spectroscopy (FTIR -Perkin Elmer model 783 Spectrophotometer).

Presence of elemental silver was detected by employing Energy Dispersive Spectroscopy (EDS -JEOL Model JED - 2300), X-Ray diffractometer (XRD) provides the crystalline nature of the particles and Atomic Force Microscopic (AFM) analysis reveals the three dimensional picture.

3. RESULTS AND DISCUSSION

3. 1. Isolation of Fungi

From the different soil samples a total of seven fungi were screened for their ability to synthesize AgNPs (*Aspergillus* sps 3 isolates, *Rhizopus* sps 2, *Fusarium* sp. 1 and *Penicillium* sp. 1). Amongst them *Aspergillus* sps which was identified as *A. niger* (Figure 1) showed better brown color. Hence, the further work was carried out with only this fungus and it was more abundant in the warmer soil samples of Hyderabad-Karnataka region. As our results correlate with Jaidev and Narasimha et al (2010) as they too reported AgNPs biosynthesis using *A. niger* isolated from South India.



Figure 1. *Aspergillus* sp. on Potato Dextrose Agar plate.

3. 2. Synthesis of Silver Nanoparticles

The biomass of fungi after 72 hrs was separated by filtration, which was yellow in colour and it turned reddish brown colour after reaction with silver nitrate. The appearance of brown colour clearly indicates the biosynthesis of silver nanoparticles (Figure 2). The colour change was caused by the surface Plasmon resonance (SPR) of AgNPs in the visible region was reported by Afreen et al (2011). Similar type of result were also observed by Dattu et al (2014) using Endophytic fungi *Penicillium* sp isolated from *curcuma longa* (turmeric).

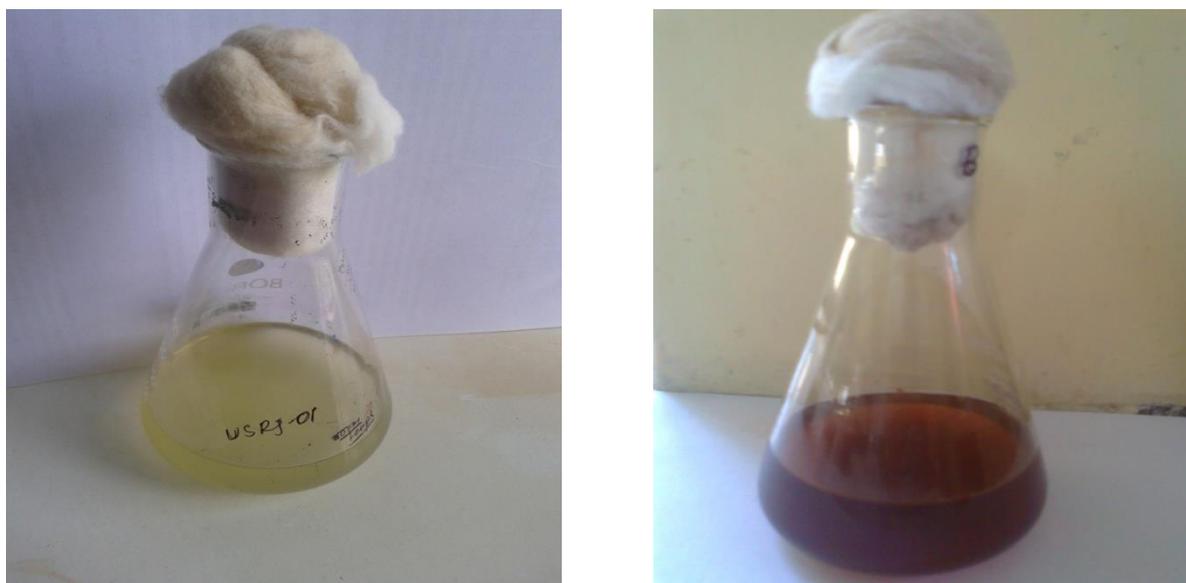


Figure 2. Colour change observed in cell free fungal filtrate of *Aspergillus* spp after exposure to silver nitrate.

3. 3. Characterization of Silver Nanoparticles

Reduction of aqueous Ag^+ with culture supernatants was observed. Visual observation of color change from light yellow to brown after addition of AgNO_3 to enzyme filtrate is the primary indication of AgNPs biosynthesis. UV- Visible spectroscopy analysis showed peak at 416 nm which confirmed the production indicating the specific surface Plasmon resonance (Figure 3).

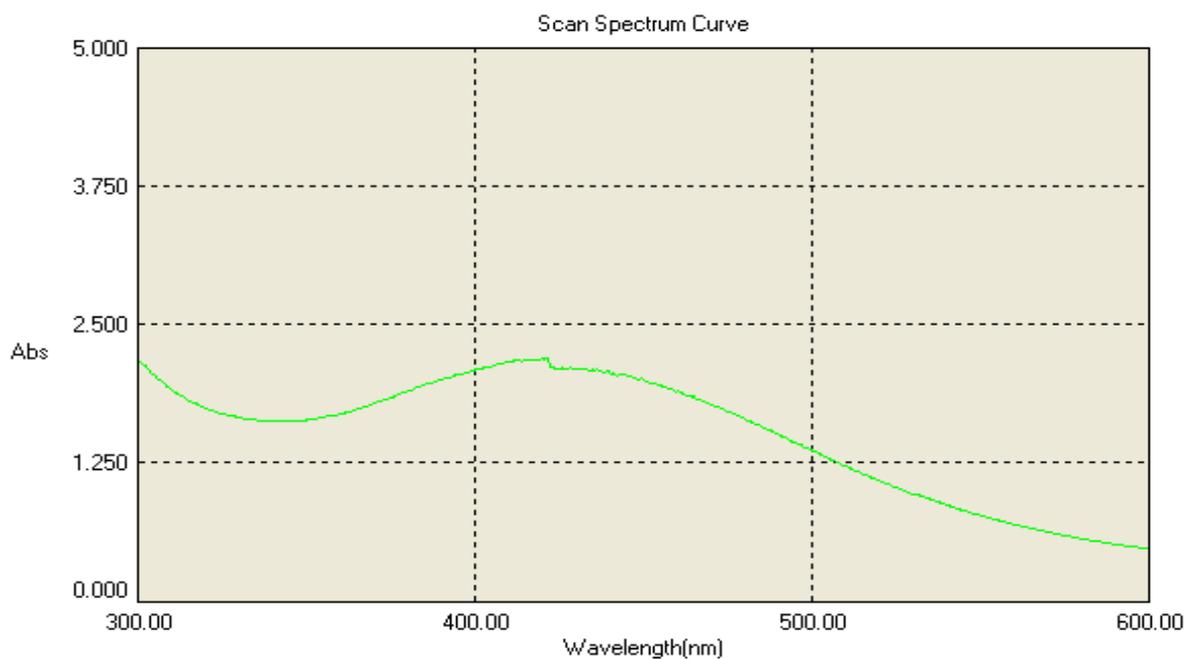


Figure 3. UV-Vis spectrum of AgNPs.

Maliszewska et al (2008) reported that the absorption spectrum of spherical AgNPs presents a maximum between 420-450 nm. Our result correlates with the reports of Dattu et al (2013) who also showed peaks at 425 nm using endophytic fungus *Penicillium* sp. and also Jyothi et al (2014) using *Rhizopus* sp. also reported an intense peak at 420 nm.

A drop of AgNPs solution was placed on the carbon coated copper grids and kept under vacuum before loading them onto a specimen holder. Then TEM micrographs were taken by prepared grids to determine the size and shape of the produced AgNPs. Figure 4 revealed the particles are spherical in shape and size of the particles is between 20-55 nm. Afreen et al (2011) dealing with *R.stolonifer* also reported the size of the particles nearing to 20-55 nm and the shape of the nanoparticle to be spherical. Guangquan et al (2012) reported the particles were spherical and polydisperse with an average size of 4.3 nm using *Aspergillus terreus*.

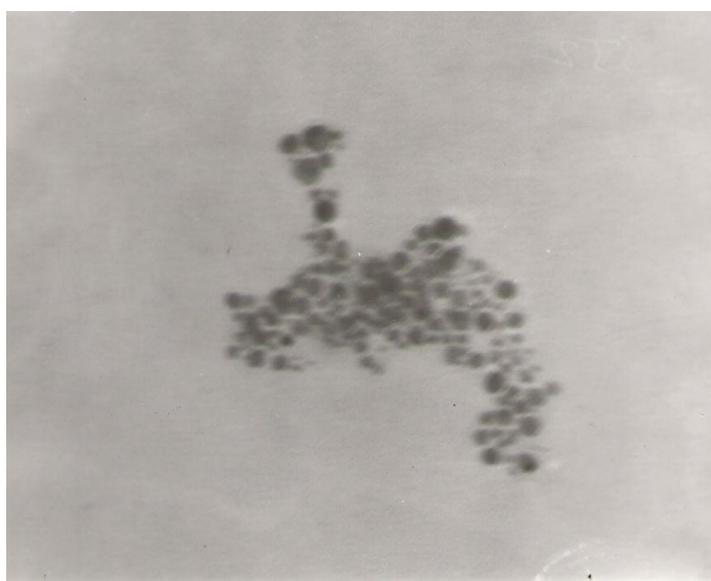


Figure 4. TEM micrograph of AgNPs.

The FTIR measurements were carried out to know the possible interaction between protein and AgNPs synthesized by *A.niger* (Figure 5). The amide linkages between amino acid residues in polypeptides and proteins give rise to well known signatures in the infrared region. T

The representative spectra in the region of 3000 to 450 cm^{-1} revealed the presence of different functional groups like 3290.73 - secondary amide (N-H stretch, H-bonded), 2928.01 - alkane (C-H stretching), 2161.23 - alkyne (C=C stretching), 1771.02 - anhydride (C=O stretching), 1613.81 - alkene (C=C stretching), 1538.60 - aromatic (C-C stretching), 1386.45, 1313.48 and 1080.10 - primary alcohol (C-O stretching) and 528.55 - alkene (=C-H bending) respectively. Proteins present in the extract can bind to AgNPs through either free amino or carboxyl groups in the proteins.

The potential biomolecule in cell free extract acts as stabilizing and capping agent. Dattu et al (2014) reported different functional groups absorb characteristic frequencies of FTIR radiation. Similar results were also presented by Afreen et al (2011).

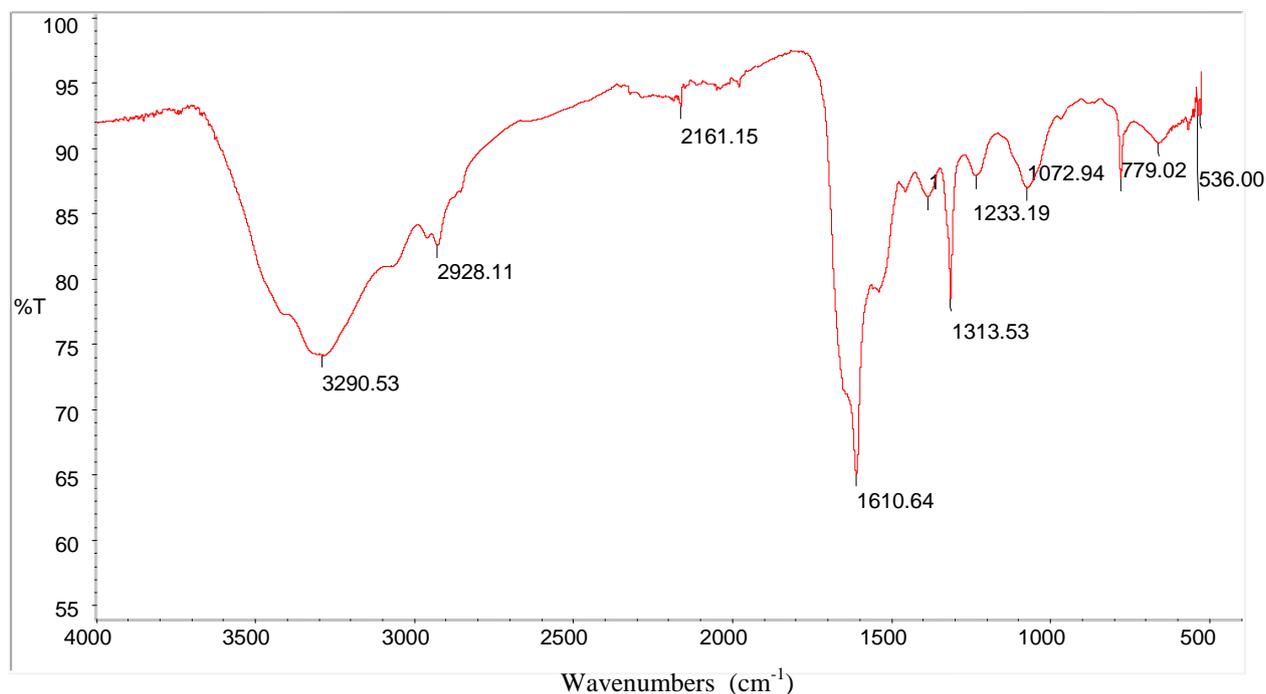


Figure 5. FTIR spectrum of AgNPs.

Energy Dispersive Spectroscopy (EDS) employed to detect elemental analysis of AgNPs. The presence of an optical absorption band at $\sim 3\text{eV}$ reveals the presence of pure metallic AgNPs along with the C and O signatures that might be from the stabilizing protein (Figure 6). Same result of EDS optical absorption peak at 3keV was also reported by Afreen et al (2011), which is typical for the absorption of AgNPs due to surface Plasmon resonance, which confirms the presence of nanocrystalline elemental silver. Jaidev and Narasimha (2010) also expressed the peak of Ag at 3keV which confirmed the elemental silver through EDS.

XRD (X-ray diffractometer) analysis reveals the crystalline nature of AgNPs. The diffracted intensities were recorded from 0 to 80 (2θ), the intense XRD peaks were observed corresponding to (111), (200) planes at 38° and 46° of 2θ (Figure 7). XRD picture revealed the particles have cubic structure. The peak at 32° might be related to AgCl which was owing to the chloride ions involved during cell free filtrate preparation. The Bragg's peak position and their intensities were compared with Standard JCPDS files.

Prema and Raju (2009) reported the X-ray diffraction pattern which showed the presence of sharp reflections at 111, 200, 220 and 311. As our results correlates with them at facets 111 and 200. While Guangquan et al also revealed the intense XRD peaks corresponding to the (111), (200), (220), (311) planes at 2θ angles of 38.28° , 44.38° , 64.54° , and 77.64° respectively. A small volume of biosynthesized AgNPs was subjected to atomic force microscopic study. AFM revealed the particles are smooth, spherical in shape (Figure 8a) and the topography of the picture shows the three dimensional structure of the nanoparticles which is depicted in figure 8b.

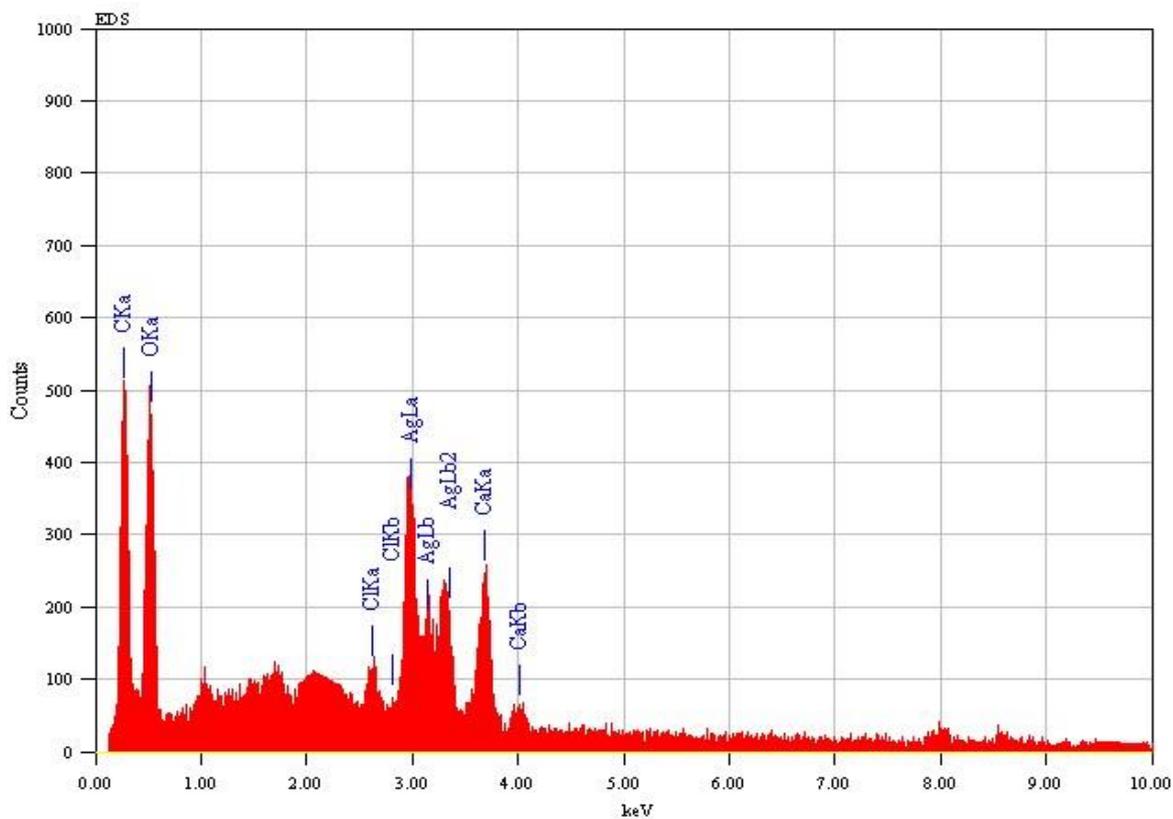


Figure 6. Electron Dispersive spectra of AgNPs.

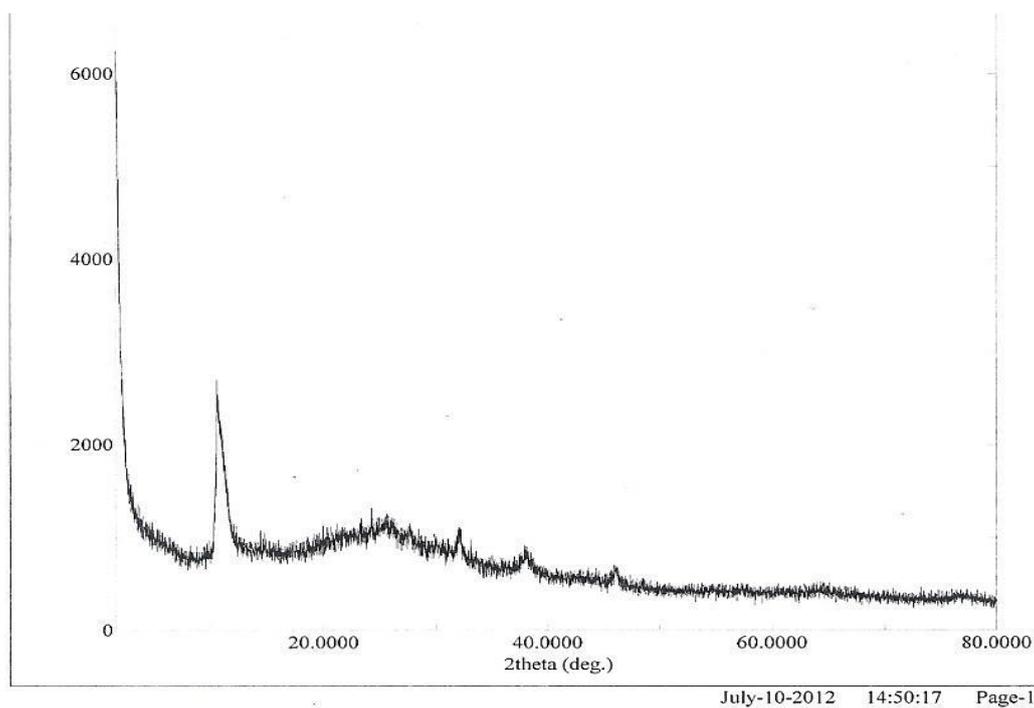


Figure 7. X-Ray Diffraction of AgNPs Synthesized from *A. niger*

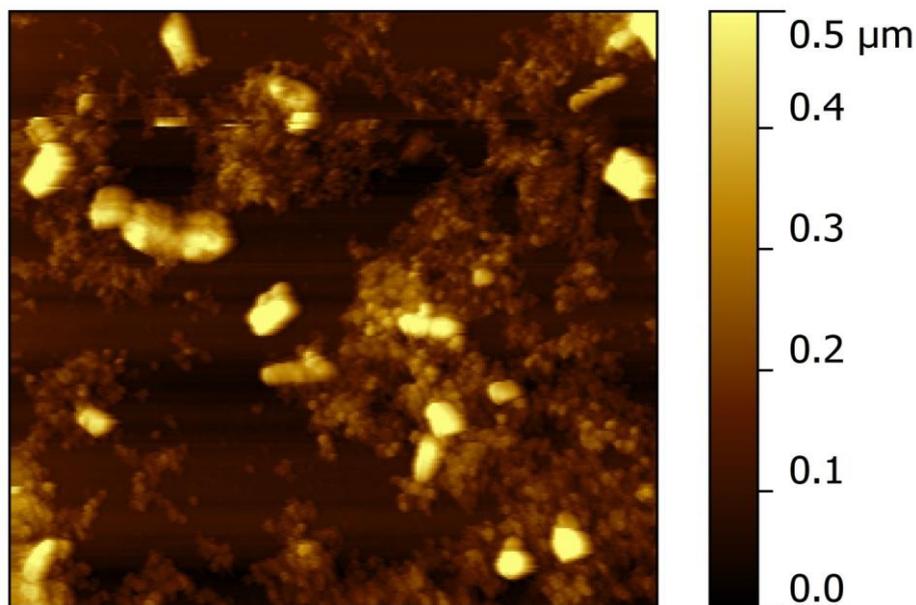


Figure 8a. Atomic Force Micrograph of AgNPs.

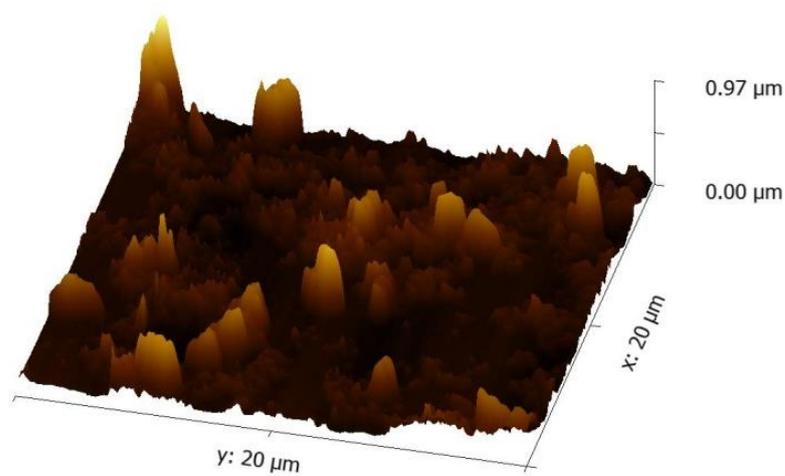


Figure 8b. Atomic Force Micrograph shows the three dimensional images of AgNPs.

4. CONCLUSION

Pharmaceutical and biomedical sectors are facing the continuous increase in the antibiotic-resistant human pathogens. Silver in the form of various compounds and bhasmas have been used in ayurveda to treat several bacterial infections since time immemorial.

Therefore, development, modification or searching for the antimicrobial compounds having bactericidal activity against pathogenic bacteria is a priority area of research. Biological synthesis of silver nanoparticles using Fungi, Bacteria and Actinomycetes provides advancement over chemical and physical synthesis as it is cost effective and environmental friendly.

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