



## RESEARCH ARTICLE

### GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING *ASPERGILLUS FUMIGATUS* AND THEIR ANTIBACTERIAL ACTIVITY

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#### ABSTRACT

Antibiotic resistance is one of the world's most pressing public healthcare problems. People who become infected with drug-resistant microorganisms usually spend more time in the hospital and require a form of treatment that uses two or three different antibiotics and is less effective, more toxic, and more expensive. Silver nanoparticles (AgNPs) are attractive option because they are non-toxic to the human body at low concentrations and have broadspectrum antibacterial actions. The biosynthesis of nanoparticles has received increasing attention due to the growing need to develop safe, cost-effective and environmentally friendly technologies for nano-materials synthesis. In this report, silver nanoparticles (AgNPs) were synthesized using a reduction of aqueous Ag<sup>+</sup> ion with the culture supernatants of *Aspergillus fumigatus*. The reaction occurred at ambient temperature and in a few hours. The bioreduction of AgNPs was monitored by ultraviolet-visible spectroscopy, and the AgNPs obtained were characterized by transmission electron microscopy and X-ray diffraction. Furthermore, the antimicrobial potential of AgNPs was systematically evaluated. The synthesized AgNPs could efficiently inhibit various pathogenic organisms, including bacteria and fungi. The current research opens a new avenue for the green synthesis of nano-materials and AgNPs have the potential to serve as an alternative to antibiotics and to control microbial infections such as those caused by multidrug-resistant pathogens.

**Key words:** silver nanoparticles; *Aspergillus fumigatus*; Antimicrobial activity.

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#### INTRODUCTION

Nanotechnology provide a platform to modify and develop the important properties of metal in the form of nanoparticles having promising applications in biomarkers, diagnostics, cell labelling, contrast agents for biological imaging, drug delivery system an antimicrobial agents and nano drugs for treatment of various disease (Sing *et al.*, 2011). Nanotechnology is a multidisciplinary field that covers wide range of physical, chemical, electrical, biological and electronics engineering. Nanotechnology is expected to be the basis of many main technological innovations in the 21st century. Research and development in this field is growing rapidly throughout the world. For the synthesis of nanoparticles, a number of chemical methods exist in the literature all these protocols involve toxic chemicals, which have been a matter of great concern for environmental reasons. Consequently, researchers in the field of nanoscale material synthesis and assembly have been eagerly looking at biological systems for an alternative (Ahmad *et al.*, 2003).

Microorganisms such as bacteria, yeast, fungi and actinomycetes have been described for the formation of nanoparticles and their applications (Sastrayet *al.*, 2003). The metabolic activity of microorganisms can lead to precipitation of nanoparticles in external environment of a cell, the fungi being extremely good candidates for synthesis of nanoparticles. The extracellular synthesis of silver and gold nanoparticles by the fungus *Colletotrichum* sp. or *Aspergillus fumigatus* has been reported. The mass production fungi is easy, for synthesis of nanoparticles. Due to their physiochemical properties, silvernanoparticles have been widely employed and currently used as anti-bacterial agents in food storage, textile and health industries and health industries, for bio labeling and biosensors. The antimicrobial activity of silver nanoparticles has now been well established and they posses anti inflammatory, anti viral and anti fungal activity (Prakash *et al.*, 2012). Filamentous fungi are more advantageous over the bacteria and algae because fungi having fungal mycelia mesh which can withstand flow pressure and agitation and other conditions in the bioreactors. The fungi like

*Fusarium solani* USM3799, *F. oxysporium*, *Aspergillus niger*, *Coriolus versicolor* are capable of synthesizing silver nanoparticles (Saha *et al.*, 2011).

## MATERIALS AND METHODS

### Screening of nanoparticles synthesizing fungal isolate from secondary metabolite producing fungi

The fungal strain was freshly inoculated on a potato dextrose broth in flask. The flask was incubated on orbital shaker at 300°C and agitated at 150 rpm for 3 days. The fungal biomass was harvested after 3 days by sieving through Whatman No 1 filter paper, later thoroughly washed with deionized water to remove the other components in the media from the biomass. Typically 20g of fresh and clean biomass was taken into Erlenmeyer flask containing 200 ml of deionized water and the flask was incubated at 30°C for 3 days and agitated at 150rpm. Later the filtrate was obtained through passage of culture media through Whatman No-1 filter paper.

Fifty milliliters of filtrate was taken into 250 ml of Erlenmeyer flask and mixed with 1 mM AgNO<sub>3</sub> (0.017 g AgNO<sub>3</sub> /100ml) as final concentration. The flasks were incubated at 30° C in dark room up to 3 days. Control was maintained (without addition of AgNO<sub>3</sub>, only cell filtrate) with the experimental flask. In order to use for future experiments the brownish yellow color Ag- NPs solution was stored in amber color bottles.

### Characterization of nano particles

The synthesized Ag-NPs were first characterized by UV-Visible spectrophotometer (UV-2450, Shimadzu) in the range of 320 - 560 nm with control as the reference. The surface plasmon resonance peaks found noted to be reliably around 420 nm region, further the Ag-NPs kept at room temperature for three months to test their stability. Analysis of Ag-NPs by FTIR (IR Prestige21, Shimadzu) through spectrum scanning range 450- 4000 cm<sup>-1</sup> at resolution of 4 cm<sup>-1</sup> was carried out. The biologically synthesized silver nanoparticles were freeze dried on lyophilizer and the powdered sample was used for X-ray diffraction (XRD) analysis. The XRD analysis was performed by X'Pert Pro A Analytical X-ray diffractometer instrument using CuK $\alpha$  radiation ( $k = 1.54056 \text{ \AA}$ ) in the range of 20-80 at 40 keV.

### Characterization of antibacterial activity

Antibacterial property was performed by using the "Nathan's Agar Well Diffusion" technique. Potato dextrose agar medium were distributed in 100ml conical flask and were sterilized. After that the media was poured in to sterilized petriplates. Chloramphenicol was taken as a positive control and DMSO was taken as negative control for antibacterial activity. Inocula were spread over the surface of agar plates with sterile glass spreader. Four wells were made at equal distance using sterile cork borer. To test the antibacterial activity of Ag-NPs sample were made to a final concentration of 100mg/ml. Aliquots of (60 $\mu$ g/ml, 80 $\mu$ g/ml) the extract was poured on each well. After completion of incubation period at 30°C and 24hrs the susceptibility was measured by considering the inhibition zone diameter around each well to the nearest mm.

## RESULTS AND DISCUSSION

### Extracellular synthesis of Ag-NPs

A comprehensive study of extracellular synthesis of Ag-NPs was carried out in this research work. The fungal biomass after 72hours incubation was filtered and the filtrate was subjected to AgNO<sub>3</sub>. The reaction was started after 24hours incubation in dark condition, the pale yellow colour of the cell free filtrate (CFF) changed to dark brownish yellow color indicating the formation of Ag-NPs (Fig-1) which correlates with the results obtained by Ingle *et al.*, (2008) and Prameela *et al.*, (2013). The filtrate showed changes in colour from almost yellow to brown; this is a clear indicator of the formation of silver nanoparticles in the reaction mixture. Formation of dark brown is due to the surface plasmon resonance property of silver nanoparticles (Yen and Mashitah, 2010; Ravishankar and Jamuna, 2011; Hemath *et al.*, 2010; Sangeetha *et al.*, 2012; Soheyla *et al.*, 2013). There is no color change noted in the control flask incubated in the same environment.

In the present study biosynthesis of silver nanoparticles were synthesized at room temperature it was compared with chemical and physical methods of synthesis, green synthesis method provides at low cost, environment friendly, easily scale up for large scale synthesis. The previous reports by Sivakumar *et al.*, 2011 gives a green synthesis method there is no need to use high pressure, energy, temperature and toxic chemicals.



Fig-1- Synthesized nanoparticles

### SEM Analysis

The shape and size of the result particles were elucidated with the SEM. Nanoparticles observed are spherical with a small percentage of elongated particles. It is a variation in particle size, and the average size was 20 nm for *Aspergillus sp.* The obtained nanoparticles are in the range of size approximately 1-50 nm and few particles are agglomerated (Narasimha *et al.*, 2013). SEM analysis suggested that most of the particles are spherical shape. Scanning electron microscopy (SEM) image of AgNPs synthesized by fungus *Aspergillus sp.* shown in Fig-2. The morphology of the nanoparticles was spherical in nature. Sadowski *et al.*, (2008) reported scanning electron microscopy has provided morphology and size details of the synthesized particles. Reported the nanoparticles synthesized in the range of 100nm and shape of the particles can be affected due to drying (Sundraramoorthi *et al.*, 2009; Ahmad *et*

al., 2002). Has reported the nanoparticles in the size range of 50-100nm. Nithya *et al.*, (2014) reported the SEM micrograph of Ag-NPs being formed using *Aspergillus niger* cell free filtrate.

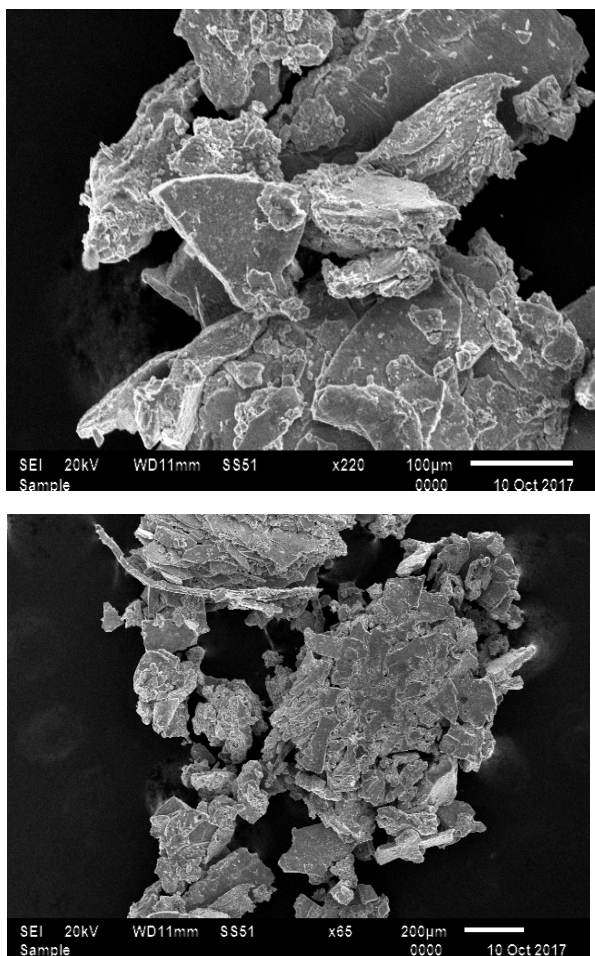


Fig. 2. SEM images of nanoparticles

### Characterization by UV- visible spectroscopy

Synthesized Ag-NPs absorption capacity was observed at every 24hrs of incubation. Fig-3 shows the absorption maxima (0.72) band at 450nm after 72hr of incubation which is surface Plasmon resonance, monitored by ultraviolet-visible spectroscopy (UV-2450, Shimadzu) of colloidal AgNPs solution. Up to some extent the  $\text{AgNO}_3$  intensity was increased with time and was clearly recorded in the spectrum. The brownish yellow color is due to the “surface of plasmon resonance of deposited silver nanoparticles” that is, “the color of the Ag- NPs is due to the coherent and collective oscillations of the surface electrons”(Link *et al.*, 2003). The peak formed between 420 to 450nm is the characteristic indication for the presence of the proteins and enzymes. These bioactive compounds are responsible for the reduction of metal ions for synthesis of nanoparticles (Wiley *et al.*, 2006).

### FTIR analysis of Ag-NPs

Silver nanoparticles were analyzed through FTIR (IR Prestige21, Shimadzu) to find out the interactions between silver and bioactive compounds produced by fungi. These bioactive compounds play major role in metal ion reduction, stabilization and synthesis of Ag- NPs. Transmission peak for the fungi containing silver nano particles were obtained at 3383, 2361, 1723, 1581, 1389, 1112, 616 showed in Fig-4.

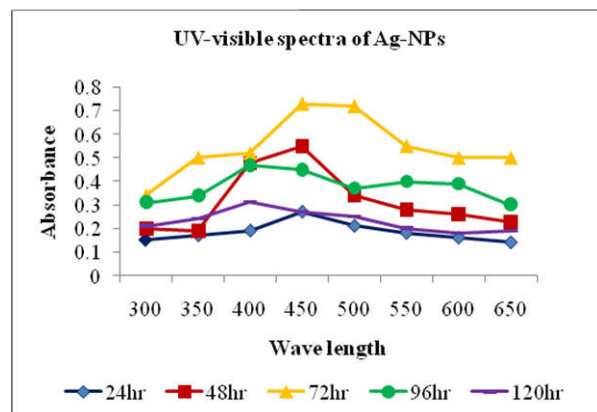


Fig. 3. UV-visible spectra of Ag-NPs synthesized by *Aspergillus fumigatus*

The absorption peaks 3383 corresponds to Amide C=O and Amine groups like wise 2361 corresponds to acid O-H, 1723 to Aldehyde, Ketone, Ester C=O, 1581 to Aromatic C=C, 1389 to C-O, 1112 to Ketones C-C stretch and 616 to Chloroalkanes C-C1 stretch. Thus the result indicates that the amide, amine, alkane, aldehyde, carboxylic acid, alkyne groups of fungi.

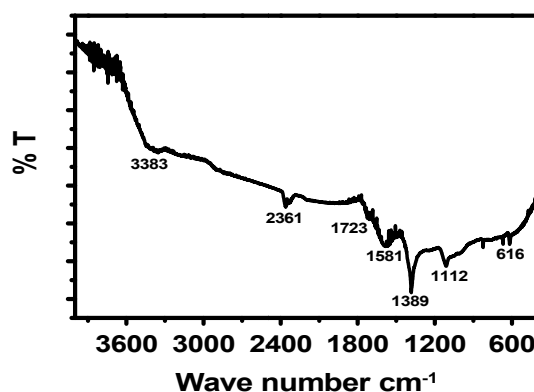


Fig. 4. FTIR analysis of Ag-NPs

### X-ray diffraction analysis

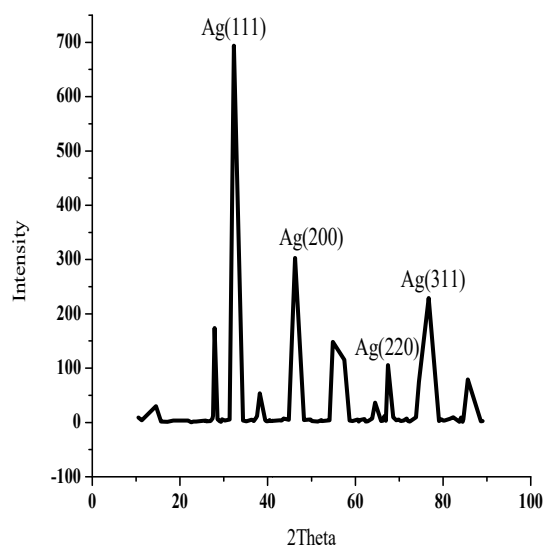


Fig. 5. X-ray diffraction analysis

The crystalline nature of the synthesized nanoparticles depicted with Bragg's peak 38.2o, 44.4o, 64.5o, 77.4o. The X-ray diffraction of silver nanoparticles using *Aspergillus fumigatus* are given in Fig-5.



XRD analysis showed three clear diffraction peaks corresponding to the (111), (200), (220) planes confirm the formation of AgNPs. Liangwei Du *et al.*, (2012) synthesized silver nanoparticles using *penicillium oxalicum* at two different pH- 8 and pH-12.61. Irrespective of the values of pH the AgNPs showed four characteristics diffraction peaks at 38.2°, 44.4°, 64.7°, 77.7°. These are corresponding at (111), (200), (220) and (311) Bragg's reflections respectively. AgNPs synthesized from *As.terreus* was examined by the XRD pattern showed 2 theta values at 32.3°, 45.1°, 75.9° assigned to the planes of (111), (200), (311) corresponds to face centered cubic structure of AgNPs. Shivaraj *et al.*, (2013) reported The XRD pattern shows peaks in the whole spectrum of 2θ values ranging from 0 to 80. A comparison of our XRD spectrum with the standard confirmed that the silver nanoparticles were in the form of nano crystals, as evidenced by the peak at 2θ value of 38 and integrated intensity value of (111) for silver. Our results correlate with Narasimha *et al.*, (2011) and Prema *et al.*, (2009). Sagar *et al.*, (2012) reported silver nanoparticles from *Aspergillus niger* confirmed by X ray diffraction analysis. The XRD peaks were observed corresponding to the (111), (200), (220), (311) planes at 2θ angles of 38.28°, 44.38°, 64.54°, and 77.64°, respectively. Some intense diffraction peaks at 2θ angles of 32.05°, 46.05°, 54.6° and 57.3°, might be related to AgCl which was owing to the chloride ions involved during preparation of the cell filtrate. Because of the biomass residue, other crystallographic impurities were also observed in the XRD profile.

## Conclusion

In this study, AgNPs were synthesized extracellularly by *A. fumigatus* at room temperature. The AgNPs were quite stable without using any toxic chemicals as capping agents. The spherical AgNPs ranged in size from 1 to 20 nm, and showed promising broad-spectrum antimicrobial activity. The ability to synthesize AgNPs as potential antimicrobial agents using *A.fumigatus* is highly promising for the green, sustainable production of nano-metals, and also enhances its widespread application as an important strategy.

## REFERENCES

Ahmed A, Mukherjee P, Senapathi D, Khan MI, Kumar R, Sastry M 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, Colloid. surf B, 28, 313-318.

Ingle A, Gade A, Pierrat S, Sonnichsen C, and Rai M 2008. *Curr Nanosci.*, 4: 141-144.

Liangwei Du, Qjuhong Xu, Meiyang Huang, Liang Xian, Jia-Xun feng, 2015. Synthesis of small silver nanoparticles under light radiation by fungus *Penicillium oxalicum* and its application for the catalytic reduction of methylene blue, *Mat.Chem. Phy.*160, 40-47.

Link S and El- Sayed MA 2003. Optical properties and ultrafast dynamics of nanocrystals, *Annu.ev Phys Chem*, 53:331.

Narasimha G, Praveen, Mallikarjuna K, Deva Prasad Raju, Mushrooms (*Agaricusbisporus*) mediated biosynthesis of silver nanoparticles, characterization and their antimicrobial activity. *Int.J.Nano Dim.* 2011. Vol. 2(1), p. 29-36.

Nithya *et al.* 2014. reported the SEM micrograph of Ag-NPs being formed using *Aspergillus niger* cell free filtrate.

Prakash R and Thiagarajan P. 2012. Syntheses and characterization of silver nanoparticles using *Penicillium sp.* isolated from soil. *International Journal of Advancer Scientific and Research* 1 138-149.

Prameela Devi T, Kulanthaivel S, Deeba Kamil, Jyothi Lekha Borah, Prabhakaran Yen SC, Mashitah MD 2012. Characterization of Ag Nanoparticles Produced by White – Rot Fungi and Its in Vitro Antimicrobial Activities. *Int. Arab. J. Antimicrob. Agents* 2:1-8

Prema, P. and Rincy, R. 2009. Fabrication and characterization of silver nanoparticle and its potential antibacterial activity. *Biotech. Biopro. Engg.* 14: 842-7.

Ravishankar RV, Jamuna BA. 2011. Nanoparticles and their potential application as antimicrobials. *Commun.Curr. Res. Technol. Adv.* 197-209.

Sadowski, Z., H.I. Maliszewska, B. Grochowalska, 21. Ghodake, I. Polowczyk and T. Kozlecki 2008. Synthesis of silver silver nanoparticles using microorganisms. *Sci. Poland*, 26(2): 419-424.

Sagar, G. and Ashok, B. 2012. "Green synthesis of silver nanoparticles using *Aspergillus niger* and its efficacy against human pathogens," *European Journal of Experimental Biology*, vol. 2, no. 5, pp. 1654–1658.

Saha S, Chattopadhyay D and Acharya K. 2011. Preparation of silver nanoparticles by bio-production using *Nigrospora oryzae* culture filtrate and its antimicrobial activity. *Digest Journal of Nanomaterials and Biostructures* 6 1519-1528.

Sangeetha G, Rajeshwari S, Venckatesh R. 2012. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International.* 6:693-700.

Sastry, M.; Ahmad, A.; Khan, I.M.; Kumar, R. 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete, *Current Sci.*, 85, 2, 162-170

Shivaraj Ningangouda, Vandana Rathod, Jyoti H, 2013. Dattu Singh, Prema K., Manzoor-ul- Haq, *Int J Pharma Bio Sci.* 4(2) 222-229.

Singh R, Singh NH. 2011. Medical application of Nanoparticles in biological imaging cell labelling Antimicrobial agents and anticancer nanodrugs, *J. Biomed. Nanotechnol.* 7,489-503.

Sivakumar J., Premkumar C., Santhanam, Saraswathi N., 2011. Biosynthesis of silver nanoparticles using *Calotropis gigantea* leaf. *African Jpurnal of Basic and Applied Science.* 3(6): 265-270.

Soheyla H, Barabadi H, Gharaei-Fathabad E, Naghibi F 2013. Green Synthesis of Silver Nanoparticles Induced by The Fungus *Penicillium citrinum*. *Trop. J. Pharm. Res.*12:7-11.

Sundaramoorthi, C., M. Kalaivani, Dhivya Mariam Mathews, S. Palanisamy and A. Kalaiselvan Rajasekaran 2009. Biosynthesis of silver nanoparticles from *Aspergillus niger* and evaluation of its wound healing activity in experimental rat model. *Int. J. PharmTech Res.*, 1(4): 1523-1529.

Wiley BJ, Im SH, McLellan J, Siekkkinen A and Xia Y 2006. Maneuvering the surface Plasmon resonance of silver nanostructures through shape-controlled synthesis, *J Phy Chem.*110 (32):15666-75.

Yen SC, Mashitah MD 2012. Characterization of Ag Nanoparticles Produced by White –Rot Fungi and Its in Vitro Antimicrobial Activities. *Int. Arab. J. Antimicrob. Agents* 2:1-8.