ISSN: 2348-8948
Vol: 2; Issue: 9

RESEARCH ARTICLE

Mycosynthesis of Silver Nanoparticles using Aqueous Extract of *Aspergillus Flavus* Mycelium and its Characterization.

R.MANIMOZHI and R.ANITHA*

*Department of Plant Biology and Plant Biotechnology, Ethiraj College For Women, Ethiraj Salai, Egmore ,Chennai -600008,India.

Date Received:	Date of Accepted:	Date Published:
14-Aug-2014	11-Sep-2014	19-Sep-2014

Abstract:
Silver nanoparticles are progressively used in various fields of biotechnology and medicine. The aim of the present study was to synthesize and characterize silver nanoparticles using filamentous fungi. Both cell free and mycelial extract of *Aspergillus flavus* were used for the study. The conditions optimal for the synthesis were standardized. The mycelia grown in Potato Carrot Broth at pH 9.5 and 100ºC produced better results. Maximum absorption at 409nm and bands at 3444cm^{-1} and 2930cm^{-1} and corresponding bending vibrations at 1640cm^{-1} were characteristics of the silver nanos in UV-Visible and Fourier Transform-InfraRed analysis. The SEM and TEM studies revealed various sizes and shapes of the nanoparticles.

Keywords: *A.flavus,* Mycelial extract, Potato Carrot Broth, Silver nanoparticles, TEM.

Introduction

Nanotechnology is the new era of science and technology which deals with matter at atomic or molecular level. It helps to modify or design properties of nanoparticles ,which has applications in diagnostics, biomarkers, imaging, cell labeling, antimicrobial agents and drug delivery for treatment of diseases [1,2] in optical devices, water treatment, production of paints, sensor technology and sunscreen lotions. They are reported to be effective mosquito repellent [3]. Nanoparticles have a unique property that they exhibit surface to volume ratio [4]. Due to the increase surface to volume ratio the properties of the nanoparticle is changed [5]. Earlier, nanoparticles were produced by physical and chemical methods using toxic chemicals which are hazardous.

Hence biosynthesis has been considered to be an alternative approach [6]. The metal-microbe interaction has contributed to the progress of bio-nanoparticles synthesis [7]. Mycosynthesis of silver nanoparticles have gained importance because it is a low cost protocol, non-toxic, less labourious and above all it is eco-friendly. Nanoparticles can be synthesized extracellularly or intracellularly. The present study involves the mycosynthesis and characterization of silver nanoparticles from both cell free and mycelial extracts of filamentous fungi *Aspergillus flavus*.

MATERIALS AND METHODS

SYNTHESIS OF SILVER NANOPARTICLES:

Aspergillus flavus culture was obtained from the culture collection maintained in the Department. It was incubated in Potato Sucrose Broth, Potato Carrot Broth, Czapek's Dox Broth and Richard's Broth for 48 hrs at room temperature 30 ±2 ºC to obtain actively growing mycelium. The separated mycelia was washed thoroughly and incubated in sterile distilled water for 48 hrs under a shake culture at 100rpm to obtain cell free extract .To obtain the mycelium extract, the mycelium was homogenized with sterile distilled water and filtered. Both the cell free and mycelial extracts of the fungi were treated with 0.05 mM of silver nitrate. It was maintained at room temperature 30±2ºC as well as boiled at 100 ºC for 5 min. Change in the colour of reaction mixture was observed.

UV-VISIBLE SPECTROSCOPY:

The culture filtrate and mycelial extract of *Aspergillus flavus* was analysed in UV –VIS spectroscopy at wavelength 300-600nm and the corresponding peaks were recorded.

EFFECT OF pH and TEMPERATURE ON SYNTHESIS OF SILVER NANOPARTICLES:

The mycelial extract was adjusted to pH 5.5 to 9.5 using 1N Sodium hydroxide and 1N Hydrochloric acid treated with 0.05mM silver nitrate were maintained at room temperature 30±2 ºC as well as boiled at 100 ºC for 5 min in a water bath. The change in the colour was used as a criteria to determine the effective pH and temperature.

FT-IR SPECTROSCOPY:

The silver nanoparticles was mixed with Potassium bromide (1:100)**.** The spectrum of samples was recorded on Shimazdu IR prestrige – 21FT-IR instrument with a diffuse reflectance mode (DRS-8000) attachment. All measurements were carried out in the range of 650-4500 cm-1 at a resolution of 4cm-1. This range was used to study the fundamental vibrations and associated rational- vibrational structure.

SCANNING ELECTRON MICROSCOPY AND TRANSMISSION ELECTRON MICROSCOPY:

The dark brown coloured silver nanoparticles were centrifuged for 20 min at 10,000g. The pellet thus obtained was washed thrice to remove any residual silver nitrate. The pellet of silver nanoparticles thus obtained was air dried, suspended in a small aliquote of ethanol and observed in Scanning Electron Microscope Hitachi 3400N and Transmission Electron Microscope Techai 10 Philips and photographed.

RESULTS AND DISCUSSION

SYNTHESIS OF SILVER NANOPARTICLES:

Both culture filtrate and mycelial extracts were capable of reducing silver nitrate of 0.05mM. The minimal concentration of silver nitrate (5mM) was sufficient for growth of silver nanoparticles [8]. Mycelial extract of *Aspergillus flavus* were more efficient in synthesizing silver nanoparticles at comparatively lesser concentration of silver nitrate. The colour change after the reduction of silver ions was intense brown Fig.(1). A similar result was reported earlier [9]. The change in colour indicates the completion of reaction with silver ions. The colour change ranged from natural colour to yellowish brown [10]. The intensity of colour is correlated with increase in the number of silver nanoparticles synthesized [11]. Mycelium of *Aspergillus flavus* grown in Potato Carrot Broth was more efficient in synthesizing silver nanoparticles. The synthesis of nanoparticles using *Schizophyllum commune* mycelium was already reported [6].

Figure- 1 Mycosynthesis of silver nanoparticles with aqueous mycelium extract of *A.flavus* at 100°C for 5mins

UV-VIS SPECTROSCOPY:

The Silver nanoparticle was confirmed with UV-Visible spectroscopy. Those synthesized using mycelial extract had maximum absorption at 409.00nm and cell free extract has maximum absorption at 404.20 Fig.(2a) and Fig.(2b) A peak between wavelength 370 and 390 nm was reported[6] In the UV-visible spectrum, a peak between 420 nm indicated the presence of silver nanoparticles. It is due to the reduction of metal ions aided by secondary metabolites in the cell [12].

EFFECT OF pH AND TEMPERATURE ON THE SYNTHESIS OF SILVER NANOPARTICLE :

Conditions for the mycosynthesis of silver nanoparticles with Aspergillus flavus mycelial extract was standardized. An alkaline pH of 9.5 was efficient; however an acidic pH of 5.5 and 6.5 were not effective in mediating the synthesis. Hence it is suggested that an alkaline condition is required for the synthesis. A similar finding was reported [13]. A boiling temperature of 100 ºC was favorable for the rapid synthesis of nanoparticles. The fungal extract incubated at 100°C resulted in more number of silver nanoparticles[13]. It is evident that under an alkaline condition high temperature initiates the

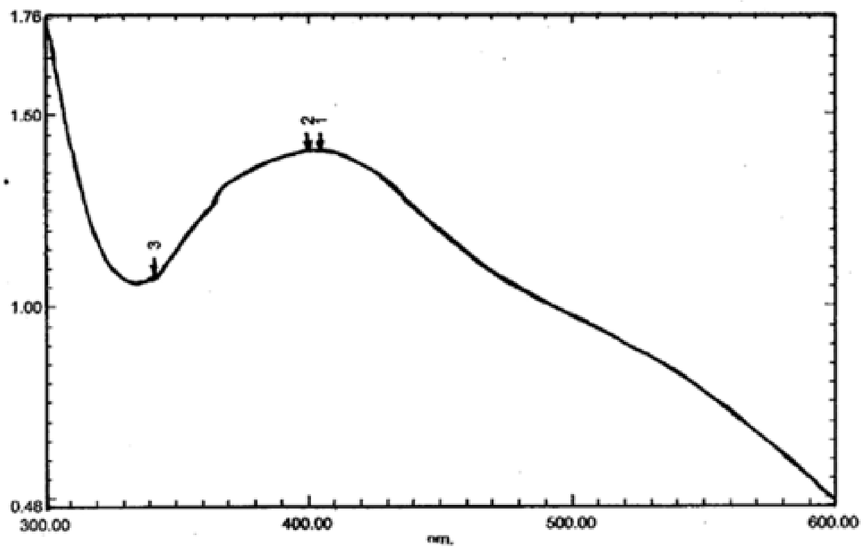

Figure- 2a. UV-Vis spectrum of silver nanoparticles obtained through mycosynthesis using aqueous extract of *Aspergillus flavus* mycelium

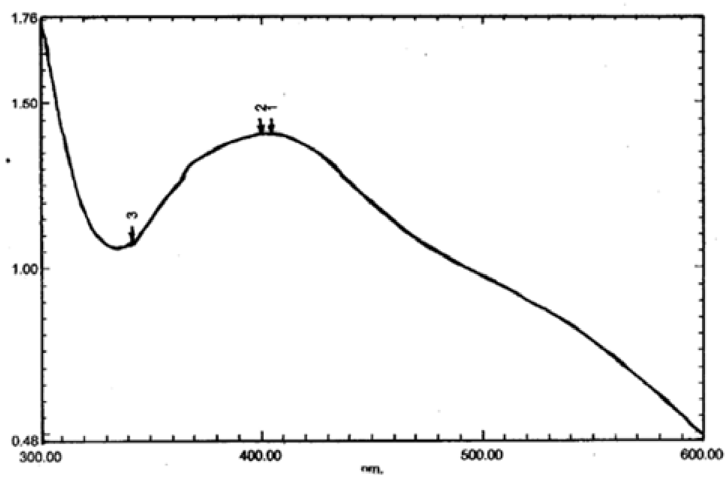

Fig 2b UV-Vis spectrum of silver nanoparticles obtained through mycosynthesis using cell free extract of *Aspergillus flavus*

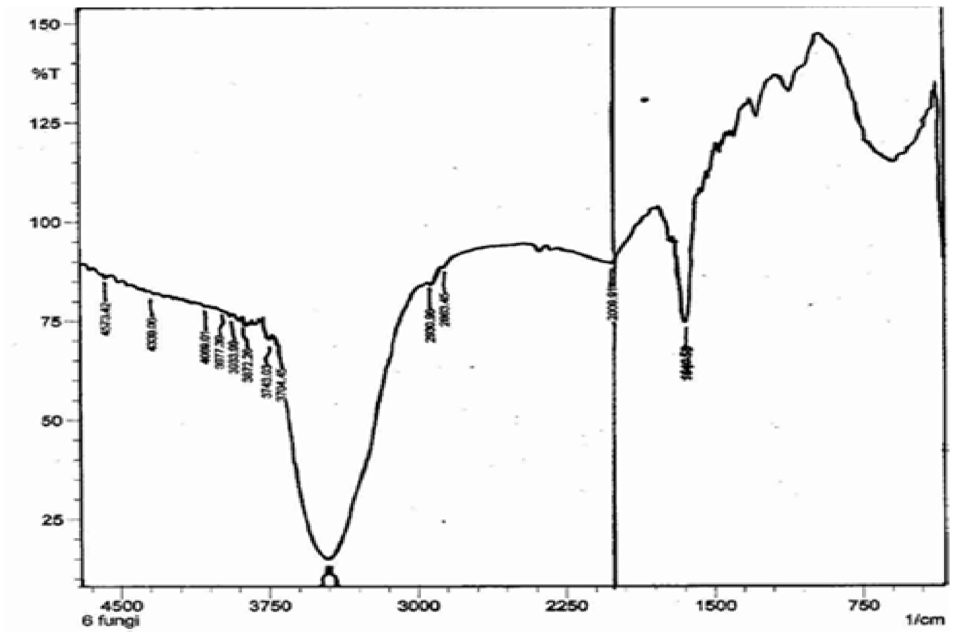

Figure-3. FT-IR spectrum of silver nanoparticles obtained through mycosynthesis using Aspergillus flavus mycelium extract

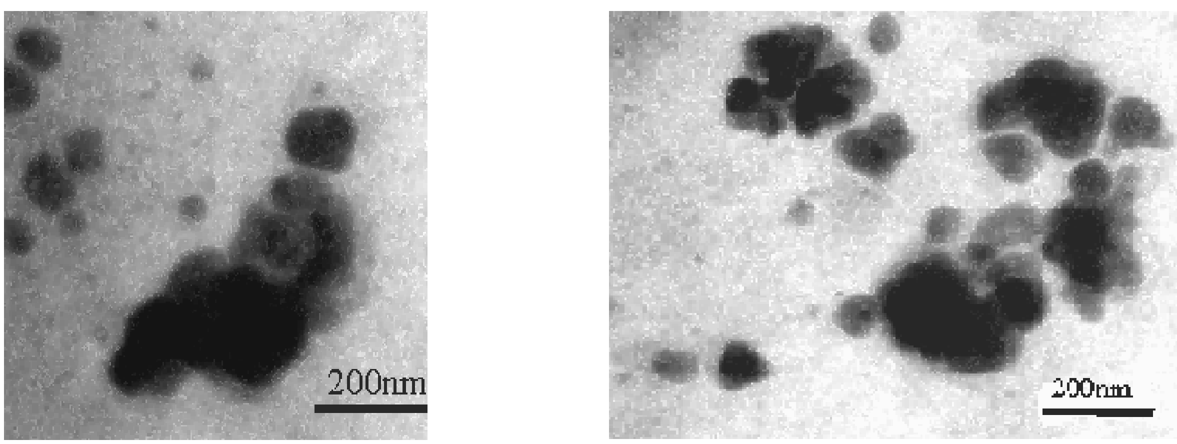

Plate-2 .Transmission Electron Microscopy image of silver nanoparticles synthesized using *Aspergillus flavus* mycelium extract reduction process. A similar observation was reported that the pH and temperature controlled synthesis of silver nanoparticles [14].

FT-IR SPECTROSCOPY:
The bands at 3444cm -1 and 2930cm -1 were the vibrations of primary and secondary amines respectively and their bending vibrations were seen at 1640cm -1 Fig. (2).The amide linkages between amino acids in proteins gives a characteristic infra red spectrum. The FT-IR spectrum showed two bands at 1625cm-1 and 1516cm -1 which correlates to the bending vibrations of the amide I and amide II of the proteins. [11].The band at about 1743 cm-1 indicates carbonyl stretch vibrations in ketones, aldehydes and carboxylic acids [15].

SCANNING ELECTRON MICROSCOPY AND TRANSMISSION ELECTRON MICROSCOPY:
The Scanning electron microscopy image Hitachi showed nanoparticles of varied shaped. It contained large spherical, irregular and few triangle shaped nanoparticles with aggregates . Spherical silver nanoparticle aggregates were reported in the study [16]. There is increase in size of the particles with increase in temperature [17]. In the present study also the size of the particles were found to be relatively larger due to high temperature. Fungi like *Fusarium oxysporum* [18], *Aspergillus niger* [19], *Coriolus versicolor* [20] are more tolerant to silver and produced nanoparticles of different sizes pre-dominantly spherical shaped [21].

The Transmission electron microscopy image showed spherical, irregular and few triangle shaped silver nanoparticles and also aggregates of nanoparticles to form rosette shape were observed. The nanoparticles were 23.88nm in size (Plate-2). The spherical and rod shaped silver nanoparticles with 30-50nm were found [10]. Silver nanoparticles less than 70nm were synthesized from *Trichophyton mentagrophy* [22]. A similar observation was reported showing silver nanoparticles with single (25-50 nm) spherical or in aggregates (100 nm) [23].

CONCLUSION
Mycosynthesis of silver nanoparticles is a low cost protocol, completely safe and provides a route for sustainable production of nanoparticles.From the present study it can be concluded that *Aspergillus flavus* mycelial extract with alkaline pH and boiling temperature is effective in the synthesis of silver of silver nanoparticles and the produced particles exhibited antimicrobial activity as well. It is suggested that it can be exploited at a larger scale production of silver nanoparticles and can be used in various biomedical applications.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

ACKNOWLEDGEMENT: The authors wish to acknowledge the Principal and The Head of the Department for their support and encouragement. We would also like to thank Mrs.Kalaiselvi of the Instrumentation Centre, Ethiraj College for Women, for her timely help.

REFERENCE
1. Le, A.T., Huy, P.T., Tam, L.T., Tam, P.D., Hieu, N., Huy, T., 2011. Novel silver nanoparticles: synthesis, properties and applications. Int. J. of Nanotechnol. 8:278-290.
2. Singh, R., Singh, N.H., 2011. Medical Application of nanoparticles in Biological Imaging, Cell Labelling, Antimicrobial Agents and Anticancer Nanodrugs. J.Biomed. Nanotechnol. 7(4): 489-503.
3. Namita Soni., Soam Prakash., 2013. Possible Mosquito Control by Silver Nanoparticles Synthesized by Soil Fungus (*Aspergillus niger* 2587). Advances in Nanoparticles. 2(2): 125-132.
4. Annamalai, A., Babu, S.T., Jose, N.J., Sudha, D., Lyza, C.V., 2011.Biosynthesis and characterization of silver and gold nanoparticles using aqueous leaf extraction of *Phyllanthus amarus* Schum & Thonn. World Appl Sci J. 13(8): 1833–1840.
5. Raimondi, F., Scherer, G.G., Kotz, R., Wokaun, A., 2005. Nanoparticles in energy technology: examples from electochemistry and catalysis. Angew Chem Int Ed. 44: 2190–2209.
6. Yen San., Chan and Mashitah., Mat Don., 2012. Instantaneous Biosynthesis of Silver Nanoparticles by Selected Macro Fungi. Australian Journal of Basic and Applied Sciences. 6(1): 222-226.
7. Muthukrishnan Lakshmipathy., Anima Nanda., 2013.Biocatalysts Of Silver Nanoparticles Synthesized Using An Environmental Isolate, *Serratia marcescens* S01. International Journal of ChemTech Research. 5(3): 1162-1168.
8. Helinando, P. de Oliveira., Leonardo, S. Cavalcanti., Noelly, B. Cavalcanti., Ismara, de S. Nascimento,, Sergio, F. Pascholati., Luis, F. P. Gusmao., Alan Greison, C. Macedo., Mateus Matiuzzi da Costa., 2013. Antimicrobial activity of silver nanoparticles synthesized by the fungus *Curvularia inaequalis.* African Journal of Biotechnology. 12(20): 2917-2923.
9. Nelson Durán,, Priscyla, D. Marcato., Gabriel, I.H. De Souza., Oswaldo, L. Alves., Elisa Esposito., 2007. Antibacterial Effect of Silver Nanoparticles Produced by Fungal Process on Textile Fabrics and Their Effluent Treatment. Journal of Biomedical Nanotechnology. 3(2): 203–208.
10. Prashant, R. Singh., Balaji raja., 2011. Biological synthesis and characterization of silver

nanoparticles using the fungus *Trichoderma harzianum.* Asian J .exp.biol.sci. 2(4): 600-605.

11. Swarup roy., Triparna mukherjee., Shatarupa chakraborty., Tapan kumar das., 2013. Biosynthesis,characterisation and antifungal activity of silver nanoparticles synthesized by *Aspergillus foetidus* MTCC8876. Digest journal of Nanomaterials and Biostructures. 8(1):197-205.
12. Panchanathan Manivasagan., Jayachandran Venkatesan., Kalimuthu Senthilkumar., Kannan Sivakumar., Se-Kwon Kim., 2013. Biosynthesis, Antimicrobial and Cytotoxic Effect of Silver Nanoparticles Using a Novel *Nocardiopsis* sp. MBRC -1. BioMed Research International Volume, Article ID 287638, 9 pages.
13. Manju bala., Vedpriya arya., 2013. Biological synthesis of silver nanoparticles from aqueous extract of endophytic fungus *Aspergillus fumigatus* and its antibacterial action. International Journal of Nanomaterials and Biostructures. 3(2): 37-41.
14. Hemath Naveen, K.S., Gaurav Kumar., Karthik L., Bhaskara Roa, K.V., 2010. Extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Penicillium sp.* Archieves of applied science research. 2(6): 161-167.
15. Saha, S., Sarkar, J., Chattopadhyay, D., Patra, S., Chakraborty, A., Acharya, K., 2010. Production of silver nanoparticles by a phytopathogenic fungus *Bipolaris nodulosa* and its antimicrobial activity. Digest Journal of Nanomaterials and Biostructures. 5(4)887-895.
16. Arpana pancholi., Ruchi kurapa., Niranjan kurane., 2013. Antibacterial activity of silver nanoparticles. International Journal of Pharmaceutical research and bio-science. 2(1): 358-371.
17. Safekordi, A.A., Attar, H., Ghorbani, H.R., 2011. Optimization of Silver Nanoparticles, Production by *E. coli* and the study of reaction kinetics. ICCEES. 346-350.
18. Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M.I., Kumar, R., Sastry, M., 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surfaces, B :Biointerfaces. 28(4): 313-318.
19. Gade, A.K., Bonde, P., Ingle, A.P., Marcato, P.D., Durán, N., Rai, M.K., 2008. Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. J Biobased Matter Bioenergy. 2(3): 243-7.
20. Sanghi, R., Verma, P., 2009. Biomimetic synthesis and characterisation of protein capped silver nanoparticles. Bioresource Technology. 100(1): 501-504.
21. Bhainsa, K.C., D'Souza, S. F., 2006. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*. Colloids and Surfaces B: Biointerface. 47(2): 160-164.
22. Maryam Moazeni, Niloofar Rashidi, Ahmad, R. Shahverdi., Fatemeh Noorbakhsh., Sassan Rezaie., 2012. Extracellular Production of Silver Nanoparticles by Using Three Common Species of Dermatophytes: *Trichophyton rubrum,Trichophyton mentagrophytes* and *Microsporum canis*. Iranian Biomedical Journal. 16(1): 52-58.
23. Hassan Korbekandi., ZeynabAshari , SiavashIravani, Sajjad Abbasi., 2013. Optimization of Biological Synthesis of Silver Nanoparticles using *Fusarium oxysporum.* Iranian Journal of Pharmaceutical Research, 12 (3):