Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

A COMPARATIVE STUDY ON BIOSYNTHESIS OF SILVER NANOPARTICLES USING FOUR DIFFERENT FUNGAL SPECIES

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Received: 08 Oct 2013, Revised and Accepted: 30 Oct 2013

ABSTRACT

This is a comparative report on biological synthesis of silvernanoparticles using four different fungal species viz. *Rhizopus nigricans, Fusarium semitectum, Colletotrichum gloeosporioides* and *Aspergillus nidulans*. The obtained nanoparticles were characterized by instrumentation techniques like UV-VIS and XRD.Morphological study of the nanoparticles was carried out by Transmission electron microscopy. In this paper we report the use of fungi for the extracellular synthesis of Silver nanoparticles from Silver nitrate ($AgNO_3$) solution i.e. through the reduction of Silver ions into Silver nanoparticles.

Keywords: Silvernanoparticles, Biosynthesis, UV-VIS, XRD, TEM.

INTRODUCTION

Nanotechnology is emerging field of science which involves synthesis and development of various nanomaterials. At present different types of metal nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate, and silver. These nano materials are used in various fields such as optical devices [1], catalytic [2], bactericidal [3], electronic [4], sensortecnolog [5], biological belling [6] and treatment of some cancers[7]. Nanotechnology involves tailoring of materials at the atomic level to attain unique properties, which can be suitably manipulated for the desired application [8]. Currently, there is a growing need to develop environmentally benevolent nanoparticles synthesis processes that do not use toxic chemicals in the synthesis protocol [9]. So the researchers in the field of nanoparticles synthesis and assembly have turned to biological inspiration. Many organisms, both unicellular and multicellular are known to produce inorganic materials either intra- or extracellularly [10].

Synthesis of Nanoparticles employing microorganisms has attracted much due to their usual optical, chemical, photoelectron chemical and electronic properties [11]. Many bacteria, fungi, yeast and plants either intra or extracellularly produce higher yield of nanoparticles with low expense [12]. Fungi are the best candidates in the synthesis of metal nanoparticles because of their ability to secrete large amount of enzyme [13], and easy to isolate from different sources like soil, air, plants etc.

In the present investigation we report the extracellular biosynthesis of silvernanoparticles employing *Rhizopus nigricans, Fusarium semitectum, Colletotrichum gloeosporioides* and *Aspergillus nidulans,* these are commonly available fungi. In the literature we have not come across use of these fungi for the production and stabilization of silvernanoparticles in aqueous system. The local environment suits for these fungi, hence, we have used these fungi in the present study. The present study includes time dependent formation of silvernanoparticles employing UV-VIS spectrophotometer, size and morphology by employing TEM, structure from powder x-ray diffraction (XRD) techniques.

MATERIALS AND METHODS

Materials

Potato dextrose agar (PDA), Silver nitrate, lacto phenol cotton blue stain, Czepak-dox Broth

METHODOLOGY

Sample collection

Infected leaves of *Pongamia* and *Sorghum* seeds were collected from Gulbarga University and from a grain shop in Gulbarga. Samples were transferred into sterile plastic bags and brought to Mycology and Plant pathology Laboratory and stored in laboratory conditions for further studies.

Isolation and inoculation

Infected leaves of *Pongamia* and seeds of *Sorghum* were surface washed by running water and kept in moist blotter for the growth of the fungi. After two days associated fungi were isolated and identified as *C.gloeosporioides, A.nidulans, F.semitectum,* and *R.nigricans* with the help of published literature. *C.gloeosporioides* and *A.nidulans* were isolated from *Pongamia* leaves whereas; *F.semitectum* and *R.nigricans* were from Sorghum. Isolated fungi were further sub cultured on PDA plates and slants in order to obtain pure culture. Pure isolates were inoculated to 250ml conical flask containing 100 ml liquid media Czepak-dox broth. The flasks were kept on rotator orbital shaker for seven days at 120rpm. Thereafter cultured material was sieved through a funnel separating media content. The biomass thus Obtained was inoculated in 250 ml conical flask containing 100ml sterilized distilled water and kept for 3 days on rotary 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 Silvernanoparticles

The fungi *R. nigricans, A.nidulans, F.semitectum* and *C. gloeosporioides* were selected for the production of sliver nanoparticles. Ten 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 treatment (without Silver nitrate, only biomass) was also run along with experimental flask. After 72 hours of time interval culture filtrate and Silver nitrate solutions turned into Orange brown due to reduction of Silver nitrate to Silver ions.

Characterization of synthesized silver nanoparticles

UV- Visible spectroscopy

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

XRD study

Powdered sample was used for X-ray diffraction; The Coherently diffracting Crystallography domain size of the Silver nano particle was calculated from the width of the XRD peaks using scherrer formula.

TEM analysis

Samples were prepared for Transmission electron microscopic Analysis (IIT Mumbai) TEM Technique was employed to see the size and shape of the synthesized silver nanoparticles

RESULTS AND DISCUSSION

It was observed that there is variation in the particle sizes around 35% of particles in 23nm range and 25% in 28nm range and 10% in 34nm ranges. The particles range from 13nm least to 74nm high, the TEM image suggests that the particles are polydispersed and are round in shape.

Four different fungal species were screened for Biological synthesis of Silver nanoparticles. Ten ml culture filtrate was treated with 1Mm Silver nitrate in 250ml conical flask the reduction of silver ion into silvernanoparticles during exposure to culture filtrate fungi was followed by changing color, colorless to brown. It is known that silvernanopatricles exhibits brown color in aqueous solution due to excitation of surface plasmonvibrations in silvernanoparticles [14] interestingly, culture filtrate of C.gloeosporioides and R.nigricans were changed the color within 30 seconds from colorless to brown whereas F.semitectum and A. Nidulans were changed the color within 300 seconds (fig1) the UV VIS-Spectroscopy of the synthesized silver nanoparticles were in the range of 425 ,430, 420 and 430 respectively.

XRD study

Obtained Silvernaonoparticles were purified by repeated centrifugation at 4000 rpm for 30 minutes by redispersing silvernanoparticles pellet into 10 ml double distilled water. After drying silvernanoparticles in room temperature structure and composition analysis was carried out by XRD (Fig2). The crystallite domain size was calculated by the width of the XRD peaks using scherrer formula $D=0.96\ \lambda/\beta \cos\theta$, where D is crystalline domain size perpendicular to reflecting planes, λ is the x-ray wavelength, β is the full width at half maximum and θ is the diffraction angle. The average particle size was 35-38 nm.

XRD analysis, peaks aasigned to the corresponding diffraction signals (111), (200), (220) and (311) facets of Silver. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the (111) plane.

TEM Analysis

Sample was prepared for Transmission electron microscopic Analysis (IIT Mumbai) TEM Technique was employed to see the size and shape of the synthesized silver nanoparticles; it is observed that there is variation in the particle sizes around 35% of particles in 23nm range and 25% in 28nm range and 10% in 34nm ranges. The particles range from 13nm least to 74nm high, the TEM image suggests that the particles are polydispersed (fig3) and are round in shape.

Table 1: UV-VIS Spectrum analysis shows time interval for changing colour of different fungal species

Fungi	Time taken for reduction after removing wrapper	UV-VIS peaks in nm	Color	Shape
A.nidulans	60 seconds	425-480	Colorless-brown	Crystalline
C.*gloeosporioides*	60 seconds	430-480	Colorless-brown	Crystalline
F.semitectum	300 seconds	420-470	Colorless-brown	Crystalline
R.nigricans	300 seconds	430-490	Colorless-brown	Crystalline

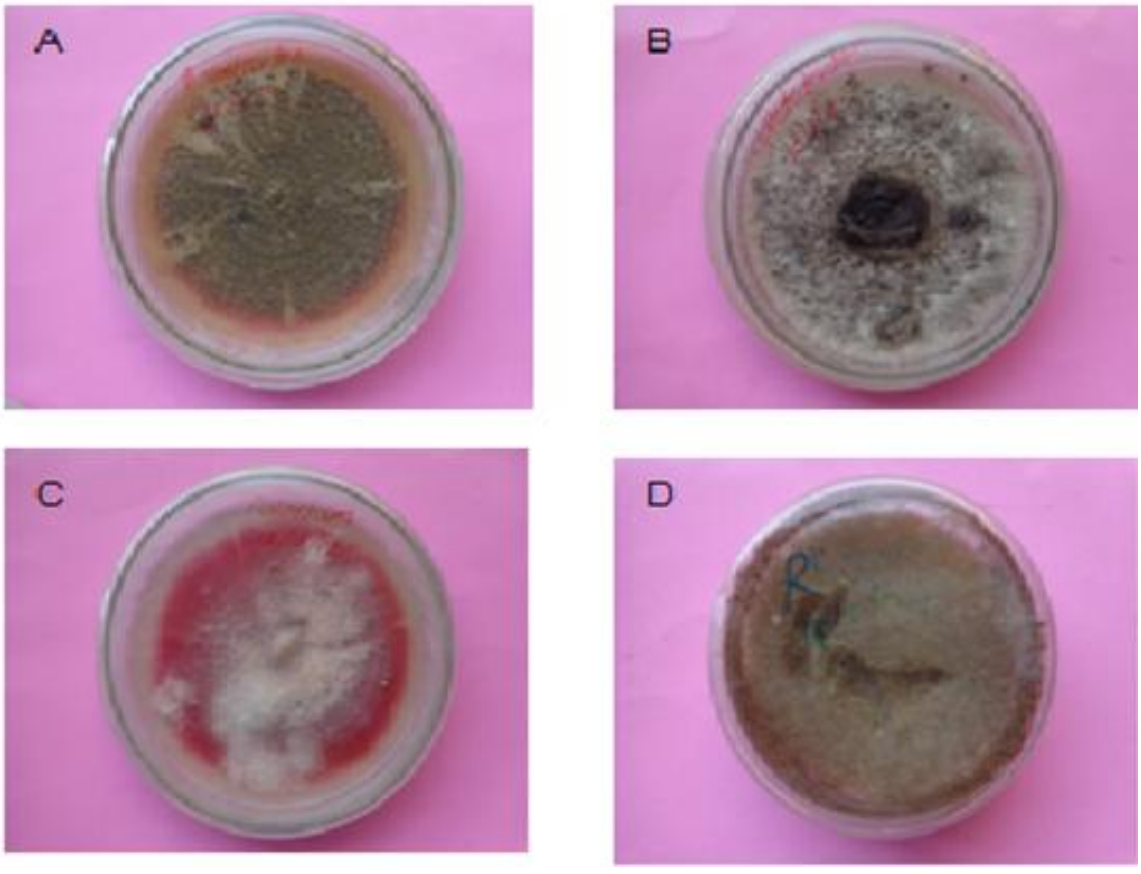

Fig. 1: Colony morphology of *A.nidulans* (A); *C.gloeosporiodes* (B), *F. semitctum*(C) *And R.nigricans* (D).

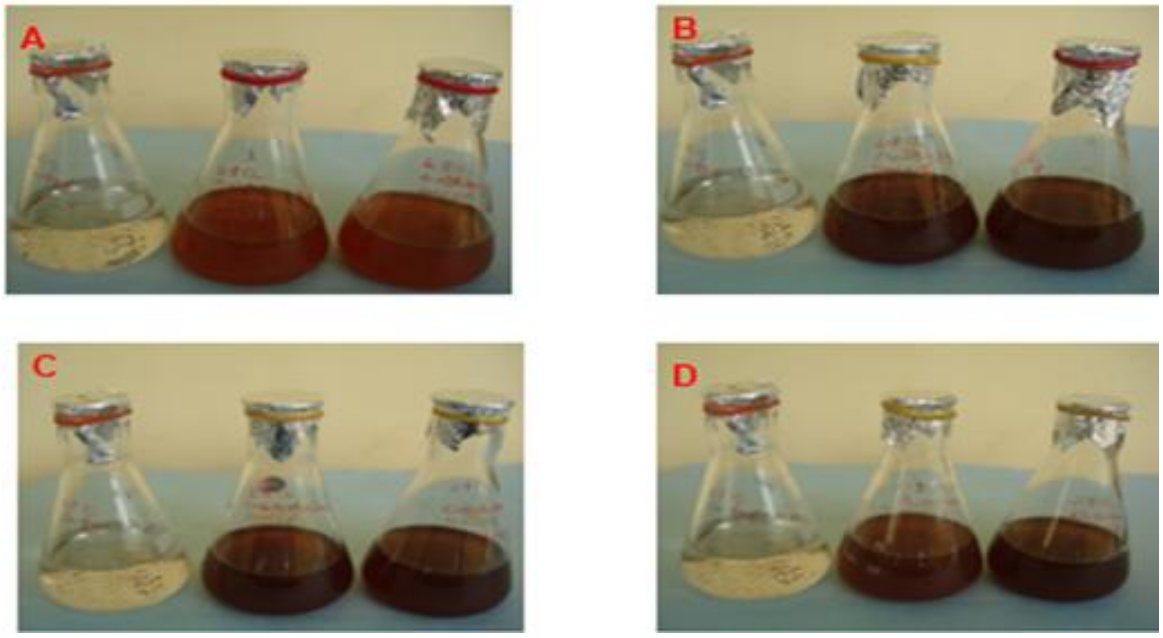

Fig 2: Biosynthesis of silver nanoparticles-color change reaction: conical flask containing the extracellular filtrate of *A.nidulans* (A) C.*gloeosporiodes* (B) *F. semitctum* (C) *and R.nigricans* (D). Biomass and conical flask containing the extra cellular filtrate of the fungal biomass exposes to silver nitrate solution for 72hrs.

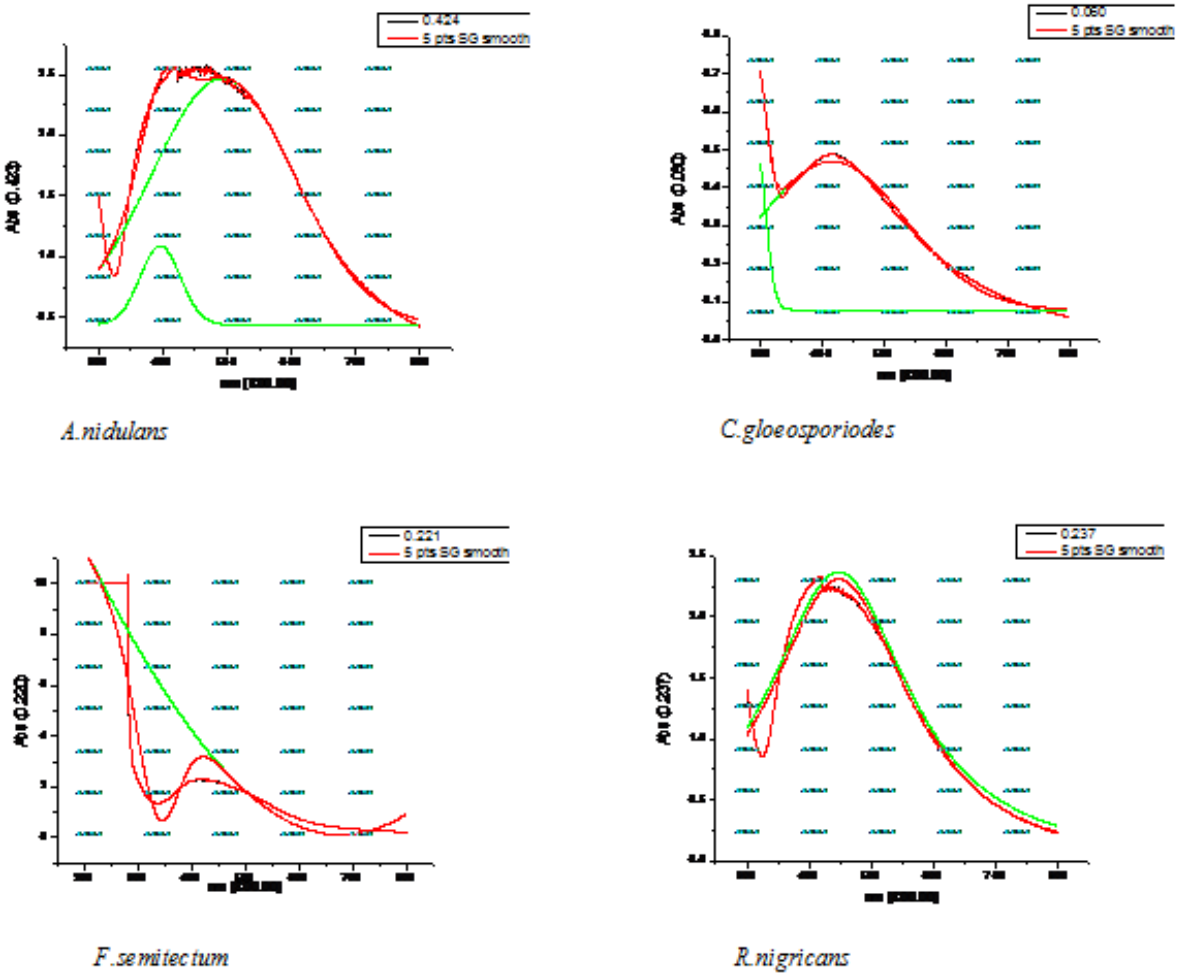

Fig 3: shows the UV-Vis spectrum of silver nanoparticles synthesized using *A.nidulans C.gloeosporiodes, F. semitctum and R.nigricans*. UV-Vis spectra recorded as function of time of reaction of an aqueous solution of 1mM silver nitrate solution with the fungal biomass filtrate. The time of reaction is indicated next to the respective curves.

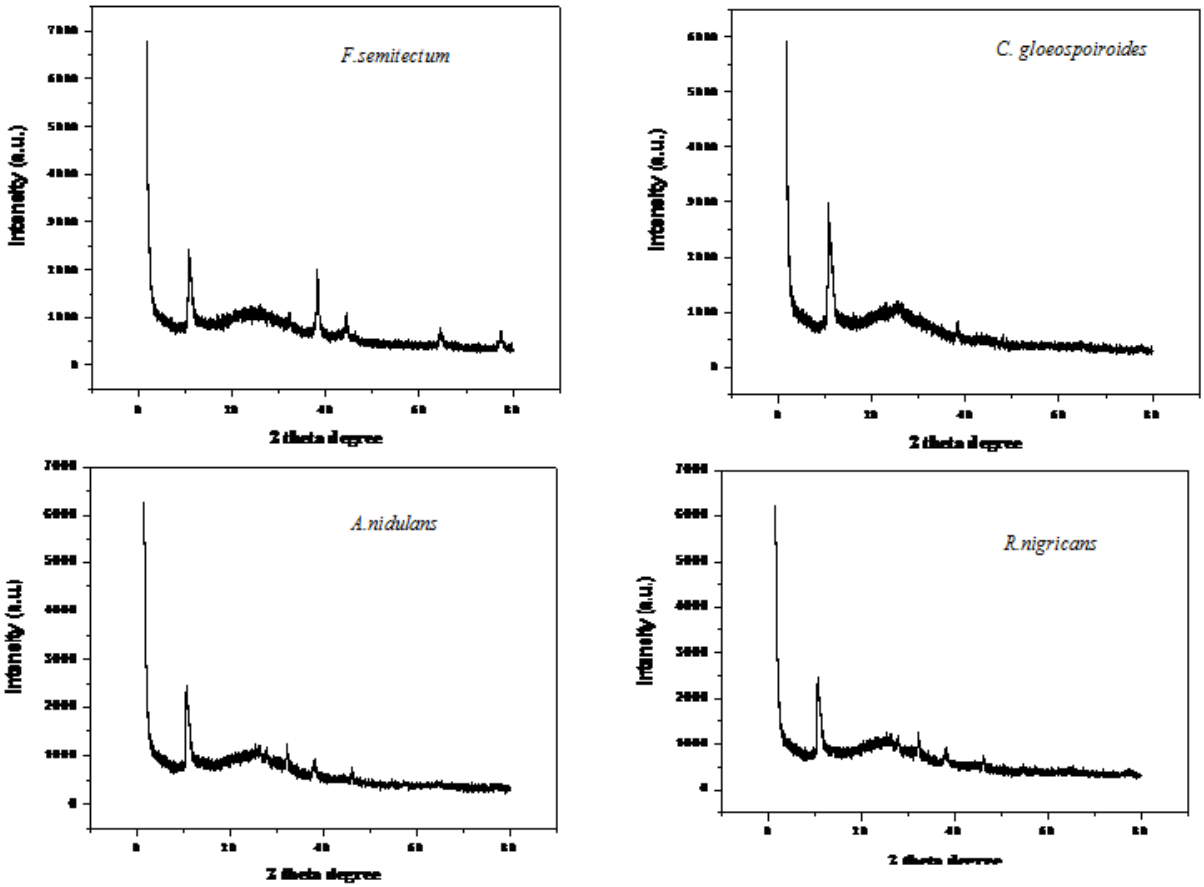

Fig 4: shows XRD analysis, peaks aasigned to the corresponding diffraction signals(111), (200), (220) and (311) facets of Silver. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the (111) plane, refractionpeaksusing the scherrer equqtion. The calculated average particle size of the silver was found to be 35-48nm.

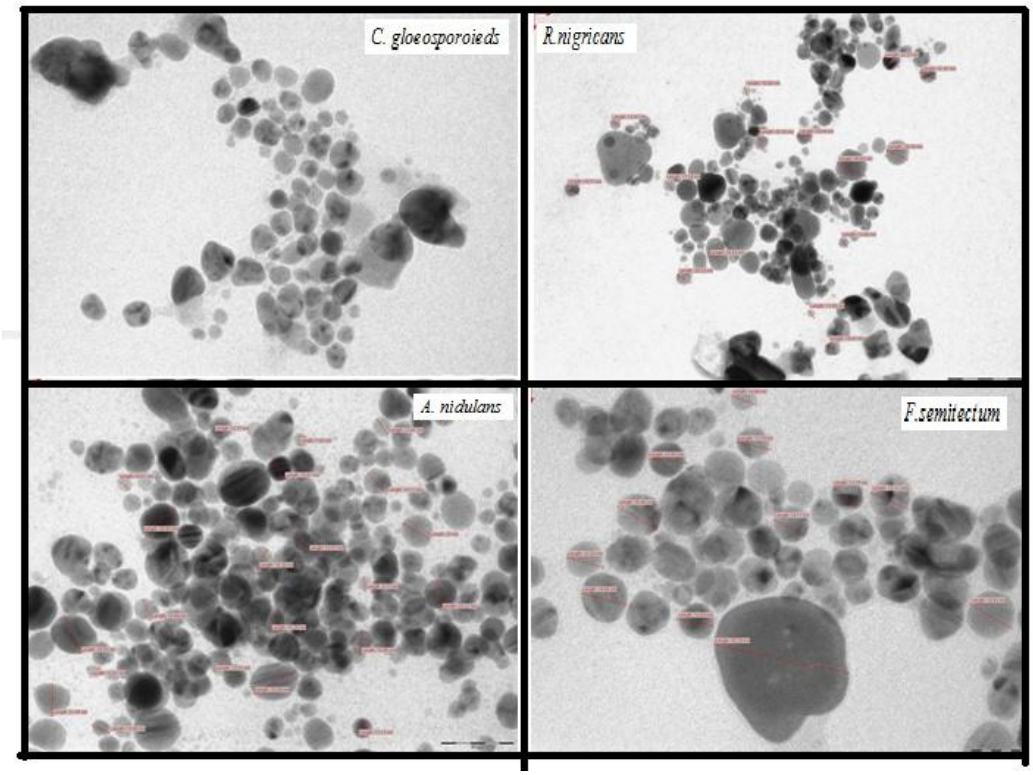

Fig 5: Transmission electron microscopic photographs of synthesized silvernanoparticles from *C.gloeosporoiedes, R.nigricans, A.nidulans* and *F.semitectum*.

CONCLUSION

In the present study nano particles were biologically synthesized using fungi *Rhizopus, Fusarium, Colletotrichum* and *Aspergillus sps* biomass isolated from leaves of Sorghum and *Pongamia pinneta* and also from *Sorghum* seed samples. The cell filtrate of fungi was challenged with 1mm Silver nitrate; change of mixture from color less to dark brown indicates the synthesis of Silver nanoparticles in the reaction mixture. And the crystallite domain size of synthesized silver nano particles was measured 35-38 nm by XRD analysis, shape and size of the silver nanoparicles was studied by TEM analysis. Results conclude that isolated fungi are prominent producer of Silver nano particles

ACKNOWLEDGEMENT

Authors wish to thank to Gulbarga University Gulbarga, Karnataka, India for providing Fellowship and also thankful to USIC and Physics departments (G.U.G) for UV spectrum and XRD analysis and IIT Mumbai for TEM analysis

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