Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2016 Vol.5 (3) July-September, pp. 40-45/Ravindra and Rajasab
Research Article

CHARACTERIZATION AND ANTIFUNGAL EFFECT OF SILVER NANOPARTICLES SYNTHESIZED FROM *COLLETOTRICUM GLOEIOSPORIOIDES*

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ABSTRACT
Biological production of nanoparticle using micro organism is a vast developing aria of nanobiotechnology, in this study *Colletotricum gloeiosporoiedes* was used for extracellular synthesis of silver nanoparticles. The silver nanoparticles were characterized by UV-VIS spectroscopy, TEM, and XRD. Studies showed the size and shape of the nanoparticles, size in the range of 8-35nm and shape round and polydispersed, reduction of silver nanoparticle into silver ion by *Colletotricum gloeiosporoied* was investigated. The nanopartical showed anti fungal activity against fungi *Stemphylium vericans*.

Keywords: *Silver Nanoparticles, Biosynthesis, UV-VIS, XRD, TEM, Antifungal*

INTRODUCTION
Nanobiotechnology is a vast growing field of science which includes production and development of various nanomaterials. Biologically synthesized silver nanoparticles have many applications, as in spectrally selected coatings for solar energy absorption, as intercelation material for electrical batteries, as optical receptors, as catalysts in chemical reactions and in bio-labeling. Silver has been recognized to have inhibitory action on microbes present in medical and industrial process (Lok *et al.,* 2007). Nanotechnology involves tailoring of materials at the atomic level to attain unique properties, which can be suitably manipulated for the desired application (Leiter, 2000).

Currently, there is a growing need to develop environmentally benevolent nanoparticles synthesis processes that do not use toxic chemicals in the synthesis protocol (Whitesides, 2003). Most recently, biosynthesis of nanoparticles using bacteria (He *et al.,* 2007; Husseiny *et al.,* 2007), fungus and plants (Raut *et al.,* 2009) so the researchers in the field of nanoparticles synthesis and assembly have turned to biological inspiration. Many organisms, both unicellular and multi cellular are known to produce inorganic materials either intra- or extracellularly (Kathiresan *et al.,* 2009).

Synthesis of Nanoparticles employing microorganisms has attracted much due to their usual optical, chemical, photoelectron chemical and electronic properties (Sastry *et al.,* 2013). Many bacteria, fungi, yeast and plant either intra or extracellularly produce higher yield of nanoparticles with low expense (Castro-longoria *et al.,* 2010).

Fungi are the best candidates in the synthesis of metal nanoparticles because of their ability to secrete large amount of enzyme (Basavaraj *et al.,* 2007), and easy to isolate from different sources like soil, air, plants etc. In this study we report the characterization, antifungal activity and synthesis of silver nanoparticles from *Colletotrichum gloeosporioides*. This is a commonly available fungus, the local environment suits for this fungus, and in the literature we have not come across use of this fungus for the production and stabilization of silver nanoparticles in aqueous system. Hence, we have used this fungus in the present study.

The present study includes time dependent formation of silver nanoparticles 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 seeds* were collected from Gulbarga University campus, 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 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, with* the help of published literature. Isolated fungus was further sub cultured on PDA plates and slants in order to obtain pure culture (figure 1). Pure isolate was inoculated to 250ml conical flask containing 100 ml liquid media Czepak-dox broth. The flask 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.

Figure 1: Shows Pure Isolate Colony Morphology of *Colletotricum Gloeosporiodes*

Biosynthesis of Silver Nanoparticles

Ten ml culture filtrate of *C. gloeosporioides* 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.

Antifungal Activity

The antifungal activity of silver nanoparticles was investigated by wells method. Potato dextrose agar plates were prepared, sterilized and solidified, after solidification fungal culture were inoculated on the plates. 2µml, 3µml silver nanoparticle was poured in the wells and kept for incubation in room temperature for five days. Zone of inhibition measured and compared with standard Bavestin and silver nitrate solution. The MIC of the silver nanoparticle was determined treating the isolated fungi

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 38nm ranges. The TEM image suggests that the particles are polydispersed and are round in shape. *Colletotrichum gloeosporioides was* 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 silver nanoparticles during exposure to culture filtrate fungi was followed by changing color, colorless to brown. It is known that silver nanopatricles exhibits brown color in aqueous solution due to excitation of surface plasmonvibrations in silver nanoparticles (Moharrer *et al.,* 2012) interestingly, culture filtrate of C. gloeosporioides was changed the color within 30 seconds from colorless to brown (figure 2) the UV VIS-Spectroscopy of the synthesized silver nanoparticles were in the range of 425, 430, 420 and 430 respectively.

Figure 2: Biosynthesis of Silver Nanoparticles- Color Change Reaction: Conical Flask Containing the Extracellular Filtrate of *Colletotricum Gloeosporiodes* Biomass after Exposes to Silver Nitrate Solution for 72 hrs

XRD Study

Obtained Silver naonoparticles were purified by repeated centrifugation at 4000 rpm for 30 minutes by redispersing silver nanoparticles pellet into 10 ml double distilled water. After drying silver nanoparticles in room temperature structure and composition analysis was carried out by XRD (Figure 3). 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 28-35nm.

XRD analysis, peaks assigned 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 (figure 4) and are round in shape.

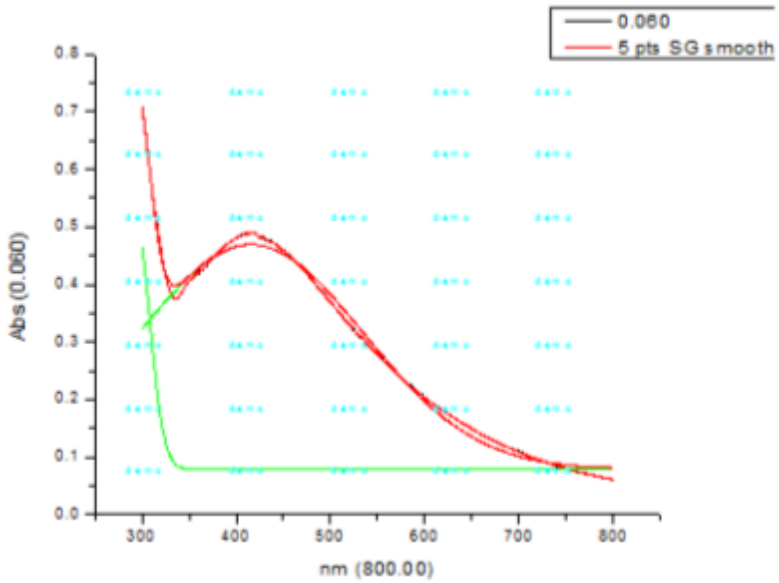

Figure 3: Shows the UV-V is Spectrum of Silver Nanoparticles Synthesized Using. *Colletotricum gloeosporiodes*. UV-V is Spectra Recorded as Function of Time of Reaction of an Aqueous Solution of 1mm Silver Nitrate Solution with the Fungal Biomass Filtrate. The Time of Reaction is Indicated Next to the Respective Curves

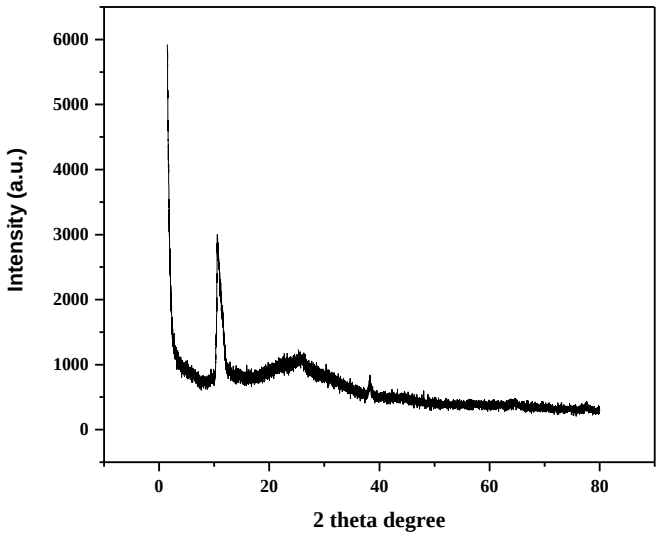

Figure 4: Shows XRD Analysis, Peaks Aasigned to the Corresponding Diffraction Signals (111), (200), (220) and (311) Facets of Silver. The Mean Particle Diameter of Silver Nanoparticles was Calculated from the XRD Pattern According to the Line Width of the (111) Plane, Refraction Peaks Using the Scherrer Equation. The Calculated Average Particle Size of the Silver was Found to be 28-35nm

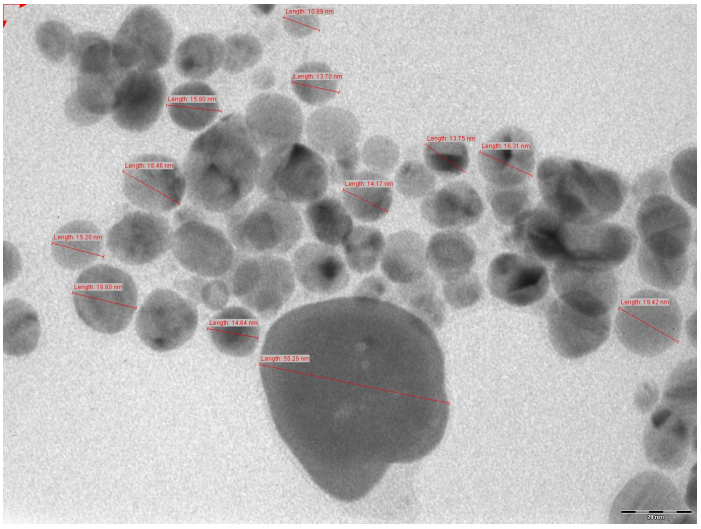

Figure 5: Shows Transmission Electron Microscopic Photographs of Synthesized Silver Nanoparticles from *Colletotricum Gloeosporoiedes*

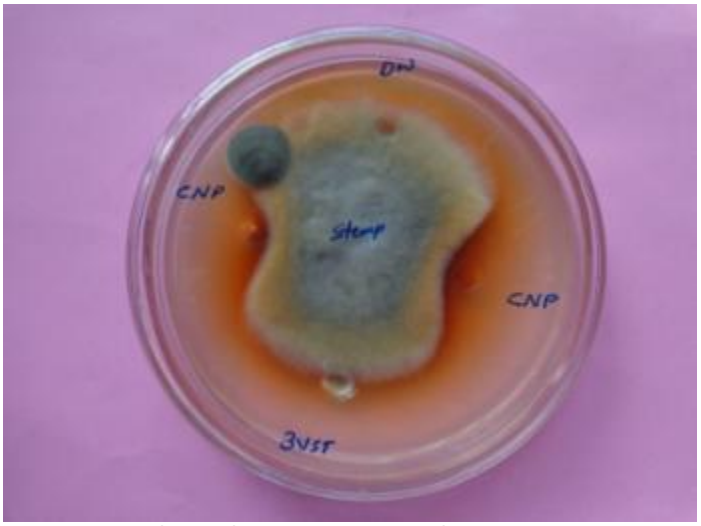

Figure 6: Shows the Photograph of Antifungal Activity of Silver Nanopatricles against *Stemphylium Vericans*

Antifungal Activity

The silver nanoparticle possess desecrate antifungal activity against isolated fungi at a concentration of 5mg/ml. the silver nanoparticles were compared favorably with standard bavistin at a concentration of the silver nanoparticls exhibited positive effect inhibiting the growth of tested fungi *Stempyliu vericans*, the MIC of silver nanoparticles were tested against which varied from 0.50 to 0.75mg/ml. whereas bavistin showed 0.60 to 80 mg/ml. The results indicated that biosynthesized AgNPs has positive antifungal effect.

Table 1: Shows Antifungal Activity of Silver Nanoparticles against *Stemphylium Vericans*

Fungi	**Zone of Inhibition**			
	Silver Nanoparticles		**Standard Bavistin**	
	0.50mg/ml	**0.75mg/ml**	**0.60mg/ml**	**0.80mg/ml**
Stemphylium Vericans	60%	65%	50%	58%

Conclusion

In the present study nano particles were biologically synthesized, characterized and investigated antifungal activity. *Colletotrichum gloeiosporoied biomass* isolated from leaves of *Pongamia pinneta*. The cell filtrate of fungus 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, antifungal activity by wells method. Results conclude that isolated fungi are prominent producer of Silver nano particles, and silver nanoparicles shows antifungal effect.

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.

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