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Bio-synthesis of antimicrobial silver nano-particles using plant pathogenic fungi *Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus* spp.

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Biosynthesis of silver nanoparticles via plant pathogenic Fungi names like *Aspergillus flavus*, *Rhizopus spp.* and *Fusarium oxysporum* were warned for latent synthesis of metal nanoparticles was examined. The hasty refuse of silver (Ag+) ions was scrutinized with UV-visible spectrophotometer and illustrated formation of silver nanoparticles within 30 minutes. Transmission electron microscopy (TEM) confirmed that the amalgamated silver nanoparticles are diversified from 13-55 nm and have the altering in shape like round, rod, uneven. Auxiliary the XRD examinations confirms the nano-crystalline period of silver structure. An FTIR examination confirms the Silver particles. The current cram, it divulges the escalating broth deliberation increases the rate of reduction and decreases the particle size. Subsequent to size, shape conformation and characterization of silver Nanoparticles, the antimicrobial activity have been detected and minimum inhibition conformed adjacent to phytopathogens.

Key words: Biosynthesis, AgNPs, UV, TEM. antimicrobial silver nanoparticles

INTRODUCTION

The countryside of nanotechnology is one of the most vigorous areas of investigation in contemporary materials science and expertise. It affords the aptitude to create materials, devices and systems with essentially new functions and properties (Karkare, 2008; Rajesh *et al.* 2009). In recent times, research in synthesis of nanoparticles using microbes and plant extracts acquisition more significance due to its eco-friendliness, elastics and main point is the avoidance of toxic chemicals (Mann, 1993). When evaluate to microbes, plant mediated synthesis is aggressively practicing by the researchers for its optimistic compensation like evasion of maintaining the microbial culture, time-consuming and cost effective (Farooqui, 2010; Arulkumar and Sabesan, 2010). The endophytic fungi *Aspergillus terreus* isolated from plant mediated can be used as a source for synthesis of silver nanoparticles and suggesting as an effective antibacterial agent. The endophytic fungi *Aspergillus terreus* isolated from *Calotropis procera* can be used as a source for synthesis of silver nanoparticles and suggesting as an effective antibacterial agent (Rani *et al.* 2017). Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. The ability to uncover the structure and function of biosystems at the nano scale, stimulates research leading to improvement in biology, biotechnology, medicine and healthcare (Arya *et al.* 2010). Nano-particle research is inevitable today not because of only application and also by the way of synthesis (Bankar *et al* 2010; Gopinath *et al.* 2012). Before hand, various microbes have been successfully used for the synthesis of biogenic metal nanoparticles (Singh *et al.* 2011).

The nanoparticles of noble metals are establish to have latent applications in various fields like microelectronics, optical devices, antibacterial effect, biological sensors, textile and filters (Elechignerra *et al.* 2005; Gajendran *et al.* 2007; Kathiresan *et al.* 2009). Amalgamation of nanoparticles employing microorganisms has

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attracted much due to their usual optical, chemical, photoelectron chemical and electronic properties. Many biological organisms, such as bacteria, fungi, yeast and plants either intra or extracellular (Castro-longoria *et al.* 2010), which are superior construction yields and with low expenses.

Fungi are the best contenders in the biosynthesis of metal nanoparticles, since of their capability to exudes large amount of enzyme (Nelson *et al.* 2005) and easy to isolate from dissimilar sources like soil, air, plants etc.,in the current report the biological schemes for the synthesis of silver nano particles using Fungi *Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus* spp. for their probable synthesis of metal nanoparticles.

MATERIALS AND METHODS

Sample collection

The Infected leaves and Sorghum seeds were collected from rural area of Mahabubnagar Dist, Telangana State, India respectively. Samples were transferred into sterile plastic bags and brought to the Palamuru University laboratory and stored in laboratory conditions for further processing.

Isolation and inoculation

Infected stems and field soil collected from agriculture area of were surface sterilized by running water and kept in moist blotter for the growth of the fungi, after two days associated fungi were isolated and identified as *Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus* spp. with the help of Barnet. Those fungi were further sub cultured on PDA plates and slants in order to obtain pure culture. Pure isolates were cultured in 250ml conical flask containing 100 ml liquid media Czepak-dox broth keeping on rotatory orbital shaker for seven days at 120 rpm. Thereafter cultured material sieved by funnel separating media content. Obtained biomass inoculated in 250 ml conical flask containing 100ml sterilized distilled water and kept for 3 days on Orbital 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 silver nanoparticles

10ml culture filtrate of the fungi was mixed with 50ml of 1 mM Silver nitrate solution in 250ml conical flask and agitated at room temperature; control (without Silver nitrate, only biomass) was also run along with experimental flask. After beginning and 72 hours of time interval culture filtrate and Silver nitrate are turned into Orange brown due to reduction of Silver nitrate to Silver ions, the formation of nanoparticles understood from the UV- Visible spectroscopy and X-ray diffraction studies.

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 spectrophotometer as a resolution of 1nm from 300 to 700 nm.

XRD study

Sample was powdered and prepared for X-ray diffraction (USIC Gulbarga university) and the target was cuk â (ë= 1.54A^0) the generator was operated as 40 KV and 30 mA current. The scanning range (20) was selected from 10 to 80 angle, scanning speed of 2.00 deg/min and chart spread of 20 mm/min were used for precise determination of lattice parameters. Highly purity selection powder was used as an internal standard. The Coherently diffracting Crystallography domain size (dxrd) of the Silver nano particle was calculated from X-ray diffraction (XRD) line broadening after subtracting the contribution from the cuk â component (Rachignor correction) and correcting the instrumental width. The integral line width was used in the Scherrer formula to calculate dxrd of the (III) plane for silver.

Agar-well diffusion technique

The assay was conducted by agar well diffusion method. About 15 to20 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. The bacterial strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 1 ml of fungal strains were spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium. Required concentration (20 mg^{-1},) of each fungi silver nano

particles were added to the wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37°C. After incubation for 48h, the plates were observed for zones of inhibition. The diameter zone of inhibition was measured and expressed in millimetres. 1mM $AgNo_3$ solution and aqueous plant extract was used negative control. Whereas positive control used Ketoconazole, Streptomycin against fungi and bacteria (1000 ìg/ml conc.). The experiments were conducted in triplicates. The same method was followed for testing antibacterial activity using nutrient agar medium incubated at 37°C for 18h.

RESULTS AND DISCUSSION

In the current work we have reported biological method for the production of silver nanoparticles using selected fungi. The size of the Silver nano particle found to be 35nm from XRD analysis.

biosynthesis initially started, whereas sample "c" shown full pledged silver nanoparticle synthesis and used for eco-friendly in nature, with climate change and protections.

Biosynthesis activity detected with Silver nitrate ions at the beginning and after 8 and 72 hours of reaction. It is observed that the color of the solution turned from colorless to brown after the 8 hour of reaction, indicating the formation of Silver nanoparticles. This arises owing to surface plasma vibration in the metal nanoparticles ((Maryam Moazeni et al. 2012; Saeed *et al.* 2012; Monroy *et al.* 2000). This important observation indicates that the reduction of Silver nitrate into Silver ions extracellularlly. Inset of Fig 3, shows distinct and fairly broad absorption band centered at 450 nm. The attendance of broad resonance indicates an aggregated structure of silver nanoparticles in the film.

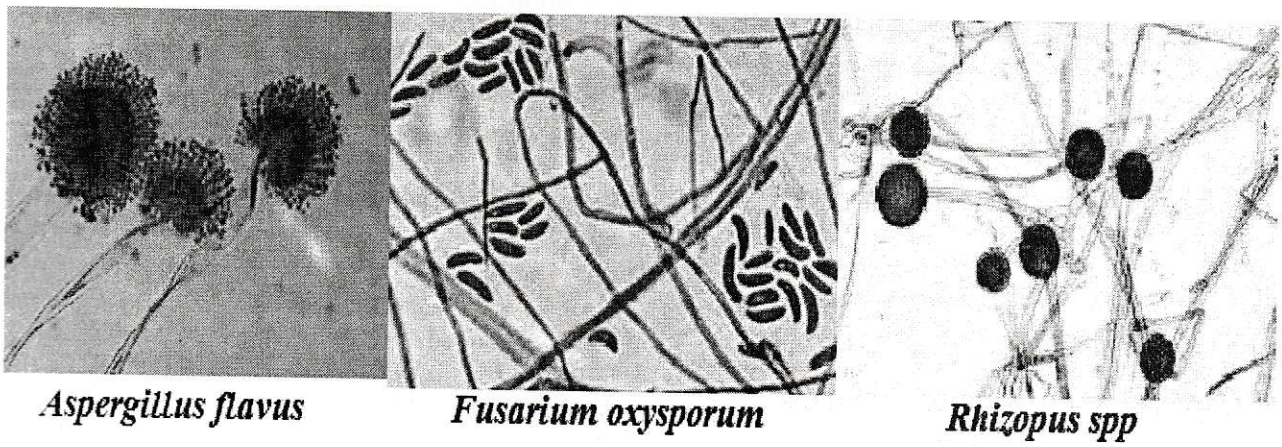


Fig. 1 : Isolated fungal mycelium plates

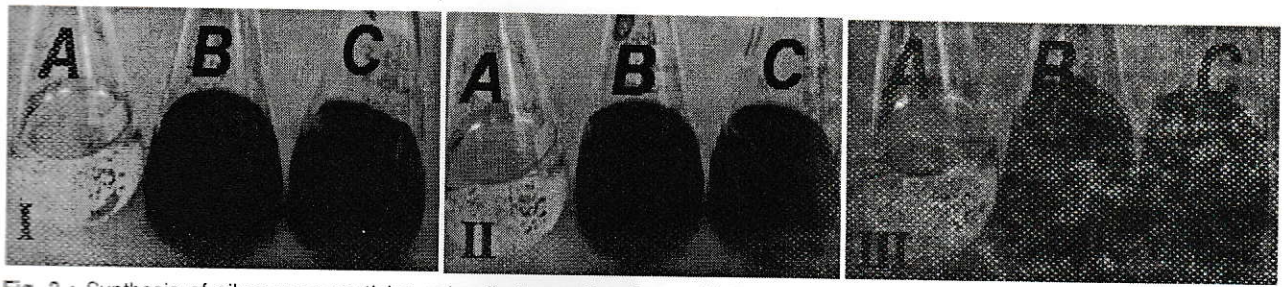


Fig. 2 : Synthesis of silver nano-particles using I) *Aspergillus flavus, II) Fusarium oxysporum III) Rhizopus* spp. aqueous extract treating with $AgNO_3$ solution at room temperature A) silver nitrate($AgNO_3$) solution, B) *Aspergillus flavus* aqueous extract, C) Formation of AgNPs.

Inset of Fig. 1 shown microscopic fungi mycelium shown clear evidence of species lvel of identification conformation. Whereas as Fig. 2 was shown I, II, III of each of samples with fungal biomass aqueous extract shown conical flask A, followed by sample treaded "B" was shown

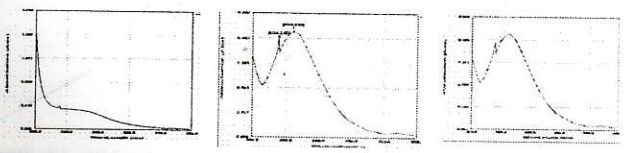


Fig. 3 : UV–vis spectrum of bio synthesized AgNPs showing surface Plasmon peak at 440 nm

UV-Vis spectra recorded from the aqueous silver nitrate solution after 8, 24, 48 and 72 hours of reaction with the biomass are shown as curve 2, 3 and 4 respectively in Fig. 2. It is clear that there is a presence of silver particles in solution, thus distinctly pointing to surface reduction of the silver ions as the most probable mechanism for the synthesis of silver nanoparticles by fungus (shown in Fig. 3).

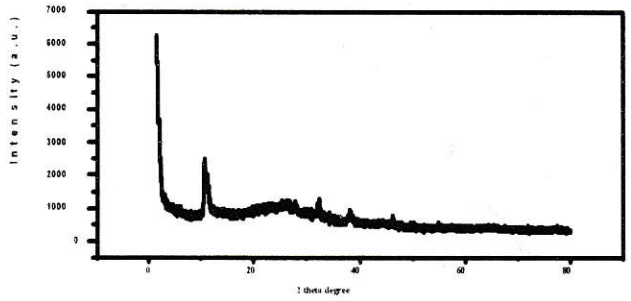


Fig. 4 : XRD patterns of bio functionalized AgNPs from *Aspergillus flavus*

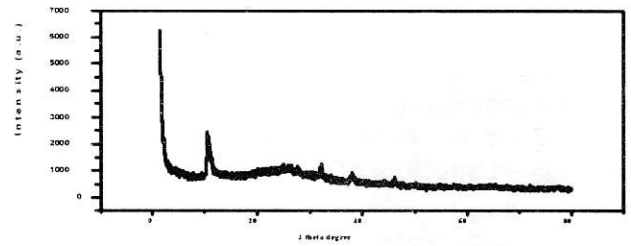


Fig.4 : XRD patterns of bio functionalized AgNPs from *Fusarium oxysporum*

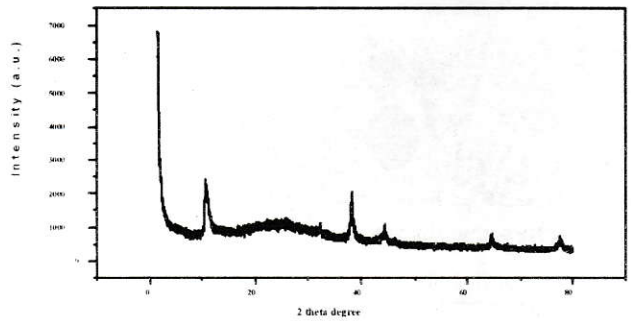


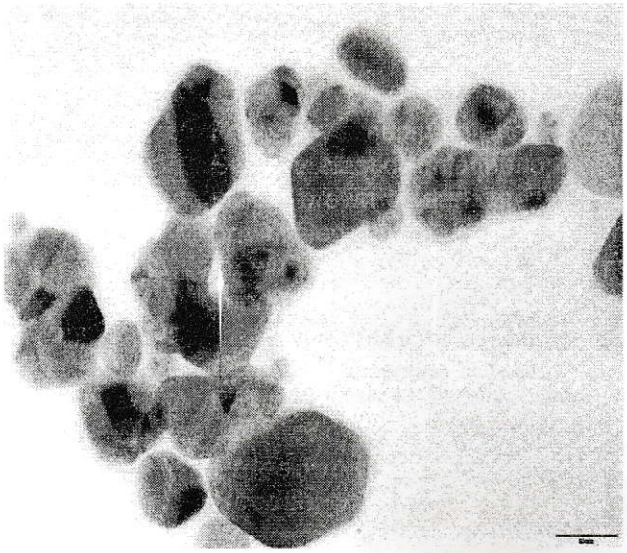
Fig.4 : XRD patterns of bio functionalized AgNPs from *Rhizopus* spp

A possible mechanism for the presence of silver nanoparticles in the fungal biomass could be the extra cellular reduction of the silver ions in solution followed by precipitation onto the cells.

Fig. 3, shows XRD analysis, peaks assigned to the corresponding diffraction signals (111), (200), (220) and (311) facets of silver. The mean particle diameter of silver nano particles was calculated from the XRD pattern according to the line width of the (111) plane, refraction peaks using the Scherer equation. The calculated average particle size of the silver was found to be 15-38nm (shown in Fig. 4).

TEM procedure was working to imagine the size and shape of AgNPs fashioned. A typical TEM image of in nature synthesized AgNPs, which advised that the particles are irregular in shape. Some are round, rod and triangular shaped particles with a changing size of 22.94–49.24 nm shown in Fig. 5A.

The FTIR analysed spectroscopy is a helpful method to learn the nucleus-casing morphology of AgNPs is as shown in Fig. 5. The two bands at



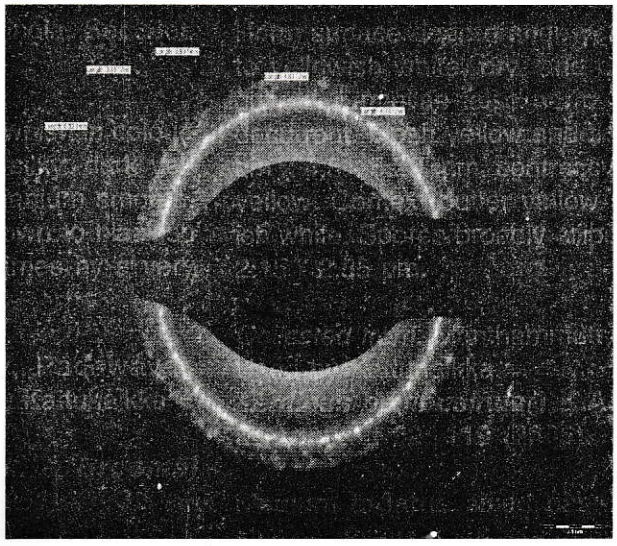


Fig. 5A : TEM image of bio functionalized AgNPs from *Aspergillus flavus*

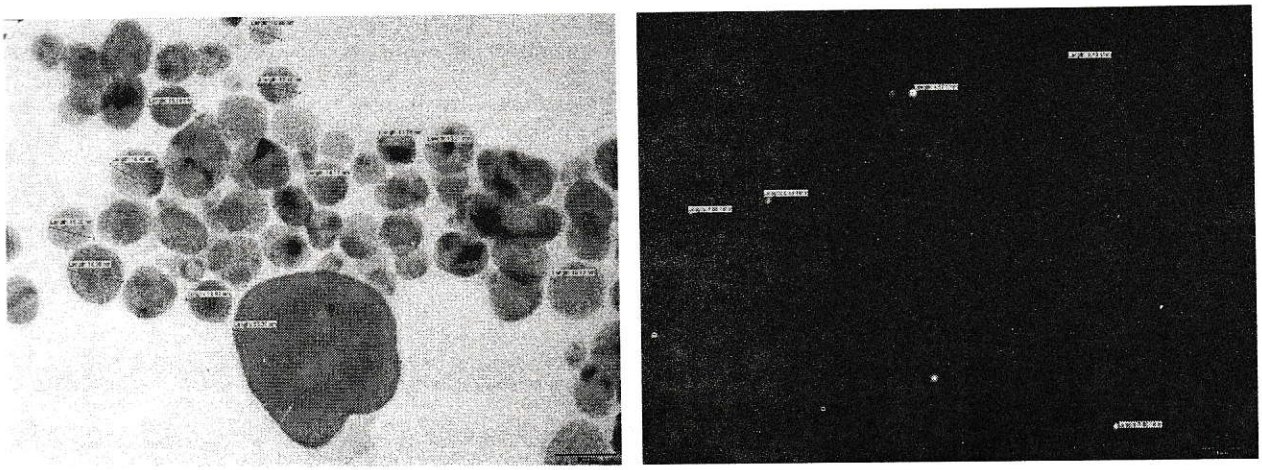


Fig. 5A : TEM image of bio functionalized AgNPs from *Fusarium oxysporum*

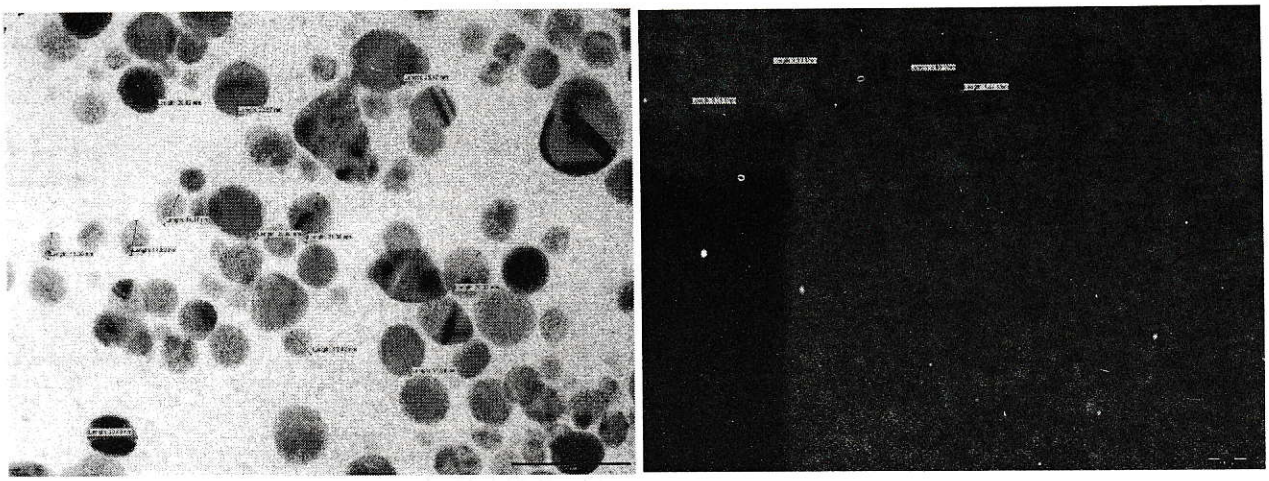


Fig. 5A : TEM image of bio functionalized AgNPs from *Rhizopus* spp

Table 1: Antimicrobial activity of silver nano particles synthesised from three phytopathogenic fungi extracts

Microbial strains Conc. 20 mg^{-1}	Different conc. of Ag NPs and inhibition zone in mm					
	AF	FO	RS	Fungi extract	1mM $AgNo_3$ Sol^n	Standard Ketoconazole/ Streptomycin sulphate 10 mg^{-1}
T. rubrum	14.00	08.00	06.00	-	-	22.00
T.tonsurans	12.00	09.00	07.00	-	-	23.00
M .gypseum	16.00	12.00	09.00	-	-	20.00
C .albicans	1800	16.00	11.00	-	-	26.00
S. aureus	22.00	17.00	12.00	--	-	32.00
B. subtilis	17.00	14.00	10.00	--	-	35.00
E. coli	20.0	18.00	14.00	-	-	30.00

AF: *Aspergillus flavus* FO: *Fusarium oxysporum* RS: *Rhizopus* spp.

_1650, 50, and 1459, 07 are pragmatic and are predictable as Ag Nanoparticles revealed yellowish brown colour in aqueous elucidation due to excitation of exterior Plasmon reverberation. On integration the extract with aqueous resolution of the Ag ion multifaceted, a modify in the colour from colourless to dark brownish was observed. It was owing to the diminution of Ag+ which point out the

configuration of Ag nanoparticle revealed in Fig. 1,2. The antimicrobial activity has been performed by using bio synthesized silver nanoparticle mentioned in Table 1.

CONCLUSION

In the current study nano-particles were biologically synthesized using isolated fungal species biomass from infected leaves field soil of *Sorghum* of Mahabubnagar district. The cell filtrate of fungi was challenged with 1mm Silver nitrate, change of mixture from color less to orange brown indicates the synthesis of silver nano-particles in the reaction mixture, and size of synthesized nano-particles was measured 25-48 nm by TEM (Fig. 5) and with XRD analysis. Results conclude that isolated fungi are prominent producer of Silver nano-particles.

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