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CURVULARIA ORYZAE AS POTENTIAL FUNGAL BIOMASS FOR SILVER NANOPARTICLE SYNTHESIS

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ABSTRACT

One of the rapidly progressing area of nano biotechnology is the microbial assisted biosynthesis of nanoparticles. Biogenic Silver nanoparticle synthesis from fungal species *Curvularia oryzae* extract was utilized to reduce the silver nitrate solution for a period of 48 hours at 439 nm of UV-Visible spectrum which corresponds to surface plasmon resonance of silver nanoparticles. The characterization of the silver nanoparticles with Transmission electron microscopy and Atomic Force Microscopy shows the size dimensions of the Nanos silver in the range of 10 nm to 15 nm. These nanoparticles exhibit effective antimicrobial activity against potential pathogenic microbial species.

INTRODUCTION

Nanotechnology had made a tremendous success in the manipulation of materials at molecular level from the past two decades. The advancement of interdisciplinary science provides large opportunity in utilization for the fabrication of nanomaterials by chemical, physical and biological methods. Recent literature states the synthesis of Nano silver would be more effective, green process and more ecofriendly than the regular chemical and physical synthetic approaches (Dahoumane *et al*., 2016; Singh *et al*., 2016).

This idea leads to utilization of microbial and plant metabolic enzymes which are more promising reactors for the synthesis of the nanoparticles. Meanwhile Nano silver gained attention in the medical field for the design of the antimicrobial agents which are coated with the Nano silver to the medical appliances and medications. Silver nanoparticles have a wide application beyond the medical field, as these are also applied in electrical, textile, cosmetics etc., (Karthik *et al*., 2016; Mohanta *et al*., 2016; Ramalingam *et al*., 2016). Silver would be a super metal towards the antimicrobial activity from the ancient periods. Hence a major interest in the silver nanoparticles was the current area of research interest (Prasad and Swamy., 2013). Previous literature showed the extracellular enzyme production form the fungal extract contains major functional elements in reducing the silver colloidal solutions (Prasad *et al*., 2012b: Bhadwal *et al*., 2014; Gopinath *et al*., 2015).

This would be the major principle in the studies of the mechanism behind the Nano silver production. Most of the currently used antimicrobial drugs are not very much effective, due to resistance developed by these microbes (Perniet *al*., 2014). Silver nanoparticles show promising inhibition of the antimicrobial susceptible microbial species (Brahmachari *et al*., 2014; Nezamdoost *et al*., 2014; Shedbalkar *et al*., 2014; Adil *et al*., 2015).

In our present study, the plant pathogen which is a major inhabitant in the paddy fields of the south Indian provinces of

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India, *Curvularia oryzae was* used as a model organism for the green synthesis of the silver nanoparticles.

MATERIALS AND METHOD

The preparation of the fungal extract from the *Curvularia oryzae,* was started with primarily by growing the fungus under the optimal conditions in 100ml Potato dextrose broth (Potato extract 4g/L, Dextrose 20g/L) media in 250ml Erlenmeyer flasks incubated for 72 hours at 25-28^{o}C at 150rpm in a shaker incubator. After proper turbidity the fungal culture was taken down from the Shaker-Incubator, followed by filtering the biomass by Whatmann filter paper No1. The fungal biomass was washed thrice with autoclaved distilled water to remove any media components. The fungal biomass was then inoculated into autoclaved distilled water, incubated for 72 hours at 25-28^{o}C at 150rpm in a shaker incubator. After incubation the fungal biomass was again filtered and the filtrate was collected.

To this filtrate, 0.1M $AgNO_3$ solution was added in 1:1 ratio and kept at 25-28^{o}C in dark for a period of three hours to identify the change in the color of the solution from the pale yellow color to dark greenish-brown color. The change in color of the solution represents the reduction of the silver nitrate to nano silver ions. In second step of the experimentation procedure, the colored solution was made to characterize to confirm the Silver Nanoparticles.

To detect the characteristic surface plasmon resonance of the nano silver, UV-visible Spectroscopy would be an essential tool to detect the presence of silver nanoparticles. As soon as a change in the color of the reaction mixture was observed from pale yellow to brown, the absorption spectrum of the sample was read in UV-Visible range. (Logeswari *et al*., 2015). To study the lattice and size dimensions of the silver nanoparticles, advanced microscopic methods such as Transmission electron microscopy was opted for the better resolution of the nanoparticles. Standard sample preparation and Focusing methods were opted from *et al*., (Agnihotri *et al*., 2014; Nalvothula *et al*., 2014; Wang *et al*., 2014).

To study the antimicrobial activity of the silver nanoparticles, the test strains *Staphylococcus aureus* and *Azotobacter vinelandii* was swabbed uniformly over a nutrient agar plate. Four wells equidistant from the center were created in the plates and the following concentrations- 25µl, 50µl, 75µl, 100µl of nanoparticles were added to each respective well. The plates were incubated at 37^{o}C for 24 hours to find the clear zones on the lawn of the bacterial strains. The plates were then screened for a zone of inhibition.

RESULTS AND DISCUSSION

The occurrence of reduction reaction was observed when the silver nitrate solution and the fungal extract were added, the solution turned pale yellow to dark yellow color (Fig 1), the time interval calculated as initial hourT_0 and final reaction hour T_1after incubation period of 20 hours from the T_0. The UV-Visible spectrum results shows a maximum absorbance at 439nm would be the characteristic for silver nanoparticles, the results were compared and inferred as suggested in the Prasad *et al*.(2013). In order to understand the complete reaction, the solution was allowed to incubate for 48 hours (T_2), here there is no change in absorption and recorded 439nm (Fig 1).

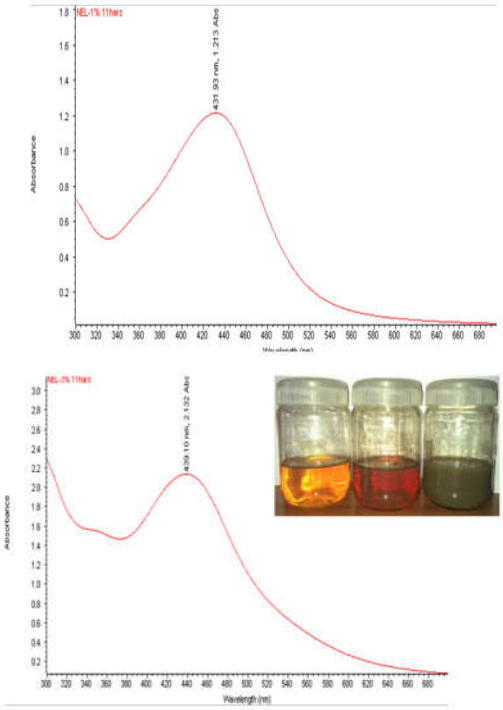

Fig 1 UV- Visible Spectrum, showing 431 nm at T1 and 439 nm at T2 which is a result of plasmonic resonance of the Silver Colloidal solution. The solution image shows the time resolution reduction of the silver nanoparticles from zero hour to 48th hour [T0-T1-T2].

The size distribution of the silver nanoparticles was polymorphic size distribution form 10-20 nm, which was inferred by using the transmission electron microscopy. Image Fig 2a- 2b shows the size distribution at scales from 50 nm-20nm size of the Silver nanoparticles. The image Fig 2c shows the spatial resolution of the individual nanoparticle which range size of 15nm in size dimension with spherical structure. While the Image Fig 2d shows the electron diffraction micrograph showing the lattice distribution of the silver atoms ion the nanoparticle. Of all these results of the TEM solution the proper inference of the nanoparticle of least size which was noticed in fewer literature which were mentioned in the Srikar *et al*., 2016.

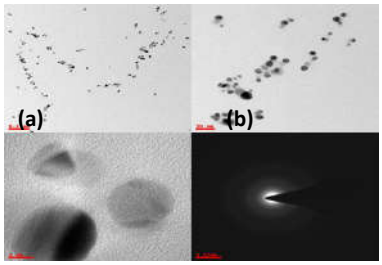

Fig 2: Transmission electron micrographs of the silver nanoparticles (a) colloidal nature of the AgNP's at 0.2 microns which shows the spherical morphology (b) 50nm range of the AgNP's (c) The size of the nanoparticle measures 10-15 nm at 5nm scale (d) EDX lattice structure

The Size distribution of the individual silver nanoparticles was observed by the Atomic force microscopy, where, the 2D image of the AFM micrograph shows the spatial resolution of the induvial silver nanoparticles as bright spots on the dark back groundas shown in Fig 3a. The Histogram Fig 3b represents the induvial sizes of the silver nanoparticles at 10-

20nm, at the maximum peak density in the Micrograph image. The comparison of the TEM and AFM data infers the spatial and size distribution of the silver nanoparticles from 10-20 nm of polymorphic and spherical in nature with a proper lattice dimension at 15nm.

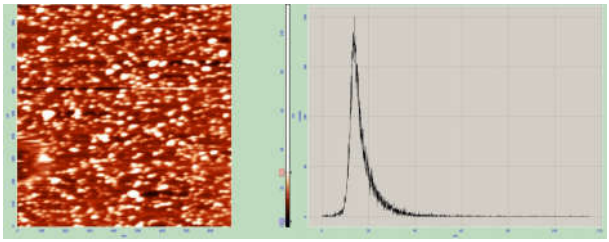

Fig 3: Atomic force microscopic images representing the size of the nanoparticles from 10 -20 nm. (a) Semi contact mode image showing the 2D image with bright spots (b) Histogram represents the distribution of the nanoparticles sizes from 10-20 nm.

The potential application of antimicrobial activity by these silver nanoparticles was showed effectively on the pathogenic microbial species *Staphylococcus aureus*, *Escherichia coli Pseudomonas aeruginosa,* which causes the skin infections and adultery skin rashes. Formation of clear zones around the soaked Whatman paper (in nanoparticles solution) discs were observed on the bacterial lawns of *E.coli* and *P. aeruginosa,* thus clearly indicating that silver nanoparticles inhibited the bacterial growth as depicted in Fig 4. The dilution dependent nanoparticles ability to inhibit the bacteria is clearly evident from the increase in the diameters of inhibition zone even at the lower dilutions. The purposes of the controls, the empty Whatman paper discs and with fungal extract are placed, although no zones of inhibitions are observed. Disk diffusion method is most predominant methodhas been employed by many researchers to confirm antimicrobial action of the AgNPs solution, inhibition zone formation around the disc reflects antimicrobial property of the nanomaterials for particular organism. (Ashokkumar *et al*., 2015; Zhang *et al*., 2013).

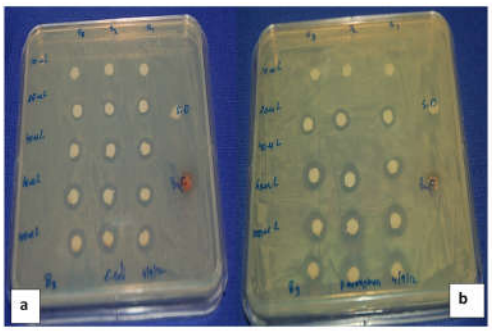

Fig 4 Anti-microbial activity of the silver nanoparticles at various dilutions ranging from 10, 20, 40, 60, and 100 µL/mL (a) *Escherichia coli* (b) *Pseudomonas aeruginosa.*

While the ecofriendly bacterial strains like *Azotobacter* inhibition was also studied with these silver nanoparticles in agar well diffusion method, with a proper zone of inhibitions were observed at different dilutions mentioned as per the *Staphylococcus aureus*. All these results were observed at lowest to highest dilutions as shown in Fig 5.In the Well diffusion method instead of using discs, small disc shaped pits are created with help of cork borer on the agar plate for filling AgNPs solution. Then inoculated plates under standard condition for the formation of clear inhibition zone (Kora *et al*., 2010; Geethalakshmi and Sarada, 2013).

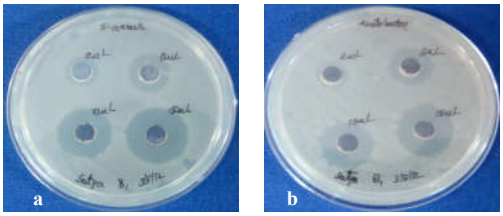

Fig 5: Anti-Microbial activity of the silver nanoparticles with well diffusion method.
(a) *Staphylococcus aureus* inhibition from dilutions from 2 µL to 15 µL
(b) *Azotobacter* inhibition from dilutions from 2 µL to 15 µL

Improper disposal of silver nanoparticles into nature will be a severe environmental threat, this can be demonstrated by the inhibition studies of nitrogenous bacterial strain *Azotobacter vinelandii*, which is environmentally so important in the ecological cycle. When the silver nanoparticles enter into the soil sources, there would be threat to these species.

CONCLUSION

With the advances in nanotechnology in upcoming future, the research should be concentrated on getting monomorphic size distribution of the nanoparticles, this is of much concern because there is no proper protocol to get a same size and dimensional nanoparticles from the biogenic methods. This should be overcome by understating the proper mechanism behind the enzymatic reactions from the extracellular secretions of the microbial species. Later the issues of proper disposal, care should be taken to minimize the risk of eradication of the ecofriendly and ecosystem food chain microbial species by these nanoparticles. Future research on microbial assisted biosynthesis of nanoparticles with unique properties is of great significance for applications in agriculture and in medicine.

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