Research Article

Synthesis and Antibacterial activity of Silver Nanoparticles from Endophytic Fungi *Phyllosticta* Sp Isolated from *Amaranthus retroflexus* - A Plant Weed

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ABSTRACT

Weeds are plants whose undesirable qualities outweigh their good points, according to man. *Amaranthus retroflexus* [pig weed] is the common name for several closely related summer annuals that have become major weeds of vegetable. Nanotechnology is manipulation of matter on an atomic, molecular, and supramolecular scale with at least one dimension sized from 1 to 100 nanometers. Silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications. For the production of silver nanoparticles, the endophytic fungi Phyllosticta Sp samples when treated with AgNP showed the change of colour in the cell filtrate from pale yellow to dark brown colour. The size of the nanoparticle was ranging from 70 nm-112 nm in cell filtrate and 62.02 nm-82.11 nm in the fungal mat. The silver nanoparticle extract showed potent activity on the human pathogenic bacteria with a zone of inhibition of upto 9 mm at 100 µl concentration. From this it can be concluded that the Silver Nanoparticles synthesized by the endophytic fungi showed potent activity against the human pathogenic bacteria. Further, it is also confirmed that the AgNPs can be synthesized using a biological source which can then be utilized for various pharmaceutical purposes.

Keywords: Endophytes, Phyllosticta, Silver Nanoparticles, Antibacterial Inhibition.

INTRODUCTION

Weeds are plants whose undesirable qualities outweigh their good points, according to man. Our human activities create weed problems since no plant is a "weed" in nature. *Amaranthus retroflexus* [pig weed] is the common name for several closely related summer annuals that have become major weeds of vegetable and row crops throughout the United States and much of the world. Most pigweeds are tall, erect-to-bushy plants with simple, oval- to diamond-shaped, alternate leaves, and dense inflorescences (flower clusters) comprised of many small, greenish flowers. Endophytes are the microorganism which is living inside plant tissues and doing substantive harm or gain benefit other than residency. Endophytes form a symbiotic relationship with their plant host. These microbes function as the biological defense for the plant against foreign phytopathogens. Antibiotics or hydrolytic enzymes can be released by endophytes prevents the colonization of microbial plant pathogens[1] or prevent insects [2].

Nanotechnology is manipulation of matter on an atomic, molecular, and supramolecular scale with at least one dimension sized from 1 to 100 nanometers. The earliest, widespread description of nanotechnology, referred to the particular technological goal of precisely manipulating atoms and molecules for fabrication of macroscale products, also now referred to as molecular nanotechnology [3]. Silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications. AgNPs play an important role in nanoscience and nanotechnology, particularly in nanomedicine. In addition, we discuss therapeutic approaches and challenges for cancer therapy using AgNPs [4].

MATERIALS AND METHODS

Collection and Preparation of Plant Sample

The fresh and healthy leaves of *Amaranthus retroflexus* were collected from Mudichur (Tambaram), Chennai. Leaves were thoroughly washed in sterile distilled water and cut into small pieces. Leaf samples are surface sterilized using 70% ethanol for 1 min, 0.1% mercuric chloride solution for 3 min, and sterile distilled water for 1 min and then allowed to surface dry on filter paper.

Isolation of endophytic fungi

The surface sterilized leaf samples are cut under sterile conditions into small pieces (2–3 cm) and inoculated in PDA medium amended with Chloramphenicol and incubated at 28±10˚C for 5 to 7 days. The fungal strains in the pure culture were preserved on potato dextrose agar (PDA) slant at 4 to 5°C with proper labeling and were sub-cultured from time to time [5].

Identification of Endophytes

The endophytic fungi were identified according to their macroscopic (front and reverse side of fungal colonies) and microscopic characteristics such as the morphology of fruiting structures and spore morphology under a bright-field microsope (10X and 40X).

Synthesis of Silver Nanoparticles

For the synthesis of silver nanoparticles, the endophytic fungus *Pestalotia* sp. was grown in 250 ml flask containing 100 ml potato dextrose broth (PDB) at 28 ˚C for 72 hrs and then harvested biomass was filtered through Whatman filter paper No.1. The fungal mat was then washed with distilled water to remove media component and suspended in 100 ml distilled water for 48 hours. After 48 hours of incubation, the cell filtrate was separated by filtration. Fungal cell filtrate was collected and treated with the $AgNO_3$ salt (final conc.1 mM).

Characterisation of Silver Nanoparticles

UV-Vis spectroscopy

UV-VIS spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability of AgNPs [6]. The spectrum was scanned at the resolution of 1 nm from 200- 800 nm for each sample.

FT-IR

FTIR is able to provide accuracy, reproducibility, and also a favorable signal-to-noise ratio. FTIR spectroscopy is frequently used to find out whether biomolecules are involved in the synthesis of nanoparticles, which is more pronounced in academic and industrial research [7]. Characterization of AgNPs was carried out by FTIR (Perkin- Elmer FTIR-1600, USA) in the range 500-4000 cm^{-1} at a resolution of 4 cm^{-1}

XRD

X-ray diffraction (XRD) is a popular analytical technique which has been used for the analysis of both molecular and crystal structures, [8,9]. The freeze-dried reaction mixture embedded with the silver nanoparticles was used for X-ray diffraction (XRD) analysis. XRD patterns were recorded on X'Pert Pro, PANalytical, USA operating at 40 kV and a current of 30 mA with Cu Ka radiation (I = 1.54A˚). The diffracted intensities were recorded from 58 to 1208 2u angles [10].

Scanning Electron Microscope (SEM)

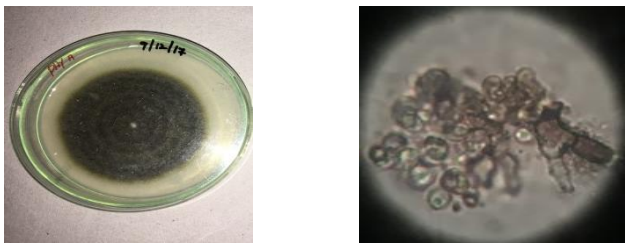
The silver nanoparticle synthesized using Fungi were allowed to dry completely by fixing the fungal mat at various percentage of acetone. Finally the fungal samples were fixed in 100% acetone for SEM analysis. Since the specimen is at high vacuum, Living cells and tissues and whole, soft-bodied organisms usually require chemical fixation to preserve and stabilize. Fixation is usually performed by incubation in a solution of a buffered chemical fixative, such as glutraldehyde. The fixed tissue is then dehydrated [11].

Antibacterial Activity of Silver Nanoparticle Synthesized Starin of *Phyllostica* sp.

Antibacterial activity of the synthesized AgNPs was studied by the standard disc diffusion method. The overnight-grown bacterial suspensions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were standardized using McFarland standard. 5 g of solidified agar was added with 50 ml of distilled water and sterilized. This mixture was poured equally into seven Petri plates. Then the bacterial suspension was swapped in each plate, well was punched on the petriplate. The wells were loaded with 50 and 100 µl of AgNps and were incubated at 37 °C and then examined for confirmation, the appearance of a clear area around the disc. The diameter of such zones of inhibition was measured using a meter ruler, and the mean value for each organism was recorded and expressed in millimeters.

RESULTS AND DISCUSSION

In the present study the inoculated leaf samples harboured different families of endophytes. Among the harboured endophytes only one coelomycetes fungi were isolated and based on the microscopic and macroscopic observation it was found to be *Phyllosticta* Sp (Fig 1).

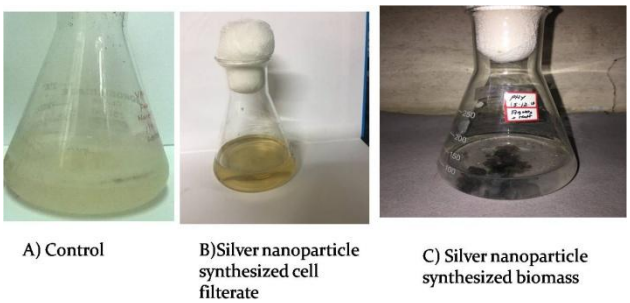


CULTURE **CONIDIA**

Figure 1: Culture and Conidia of *Phyllosticta* Sp.

Synthesis of Silver Nanoparticles

For the production of silver nanoparticles, the fungal samples when treated with AgNP showed the change of colour in the cell filtrate from pale yellow to dark brown colour which is the preliminary indication of silver nanoparticle production. The fungal biomass was also subjected to the production of silver nanoaprticles which showed change in colour of the fungal mat. (Fig 2).



A) Control B)Silver nanoparticle synthesized cell filterate C) Silver nanoparticle synthesized biomass

Figure 2: Synthesis of silver nanoparticles by the Endophytic Fungi

UV- VIS ANALYSIS

The cell filtrate samples when treated with AgNPs showed the peak of absorbance at 400 -420 nm indicates the presence of Silver Nanoparticles and appeared due to plasmon resonance (Figure 3).

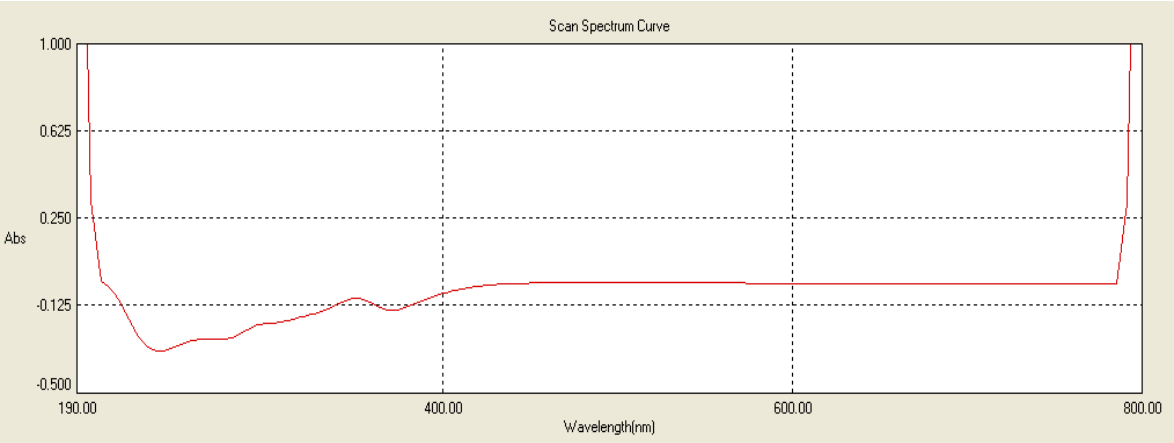


Figure 3: UV Spectra of silver nanoparticle synthesized cell filtrate

FT-IR Spectra of silver nanoparticle synthesized cell filtrate

The peaks at 3318 indicates the presence of hydroxyl amine or nitro groups. The peak at 2110 is due to the presence of CH stretching. The peak at 1636 shows the presence of C=O stretching which is a carbonyl group (Fig 4). FTIR spectroscopy has confirmed that amino acid residues and peptides of proteins has the stronger ability to bind with metal, so that the proteins could most possibly form a coat covering the metal nanoparticles i.e. (capping of silver nanoparticles) to prevent agglomeration of the particles and stabilizing in the medium [12].

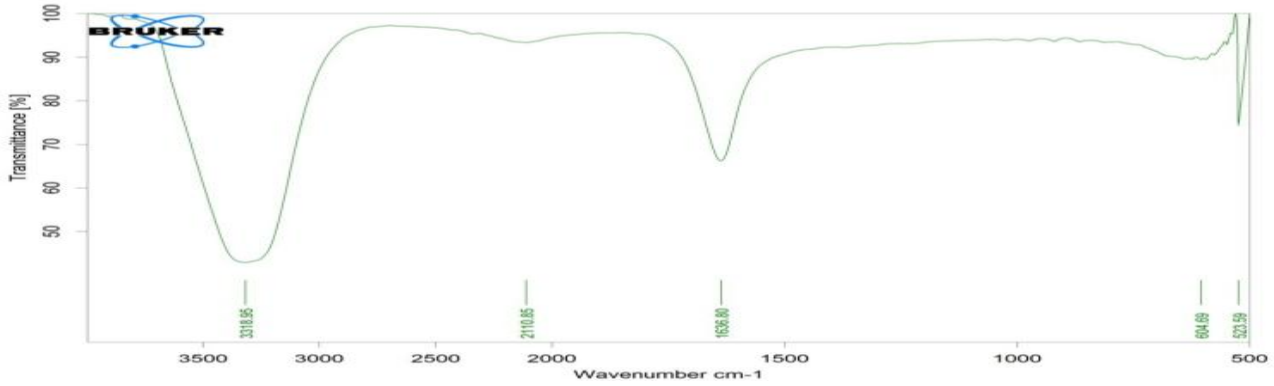
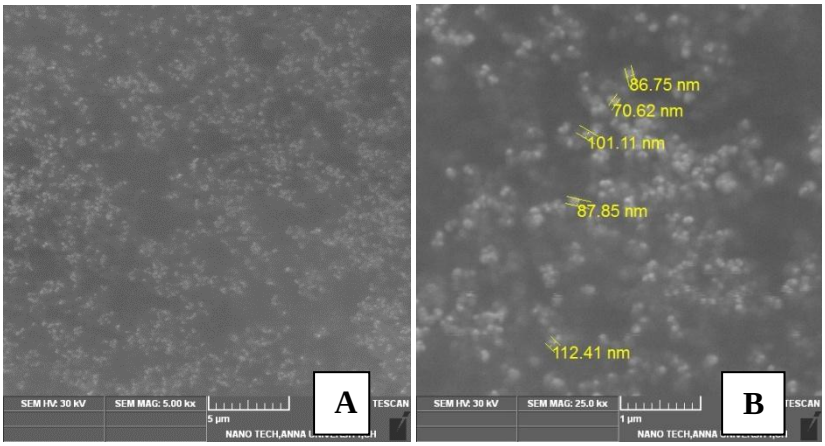


Figure 4: FT-IR Spectra of synthesized silver nanoparticles.

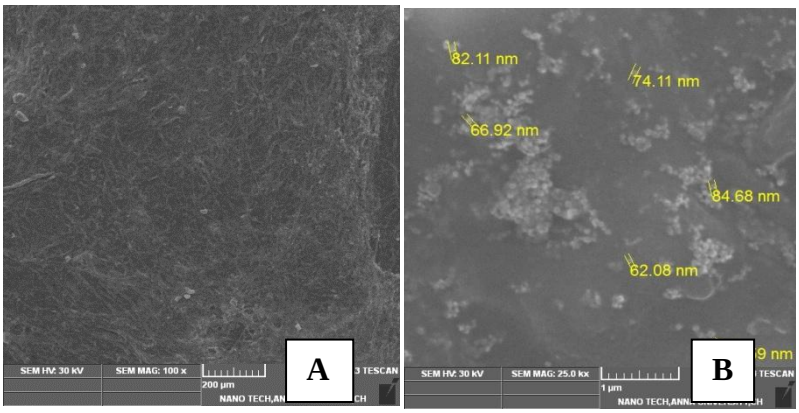
Scanning Electron Microscope (SEM)

The fungal cultures after incubation in the nanoparticle amemded media, was subjected to SEM analysis to find out the size of the silver nanoparticle synthesized by the test fungus. Both the cell filtrate and the fungal biomass when analysed using SEM showed the presence of Nanoparticle of Size ranging from from 70 nm-112 nm (Fig 5) and 62.02 nm-82.11 nm. (Fig 6) respectively.



Control Fungal extract

Figure 5: SEM CELL FILTRATE: The silver nanoparticle synthesized from cell filtrate is ranging from 70 nm-112 nm.



Control Fungal biomass

Figure 6: SEM FUNGAL BIOMASS: The silver nanoparticle synthesized from fungal biomass is ranging from 62.02 nm-82.11 nm

XRD

The phase evaluation of the dried Ag nanoparticles were analysed through X-Ray Diffractometer with Cu-Kα as a radiation source. The diffracted intensities were recorded from 10° to 80° of 2θ angles. The intense peak at 20 values of 38°, 40°, 65° correspondes to the indices(111), (200), (220) were recorded. The synthesized silver nanopaticles from the endophytic fungi was crystalline in nature which was represented by narrow and strong peak (Fig 7).

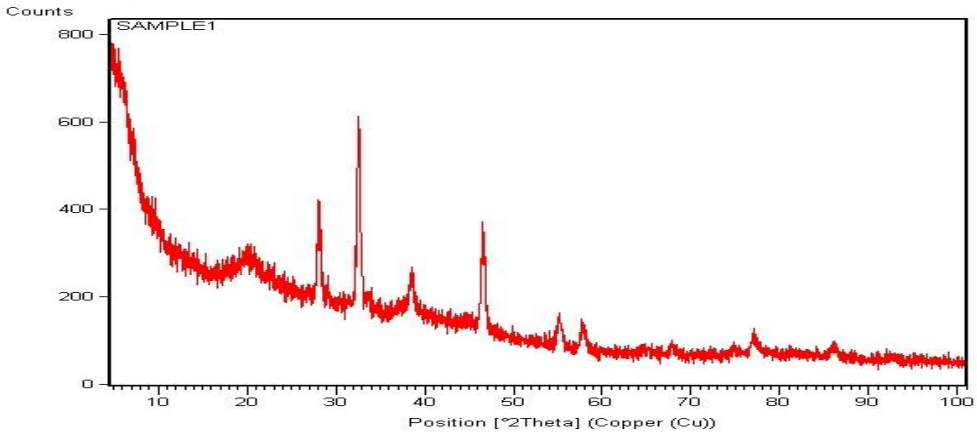


Figure 7: XRD pattern of silver nanoparticle synthesized by the test fungus.

Antibactterial Acivity

In the present study the antibacterial activity was performed using the organisms *Pseudomonas, Staphylococcus*. The silver nanoparticle synthesized fungal extract shows activity against the organisms. The concentration used to measure the zone of inhibition was 50µl and 100µl. In both concentration *Pseudomonas* shows high zone of inhibition of 8 mm and 9 mm respectively (Table 1).

Table 1: Antibacterial Activity of Silver Nanoparticles synthesized by Endophytic fungi.

Name of organisms	Concentration of the sample(µl)	Zone of inhibition(mm)
Staphylococcus aureus	50µl	6mm
Pseudomonas aeruginosa	50µl	8mm
Staphylococcus aureus	100µl	7mm
Pseudomonas aeruginosa	100µl	9 mm

DISCUSSION

In this study endophytic fungi were isolated from the weed, *Amaranthus Retroflexus*, and it was identified as *Phyllostica* Sp. The silver nanoparticles have been produced by endophytic fungal extracts. The sizes of the nanoparticles were ranging from 70 nm-112 nm in cell filtrate and 62.02 nm-82.11 nm in the fungal biomass. The antibacterial activity was performed using disc diffusion method. The zone of inhibition found in the antibacterial test indicated the silver nanoparticles synthesized, in this process has the efficient antibacterial activity against the organism *Pseudomonas,* which

showed the zone of inhibition of 8 mm and 9 mm at the concentration of 50 and 100 µl respectively. From this it can be concluded that the Silver Nanoparticles synthesized by the endophytic fungi showed potent activity against the human pathogenic bacteria. Further, it is also confirmed that the AgNPs can be synthesized using a biological source which can then be utilized for various pharmaceutical purposes.

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