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Efficacy of biosynthesized AgNPs from *Alternaria chlamydospora* isolated from indoor air of vegetable market

***[1]B. K. Nayak, [2]Chitra N. and [2]Anima Nanda**

[1]Department of Plant Science, K. M. Centre for P.G. Studies (Autonomous) Lawspet, Pondicherry-605008, India
[2]Department of Biomedical Engineering, Sathyabama University, Rajiv Gandhi Salai, Chennai - 600119, India

***Corres. author: bijuknayak@gmail.com**
Tel.: +91 9443653844

Abstract: Importance of silver nanoparticles against pathogenic bacteria has been known from time immemorial. In recent trends, bacteria are getting resistant to varied antibiotics based on their wide adaptability nature. A green, simple and effective approach was performed to synthesize potent silver nanoparticles (AgNPs) from an airborne allergenic fungus, *Alternaria chlamydospora* using both reducing and stabilizing agent. The appearance of yellowish brown color in the conical flask suggested the formation of AgNPs. The supernatant of the fungus culture changed the solution into brownish color upon the completion of the 10 minute reaction. The characterization of silver nanoparticles was confirmed by Uv-Vis spectrophotometer, Field emission scanning electron microscopy (FESEM) and XRD analysis. Size of the nanoparticles was measured in between 20nm to 33nm by FESM. Silver nanoparticles showed good antimicrobial activity against the selected pathogens studied herewith.

From the Clinical Editor: In this study, *Alternaria chlamydospora* has been used for the synthesis of silver nanoparticles is quite fast, biocompatible, simple and free from any toxic chemicals The antibacterial efficacy of various antibiotics was found to be enhanced in the combination of silver nanoparticles against varied human bacterial pathogens.

Key words: AgNPs, *Alternaria chlamydospora*, FESEM, XRD, Uv-Vis Spectrophotometer.

Introduction

Nanotechnology is one of the promising interdisciplinary fields of science that offers valuable nanomaterials with anticipated applications in the medical, electrical, mechanical, catalysis, photonics, molecular computing and structural material fields. Studies on nanotechnology, particularly silver nanoparticles have wide range of applications in the healthcare sectors. For a long run, silver has been known for its disinfectant property and were in the use of various traditional medicines as well as antimicrobial drugs[1]. It was found that silver is safe in low concentration for human cells but lethal for bacteria and viruses[1]. The antibiotic property of silver nanoparticles and its related compounds are studied by earlier workers[1,2] and it is found that they also posses anti inflammatory, antiviral, anti-platelet and antifungal activity[2,3]. Recent studies on the metallic nanoparticles confirm that silver have good antimicrobial activities[2] and its combined effect with antibiotics has also been reported by different workers[4]. Silver nanoparticles are used to be synthesized by physical, chemical and biological methods but physical and chemical methods are costly, time consuming and

toxic[4]. Synthesis of nanoparticles by biological way is wiser in the sense of cost effective, convenient and free from any chemicals[5]. The ability of the fungi and bacteria in order to synthesize nanoparticles in a controlled manner to form new materials is cost worthy[4,6,7].

The aims and objectives of the recent study is to biosynthesize silver nanoparticles by extracellular method from an aero-allergenic fungus, *Alternaria chlamydospora* isolated from vegetable market in order to confirm the formation of silver nanoparticles by UV-Vis spectroscopy followed by various microscopic characterization and to evaluate its (silver nanoparticles) efficacy as a bactericide in order to combat the growth of selected bacterial pathogens viz., *Staphylococcus aureus, S. epidermidis, Bacillus cereus, Proteus vulgaris* and *E. coli.*

Materials and Methods

Isolation of *Alternaria chlamydospora*

The airborne fungi were collected from indoors of a vegetable market by exposing Sabouraud Dextrose agar for 5 minutes on media plates based on gravitation method and the plates were incubated in BOD incubator at 25±3°c for 3-7 days in the Microbiology research Laboratory, Sathyabama University, Chennai for their enumeration. *Alternaria chlamydospora* was isolated and identified from the mixed culture of airborne fungi [8, 9], put on pure culture and stored in a refrigerator at 4°c for further studies.

Synthesis of silver nanoparticles

Isolated *Alternaria chlamydospora* fungus was subjected to biosynthesis of silver nanoparticles. Fungal biomass was grown aerobically in a specific liquid medium containing (g/L): KH_2PO_4 7.0; 2.0 K_2HPO_4 $MgSO_4$. $7H_2O$ 0.1; $(NH_4)2SO_4$ 1.0; yeast extract 0.6; glucose 10.0 at 25±3 ^OC and incubated at 25^OC in a shaker at 140 rpm for 72 hours. After incubation, the biomass was filtered using Whatman filter paper No.1 and extensively washed with distilled water to remove all residual media components. The resulting fresh and clean biomass was taken into the Erlenmeyer flasks, containing 100ml of deionized Milli-Q water. The flask was again incubated at 25^{O}c in a shaker at 140 rpm for 72 hours. The biomass was filtered again with Whatman filter paper No.1 and the cell free extract was used in the following experiment. 1mM $AgNo_3$ was prepared and 50ml was added to the cell free extract and kept in a dark condition for 48 hrs.

Characterization of silver nanoparticles

The solution in the flask was observed for color change and maximum absorbance was analyzed using UV- spectrophotometer. 1ml of sample supernatant was taken after 24hours and absorbance was measured by using UV-visible spectrophotometer (T-60, PG Instruments Ltd. *Lutterworh,* United Kingdom) between 300-600nm.

FESEM analysis was used to determine the surface morphology and particle size of the silver nanoparticles. The AgNPs samples were sonicated and later centrifuged at 15000 rpm for 20 minutes. Before the process for FESEM analysis, the samples were further sonicated to get the uniformity and better observation. Later the supernatant were discarded and pellet was washed with the Milli-Q water for three to four times. Later on the sample were transferred into the Petriplate and dried for about two hours at 50^{0}c, after that the sample were subjected to FESEM analysis.

XRD analysis was used to determine the crystallinity, metallic nature and face centered cubic structure of silver nanoparticles. For XRD analysis, the sample was prepared by centrifugation of the silver nanoparticle solution at 15000 rpm for 20 minutes. The supernatant was discarded and the pellet was washed with Milli-Q water three to four times and then dried in Petriplates. The powder form of the sample was subjected for XRD analysis at International Research Centre, Sathyabama University, Chennai, Tamilnadu, India.

Antibacterial study of AgNPs

The silver nanoparticles were checked for its antibacterial activity by disc diffusion method[10]. The antimicrobial activity of the prepared silver nanoparticles from *Alternaria chlamydospora* was tested against the pathogenic bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Proteus vulgaris* and *Escherichia coli*. The $AgNo_3$ and Amoxicillin 30mcg were taken separately as control parallel to

the AgNPs to find a comparative assessment of the antibiotic efficacy over the pathogenic bacteria. The zone of inhibition was measured after overnight incubation at 37^0C.

Results and Discussion

The *Alternaria chlamydospora* was isolated from indoor air of the vegetable market and used in the present study for the biosynthesis of silver nanoparticles. AgNPs were synthesized from Ag+ ions by treating the supernatant of *A. chlamydospora*. The appearance of yellowish brown color in the conical flask suggested the formation of AgNPs[3,4]. The supernatant of the *A. chlamydospora* culture changed the solution to a brownish color upon the completion of the 5 minute reaction with Ag+ (Fig 1). The AgNPs were characterized by UV-Vis spectroscopy, which has proved to be very useful for the analysis of nanoparticle[7.8]. As illustrated in Fig 2, Uv-Vis spectra, a strong surface plasmon resonance were centered at approximately 420nm indicated the presence of silver nanoparticles.

(A) (B)

Fig 1: Synthesis of silver nanoparticles from *Alternaria chlamydospora*.
(A) Without $AgNo_3$ treatment (B) With $AgNo_3$ treatment

The exact mechanism for the synthesis of silver nanoparticles has not been clear yet but it has been suggested that the fungal biomass contain the NADH dependent nitrate reductase enzyme, when the silver ions comes in contact with the cell wall of the fungal biomass, the nitrate reductase secreted by the fungus causes the reduction of silver ions into silver nanoparticles[11].

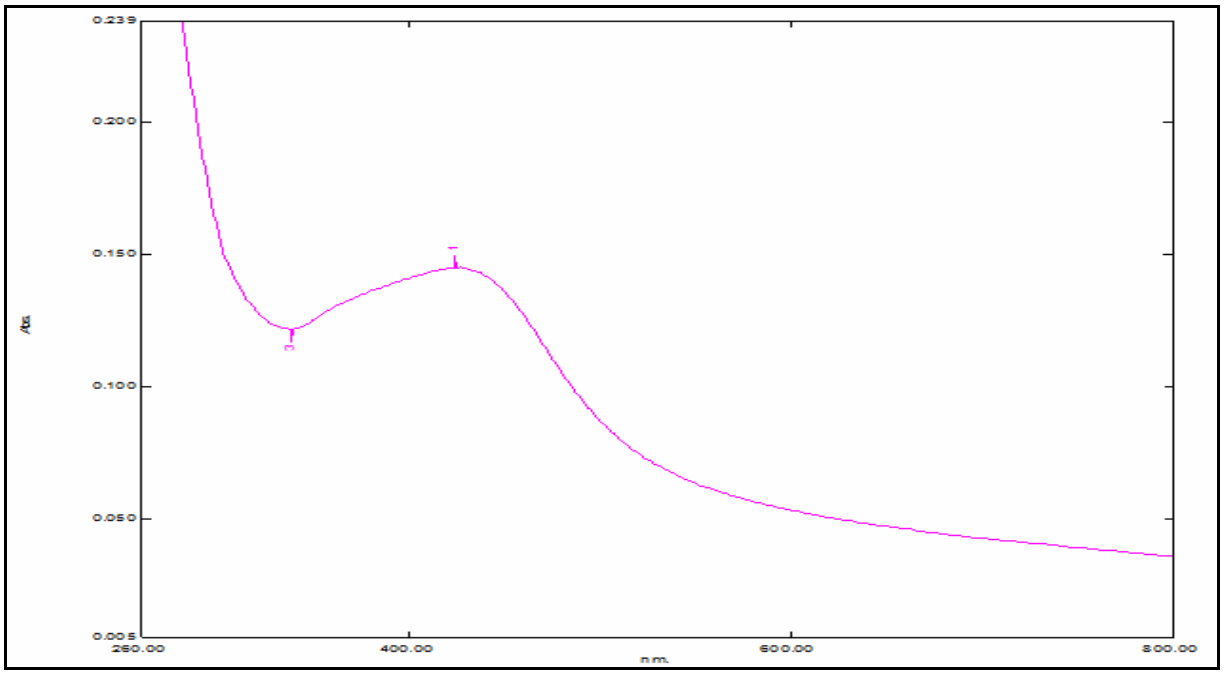


Fig. 2: Uv–Vis spectrum of silver nanoparticles synthesized from *Alternaria chlamydospora*.

Field emission scanning electron microscopy (FESEM) was used to understand the surface topology and the size of silver nanoparticles and it showed the silver nanoparticles are spherical and well dispersed with the diameter ranges between 20 nm and 33 nm from (Fig 3).

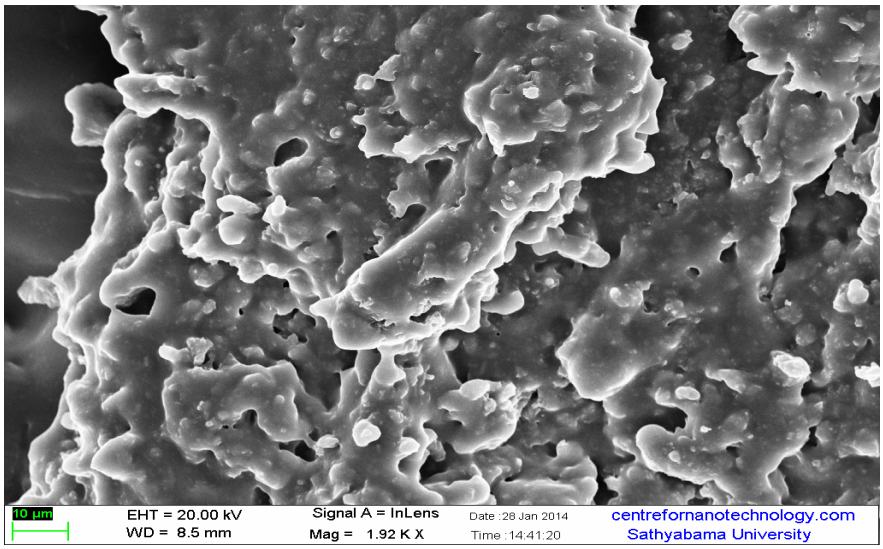


Fig. 3: FESEM analysis of silver nanoparticles synthesized from *A. chlamydospora*.

These biologically silver nanoparticles were further characterized by X-ray diffraction (XRD) technique which is used to determine the metallic nature of nanoparticles. X rays are actually electromagnetic radiations with photon energy in the range of 100 eV – 100 KeV. These highly energetic X-rays which have high penetration power enter deep into the material and provide the detailed information about the material. XRD showed the diffraction peak of the values at 24, 26, 32, 39, 47, 62 and 70 respectively which determined the metallic nature of nanoparticles and peak was specific for the silver nanoparticles (Fig 4). These results were agreed with the previous studies made by the following workers [12,13].

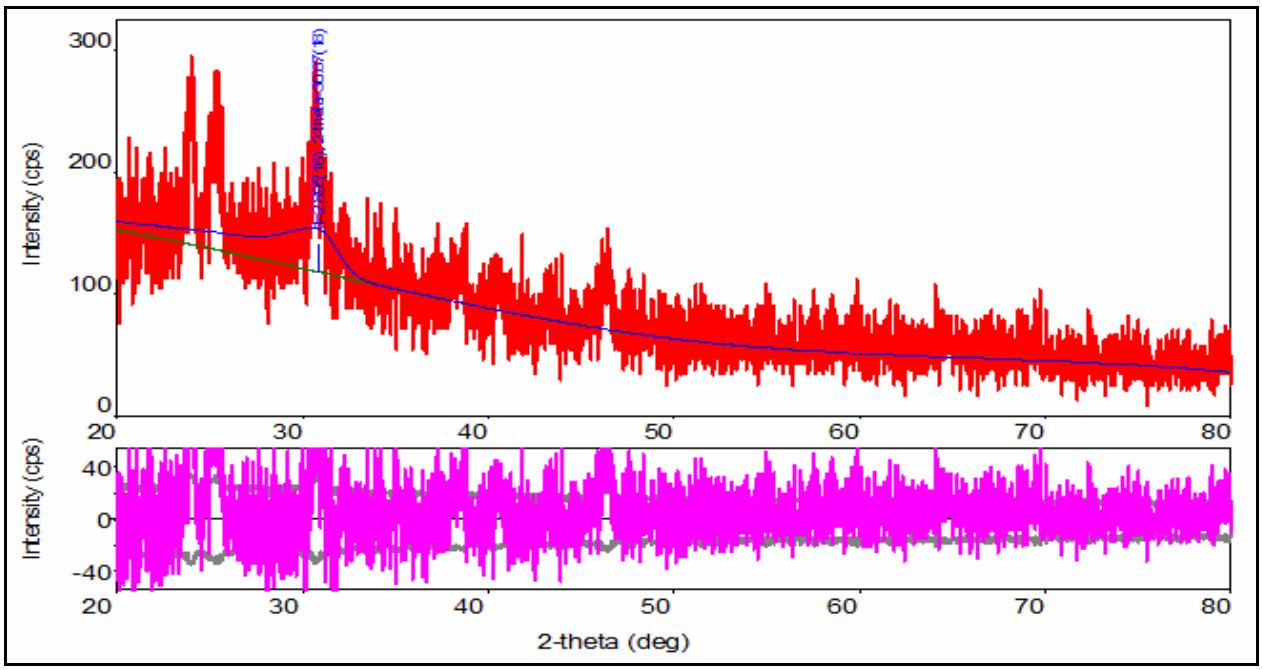


Fig 4: XRD analysis of silver nanoparticles synthesized from *A. chlamydospora*

The antimicrobial activity of synthesized silver nanoparticles was studied by disc diffusion method against different clinically isolated pathogens viz., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Proteus vulgaris* and *Escherichia coli*. Synthesized silver nanoparticles showed good antimicrobial activity against the selected pathogens but the antibiotics (amoxicillin) and $AgNo_3$ on their own

didn't show any impressive result over the test pathogens. 25µl of the biosynthesized nanoparticles from *Alternaria chlamydospora* was found to be more effective than the other two dilutions of 10 & 15µl against the bacteria studied. *Staphylococcus epidermidis* was found to be more susceptible followed by *Bacillus cereus* and *E. coli* (Table 1& Fig 5). *Proteus vulgaris* was the least affected bacteria among five pathogens seem to resistance to the AgNPs. In our study, the biological method for the synthesis of nanoparticles was found safe, ecofriendly and can be readily used in the field of biomedicine [14,15]. Further investigation is required to check the exact mechanism of the biosynthesis of silver nanoparticles from different microbes and to study its effect in vivo including cytotoxicity studies are necessary in order to find out its biocompatibility with the animals and human beings before using it as antimicrobial drug.

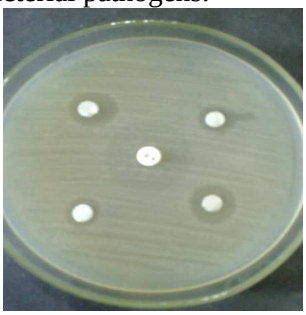
Table 1: Zone of inhibition (mm), Antibacterial Activity of AgNPs against bacterial pathogens

Sl. No.	Pathogenic Bacteria	15µl AgNPs	20µl AgNPs	25µl AgNPs	15µl $AgNo_3$	Amoxicillin (30mcg)
1	*S. aureus*	12	15	21	11	13
2	*S. epidermis*	11	14	27	10	09
3	*B. cereus*	10	14	25	10	13
4	*P. vulgaris*	11	13	13	09	15
5	*E. Coli*	11	15	25	07	15

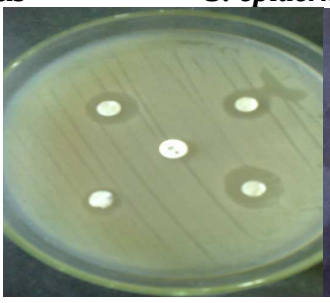
Fig 5: Comparative analysis of antibiotic efficacy of AgNPs from *A. chlamydospora*, $AgNo_3$ and amoxicillin (control) against clinical bacterial pathogens.

S. aureus

S. epidermidis



B. cereus



P. vulgaris

E. coli

Conclusion

During our study, in vitro biosynthesis of silver nanoparticles was made by extracellular method from *Alternaria chlamydospora*. The appearance of yellowish brown color in the conical flask confirmed the formation of AgNPs. The supernatant of the fungus culture changed the solution to a brownish color upon the completion of the 5 minute reaction. Size of the nanoparticles was measured in between 26 nm to 33 nm by FESEM. Synthesized silver nanoparticles showed good antimicrobial activity against the selected pathogens but the antibiotics, amoxicillin and $AgNo_3$ didn't show any effect over the test pathogens. 25µl of the biosynthesized nanoparticles from *Alternaria chlamydospora* was found to be more effective than the other two dilutions of 10 & 15µl against the bacteria studied. But silver nanoparticles needs animal trial to check its cytotoxicity before its availability in the market.

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