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# RESEMBLANCES BETWEEN THE BIOSYNTHESIZED SILVER NANOPARTICLES OF *ASPERGILLUS NIGER* AND *TRICHODERMA VIRIDE* AND THEIR ANTIOXIDANT ACTIVITY

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**ABSTRACT**
Bacterial and fungal synthesized silver nanoparticles are imperative in nanotechnology. In present report resemblances between the *Aspergillus niger* and *Trichoderma viride* synthesized silver nanoparticles is described and also assess their antioxidant property. These biosynthesized silver nanoparticles were evidenced by UV-vis spectrum showing the absorbance between 415-425 nm. The extracellular silver nanoparticles were characterized by Transmission electron microscopy (TEM) and X-ray Diffraction (XRD). TEM showed spherical shape of the nanoparticles of a specific average size 10–50 nm. XRD confirmed the presence of crystalline silver nanoparticles in the sample. The reduction of the silver ions might have occurred by a nitrate-dependent reductase enzyme. *A. niger* and *T.viride* were found to be quite competent for the purpose of commercial silver nanoparticle production, since it is synthesized extracellularly as well as rapidly. This work also presents the silver nanoparticles have remarkable antioxidant activity against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria and consequently it may discover potential applications in the treatment of the diseases.

**KEYWORDS:** Silver nanoparticles; *Aspergillus niger*; *Trichoderma viride*; UV-vis spectrum; Transmission Electron Microscopy (TEM); X-ray Diffraction (XRD).

## 1. INTRODUCTION

In the field of nanotechnology the basic need is the development of reliable and eco-friendly techniques for synthesis of metal nanoparticles because of its impact on different fields like agriculture, chemicals, medicine, electronics and space industry.[1-3] To accomplish this need the fungal system has emerged as an competent living factory for synthesis of metal nanoparticles. Cluster atoms of nanoparticles are 1–100 nm in the size. These nanoparticles have defined chemical, optical and mechanical properties.[4, 5] Not only during ancient time but also present day silver is use for treatment of different diseases. Currently, microorganisms such as bacteria, fungi and yeast perform an imperative part in the remediation of toxic metals through reduction of metal ions and act as interesting nanofactories.[6] These microorganisms are particularly good candidates in the synthesis of silver and gold nanoparticles.[7, 8]

The several reports on the biosynthesis of AgNPs using fungi, including *Fusarium oxysporum.*[9], *Fusarium acuminatum.*[10], *Penicillium fellutanum.*[11], *Aspergillus clavatus.*[12], *F. solani.*[13], *Aspergillus niger.*[14], *Alternaria alternata*[15] etc. have been successfully used for the synthesis of silver nanoparticles. The extracellular synthesis of silver nanoparticles by exploiting the biomass of endophytic fungus with 1mM silver nitrate was found to have an additional antimicrobial activity.[16] Morones *et al.*,[17] defined the antibacterial activity of silver nanoparticles against four types of Gram negative bacteria *Escherichia coli, Vibriocholera, Pseudomonas aeruginosa* and *Salmonella typhus* and suggested that silver nanoparticles attach to the surface of the cell membrane penetrate bacteria and disturb its function by releasing silver ions.[18,19] Silver ions and silver-based compounds are highly toxic to living organisms when it is a higher concentration in the cell, death occurs due to more reactive silver ions, but when it is in small concentration i.e. in nanoparticle which is useful to living organisms.

Furthermost of the organisms both uni and multicellular possess the capability to synthesis nanoparticles either intracellularly or extracellular.[20] Synthesis of nanoparticles using biological means, especially fungi is biocompatible as they secrete functional biomolecules which actively reduce metal ions.[21- 23] Moreover, fungi as a biological agent are eco-friendly and so are the reducing and capping agents involved in the synthesis process.[24]

In this article, the cell filtrate of this fungus *Aspergillus niger* and *Trichoderma viride* was used for the synthesis of silver. In both fungi silver nanoparticles were observed within 30 min after incubation with AgNo3 solution in to cell filtrate. However to study the effect of biosynthesized silver nanoparticles on human bacteria was also done.

## 2. MATERIALS AND METHODS

### 2.1 Collection of materials

*Aspergillus niger* and *Trichoderma viride* were isolated from soil as well as infected fruits and maintained on potato dextrose agar (PDA) medium at 28°C. The isolated fungi were identified by lacto phenol cotton blue mounting by morphological and microscopic observation. Clinically isolated gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria were used for check the antibacterial activity.

### 2.2 Biosynthesis of AgNPs

For the synthesis of silver nanoparticles, the biomass of each fungus were prepared by growing aerobically in glucose nitrate broth containing glucose-10 gm, KNo3-2.5 gm, KH2Po4-1 gm, MgSo4-0.5 gm and 1000 ml distilled water. The inoculated flasks were incubated on orbital shaker at 30°C and agitated at 120 rpm for 96 hr. The biomass was harvested after six days incubation by filtering followed by repeated washing with distilled water to remove any medium component from the biomass. In a 250 mL Erlenmeyer flask five g (wet weight) was brought in contact with 100 mL of double distilled water for 3 days at 30°C and agitated again at 120 rpm. The cell filtrate was obtained by filtering it through Whatman filter paper No. 1. The 5 mL filtrate was treated with 5 mL of 1 mM AgNo3 solution in an Erlenmeyer flask and incubated at room temperature in dark. Control containing cell-free filtrate without silver nitrate solution was run simultaneously as standard with the experimental flask. All experiments were done in three replicates.

## 3. CHARACTERIZATION OF AgNPs

### 3.1 UV-visible spectroscopy analysis

Change in color of the cell free filtrate incubated with silver nitrate solution was visually observed over a period of time. Silver ion bio-reduction was monitored by sampling of aliquots (1 mL) at different time intervals. Absorption measurements were carried out on UV-visible spectrophotometer (Cystronics UV-Vis spectrophometer 117). UV-Visible analysis of several weeks old samples was also carried out to check the stability of synthesized AgNPs.

### 3.2 Transmission electron microscope (TEM)

For TEM measurements, a drop of synthesized AgNPs was placed on the carbon coated copper grids and kept for dry. After dryness of sample grid loaded on to a specimen holder. TEM micrographs of the sample were taken using the Morgagni 268D TEM instrument (AIIMS, New Delhi).

### 3.3 X-Ray Diffraction (XRD)

XRD Measurements was done to know the crystallographic information of nanoparticles. The biosynthesized silver nanoparticles suspension was centrifuged at 10,000 rpm at 4 °C for 10 min to obtain a pellet of pure nanoparticle for XRD analysis. X-Ray Diffraction (XRD) patterns were carried out on a Philips-X'Pert MPD X-ray diffractometer. The pattern was recorded by Cu-Kα 1 radiation, with λ of 1.5406 Å and a nickel monochromator filtering the wave at a tube current of 30 mA and tube voltage of 40 Kv. In the region of 2θ the scanning was done from 30° to 80°, at the time constant 2 s. and 0.02°/min.

### 3.4 Antimicrobial activity

The antimicrobial activity of the synthesized AgNPs was assessed against two tests micro-organisms, viz., *Staphylococcus aureus* and *Escherichia coli*. The overnight grown test bacterial cultures were plated on Glucose nutrient agar. Three wells were cut on the plates using 4 cm width cork borer and 50 µL of AgNPs of *A. niger* and *T. viride* solution was immersed in two well while as in the 3rd well antibiotic streptomycin drug was dispensed with the help of micropipette. The plates were incubated overnight at 37°C for 24 hr, and observed for the presence of zones of inhibition.

## 4. RESULT AND DISCUSSION

### 4.1 Biosynthesis of AgNPs

When cell-free filtrates of two fungal isolates were incubated with silver nitrate salt, the color of cell filtrates were exhibited a gradual change to brown color under dark conditions. The color of the culture filtrate with silver nitrate salt changed to intense brown after 24 hr of incubation which show little bit color difference in both fungal nanoparticles suspension, whereas the control (without silver nitrate salt) did not exhibit any color change (Fig.1).



**Fig. 1: Color of sample A) 1 mM Silver nitrate solution B) Cell free filtrate before immersion of AgNo3 C) Cell free filtrate after immersion of AgNo3.**

### 4.2 Characterization of AgNPs

The UV-visible spectra of fungal cell filtrate of *A. niger* and *T. viride* treated with the silver nitrate solutions showed a characteristic surface plasmon absorption band at 422 and 419 nm respectively, and the maximum color intensity was obtained after three days. Beyond three days of incubation, no further increase in intensity was recorded indicating complete reduction of silver ions by the fungal cell filtrate. The reduction of the silver ions might have occurred by a nitrate-dependent reductase enzyme. Synthesized AgNPs was extremely stable at room temperature without agglomeration after 60 days was monitored regularly by UV-visible spectrophotometer. This indicated that the nanoparticles were well dispersed in the solution without aggregation (Fig. 2).

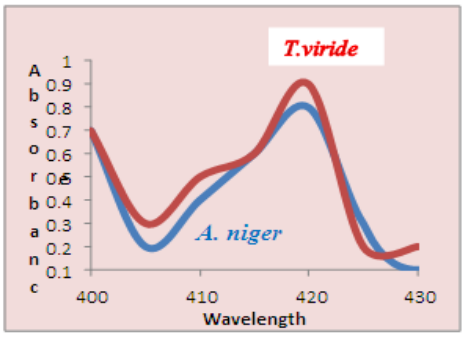


**Fig. 2: UV-Vis spectra recorded after the exposure of 1mM silver nitrate solution in crude cell filtrate of *A. niger* and *T. viride.***

### 4.3 Transmission electron microscope (TEM)

TEM micrograph provided detailed morphology of silver nanoparticles. The data obtained from micrograph images showed distinct shape and size of polydisperse nanoparticles. Mostly both fungal nanoparticles were spherical but some are ellipsoidal in shape in the range of 9-60 nm without significant agglomeration (Fig. 3).

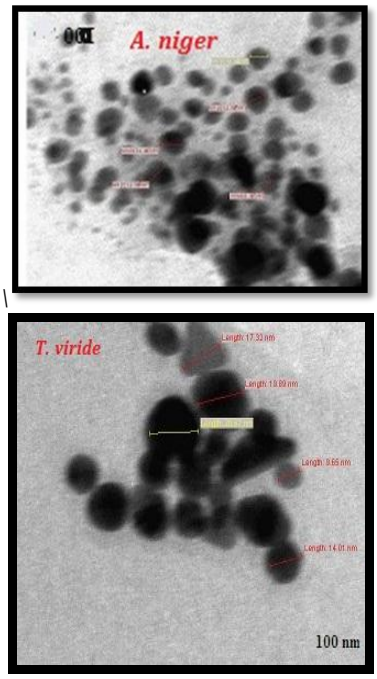


**Fig. 3: TEM micrograph of silver nanoparticles synthesized by *A. niger* and *T. viride.***

### 4.4 X -Ray Diffraction

A comparison of the XRD data between two species nanoparticles with the confirmed that the particles formed in our experiments were silver nanocrystals. They can be revealed by the peaks at 2θ values of 38, 45, 64, 69 and 77 corresponding to 111, 200, 220 and 311 planes for silver respectively in both species. This XRD pattern confirms th crystallinity of SNPs.

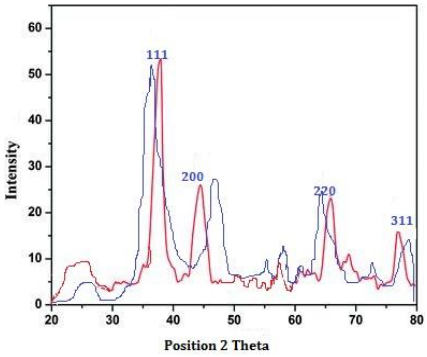


**Fig.4: XRD Pattern of Silver Nanoparticles.**

### 4.5 Antimicrobial activity

The fungus synthesized AgNPs were tested against *Staphylococcus aureus* and *Escherichia coli* for the antimicrobial efficacy which resulted in formation of varying zone of inhibitions compared with inhibition zone of antibiotic streptomycin. The diameter of *A. niger* and *T. viride* synthesized AgNPs revealed significant increase in the zone of inhibition against *S. aureus* as compared to streptomycin than *E. coli* (Table 1). (Fig. 5).

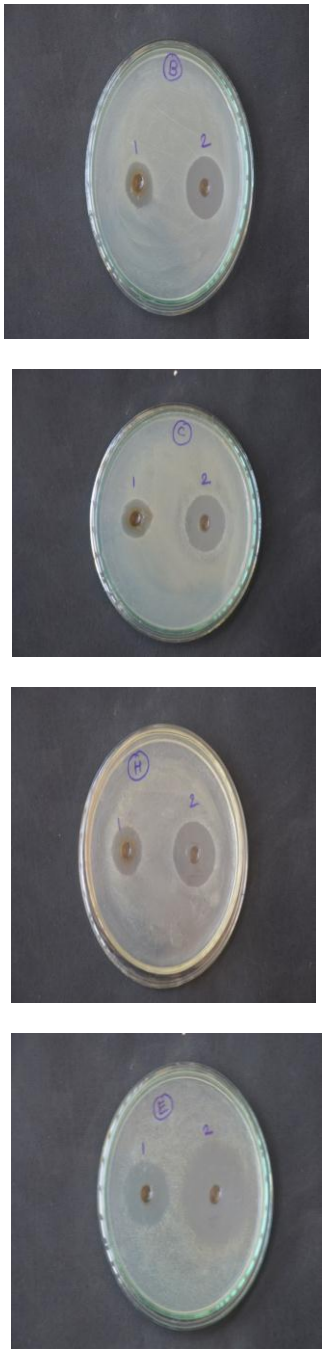

**Fig. 5: Antibacterial activity of streptomycin against silver nanoparticles**
**1. *A. niger* – B) *E. coli,* C) *S. aureus***
**2. *T. viride*- H) E. *coli,* E) *S. aureus***

**Table: 1. Antimicrobial activity observed as zones of inhibition (mm) produced by mycosynthesized silver nanoparticles (AgNPs) against the test organisms.**

| Silver nanoparticles of fungus | Zone of inhibition in diameter (cm) | |
|---|---|---|
| | *E. coli* (B, C) | *S. aureus* (H, E) |
| *A. niger* | 1.6 | 1.8 |
| *T. viride* | 1.7 | 2.4 |
| *Streptomycin* | 1.4 | 1.6 |

## 5. CONCLUSION

The two species of *A. niger* and *T. viride* genus observed absorbance peak at 422 and 419 nm which are nearby similar indicating the synthesis of silver nanoparticles. Silver nanoparticles reveal resemblances between the size and shape in both species provided by TEM micrographs which are similar in spherical but some are ellipsoidal in shape in the range of 9-60 nm. Silver is known to have broad-spectrum antimicrobial activity against bacteria, viruses and eukaryotic micro-organisam.[25-27] Both fungus synthesized silver nanoparticles observed potent antimicrobial activity against gram positive bacteria *E. coli* and gram negative bacteria *S. aureus.* This study demonstrates that the *A. niger* and *T. viride* mycosynthesized AgNPs disclosed similitudes between brown color, peak formation, size, shape, XRD pattern and potent antimicrobial activity. Therefore this experiment concludes that the *A. niger* and *T. viride* synthesized silver nanoparticle are near about analogous in each point of view.

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