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# Silver Nanoparticles Biosynthesis by *Fusarium oxysporum* and Determination of Its Antimicrobial Potency

**Nida Tabassum Khan*, Mahum Jameel and Jibran Jameel**

*Department of Biotechnology, Balochistan University of Information Technology Engineering and Management Sciences (BUITEMS), Quetta, Pakistan*

## Abstract

Nanotechnology encompasses the engineering of nanoparticles with enhanced functionality and improved stability which is quite different from the bulk form of the same material. Fungi as bionanofactories for the production of diverse metallic nanocrystals is the right choice because it not only offers an ecofriendly and cost effective procedure but also provide easy and simple down streaming for product recovery. Fungal filtrate of *Fusarium oxysporum* isolated from banana fruit was used for the amalgamation of silver nanoparticles. Appearance of light brown colour of fungal filtrate upon incubation with $AgNO_3$ indicates silver nanoparticle formation with strong absorption in the visible region at 440 nm as determined by ultra violet visible spectroscopy. Disc diffusion assay showed enhanced antibacterial and antifungal activity of silver nanoparticles against pathogens like *Candida albicans*, *Escherichia coli*, *Candida krusei*, *Staphylococcus aureus* and *Aspergillus flavus*.

**Keywords:** Green synthesis; *Fusarium oxysporum*; Silver nanoparticles; Ultra violet spectroscopy

## Introduction

Now a days, silver nanoparticles have gained significant consideration due to their unique characteristics and diverse application like electrical conduction [1], surface-enhanced Raman scattering [2], nucleotide sequencing [3], antibacterial and antifungal activities etc. [4,5]. Numerous methods were available for the fabrication of silver nanoparticles such as heat decomposition in organic solvents [6], reduction with or without the presence of stabilizers [7], photo-reduction [8,9], and electromagnetic reduction [10-12] etc. But these above mentioned approaches are costly and involve the use of compounds which may pose potential environmental risks. Therefore there is a need to develop an eco-friendly procedure that applies biological principles in nanoparticle formation i.e., biomimetic approach [13-15] such as the use of fungus [16], proteins [17], plants or plant extracts [18-20] for the production of metallic nanoparticles that do not employ harmful compounds. Silver nanoparticle can be produced by a variety of biological systems such as bacteria, plants, fungi but among these, eukaryotic fungi is the suitable candidate with unique features like increased growth and rapid reproduction by virtue of mycelial branching, capability to produce number of enzymes with the ability to bio-accumulate different metal nanoparticles by bioreduction, ability to grow under extreme conditions etc. make them suitable for such purposes [21-23]. In current study silver nanoparticles were produced from *Fusarium oxysporum* under controlled *in vitro* conditions and the mycosynthesized silver nanoparticles were than tested for their antimicrobial activity against different disease causing pathogens.

## Materials and Methods

*Fusarium oxysporum* was isolated from banana fruit on PDA (potato dextrose agar) at 28°C for three to four days. The fungus was identified using morphological characteristics such as colony colour, texture of mycelia etc. *F. oxysporum* was grown on CD (cezapex Dox) broth (glucose (10 g), magnesium sulphate (0.5 g), yeast extract (1 g), Potassium dihydrogen phosphate (1 g), calcium chloride (0.5 g), zinc sulphate (0.01 g), ferrous sulphate (0.01 g) and Sodium nitrate (2 g) dissolved in one liter of distilled water and autoclaved for 15 min at 121°C at 15 psi (pound/square inches). The flasks containing CD medium inoculated with *F. oxysporum* was kept for 5 days on rotatory shaker at 25°C at 150 rpm. The mycelia were harvested using Whatman's filter paper no. 42 followed by extensive washing twice to remove media components. 50 ml of obtained fungal filtrate was then incubated with 10 ml of $AgNO_3$ (10 mM) at 28°C for 24 hrs in dark to avoid any photochemical reactions. Freshly prepared CD broth with silver nitrate solution was run as the control. Confirmation of silver nitrate formation is indicated by a colour change in the experimental flask. Bioreduction of Ag metal ions in the aqueous solution was monitored by means of ultra violet spectroscopy (JENWAY 6305) from 400 to 500 to determine optimum wavelength. Antimicrobial activity of the silver nanoparticles were determine using dis diffusion assay carried on Yeast Malt extract agar (YM) against *Candida albicans*, *Escherichia coli*, *Candida krusei*, *Staphylococcus aureus* and *Aspergillus flavus* by placing one cm sterile filter paper disc impregnated with 60 µL of colloidal siver nanoparticle. Ruler scale was used to estimate the diameter of inhibition zone.

## Results and Discussion

Sufficient biomass of *Fusarium oxysporum* was obtained in CD broth because of utilization of the sugar source glucose and all the other essential nutrients required by the fungal mycelia to grow and continuous agitation helps in uniform distribution of all the nutrients available in the CD medium (Figure 1) [20].

Colour change of the filtrate incubated with $AgNO_3$ to brown depicted silver nanoparticle formation (Figure 2). Silver nanoparticles were found to be quite stable in the fungus supernatant [24]. Silver

***Corresponding author :** Nida Tabassum Khan, Department of Biotechnology, Faculty of Life Sciences and Informatics, Balochistan University of Information Technology Engineering and Management Sciences (BUITEMS), Quetta, Pakistan, Tel: +92-3368164903; E-mail: nidatabassumkhan@yahoo.com

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nanoparticle exhibits strong absorption in the visible region. Ultraviolet visible spectrum of the sample revealed absorbance peak at 440 nm which is specific for silver nanoparticles [10]. A single peak indicates the formation of spherical nanoparticles with wide spread distribution (Figure 3). However no change in colour was observed in freshly prepared CD media incubated with silver nitrate indicating absence of silver nanoparticles.

Antimicrobial potency of silver nanoparticles were tested against various pathogens and compared with the controls. The diameter of inhibition zone is shown in Table 1.

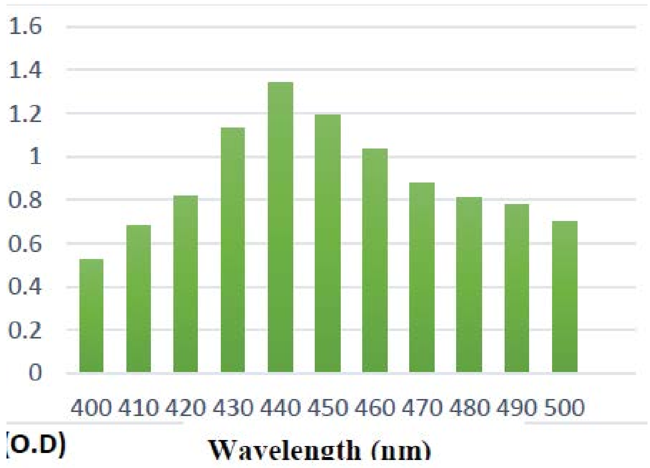


**Figure 1:** Absorbance peak at 440 nm.


**Figure 2:** Colour change from yellow to brown.

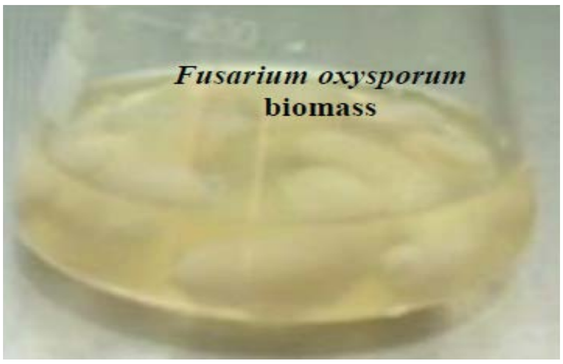


**Figure 3:** *Fusarium oxysporum* biomass in Cezapex Dox broth after 5 days.

| S.no | Test pathogens | Zone of inhibition (cm) Silver nanoparticles (60 µL) |
|---|---|---|
| 1 | *Staphylococcus aureus* | 0.9 |
| 2 | *Candida albicans* | 0.8 |
| 3 | *Candida krusei* | 0.8 |
| 4 | *Aspergillus flavus* | 0.8 |
| 5 | *Escherichia coli* | 1.0 |

**Table 1:** Silver nanoparticle antimicrobial activity against various pathogens.

Silver nanoparticles could inhibit two different pathogenic bacteria including *Escherichia coli* and *Staphylococcus aureus*. Thus displayed broad antibacterial spectrum as increased activity was seen against these two bacteria Staphylococcus aureus and Escherichia coli with a wide clear inhibition zone of 0.9 cm and 1 cm around the sterile discs was observed. Enhanced antifungal potency of these mycosynthesized silver nanoparticles was seen against the pathogenic *Candida* species such as *Candida krusei* and *Candida albicans*. Similar results were reported while using silver nanoparticles synthesized at different physioculture conditions by Nayak et al. [25]. Silver nanoparticles produce from *F. oxysporum* were also found to be effective against *Aspergillus flavus*. Therefore because of their effective antimicrobial properties silver nanoparticles could have potential clinical applications.

## Conclusion

Green mycofabrication of Silver nanoparticles using *Fusarium oxysporum* is an ecofriendly and cost effective process which can be easily achieved in lab under standard conditions forming stable colloidal silver nanoparticles of spherical morphology with a potential to be used as anti-bacterial and antifungal agents.

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