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**RESEARCH ARTICLE**

# SYNTHESIS, CHARACTERIZATION AND STABILITY OF GOLD NANOPARTICLES USING THE FUNGUS *FUSARIUM OXYSPORUM* AND ITS IMPACT ON SEED GERMINATION

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**ABSTRACT**

This study was conducted to explore the possibility of biosynthesis of gold nanoparticles by using the fungus *Fusarium oxysporum*. The cell filtrate of *Fusarium oxysporum* reacted with HAuCl4 ions, resulting formation of gold nanoparticles within 3 hours. The gold nanoparticles were characterized by Visual analysis, UV-Vis absorption spectroscopy and Transmission electron microscopy (TEM). The gold nanoparticles exhibited maximum absorbance at 550 nm in UV-Vis spectroscopy. UV-spectral reading clearly showed that stability of gold nanoparticles also constant after 3 month. TEM showed polydisperse spherical and ellipsoid nanoparticles in the size range from 18-24 nm and average size 21.82 nm.T he AuNPs were evaluated for their impact on viability of seed. ***F. oxysporum* synthesized gold** nanoparticles observed optimistic effect on seed germination. Therefore biosynthesized gold nanoparticles have biological assay used in agricultural purposes to increases the viability of seeds.



## INTRODUCTION

Nanotechnology has emerged as one of the most promising and attractive research field, with applications ranging from aerospace to health industries (Jochen*et al*, 2003). Now different types of metal nanomaterial are being produced using Copper, Zinc, Magnesium, Silver and Gold. Eco friendly nanoparticle synthesis process has been great demand in current and future generation. Large numbers of organisms, both unicellular and multicellular are able to produce inorganic nano materials either intracellular or extracellular (Dickson and Magn, 1999). Microorganism like bacteria, fungi and actinomycetes are the important tool andattractive alternative for synthesis of nanoparticles (Klaus-Joerger *et al*, 1999; Mukherjee *et al*, 2001). Gold nanoparticles are of interest mainly due to their stability under atmospheric conditions, resistance to oxidation, and biocompatibility (Gericke and Pinches, 2006; Huang and Sun,2007). Therefore, development of techniques for synthesis of gold nanoparticles, of well-defined size and shape, is of great challenge. Recently, bacterial cell supernatant of *Pseudomonas aeruginosa* was used for the reduction of gold ions resulting in extracellular biosynthesis of gold nanoparticles (Husseiny *et al*, 2007). Buttermilk Lactobacillus strains was used for the formation of gold, silver, and gold-silver alloy crystals (Nair and Pradeep, 2002).The extremophilic actinomycete, *Thermomonospora sp*. when exposed to gold ions reduced the metal ions extracellularly, yielding gold nanoparticles with a much improved polydispersity (Sastry *et al*, 2003). *Colletotrichum sp*. growing in the leaves of geranium was used for the extracellular synthesis of stable and various shaped gold nanoparticles (Shanker *et al*, 2003). The *Rhizopus oryzae* synthesize of gold NPs at room temperature by reduction of gold ions (Das and Marsili, 2010). The intracellular synthesis of gold nanoparticles by using the fungus *Verticillium sp*. was also reported (Mukherjee *et al*, 2001). However extracellular formation of gold nanoparticles by treatment of the fungus *Fusarium oxysporum* with aqueous AuCl4- ions has also been reported (Mukherjee *et al*, 2002). Nanotechnology promises considerable help in agriculture throughout the world (Dhoke *et al*, 2013) consequently it play significant role in agriculture. The various functions of nanoparticles on seed germination and growth, biomass yields ofseedling depend in a multipart way on magnetic instability densities, frequencies the prehandling of the material, and treatment duration (Najafi *et al*, 2013).

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Seed is most important input determining productivity of any crop. (Kumar *et al,* 2014) explore possible effects of silver nanoparticles synthesized with the mediation of *Euphorbia hirta*on seed germination and seedling growth. In this article, the fungus *Fusarium oxysporum* was used to synthesize gold nanoparticles observed within 6 hours after AuCl4- solution was added to the cell filtrate and stable after 3 month. Gold nanoparticles found optimistic effect on seed germination.

## MATERIALSAND METHODS

### *Collection of Materials*

The fungus *Fusarium oxysporum* was isolated from faded vegetables like tomato and maintained on potato dextrose agar (PDA) medium at 30Cº. Macroscopic and microscopic observation through mounting by lacto phenol cotton blue was used identified isolated fungus. Pure culture was maintained on potato dextrose agar slants in triplicate at 30Cº.

### *Biomass Preparation*

The biomass of fungus *Fusarium oxysporum* was prepared in Glucose nutrient broth (GNB). The flask was inoculated with spores of pure slants of *F. oxysporum*and incubated at 28ºC on a rotatory shaker (120 rpm) for 6 days (Fig1). The biomass was harvested by filtration through Whatman filter paper 1 and then washed with distilled water to remove any components of the medium. The fresh, clean *F. oxysporum* biomass was brought in a 250 mL Erlenmeyer flask in contact with 100 mL of double distilled water for 3 days at 30°C and agitated again at 120 rpm. The crude cell filtrate was obtained by filtering through Whatman filter paper 1 which was used for further experiment.

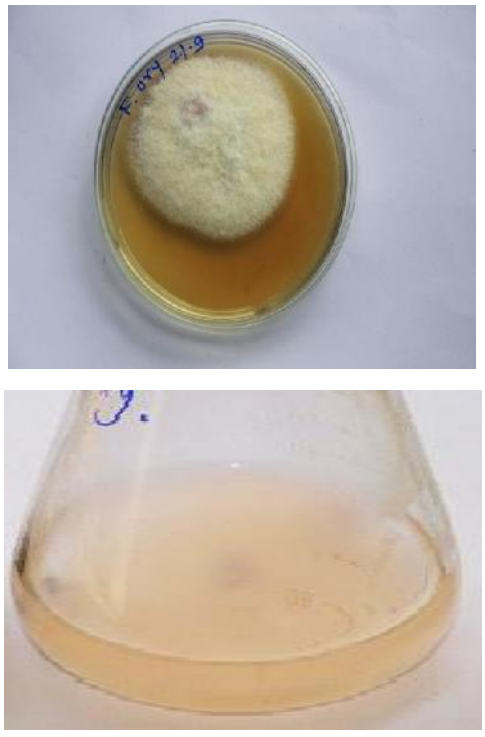


**Figure 1**Pure culture in PDB media and Biomass production in GNB media of *F. oxysporum.*

### *Biosynthesis of Gold Nanoparticles*

The crude cell filtrate was treated with 1 mM HAuCl4 solution in Erlenmeyer flask as 1:1 proportion and incubated at room temperature in dark condition. Control containing cell-free filtrate without Chloroauric acid solution was run simultaneously as standard with the experimental flask. All experiments were done in duplicate.

### Characterization of gold Nanoparticles

### UV-visible spectroscopy analysis

The color of crude cell filtrate becomes changed after the incubation of Chloroauric acid solution was visually observed over a different period of time i.e. 1, 24, 48, 72 and 96 hr. Gold 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 3 month old samples was also carried out to check the stability of synthesized Au NPs and absorbance was measured between 350-750 nm.

### Transmission electron microscope (TEM)

TEM micrographs of the sample were taken using the Morgagni 268D TEM instrument AIIMS, New Delhi. For TEM measurements, *F. oxysporum* synthesized AuNPs drop was placed on the carbon coated copper grids and kept for few min. to dry. Then sample dried copper grid loaded on to a specimen holder for TEM images.

### Effect of biosynthesized silver nanoparticles on seed germination

Seeds of sunflower were surface sterilized with 1% mercuric chloride solution for 1 min. and rinsed several time in sterile distilled water. Clean seeds were per-soaked in 3 days old gold nanoparticles solution of *F. oxysporum* for varying period of time (4hr and 6hr) in undiluted solution. Control containing water in which seeds were pre-soaked was run simultaneously as standard with the experimental flask.

## RESULT AND DISCUSSION

### Visual Analysis

Color of crude cell filtrate turns into purple color, after the addition of Chloroauric solution, it is the preliminary test for the presence of gold nanoparticles. The color Chloroauric acid solution was golden while *F. oxysporum* crude cell filtrate was colorless. After the exposure of 1:1 proportion of 1 mM aqueous solution of HAuCl4 in to crude cell filtrate, the color completely turns into purple color.

It is observe that the color of gold nanoparticles change from colorless to purple which clearly indicates the synthesis of gold nanoparticles (Fig2).

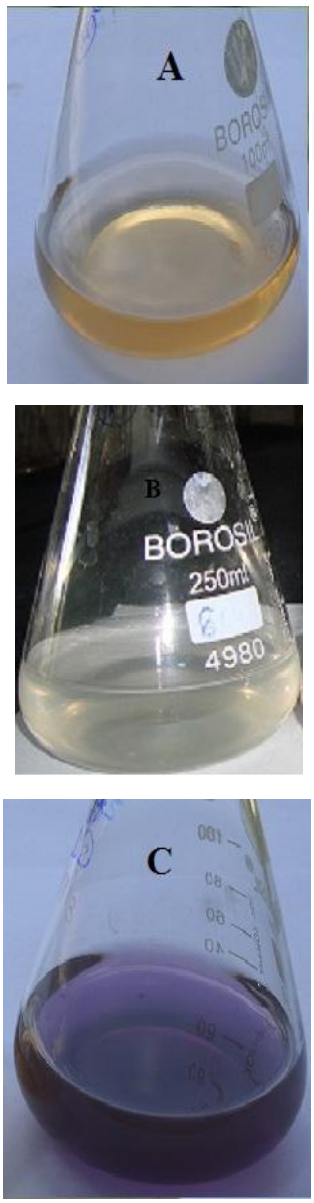
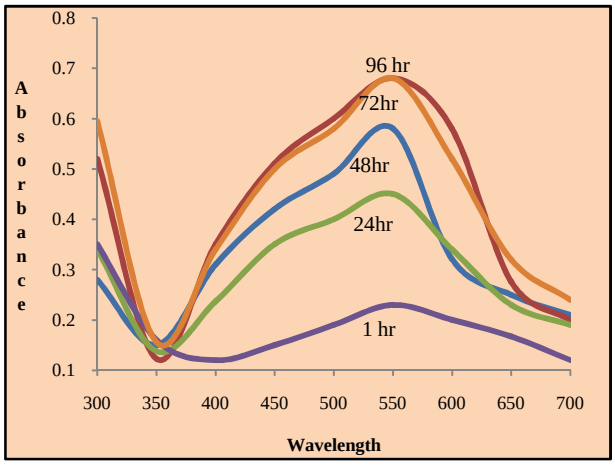


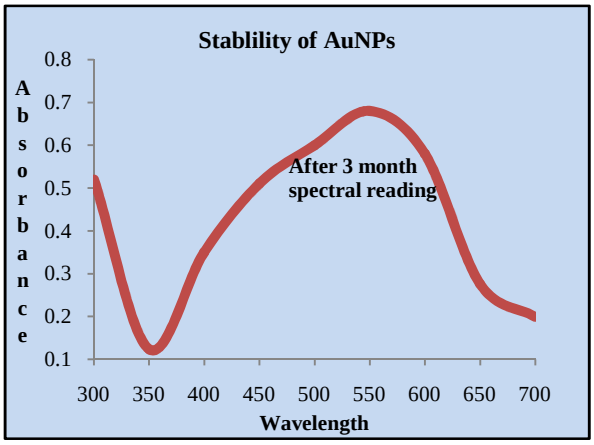

**Figure 2 A**. Conical flask shows color the 1 mM Chloroauric acid solution, **B**. Color of crude cell filtrate of *F.oxysporum* before the immersion of 1 mM HAuCl4 and **C**. Purple color of crude cell filtrate after immersion of 1mM aqueous solution of HAuCl4.

## UV Spectrophotometer Analysis

Synthesis of gold nanoparticles was monitored by UV-visible spectroscopic analysis. The UV-visible spectra of fungal cell filtrate of *F. oxysporum*treated with the Chloroauric acid solution showed a characteristic surface plasmon absorption band at 550 nm,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 gold ions by the fungal cell filtrate (Graph 1). Synthesized AuNPs was extremely stable at room temperature, without agglomeration after 90 days was monitored regularly by UV-visible spectrophotometer. This indicated that the nanoparticles were well dispersed in the solution without aggregation, which indicates the formation of gold nanoparticles and they are stable after three months (Graph 2).




**Graph 1** UV-visible spectra recorded peak formation from fungal cell free extract after the immersion of 1mM HAuCl4 solution after different time interval (1, 24, 48, 72 and 96 hours).




**Graph 2**UV-visible spectra recorded peak formation from fungal cell free extract after 3 month incubation.

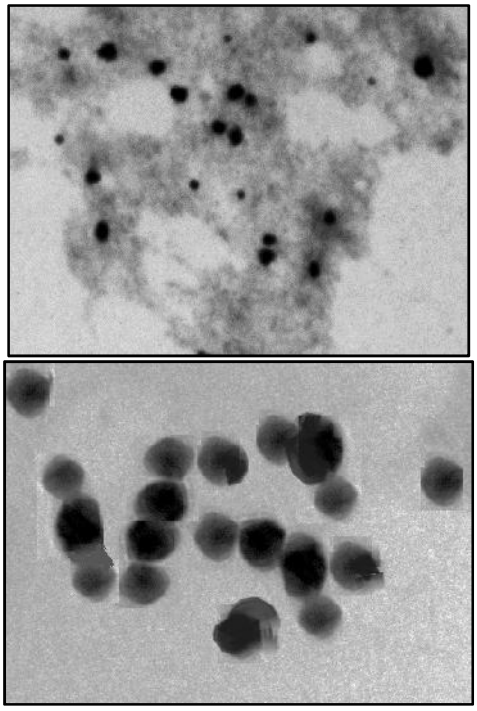


**Figure 3**TEM micrograph (200nm and 50nm) of gold nanoparticles synthesized by *F. oxysporum.*

**TEM Analysis**

TEM micrograph showed details morphology of gold nanoparticles. The data obtained from micrograph images found distinct shape and size of polydisperse nanoparticles. Mostly particles were spherical and ellipsoidal and in shape in the range of 18-24 nm and average size 21.82 nm in size without significant agglomeration (Fig3).

**Effect of biosynthesized silver nano-particles on seed germination**

It is clear that gold nanoparticle solution of *F. oxysporum* found optimistic effect on germination sunflower. As compered control, *Helianthus annuus* shows maximum germination irrespective of gold nanoparticles solution. Results indicates that increasing the soaking period of gold nanoparticles solution, was increase the germination of sunflower which indicates that's seed germination is directly proportional to soaking period of gold nanoparticles solution. Gold nanoparticles soaking sunflower seed found maximum root shoot length as compared to control in 4hr and 6hr soaking period (Fig4).

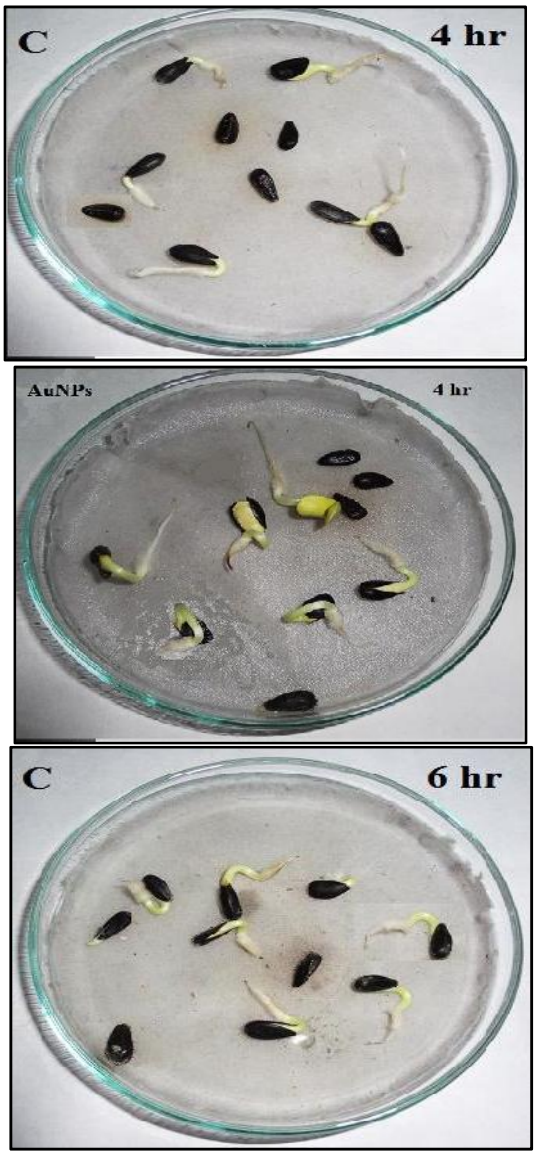




**Figure 4** *Helianthus annuus* seedling and seed germination percentage in controland AuNPs solution at 4 hr. and 6hr. soaking periods.

## CONCLUSION

The synthesis of AuNPs using a cell free extract of *F. oxysporum* appears to be simple and a cost effective method however this approach would be suitable for developing a biotechnological process for large-scale production of small AuNPs.The present study demonstrated the biosynthesis of gold nanoparticles by cell free extract of *F. oxys porum*using 1 mM Chloroauric acid. These gold nanoparticles are found to have strong absorption peak at 550 nm and stable after long time without any clamping. The TEM result shows the synthesis of polydisperse spherical and ellipsoidal gold nanoparticles of the size range 18-24 nm with no agglomeration. Nevertheless, on the basis of the results we are reporting, it is recommended that the influence of nanoparticles be assessed in order to encourage seed germination and seedling growth of a range crop and plant species the which are well known for poor seed germination due to high rates of dormancy. Therefore these result concluded that *Fusarium oxysporum*is a prominent producer of gold nanoparticles which found optimistic effect on seedling and seed germination.

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