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Myco-synthesis of silver nanoparticles from *Trichoderma harzianum* and its impact on germination status of oil seed

Gitanjali B. Shelar[1]* and Ashok M. Chavan[2]

[1, 2] *Seed Pathology and Fungal Biotechnology Lab, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004. (M.S), India.*

E-mail: gitanjalibshelar@gmail.com

ABSTRACT

The present study highlighting on the biosynthesis of silver nanoparticles by using the fungus *Trichoderma harzianum*. The cell filtrate of *Trichoderma harzianum* reacted with 1 mM silver nitrate solution, resulting the formation of silver nanoparticles within 3 hours. The silver nanoparticles were characterized by Visual analysis, UV-Vis absorption spectroscopy and Transmission electron microscopy (TEM). Biosynthesized silver nanoparticles exhibited maximum absorbance at 440nm in UV-Vis spectroscopy. TEM showed polydisperse spherical and occasionally ellipsoid nanoparticles in the size range from 19-63 nm and average size 34.77 nm. Disease free healthy looking seeds of Sunflower (*Helianthus annuus*) and Soybean (Glycine max) per-soaked in 3 days old silver nanoparticles solution of *Trichoderma harzianum* for 2hr and 5hr soaking period and. It is clear form the result that percentage of seed germination was enhanced irrespective of the myco-synthesized silver nanoparticles solution. *T. harzianum* synthesized silver nanoparticles showed increase in percentage of seed germination with increased in soaking time of silver nanoparticles solution. *T. harzianum* synthesized silver nanoparticles observed optimistic effect on seed germination. Therefore biosynthesized silver nanoparticles have biological assay used in agricultural purposes to increases the viability of seeds.

Key words: Silver nanoparticles, *Trichoderma harzianum*, Transmission electron microscopy, Seed germination, biological assay.

INTRODUCTION

Nanotechnology is the science and technology of small things i.e. ‘nano’. This term originate from Greek word meaning ‘dwarf”. This term was firstly used by ‘Richard Feynman’ in 1959. Therefore nanotechnology term was used from last 53 years. Microbes play an important role in nanotechnology due to the synthesis of nanoparticles by biological method. Microbes are the microscopic organisms which are single or multicellular found in all universes. They include the bacteria, algae, fungi and protozoa. Microbes play an important role in balancing the ecosystem by various processes like soil fertility bio-degradation, sewage treatment and improving agricultural productivity. Microbes are known as decomposer in the ecosystem especially fungi well it grows on living or on dead organism.

A fungus produces several hydrolytic enzymes like Amylases, proteases, Lactase, Pectinases, Catalase, Penicillinase, Glucosidases etc. Mycotoxins like Aflatoxin, Zearalenone, Ochratoxin, Citrinin, T-2 toxin, Fumonisins etc. Pigments like qunonine, phynolic group, and also synthesizes the Nanoparticles like Silver (Ag), Gold (Au), Platinum (Pt), Copper (Cu) (Ahmed et al. 2002; Ahmed et al.2003; Bansal et al. 2004). Biological synthesis of nanoparticles

has gained more attention by the researchers for its potential applications (Narayanan and Sakthivel, 2008; Philip D. 2010). There are also have been several reports on the biosynthesis of AgNPs using fungi, including *Fusarium oxysporum* (Ahmad et al. 2003), *Fusarium acuminatum* (Ingle et al. 2008), *Penicillium fellutanum* (Kathiresan et al. 2009), *Aspergillus clavatus* (Verma et al. 2010), *F. solani* (Ingle et al. 2011), *Aspergillus niger Gade* et al. 2008), *Alternaria alternata* (Gajbhiye et al. 2009) etc. have been successfully used for the synthesis of silver nanoparticles. Silver ions and silver-based compounds are highly toxic to living organisms because 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.

In this article, the cell filtrate of this fungus *Trichoderma harzianum* was used for the synthesis of silver nanoparticles. Silver nanoparticles were observed within 1hr after incubation with $AgNo_3$ solution in to cell filtrate. However to study the effect of biosynthesized silver nanoparticles on germination of oil seeds was also done.

MATERIALS AND METHODS

Collection of Materials:

T. harzianum fungi was isolated from agriculture soil and maintained on potato dextrose agar (PDA) medium at 28Cº. The isolated fungus was identified by lacto phenol cotton blue mounting by morphological and microscopic observation. Pure culture was maintained on potato dextrose agar slants at 28Cº. Healthy sunflower and soybean seed were collected from market to check the effect of silver nanoparticles on seed germination.

Biosynthesis of Silver Nanoparticles:

Glucose nutrient broth medium (GNB) was used for biomass preparation of *T. harzianum*. 25gm of clean fresh fungal biomass was again inoculated in 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 and the cell free filtrate was collected for experiment. The 10 mL filtrate was treated with 10 mL of 1 mM $AgNo_3$ 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 duplicate.

Characterization of Silver Nanoparticles:

UV-visible spectroscopy analysis

Color of the cell free filtrate changes after the incubation of silver nitrate solution was visually observed. 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) and absorbance was measured between 300-600 nm.

Transmission electron microscope (TEM)

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

Effect of biosynthesized silver nano-particles on seed germination:

Seeds of soybean and safflower 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 silver nanoparticles solution of *T. harzianum* for varying period of time (2hr and 4hr) in undiluted solution. Control containing water in which seeds were pre-soaked was run simultaneously as standard with the experimental flask.

RESULTS AND DISCUSSION

Biosynthesis of AgNPs:

When cell-free filtrate of *T. harzianum* isolates was incubated with silver nitrate salt, the color of cell filtrate was exhibited a gradual change to

brown color under dark condition. The color of the culture filtrate with silver nitrate salt changed to intense brown after 24hr of incubation whereas the control (without silver nitrate salt) did not exhibit any color change (Figure-1).

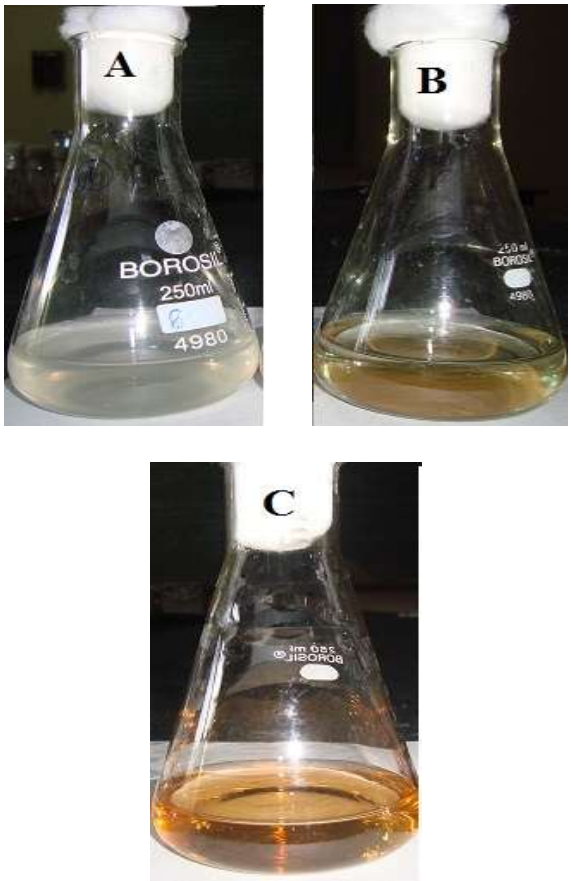

Figure-1. Color of sample A) 1 mM Silver nitrate solution B) Crude cell filtrate of T. harzianum before immersion of $AgNo_3$ C) after immersion of $AgNo_3$.

Characterization of AgNPs:

The UV-visible spectra of *T. harzianum* fungal cell filtrate of treated with the silver nitrate solutions showed a characteristic surface plasmon absorption band at 440nm which are nearby similar to result of Mukherjee et al. (2008) reported an intense peak at 410nm. It is reported that the absorption spectrum of spherical silver nanoparticles presents a maximum between 420nm and 450nm (Maliszewska et al. 2008). Banu and Rathod, (2011) indicating the synthesis of silver nanoparticles 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. Synthesized AgNPs was extremely stable at room temperature, without agglomeration was monitored regularly by UV-visible spectrophotometer. This indicated that the nanoparticles were well dispersed in the solution without aggregation (Figure-2).

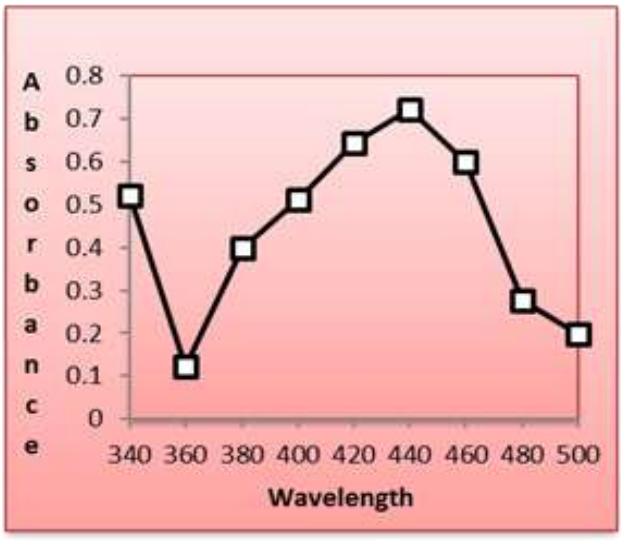

Figure-2. UV-Vis spectra recorded after the exposure of 1mM silver nitrate solution in crude cell filtrate of *T. harzianum*.

TEM micrograph provided detailed morphology of silver nanoparticles. The data obtained from micrograph showed distinct shape and size of polydisperse nanoparticles. Mostly particles were spherical but some are ellipsoidal in shape and 19-63 nm and average size 34.77 nm in size without significant agglomeration (Figure-3).

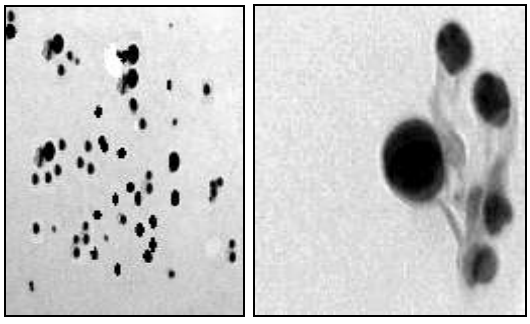

Figure-3. TEM micrograph (200nm and 50nm) of silver nanoparticles synthesized by *T. harzianum.*

TEM provided confirmation of presence of silver nanoparticles with detailed size and shape which are similar to the results of Anand KR Thakur, (2014) of Estari Mamidala et al (2014).

Effect of silver nanoparticles on seed germination:

From Table no-1 it is clear that silver nanoparticle solution of *T. harzianum found* optimistic effect on germination of soybean and sunflower as compared to control. Silver nanoparticles solution showed maximum effect on germination status of soybean and sunflower. As compered soybean, sunflower shows maximum germination irrespective of silver nanoparticles solution. Results indicates that increasing the soaking period of silver nanoparticles solution, will increase the germination of soybean and sunflower which indicates that's seed germination is directly proportional to soaking period of silver nanoparticles solution (Figure-4).

Seed	Time		
	Control	2hr	4hr
Soybean	**10**	**13**	**19**
Sunflower	**13**	**16**	**19**

Table-1. Effect of different soaking periods on germination % of soybean and sunflower.

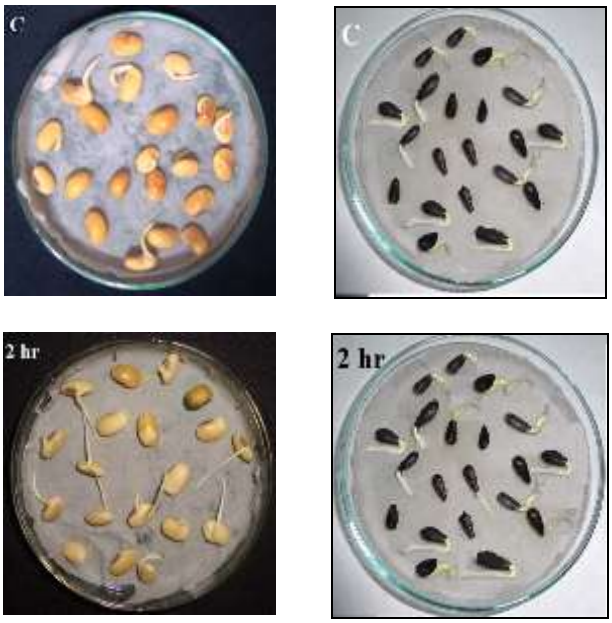

Figure-4. Germination percentege of soybean and sunflower at 2 hr and 4 hr soaking periods and control.

CONCLUSION

From above result it can be concluded that *T. harzianum* secrete large amount of proteins and enzymes so there is no need of reducing agent and stabilizer like as chemical method. Thus the present study has reported the biological process for the synthesis of silver nanoparticles using *Trichoderma harzianum.*

Characterization was made by UV-Visible absorption spectroscopy which shows maximum absorption at 440nm, Transmission Electron Microscope (TEM) revealed the formation of spherical and ellipsoid nanoparticles with size ranging between 19-63 nm and average size 34.77 nm with no agglomeration.

Biosynthesized silver nanoparticles have optimistic effect of seed germination. Thus, results conclude that maximum soaking period of silver nanoparticles will allow the maximum soaking of silver nanoparticles through seed coat and scutellum which ultimately effect on physiology of the seed. Therefore biosynthesized silver nanoparticles have biological assay used in agricultural purposes to increases the viability of seeds.

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