Research Article

Extracellular Myco-synthesis of Silver Nanoparticles from *Trichoderma virens* and *Metarhizzium anisopliae*

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Abstract

Application of nanotechnology in the field of agriculture enhance the efficacy of input use to increase the yield. In the present investigation a total of six isolates of *Trichoderma virens* and two isolates of *Metarhizzium anisopliae* were used to check its reducing properties of silver into nanosilver particle. All the isolates produced silver nano particle but the intensity varied, among the tested isolates. The isolate, *T. virens* (T4) could produce maximum nanoparticles as evident from the UV-Vis study. The absorbance was recorded at 420 nm at every 24 h interval and maximum intensity was noticed at fifth day in *T. virens* (T4) (0.142) followed by *Metarhizium* ITCC 5414 (0.120) but on first day. Further, the high resolution transmission electron microscopy (HRTEM) provided the shape and size of nanopartilce synthesized. The nanoparticls were found single and also as aggregated form with more or less uniform in shape and size of 8-60 nm. Thus the result confirms the production of silver nanoparticles through reduction by extracellular culture filtrate.

Key words : *Metarhizzium anisolpiae*, silver nanoparticles, *Trichoderma virens*

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With the past two decade, agricultural science has started up in the field of nanotechnology and has become a novel thrust area of research for greater input use efficiency. Currently, objects in the nano sizes are playing a major role in new drug discovery, agrochemical and biotechnological industry (Vijayakumar et al 2013). Synthesis of nano particles is emerging as one of the growing field of research due to their enhanced physical, chemical and biological properties. The use of reducing agents in the nanoparticles synthesis has opened a crucial pathway which threatens the environmental sustainability and also limited the uses of these noble materials towards biological applications (Firdhouse and Lalitha 2015). Researcher across the world has turned to biological system for synthesis of nanoparticles as alternatives to chemical and physical methods.

Several research works on microbial synthesis of nanoparticles has been granted with patents, as evidence in the synthesis of AgNPs (5–50nm) and (10-60nm) harnessing wet biomass of *Trichoderma reesei* fungus at 28C after 120 hours of continuous shaking (Vahabi et al 2011). The spherical, semipentagonal, and hexahedral structures (10–60nm) of silver nanoparticles were formed using *Bipolaris nodulosa* (Saha et al 2010). Mukherjee et al (2001) found that the reduction of silver ions is the result of presence of reducing enzymes on the surface of *Verticillium*. Thus, the microbe assisted syntheses of silver nanoparticles have developed the bio mimetic conduit towards plant species. Attempts have been made successfully to use the fungal filtrate for biosynthesis of nanosilver particles by using five different species of *Trichoderma viz., T.virens, T.asperellum, T.harzianum, T.longibrachiatum, T.pseudokoningii* (Prameela Devi et al 2013). Reseachers have used several species of fungus to produce nano metal particle (Thirumurugan et al 2009; Jaidev and Narsimha 2010; Jain et al 2011; Verma et al 2011). In the present study an attempt has been made to synthesis the nano silver particle using *T. virens* and *M. anisopliae*.

Materials and Methods

Fungal Cultures. Six isolates of *T. virens* (T1- ITCC 6123, T2-ITCC 6461, T3-ITCC 6411, T4-ITCC 6471, P12 R36-ITCC 7358, P27 R36 ITCC 7359) and two isolates of *M. anisopliae* (ITCC 7805, ITCC 5414) used in the study were obtained from Indian

Type of culture collection (ITCC), Division of Plant Pathology, ICAR-IARI, New Delhi. All the isolates were cultured in Potato Dextrose Broth and incubated at 25C in the BOD incubator for 5 days and used for the extracellular synthesis of silver nanoparticles.

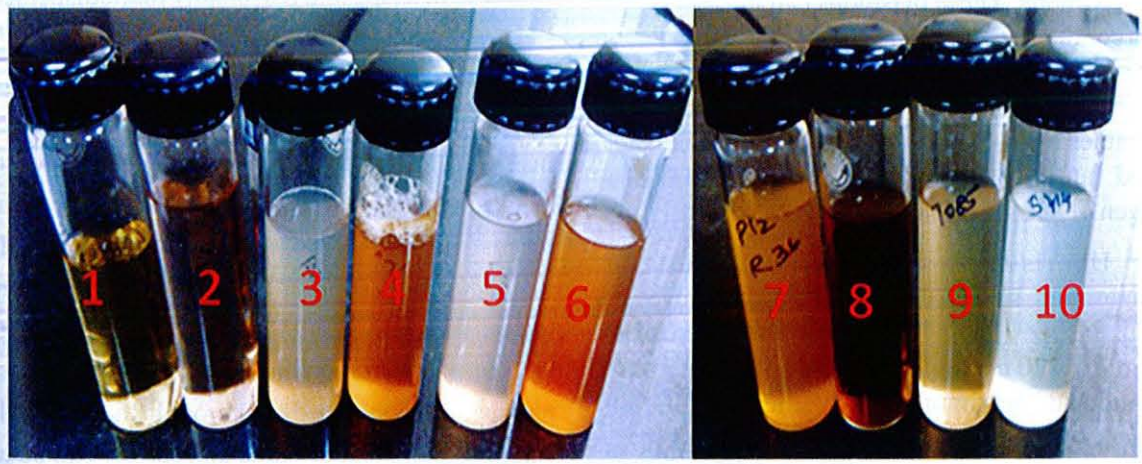
Extracellular biosynthesis of silver nanoparticles using culture supernatant. For the synthesis of silver nanoparticles extracellularly, 50 mL aqueous solution of 1 mM silver nitrate (dissolve 0.17 g of silver nitrate in 1000 ml of double sterile distilled water) was treated with 50 mL of *T. virens* and *M. anisoliae* supernatant solution collected after centrifugation at 5000 rpm in a 250 mL conical flask (pH adjusted to 8.5). Whole mixture was treated at 40C (200 rpm) for 5 days and maintained in the dark. Control experiment were conducted with uninoculated set.

UV visible studies. The reduction of silver ions was monitored by measuring the UV-VIS spectrum of the reaction medium at 24 h with time interval upto 120 h and their absorbance were recorded at 420 nm using UV-Visible Spectrophotometer (Varian Cary 50).

High resolution transmission electron microscopy analysis (HRTEM). The HRTEM analysis of extracellular synthesis of silver nanoparticle was prepared by drop coating biosynthesized silver nanoparticles solution on carbon coated TEM grids (40 × 40 µm mesh size). Samples were dried and kept under vacuum ion desiccators before loading them onto a specimen holder. HRTEM measurement were performed on a LOEL model 1200EX electron microscope operated an accelerating voltage at 120 KV.

Results and Discussion

Six *T. virens* isolates and two *M. anisopliae* were used for the synthesis of silver nanoparticles. It was observed that the fungal supernatant (positive control) retained its original colour but silver nitrate treated fungal supernatant turned dark brown at 24 hr due to deposition of silver nanoparticles. (Fig.1). Differential reaction was observed in the intensity of colour produced by each isolate, from the result it can be concluded that not all isolates can produce the nanoparticle with the same intensity and more over the colour produced does not guaranteed that silver particle has reduced. The color change is due to the excitation of surface plasmon vibrations in the metal nanoparticles (Honary et al 2013). Several metals can be treated as free-electron systems. These metals are



1- Broth alone
2- Silver nitrate + Broth
3- Silver nitrate + *T. virens* (T1)
4- Silver nitrate + *T. virens* (T2)
5- Silver nitrate + *T. virens* (T3)
6- Silver nitrate + *T. virens* (T4)
7- Silver nitrate + *T. virens* (P12 R36)
8- Silver nitrate + *T. virens* (P27 R36)
9- Silver nitrate + *M. anisopliae* (7085)
10- Silver nitrate + *M. anisopliae* (5414)

Figure 1. Cylindrical tubes containing silver nanoparticles

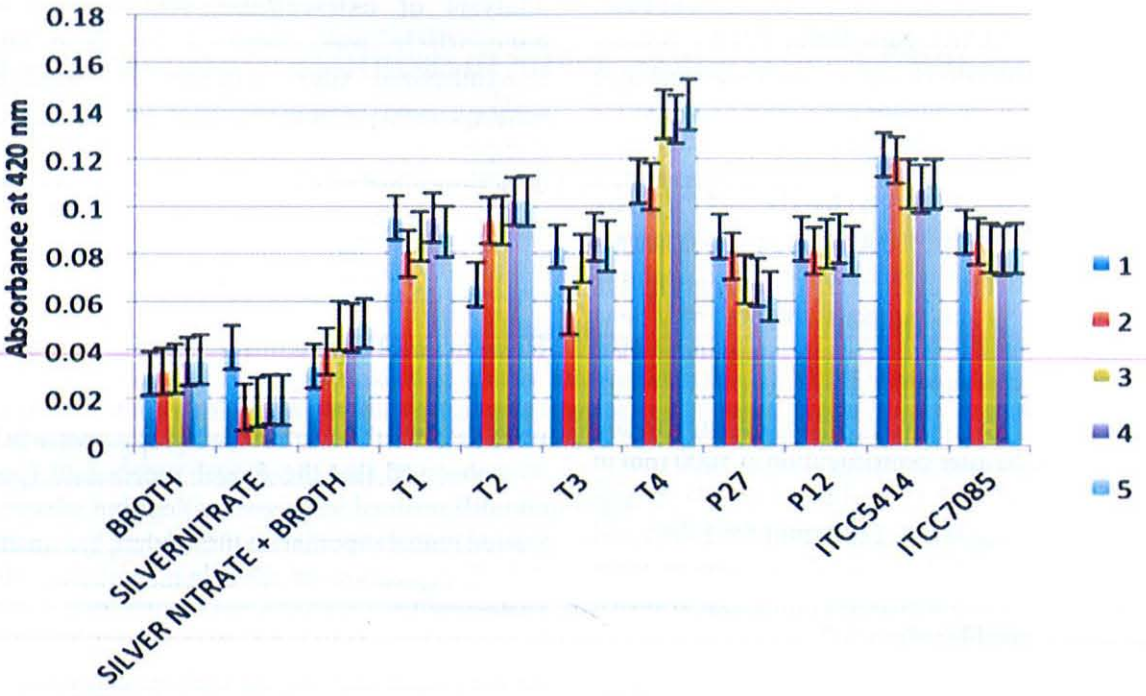


Figure 2. UV visible spectra of fungal filtrate at 24 hour interval at 420 nm

called as plasma which contains equal numbers of positive ions and conduction electrons. If irradiated in an electromagnetic wave, the free electrons are driven by the electric field to vacillate coherently. These collective oscillations of the free electrons are called plasmons. These plasmons can interact, under certain conditions, with visible light in a phenomenon called surface plasmon resonance (SPR) (Pitark et al 2005). Shyla et al (2014) used chemical methods for the synthesis of Silver and Titanium which also showed antifungal activity against soil borne pathogen *Macrophomina phaseolina.*

UV-Vis spectroscopy can be used to examine the size and shape controlled nanoparticles in aqueous suspension, in the present study the spectra measured at 420 nm region showed an increase in the absorption and maximum was obtained on 72 hr post treatment with culture filtrate. The highest absorption band was found for *T. virens*. (T4) (0.142) on 3rd day followed by *M. anisopliae* ITCC 5414 (0.120). Change in colour intensity was noticed (Fig. 2). Honary et al (2013) has also reported that UV-vis spectrum of silver nanoparticles produced by *Penicillium citrinum* exhibited an absorption band at around 400 - 420 nm which is a typical plasmon band, suggesting the formation of silver nanoparticles.

In order to check the size of nanoparticle, TEM images of nano particles extracted through the culture filtrate of *T. virens* (T4) and *M. anisopliae* ITCC 5414 was performed. In general particles are isotropic in shape and reasonably monodisperse. The sizes of silver nanoparticle were found to be 15-70nm (Fig. 3). The particles were present as individual nano silver form or as an aggregates, provided they are not in direct contact even within the aggregates suggesting the stability of nanoparticles by caping agents. The synthesis of nanoparticle is largely due to the reductase enzymes such as NADH dependant nitrate reductase which acts as nucleating agents. Hence the present study showed that the silver nanoparticles synthesized through the extracellular fungal supernatant is stable and further can be tested against major plant pathogens.

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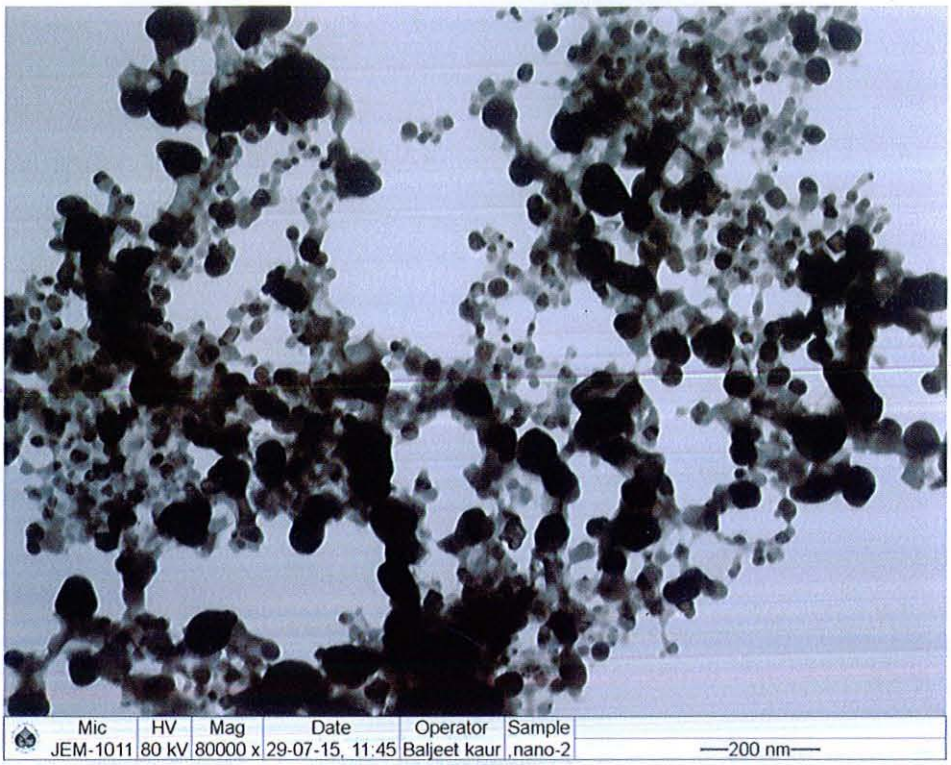


Figure 3. HRTEM photographs of nanosilver particle synthesized from *T. viride*

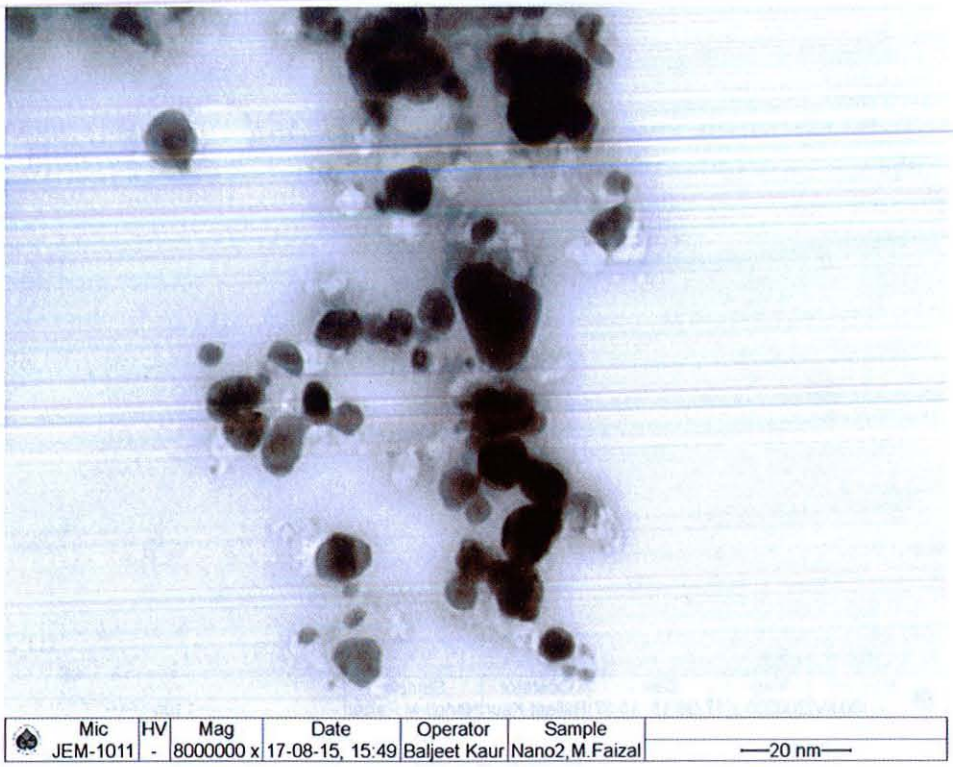


Figure 3a. Single silver nanoparticle seen on *T. virens* (T4)

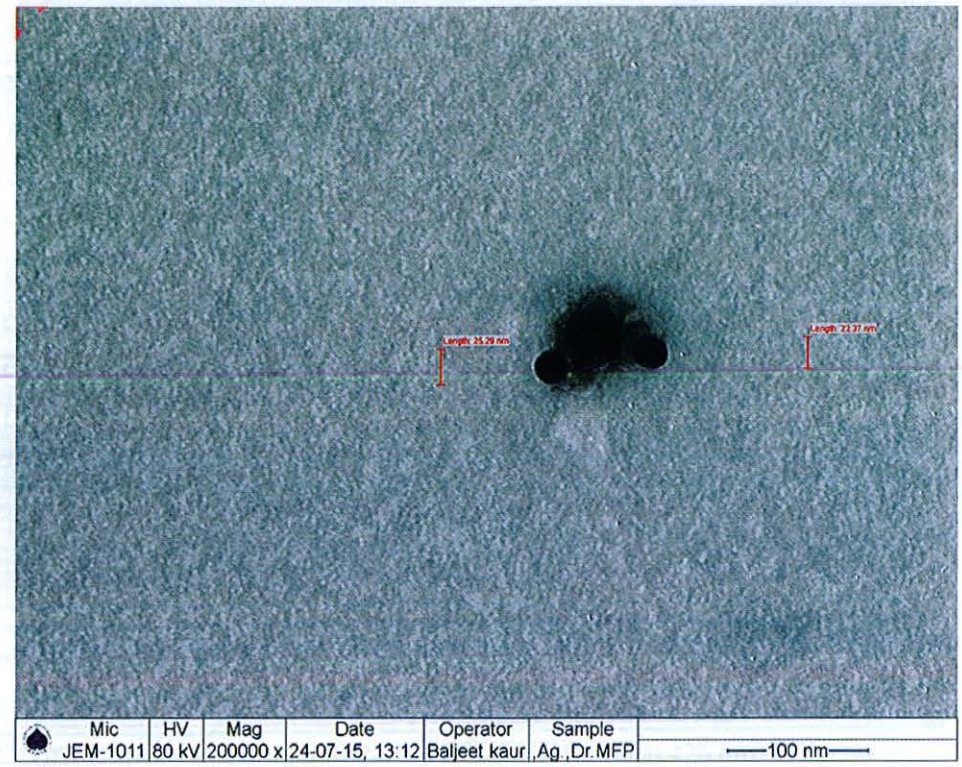
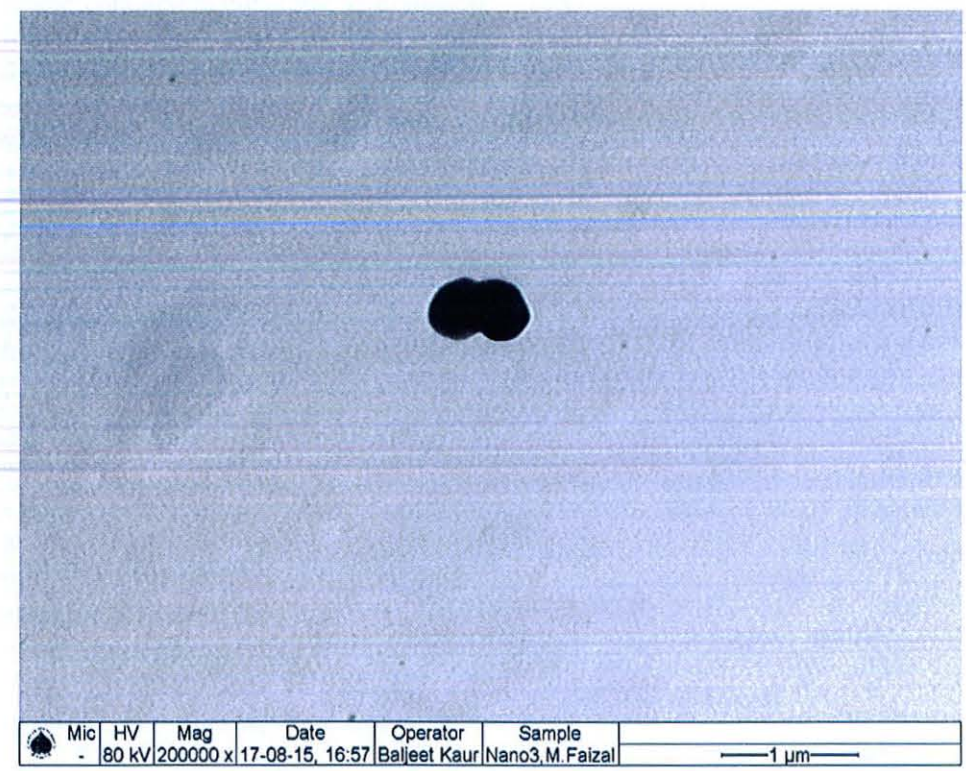


Figure 3b. Single silver nanoparticle seen on *T. virens* (T4)



igure 3c. Silver nanoparticles reduced by *M. anisopliae* 5414

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