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Synthesis And Charactererization Of Bio Nano Particles Of Silver Nitrate Using Bio Control Agents

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ABSTRACT
In the present investigation bio silver nano conversion was attempted using Potato Dextrose Broth (PDB) culture filtrates of two isolates of Trichoderma (ET-1 and RT-4), Nutrient Broth (NB) culture filtrates of two isolates of P. fluorescens (PF-2 and PF-5), only mycelial mat and spore suspension (10^{-8} spores/ml) of ET-1 isolate. Bionano conversion was also attempted using uninoculated autoclaved PDB and NB for comparison. Conversion of silver nitrate into silver nano particles was also assessed using culture filtrate of rice sheath blight pathogen R. solani. The result indicated that ability to convert silver nitrate to nano silver varied with type of microbe and age of the culture filtrate. With P. fluorescens conversion occurred with 5 day culture filtrate while 10 and 15 day old culture filtrates failed to convert silver in to nano silver efficiently. Within Trichoderma isolates, ET-1 was effective with 10 and 15 day old culture filtrates while RT-4 with all the culture filtrates.When washed mycelial mat of ET-1 was exposed to silver nitrate solution, nano conversion was observed indicating that the ability of nanoconversion is instantaneous. However, accumulated effort of an organism in converting silver in to nano silver was found more efficient as absorbance peaks were quite faint with mycelial mats assessed just after 24 hours of mixing with silver nitrate solution.
Key words: *Potato Dextrose Broth, Nutrient Broth, Trichoderma, Silver nano particles, Mycelial mat.*

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INTRODUCTION:

Nanotechnology is an emerging field in the area of interdisciplinary research especially in biology. The advancement of nanotechnology mainly requires the development of reliable and ecofriendly protocols for the synthesis of nanomaterial over a range of biological composition, sizes, shapes and high monodispersity. Nanoparticles possess exceptional physical and chemical properties which lead to rapid commercialization. Nanoparticles are considered as fundamental molecular building blocks for nanotechnology. They are the pre-requisites for preparing many nanostructure materials and devices. Biosynthesis of nanoparticles is an attractive possibility of advancement of green nanotechnology which has potential to find numerous applications in biology - agriculture in particular.

Specific antimicrobial mechanisms of silver are still not completely understood though the toxic effect is postulated to be through inhibiting the expression of proteins associated withATP production (Yamanaka *et al*., 2005). Nano silver particles are used for control of various plant pathogens and compared with synthetic fungicides (Min *et al*., 2009). Jo *et al*. (2009) studied the effect of various forms of silver nanoparticles on two plant pathogenic fungi, *Bipolaris sorokiniana* and *Magnaporthe grisea.* Since agriculturally important microorganisms are environmental friendly and they are well known for their formation of extracellular enzymes and metabolites in very large amounts, utilizing these bioagents could be an excellent method for production of silver nanoparticles. However, mechanism of silver nano conversion using bioagents and role of silver nano particles in plant disease management with or without microbiological assistance is yet to be worked out.

MATERIAL AND METHODS:

Culture filtrates of test fungal and bacterial cultures were used to study extra cellular synthesis of silver nanoparticles from silver nitrate. Attempt was also made to synthesize bio nano silver using mycelia mat of fungal cultures

2.1 Production of Culture Filtrates of Fungal and Bacterial Cultures. Seven day old pure culture of selected isolates of *Trichoderma* spp,, *P. fluorescens* and one isolate of *R. solani*were individually inoculated in 250 ml conical flasks containing 100 ml of Potato Dextrose Broth (PDB) for fungal cultures and Nutrient broth for bacterial cultures. The culture flasks were incubated at 28 ± 1°C. The culture filtrate obtained at 5 DAI, 10 DAI, and 15 DAI through 2 layers of sterilized Whattman No-1 filter paper and used for the synthesis of silver nanoparticles. Mycelial mat of fungal cultures after filtration was collected and used for bionano silver synthesis.

2.2 Synthesis of Silver Bionano Particles 90 ml of aqueous solution of 1mM Silver nitrate ($AgNO_3$)(equivalent to 170 ppm) was mixed with 10 ml of either *Trichoderma* spp, *R. solani*,or *P. fluorescens* culture filtrates for the extra cellular synthesis of silver nanoparticles in a 250 ml conical flask. For mycelia mats, mycelia mat obtained from one 250 ml flask was mixed with 90 ml of silver nitrate solution. The whole mixture was incubated at room temperature for 24 hrs. The color change of silver nitrate from colorless to brown color was considered as indicator of formation of silver nanoparticles through reduction of silver ions from Ag^+ to Ag^0.

2.3 Characterization of synthesized silver bionano particles by UV-Visible Spectroscopy The spectra of the surface Plasma resonance of AgNPs in the reaction mixture were recorded using UV–Vis spectrophotometer (Shimodzu, UV-2450) at wavelengths between 200 to 800 nm.

RESULTS AND DISCUSSION:

Silver is being used as an antimicrobial agent. Several formulations are in the market using silver nano particles for medical use. The size of the particles to be called as nano should be less than 100 nm. Nano particles were synthesized using chemical and physical means. However, due to drawbacks in synthesis and utility, recently nano particles are being synthesized using microbial agents. Microbes have the tendency to convert silver nitrate ions into nano silver particles through intracellular and extracellular means.

In the present investigation, two potential antagonistic isolates of *P.fluorescens, i. e.,* PF-2 and PF-5, and two potential antagonistic isolates of *Trichoderma, i. e.,* ET-1 and RT-4 were evaluated for their ability to convert silver in to nano silver.

Silver nitrate (170 ppm) in aqueous solution had an absorbance peak between 216nm and 310nm when measured using UV–VIS spectrophotometer. Up on storage, silver in silver nitrate solution settled down after 24 hours with black colour agglomerated particles. On day-1, *i.e*., immediately after preparation, absorbance peak was obtained at 310 nm while on day-15 absorbance peak was obtained at 216 nm with a progressive decline in the absorbance peak wavelength from first day to fifteenth day.

The bio silver nano conversion was attempted using Potato Dextrose Broth (PDB) culture filtrates of two isolates of *Trichoderma* (ET-1 and RT-4), Nutrient Broth (NB) culture filtrates of two isolates of *P. fluorescens* (PF-2 and PF-5), only mycelial mat and spore suspension (10^{-8} spores/ml) of ET-1 isolate. Bionano conversion was also attempted using uninoculated autoclaved PDB and NB for comparison. Conversion of silver nitrate into silver nano particles was also assessed using culture filtrate of rice sheath blight pathogen *R. solani*.

Three absorbance peaks were obtained when tested using UV-VIS spectrophotometer, *i.e.,* one at 220 nm indicating pure silver nitrate, second at 260 nm indicating partial conversion and third at 400-450 nm indicating nano particles presence.

When culture filtrate of *Trichoderma* isolate ET-1 (10 ml) was mixed with 90 ml of 170 ppm silver nitrate solution, change in colour was observed from initially transparent solution to colloidal brown solution in 24 hours(Plate 3.1). When absorbance peak was observed using UV-VIS spectrophotometer, result indicated that the conversion was maximum with fifteen day old culture filtrate while the conversion was initiated with five day old culture filtrate with absorbance maximum at around 450 nm wavelength (Fig 3.1 a to c). Raja *et al*. (2008), Singh and Raja (2011) and Devi *et al*. (2013) reported that conversion of silver nitrate into nano silver is judged by the brown coloured colloidal formation with absorbance peak from 400 nm to 450 nm.

In case of *Trichoderma* isolate RT-4 similar observations were made with detection of silver nano particles at around 450 nm and colour of the solution turning brown.

In case of *P. fluorescens* isolate PF-2 absorbance peak was obtained pertaining to nano silver particles, *i.e*., at 420 nm, only with 5 day old culture filtrate. With 10 day and 15 day old culture filtrates, absorbance peak was at <400nm. However, colour change was observed even with 10 day old and 15 day old culture filtrates indicating conversion to a lesser extent (Fig. 3.2 a to c).

Similar result of absorbance peak at 420 nm was observed with *P. fluorescens* isolate PF-5 from 5 day old culture filtrate but not from 10 or 15 day old culture filtrate.

Thus, the result indicated that ability to convert silver nitrate to nano silver varied with type of microbe and age of the culture filtrate. With *P. fluorescens* conversion occurred with 5 day culture filtrate while 10 and 15 day old culture filtrates failed to convert silver in to nano silver efficiently. Within *Trichoderma* isolates, ET-1 was effective with 10 and 15 day old culture filtrates while RT-4 with all the culture filtrates.

Sastry *et al*. (2003), Singh and Raja (2011), Roy *et al*. (2013) and Yousuff *et al*. (2013) reported such variation in converting silver nano in to nano silver depending upon type of species used and their age.

Davet (1981), Jo *et al*. (2009), Min *et al*. (2009) and Vahabi *et al*. (2011) reported conversion of silver in to nano silver by microbes is to avoid stress due to high metal (silver) toxicity. Under such circumstances, *R. solani*, a soil borne plant pathogen, also may have the ability to convert silver into nano silver to avoid toxicity. In order to confirm the above statement culture filtrate of *R. solani* was also assessed for nano conversion. The result indicated that *R. solani* culture filtrate, irrespective of its age, possessed the ability to convert silver in to nano silver as evidenced from absorbance peak at 450 nm with all the three culture filtrates *i.e*., 5, 10 and 15 day old (Fig. 3.3 a to c).

Further, it may be noted here that all the three test fungal cultures *i.e*., two isolates of *Trichoderma* and one isolate of *R. solani* had the ability of nano conversion even up to 15 days of age. However, *P. fluorescens* isolates had the maximum ability with 5 day old culture filtrate indicating bacterial growth in exponential form is suitable for nano conversion.

In order to assess whether ability of nano conversion is an accumulated effort or an instantaneous effort, nano convertibility was assessed with mycelial mat and spore suspension of *Trichoderma* isolate ET-1 (Fig. 3.4 a and 3.4 b). The results indicated that with mycelial mat too an absorbance peak at 420 nm appeared though the peak was a fainted one. Further, several faint peaks obtained at and above 450 nm indicated agglomeration of converted nano silver. Similar observations were made with spore suspension. The nano conversion was also confirmed with colour change from transparent to brown within 24 hours of mixing mat or spore suspension with silver nitrate solution indicating instantaneous ability of the organism used for nanoconversion. However, accumulated effort causes better nanoconversion as the nano convertibility in the present investigation was done just after 24 hours of exposing mycelial mat with silver nitrate solution.

The experiment was also done to assess ability of the nutrient media used for preparing culture filtrates such as autoclaved PDB and NB alone (Fig. 3.5 a and b). It may be noted here that autoclaved PDB alone also could convert silver into nano silver giving an absorbance peak at 420 nm. Similar result was obtained with Nutrient Broth with an absorbance peak at 420 nm. It may be noted here that PDB contains potato and NB contains beef extract which are plant and animal derived respectively. It is interesting to note that the principle involved in nano conversion appeared to be thermo stable as nano conversion was obtained with autoclaved PDB and NB.

The present investigation gave an indication that nano conversion is a common biological process. Reports are available on synthesis of nanoparticles using fungal spp. (Mandal *et al*., 2006; Mukherjee *et al*., 2001a; Mukherjee *et al.,* 2001b; Duran *et al*., 2005), bacteria (Klaus-Joerger *et al*., 2001), plant extracts (Shankar *et al.,* 2004) and even human cells (Anshup *et al*., 2005).

Summery and Conclusion:

Pure silver nitrate solution (170ppm) had absorbance peaks ranged from 216nm to 310nm when measured with UV-Vis spectrophotometer over a period of 15 days in storage under ambient conditions. Colour of the solution turned transparent to black after 24 hours of storage with settlement of particles at the bottom of the flask.

When the cell free culture filtrates of ET-1, RT-4 (prepared in PDB), PF-2 and PF-5 (prepared in NB) were mixed with silver nitrate solution (170ppm) @ 10:90 (culture filtrate: silver nitrate solution), with all the mixtures, colour of the silver nitrate solution changed to light brown colour in 48 hours with colloidal appearance indicating conversion of silver nitrate to bionano silver. The conversion was further confirmed using UV-Vis spectrophotometer when one of the three absorbance peaks, *i. e.*, between 400 to 450nm corresponded to bionano silver. It is interesting that similar observations were made even with the culture filtrate of *R. solani*, autoclaved-uninoculated Potato Dextrose broth and autoclaved-uninoculated Nutrient broth indicating that every organism (potato in PDA and beef extract in NB) has the ability of nanoconversion of heavy metals like silver.

When nano conversion was attempted with three different ages of culture filtrates (*i.e*., 5, 10 and 15 days), the result indicated that ability to convert silver nitrate to nano silver varied with type of microbe and age of the culture filtrate. With *P. fluorescens* conversion occurred with 5 day culture filtrate while 10 and 15 day old culture filtrates failed to convert silver in to nano silver efficiently indicating that exponential growth phase is necessary for nano conversion. Within *Trichoderma* isolates, ET-1 was

effective with 10 and 15 day old culture filtrates while RT-4 could convert silver in to bionano silver with all the culture filtrates.

When washed mycelial mat of ET-1 was exposed to silver nitrate solution, nano conversion was observed indicating that the ability of nanoconversion is instantaneous. However, accumulated effort of an organism in converting silver in to nano silver was found more efficient as absorbance peaks were quite faint with mycelial mats assessed just after 24 hours of mixing with silver nitrate solution.

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Plate 3.1 Colour development in biosynthesized silver nano solution after 24 hours

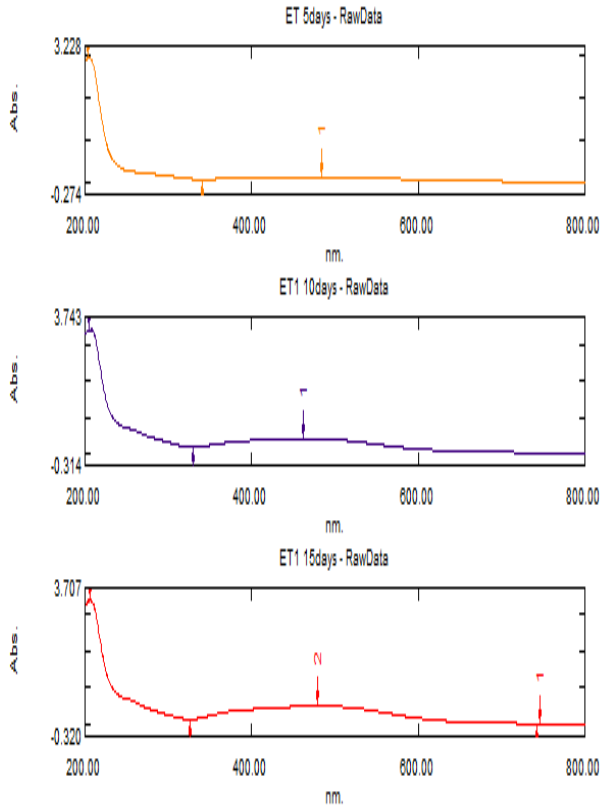

Fig. 3.1. a to c. UV VIS Absorption spectrum of bionano silver prepared from culture filtrate of *Trichoderma* isolate ET-1.

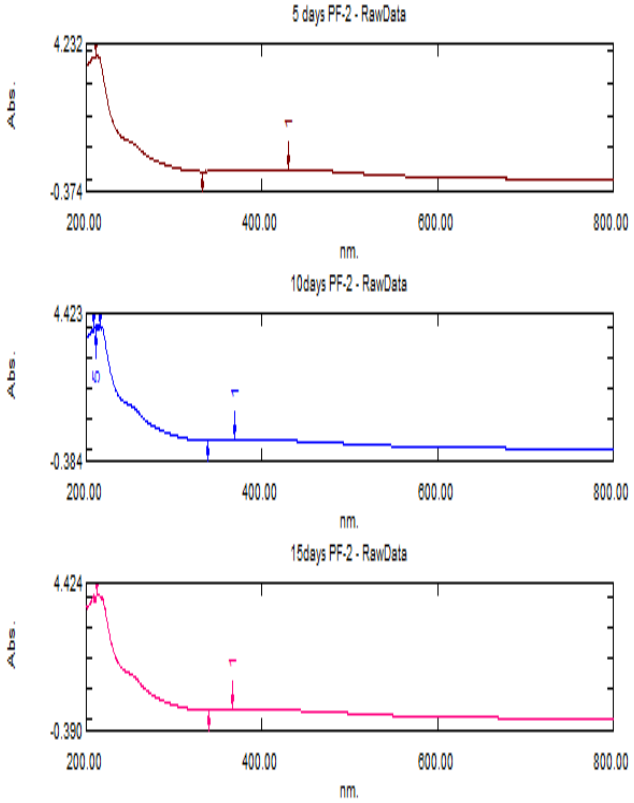

Fig. 3.2. a to c. UV VIS Absorption spectrum of bionano silver prepared from cell free culture filtrates of *P. fluorescence* PF-2.

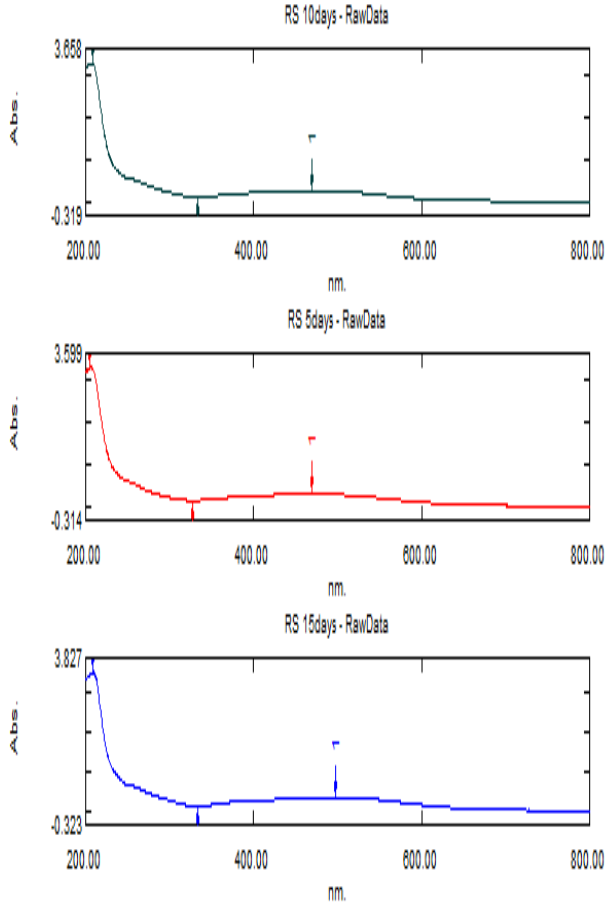

Fig. 3.3. a to c. UV VIS Absorption spectrum of bionano silver prepared from culture filtrate *R. solani*

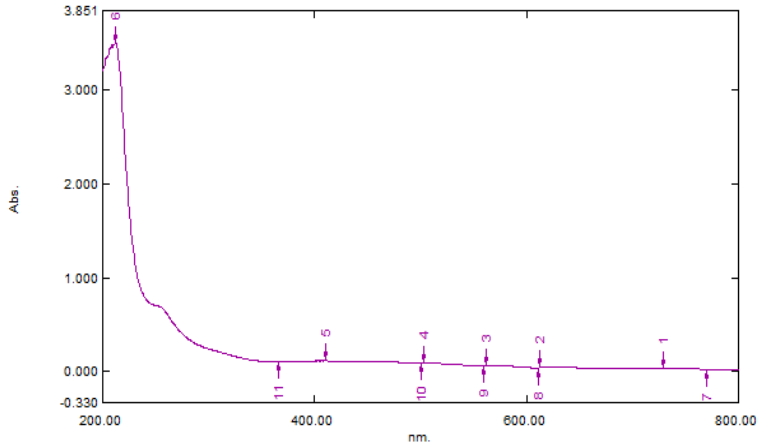

Fig. 3.4.a. UV VIS Absorption spectrum of bionano silver by *Trichoderma* ET-1 mycelial mat

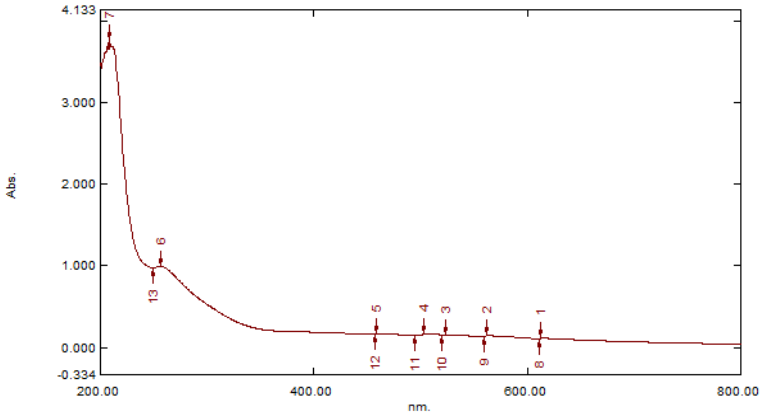

Fig. 3.4. b. UV VIS Absorption spectrum of bionano silver by *Trichoderma* ET-1 spore suspension

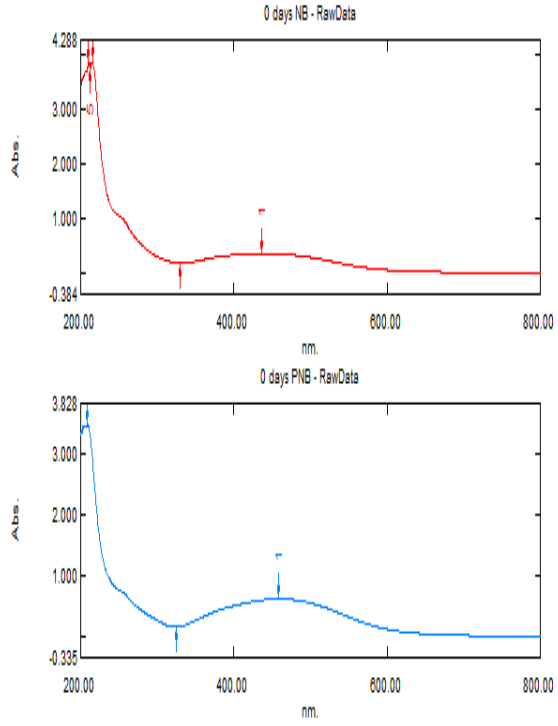

Fig .3.5a and b. UV VIS Absorption spectrum of bionano silver by NB and PDB

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