Biosynthesis of Silver Nanoparticles by *Trichoderma harzianum* and its Effect on the Germination, Growth and Yield of Tomato Plant

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Myconanotechnology is a branch of science which deals with the study of nanoparticle synthesis by using fungi. Fungus mediated synthesis of nanoparticles is a reliable and ecofriendly technique. In the present work T.harzianum was used for the synthesis of silver nanoparticle.The synthesized nanoparticles were characterized using UV-Spectrophtometer, Transmission Electron Microscope (TEM) and Fourier Transform Infrared Spectroscopy (FTIR). Through TEM analysis shape and size of the synthesiszed nanoparticles was determined. The size of the synthesised nanoparticles was 12 nm. The synthesiszed nanoparticles were tested in glass house conditions against the Fusarium wilt of tomato. Nanoparticle treated plants showed promotory effect on the all tested paparmeters (germination, plant height, root length, total yield and root dry weight) as compared to control and Trichoderm formulation treated plants.

Keywords: Nanoparticles, Nanotechnology, Trichoderma harzianum.

Nanotechnology is a branch of science which deals with various aspects of research and technology. Myonanotechnology is a word which is formed by combining mycology and nanotechnology together. Myonantechnology is having a large scope in present era as we have a large fungal diversity (Rai, M., 2009).There are many advantages associated with fungus mediated synthesis of nanoparticle (Deendayal M *et al* 2006). Fungi secrete some enzymes that are able to hydrolyze metal ions, they are easy to isolate and culture and the mass productivity is also higher. Downstream processing and the handling of fungal biomass are less complex than the synthetic methods.

When we talk about fungus nanoparticles the synthesiszed nanoparticles are of good monodispersity. Green nanotechnology is getting popularity as it utilizes biologic materials for the synthesis of nanoparticles, that are environmental friendly and do not produce toxic wastes in their synthesis process (M. Anandaraj, 2011). Certain bacteria, fungi and yeast has been found to play a major role in the bioremediation of metal pollution. So, these microorganisms could minimize the toxicity of metal ions in the process of metallic nanoparticle formation, by the reduction of the metal ions or by the formation of insoluble complexes with metal ions in the form of colloidal particles.

Myofabrication is a world which can be defined as the synthesis of nanoparticles by the use of fungi. In recent times fungal genera has been emerged as nanofactories for the production

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of nanoparticles (Mahendra R *et al.*, 2009) . Various metals like silver, gold, copper and platinum are accumulated by fungi by various physical chemical and biological mechanisms. Fungi are excellent secretors of proteins resulting in the higher yield of biomass. Due to these properties fungi could be used for rapid and large scale production of ecofriendly nanoparticle (Raveendran P, 2003).

This study involves the study of synthesis of *Trichoderma harzianum* silver nanoparticles, their characterization and their effect on the tomato plant seedlings.

MATERIALS AND METHODS

Nanopaterial synthesis from *Trichoderma harzianum*

For nanoparticle synthesis fungal inocula was prepared in PDB media. For nanoparticle synthesis fungus grown in 200ml bottle containing 100 ml PDB media incubated at 28°C for 72 hours at 150 rpm. After incubation process the contents of the flasks were filtered through filter paper and the settled biomass was washed thrice with distilled water. The harvested biomass was then used for the synthesis of silver nanoparticles. For the synthesis 10 g mass mixed with 100 ml aqueos solution of 1mM silver nitrate, and then the mixture is placed in dark (T Prameela D, 2013, Sanghi R& Verma P ,2009). During this process the silver nanoparticles were produced by the reduction of silver ions to metallic silver.

Characterization of nanoparticles

The reduction of silver ions was routinely measured by the physical evaluation of the solution. Silver Nanoparticles were synthesized using *Trichoderma harzianum* strain. After nanoparticle synthesis we done their FTIR , TEM (All these two analysis were done by Sandor life science, Hyderabad) and UV- Visible Spectroscopy analysis.

Fourier Transform Infrared Spectroscopy

FTIR spectroscopy analysis was carried out to identify the potential bio molecules in the extract responsible for the reduction and also the capping reagent responsible for the stability of the bio reduced silver nanoparticles.

For FTIR measurements, the Silver nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/ enzymes that are not capping the silver nanoparticles. The samples were dried and grinded with KBr pellets and analysed on a Bruker Optics (Germany made) Tensor 27model in the diffuse reflectance mode operating at a resolution of 0.4 cm^{-1}.

TEM analysis

TEM grids were prepared by placing a drop of the particle solution on a carbon-coated copper grid and drying under lamp. The 200 kV Ultra High Resolution Transmission Electron Microscope (JEOL-2010) has been used.

UV- Visible Spectroscopy analysis

Silver ion reduction was monitored by measuring UV-VIS spectrum of the reaction medium at 24h with time interval upto 120 h and their absorbance was teken at 420 nm using spectrophotometer (BioRad, USA).

Effect of biosynthesized silver nano-particles on tomato plants

This study was conducted to examine the promotory and inhibitory effect of *Trichoderma harzianum* silver nanoparticles based bio-formulation on the plant growth, development and final yield of tomato plants. This study was initiated to generate new information about the efficacy of *Trichoderma* silver nanoparticles bioformulation on the growth and development of tomato plant. Different doases of the *Trichoderma* silver nanoparticle based bioformulation were tested to optimize the concentration. Total 7 treatments were used for tomato crop. For *Trichoderma herzianum* nanoparticle based bioformulation preparation talcum powder and synthesissed nanoparticles were mixed in 9:1 ratio and used for the experiment.

RESULTS

Synthesis of *Trichoderma* silver nanoparticles

It is evident from the figure 1 that the silver nitrate trated *T.harzianum* supernatant changes to brown colour due to the deposition of silver nanoparticles while the non treated *Trichoderma* supernantant (control) did not show the appearance of brown colour.The appearance of a yellowish-brown color in solution containing the biomass is a clear indication of the formation of silver nanoparticles in the reaction mixture. The brown color of the solution is due to the excitation of surface plasmon vibrations (essentially the vibration of the group conduction electrons) in the silver nanoparticles.

Fourier Transform Infrared Spectroscopy

From the obtained FTIR spectra of silver nanoparticles (Fig2) the appered bands at 1642 and 3359 which are due to hydroxyl and amide-1 and which are responsible for reducing AG^{+} ions to atoms and suppressed bands at 21341 and 1355 are responsible for stabilizing nano particles.

Transmission electron microscopy

Transmission electron microscopy was done in order to study the shape and size of synthesiszed *Trichoderma* silver nanoparticles.The TEM images of the *Trichoderma* silver nanoparticle solution deposited on carbon coated copper tem grid is given below (Figure 3). These picture shows individual as well as aggregates of nanoparticles. The Average size of nanoparticles were also calculated by using J image software.(Figure3)

The average size of the nan-particles were calculated by J-image software is 12 nm and number of nanoparticles per 1ml volume is $\sim 10^{12}$ (Figure 4).

UV- Visible Spectroscopy analysis

UV visible spectroscopy analysis of the *Trichoderma* nanoparticle solution (Figure 5) indicates that after 72 hours there is no appreciable changes in the absorbance. In other words we can say that after 72 hours the reaction attained equilibrium. It should be noted that the reaction mixture was monitored for about one month and it was found that solution was extremely stable even after a month of reaction, without any aggregation and precipitation

Trichoderma culture grown on PDB medium | 1mM Silver Nitrate solutin | *Trichoderma* cell mass+Silver nitrate solution | Formation of Nanoparticles

Fig. 1. Processs of *Trichoderma* nanoparticle formation

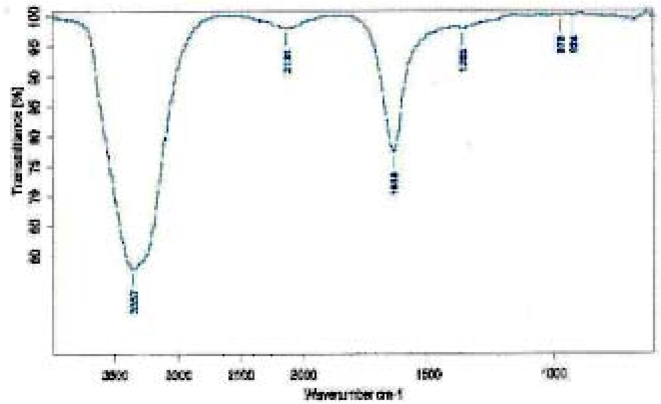

Fig. 2. FTIR Spectrum showed in the range of 1000 to 3500

Glass house Experiment (TOMAT0)

Study was initiated to examine the effect of *Trichoderma* silver nanoparticles based bio-formulation on the plant growth, development and yield of tomato plants. Different doses of the *Trichoderma* silver nanoparticle based bioformulation were tested to optimize the concentration.

Addition of Silver nano base bioformulation of *Trichoderma harzianum* (Th. azad) in tomato (T5) has been found to reduce disease incidence and they play an important role in growth promoting character (Figure 6). It was also observed that the antagonistic activity of *Trichoderma harzianum (Th. azad)* was stimulated by Silver coated *Trichoderma* spore when apply against *Fusarium oxysporum.* Nano particle treated plants showed increase in all tested paprameters over control. Nanoparticle treated tomato plants (5% formulation) grown under glass house revealed increased plant yield 41.49 to 219.66 per cent (Table 1).

DISCUSSION

In todays world nanoparticles are using extensively in every field. Silver nanoparticles are synthesized by using various physical, checal and biological methods. Biological method of nanoparticle synthesis would help to remove harmful residues generated during the processing of nanoparticle. Large number of microbes have been found capable of synthesisning nanoparticles. Fungal medidate sunthesis of metallic nanoparticles is very economical and easy. Various fungal genera such as *Verticillium, Fusarium, Aspergillus, Trichoderma* (Mukherjee P *et al*., 2001,16.Verma V C *et al* 2011, Bhanisa KC. & DÈsouza 2006 and Mukherjee P., *et al* 2008*)* etc. are extensively used in nanoparticle formation.

In the present investigation *Trichoderma herzianum* was used for the production of nanoparticles. Naoparticle production was confirmed from thechange of colour from yellow to brown.The synthsesd nanoparticles were characterized Through TEM, FTIR and UV-visible spectra. The FTIR spectra confirms the stability of synthesissed nanoparticles. uv-visible spectra of T.herzianum nanoparticle solution showed a characteristic surface plasmon absorption band at420 nm. which are similar to the results of Mukherjee *et al* 2008 which reports an intense peak at 410nm and Gitanjali B. Shelar1 *et al*l., 2014 which found intense peak at 440 nm. It has ben found that the absorption spectrum of spherical silver nanoparticles presents a maximum between 420nm and 450nm (Maliszewska *et al*. 2008). Banu and Rathod,(2011) investigated that silver

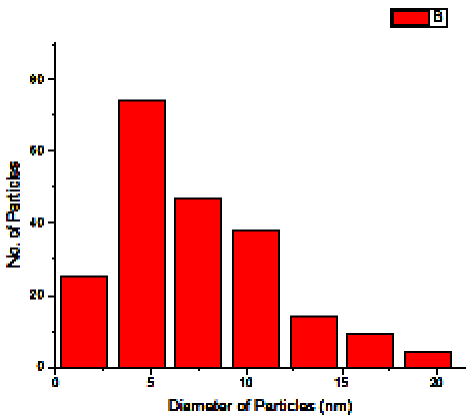

Fig. 4. Aaverage size of silver nanoparticle

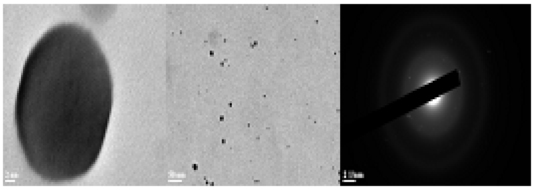

Fig. 3. TEM images of *Trichoderma* nanoparticle solution

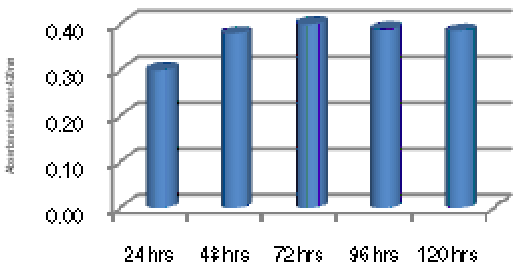

Fig. 5. UV Visible spectra recorded at various time interval (24-120 hrs)

nanoparticles showed maximum colour intensity after three days of incubation, after this incubation the reaction attains equilibrium. Synthesised nanoparticles were stable at room tempertatre without agglutination.This indicated that the nanoparticles were well dispersed in the solution without aggregation.

In present styudy we showed the potential of *T.herzianum* for the production of nanoparticles. The synthesized nanoparticles were characterized by TEM, FTIR and UV-visible spectroscopy . TEM analysis of the synthesiszed nanoparticles confirms their clear lattice fringe and uniform distribution.The average number of nanoparticles present in 1 ml of the solution was 10^{12}. In present time the main problem associate with crop protection is the harmful efffect of residues which are left after chemical pesticides application. Nanoparticles based formulation which are produced by biocontrol fungi will be a better alternative to avoid soil pollution and to maintain soil frtility.

From the table 1 it is clear that *Trichoderma* silver nanoparticle based formulation

Table 1. Pot culture experiment under glass house conditions

Treatments	No. of plant d germinate				Plant height (cm)				Avg. root length (cm)	Root dry weight (g)	Yield per pot (g)	% increase
	R_1	R_2	R_3	(%)	R_1	R_2	R_3	Avg.				
T_1 *F.o.l* 5% (Control)	3	2	2	58.33	20	24	22.5	22.16	3.27	0.21	76.33	
T_2 (Th 5% + *F.o.l* 5%)	4	3	4	91.66	25	28	30.0	27.6	6.0	1.01	228	198.70
T_3 (Th 10% + *F.o.l* 5%)	3	4	2	75.00	25	26	27.5	26.16	5.23	0.95	159	108.30
T_4 (Th 20% + *F.o.l* 5%)	3	4	2	75.00	25	28	28.0	27.00	3.93	0.63	145	89.96
T_5 (Th nanoparticle 5% + *F.o.l* 5%)	4	4	4	100.00	32	33	31.5	32.16	6.17	1.04	244	219.66
T_6 (Th nanoparticle 10% + *F.o.l* 5%)	4	3	3	83.33	30	22	22.5	30.00	5.33	0.71	183	139.74
T_7 (Th nanoparticle 20% + *F.o.l* 5%)	4	3	3	83.33	31	31.5	30.0	30.83	4.5	0.21	108	41.49
CD @ 5%									0.784	0.118	8.79	
SE (d)									0.362	0.054	4.059	

A-*T. harzianum* Nano particle based formulation (5%) A

B-Control B

C-*T. harzianum* Nano particle based formulation (5% + control)

Fig. 6. Pot culture experiment showing A-higher plant growth after *Trichoderma* silver nanoparticle based formulation (5%), B- Control plant without any treatment and C- T. harzianum Nano particle based formulation (5%) + control

treated plants showed promotory effect on the all tested paparmeters of tomato plants as compared to control and *Trichoderm* formulation. Prasad T.N.V.K.V *et al* in 2012 conducted an experiment and found that nanoscale zinc oxide treated peanut plants showed higher germination, growth and yield ovber the control plants..Shelar B. G and Chavan A.M.., 2014 found that nanoparticle solution of *T.herzianum* pose a positive effect on the germination of soyabean and sunflower seeds.

In order to understand the possible benefits of nanoparticles in agriculture sector, it is important to understand the penetration and transport route of nanoparticles in plants.Size of the nanoparticle plays an important role in reactivity, stability and behaviour of nanoparticles. Detailed analysis should be done to understand the the mechanism of action of nanoparticles.

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