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Effects of green synthesized silver nanoparticles on soil properties

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Abstract
Silver nanoparticles (Ag NPs) were synthesized using culture filtrate of fungal antagonist *Trichoderma asperellum* for which silver nitrate was used as the precursor. The Ag NPs thus formed were then characterized by using UV-Vis spectrophotometer, Dynamic Light Scattering (DLS), Zetasizer, Transmission Electron Microscope (TEM) and Energy Dispersive X-Ray Analysis (EDX). The UV-Vis spectroscopy showed a characteristic Surface Absorption Band at 420 nm which confirmed the formation of silver nanoparticles. DLS and TEM study revealed the uniform and well-dispersed nature of the biosynthesized nanoparticles with a spherical shape. The average particle size recorded was 8.26 nm with polydispersity index of 0.857. The charge of silver nanoparticles determined by zeta sizer had a negative potential value of -1.34 mV which indicated stability on dispersion. EDX results showed biosynthesized material contained 32.18% silver, 10.16% oxygen, and 57.66% carbon. The silver nanoparticles were applied at 100% concentration to the collected soil samples from tea garden and a few soil parameters *viz.* soil pH, soil microbial count, soil organic carbon and soil microbial biomass carbon were evaluated in both treated and untreated soil samples. All the parameters showed increased values, except, the soil microbial count which was seen decreased, in the Ag NPs treated soil samples.

Keywords: Silver nanoparticles, *Trichoderma asperellum*, soil properties, soil microbes

1. Introduction
Nanoscience and nanotechnology though a new field of science is emerging as the fastest growing field of research with new outcomes every day. The metal nanoparticles which differ from their parent components in many properties have attracted the interest of scientists from various fields. Silver nanoparticles (Ag NPs) are among the most widely used engineered NPs in a wide range of consumer products and are expected to enter natural ecosystems including soil via diverse pathways [1]. Also, silver ions and silver based composites are highly toxic for microorganisms. Therefore, silver nanoparticles have been used in various types of pesticide formulations. However, the role of nanoparticles in agriculture and environmental science is still limited as compared to that of technology and manufacturing sectors. In agriculture, nanosizing of chemicals with an aim to improve efficacy and thus enabling a reduction in the use of pesticides, biocides may develop a better control of applications as in slow-release pesticides [2]. The agricultural chemicals are mostly soil applied; hence, the nano sized agro chemicals must be studied for their effects on soil properties. These nanoparticles will enter the plant system through soil which will further alter the mechanisms inside plants. But only a few researches have been done on this field and very limited information is available on effects of metal nanoparticles, mostly the ones synthesized biologically, on various soil properties. Earlier studies found that the nanoparticles did not change the total amount of organic materials in the soil or the total organic carbon in the soil extract; however, three-dimensional fluorescence spectroscopy demonstrated changes in humic substances. The nanoparticles also affected the soil bacterial community composition [3]. TiO_2 and CuO NPs decreased soil microbial biomass and enzymatic activities, affected the community structures in flooded paddy soil [4]. Similar results were obtained when the effect of ZnO, TiO_2, CeO_2 and Fe_3O_4 NPs on soil enzymatic activities (invertase, urease, catalase, and phos-phatase) and bacterial communities of saline-alkali and black soils were studied [5]. CuO NPs changed the soil properties by increasing the pH and Eh of the lower organic matter soil rather than those of the higher organic matter soil. Furthermore CuO NPs accelerate the degradation or mineralization of the organic matter, as well as the Fe reduction process, by increasing the Fe (II) content after flooding for 60 days in the lower organic matter soil.

The microbial biomass in both soils was severely inhibited by CuO NPs and the organic matter partly mitigates the negative effects of CuO NPs [6].

2. Materials and Methods

All the reagents and chemicals were of analytical grade and used without further purification. The pure culture of *Trichoderma asperellum* (ITCC no. 8886.13) was collected from preserved culture in *Surakshit* (A long-term preservation method of fungal biocontrol agents) from Department of Plant Pathology, AAU, Jorhat, Assam. Silver nitrate (HiMedia) was used as the precursor for synthesis of silver nanoparticles.

2.1 Synthesis of silver nanoparticles from culture filtrates of *T. asperellum*

Synthesis of silver nanoparticles was done by standardized method [7]. Freshly grown 7 days old fungal mat of *T. asperellum* was harvested and centrifuged at 5000 rpm for 10 minutes at 4˚C. 50 ml of *Trichoderma* supernatant was taken and treated with 50 ml of 1 mM Silver nitrate ($AgNO_3$) aqueous solution (as precursor) in a 250 ml Erlenmeyer flask. After adjusting the pH at 10, the whole mixture was kept in an orbital shaking incubator for 5 days under dark condition. Control experiments were conducted without the precursor. The formation of silver nanoparticles was monitored by using UV-Vis spectroscopy.

2.2 Characterization of silver nanoparticles

Characterization of silver nanoparticles was done by different type of equipments like UV-VIS Spectrophotometer (Eppendorf Biospectrometer), DLS (ZETA sizer, Nano series, Malvern instrument Nano Zs, 2000), Zeta sizer (ZETA sizer, Nano series, Malvern instrument Nano Zs, 2000), EDX and Transmission Electron Microscopy (JEM-2100) study at different institutes like Dept. of Plant Pathology, Assam Agricultural University, Jorhat, Assam, Department of Material Science, NEIST, Jorhat, Assam and SAIF, NEHU, Shillong, Meghalaya.

2.3 Collection of soil samples

Soil samples were collected from Experimental Garden for Plantation Crops (EGPC), AAU, Jorhat. Samples were collected from 20 different locations in a zig zag pattern at a depth of 60 cm with the help of soil auger and spade. The bulk was reduced to half a kilogram by quartering, and put in polythene bags, labeled and stored for further study.

2.4 Application of Ag NPs to soil samples

The collected bulk of soil was separated into two parts and one of the parts was treated with 100% silver nanoparticles while the other was kept untreated.

2.5 Estimation of soil properties

After 15 days of treatment both the treated and untreated soil samples were estimated for the following parameters maintaining six replicates:

2.5.1 Soil pH

pH of the soil was determined in 1: 25 soil-water suspension using a glass electrode pH meter (Eutech Instruments, pH 700) by following the standard procedure [8].

2.5.2 Soil Organic Carbon (OC)

Organic carbon was determined by the standard wet digestion method [9]. Organic carbon (%) in the soil was calculated by using the following equation:

% organic carbon in soil= [0.5 X (B-S) X1 X0.003 X 100]/W

Where, B= Volume of 0.5 N FAS used for titration of blank (ml)
S= Volume of 0.5 N FAS used for titration of sample (ml)
W= Weight of soil taken (gm)

2.5.3 Microbial Biomass Carbon (MBC)

MBC was determined by chloroform fumigation extraction technique [10]. Following equation was followed to get the results:

$C\ (\mu g\ ml^{-1}) = [(HB-S)/CB]\ X\ N\ X\ (V_D/\ V_S)\ X\ E\ X\ 1000$

Where, S= Consumption of titration solution by the sample (ml)
HB=Consumption of titration solution by the hot blank (ml)
CB= Consumption of titration solution by the cold blank (ml)
N= Normality of $K_2Cr_2O_7$
V_D= Added volume of $K_2Cr_2O_7$ solution (ml)
V_S = Added volume of sample (ml)
E= 3 i.e. conversion factor of Cr^{+4} to Cr^{+3}
All organic carbon is as [C (O)]

$C\ (\mu g\ g^{-1}\ soil) = C\ (\mu g/ml)\ X\ (V_K+S_W)/D_W$

Where, V_K = Volume of K_2SO_4 solution added (ml)
S_W = Volume of soil water (ml)
D_W= Dry weight of soil (gm)
Biomass Carbon (BC) = E_C/K_{EC}

Where, E_C = Organic carbon extracted from fumigated soil - Organic carbon extracted from non-fumigated soil
K_{EC} = 0.38 i.e. extractable part of microbial biomass carbon

2.5.4 Soil Microbial Count

The enumeration of soil microbial count was done by using the standard protocol [11]. The collected soil samples were serially diluted from 10^{-1} to 10^{-3} dilutions using sterile distilled water as a blank and the 10^{-3} dilution was inoculated on PDA media by pour plate technique under aseptic conditions. After 48 hours of incubation at 25±1°C, the colonies were counted (Cfu/ ml = Cfu/plate x dilution factor x 1/aliquot) using colony counter and recorded.

3. Results and Discussion

3.1 Green synthesis of silver nanoparticles and its characterization

For the green synthesis of silver nanoparticles, when supernatant of *T. asperellum* was exposed to 1mM aqueous solution of Ag NO_3 the color of supernatant changes from green to yellowish brown to brown after 192 hours of reaction (Plate 1b). The fungal supernatant of *T. asperellum* without Ag NO_3 retains its original color (Plate 1a). The color change in the supernatant from green to brown confirms the formation of silver nanoparticles. The color change observed during this study was due to the Surface Plasmon Resonance (SPR) phenomenon. A possible mechanism for the conversion of silver ions into nano form by using fungal biomass could be the extracellular reduction of silver ions in the solution followed by precipitation on to the cells. This may be the reason for the gradual change in color of the silver nitrate treated *Trichoderma* supernatant from green to brown [12, 13].

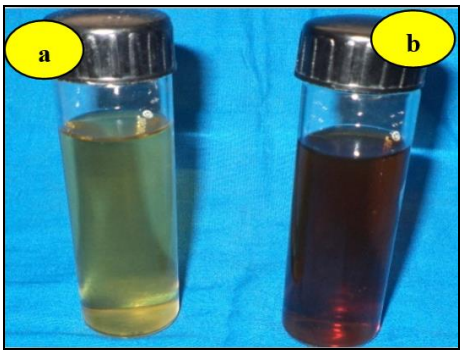

Plate 1: Vials containing the supernatant of *T. asperellum* in aqueous solution of 1 mM Ag NO3 at the beginning of the reaction (a) and after 5 days of reaction (b)

UV-VIS Spectroscopy of the Ag NO_3 treated with *T. asperellum* was carried out at different wavelengths and showed maximum absorption at the critical wavelength (300-500 nm). In the present study a characteristic, SPR absorption band was observed in the supernatant of *T. asperellum* treated with 1mM Ag NO_3 at 420 nm. No absorption band was observed in control i.e. supernatant of *T. asperellum* without 1mM Ag NO_3. DLS was used in present study to determine the size distribution profile of nanoparticles present in the final solution after ultrasonication and also polydispersity, hydrodynamic sizes and aggregation of particles in the suspension. DLS analysis showed that biosynthesized nanoparticles have an average size of 68 nm with a polydispersity index (PDI) of 0.857, indicating the nanoparticles were poly dispersed in nature [14]. To study the stability of the biosynthesized silver nanoparticles Zeta potential was determined and the charge was recorded as -1.34 mV. It indicated that synthesized silver nanoparticles were highly stable and do not have an affinity to agglomerate. Nanoparticles with Zeta Potential values between +30 mV and -30 mV typically have high degrees of stability and the large negative zeta potential value (above -0.50 mV) indicates higher electrostatic repulsion among silver nanoparticles and stable on their dispersion [15]. Transmission Electron Microscopy (TEM) micrographs at 20,000 X magnifications revealed that the average size of nanoparticles were 8.26 nm with roughly spherical shape. TEM micrographs also indicated that nanoparticles were relatively uniform in nature and well separated from each other having no agglomeration. Energy Dispersive X-ray analysis (EDX) was done at an accelerating voltage of 200 kV using the TEM. EDX spectrum revealed that the synthesized nanoparticles contain elements *viz.* silver (32.18%), oxygen (10.16%) and carbon (57.66%) [16]. The results were in accordance with the earlier works done by scientists [17]. The fungal media i.e. PDB used for culturing *T. asperellum* might be the source of carbon and oxygen in the biosynthesized material.

3.2 Estimation of soil properties

3.2.1 Soil pH

Data presented in below table shows that there was a slight increase in pH after 15 days of treatment with silver nanoparticles. For the untreated soil sample pH was recorded to be 5.78 and the pH increased to 5.88 in the treated sample. This increase in pH might be due to the precursor used for synthesis of silver nanoparticles, and during the reduction process silver cations (Ag^+) are formed from the silver nitrate.

3.2.2 Soil Organic Carbon (OC)

Soil organic carbon in untreated samples was recorded to be 0.48% which was found 0.98% in the silver nanoparticle treated samples. (Table1).

3.2.3 Soil Microbial Biomass Carbon (MBC)

Data presented in Table 1, showed that the application of silver nanoparticles had lead to an increase in the soil MBC content. Soil MBC recorded for treated and untreated samples were 1.232% and 0.093% respectively.

The increased values of soil OC and MBC observed in the present study might be due to the application of green synthesized silver nanoparticles using *T. asperellum*. Further, the EDX analysis done to determine the elemental composition of green synthesized silver nanoparticles showed that the synthesized nanomaterials contain a higher amount of carbon, which aid in increasing the soil OC and MBC.

3.2.4 Soil Microbial Count

This study showed that microbial population was less in the soil samples treated with silver nanoparticles compared to that of untreated soil (Plate 2a and 2b). In untreated samples fungal colonies were log 5.04 cfu/gm of soil and bacterial colonies were log 4.11 cfu/gm of soil. But in treated samples the values recorded were log 4.02 cfu/gm of soil for fungus and log 3.88 cfu/gm of soil for bacteria. The decrease in microbial population may be due to the antibacterial property of silver nanoparticles as reported earlier by many workers. [18, 20].

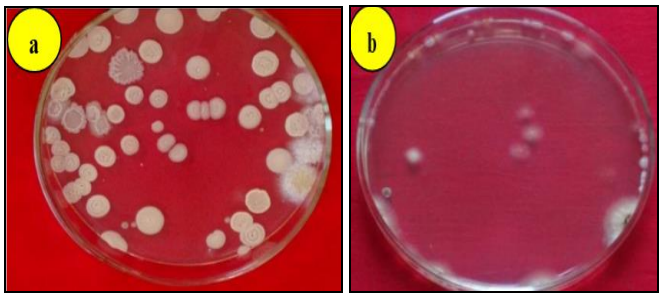

Plate 2: Soil microbial count (a) Untreated soil sample (b) Soil sample treated with Ag NPs

Table 1: Effects of green synthesized silver nanoparticles on soil Ph, soil microbial count, soil organic carbon and soil microbial biomass carbon (MBC)

Treatment	Soil pH	Soil organic carbon (%)	Soil MBC (%)	Soil microbial count (log cfu g^{-1} soil)	
				Bacteria	Fungi
T_a: Untreated soil sample	5.88	0.98	1.232	3.88	4.02
T_b: Treated soil sample	5.78	0.48	0.093	4.11	5.04

*Data are mean of six replications

4. Conclusion

An attempt was made to study effects of biosynthesized silver nanoparticles on soil and it was observed that soil pH, soil organic carbon and soil microbial biomass carbon was found to be higher in Ag NP treated sample than those of untreated sample. However, the soil microbial population is negatively affected by the application of silver nanoparticles. So, it opens up a future line of research focusing on analysis of harmful

and beneficial microbes that are being affected by the application of silver nanoparticles on soil. The information provided from this study may help in formulation of nano sized soil chemicals or agrochemicals. Also it attempts to emphasize the use of biologically synthesized nanoparticles rather than the physically or chemically synthesized nanoparticles, in the field of agriculture.

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