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Evaluation of the Effect of Mycogenic Silver Nanoparticles on Soil Exo-Enzymes in Groundnut Growing Soils

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ABSTRACT

Soil exo-enzymes like acid phosphatase, alkaline phosphatase and dehydrogenase activity in groundnut growing soils with the application of mycogenic silver nanoparticles synthesized from Trichoderma viride (GRT-1) was measured after 0 days (at the time of sowing), 15 days and 30 days after sowing. The activity of acid phosphatase, alkaline phosphatase and dehydrogenase was observed to be significantly high at 30 days after sowing compared to control. Physiological parameters like plant height and number of leaves per plant were recorded to be significantly high in groundnut plant growing in silver nanoparticles treated pots at 30 days after sowing compared to control. Among all the treatments, treatment at 150 ppm concentration showed higher values compared to treatment at 100 and 50ppm. The plant height and number of leaves per plant were observed to be increased from 10 days, 20 days and 30 days of sowing. Root and shoot length, fresh and dry weight of groundnut plant were recorded to be significantly higher at 30 days after sowing. Total chlorophyll content was measured in all the treatments and recorded higher values compared to control. Among all the treatments, treatment at 150ppm conc. showed higher chlorophyll content when compared to treatment at 100 and 50ppm. The chlorophyll a was observed to be higher, when compared to chlorophyll b.

Key words: *Soil exo-enzymes, acid phosphatase, alkaline phosphatase and dehydrogenase*

INTRODUCTION

Soil enzymes play a vital role in maintaining soil health and most of the soil enzymes depend on the microorganisms present in the soil. These enzyme activities show high potential for biological factors of soils due to the rapid changes in soil management[1]. Soil contains a group of enzymes, which determines the soil metabolic protocols[4].

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And these enzymatic levels vary depending on the organic matter present in the soil, microbial activity and soil biological processes. Most of the enzymes like phosphatases and dehydrogenases are generally present in the superficial layers of the soil. The enzyme activities of the soil viz. acidic phosphatase activity, alkaline phosphatase activity, dehydrogenase activity directly imply the soil fertility and influenced by the soil temperature and moisture content.

MATERIAL & METHODS

Soil enzymes activities and physiological growth parameters of 20 pots in pot-culture = 3 treatments (3t) and 1 control (1c) of 5 replications (5r) synthesized from Trichoderma silver nanoparticles applied which equals to {(3t+1c) ×5t} i.e. 20 pots.

Treatment-1: 150 ppm concentration

Treatment-2: 100 ppm concentration

Treatment -3: 50 ppm concentration

Pre soaking of groundnut seeds in mycogenic silver nanoparticles at 50, 100 and 150ppm concentration was done for 12 hrs. After that, groundnut seeds along with silver nanoparticles were mixed in pots at the time of sowing. Soil was taken from each peanut pot for the estimation of soil phosphatase (acidic and alkaline) activity and soil dehydrogenase activity at 0 days, 15 days and 30 days of sowing period in all treatments by maintaining controls.

Assay of soil phosphatase activity

The phosphatases catalyze the hydrolysis of phosphate and have broad substrate specificity. The phosphor-mono-esterase has been extensively studied because they catalyze the hydrolysis of organic phosphor-mono-esterase to inorganic phosphorous that can be taken up by the plants. According to the pH optima, these are classified as acidic or alkaline phosphatases. Both acidic and alkaline phosphatases have been found in soil. Acid phosphatase is pre-dominant in acid soils, while alkaline phosphorous prevail in alkaline soils[7].

Assay of dehydrogenase activity

Dehydrogenase activity is based on the estimation of the Tryphenyl Tetrazolium Choloride (TTC) reduced rate of Tri Phenyl Formation (TPF) in soils after incubation at 300C for 24 hr[8].

Physiological traits

Physiological growth parameters like plant height, number of leaves were recorded in three regular time intervals of 10 days, 20 days and 30 days of sowing period. Root length, shoot length fresh weight and dry weight were recorded after 30 days of sowing period. After 30 days of sowing period, groundnut plants from the pots were removed and each plant was differentiated into its root and shoot. Fresh weight of roots and shoots were measured immediately after differentiating the plants into roots and shoots. Dry weights were measured after incubating the roots and shoots in an oven for 36 hrs at 650C. At the end of pot culture chlorophyll content and protean content present in leaves of the groundnut plants were also estimated.

Estimation of chlorophyll content in leaves

1 gm. of leaf sample was cut in to bits and 100 mg of sample was taken into 20 test tubes. 7 ml of Dimethyl sulphoxide (Ds) was added to the tubes and kept under dark for 24 hrs. Later, the solutions were filtered and the final volume was adjusted to 10 ml with distilled water. The absorbance at 440 nm was measured against dimethyl sulphoxide as blank.

Statistical analysis

Experiments were conducted in Completely Randomized Design (CRD) or Factorial CRD. Data was analyzed following the statistical methods outlined by Gomez and Gomez (1984).

Results and Discussion

Estimation of acid phosphatase activity in soil

Soil enzyme activities have the potential to provide aunique integrative and reliable biological assessment of soils because of their relationship to soil biology andrapid response

to changes in soil organic matter (SOM) and soil management[1].

For the estimation of the effect of mycogenic silver nanoparticles synthesized from *Trichoderma viride* (GRT-1) on acid phosphatase activity in groundnut growing soils, groundnut (Var. Narayani) seeds were treated with 50, 100, 150 ppm concentration of silver nanoparticles and control (without silver nanoparticles) were sown in pots and results were presented in Table 1 The activity was measured after 0 days (at the time of sowing), 15 days and 30 days after sowing. The activity of acid phosphatase was observed to be significantly high when compared with the control and recorded 3.84 µg, 3.65µg and 3.43 µg of p-nitrophenol released g-1 of soil h-1at 150, 100, 50ppm concentration respectively at the time of sowing. At 15 days (8.77µg, 8.48µgand 8.27 µg of p-nitrophenol released g-1 of soil h-1), at 30 days (10.82µg, 10.64µg and 10.43 µg of p-nitrophenol released g-1 of soil h-1) were recorded when compared to the controls (2.86µg at the time of sowing, 7.12µg at 15 days and 9.83 µg of p-nitrophenol released g-1 of soil h-1at 30 days). Overall, the acidic phosphatase activity increased with the time and maximum activity was recorded with 150ppm concentration of AgNPs at 30days.

Table 1: Estimation of acid phosphatase activity in soil with the application of different concentrations of silver nanoparticles synthesized from one month culture filtrate of *Tricoderma viride* (GRT-1)

S. No	Treatment	0 days	15 days	30 days
		(µg of p-nitrophenol released g^{-1} of soil h^{-1})		
1	T1(150 ppm)	3.84	8.77	10.82
2	T2(100 ppm)	3.65	8.48	10.64
3	T3(50 ppm)	3.43	8.24	10.43
4	Control	2.86	7.12	9.83
	CD	0.022	0.073	0.021
	SE (m)	0.007	0.024	0.007
	SE (d)	0.010	0.034	0.010
	CV	0.479	0.664	0.145

Estimation of alkaline phosphatase activity in soil

Alkaline phosphatase activity was measured after 0 days (at the time of sowing), 15 days and 30 days after sowing and results were presented in Table 2 The activity of alkaline phosphatase was observed to be significantly high compared to control and recorded 4.68µg, 4.55µgand 4.34µg of p-nitrophenol released g-1 of soil h-1at 150, 100, 50ppm concentration respectively at the time of sowing. At 15 days (7.72µg, 7.54and 7.35µg of p-nitrophenol released g-1 of soil h1), at 30 days (11.65µg, 11.34µg and 11.14µg of p-nitrophenol released g-1 of soil h1) were recorded when compared to the controls (3.85µg at the time of sowing, 6.92µg at 15 days and 10.24µg of p-nitrophenol released g-1 of soil h-1) at 30 days.

Similar reports were given by Durgapramela *et al*[2]., In soil ecosystems, phosphatases were believed to play vital role in P-cycling[6] as they were correlated to P stress and plant growth. Apart from being good indicators of soil fertility, phosphatase also plays a key role in the maintenance of soil system[3,7]. The assay performed on ALP and ACP activities of soil with the application of m-AgNPs are evident from the results that enzymatic activity at zero day increased in all the treatments except for 50 ppm. However, inhibition of the activity was observed at first day, but on tenth day and30 days of incubation at all the higher concentrations of AgNPs (50, 100, 150 ppm) the enzymatic activity increased. It has been reported in the literature that adsorption by SOM reduces the mobility of Engineered Nanoparticles (ENPs) in the soil matrix is curtailed and hence their influence on the

microbial populations was drastically reduced. ENPs can be strongly sorbed to soil surfaces and SOM making them less mobile or are small enough to be trapped in the inter-spaces of soil particle sand might therefore travel farther than larger particles before becoming trapped in the soil matrix. The strength of sorption would, however, depend on the size, chemistry, aggregation behavior, conditions under which it is applied, etc. In fact, whether an ENP can be hazardous in soildepends not only on its concentration, but also on the likelihood of it ever coming into contact with microbial cells. It may also be noted that natural colloids and ENPs in the environment can interact with one another and also with other larger particles.

Table 2: Estimation of alkaline phosphatase activity in soil with the application of different concentrations of silver nanoparticles synthesized from one month culture filtrate of *Tricoderma viride* (GRT-1)

S. No	Treatment	0 days	15 days	30 days
		(µg of p-nitrophenol released g^{-1} of soil h^{-1})		
1	T1(150 ppm)	4.68	7.72	11.65
2	T2(100 ppm)	4.55	7.54	11.34
3	T3(50 ppm)	4.34	7.35	11.14
4	Control	3.85	6.932	10.24
	CD	0.086	0.025	0.024
	SE (m)	0.028	0.008	0.008
	SE (d)	0.040	0.012	0.011
	CV	1.452	0.250	0.160

Estimation of dehydrogenase activity in soil

The dehydrogenase enzyme activity is also commonly usedas an indicator of biological activity in soils to support biochemical processes that are essential to maintain soil fertility as well as soil health. This enzyme exists as an integral part of intact cells but does not accumulate extra cellularly in the soil. Dehydrogenase enzyme is known to oxidize SOM by transferring protons and electrons from substrates to acceptors. Metal nanoparticles, such as Ag have been proven to be toxic to soil micro biota several researchers demonstrated the toxic effect of Ag on soil dehydrogenase activity as severe and bacterial colony growth was inhibited at levels between 0.1 and 0.5 mg Ag kg-1 soil. These finding also suggests that soil denitrifying bacteria are susceptible to inhibition by Ag. For the estimation of dehydrogenase activity of mycogenic silver nanoparticles synthesized from *Trichoderma viride* (GRT-1), groundnut (Narayani variety) seeds were treated at 50, 100, 150ppm concentration of silver nanoparticles and control without silver nanoparticles were sown in pots and results were presented in Table 3 The dehydrogenase activity was measured after 0 days (at the time of sowing), 15 days and 30 days after sowing. The activity was observed to be significantly high and recorded 0.25µg, 0.23µg and 0.21µg of TPF g-1 of soil h-1at 150, 100, 50ppm concentration respectively at the time of sowing. At 15 days (1.26µg, 1.23µg and 1.21µg of TPF released g-1 of soil h-1), at 30 days (2.27µg, 2.25 µg and 2.23µg of TPF released g-1 of soil h-1) were recorded when compared to the controls (0.10 µg at the time of sowing, 1.0 µg at 15 days and 2.14µg TPF released g-1 of soil h-1) at 30 days.

Table 3: Estimation of dehydrogenase activity in soil with the application of different concentrations of silver nanoparticles synthesized from one month culture filtrate of *Tricoderma viride* (GRT-1)

S. No	Treatment	0 days	15 days	30 days
		(µg of TPF released g^{-1} of soil h^{-1})		
1	T1(150 ppm)	0.25	1.26	2.27
2	T2(100 ppm)	0.23	1.23	2.25
3	T3(50 ppm)	0.21	1.21	2.23
4	Control	0.10	0.04	2.14
	CD	0.013	0.038	0.039
	SE(m)	0.004	0.013	0.013
	SE(d)	0.006	0.018	0.018
	CV	4.529	2.386	1.286

Measurement of physiological growth parameters of groundnut plants (plant height and no. of leaves)

Plant height and number of leaves per plant were recorded in silver nanoparticles treated (50, 100 and 150ppm conc.) pots at 10 days, 20 days and 30 days after sowing in comparison with control without treatment with silver nanoparticles and results were presented in Table 4 At 10 days after sowing, plant height of all the treatments(10.16 cm, 9.16 cm and 8.14 cm) showed significantly higher values compared to the control (7.12 cm) and the no. of leaves per plant of the treatments (11.60, 10.40 and 9.00) in compassion with control (8.00/plant).

At 20 days after sowing the treatments showed significant values of plant height and no. of leaves per plant compared to the control. Plant height of the treatments (18.54 cm, 17.50 cm and 16.44 cm) showed significantly higher values compared to the control (15.52 cm) and the no of leaves per plant (24.60, 23.00, and 22.00) also showed significant values compared to control (21.00/plant).

At 30 days after sowing, all the treatments showed significant values of plant height and no. of leaves per plant compared to the control. Plant height of the treatments (33.16 cm, 32.20 cm and 32.18 cm) showed significantly higher values compared to the control (30.16 cm) and number of leaves per plant (42.40, 40.60 and 39.40) also showed significant values compared to control (37.40/plant).

Table 4: Physiological growth parameters of groundnut with the application of different concentrations of silver nanoparticles synthesized from one month culture filtrate of *Tricoderma viride* (GRT-1)

S. No	Treatment	Plant height (cm)			Number of leaves per plant		
		10 days	20 days	30 days	10 days	20 days	30 days
1	T1(150 ppm)	10.16	18.54	33.16	11.60	24.60	42.40
2	T2(100 ppm)	9.16	17.50	32.20	10.40	23.00	40.60
3	T3(50 ppm)	8.14	16.44	32.18	9.00	22.00	39.40
4	Control	7.12	15.52	30.16	8.00	21.00	37.40
	CD	0.071	0.132	0.113	0.524	0.370	0.741
	SE (m)	0.023	0.044	0.037	0.173	0.122	0.245
	SE (d)	0.033	0.062	0.053	0.245	0.173	0.346
	CV	0.607	0.573	0.263	3.972	1.209	1.371

Among all the treatments, treatment at 150ppm concentration showed higher values compared to treatment at 100 and 50ppm. The plant height and no. of leaves per plant was observed to be increased from 10 days, 20 days and 30 days of sowing (fig a & b).

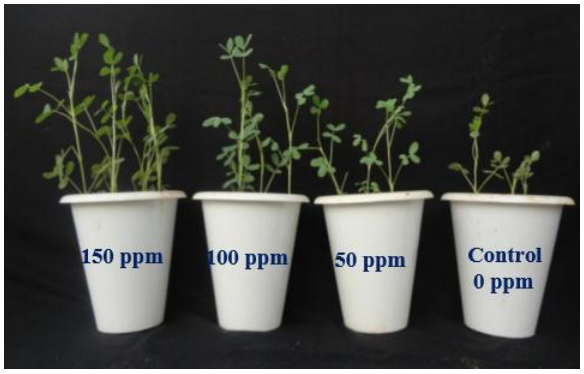

Fig a. Effect of seed treatment of groundnut seeds with different concentrations of AgNPs on physiological parameters at 15 days after sowing

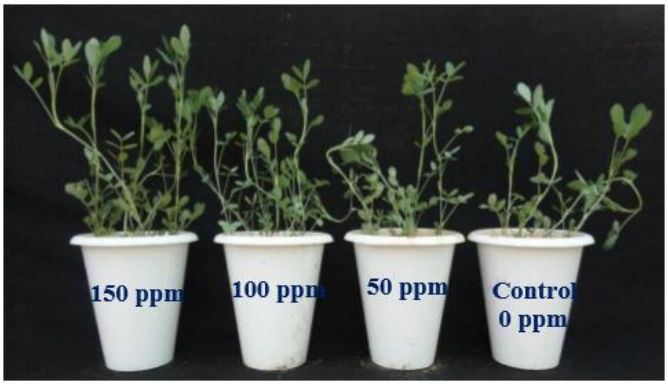

Fig b. Effect of seed treatment of grondnut seeds with AgNPs on physiological parameters at different concentration after 30 days

Root and shoot length of groundnut plants

Root and shoot length of groundnut plant was measured at 30 days after sowing and presented in Table 5. All treatments showed significantly higher values compared to control. Root length (14.64 cm, 13.46 cm, 13.18 cm) and shoot length (32.14 cm, 31.34 cm, 30.18 cm) showed higher values over control 12.14 cm and 29.18 cm respectively.

Table 5: Root length and shoot length after 30 days with the application of different concentrations of silver nanoparticles synthesized from one month culture filtrate of *Tricoderma viride* (GRT-1)

S. No	Treatment	Root length (cm) 30 days	Shoot length (cm) 30 days
1	T1 (150 ppm)	14.64	32.14
2	T2 (100 ppm)	13.46	31.34
3	T3 (50 ppm)	13.18	30.18
4	Control	12.14	29.18
	CD	0.085	0.096
	SE (m)	0.028	0.032
	SE (d)	0.040	0.045
	CV	0.473	0.232

Fresh weight and dry weight of groundnut plants

Fresh and dry weight of groundnut plants was measured at 30 days after sowing and presented in Table 6 Fresh and dry weight of groundnut plant was observed to be significantly higher in all treatments (fresh weight 13.26 gm, 12.42 gm, 11.28 gm and dry weight 4.26 gm, 3.22 gm, 2.28 gm), when compared to the control (fresh 10.26 gm and dry weight 1.44 gm).

Table 6: Fresh weight and dry weight of groundnut plants after 30 days with the application of different concentrations of silver nanoparticles synthesized from one-month culture filtrate of *Tricoderma viride* (GRT-1)

S. No	Treatment	Fresh weight (gm)	Dry weight (gm)
1	T1 (150 ppm)	13.26	4.26
2	T2 (100 ppm)	12.42	3.22
3	T3 (50 ppm)	11.28	2.28
4	Control	10.26	1.44
	CD	0.107	0.107
	SE (m)	0.035	0.035
	SE (d)	0.050	0.050
	CV	0.669	2.823

Recent studies have reported that a plant's response to AgNPs, enhancement or inhibition of growth, depends on the AgNPs dosage. Exposure to specific concentrations of AgNPs could enhance plant growth compared with non-exposed plants, whereas higher and lower concentrations could affect plant growth negatively. Of the reported AgNPs concentrations used (0, 25, 50, 100, 200 and 400 ppm); a 50-ppm treatment has been determined to be optimal for growth response in Brassica juncea seedlings. The fresh weight, root and shoot length, and vigour index of seedlings were positively affected at this concentration. This dose was found to induce a 326% increase in root length and a 133%increase in the vigour index of the treated seedlings[5] (Sharma *et al*. 2012). Using10 mg/L of polyvinylpyrrolidine-coated AgNPs (PVP-AgNPs) also found to increase root elongation in Erucasativa[9].

Estimation of chlorophyll content

Total chlorophyll content was measured in all treatments and recorded (Table 7) chlorophyll content (2.82 mg/g, 2.66 mg/g, 2.58 mg/g) but higher values compared to control (1.03mg/g). Among all the treatments, 150ppm conc. showed higher chlorophyll content when compared to 100 and 50ppm conc. The chlorophyll a was observed to be higher, when compared to chlorophyll b, which may be due to the relative concentration of pigments present in chlorophyll.

Table 7: Estimation of chlorophyll content in groundnut with the application of different concentrations of silver nanoparticles synthesized from one-month culture filtrate of *Tricoderma viride* (GRT-1)

S. No	Treatment	Total chlorophyll (mg/g)	Chlorophyll (a/g) tissue (mg)	Chlorophyll (b/g) tissue (mg)	Chlorophyll (a/b) tissue (mg)
1	T1(150 ppm)	2.82	1.98	0.84	2.36
2	T2(100 ppm)	2.66	1.87	0.79	2.35
3	T3(50 ppm)	2.58	1.81	0.74	2.45
	Control	1.03	0.68	0.35	1.93

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