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Microbial population and soil enzyme activities in the rhizosphere of groundnut plants treated with compost enriched with optimum levels of microbial inoculants

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An investigation was carried out under green house condition to know the effect of enriched compost with different levels of *Aspergillus awamori*, *Trichoderma harzianum* and *Glomus mosseae* on microbial population and soil enzymatic activities in the rhizosphere of groundnut plants. Results revealed that acid phosphotase activity and dehydrogenase activity was recorded maximum (35.83 and 198.05 micro grams PNP/g soil, respectively) in the rhizosphere soil of plants inoculated with *Aspergillus awamori*(10^{-8}/ml)+ *Trichoderma harzianum*(10^{-4})+ *Glomus mosseae*(40spores/10gm soil inoculum)+compost(4g/kg soil),also more bacterial population($14X10^4$CFU/ml)and maximum per cent mycorrhizal colonization(80.5%)was recorded in the root bits of plants, however maximum mycorrhizal spore number (127 spores/50g soil)was recorded in the rhizosphere soil of plants treated with *Aspergillus awamori* (10^{-8}/ml)+ *Trichoderma harzianum*(10^{-4})+ compost(4g/kg soil)followed by *Aspergillus awamori*(10^{-8}/ml)+ *Trichoderma harzianum*(10^{-4})+ *Glomus mosseae*(40spores/10gm soil inoculum)+compost(4g/kg soil).where as alkaline phosphotase activity was recorded maximum (34.27micro gram PNP/g soil)in the soil treated with *Aspergillus awamori*(10^{-8}/ml)+ *Trichoderma harzianum*(10^{-4})+ compost(4g/kg soil).Interestingly,maximum fungal population ($10X10^2$ CFU/ml) recorded in the treatments included mycorrhizal fungi. In general, actinomycetes population was recorded in all the inoculated plants, but in 10^{-2} dilution maximum population recorded in the plants treated with *Aspergillus awamori*(10^{-8}/ml)+ *Trichoderma harzianum*(10^{-4})+ *Glomus mosseae*(40spores/10gm soil inoculum)+compost(4g/kg soil). Least were recorded in uninoculated control plants.

Key words : Rhizoephere, Microbial inoculants, Soil enzime, Groundnut

INTRODUCTION

Groundnut (*Arachis hypogla*) is an annual legume with 3-4 foliate leaves is an important contributor of vegetable oil in India. It occupies a prime place among oil seeds of our country. About 80% of the production is used up for oil alone. The average pod yield for India is around 1 t ha^{-1} during the *kharif* season and 1.7 t ha^{-1} for the *rabi* season. Seedling diseases caused by, Fusarium, is the economically important disease of ground nut (Ali *et al.*,2000). Biological control of this disease will be an ecofriendly approach with no deleterious effect on the environment. The use of biocontrol strategies offers several advantages over chemical control, as it is economical, self-perpetuating and usually free from residual side effects. There have been some scientific reports suggesting that dual inoculations with arbuscular mycorhizal(AM)and saprophytic soil fungi may cause an additive or synergetic growth enhancement of the inoculated host plant.Vanitha and Anasuya(1998) showed significant rise in P content of soil and plants when inoculated with compost along with *Aspergillus awamori* and AM fungi.Some *Trichoderma* spp. have shown antagonistic potential against pathogenic fungi and a beneficial effect on plant growth. Different mechanisms have been suggested as being responsible for their biocontrol activity,which include competition for space and nutrients,secretion of chitinolytic enzymes,mycoparasitism and production of inhibitory compounds(Haram *et al.*, 1996; Zimand *et al.*, 1996). The rhiozosphere is a volume of soil as influenced by plant roots (Hiltner, 1940) and it is a region where the plants and microorganisms interact very closely. The nutrient accumulation in the soil is due to the action of microorganisms that decomposes the organic matter in the soil and thus promotes the plant growth. The biological activity in soil provides better insight in understanding the decomposition and transformation of organic matter.The interactions between microorganisms and their influence on mycorrhizal root colonization, disease control and plant growth enhancement, the changes produced in the soil microbial

activity were evaluated in the present study. So the present investigation was carried out to evaluate the microbial status, AM association and enzymatic activities in the groundnut rhizosphere.

MATERIALS AND METHODS

Groundnut nut (*Arachis hypogia*) seeds were sowed in the pots containing 4 kg of soil (soil type-red sandy clay loam P^H6.51) which was made sick by Fusarium {10ml(10-3 CFU/ml)/kg soil) two weeks before the sowing. After sowing soil was treated with compost enriched with different levels of *Aspergillus awamori*,*Trichoderma harzianum* maintained in the laboratory and *Glomus mosseae* was obtained from rhizosphere of *Chloris gayana* maintained in the glass house. A best level of each inoculum was standardized in the preliminary growth studies.

Inoculum preparation: *Aspergillus awamori* and *Trichoderma harzianum* were grown in potato dextrose broth in a one liter flask each containing 500 ml medium it was placed on a rotary shaker for 7 days and macerated by using a homogenizer. Tested or the population density in the inoculum. Ten-ml/kg soil of each of this inoculum was added to each plant in a pot containing 4 kg of soil.

Experimental details:

T_1: Control (only compost), T_2: *Aspergillus awamori* (10^{-8}CFU/ml)+compost (4g/kg soil), T_3: *Trichoderma harzianum* (10^{-4}CFU/ml)+compost(4g/kg soil), T_4: *Glomus mosseae*(10g(40 spores) soil inoculum)+ compost (4g/kg soil), T_5: *Aspergillus awamori* (10^{-8}CFU/ml)+ T. harzianum(10^{-4}CFU/ml)+compost(4g/kg soil), T_6: *A. awamori* (10^{-8}CFU/ml) + G.mosseae (10g(40spores) soilinoculum) + compost (4g/kg soil), T_7: *T. harzianum* (10^{-4}CFU/ml)+*G.mosseae*(10g(40spores)soilinoculum compost(4g/kg Soil), T_8:A.awamori(10^{-8}CFU/ml)+ *T. harzianum* (10^{-4}CFU/ml)+ *G.mosseae*(10g(40spores/10g soil inoculum+Compost (4g/Kg soil)

Microbial count:

Populations of different groups of microbes in soil samples were assessed by standard dilution plate technique (Jensen, 1968). The results were expressed in colony forming units (cfu) per gram of soil sample. Enumeration of the soil microorganisms was done by the dilution plate method. bacteria was grown on nutrient agar medium containing (gl^{-1}) peptone (10.0), beef extract (10.0), sodium chloride (5.0) and dextrose (20.0); fungi on Rose Bengal agar containing dextrose (10.0),soya peptone(5.0),monosodium potassium phosphate (1.0), magnesium chloride(0.5) and rose bengal(0.5); Actinomycetes on actinomycetes agar containing sodium propionate (4.0),sodium caseinate (2.0),dipotassium phosphate (0.5),L-Aspergine (0.1),magnesium sulphate (0.1) and ferrous sulphate (0.001).The plates were in

Enzyme activities:

Enzyme activities such acid phosphatase, alkaline phosphatase and dehydrogenase of soil were assayed as described by Eivezi and Tabatabai(1977)

Acid and Alkaline phosphatase:

One gram of soil was placed in a 50ml Erlenmeyer flask. Then 0.2ml of toluene, 4ml of modified uuniversal buffer (p^H 6.5 for assay of acid phosphatase and p^H 11 for assay of alkaline phosphatase) and 1ml of P-nitrophenyl phosphate solution (made in the same buffer) were added to it. The flasks were stoppered, swirled for a few seconds and incubated at 37^0 C in an incubator for I hour. After incubation, I ml of 0.5 M $CaCI_2$ and 4ml of 0.5 m Na 011 were added to flask, mixed well for a few seconds.

Supernatant was filtered through whatman No.2 tilter paper. The yellow color compound was measured using I cm cuvette in a spectrometer (Shimadzu UV –visible) at 420 nm. The amount of P- nitrophenol (PNP) released was calculated by referring to a calibration graph. Controls were prepared using the same procedure but I ml of P- nitrophenol was added after the incubation period before filtration results were expressed as u PNP/g soil/ hour.

Dehydrogenase activity:

Two grams of soil and 0.2 g of $CaCO_3$ (AR grade), Iml of 2% 2,3,5-triphenyl tetrazolium chloride (TTC) and a column (I cm) of distilled water were added to screw cap test tube and incubated at 37^0 C for 24 hours. After incubation, the contents were faltered using whatman No.2 filter paper with washings of methanol unit a colorless filtrate was obtained. The filtrate was made up to 100 ml with methanol in a volumetric flask and the absorbance was read at 485 nm on spectronic –20 using methanol as a blank. The absorbance units were converted to concentrations of tri phenylformazon (TPF) from a standard curve prepared from 5,10,15 and 20 ml of TPF. The results recorded were expressed as Tpf/G Soil/Hour.

AM root colonization and spore count:

Spore in the rhizosphere soils were counted by subjecting the soil to the wet sieving and decanting were method (Gerdemann and Necolson 1963). Fifty grams of

root zone soil samples were addded top 500ml water and stirred thoroughly. The suspension was allowed to stand for 1min and then made to pass through a series of series of sieves measuring 1mm, 450µm, 350µm, 250µm, 105µm, and 45µm.the spore from the two sieves of smaller pore sizes were transferred on to a nylon mesh of 45µm, which was then placed in a plate and then counted using a stereomicroscope.

The degree of colonization was determined after clearing the roots with 10%KOH and staining with acid fuschin in lacto glycerol (Giovanetti and Mosse,1980).the data collected in this study was subjected to statistical analysis suitable to CRD Duncun multiple range test (DMRT)to separate the means (Sunderaj *et al.*)

RESULTS AND DISCUSSION

Results indicated that, in general the microbial load and enzymatic activity in the rhizosphere soils of groundnut found to be significantly high in inoculated plants when compared to control plants. More bacterial population ($14x10^4$ cfu/ml) recorded in the rhizosphere soil of T_8.Maximum fungal population ($10x10^2$ cfu/ml) recorded in the treatments included mycorrhizal fungi. In general, actinomycetes population was recorded high in all the inoculated plants, but in 10^{-2} dilutions maximum population was recorded in the plants treated with T_8 and T_7.Least were recorded in the uninoculated control plants. Interestingly, more microbial population recorded in the treatments, which receives mycorrhizal fungi. Barea *et al.* (1975) pointed out that since root growth and metabolism are altered by infection with AM fungi, it is likely that the root exudates and in turn the rhizosphere population could be affected.

Soil enzyme activity is a measure of soil metabolic activity and soil health. they play an important role in describing and making predictions about an ecosystem productions, quality and interactions among the subsystems both microbes and plants release enzymes into the environment (Juma and Tabatabai,1988).in the present study, enzyme activities varied in different

Table 1 : Effect of enriched compost with optimum inoculum levels of *A.awamori, T. harzianum* and *G.mosseae* on microbial load of rhizosphere soil of groundnut

Treatments	Bacteria		Fungi		Actinomycetes	
	10^4CFU/ml	10^5CFU/ml	10^2CFU/ml	10^3CFU/ml	10^1CFU/ml	10^2CFU/ml
Control	8.67[b]	3.67[c]	5.50c	3.83[b]	6.00[cd]	2.33 [b]
*A.awamori(*H)+Compost	9.50[b]	4.00[c]	8.17 [abc]	6.33 [a]	5.67[d]	2.83 [b]
*T. harzianum(*M)+Compost	8.33[b]	5.83[abc]	5.83[bc]	5.00 [ab]	10.33 [ab]	3.17 [b]
*G.mosseae(*L)+Compost	10.50[b]	4.83[bc]	10.83[a]	6.17[a]	11.83[a]	3.50 [b]
A(H)+T(M)+Compost	10.33[b]	6.00 [abc]	7.00[bc]	5.00 [ab]	12.17 [a]	5.67 [b]
A(H)+V(L)+Compost	9.33[b]	6.00 [abc]	8.33 [abc]	4.17 [b]	8.33[bc]	4.17 [b]
T(M)+V(L)+Compost	10.83[b]	6.83[ab]	7.00 [bc]	5.00 [ab]	11.00 [a]	6.17 [a]
A(H)+T(1)+V(L)+Compost	14.17[a]	8.17 [a]	9.33 [ab]	3.83 [b]	11.50 [a]	7.50 [a]

Means in the same column followed by the same super script donot differ significantly according to Duncan's Multiple Range Test ($p<0.05$)

Table 2: Effect of enriched compost with optimum inoculum levels of *A.awamori, T. harzianum* and *G.mosseae* on microrhizal parametrers and enzymatic activity of rhizosphere soil of groundnut

Treatments	Percent micorrhizal colonization	Mycorrhizal spore / 50gm air dried soil	Acid phosphatase Micrograms PNP / gm soil	Alkaline phosphatase Micrograms PNP / gm soil	Dehydrogenase Micrograms / h / gm soil
Control	59.67[c]	116.83 [ab]	23.77 [b]	66.23 [b]	154.20 [c]
*A.awamori(*H)+Compost	61.83[c]	103.67[b]	27.48 [b]	86.25 [ab]	166.35 [bc]
*T. harzianum(*M)+Compost	64.33[bc]	117.83 [ab]	25.90 [b]	70.47 [b]	157.55[c]
G.mosseae(L)+Compost	68.50[abc]	121.83 [ab]	25.67 [b]	116.04 [a]	174.80 [b]
A(H)+T(M)+Compost	61.50 [c]	143.67 [a]	26.06 [b]	116.61 [a]	175.37 [b]
A(H)+V(L)+Compost	76.83[ab]	112.17 [b]	26.78 [b]	87.04 [ab]	178.92 [b]
T(M)+V(L)+Compost	71.50 [abc]	127.50 [ab]	32.69 [a]	91.12 [ab]	201.10 [a]
A(H)+T(1)+V(L)+Compost	80.50[a]	122.83 [ab]	35.83 [a]	96.98 [ab]	198.05 [a]

Means in the same column followed by the same super script do not differ significantly according to Duncan's Multiple Range Test ($p<0.05$)

treatments studied. Acid phosphatase activity and dehydrogenase activity was recorded maximum (35.83 and 198.05 µg PNP/g soil, respectively) in the rhizosphere soil of plants inoculated with T_8.where as Alkaline phosphatase activity was recorded maximum (34.27µg PNP/g soil) in the soil treated with T_5.All other treatments showed good enzymatic activity and significantly superior over the control. The individual enzyme assays are useful as indicators of microbial activity. Similarly, Singh (2002) observed soil microbes and soil enzymes as an indicator of soil fertility in groundnut. These enzymes adsorbed on the organic and mineral soil particles are important for their activity in the rhizosphere soils (Ruggiero and Radogna, 1988). Taylor *et al.* (2002) described positive correlation between enzyme activities and organic matter content.

Mycorrhizal fungi interact with a wide range of soil microorganisms in the rhizosphere soils. They influence the plant growth by their mutualistic symbiotic associations with the plants. There was no direct correlation between the mycorrhizal spore number and colonization.Maximum per cent mycorrhizal colonization (80.5%) was recorded in the root bits of plants treated with T8,however maximum Mycorrhizal spore number(127 spores/50 g soil)was recorded in the rhizosphere soil of plants treated with T_5 followed by T_8. The interesting aspect of Mycorrhizal fungi is that they not only survive in the most stressful environment but they also make the plants to do so. Significant increase in the production of G.mosseae spores in the presence of *Trichoderma* spp. Was observed by Calvet *et al*. (1990) enhanced Mycorrhizal spore production was later reported to be due to production of volatile compounds by *Trichoderma* (Calvet *et al.*, 1990). Increase in the root colonization levels of plants grown in unsterile soil inoculated with AM fungi have been observed earlier (Bhagyaraj and Manjunath, 1980). Least recorded in the uninoculated control plants, this suggests the possibility of number of infective propagules in the substrate being low or the ineffectivity of native AM fungi.

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