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INFLUENCE OF ORGANIC AMENDMENTS ON THE DEVELOPMENT OF ECTOMYCORRHIZAE AND THEIR EFFICIENCY IN P UPTAKE AND SEEDLINGS GROWTH OF *PINUS KESIYA*

C.S. Rao, G.D. Sharma and A.K. Shukla*
Department of Botany, North-Eastern Hill University, Shillong- 793022, India.

Abstract

Mycorrhizal infection with seedlings was maximum by *Suillus luteus* fungus in grass litter amended soil and minimum by *Pisolithus tinctorius* in fresh pine needle amended soil. Production of mycorrhizae was maximum by *S. luteus* fungus in grass litter amended soil and minimum in those seedling inoculated with *P. tinctorius* grown in unamended soil. Root phosphatase activity was more with mycobionts inoculatese activity of root was observed in seedlings inoculated with *S. luteus* and grown in grass litter amended soil. In general nutrient concentration recorded higher in the seedlings inoculated with the mycobionts grown in amended soil than uninoculated one.

Introduction

Organic matter is the main energy source for the growth and multiplication of microbes and also supports the growth of green plants. The level of organic matter in soil determines a multiplicity of microorganisms and make a system more dynamic (Prescott *et al.*, 1993). Mycorrhizal fungi depends on their host for the supply of energy source. However, mineralization of litter may determine the availability of inorganic salts to them and to their hosts. Therefore, litter quality and quantity may regulate the nutrient suppluy. In such situation, colonization and efficiency of mycorrhizal fungi may be influenced (Rose *et. al.*, 1983).

Fungal growth and mycorrhiza formation depends on its genotype, host (Rosado *et al.*, 1994) and also on external factors including availability of nutrients such as carbohydrates, nitrogen and phosphorus (Gibson and Deacon, 1990). Study on the effect of forest degradation on the infection by mycorrhizal fungi is available (Jha, 1990) but information on the qulaity of litter, which may influence the development of mycorrhiza is not available. This study was undertaken to help in filling the gap in our knowledge by evaluatign the effect of various levels of organic amendmens on the development of ectomycorrhizae and their efficiency in P uptake and growth of seedlings.

Materials and Methods

Soil was collected from the Botanical Garden of North-Eastern Hill University, Shillong, India. The soil is sandy loam (sand 73%, silt 15%, clay 12%). The physico-chemical characteristics of soil were: pH 5.2, organic carbon 2.1%, total nitrogen 0.18%, available phosphorus 0.021% and potassium 0.016%. The soil was steam sterilized at 15 psi twice at an interval of 24 hr. After that soil was mixed with autoclaved building sand in a ratio of 1:1, then 3 kg of sand-soil mixture was placed in plastic pots (diameter 16 cm) with a drainage hole.

The sterilized soil was amended with the various organic materials, viz. (i) Fresh pine litter (ii) Pine duff (iii) Grass litter, and (iv) Grass litter + pine duff. Fresh and duff pine needles were collected from the floor of Khasi pine (*Pinus kesiya*) stand after litter fall in the month of January. For each amendment, 30 g of oven dried (at 60 °C) fresh fine, pine duff, grass litter and 15 g of pine duff + 15 g of grass litter were mixed separately in the previously sterilized soil kept in the plastic pots. Litter was sterilized in autoclave prior to amendment in soil. 30 pots were maintained for each

Present address: Department of Life Sciences (Botany), Arunachal University, P.G. Centre, JNC, Pasighat, Arunachal Pradesh.

amendment. The control set did not receive any litter.

Sterilized pine seeds were germinated at 30 °C. Eight seedlings (3 cm radicle) were transplanted in each pot and maintained in greenhouse for 6 months. Two mycorrhizal fungi, i.e., one indigenous *Suillus luteus* and an exotic *Pisolithus tinctorius* grown previously for 2 months on Modified Melin Norkan's medium (MMN) were used. 20 ml of mycelial slurry of each mycobiont was inoculated 2 cm below the soil surface near the root system of the seedlings in each pot. Ten sets of pot for each amendments were maintained. Control set received the same quantity of autoclaved inoculum which were without amendment. Eigth seedlings per amendment per treatment were harvested after 180 days of transplantation. Seedlings along with their root systems were brought to the laboratory for further observations. Roots of seedlings were washed under running tap-water. Percentage ectomcorrhizal infection was determined as suggested by Beckjord *et al.* (1984).

$$\text{Ectomycorrhizae (\%)} = \frac{\text{No. of mycorrhizae lateral rootlets}}{\text{Total No. of lateral rootlets}} \times 100$$

Shoot height, root length and root collar diameter of seedlings were measured. Shoot and root dry weight of seedlings were determined by drying them at 60 °C for 72 h in hot air oven. Seedling volume was calculated as C [(root collar diameter)^2 x Height or D^2H] (Marx, 1980).

Phosphatase activity of lateral rootlets was measured by the method of Dodd *et al.* (1987) using p-nitrophenyl phosphate as substrate. For the chemical analysis oven dried seedlings were milled in the laboratory and analysed for total contents of the elements N, P and K. Nitrogen was determined by semimicro kjeldahl procedure (Allen, 1974). After an acid wet oxidation in HNO_3+H_2SO_4+$HC0_4$, analyses were performed for phosphorus and potassium as suggested by Allen (1974). Per cent phosphorus translocatin to the shoot was calculated as described by Theodorou and Bowen (1993).

$$\text{Per cent P Translocation to shoot} = \frac{\text{Shoot p (mg)}}{\text{Total P (mg)}} \times 100$$

Results

Mycorrhizal infection was maximum in the seedlings inoculated with *Suillus luteus* and grown in grass litter amended soil and minimum in *Pisolithus tinctorius* inoculated one in fresh pine needle amended soil. In general seedlings inoculated with *S. luteus* showed better infection than the *P. tinctorius*. Mycorrhizal production was also found maximum in the seedlings inoculated with *S. luteus* under grass litter amendment and minimum with *P. tinctorius* grown in fresh pine needle amended soil. D^2H value of seedlings was always higher in organic matter amended soil with mycobionts than those in unamended soil without mycobionts. Among two mycobionts maximum D^2H value was recorded for *S. luteus* seedlings grown in grass litter amended soil and minimum for *P. tinctorius* grown in pine fresh amended soil. An enhancement in growth of seedlings was found after inoculation of mycobionts. Maximum shoot:root ratio was observed in *P. tinctorius*, unamended soils seedlings and minimum in pine fresh amended soil without inoculum (Table-1 & Fig. 1). Biomass of the seedlings also showed the same trend as mycorrhizal infection and productivity under various treatments.

Root phosphatase activity was more in mycobiont inoculated seedlings in organic matter amended soil than in the control. Maximum activity was in grass litter amended soil with *S. luteus* inoculated seedlings and minimum in pine duff amended soil with *P. tinctorius* (Table-2). Significant variation was observed between the inoculated and uninoculated ones and amended and unamended soils (3.89 at P=0.05) but not between the various organic matter amendments.

Maximum nitrogen content in seedlings was observed with *S. luteus* on grass litter amended soil and minimum in unamended uninoculated ones (Table-2). Potassium was maximum in shoot and root of *S. luteus* inoculated seedlings grown in grass litter amended

Table-1: Growth parameters, dry matter production and productivity of mycorrhizae in Pine seedlings inoculated with different mycobionts under various organic amendments

Treatments	Parameters	Unamended Soil	Pine fresh + Soil	Pine duff +Soil	Grass litter + Soil	Grass + Pine duff + Soil	L.S. D. P=0.05
Control	Mycorrhizal infection (%)	-	-	-	-	-	-
	Mycorrhizal production (mg)	-	-	-	-	-	-
	Shoot/Root ratio	0.42	0.41	0.64	0.42	0.33	0.10
	Root Collar Diameter (cm)	0.23	0.25	0.22	0.24	0.23	0.09
	Seedling Volume (cm^3)	0.58	0.69	0.56	0.71	0.65	0.20
	Seedling Biomass (g)	0.31	0.30	0.42	0.55	0.47	0.17
P. tinctorius	Mycorrhizal infection (%)	63.0	61.0	65.0	79.0	66.0	18.65
	Mycorrhizal production (mg)	74.0	72.0	88.2	181.6	100.1	17.32
	Shoot/Root ratio	0.92	0.47	0.46	0.54	0.48	0.25
	Root Collar Diameter (cm)	0.29	0.28	0.26	0.27	0.24	0.08
	Seedling Volume (cm^3)	0.84	0.9[illegible]	1.11	1.37	1.27	0.16
	Seedling Biomass (g)	0.46	0.47	0.66	0.96	0.74	0.28
S. luteus	Mycorrhizal infection (%)	76.0	69.0	74.0	90.0	82.0	7.82
	Mycorrhizal production (mg)	101.0	99.6	158.9	255.0	186.0	14.80
	Shoot/Root ratio	0.44	0.51	0.58	0.54	0.50	0.05
	Root Collar Diameter (cm)	0.26	0.25	0.23	0.28	0.24	0.10
	Seedling Volume (cm^3)	0.98	1.00	1.11	1.74	1.51	0.36
	Seedling Biomass (g)	0.57	0.50	0.80	1.08	0.98	0.24

soil and minimum in the roots of unioculated seedlings with fresh pine needle amended soil. Phosphorus content of shoot as well as in root was maximum in *S. luteus* inoculated seedlings grown on grass litter amended soil and minimum in fresh pine amended uninoculated ones (Table-2). Significant variation in P contents of seedlings was observed between treatments (5.99 at P=0.05; Table-3). While considering the translocation of phosphorus from soil to the shoot on per gram basis of seedlings it was higher in *S. luteus* inoculated amended with grass litter seedlings and minimum in uninoculated grown in unamended soil. No significant relationsship was observed between various amendments. However, a significant relationship was observed between the fungal inoculants.

Discussion

The results depict that mycorrhizal infection and production was better in the seedlings inoculated with the indigenous mycobiont *S. luteus* than the exotic *P. tinctorius*. Amendment of grass litter to both types of mycorrhizal seedlings showed an improvement in their infection and growth of the seedlings than those either amended with fresh pine or unamended ones which was attributed to the presence of insoluble and toxic substances in pine litter than in grass litter

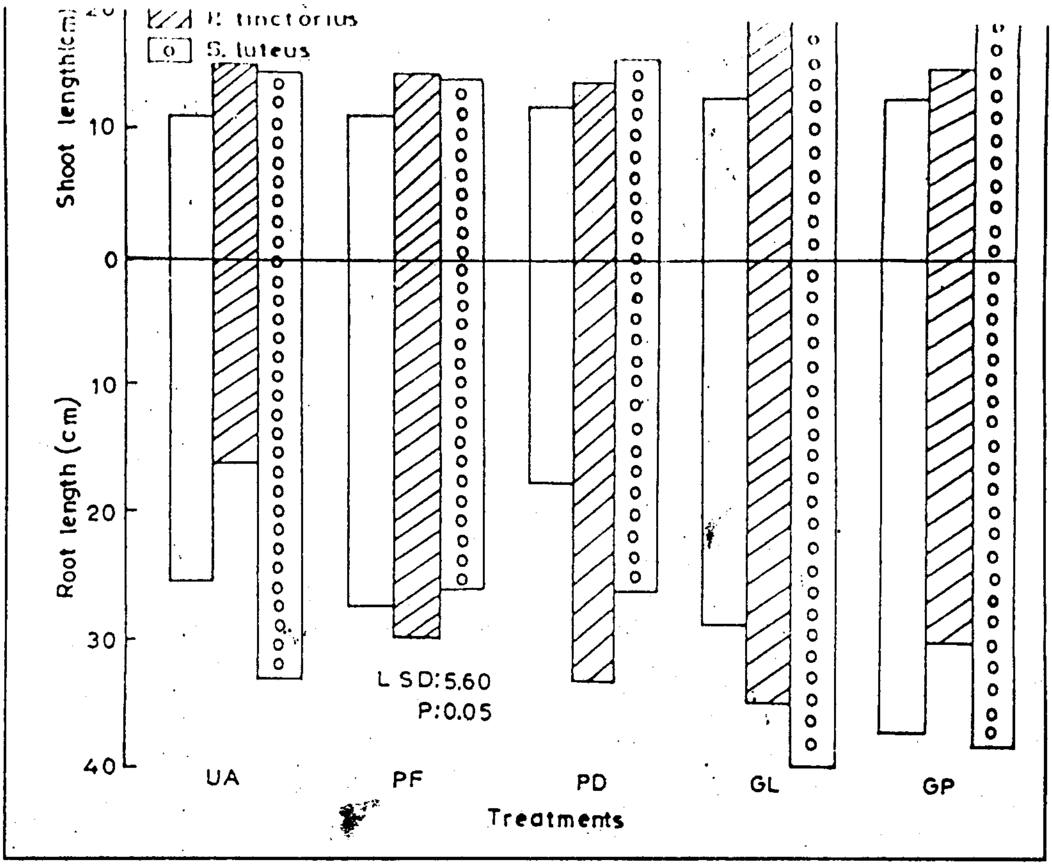

Fig. 1: Shoot height and root length of pine seedlings inoculated with different mycobionts in various soil amendments (UA- Unamended; PF-Pine fresh; PD-Pine duff; GL- Grass litter; GP-Grass + pine duff).

(Berg and McLaugherty, 1989). Release of nitrogen, phosphorus differed between fresh and duff coniferous foliage litter. Among the two litters, more mobilization of nutrients, growth response and mycorrhizal infection may be comparatively poor due to absence of leaching phase during decomposition in conifer needles (Berg, 1988). Better development of ectomycorrhiza with *S. luteus* and *P. tinctorius* on grass litter and pine duff than the uninoculated and unamended ones and was releated to the improved physico-chemical characteristics of the soil (Riffle, 1977).

Improvement of growth and accumulation of more dry matter in mycorrhiza pine seedlings than uninoculated ones were related to the enhanced nutrient uptake by earlier than the latter ones (Griffiths *et al.,* 1984). Better shoot and root growth of the mycorrhizal seedlings on various organic amendments was attributed to the improved mycelial strands production by the mycobiont. It conferred high penetration of large inter root distances and had a positional advantage for competition with other micro-organisms for both inorganic and organic nutrients. Hacskaylo (1973) found cellulolytic enzymes in *S. luteus, S. varieagatus* and *S. subtomentosus*, which were able to attack hemicelluloses of the litter, and other naturally occurring complex carbohydrates and obtain their required carbon compounds. Poor growth of seedlings with fresh pine litter was due to the

Table-2: Nutrient concentration (Nitrogen-N, Phosphorus-P, Potassium-K) and Phosphatase activity (µg/g/dry wt/h) in pine seedlings inoculated with different mycobionts under various organic amendments (UA - Unamended; PF- Pine fresh; PD-Pine duff; GL - Grass Litter; GP-Grass + Pine duff)

Treatments	Parameters	Unamended Soil	Pine fresh + Soil	Pine duff +Soil	Grass litter + Soil	Grass + Pine duff + Soil	L.S. D.
	Shoot N (%)	2.70	2.70	2.77	2.81	2.84	0.36
	Root N(%)	1.00	1.09	1.08	1.04	1.06	0.16
	Shoot K(%)	1.83	1.87	1.81	1.89	1.84	0.20
	Root K(%)	0.78	0.79	0.76	0.85	0.82	0.07
Control	Shoot P(%)	0.29	0.28	0.29	0.32	0.33	0.03
	Root P(%)	0.21	0.21	0.21	0.23	0.21	0.03
	Phosphatase	369.0	356.0	343.0	387.0	366.0	36.42
	% P translocation to the shoot	41.96	43.63	53.41	56.22	54.63	4.72
	Shoot N (%)	2.74	2.71	2.75	2.81	2.79	0.31
	Root N(%)	1.15	1.21	1.19	1.28	1.29	0.23
	Shoot K(%)	2.12	2.10	2.14	2.13	2.11	0.29
	Root K(%)	0.86	0.82	0.89	0.98	0.96	0.08
P.tinctorius	Shoot P(%)	0.31	0.29	0.31	0.33	0.32	0.01
	Root P(%)	0.26	0.25	0.28	0.33	0.32	0.02
	Phosphatase	892.0	888.0	819.0	898.0	868.0	87.30
	% P translocation to the shoot	48.35	48.35	61.27	75.95	62.90	23.92
	Shoot N (%)	2.02	2.18	2.47	2.88	2.82	0.29
	Root N(%)	1.01	1.19	1.30	1.30	1.29	0.17
	Shoot K(%)	2.48	2.50	2.60	2.71	2.68	0.23
	Root K(%)	1.01	1.11	1.16	1.20	1.18	0.15
S. luteus	Shoot P(%)	0.32	0.31	0.34	0.39	0.38	0.03
	Root P(%)	0.32	0.36	0.41	0.47	0.42	0.04
	Phosphatase	863.0	854.0	888.0	902.0	896.0	88.08
	% P translocation to the shoot	56.10	58.40	71.02	84.36	76.66	6.94

suppressed the growth of *C. graniforme* and *L. forninos* (Mikola, 1948).

Table-2: Analysis of varianc (F) of various amendments and treatments

Parameters	Between amendments	Between treatments
Mycorrhizal infection	5.56*	6.56*
Mycorrhizal productivity	4.96*	6.02*
Shoot height	3.76*	9.16**
Root length	NS	NS
Shoot/Root ratio	NS	NS
Seedling volume	3.63*	4.36*
Biomass of seedlings	NS	NS
Shoot nitrogen	NS	NS
Root nitrogen	NS	NS
Shoot potassium	NS	5.60*
Root potassium	NS	28.71**
Shoot phosphorus	5.70*	4.46*
Root phosphorus	3.87*	5.99*
Root phosphatase	4.77*	3.89*

NS-Not significant; *-Significant at $P<0.05$; **-Significant at $P<0.01$

Increased growth and dry matter in mycobionts inoculated seedlings with grass litter was related to the presence of easily degradable composts and more easily available nutrients which increased the growth of mycorrhizal fungi (Schisler and Linderman, 1989). The enhanced growth of conifer seedlings amended with litter than unamended ones was attributed to biological rather than nutritional factors (Parke *et al.*, 1983). Presence of more phosphorus in the mycorrhizal seedlings than in the non-mycorrhizal ones was supported by Stribley *et al.* (1980). Higher nutrient uptake by mycorrhizal plants was due to improved hyphal growth, better exploitation of the soil volume by *S. luteus* over *P. tinctorius.*

correlated to higher rate of phosphatase activity in mycorrhizal than non-mycorrhizal ones (Tarafdar and Marschner, 1994). The results have suggested that the inoculation of the indigenous mycobiont *S. luteus* along with organic amendment of grass litter which grows at the early stage of pine seedlings in natural condition regeneration is better than the introduction of exotic mycobiont *P. tinctorius* in both unamended or amended soils. Addition of pine litter to the pine nursery may have detrimental effect on the development of mycorrhiza as well as on the growth of the seedlings.

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