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Response of Pine Seedlings with Various Ectomycorrhizal Fungi in Organic Amended Soils

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Abstract: ***Seedlings of Pinus kesiya were inoculated with mycorrhizal fungi namely C. rachata L. laccata, P. tinctorius and S. luteus grown in organic amended soils using pure culture techniques. Mycorrhizal infection was maximum in the seedlings inoculated with S. luteus in grass litter amended soil and minimum with seedlings inoculated with P. tinctorius. Similarly Production of mycorrhizae was higher by S. luteus fungus in grass litter soil than any other mycorrhizal fungi in organic amended soil. In general mycorrhizal fungi inoculated pine seedlings harboured higher nutrient concentration as well as shoot weight, root weight seedling volume and seedling biomass than uninoculated seedlings in different organic amended soils.***

Keywords: Pinus, Ectomycorrhiza, Fungi, Organic soil, *Laccaria laccata, Colibia collata, Pisolithus tictorius, Suillus luteus*

1. Introduction

The mycorrhizal relationship is essential to the healthy growth of trees, especially where soil nutrients are limiting or environmental conditions are harsh (Harley and Smith, 1983). The importance of ectomycorrhizal fungi in the establishment of conifer seedlings have been demonstrated by various workers (Harley and Smith, 1983; Gbdegesin, 1990). Ectomycorrhizal fungi are known to provide a much larger physiologically active, root fungus area for nutrient and water absorption. These fungi absorb and accumulate nitrogen, phosphorus. potassium and calcium in the fungus mantle more rapidly and for a longer period of time than non-mycorrhizal feeder roots. They help in breaking down certain complex minerals and organic substances in the soil and transmit nutrients to the plant (Marx, 1980). Seedling growth responses vary according to soil 'characteristics as well as the fungal inoculum used (Marx and Cordell 1988).

Organic matters are main energy source for the growth and multiplication of microbes and also support the growth of green plants. The level of organic matter in soil determines a multiplicity of microorganisms and makes a system more dynamic (Prescott et al 1993)

Mycorrhizal fungi depend *on* their host for the supply of energy source. However mineralization of litter may determine the availability of inorganic salts to them and their hosts. Therefore, litter quality and quantity may regulate the nutrient supply**.** In such situation colonization and efficiency of mycorrhizal fungi may *be* influenced (Rose et al 1983). This study was undertaken to work out the effect of various level of organic amendments along with different mycorrhizal fungi on the pine seedlings growth and P uptake in the nursery.

2. Materials and Methods

Soil was collected from the Botanical Garden. 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 15psi twice at an interval of 24 hrs. After that soil was mixed with autoclaved 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 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 pine stand after litter fail in the month of January. For each amendment, 30g of oven dried (at 60 °C) fresh pine,.pine duff, grass litter and 15g of pine duff + 15g 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 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 green house for six months. The mycorrhizal fungi i.e. *Laccaria laccata, Colibia collata, Pisolithus tictorius* and *Suillus luteus* grown previously for two months on Modified Melin Nockan's medium (MMN) were used. 20 ml of mycelia' slurry of each mycobiont was inoculated 2cm below the soil surface near the root system of the seedlings era each pot. Ten sets of pot for each amendment were maintained. Control set received the same quantity of autoclaved inoculum which was without amendment. Eight 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 ectomycorrizal infection was determined as suggested by Beckjord et al. (1984).

$$\text{Ectomycorrhizae (\%)} = \frac{\text{Number of mycorrhizal lateral rootlets}}{\text{Total number of lateral rootlets}} \times 100$$

Shoot height, root length and root collar diameter of seedlings were measured. Shoot and root dry weight of seedlings was determined by drying them at 60 °Cfor 72 h

in hot air oven. Seedling volume was calculated as C [(root colar diameter)^2 X Height or D^{2}H] (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 ground in the laboratory and analysed for total contents of the elements N, P and K. Nitrogen was determined by semi micro-kjeldahl method (Allen, 1974). After an acid wet oxidation in HNO_3+H_2SO_4+$HCOl_4$, analyses were performed for phosphorus and potassium as suggested by Allen (1974). Percent phosphorus translocation to the shoot was calculated as described by Theodorou and Bowen (1993).

$$\text{Percent P translocation to shoot} = \frac{\text{Shoot P (mg)}}{\text{Total P (mg)}} \text{X } 100$$

3. Results

Pine seedlings grown in grass litter amended soil and inoculated with *Suillus luteus* harboured maximum mycorrhizal infection and minimum in *Pisolithus tictorius* inoculated one in fresh pine needle amended soil. In general seedlings inoculated with *S. luteus* showed better infection than other mycorrhizal fungi. 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^{2}H value of seedlings was always higher in organic matter amended soil with mycobionts than those in unamended soil without mycobionts. Among all mycobionts maximum D^{2}H value was recorded for *S. luteus* seedlings grown in grass litter amended soil and minimum for *P. tinctorius* grown in fresh pine needle 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). Biomass of the seedlings also showed the same trend as mycorrhizal infection and productivity under various treatments.

Root phosphatase activity was more in mycobionts 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.* Significant variation was observed between the inoculated and uninoculated ones and with amended and unamended soils. There was not much variation in the activity among organic matter amended pots.

Maximum nitrogen content was observed in the seedlings inoculated with *S. luteus* grown in 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 soil and minimum in the roots of uninoculated seedlings with fresh pine needle amended soil. Phosphorus content of shoot as well as in root was higher in S. *luteus* inoculated seedlings grown with grass litter amended soil and minimum in fresh pine amended uninoculated ones. Significant variation in P content of seedlings was observed between treatments (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 relationship was observed between various amendments. However, a significant relationship was observed between the fungal inoculants.

Table 1: Growth parameters, dry matter production and productivity of ectomycorrhiza in pine seedlings inoculated with different mycobionts under various organic amendments (UAS: Unamended soil; PFS: Pine fresh+ soil; PDS: Pine duff+ soil; GLS: Grass litter + soil; GPS: Grass +Pine duff+ soil).

Fungi	Amendments	UAS	PFS	PDS	GLS	GPS	L.S.D. (P=0.05)
Control	Mycorrhizal infection (%)	-	-	-	-	-	-
	Mycorrhizal production (mg)	-	-	-	-	-	-
	Shoot/Root ratio	042	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
C. radiate	Mycorrhizal infection (%)	65.0	62.0	70.0	81.0	68.0	8.61
	Mycorrhizal production (mg)	88.0	76.0	110.0	202.0	123.0	16.26
	Shoot/Root ratio	0.68	0.50	0.52	0.53	0.51	0.12
	Root Collar Diameter (cm)	0.27	0.26	0.25	0.26	0.24	0.09
	Seedling Volume (cm^3)	0.89	0.91	0.93	1.46	1.28	0.23
	Seedling biomass (g)	0.50	0.48	0.70	0.97	0.82	0.17
L. laccata	Mycorrhizal infection (%)	70.0	64.0	72.0	84.0	70.0	7.76
	Mycorrhizal production (mg)	95.0	84.0	140.0	240.0	170.0	15.38
	Shoot/Root ratio	0.51	0.49	0.53	0.52	0.51	0.11
	Root Collar Diameter (cm)	0.27	0.26	0.24	0.26	0.25	0.08
	Seedling Volume (cm^3)	90.0	93.0	1.07	1.70	1.30	0.39
	Seedling biomass (g)	0.53	0.49	0.76	0.96	0.89	0.16
P. tinctorius	Mycorrhizal infection (%)	63.0	61.0	66.0	79.0	66.0	18.65

	Mycorrhizal production (mg)	74.0	72.0	98.2	181.6	100.1	17.32
	Shoot/Root ratio	0.92	0.47	0.46	0.56	0.49	0.06
	Root Collar Diameter (cm)	0.29	0.28	0.26	0.27	0.24	0.08
	Seedling Volume (cm^3)	0.84	0.92	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)	100.0	99.5	157.9	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	0.26	0.24	0.10
	Seedling Volume (cm^3)	98.0	1.00	1.11	1.74	1.51	0.35
	Seedling biomass (g)	0.57	0.50	0.80	1.08	0.98	0.25

Table 2: Nutrient concentration (N: Nitrogen; K: Potassium; P: Phosphorus) in pine seedlings inoculated with different mycobionts under various organic amendments (UAS: Unamended soil; PFS: Pine fresh+ soil; PDS: Pine duff+ soil; GLS: Grass litter + soil; GPS: Grass +Pine duff+ soil).

Mycobionts	Nutrients	UAS	PFS	PDS	GLS	GPS	L.S.D. (P=0.05)
Control	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
	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
C. radiate	Shoot N (%)	2.76	2.45	2.46	2.74	2.80	0.37
	Root N (%)	1.11	1.18	1.21	1.25	1.23	0.16
	Shoot K (%)	2,14	2.12	2.17	2.17	2.16	0.21
	Root K (%)	0.89	0.86	0.91	0.97	0.96	0.08
	Shoot P (%)	0.29	0.29	0.30	0.32	0.30	0.01
	Root P (%)	0.24	0.23	0.26	0.29	0.29	0.02
L. laccata	Shoot N (%)	2.82	2.50	2.51	2.79	2.80	0.32
	Root N (%)	1.13	1.20	1.23	1.26	1.24	0.18
	Shoot K (%)	2.15	2.11	2.20	2.23	2.19	0.11
	Root K (%)	0.91	0.90	0.96	0.99	0.98	0.07
	Shoot P (%)	0.29	0.28	0.31	0.34	0.32	0.05
	Root P (%)	0.27	0.30	0.30	0.37	0.34	0.03
P. tinctorius	Shoot N (%)	2.74	2.71	2.75	2.81	2.79	0.31
	Root N (%)	1.15	11.21	1.19	1.26	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	0.09
	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	0.03
S. luteus	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	2.66	0.23
	Root K (%)	1.01	1.11	1.16	1.20	1.18	0.15
	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

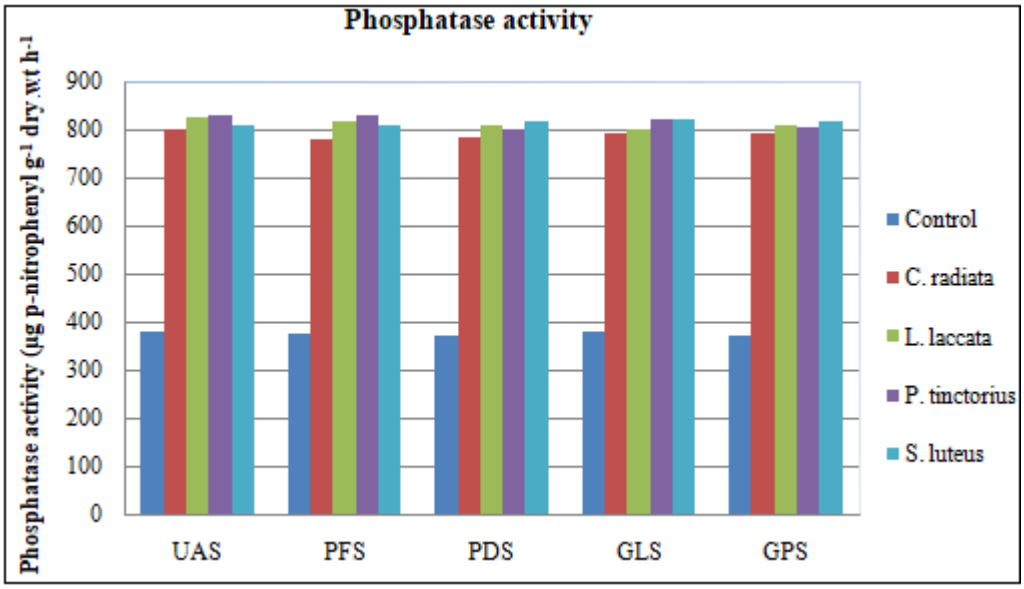

Figure 1: Phosphatase activity in pine seedlings inoculated with different mycobionts under various organic amendments (UAS: Unamended soil; PFS: Pine fresh+ soil; PDS: Pine duff+ soil; GLS: Grass litter + soil; GPS: Grass +Pine duff+ soil).

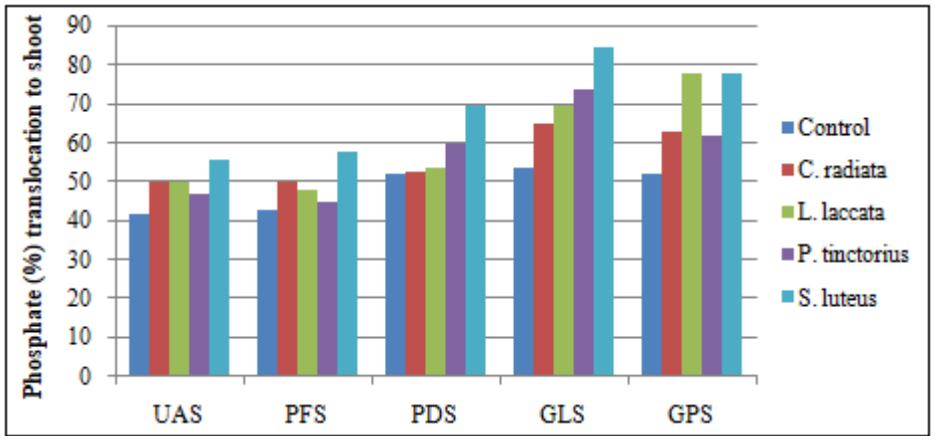

Figure 2: Percent P translocation to the shoots in pine seedlings inoculated with different mycobionts under various organic amendments (UAS: Unamended soil; PFS: Pine fresh+ soil; PDS: Pine duff+ soil; GLS: Grass litter + soil; GPS: Grass +Pine duff+ soil).

4. Discussion

It is evident from the results that mycorrhizal infection and production was always higher in the seedlings inoculated with the indigenous mycobionts *S. luteus* than other mycobionts. Amendment of grass litter to all the 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 (Berg and McLaugherty, 1989). Release of nitrogen, phosphorus differed between fresh and duff coniferous foliage litter. Between 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 mycobionts inoculated seedlings on grass litter and pine duff than the uninoculated and unamended ones was related to the improved physicochemical characteristics of the soil (Riffle, 1977).

Table 3: Analysis of variance (F) of various amendments and treatments (NS: Not significant; * significant at P< 0.05; ** significant at P< 0.01

Parameters	Between amendments	Between treatments
Mycorrhizal infection	5.86*	6.76*
Mycorrhizal production	5.12*	6.21*
Shoot/Root ratio	NS	NS
Seedling Volume	3.89*	4.52*
Seedling biomass	NS	NS
Shoot N	NS	NS
Root N	NS	NS
Shoot K	NS	4.12*
Root K	NS	9.71**
Shoot P	6.08*	5.97*
Root P	4.05*	5.85*
Root phosphatase	4.49*	3.92*

Improvement of growth and accumulation of more dry matter in mycorrhizal pine seedlings than uninoculated ones were related to the enhanced nutrient uptake by earlier than the later ones (Griffiths etal., 1984). Better root and shoot growth of the mycorrhizal seedlings on various organic

amendments was attributed to the improved mycelial strands production by the mycobionts. it conferred high penetration of large inter root distances and had a positional advantage for competition with other microorganisms for both inorganic and organic nutrients. Hacskaylo (1973) found cellulolytic enzymes in *Suillus* species, 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 inhibitory phenols and organic acids that suppressed the growth of C. *graniforme* and *L. forninos* (Mikola. 1973).

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 the biological rather than nutritional factors (Parke *et al.*, 1983). Stribley *et al.* (1980) supported presence of more phosphorus in the mycorrhizal seedlings than in nonmycorrhizal ones. Quality of external mycelium and sheath determine the rate of uptake of P and other mineral nutrients. Perhaps because of this reason there was variation in P uptake by various mycobionts. Mycobionts inoculated seedlings roots contain higher amount of P than control; this may be attributed to the capability of sheath for storing large quantity of P usually in the form of polyphosphate (MacFall et al., 1992). Higher nutrient uptake by mycorrhizal plants was due to improved hyphal growth, better exploitation of the soil volume by *S. Iuteus* over other mycobionts. Higher phosphate uptake by the seedlings was correlated to higher rate of phosphates activity in mycorrhizal seedlings than non-mycorrhizal ones (Tarafdar and Marschner, 1994). The results have suggested that the inoculation of the indigenous mycobionts *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 other mycobionts in both unamended and amended soils. Addition of pine litter to the pine nursery may have detrimental effect on the development of mycorrhizal as well as on the growth of the seedlings. It may be concluded that ectomycorrhizal fungi significantly enhanced the seedling growth and P uptkake. A suitable mycobiont may be selected after more observation for inoculation in nurseries.

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