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Ectomycorrhizal Efficiency of Various Mycobionts with *Pinus kesiya* Seedlings in Forest and Degraded Soils

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Efficiency of various mycobionts was estimated by inoculating them onto *Pinus kesiya* seedlings grown in forest and degraded soils. Mycorrhizal colonization was maximum in *Scleroderma aurantium* inoculated seedlings grown in forest soil and in *Suillus luteus* inoculated ones in degraded soil. Productivity of mycorrhizae was maximum with *S. aurantium* and minimum with *Cenococcum graniforme* in forest soil. Whereas, in degraded soil maximum and minimum production of mycorrhizae was observed in *S. luteus* and *C. graniforme* inoculated seedlings respectively. All the mycorrhizal symbionts exhibited a promotory effect on the growth of pine seedlings in forest and degraded soils. Enhancement in seedling growth followed a definite trend *S. aurantium* > *L accaria. laccata* > *S. luteus* > *C. radiata* > *P. tinctorius* > *C.graniforme* in forest soil; in degraded soil, the order was *S. luteus* > *L. laccata* > *S. aurantium* > *C. radiata* > *P. tinctorius* > *C. graniforme*. Seedling's growth was three times higher in forest soil than in degraded soil with all the mycobiont treatments.

Key Words : Ectomycorrhizae, Fungi, Mineral contents, *Pinus kesiya*

Introduction

Ectomycorrhizae are known to increase the survival and growth of the seedlings of tree species (Koide & Lu 1995). Nonmycorrhizal pine seedlings can be grown in the greenhouse in nutrient rich conditions to minimize stress. However, such seedlings often fail to survive or grow on natural planting sites, especially during period of environmental stress (Anderson & Rygiewicz 1991). Artificial inoculation of host plants with specific mycorrhizal fungi has shown that plant growth may depend upon the efficiency of mycobionts on conifer roots and their competitive ability under varied ecological conditions (Browning & Whitney 1993). Mycorrhizal inoculation improves plant productivity especially in soils with a

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low nutrient status. The disinfection often leads to retarded plant growth due to low water and nutrient availability which can often be overcome by introducing suitable mycorrhizal fungi into the soil (Sharma et al. 1995). Response of pine seedling to infection with ectomycorrhizal fungi has been demonstrated but there are few studies of the benefits of the specific mycobionts on the Khasi pine (*Pinus skesiya*) seedlings (Kumar 1990). The aim of the present study was to investigate the efficiency of different mycorrhizal fungi with pine seedlings for their survival, growth and nutrient-uptake in forest and degraded soils.

Materials and Methods

Forest and degraded soils (with scanty grasses) were collected from the Nongstoin, West Khasi Hills (Himalaya foothills), Meghalaya. The physico-chemical properties of the forest soil were: sand 65.2%, silt 19.1%, clay 15.7%; *p*H 5.8; water holding capacity 73.2%; organic carbon 3.6%. Degraded soil was taken from an adjacent barren land. The physico-chemical characteristics of the degraded soil were: sand 79.2%; silt 10.6%; clay 10.2%; *p*H 5.5; water holding capacity 40.7%; organic carbon 1.7%. Soils from both the sites were collected from 0-15 cm depth at 10 separate locations and mixed to get homogenous mixture of each type. The soils were steam sterilized at 15 psi. Sterilization was repeated twice with an interval of 24hr. The soils were allowed to dry and were mixed with autoclaved building sand in a ratio 1 : 1, then 3 kg of the sand-soil mixture was used to fill plastic pots (diameter 16 cm) with a drainage hole.

Seeds of pine were surface sterilized in 0.1% $HgCl_2$ for 5 minutes and washed in sterilized water several times. They were germinated in sterilized sand at 30°C in a growth chamber. Six ectomycorrhizal fungi, based on their dominance in nature i.e. *Pisolithus tinctorius*, *Cenococcum graniforme*, *Collybia radiata*, *Laccaria laccata*, *Suillus luteus* and *Scleroderma aurantium* were selected for the experiment. Cultures of mycorrhizal fungi were obtained from Forest Research Institute. Dehradun and maintained on MMN medium and also tested for their mycorrhizae synthesis. Twenty ml of inoculum culture was inoculated near the roots of pine seedlings separately in each pot after 15 days of seedlings transplantation. Pine seedlings treated as control received same amount of inoculum in sterilized form. Pots were watered regularly to maintain 15-20% moisture in soil. Ten pots of each treatment were maintained. Twelve seedlings for each sampling date were harvested. Seedlings were brought to the laboratory for further studies along with their root system and attached soil. Roots of seedlings were washed under running water. Percentage mycorrhizal colonization was calculated as follows :

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

At each harvest date productivity of ectomycorrhizal roots was measured on dry weight basis and expressed as mg/day for different harvests. 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 24 hr. Seedling volume was calculated as [(root collar diameter)^2 × height or D^2H]. Oven-dried shoots and roots of seedling of each treatment were ground

separately and sieved through 0.2 mm sieve for nutrient analyses. Nitrogen was determined by semi-micro Kjeldahl procedure (Allen 1974). After an acid wet oxidation in HNO_3 + H_2SO_4 + $HClO_4$, analyses were performed for phosphorus and potassium as suggested by Allen (1974). The data were processed by analysis of variance (ANOVA).

Results

Mycorrhizal colonization was maximum in *Scleroderma aurantium* inoculated seedlings grown in forest soil and in *Suillus luteus* inoculated one in degraded soil. Minimum colonization was observed with *Cenococcum graniforme* inoculated seedlings in both the soils (table 1). Mycorrhizal productivity of

Table 1 *Growth parameters, mycorrhizal colonization (MC) and mycorrhizae productivity (MP) in pine seedlings inoculated with different mycobionts at different time intervals in forest and degraded soils*

Mycobionts	Forest soil					Degraded Soil				
(days)	Root collar diameter (cm)	Shoot/root ratio	Seedling volume (cm^3)	MC (%)	MP (mg/day)	Root collar diameter (cm)	Shoot/root ratio	Seedling volume (cm^3)	MC (%)	MP (mg/day)
Control	0.18	1.59	0.25	0	0.0	0.11	1.10	0.09	0	0.0
Pisolithus tinctorius	0.15	1.65	0.33	11	21.0	0.11	1.37	0.11	11	28.0
Cenococcum graniforme	0.19	1.66	0.26	10	19.8	0.12	1.4	0.13	10	25.0
Collybia radiata	0.19	1.96	0.46	27	43.7	0.13	2.03	0.17	18	41.0
Laccaria laccata 90	0.15	1.76	0.38	30	48.7	0.14	1.41	0.19	21	49.0
Suillus luteus	0.12	1.76	0.43	25	78.4	0.13	1.67	0.20	28	65.3
Scleroderma aurantium	0.15	1.78	0.56	36	92.2	0.14	1.4	0.19	26	61.1
Control	0.22	0.42	0.82	0	0.0	0.17	0.30	0.26	0	0.0
Pisolithus tinctorius	0.22	0.46	0.98	39	116.3	0.18	0.38	0.32	69	139.2
Cenococcum graniforme	0.21	0.48	0.86	36	110.1	0.17	0.54	0.27	55	111.4
Collybia radiata	0.25	0.60	1.25	64	135.0	0.19	0.32	0.36	71	141.2
Laccaria laccata 180	0.21	0.51	1.18	82	144.0	0.18	0.39	0.42	81	158.1
Suillus luteus	0.22	0.52	1.03	82	144.0	0.19	0.41	0.46	88	164.0
Scleroderma aurantium	0.23	0.67	1.52	90	149.0	0.19	0.45	0.38	76	147.0

different mycobionts followed same trend as shown by mycorrhizal colonization. Productivity of mycorrhizae was maximum with *S. aurantium* and minimum with *C. graniforme* in forest soil. Degraded soil harboured maximum and minimum production of mycorrhizae in *S. luteus* and *C. graniforme* inoculated seedlings respectively. Seedling volume was maximum and minimum with *S. aurantium* and *C. graniforme* inoculated seedlings in forest soil. In degraded soil it was maximum in *S. luteus* and minimum in *C. graniforme*. Volume of seedlings was significantly higher in forest soil compare to degraded soil. There was not much difference in the shoot : root ratio of forest and degraded soils. Among different mycobionts it does not show much variation.

There was great variation in shoot height of seedlings grown in forest and degraded soils. Average root length was more than the shoot height (figure 1). Biomass of seedlings inoculated with mycobionts was always higher than uninoculated ones under both the soils (figure 2). In general mycorrhizal fungi exhibited a promotory effect on the growth of pine seedlings. Stimulation in seedlings growth followed a trend *S. aurantium* > *L. laccata* > *S. luteus* > *C. radiata* > *P. tinctorius* > *C. graniforme* in forest soil. Whereas in degraded soil, the order was *S. luteus* > *L. laccata* > *S. aurantium* > *C. radiata* > *P. tinctorius* > *C. graniforme*.

Higher amount of nitrogen, phosphorus and potassium content was recorded in

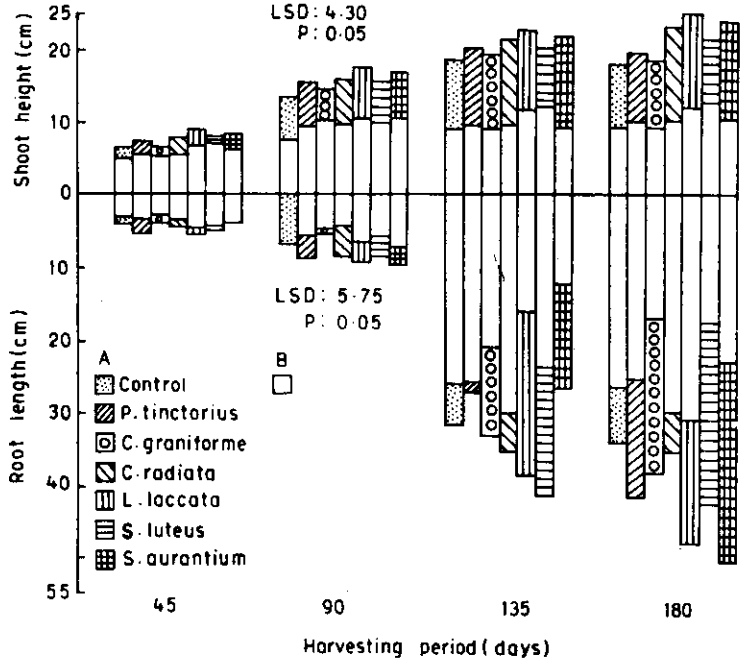

Figure 1 Shoot height and root length of pine seedlings at various sampling periods inoculated with different mycobionts in (A) forest and (B) degraded soils.

seedlings inoculated with various mycobionts than uninoculated ones (table 2). Shoot nitrogen and phosphorus were maximum in *S. aurantium* inoculated seedlings grown in forest soil. Amount of N, P and K contents were higher in the shoot of *S. luteus* inoculated seedlings grown in degraded soil. Seedlings grown in degraded soil have more amount of N and P in roots inoculated with *S. luteus*. Concentration of potassium in roots of seedlings was maximum with *C. radiata* inoculated and minimum with *S. aurantium* in degraded soil. In general the mineral contents of shoot and root were higher in seedlings grown in forest soil than degraded soil.

Discussion

At initial stage there was no significant variation in seedling growth of pine between inoculated and control treatments in forest and degraded soils. Drain of photosynthates from the host to the fungus, may be responsible for invisible enhanced growth by the different symbionts at an early safe of seedling growth (Cairney 1992). However, the enhanced growth of seedlings by ectomycorrhizal fungi in forest and degraded soils at later part of seedling development was attributed to the increase in root absorbing surface area due to extensive network of fungal mycelium (Colinas et al. 1994). The growth of 180 days old pine seedlings was observed significantly more in forest soil than in degraded soil, which was attributed to the high amount of soil organic matter, which favoured better formation of mycorrhizae. Maximum colonization of pine

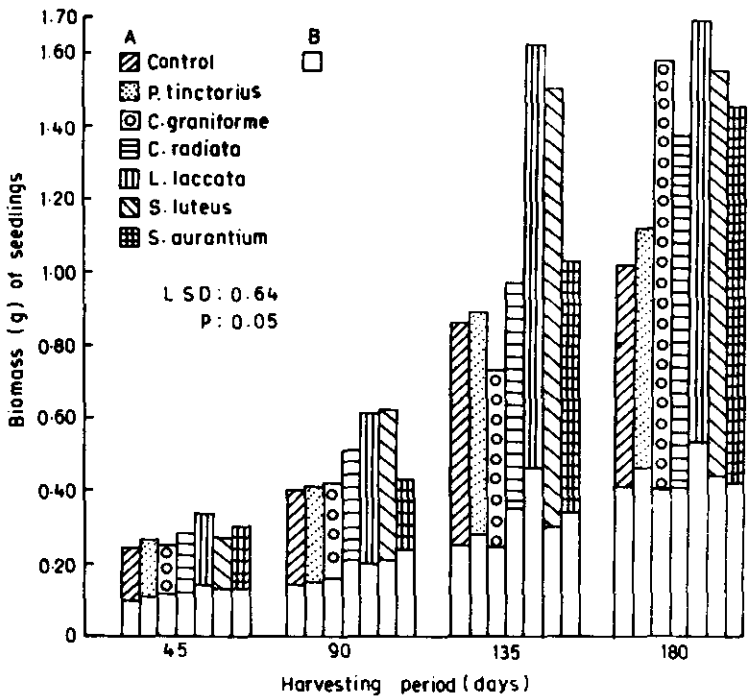

Figure 2 Biomass of pine seedlings at various sampling periods inoculated with different mycobionts in (A) forest and (B) degraded soils.

Table 2 *Nutrient concentration (Nitrogen-N, Phosphorus-P, Potassium -K) % in Shoot and root of pine seedlings inoculated with different mycobionts in forest and degraded soils*

Mycobionts	Forest Soil						Degraded Soil					
	Shoot			Root			Shoot			Root		
(days)	N	P	K	N	P	K	N	P	K	N	P	K
Control	1.34	0.23	1.40	0.80	0.20	0.75	1.71	0.20	1.72	0.88	0.21	0.91
P. tinctorius	1.81	0.31	1.79	0.99	0.27	0.92	1.81	0.28	1.89	0.98	0.33	1.07
C. graniforme	1.82	0.29	1.81	1.11	0.24	0.89	1.81	0.32	1.92	1.00	0.29	0.99
C. radiata 90	1.89	0.30	1.77	1.03	0.29	0.96	1.87	0.33	1.96	1.07	0.29	1.02
L. laccata	1.92	0.36	1.89	1.10	0.36	0.99	1.85	0.31	1.92	1.01	0.32	0.99
S. luteus	1.88	0.35	1.82	0.99	0.25	0.93	1.89	0.37	1.99	1.07	0.34	1.09
S. aurantium	1.96	0.49	1.86	1.09	0.36	1.0	1.81	0.33	1.92	1.02	0.30	1.00
Control	1.49	0.26	1.63	1.00	0.24	0.89	1.75	0.25	1.86	1.01	0.26	0.99
P. tinctorius	2.02	0.38	1.90	1.19	0.36	1.16	1.83	0.33	1.98	1.03	0.37	1.12
C. graniforme	2.29	0.47	1.95	1.32	0.36	1.16	1.86	0.35	1.96	1.08	0.36	1.07
C. radiata 180	2.13	0.38	1.93	1.21	0.34	1.12	1.82	0.34	2.01	1.11	0.31	1.16
L. laccata	2.32	0.54	1.99	1.25	0.42	1.18	1.91	0.39	2.03	1.11	0.38	1.09
S. luteus	2.01	0.39	2.02	1.31	0.35	1.09	1.96	0.46	2.04	1.17	0.39	1.10
S. aurantium	2.69	0.64	2.00	1.25	0.48	1.14	1.88	0.38	1.97	1.13	0.32	1.04

root by *S. luteus* in degraded soil could be related to its affinity with low organic matter content, favourable moisture and aeration levels which favoured the development of mycorrhizae compare to the forest soil where nutrient concentration in the substrate restricted mycorrhizal colonization (Kumar 1990). Mycorrhizal colonization was minimum with *C. graniforme* in forest and degraded soils which ultimately reflected the reduction in NPK contents, biomass and seedling volume. The difference between these two may be attributed to the fertility status of the soils. *S. luteus* demonstrated its better compatibility with *Pinus kesiva* in degraded soil by colonizing more of the root system than the other fungi and by mediating a far superior growth response. Seedlings with shorter root length and shoot height in the degraded soil could be sacrificed to the good development of mycorrhizae and mycorrhizal production than the seedlings with better growth characteristics but with less mycorrhizae in forest soil (Rousseau et al. 1994). A decrease in shoot root ratio in degraded soil in comparison to the forest soil is similar to the application of fertilizers leading to the high fertility of the substrate (Thomas et al. 1994). The difference in seedling growth caused by different

Table 3 *Analysis of variance (f) of various mycobionts and sampling period with various parameters in forest and degraded soil*

Source of variance	Forest soil		Degraded soil	
	Variation Between mycobionts	Variation Between Sampling periods	Variation Between mycobionts	Variation Between Sampling periods
Shoot height	NS	108.82**	NS	23.47**
Root length	NS	82.71**	NS	28.1**
Seedling volume	NS	33.32**	NS	38.9**
Mycorrhizal infection	NS	8.89**	NS	65.88**
Mycorrhizal productivity	NS	NS	NS	52.18**
Shoot Nitrogen	NS	13.35**	NS	NS
Shoot Phosphorus	NS	6.87**	5.67**	3.40*
Shoot Potassium	5.96**	4.03*	NS	6.10**
Root Nitrogen	NS	15.02**	3.3*	6.43**
Root Phosphorus	NS	10.31**	7.08**	3.04*
Root Potassium	NS	12.92**	4.31**	4.74**

*= Significant at $p < 0.05$ level;
** = Significant at $p < 0.01$ level

mycobionts was attributed to ectomycorrhizae formed by them during the growing season and variation in nutrient and water absorption. In the low fertile degraded soil, the active fungus of the mycorrhizal seedlings utilized considerably high amount of host photosynthate. The differences between mycorrhizal fungi in nourishment of the host were related to overall growth of the host and nutrient content.

The present study clearly indicates that seedlings with more quantity of ectomycorrhizae are able to rapidly regenerate numerous new lateral roots of greater length and thereby, utilize available water and nutrients more effectively. Reclamation and reforestation of the degraded sites in this area can be expedited by using pine seedlings tailored with mycorrhizae formed by native fungi like

S. luteus and *S. aurantium*, capable of growing under adverse conditions, which were physiologically and ecologically adapted to the adverse conditions.

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