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Effect of Ectomycorrhizal Development on Growth in Pine Seedlings

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Abstract: A study was carried out with the aim to evaluate the effect of different physical factors viz., temperature, light and relative humidity on the pine seedlings growth and ectomycorrhizal development. Moderate and high light intensities favoured mycorrhizal colonization and seedling growth. Pine seedlings inoculated with *Pisolithus tinctorius* compared to other fungi attained maximum growth. Survival of pine seedlings was higher under moderate light intensity than low and high light intensity. Seedling growth and mycorrhizal colonization was better at 25°C than 10°C. Variation in humidity did not show much difference in mycorrhizal colonization and seedling growth. However, seedling survival was greater at high than at low humidity. Pine seedlings showed best survival with ½ P level (46.153 mg Phosphorus kg^{-1} soil) of phosphorus at 25°C temperature and under moderate light intensity. Among the mycorrhizal fungi used *P. tinctorius* was the most effective endophyte and was followed by *Laccarai laccata*, *Rhizopogon luteolus* and *Collybia radicata* under various physical factors. On the basis of performance shown by *P. tinctorius*, *L. laccata* and *R. luteolus*, it may be stated that these fungi can be utilized for inoculation with pine seedlings in nursery under aforestation programme.

Key words: Ectomycorrhiza, *Pinus kesiya*, temperature, humidity, light

INTRODUCTION

The symbiotic association of ectomycorrhizal fungi with various plants and their effects on growth, biomass and plant productivity has been reported (Herrmann *et al*., 2004; Rao *et al*., 1996; Wallander *et al*., 2005). The most consistent and important nutritional effect of mycorrhizal fungi is to improve uptake of immobile nutrients such as P, Cu and Zn (Bucher, 2007; Taylor and Peterson, 2005). They are also very useful to plants that inherently lack morphological and physiological mechanisms for efficient P uptake (Anderson and Cairney, 2007; Nara, 2006). Assuming a radial distribution of fungal hyphae around roots, the volume of soil explored by mycorrhizae exceeds that explored by nonmycorrhizal roots a hundred fold (Leake *et al*., 2004; Wallander *et al*., 2005). Moreover, the much smaller diameter of fungal hyphae allows them to explore soil micro sites that are not accessible to roots. The degree to which mycorrhizal fungi enhance the nutrition and health of their host plants depends on many biotic and abiotic soil factors, as well as other environmental factors that influence the host, endophyte and their association. Physical factors like light intensity, temperature and humidity may also be important for the development of the mycorrhiza it self. Mycorrhizal fungi require additional energy from their host in the form of carbon compounds for their growth (Cheng *et al*., 2005; Grunze *et al*., 2004). Hence, light is an important factor in addition to N and P in influencing the amount of free soluble sugar in the roots which regulates the development of mycorrhizal infection. Intensity of mycorrhizal infection is influenced by soil nutrients. Thus, excess

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phosphate at high light intensity was found to reduce the development of mycorrhiza (Son and Smith, 1988). This study was made to evaluate mycorrhizal development in pine seedling roots in response to physical factors such as temperature, humidity and light intensity.

MATERIALS AND METHODS

Four fast-growing ectomycorrhizal fungi, *Collybia radicata*, *Laccaria laccata*, *Pisolithus tinctorius* and *Rhizopogon luteolus* were selected and cultured on modified Melin Norkran's (MMN) nutrient agar medium. Mycelium cultures of these fungi were used for inoculation of pine seedling roots under various environmental conditions. Pine seedlings were grown in sterilized sand from seeds, on attaining 2 cm in size, they were transferred to pots. To prepare soil amendments with different levels of P a mixture of sand and soil (1:1; w/w) was autoclaved at 1 kg cm^{-2} two times (24 h interval). The soil used had the following properties: pH, 5.5; organic carbon, 2.27%; total N, 0.12%; available P, 5 ppm. Four levels of available P (0P, ½P, 1P, 2P) were prepared using 30 kg P ha^{-1} as the 1P level. Amounts of P (as KH_2PO_4) were added to each pot to get the final level of P at the rate of 46.153 mg kg^{-1} soil for ½ P, 92.308 mg kg^{-1} for 1P, 184.615 mg kg^{-1} for 2P and no addition for the 0P control. Earthen pots (10×9.5 cm) were filled with the mixture (200 g pot^{-1}) in fourteen replicates for each fungal species under each light intensity, temperature, humidity and phosphorus level separately. Five pine seedlings were planted per pot and inoculated with 10 mL homogenized solution of fungal mycelium for each mycorrhizal fungus separately. The pots were watered regularly.

To study the effect of light intensity three levels i.e., 30000, 1000 and 500 lux (high, medium, low) were maintained in a net house. The different light intensities were adjusted by keeping sets of experimental units under direct sunlight, in a light chamber made of white cheese cloth and in a light chamber made of black cloth respectively. Two levels of temperature (10 and 25°C; 12 h light period) and a two levels of relative humidity (high, 90 to 100%; low, 50 to 65%) were maintained in a growth chamber. These levels of humidity were maintained at 25°C with a 12 h photoperiod. Ectomycorrhizal development was estimated following the procedure as outlined by Sharma (1981). Percentage ectomycorrhizal infection was determined with the help of following formula.

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

Survival of the mycorrhizal and non-mycorrhizal seedlings was observed for each treatment separately and subsequently percentage survivorship was calculated. The growth of seedlings was expressed in terms of shoot and root length. Fresh and dry weight of shoots and roots was also determined considering one of the indices for growth. Observations were recorded for all the parameters on 90-day-old pine seedlings. A statistical analysis of data was performed using Least Significance Difference (LSD).

RESULTS

Impacts of light intensities on shoot and root length as well as mycorrhizal colonization are shown in Table 1 and 3. Maximum shoot length of seedlings was recorded at moderate light intensity grown seedlings for all the treatments. Seedlings inoculated with *P. tinctorius* consisted more shoot length, fresh and dry weight among the mycorrhizal inoculated one under moderate and high light intensity. Seedling growth was extremely slow under low light intensity and Mycorrhizal fungal colonization was also very poor. There was not significant variation in shoot length, fresh and dry weight of seedlings inoculated with different mycorrhizal fungi at low light intensity. Profuse growth of needles was

Table 1: Impact of light intensities on the length and weight of shoots and roots of pine seedlings

Fungi and different doses of phosphorus	High light intensity						Moderate light intensity						Low light intensity					
	Lnt (cm)		Weight (mg)				Lnt (cm)		Weight (mg)				Lnt (cm)		Weight (mg)			
			Shoot		Root				Shoot		Root				Shoot		Root	
	SL	RL	FW	DW	FW	DW	SL	RL	FW	DW	FW	DW	SL	RL	FW	DW	FW	DW
C. radicata																		
½	14	20	512	238	104	26	24	9	610	119	89	10	7	3	26	12	12	4
1	15	23	556	260	180	43	25	10	656	206	103	15	8	4	31	14	12	4
2	12	23	655	325	195	45	21	10	760	271	143	23	10	4	33	15	15	4
L. laccata																		
½	13	20	425	257	150	34	31	9	759	150	75	8	6	3	28	10	10	3
1	17	22	610	259	192	44	32	9	775	231	81	11	6	3	31	14	12	5
2	21	23	780	346	212	46	32	10	790	270	192	24	7	3	30	13	14	4
P. tintorius																		
½	15	20	580	310	252	89	31	9	730	200	59	9	9	3	39	19	14	4
1	19	21	805	305	250	90	35	10	810	370	96	14	8	3	36	16	14	4
2	18	22	682	275	378	101	32	10	760	296	182	25	6	3	30	15	12	3
R. luteolus																		
½	15	22	670	280	207	52	26	8	710	295	63	11	8	5	34	15	11	3
1	16	24	630	305	218	59	28	9	730	337	83	13	10	3	35	15	11	3
2	20	26	763	342	243	81	32	11	807	360	163	22	7	4	33	14	17	5
Control	12	20	308	198	160	38	12	8	686	109	97	10	6	3	25	11	10	3
LSD (p = 0.05)	2.1	1.6	45.2	24.1	26.9	6.5	2.4	1.0	69.6	19.8	14.9	1.7	0.9	0.6	4.3	1.8	1.4	0.4

Lnt: Length, SL: Shoot Length, RL: Root Length, FW: Fresh Weight, DW: Dry Weight

noticed under moderate light intensity compare to low light intensity while dense clusters of needles were apparent on the terminal shoot at high light intensity. Fresh and dry weight of shoot were also higher under moderate light intensity than high light intensity with 2P level except in case of *P. tinctorius* inoculated seedlings where fresh and dry weight of shoot were found more under moderate light intensity with 1P level. Root length, fresh and dry weight of root was also affected by light intensities. Root length as well as fresh and dry weight of seedlings was higher at high intensity and followed by moderate light intensity (Table 2). Root growth was also poor at low light intensity. In general an enhanced of pine seedlings growth was observed with 2P level compared to 1P and ½ P levels when inoculated with ectomycorrhizal fungi. Relative growth of seedlings with 2P level was higher at moderate light intensity except in case of *P. tinctorius* inoculated seedlings those obtained higher growth at 1P level. Under low light intensity marked variation in the growth of pine seedlings at different levels of phosphorus was not observed.

Mycorrhizal colonization and growth of pine seedlings was found maximum at 25°C temperatures. Slow seedling growth was obtained at 10°C. Colonization of mycorrhizae was also slow at this temperature. Mycorrhizal colonization was higher in *P. tinctorius* inoculated seedlings under high and moderate light intensities. At low light intensity colonization was very poor by all the mycorrhizal fungi. The phosphorus level at ½ P was found most favourable to promote colonization of mycorrhiza and the growth of seedlings. Maximum shoot height was obtained by the seedlings with *P. tinctorius* under 1P level at 25°C. Minimum shoot height was obtained by seedlings inoculated with *R. luteolus* at this temperature under ½ P level. At 10°C temperature seedlings with *L. laccata* obtained highest percentage of mycorrhizal colonization and growth under ½ P level. It was followed by *R. luteolus* and *L. laccata* while, minimum seedling growth was obtained by seedlings with *P. tinctorius*. Root and shoot dry matter production was found affected by the temperature and in general growth of seedlings was better at 25°C compare to 10°C.

Table 2: Impact of temperature and humidity on the length and weight of shoots and roots of pine seedlings

	Temperature 10°C						Temperature 25°C					
			Weight (mg)						Weight (mg)			
Fungi and different doses of phosphorus	Lnt (cm)		Shoot		Root		Lnt (cm)		Shoot		Root	
	SL	RL	FW	DW	FW	DW	SL	RL	FW	DW	FW	DW
C. radicata												
½	9	23	215	50	160	25	13	15	397	76	183	26
1	8	10	222	52	115	20	12	16	360	72	196	30
2	7	12	180	43	100	18	12	17	382	70	144	20
L. laccata												
½	11	14	251	61	222	30	19	12	491	99	194	30
1	10	14	230	54	211	28	19	11	450	89	168	24
2	9	9	197	46	180	25	20	12	460	88	152	23
P. tintorius												
½	7	14	201	49	106	19	21	14	498	98	258	36
1	7	13	200	49	168	24	22	14	508	98	262	37
2	5	10	115	38	96	17	18	12	458	95	233	32
R. luteolus												
½	8	16	247	59	173	25	15	13	395	80	255	34
1	7	11	221	52	193	95	14	15	390	79	197	29
2	6	12	175	41	119	21	14	13	388	74	169	22
Control	5	17	110	38	97	15	10	11	286	63	122	13
LSD (p = 0.05)	0.8	2.6	18.2	7.2	15.9	4.2	1.7	2.1	33.2	9.2	21.2	3.7
	Low humidity						High humidity					
			Weight (mg)						Weight (mg)			
Fungi and different doses of phosphorus	Lnt (cm)		Shoot		Root		Lnt (cm)		Shoot		Root	
	SL	RL	FW	DW	FW	DW	SL	RL	FW	DW	FW	DW
C. radicata												
½	11	14	399	76	178	30	11	12	375	69	175	33
1	12	15	411	79	188	32	12	11	382	71	140	27
2	10	11	295	70	151	21	10	10	370	67	112	25
L. laccata												
½	11	15	390	74	195	27	11	14	371	70	233	36
1	10	19	370	72	260	34	10	16	366	66	270	38
2	8	17	265	60	210	32	9	12	353	65	180	29
P. tintorius												
½	12	14	399	79	210	28	12	13	390	74	189	26
1	11	12	430	83	185	26	11	12	310	58	172	24
2	11	10	410	81	166	21	11	12	292	52	199	27
R. luteolus												
½	11	12	420	79	217	22	12	13	394	70	200	29
1	10	12	350	69	211	21	9	15	309	63	208	30
2	9	10	261	57	160	17	10	12	325	67	192	26
Control	10	12	252	51	197	19	10	13	297	52	162	22
LSD (p = 0.05)	1.8	1.3	35.1	7.2	26.8	3.6	1.9	2.2	35.9	7.3	22.5	3.6

Lnt: Length, SL: Shoot Length, RL: Root Length, FW: Fresh Weight, DW: Dry Weight

At both levels of humidity mycorrhizal development and growth of seedlings was more or less similar. However, Seedlings with *P. tinctorius* attained maximum growth compared to others (Table 4). The ½ P level of phosphorus was found most suitable for the mycorrhizal development and the growth of seedlings at the both levels of humidity. 1P and 2P levels of phosphorus did not exhibit any significant variation both on the development of mycorrhiza and growth of seedlings. Maximum shoot and root dry matter was produced by *P. tinctorius* followed by *L. laccata* and *R. luteolus*.

Table 3: Impact of light intensities on the Ectomycorrhizal (Ect.) development and survival (Srv.) of seedlings

Fungi and doses of phosphorus	High		Moderate		Low	
	Ectomycorrhiza (%)	Survival (%)	Ectomycorrhiza (%)	Survival (%)	Ectomycorrhiza (%)	Survival (%)
C. radicata						
½	52	80	46	85	15	60
1	60	80	45	80	14	70
2	55	85	55	85	13	80
L. laccata						
½	60	85	51	85	18	60
1	65	85	59	90	17	65
2	69	90	50	90	12	70
P. tintorius						
½	63	90	68	95	19	60
1	62	90	68	95	17	70
2	65	95	75	100	13	70
R. luteolus						
½	51	75	47	80	15	80
1	54	80	48	80	14	75
2	50	85	50	85	11	75
Control	0	36	0	40	0	20
LSD ($p = 0.05$)	6.5	16.3	7.4	11.3	3.4	6.1

Table 4: Impact of temperature and humidity on the ectomycorrhizal (Ect.) development and survival (Srv.) of seedlings

Fungi and doses of phosphorus	10°C		25°C		Low humidity		High humidity	
	Ectomy corrhiza (%)	Survival (%)	Ectomy corrhiza (%)	Survival (%)	Ectomy corrhiza (%)	Survival (%)	Ectomy corrhiza (%)	Survival (%)
C. radicata								
½	40	100	45	100	39	85	45	100
1	42	100	42	100	32	85	38	100
2	24	67	32	67	27	60	28	80
L. laccata								
½	50	100	62	100	43	80	50	100
1	41	100	55	100	38	80	45	90
2	29	80	31	67	24	50	31	70
P. tintorius								
½	51	100	63	100	55	90	55	100
1	43	100	68	80	49	90	51	100
2	30	100	61	100	28	60	32	70
R. luteolus								
½	30	100	52	100	38	90	40	100
1	28	90	58	100	35	85	35	95
2	22	80	50	100	32	60	25	80
Control	0	60	0	35	0	50	0	65
LSD ($p = 0.05$)	4.6	5.7	7.4	11.5	6.8	5.9	4.7	9.2

Survival of seedlings was higher under moderate light intensity than high light intensity. Lowest rate of seedling survival was obtained under low light intensity. Different levels of phosphorus did not affect the survival of pine seedlings significantly. Maximum survival percent was noticed with *P. tinctorius* which was followed by *L. laccata* inoculated seedlings. Minimum survival percent was noticed in non-mycorrhizal seedlings under all the light intensities. At different temperatures survivality of seedlings was affected by the levels of soil phosphorus. Survival of seedlings was found highest in ½ P level and lowest was obtained at 2P level. However minimum survival percentage was observed in non-mycorrhizal seedlings at all the levels of phosphorus. Low level of humidity decreased the survivorship of the seedlings compared to high level. ½ P level of the soil phosphorus was found to enhance the survivorship of seedlings in this case also. Survivorship was noticed minimum with *L. laccata* and maximum with *P. tinctorius* at low level of humidity while survivorship was not found significantly varied with high level.

DISCUSSION

Higher rate of mycorrhizal development at high light intensity may be attributed to the increased amount of photosynthate available to the mycorrhizal fungi. Regulation of soluble sugars in the root tissue of the host plant may be influenced by the light intensity which promoted better mycorrhizal development under high light intensity than at low light regime. The hypothesis on the regulation of carbon energy in heterotrophs due to light has also been supported by other workers (Son and Smith, 1988; Choi *et al*., 2005). Studies revealed that active photosynthesis was necessary for the development of mycorrhizae only after the primary leaves are developed (Herrmann *et al*., 2004; Nara, 2006). Considerably lower levels of colonization under low light intensity have also been attributed to the slow rate of photosynthate availability to the mycorrhizal fungi which was directly proportional to the amount of soluble sugars in the root tissue. Less of mycorrhizal infection under low light intensity may be due to inability of the fungal auxins to induce the specific physiological and metabolic changes in the roots which are required for establishment of the symbiotic relationship. Less mycorrhizal at low light intensity can therefore, be coupled with deficiency of soluble sugars and auxins required to induce the changes in morphology of roots (Karoliina *et al*., 2005). Different levels of phosphorus have also not induced the development of mycorrhizae probably due to limited supply of carbon especially at low irradiation. However, better mycorrhizal development with pine seedlings at ½ P level under moderate light intensity suggested that some amendment of low fertile soil was essential to improve the efficiency of mycorrhizal fungi. Tester *et al*. (1985) have also reported low uptake under low light intensity. Maximum colonization by mycorrhizal fungi and growth of pine seedlings at 25°C showed adaptability of mycobionts. Bowen (1970) correlated the catalyst of root metabolism and exudation to the development of mycorrhizae at 25°C than 10°C which might have produced insufficient root exudates for the colonization. However, colonization process may also be influenced by different fungal species (Marx *et al*., 1977). Dixon *et al*. (1981) stated that influence of temperature on the development of mycorrhizae vary from species to species. Temperature can have a profound effect on the growth of certain mycorrhizae. Optimum temperatures for mycelial growth lie between 18 and 27°C for the majority of fungal species. No significant difference in colonization and growth of pine seedlings was found at the two levels of relative humidity. The possible reasons for this may be either due to a less difference between the high (70-100%) and low (50-65%) levels of relative humidity. Application of double dose (2P) of phosphorus to the seedlings under high light intensity enhances the mycorrhizal colonization as well as the seedling growth which has contradicted the findings of Hacskaylo and snow (1959). Who were of the opinion that application of fertilizers above moderate level reduced the mycorrhizal development under high light intensity. However, exact interpretation of these results may be obtained only after getting his light exposure data. In environment with low nutrient concentrations, plants are stressed by the lack of adequate nutrients and they survive by stress tolerance mechanism but an environment with more nutrients has the potential to produce more plant biomass as plants grow (Grime, 1979). Mycorrhizal colonization was slightly increased at ½ P level of phosphorus than the 1P under moderate light intensity which suggest that better carbon supply to mycorrhizal fungi at a threshold level of phosphorus may be most suitable for maintaining the phosphorus uptake. There are critical ranges of soil solution phosphorus concentration at which the host and fungus association is truly mutualistic (Fitter, 2006). Low phosphorus uptake by mycorrhizal seedlings under low light intensity has been reported by some workers.

Mycorrhizal fungi enhance the survival and growth rate of seedlings under different climatic conditions (Buscot *et al*., 2000). In the present study, seedlings with *P. tinctorius* produced marked growth compared to others. Similar superiority of this mycorrhizal fungus has also been noticed elsewhere (Marx *et al*., 1977). Karoliina *et al*. (2000) and Rao *et al*. (1999) supported the findings that certain species of fungal symbionts exert more beneficial effect than others. It may, therefore, be suggested that *P. tinctorius, L. laccata* and *R. luteolus* can be exploited in reforestation programme; however, few more trials regarding their field performance are required as they may show some variability in inducing growth and nutrient uptake in pine seedlings in different field condition.

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