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Performance of *Pinus Roxburghii* Inoculated with Pure Culture of Four Indigenous Ectomycorrhizal Fungi

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Abstract
Many ectomycorrhizal fungi were isolated from the spores and sporocarps collected from different pine forests of Nepal. Among them, *Astraeus hygrometricus*, *Boletus* sp., *Lycoperdon pyriforme* and *Pisolithus arrijus* were compared for their effectiveness under natural field conditions. Many of them were subjected for pure culture synthesis and selected from the pot culture experiment. Four fungi were inoculated in pine nursery (*Pinus roxburghii*) for their performance at two sites, Godawari and Chalnakhel. Their performance were observed after six, nine and twelve months of transplantation. The results revealed that inoculated seedlings attained better shoot height and had better root length than their non mycorrhizal counterparts. More lateral and mycorrhizal roots, more shoot weight and more root weight than non inoculated ones. The inoculated transplants had higher survival rate in the field as compared to those which were not inoculated. Among the above fungi, *Pisolithus arrijus* performed better as regards to growth and survival of seedlings.

Key words : *Asterius hygrometricus, Boletus* sp., *Lycoperdon pyriforme, Pisolithus arrijus*

Introduction
Chir pine (*Pinus roxburghii*) is an important multipurpose species which establishes itself well on new clearings, landslides and in eroded areas (Mohan & Puri. 1956) and has been successful in checking erosion in areas where grazing is no controlled (Shrestha 1975).

The ectomycorrhizal (EM) fungi help the plants in their nutrition by facilitating the absorption of mineral nutrients. The important role of mycorrhiza plays as regards to the growth and development of plant species has already been reviewed widely (Melin & Nilsson 1958, Mosse *et al.* 1981). Pines cannot grow without mycorrhizae (Mikola & Gaillard 1978). In many countries the use of natural mycorrhizal fungi in the raising of pine seedlings has still been a common practice (Semakhanova 1962, Mikola 1970, Bakshi 1980). These measures have been and are still adopted in Nepal too. This method may carry diseases and pests and is erratic. Mycorrhiza formation is required for the survival and growth of many forest tree species (Marx & Keny 1991, Smith & Read 1997). The fungi increase the uptake of nutrients especially in infertile soils by extending the absorbing surface of the roots (Hatch 1937). The hyphae of mycorrhizal fungi are widely distributed through the soil and contribute significantly towards nutrient uptake and cycling in the soil and in many forest ecosystems (Brundrett, *et al.* 1996). They also provide resistance to plants against adverse soil conditions and some root pathogens (Zak 1964, Bakshi & Kumar 1968, Marx 1989, Marx & Cavey 1969). The survival and success of plantation has been low probably due to the lack of proper EM fungal inoculation. So, mycorrhizal associations are considered as key factors in forest ecosystem. Therefore the studies were initiated using pure cultures of ectomycorrhizal fungi for inoculation. This has been successfully used on field scale by a number of investigators (Moser 1961, Hacskaylo & Vazzo 1967, Marx & Bryan 1975) and seedling quality field performance is largely governed by processes occurring under the soil surface in the root zone of seedlings (Charles *et al.* 1987).

There is a growing concern in Nepal about the serious degradation in the quality of the forests and significant reduction in the area of the forest land. So, Nepal immediately needs intensive reforestation activities.

Methodology

Sporocarps were collected from pine forests of three districts (Dadeldhura, Makwanpur and Palpa) of pine forest of Nepal in spring and were identified according to Adhikari (2000) and Brundrett *et al.* (1996). Among different types of ectomycorrhizal fungi, *Astraeus hygrometricus*, *Boletus* sp., *Lycoperdon pyriforme* and *Pisolithus arrijus* were selected from pot experiment for field trials according to Shrestha (1999a). The selection of mycorrhizal fungi is extremely important for pure culture inoculation on large scale Shrestha (1999b).

Description of the Experimental Sites:

Two sites namely Chalnakhel and Godawari. were selected for field trials. Chalnakhel lies in Kathmandu district while Godawori in Lalitpur district.

The altitude of Godawori is 1515 m. The mean maximum temperature ranges between 28 °C to 18 °C and minimum temperature is between 16 °C to -2 °C. Total rainfall recorded at Godawori is 1863.5 mm. Godawori valley, situated at the foot hills of Phulchoki, is more humid and cooler than Kathmandu valley during summer and winter months. The deviation in rainfall could be attributed to the favourable arrangement of the folds of Phulchoki mountain for bringing more precipitation down to Godawori valley. Monsoon period starting from early June and ending by late September has over 80% of the total rainfall. Few spells of rains occur during winter from January to February. Godawori valley commonly experiences frosts in early December to February. In this place *Pinus roxburghii* and *Pinus wallichiana* occur.

The altitude of Chalnakhel is 1288 m. It is characterized by typical monsoon climate with rainy summer and dry winter. The mean maximum temperature ranges between 32 °C to 20 °C and the mean minimum temperature is between 18 °C to 0 °C. The total rainfall recorded at Chalnakhel is 1301.9 mm. While at Kathmandu valley,frosting is less common. In Chalnakhel, *Pinus roxburghii* and *Pinus patula* occur due to their altitude difference.

Chemical analysis . Chemical analysis of the soil samples from Chalnakhel and Godawori were performed to check their pH, nitrogen, phosphorus, potassium and organic matter. Division of Soil Science, Nepal Agriculture Research Council (NARC) performed these analysis according to the following methods:

- Total Nitrogen (N) by Kjeldhal method
- Available Phosphorus (P) by modified Olsen's Bicarbonate method
- Available Potassium (K) by Flame Photometer method .

Experiment 1

Hundred seedlings of *P. roxburghii* inoculated with 2mg of each of *Asterius hygrometricus*, *Boletus* sp. *Lycoperdon pyriforme*, *Pisolithus arrijus* and natural mycorrhiza were mixed with soil seperately and then were kept in polypropylene bags. The soil inoculum having natural mycorrhiza was inoculated in 1:10 ratio. The seeds of *P. roxburghii* were planted in polypropylene bags. Seedlings that had been aseptically raised in the glass house and were allowed to develop until they mostly attained a height of 10-30 cm and then planted out in the field. All the seedlings were numbered so that their field performance could be followed on each site.

Results

The seedlings were harvested after seven months and examined for shoot height , root length, mycorrhizal root and oven dry weight of shoots and roots. Results have been shown in Table 1.

Table 1(a). Effect of different ectomycorrhizal fungi within seven months

Treatments	Shoot height (cm)	Root length (cm)	Lateral root per plants	Mycorhizal root (%)	Oven dry wt. per plant	
					shoot (g)	root (g)
T1	35.00	23.5	15	38	0.712	0.389
T2	32.00	23.1	13	35	0.659	0.208
T3	33.50	24.5	14	36	0.768	0.301
T4	36.00	24.8	16	41	0.894	0.435
T5	22.50	18.2	10	31.5	0.592	0.135

T1 = *Astraeus hygrometricus*, T2 = *Boletus* sp., T3 = *Lycoperdon pyriforme*, T4 = *Pisolithus arrijus*, T5 = Natural mycorhiza

In case of *P. roxburghii* seedlings, *Pisolithus arrijus* fungi as inoculum had a significantly more height growth, mycorrhizal root and dry weight of shoot and root compared to those *Astraeus hygrometricus*, *Boletus* sp. *Lycoperdon pyrforme*, *Pisolithus arrijus* and natural mycorrhiza

Anova Table

Table 1(b). Significance value (f) of various treatments between column and row.

Source of Variation	Sum of square	Degree of freedom	Means square	Ratio of variance	F test
Between column	16004.9	5	3200.98	3200.98	
Between	2.7	11	0.24545	0.35099	9121.96
Residual	19.3	55	0.35099	0.35099	
				0.24545	1.42998
Between column	13807.128	5	2761.4256	2761.4256	
Between	4.07	11	0.37	63.025	43.81
Residual	3466.42	55	63.025	63.025	
				0.37	170.337
Between column	14820.78	5	2964.156	2964.156	
Between	3.58	11	0.3254	0.4078	7268.65
Residual	22.43	55	0.4078	0.4078	
				0.3254	1.2532
Between column	17785.364	5	3557.072	3557.072	
Between	9.087	11	0.826	0.4968	7159.96
Residual	27.326	55	0.4968	0.826	
				0.4968	1.6626
Between column	9441.787	5	1888.3574	1888.3574	
Between	11.268	11	1.02436	0.4391	4300.51
Residual	24.153	55	0.4391	1.02436	
				0.4391	2.3328

Significance level of 0.995, the critical value of F is 1.9174, 2.3683 (by taking the value of F corresponding to 5, 11 and 60 degree of freedom) as there is no value of 5.5 in denominator the closest value for 60 considered. In the above calculation, it has shown two different type column wise and row wise. In the above calculation, it has shown two different type column wise and row wise. In the above calculation all the column wise, the calculated value of X^2 (all treatments) are more than the tabulated value. All the treatments are considered to be poor in other the calculated as row wise.

Among these calculated value of X^2 (all treatment) 4 is best due to lesser tabulated value.

$V_1 = (6 - 1) = 5$

$V_1 = (12 - 1) = 11$

$V_2 = [72 - (5 + 11) = 55$

$V_1 = 5$ and V2 = 55 nearest 60 the tabulated value = 2.37

$V_1 = 11$ (nearest 12) and $V_2 = 55$ nearest to 60 the tabulated value = 1.92

Experiment 2

In this experiment only 50 seedlings of *P. roxburghii* were used and the same mycorrhizae as in experiment 1 were used and rest of the method was also same.

The height of each plant was recorded on the same day after planting in May 1999. All plots were regularly weeded by hand in accordance with the usual practice in pine plantation (Momoh & Gbadegesin 1980). The soil samples of the two sites were analysed for pH, Nitrogen, Phosphorus, Potassium and organic matter before planting (Table 2).

Table 2. Physico-chemical properties of the soil samples collected from the study sites.

Sampling Area	pH	N %	P_2O_5 kg/ha	K_2O kg/ha	OM %
Chalnakhel	4.4	0.201	33.48	824	6.16
Godawari	4.9	0.138	18.26	824	4.15

In above table it has shown that the chemical properties of soil (N, P and organic matter) of

Chalnakhel has shown higher than that of Godawori soil but K is same in both sites and pH is acidic in Chalnakhel than that of Godawori.

The experiment was done for twelve months from May 1999 to May 2000. The first assessment of the experiments were done on same day of planting. The second assessment was done in November, 1999. The third and fourth assessments were done in February 2000 and May 2000. Within this one year period, the plants had survived one rainy season and one dry season in the field and had a total of twelve months in the field.

The results clearly showed that the survival rate of the plants was higher on inoculated with *P. arrijus* than *A. hygrometricus, L. pyriforme, Boletus* sp. and natural mycorrhizal soil inoculum in Chalnakhel than in Godawori (Fig.1and 2). Similarly, *P. arrijus* showed better performance than the other species of mycorrhizae at both sites. All the three tested mycorrhizae performed better in Chalnakhel than in Godawori (Fig. 3 and 4).

Discussion

In this present study *P. arrijus* was found to lead to better survival and better growth of *P. roxburghii* in both sites than *A. hygrometricus, L. pyriforme, Boletus*

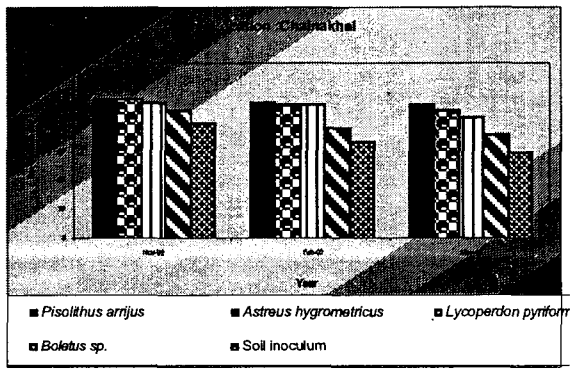

Fig. 1. *P. roxburghii* inoculated with *P. arrijus*

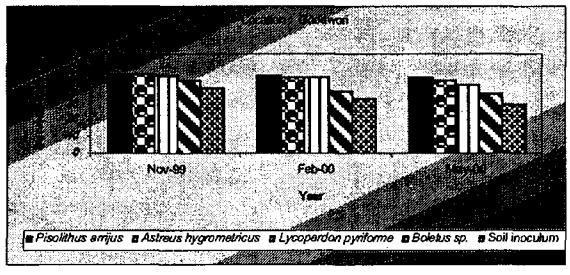

Fig. 2. *P. roxburghii* inoculated with *P. arrijus*

sp. and natural mycorrhiza which was also reported by Trappe (1962b). *Boletus* sp.has shown a

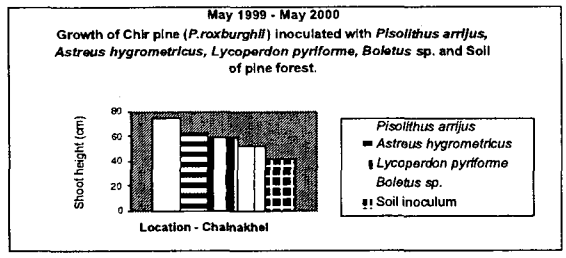

Fig. 3. *Pinus roxburghii* inoculated with *P. arrijus*

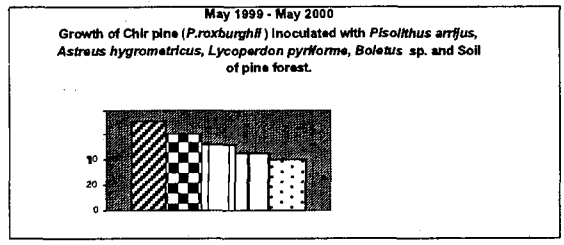

Fig. 4. *P. roxburghii* inoculated with *P. arrijus*

mycorrhizal fungus (Bakshi 1974).

Bery and Marx (1976) also discovered that the fungus gave better growth of pinus taeda than other naturally occuring fungi in the southern United States. Marx (1990) also found that ectomycorrhizal development on seedlings was encountered among nurseries and development of ectomycorrzae depends on the types of the soil also. In the present study also it is clearly seen that all ectomycorrhizae grew better in Chalnakhel than in the soil of Godawori nursery due to higher phosphorus, nitrogen and organic matter contained in the soil of Chalnakhel than in the soil of Godawori nursery. The quality of the soil for plants as well as for the mycorrhizae has also been reported by (Shrestha 2000). Similar results were reported by Gray & Gerdman 1973, Jackson *et al.* 1972 in which mycorrhizal plants have been found to thrive well and grow much better than the non-mycorrhizal plants in soils with low quantities of available phosphorus. Marx and Kenny (1991) have reported that the trees of many species including pines will not grow and develop normally without fungi. Marx & Cordell (1988) have found that pine seedlings with abundant *Pisolithus* ectomycorrhizae formed in the nursery survived and grew better after out planting than seedlings with natural occurring fungi. The present study has also found similar results. *P. arrijus* show better performance in the field. By exploring such fungi we can substitute chemical fertilizers and can use them for rehabilitation of the forest as well as for biological control.

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