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Effect of mycorrhizal inoculation on morphological characteristics of seedlings of Himalayan Cypress (*Cupressus torulosa* Don) provenances in Garhwal Himalayas

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ABSTRACT : Significant variations in growth of mycorrhizal and non-mycorrhizal seedlings i.e., shoot length, collar diameter, number of needles, root length and root-shoot dry weight etc. were noticed in different provenances of *C. torulosa* under nursery conditions. The maximum total biomass after 6 months growth in mycorrhizal seedlings was 0.075 g / seedling, which was considerably higher than that of non-mycorrhizal seedlings (0.051 g/ seedling). In mycorrhizal seedlings of *C. torulosa*, a highly significant ($p < 0.001$) correlation was observed between number of needles *vs.* shoot biomass ($r = 0.85$) and root biomass ($r = 0.70$). From the present study it is clear that inoculation with mycorrhizae is beneficial for the growth and survival of *C. torulosa* seedlings and hence recommended for successful afforestation programmes.

Key words: Biomass, Correlation coefficient, Feeder roots, Inoculation, Seedlings growth.

Mycorrhizae are a composite association between fungus and the root of plants, which are generally accepted as necessary for good survival and growth of forests. This is especially true when afforestation of various species is done (Garcia *et al.*, 2000 and Caravaca *et al.* 2002). Mycorrhizae are formed by most species of angiosperms, gymnosperms and pteriodophytes (Rudawska *et al.* 2001). They exist in the interface of the soil and root and by absorption or release of certain ions they may change the pH (Deirdre and Derek 1999). However, they may also alter the soil environment and the availability of chemical compounds in the root region (Smith and Read 1997). The immediate source of mycorrhizae is the soil solution with which both hyphal surfaces and roots surfaces are in contact. The hyphae typically ramify through the soil producing a large exploited volume for mycorrhizal plants, which varies in importance, depending upon the nutrients, their concentration and mobility in the soil and demand for uptake by the plants (Cathy 2001). The degree of mycorrhizal dependency varies with environmental conditions (Colpaert and Van Tichelen 1996; Srivastava *et al.* 1996), while dependence of plants on mycorrhizae is for uptake of nutrients and other soil derived substances (Ouahmane *et al.* 2006).

During the process of deforestation, the soil becomes infertile and it is difficult for plants to establish themselves, without an appropriate mycosymbiont (Rhodes 1980). It is now a well accepted fact that in a natural ecosystem almost all the plants are mycorrhizal. Mukerji and Sharma (1996) have reported seven different types of mycorrhizal associations, of which the most commonly reported, were ectomycorrhiza and endomycorrhiza. Ectomycorrhizal developments are mainly reported from temperate regions formed by fungi belonging to Ascomycotina and Basidiomycotina, whereas Endomycorrhiza or Vesicular Arbuscular Mycorrhiza (VAM) are formed by fungi belonging to order Glomales of Zygomycotina (Harley and Smith 1983; Mukerji 1996). The role of mycorrhizae in rejuvenation and reclamation of wastelands deficient in nutrients and organic matter, has been successfully explored (Miyasaka *et al.* 2003). Mycorrhizologists in Europe and USA have demonstrated that the performance of seedlings inoculated with specific mycorrhizal fungus is better than that of normally raised seedlings. The importance of ectomycorrhizae in coniferous forests is well recognised and some implications of forest management to the maintenance of ectomycorrhizal diversity and function have also been reported by Brundrett (1991) and Molina *et al.* (1992).

MATERIAL AND METHODS

The study was conducted to investigate the germination behaviour of mycorrhizal and non-mycorrhizal seedlings of *C. torulosa* after collecting seeds from five different seed sources, distributed in three districts i.e., Pauri, Chamoli and Tehri of Garhwal Himalaya (Latitude 29°26' to 31°28'N and longitude 77°49' to 80°06'E). The rainfall in the region varied from 1336 mm to 1892 mm (from June to September), and is represented by sub-tropical to temperate climates. Detailed geographical attributes of the locations of various provenances are given in Table 1.

Table 1. *Geographical and meteorological description of the seed sources of C. torulosa.*

Provenance	District	Altitude (m)	Latitude (N)	Longitude (E)	Temperature (°C)		Mean annual rainfall (mm)
					Min.	Max.	
Gwaldam	Chamoli	1960	30°20'	79°34'	-0.53	29.6	1336.00
Mandal	Chamoli	1768	30°23'	79°15'	-0.60	28.9	1750.00
New Tehri	Tehri	1675	30°33'	78°29'	1.23	29.7	1632.00
Pauri	Pauri	1660	30°9'	78°48'	-0.48	26.30	1792.00
Tapovan	Chamoli	2798	30°31'	79°36'	-0.84	24.5	1892.00

The effect of mycorrhizal inoculation on the growth and development of different seed sources (five provenances of *C. torulosa*) was monitored regularly for six months. The soil used for this experiment was collected from a barren area without vegetation cover. It was crushed, passed through a 2 mm sieve and autoclaved at 140^{0}C for 40 minutes to eliminate native micro-organisms. After autoclaving the soil was filled into earthen pots. These pots were inoculated with ectomycorrhizae fungi, collected from the *C. torulosa* forests, which were cultured in an agar medium and analysed under a microscope as per Ingleby *et al.* (1990). Half of the filled pots were subjected to inoculation with ectomycorrhizal fungi and remaining half were treated as control. For observing the effect of mycorrhizae on the growth and development of seedlings of *C. torulosa*, another experiment consisting of 20 seedlings of each provenance were sown in earthen pots, containing soil, taken out from an undisturbed forest of *C. torulosa*. After 3 weeks of germination the pots were maintained with ten seedlings of each provenance. The inoculation was again supplemented by adding 3g of fresh ectomycorrhizal inoculum, consisting of short roots and infected soil.

Observations were recorded at two months interval on various growth parameters. Observations with respects to seedlings height, circumference and number of needles of mycorrhizal and non-mycorrhizal seedlings were also taken. Data pertaining to length of tap roots and biomass of seedlings were recorded by the destructive method after washing the root system with clean water. Shoot portion of each of the selected seedling was cut at the collar height and fresh weight of shoot and root was recorded separately. They were further dried inside an oven at 80^0 till a constant weight, for determination of the seedlings biomass on dry weight basis. The seedlings were harvested and the shoot length, root length (as per Tennant 1975) and total seedlings dry mass (oven drying at 80^{0}C for 24 hours) were determined. Mycorrhizal association was confirmed under the microscope. The mycorrhizal formation in the root zone was evaluated simultaneously by counting the number of dichotomous branching formed by the lateral roots.

Table 2. *Variation in morphological characters and biomass production in mycorrhizal and non- mycorrhizal seedlings of Cupressus torulosa at nursery stage.*

Provenance	Shoot length (cm)		Collar diameter (cm)		No. of needles		Shoot biomass (g/seedling)		Root length (cm)		No. of lateral roots		Root biomass (g/seedling)		Total biomass (g/seedling)		Root-shoot ratio	
	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM
After two months																		
Gwaldam	4.4	3.6	0.20	0.16	34.0	30.4	0.016	0.009	4.4	3.8	7.0	7.0	0.010	0.004	0.026	0.013	0.62	0.44
Mandal	5.2	4.6	0.21	0.17	34.2	35.0	0.010	0.009	5.8	4.9	5.4	14.0	0.009	0.008	0.019	0.017	0.90	0.88
New Tehri	5.2	3.6	0.22	0.16	36.6	35.0	0.016	0.008	4.9	4.6	7.2	13.0	0.009	0.007	0.025	0.015	0.56	0.87
Pauri	5.5	5.3	0.22	0.19	39.4	45.6	0.019	0.008	2.5	2.3	8.0	5.7	0.008	0.003	0.027	0.011	0.42	0.37
Tapovan	7.1	6.0	0.25	0.23	54.8	49.2	0.021	0.013	4.8	4.8	9.0	8.6	0.017	0.007	0.038	0.020	0.80	0.53
After four months																		
Gwaldam	5.2	4.3	0.23	0.18	43.0	37.6	0.024	0.012	5.4	6.4	10.8	29.6	0.012	0.006	0.036	0.018	0.50	0.50
Mandal	6.0	5.2	0.24	0.21	40.2	48.2	0.014	0.012	6.6	6.3	9.8	25.6	0.012	0.010	0.026	0.022	0.75	0.83
New Tehri	6.3	4.3	0.24	0.18	43.6	45.6	0.013	0.011	5.5	5.1	12.8	21.0	0.013	0.010	0.026	0.021	1.00	0.90
Pauri	6.8	6.0	0.25	0.23	43.2	46.8	0.025	0.014	4.3	3.8	16.8	8.4	0.012	0.004	0.037	0.018	0.48	0.28
Tapovan	8.3	6.7	0.28	0.26	63.2	53.4	0.027	0.018	5.8	5.0	24.0	25.8	0.017	0.013	0.044	0.031	0.62	0.72
After six months																		
Gwaldam	6.6	5.9	0.24	0.20	50.0	41.6	0.037	0.027	7.0	7.1	17.4	36.6	0.021	0.014	0.058	0.041	0.56	0.51
Mandal	8.0	7.2	0.28	0.30	47.8	54.2	0.039	0.031	8.3	7.4	17.6	34.4	0.024	0.015	0.063	0.046	0.61	0.48
New Tehri	8.2	6.3	0.30	0.21	49.4	56.8	0.041	0.029	8.2	7.9	16.2	29.0	0.027	0.019	0.068	0.048	0.65	0.65
Pauri	7.3	6.4	0.27	0.26	60.4	53.6	0.040	0.028	9.0	5.5	26.0	16.2	0.026	0.013	0.066	0.041	0.65	0.46
Tapovan	9.6	8.4	0.35	0.32	74.0	60.8	0.046	0.034	8.5	5.9	28.0	32.2	0.029	0.017	0.075	0.051	0.63	0.50

M = Mycorrhizal seedlings, NM= Non - Mycorrhizal seedlings

RESULTS AND DISCUSSION

The maximum height of mycorrhizal and non-mycorrhizal seedlings after 2 months was registered as 7.1 cm and 6.0 cm respectively, which after 4 months were 8.3 cm and 6.7 cm and after 6 months, 9.6 cm and 8.4 cm respectively for Tapovan provenance (Table 2). The number of needles was also recorded to be maximum for Tapovan provenance. Similarly the root length for mycorrhizal and non-mycorrhizal seedlings after 2 months was recorded as 5.8 cm and 4.9 cm for Mandal provenance, which after 4 months were 6.6 cm and 6.3 cm. After six months, the maximum root length in mycorrhizal seedlings was recorded in seedlings collected from Pauri (9.0 cm) and the minimum (7.0 cm) from Gwaldam. In non-mycorrhizal seedlings, the maximum root length was recorded in seedlings collected from New Tehri (7.9 cm) & minimum in those collected from Pauri. These results indicate the variation between mycorrhizal and non-mycorrhizal seedlings (Table 2).

The results pertaining to dry weight/ biomass parameters viz. shoot biomass; root biomass and total seedlings biomass revealed significant variations among the mycorrhizal and non-mycorrhizal seedlings of various provenances. The maximum values of all these biomass parameters after six months of age were recorded for Tapovan provenance. An increment of 47% to 41% (in shoot biomass), 41% to 23% (in root biomass) and 41% to 39% (in total seeding biomass) was recorded respectively in the growth of mycorrhizal and non-mycorrhizal seedlings after six months as compared to 4 months (Table 2).

In the mycorrhizal seedlings of *Cupressus torulosa*, a positive significant correlation was observed between shoot length vs. shoot biomass (r = 0.94), root biomass (r = 0.86), number of needles (r = 0.66), whereas a negative correlation was recorded between shoot length vs. number of lateral roots (r = -0.27). Collar diameter showed a significant positive correlation with root length (r = 0.43) and number of lateral roots (r = 0.49). However a negative correlation with number of needles (r = -0.59) and root biomass (r = -0.43) was observed. A highly significant ($p<0.001$) correlation was recorded between number of needles vs. shoot biomass (r = 0.85) and root biomass (r = 0.70); between shoot biomass vs. root biomass

Table 3. *Correlation (r) among average non-mycorrhizal and mycorrhizal seedlings traits of various provenances of Cupressus torulosa.*

Variables	01	02	03	04	05	06	07	08	09	10	11	12	13
Seedling length (Non-mycorrhizae)	-												
Seedling length (Mycorrhizae)	0.90	-											
Collar diameter (Non-mycorrhizae)	-0.45	-0.15	-										
Collar diameter (Mycorrhizae)	-0.49	-0.59	0.32	-									
No. of needles (Non-mycorrhizae)	0.73	0.89	0.22	-0.26	-								
No. of needles (Mycorrhizae)	0.73	0.66	-0.11	-0.59	0.55	-							
Root length (Non-mycorrhizae)	-0.23	-0.01	0.06	0.21	0.03	-0.75	-						
Root length (Mycorrhizae)	0.09	0.29	0.38	0.43	0.56	-0.35	0.70						
No. of lateral roots (Non-mycorrhizae)	0.17	0.10	-0.80	-0.41	-0.27	-0.25	0.42	-0.16	-				
No. of lateral roots (Mycorrhizae)	-0.13	-0.27	0.47	0.49	0.01	0.29	-0.66	-0.12	-0.88	-			
Shoot biomass (Non-mycorrhizae)	0.98	0.94	-0.42	-0.51	0.77	0.65	-0.07	0.20	0.24	-0.26	-		
Shoot biomass (Mycorrhizae)	0.84	0.94	-0.02	-0.67	0.85	0.85	-0.29	0.06	-0.10	-0.05	0.84	-	
Root biomass (Non-mycorrhizae)	0.29	0.64	0.15	-0.57	0.57	0.05	0.55	0.43	0.28	-0.67	0.43	0.53	-
Root biomass (Mycorrhizae)	0.67	0.86	0.32	-0.43	0.94	0.70	-0.16	0.34	-0.37	0.10	0.69	0.92	0.57

(r = 0.92); whereas, negative significant correlation was observed between number of needles vs. root length (r = -0.35) (Table 3).

Results pertaining to growth (plant height, number of needles, root length and number of lateral roots) and biomass production (shoot, root and total seedling dry weight) have shown that the values of these parameters differ significantly among the provenances of *C. torulosa* (Table 4). It was evident from the results that there was improved growth in the mycorrhizal seedlings as compared to non-mycorrhizal seedlings, throughout the observation period. All the mycorrhizal seedlings had number of feeder roots, whereas the roots of non-mycorrhizal seedlings were shorter. The growth of mycorrhizal seedlings might have been influenced to some extent by seed sources (Wright and Ching 1962). There is evidence of influence of seed origin on mycorrhizae formation in *Pinus* spp. as reported earlier by Lundeberg (1968) and Marx and Bryan (1971).

The effectiveness of the mycorrhizal fungi to stimulate plant growth in the field is detected after a relatively long period of time, therefore long term studies are necessary to determine the benefit of inoculation effectively (Pera *et al.* 1999). It is evident from the present results that the Tapovan seed source gave the best performance in terms of growth and biomass production amongst the seedlings of *Cupressus torulosa* under ordinary nursery conditions. It is worth to mention here that the Tapovan area is situated in the core zone of Nanda Devi Biosphere reserve and therefore, is free from biotic disturbances. It has been reported earlier also that the seedlings of different provenances, when grown under common nursery environmental conditions, often displayed different patterns of shoot growth (Dormling 1979; Rehfeldt and Wycoff 1981).

The recovery of soil structural stability is a precondition for successful afforestation programme in semiarid environments (Caravaca *et al.* 2002).Soil amendment with organic materials prior to afforestation as well as the use of mycorrhizal inoculation are advisable practices in afforestation of semiarid areas (Garcia *et al.* 2000). The effect of soil inoculation with an arbuscular mycorrhizal fungus, *Glomus fasciculatum*, isolated from gypsum mine spoil, on dry matter production, nutrient uptake rhizosphere microbial activity in five tree species growing in gypsum mine spoil was studied by Rao and Tak (2001). Dry matter production was significantly enhanced upon inoculation to varying degrees depending on tree species. It was also noticed that arbuscular mycorrhizal fungi were important symbionts for use in revegetation of gypsum mine spoils. Rincon *et al.* (2006) have indicated two techniques which are to be used in soil restoration: (i) inoculation with selected microbial strains, and (ii) soil amendments. They have also emphasized on the doses of amendments, to assure the establishment and development of plants and to keep the ecological equilibrium of soil. Growth and ectomycorrhizal development of Loblolly pine seedlings in fumigated and nonfumigated nursery soil, infested with different fungal symbionts was studied by Marx *et al.* (1978). Significant increase in the number (75 to 155 percent) and total fresh weights (24 to 125 percent) of plantable seedlings was observed. Further inocula of all fungi were reported to be more effective in forming ectomycorrhizae in fumigated than in non-fumigated soil. Menkins *et al.* (2007) studied the afforestation of abandoned farmland with conifer seedlings inoculated with three ectomycorrhizal fungi and found that after the first year, natural mycorrhizal infections prevailed in the inoculated root systems. The pre-inoculated seedlings showed significantly higher survival and growth as compared to controls. Menkins *et al.* (2005) have observed the fungi colonising root tips of *Pinus sylvestris* and *Picea abies* grown

Table 4. *ANOVA for seedling growth of different provenances of Cupressus torulosa.*

Cupressus torulosa (non-mycorrhizae)								
Monthly Seedlings growth[Shoot]	1.22	2	0.61	7.62*	4.45	8.65	0.53	0.76
Provenances	2.84	4	0.71	8.86**	3.83	7.01	0.41	0.60
Error	0.64	8	0.08					
Total	5.523	14						
Cupressus torulosa (mycorrhizae)								
Monthly Seedlings growth[Shoot]	2.49	2	1.245	20.33	4.45	8.65	0.46	0.67
Provenances	3.78	4	0.945	15.43	3.83	7.01	0.36	0.52
Error	0.49	8	0.061					
Total	6.76	14						

*Significant at 5% and **significant at 1%

under four different seedling cultivation systems by morphotyping, direct sequencing and isolation methods. The cultivation system had a marked effect on the level of mycorrhizal colonisation. Similarly, Kahr and Arvedy (1986) have experimented upon Scots pine (*Pinus sylvestris*) and Norway Spruce (*Picea abies*) seedlings and recorded that the fungi infected the short roots within a few days and formed typical mantles and Hartig nets. On the other hand ectomycorrhiza formation, survivability and physiognomic characteristics were assessed for conifer seedlings, encountered in 1 and 2 years post fire environment in Huck burn site near Grand Teton National Park by Miller *et al.* (1998). The number of ectomycorrhizae was positively correlated with the number of primary needles and the root/shoot ratio.

CONCLUSION

These results highlight the importance of mycorrhizae to conifer seedling survival during the initial growing season and point to alteration of carbon allocation as a primary mechanism affecting seedling survival. Therefore, on the basis of present study it is emphasized that C. torulosa seedlings be inoculated with compatible mycorrhizal species under nursery conditions prior to planting out, for achieving successful afforestation particularly on the disturbed sites.

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