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Studies on Fungal Associates of *Quercus leucotrichophora*

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In the present communication, fungal associates of *Quercus leucotrichophora* are being reported. Rhizosphere soil samples of *Quercus leucotrichophora* revealed the presence of seventeen species of fungi belonging to ten genera. *Penicillium* (represented by 3 species) was the dominant fungal genus followed by *Aspergillus*, *Cladosporium*, *Fusarium* and *Trichoderma* (each represented by 2 species). Maximum number of isolated genera belong to subdivision Deuteromycotina. On screening these soil samples for the presence of VAM fungal spores, twenty four species belonging to five genera (i.e. *Acaulospora*, *Endogone*, *Entrophospora*, *Gigaspora* and *Glomus*) were isolated. 143 VAM fungal spores were isolated from 100 gm of rhizosphere soil of *Quercus leucotrichophora*. The percent root colonization of the VAM infected roots was observed to be 78%. Further, five species of fungal endophytes belonging to four genera (i.e. *Gliocladium*, *Penicillium*, *Rhizopus* and *Trichoderma*) were isolated from the roots, leaves and bark of *Quercus leucotrichophora*.

Key words: Rhizosphere, VAM, Endophytes.

Fungi are a diverse group of organisms comprising both single - celled and multicellular filamentous forms. Fungi are known to play a vital role as decomposers, symbionts of plants and animals and as parasites of plants in different ecosystems[1].

The symbiotic association between plant roots and fungi is called "Mycorrhiza"[2]. Vesicular arbuscular mycorrhizal (VAM) fungi improve plant growth under low fertility conditions, confer tolerance to some plant pathogens, contribute to the formation of soil structure and also help plants to become established in new areas[3]. Metting[4] defined rhizosphere as the volume of soil that is adjacent to and influenced by the plant roots. It is regarded as a hot spot for microbial colonization. Rhizosphere microorganisms increase the ability of plants to acquire nutrients from soil by either increasing the extent of the root system (e.g. through fungal hyphae) or by solubilizing macronutrients such as phosphorus or sulphur[5]. Endophytes are the microbes residing in the plant host tissues without causing any overt symptoms[6]. The practical applications of these endophytes are manifold: as potential biocontrol agents, sources

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of novel metabolites for therapeutics, plant protection, other industrial applications and as model systems for studying ecosystems[7].

Rhizosphere, VAM and endophytic fungi of agriculture and forest trees have been extensively studied (Sagar *et al*., Kumar and Sagar, Thakur and Sagar)[8,9,10] but studies on *Quercus leucotrichophora*, a prominent tree of Himachal Pradesh, are lacking. Hence, keeping in view the beneficial role of rhizosphere, VAM and endophytic fungi in the growth and development of plants, it was considered worthwhile to study the fungal associates of *Quercus leucotrichophora*.

MATERIALS AND METHODS

Materials used in the present study were roots, leaves, bark and soil samples from the rhizosphere of *Quercus leucotrichophora.* Collections were made from the forest area of Summer Hill, Shimla.

For the isolation of rhizosphere fungi, dilution plate method[11,12] was followed. Medium used for culturing rhizosphere fungi was Potato Dextrose Agar[13]. For isolating VAM spores from rhizosphere soil, Wet Sieving and Decanting Technique[14] was used. VAM infection in the roots was also assessed[15]. Percent root infection was calculated by following the method of Giovannetti and Mosse[16]. For the isolation of endophytes, Hot Water Treatment and Three Step Method was followed. In Hot Water Treatment, small pieces of roots, leaves and bark were washed with hot water (60°C) for 15 minutes in a test tube. Then these pieces were inoculated on Petriplates containing PDA medium of half strength for endophytic fungal growth. In Three Step Method, samples were washed with distilled water. Then these were surface sterilized with 25% methanol for 5 minutes, followed by 50% methanol for 3 minutes, again followed by 75% methanol for 2 minutes. Finally these samples were washed in sterilized water for 5 minutes and then these samples were inoculated on Petriplates containing PDA medium of half strength for the growth of endophytic fungal colonies. Rhizosphere and endophytic fungi were identified following Nagamani *et al.*[17] while VAM fungal spores were identified following Manoharachary[18].

RESULTS AND DISCUSSION

17 species of fungi belonging to 10 genera (i.e. *Absidia*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Gliocladium*, *Humicola*, *Penicillium*, *Phoma* and *Trichoderma*) were isolated from the root adhering soil samples of *Quercus leucotrichophora* during winter, spring, summer and rainy seasons (Table 1). *Penicillium* was the dominant genus (represented by *P. chrysogenum*, *P. funiculosum* and *P. griseofulvum*) followed by genera *Aspergillus*, *Cladosporium*, *Fusarium* and *Trichoderma* (each represented by 2 species i.e. *Aspergillus flavus*, *A. niger*, *Cladosporium cladosporioides*, *C. sphaerospermum*, *Fusarium moniliforme*, *F. oxysporum*, *Trichoderma pseudokoningii* and *T. viride*). Maximum number of fungi were isolated during the rainy season followed by spring, summer and winter season (Table 1). Maximum number of isolated genera belong to subdivision Deuteromycotina.

Sagar and Lakhanpal[19] isolated fungal species belonging to genera *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Humicola*, *Penicillium* and *Trichoderma* as the predominant fungi from the mycorrhizosphere of *Pinus wallichiana*. Thakur and Sagar[10] reported genera *Absidia*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Phoma*, *Humicola* and *Trichoderma* from the rhizospheres of *Terminalia bellirica*, *Terminalia chebula* and *Emblica officinalis.*

143 VAM fungal spores belonging to 5 genera (i.e. *Acaulospora*, *Endogone*, *Entrophospora*, *Gigaspora* and *Glomus*) were isolated per 100 gm of rhizosphere soil of *Quercus leucotrichophora* (Table 2; Figs.1, 2 and 3). *Acaulospora* (represented by 8 species) and *Glomus* (represented by 9 species) were the dominant fungal genera. VAM infection was observed in the roots of *Q. leucotrichophora*. The roots were found to be extensively colonized by fungal hyphae and vesicles (Fig.4F). The percentage of VAM infection in the roots was observed to be 78%. Thapar *et al.*[20] observed the roots of important tree species growing in Dehradun to be colonized by VAM fungal hyphae, vesicles and arbuscules. Verma and Jamaluddin[21] isolated *Acaulospora scrobiculata*, *Gigaspora*

Table 1. List of Fungi Isolated from Rhizosphere Soil Samples of *Quercus leucotrichophora* and their Seasonal Distribution

S. No.	Name of Fungus Isolated	Winter	Spring	Summer	Rainy
1.	*Absidia cylindrospora*	-	-	+	+
2.	*Aspergillus flavus*	-	+	+	-
3.	*Aspergillus niger*	+	-	-	+
4.	*Cephalosporium acremonium*	-	+	-	+
5.	*Cladosporium cladosporioides*	-	+	-	+
6.	*Cladosporium sphaerospermum*	-	-	+	-
7.	*Fusarium moniliforme*	-	+	-	+
8.	*Fusarium oxysporum*	-	+	-	+
9.	*Gliocladium catenulatum*	-	-	+	-
10.	*Humicola grisea*	+	+	-	+
11.	*Penicillium chrysogenum*	+	-	-	+
12.	*Penicillium funiculosum*	-	+	+	+
13.	*Penicillium griseofulvum*	-	+	-	-
14.	*Phoma* sp.	-	-	-	+
15.	*Trichoderma pseudokoningii*	+	-	+	-
16.	*Trichoderma viride*	-	-	-	+
17.	Non-sporulating mycelium	-	+	-	-

+ = Present – = Absent

Table 2. List of VAM Fungal Spores Isolated from the Rhizosphere Soil of *Quercus leucotrichophora*

S. No.	Name of VAM Fungal Spores
1.	*Acaulospora delicata*
2.	*Acaulospora foveata*
3.	*Acaulospora laevis*
4.	*Acaulospora mellea*
5.	*Acaulospora myriocarpa*
6.	*Acaulospora scrobiculata*
7.	*Acaulospora spinosa*
8.	*Acaulospora tuberculata*
9.	*Endogone* sp.
10.	*Entrophospora* sp.
11.	*Gigaspora albidum*
12.	*Gigaspora calospora*
13.	*Gigaspora decipiens*
14.	*Gigaspora margarita*
15.	*Gigaspora ramisporophora*
16.	*Glomus aggregatum*
17.	*Glomus deserticola*
18.	*Glomus fasciculatum*
19.	*Glomus formosum*
20.	*Glomus geosporum*
21.	*Glomus intraradices*
22.	*Glomus macrocarpum*
23.	*Glomus mosseae*
24.	*Glomus rubiformis*

Table 3. List of Fungal Endophytes of *Quercus leucotrichophora*

S. No.	Name of Fungal Endophyte
1.	*Gliocladium fimbriatum*
2.	*Penicillium chrysogenum*
3.	*Rhizopus oryzae*
4.	*Trichoderma* sp.
5.	Non-sporulating mycelium

spp., *Glomus aggregatum*, *G. fasciculatum*, *G. intraradices* and *G. macrocarpum* from the rhizosphere of *Tectona grandis.* Vogeti *et al*.[22] reported *Acaulospora foveata*, *A. laevis*, *A. scrobiculata*, *Entrophospora* sp., *Gigaspora* spp., *Glomus aggregatum*, *G. fasciculatum*, *G. geosporum*, *G. intraradices* and *G. mosseae* from the rhizosphere soil of sweet potato.

5 species of endophytic fungi belonging to 4 genera (i.e. *Gliocladium*, *Penicillium*, *Rhizopus* and *Trichoderma*) were isolated from the roots, leaves and bark of *Quercus leucotrichophora* [Table 3; Figs.4 (A-E)]. Mahesh *et al.*[23] reported *Penicillium* spp., *Trichoderma* spp. and sterile mycelia as endophytes from *A. indica*. Monnanda *et al*.[24] reported *G. delequescens* as fungal endophyte of *C. magna*. *Trichoderma* spp.

are reported to have growth promoting activity when cultivated with rice seedlings[25]. Thakur and Sagar[10] isolated *Penicillium* sp. and *Rhizopus oryzae* as endophytes of *E. officinalis* and *T. chebula*; and *T. harzianum* and *Trichoderma* sp. as endophytes of *T. bellerica*. The variations in the incidence of mycoflora under different parameters like rhizosphere, VAM fungi and endophytic studies may be due to difference in the root exudates[26], microbial competition, antagonism and succession.

Although these findings are preliminary in nature, they have established a base for the further extension of this study to screen the endophytes for secondary metabolites of pharmaceutical importance and exploit the potential of rhizosphere and VAM fungi for the growth and development of *Q. leucotrichophora*.

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