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Seasonal Fluctuations among the Fungal Associates of *Rhododendron campanulatum* D.Don.

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A B S T R A C T

The present study was undertaken to investigate the fungal associates of *Rhododendron campanulatum* D. Don from Churdhar region of District Shimla and Sirmaur in Himachal Pradesh. Studies revealed the presence of seven species of fungi belonging to six genera (*Absidia*, *Aspergillus*, *Curvularia*, *Fusarium*, *Pythium* and *Trichoderma)* from mycorrhizosphere soil of *Rhododendron campanulatum. Fusarium* was the most dominant genus among rhizosphere soil fungi. Twenty seven species of AM fungal spores belonging to eight genera (*Acaulospora*, *Dentiscutata*, *Endogone*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*) were isolated from the rhizosphere soil of *Rhododendron campanulatum. Glomus* was the most abundant genus recorded. Twenty species of endophytic fungi belonging to thirteen genera (*Alternaria*, *Aspergillus*, *Baratalinia*, *Cephalosporium*, *Cunninghamella*, *Fusarium*, *Gliocladium*, *Macrophomina*, *Myrothecium*, *Penicillium*, *Phoma*, *Pythium* and *Trichoderma*) were isolated from the leaves, bark, stem and roots of *Rhododendron campanulatum. Penicillium* was the most dominant genus among the endophytic fungi. Maximum numbers of endophytic and rhizospheric fungi were observed during the rainy season followed by summer and winter season. Maximum numbers of VAM spores were observed during the rainy season followed by winter and summer season.

Introduction

The microbial biomass constitutes an important component of soil organic matter and it can sensitize to any change in organic inputs (Powlson *et al*., 1987). Interactions between plants and soil microorganisms have long been recognized for their importance in plant mineral nutrition and nutrient cycling. Mycorrhizae are beneficial for the growth because of the enhanced nutrient uptake. Endophytes are the microbes such as Fungi and bacteria that survive with their hosts without producing any apparent symptoms or negative effects (Hirsch and Braun, 1992). Fungi are most frequently isolated endophytes and known to produce polysaccharides, enzymes or proteins and secondary metabolites. One more group of fungi is VAM which enhance

the uptake of nutrients, especially phosphorus (Hayman, 1975), promote the plant growth, improve plant tolerance to drought and toxicity of pollutants (Bedini *et al*., 2007: Zareri *et al*., 2010), resistance against pathogens (Davis and Menge, 1982) and increase the absorptive surface area of roots.

One of the most fascinating hot spots of activity and diversity in the soil is rhizosphere (Hinsinger, 2005). The plant rhizosphere favour the growth of micro-organisms and provide a critical link between the plant and soil environments (Qian *et al*., 1998). Rhizosphere microorganisms produce plant growth hormones such as Indole Acetic acid (IAA), Gibberllins and Cytokinins (Tien *et al*., 1979).

A brief review of literature revealed the work on rhizosphere fungi, endophytes and VAM association of different plants like *Adhatoda vasica, Picea smithiana, Ocimum sanctum, Rhododendron arboreum* and *Taxus baccata* but the fungal associates of *Rhododendron campanulatum* are not fully explored. *Rhododendron campanulatum* D.Don belongs to family Ericaceae is economically and medicinally very important in North-West, India.

Rhododendrons and their habitats are facing tremendous threats both due to natural as well as anthropogenic reasons. Flower is edible and harvested for making chutney and squash, consequently seed formation and seed dispersal is effected resulting significant decline in populations and species diversity of *Rhododendrons.* As a result more species will become endangered, rare and threatened in future. Hence the present work was undertaken to investigate the fungal associates of *Rhododendron campanulatum* D.Don

Materials and Methods

Materials

Native to India *Rhododendron campanulatum* is a shrub or small tree upto 5m high, and widely distributed in Himalayan regions from Jammu & Kashmir to Sikkim, at an altitudes between 2400 and 5200m. Material used in this study were roots, leaves, stem, bark and soil samples from rhizosphere of this plant. The samples were collected form Churdhar region of District Sirmaur and Shimla, Himachal Pradesh. The collections were made during summer, rainy and winter season.

Methodology

Isolation of Rhizosphere and Endophytic Fungi

For the isolation of rhizosphere fungi, dilution plate method of Wakesman (1927) and Warcup (1950) was followed. The media used for culturing rhiozospheric fungi were Czapeks Dox (Raper and Thom, 1949) and Potato Dextrose Agar (Rawling, 1933). Fungal endophytes were isolated from leaf, stem, bark and root samples of *Rhododendron campanulatum* following three step method of Suryanarayanam and Rajagopal (2000). The isolated fungi were identified following Nagamani *et al*. (2006)

Isolation of AM Fungal Spores

"Wet Sieving and Decanting Technique" (Gerdman and Nicolson, 1963) was used for isolation of AM spores. The criteria employed for identification were colour, size, shape, wall characteristics, contents and surface ornamentation of spores. The identification was done following Trappe (1982) and Schenck and Perez (1988). AM infection in roots was assessed by following

the method of Philips and Hayman (1970).

Results and Discussion

Seven species of fungi belonging to six genera (*Absidia cylindrospora, Aspergillus niger, Curvularia prasadii, Fusarium moniliforme, Fusarium solani, Pythium* sp. and *Trichoderma viride)* were isolated from the mycorhizosphere soil samples of *Rhododendron campanulatum* (Table1). *Fusarium* was most predominant genus reported in present investigation. Lakhanpal and Kumar (1984) isolated *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp. from the mycorrhizosphere of *Picea smithiana.* Thakur and Sagar (2007) studied the microbial associates of *Terminalia chebula* and *Embilica officinalis* and isolated *Aspergillus, Fusarium, Trichoderma* and *Penicillium.* Similar genera have been recorded in the present investigation. Sagar *et al.* (2015) screened rhizospheric soil samples of *Triticum aestivum* and revealed the presence of 18 species of fungi belonging to genus *Aspergillus, Fusarium* and *Absidia* and maximum genera isolated were belongs to division Deuteromycota.

Twenty species of endophytic fungi belonging to thirteen genera (*Alternaria alternata, Aspergillus niger, Baratalinia sp., Cephalosporium acremonium, Cunninghamella elegans, Fusarium moniliforme, Fusarium oxysporum, Fusarium solani, Gliocladium catenulatum, Macrophomina phasioli, Myrothecium roridum, Penicillium chrysogenum, P. citrinum, P. griseofulvum, P. purpurogenum, P. restrictum, Phoma sp., Pythium sp., Trichoderma harzianum* and *T. viride)* were isolated from the bark, roots, stem and leaves of *Rhododendron campanulatum* (Table 2). *Penicillium* was found to be the most dominant genera in the present investigation.

The rhizospheric and endophytic fungal isolates were further grouped into Eumycota, Zygomycota, Ascomycota and Deuteromycota. The maximum isolates from the present study belongs to Deuteromycota. This could be attributed to the reason that 'Fungi imperfecti' can tolerate wider environmental conditions as compared to other fungal populations (Behra and Mukerji, 1984).

A comparison of seasonal distribution of these rhizospheric and endophytic fungal isolates from *Rhododendron campanulatum* revealed that maximum number of fungi were recorded in rainy season, followed by summer season and winter season respectively (Fig. 1). Sagar and Kaur (2010) isolated the rhizospheric fungi of *Aesculus indica* and recorded maximum number of fungi during rainy season, followed by spring, winter and summer season. It is attributed to the fact that variation in individual fungal species distribution depends upon the type of soil, moisture content of soil, depth, season of the year, concentration of organic matter. Isolation procedure employed also influences the microbial distribution around the root surface (Atkinson, 1980; Subrahmanyam, 1990 and Mohan *et al*., 1995).

Twenty seven species of VAM fungal spores (*Acaulospora delicata, A. foveata, A. minuta, A. scrobiculata, A. tuberculata, Dentiscutata nigra, Endogone sp., Entrophospora colombiana, Gigaspora gigantea, G. margarita, G. rosea, Glomus ambisporum, G. clarum, G. clavoideum, G. fasiculatum, G.glomerculatum, G. halon, G. macrocarpum, G. microsporum, G. mosseae, G. occultum, G. rubiforme, G. spurcum, G. verrucosa, Sclerocystis sp.* and *Scutellospora minuta)* were isolated from the root adhering soil of *Rhododendron campanulatum* (Table 3).

Table.1 List of Fungi Isolated from Rhizosphere Soil Samples of *Rhododendron campanulatm* D. Don

Sr. no.	**Division**	**Name of fungus isolated**
1.	Eumycota	*Pythium sp.*
2.	Zygomycota	*Absidia cylindrospora*
3.	Ascomycota	*Aspergillus niger*
4.	Deuteromycota	*Curvularia prasadii, Fusarium moniliforme, F. solani, Trichoderma viride.*

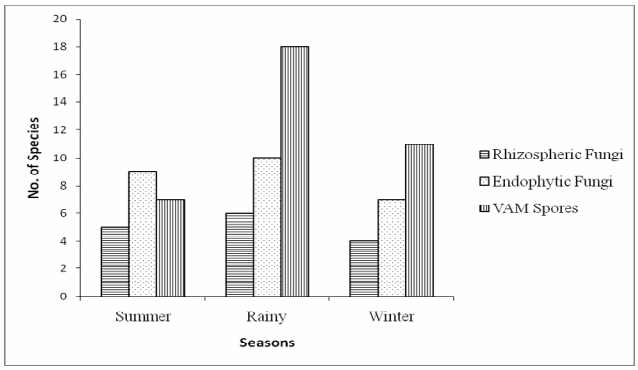
Table.2 List of Endophytic Fungi Isolated from Bark, Leaves, Roots and Stem of *Rhododendron campanulatum* D. Don

Sr.no.	**Division**	**Name of fungus isolated**
1.	Eumycota	*Pythium sp.*
2.	Zygomycota	*Cunninghamella elegans*
3.	Ascomycota	*Alternaria alternata, Aspergillus niger, Penicillium chrysogenum, P. citrinum, P. griseofulvum, P. purpurogenum, P. restrictum, Phoma sp.*
5.	Deuteromycota	*Baratalinia sp., Cephalosporium acremonium, Fusarium moniliforme, F. oxysporum, F. solani, Gliocladium catenulatum, Macrophomina phasioli, Myrothecium roridum, Trichoderma harzianum, T. viride.*

Table.3 List of VAM Fungal Spores Isolated from the Rhizosphere Soil Samples of *Rhododendron Campanulatum* D. Don

Sr. No.	**Genus**	**Species**
1.	*Acaulospora*	*A. delicata, A. foveata, A. laevis, A. minuta, A. scrobiculata, A. tuberculata*
2.	*Dentiscutata*	*D. nigra*
3.	*Endogone*	*Endogone sp.*
4.	*Entrophospora*	*E. colombiana*
5.	*Gigaspora*	*G. gigantea, G. margarita,G. rosea*
6.	*Glomus*	*G. ambisporum,G. clarum, G. clavoideum, G. fasiculatum, G. glomerculatum, G. halon, G. macrocarpum, G. microsporum, G. mosseae, G. occulatum, G. rubiforme, G. spurcum, G. verrucosa*
7.	*Sclerocystis*	*Sclerocystis sp.*
8.	*Scutellospora*	*S.minuta*

Fig.1 Seasonal Distribution of Rhizospheric Fungi, Endophytic Fungi and VAM Spores of *Rhododendron campanulatum* D. Don



These fungal isolates were further grouped into Zygomycota and Glomeromycota. The maximum isolates from the present study belongs to the Glomeromycota and genus *Glomus*. Kaur *et al*. (1997) studied the VAM associates of *Celtis australis* and *Grewia optiva* and reported the genus *Glomus* to be more dominant in Himachal Pradesh soils. Tamuli and Boruah (2002) isolated two genera *Glomus* and *Sclerocystis* from the rhizosphere soil samples of Agarwood. They found *Glomus* as most frequent VAM fungus in their investigation. Rani *et al*. (2008) isolate 29 species of AM fungi associated with 15 medicinal and ornamental plants belonging to Asclepidaceae. *Glomus* was found to be the most dominant genus and represented by15 species. Sagar *et al*. (2015) isolated 15 species of VAM fungal spores belonging to six genera with *Glomus* as dominant (*Acaulospora, Glomus, Claroideoglomus, Dentiscutata, Scutellospora* and *Gigaspora*) from the soil samples of *Triticum aestivum* from normal and disturbed field of Darlaghat, Himachal Pradesh, India

A comparison of seasonal distribution of these isolates revealed that maximum numbers of fungal spores were recorded in rainy season, followed by winter season and summer season respectively (fig. 1). This variation may be attributed to the fact that VAM colonization is found to be decreased in winter and summer and reached to maximum in the rainy season (Sharma *et al*., 2005).

The present investigations are of preliminary type, yet have established a base for future exploitation of these fungal associates (rhizosphere and VAM fungi) for mass multiplication of nursery seedlings, growth and productivity of *R. campanulatum* as well as commercial production of secondary metabolites from endophytes.

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