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SEASONAL POPULATION DYNAMICS OF VAM, ENDOPHYTIC AND RHIZOSPHERE FUNGI ASSOCIATED WITH *RHODODENDRON ARBOREUM* SM.

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Abstract

In the present study, the fungal associates of *Rhododendron arboreum* were investigated. Studies revealed the presence of twelve species of endophytic fungi and fifteen species of rhizospheric fungi belonging to different genera (*Aspergillus, Cladosporium, Fusarium, Penicillium, Rhizoctonia, Rhizopus, Stachybotrys, Trichoderma* and a sterile mycelium) from the leaves, stem, bark, roots and rhizosphere soil of *Rhododendron arboreum*. *Aspergillus* was the most abundant genus among the endophytic and rhizospheric fungi and maximum number of fungi was observed during rainy season followed by winter and summer. Twenty two species of AM fungal spores belonging to five genera (*Acaulospora, Dentiscutata, Entrophospora, Gigaspora* and *Glomus*) were isolated from mycorrhizosphere soil samples of this plant. *Glomus* was found most abundant genus among the AM fungal spores. Maximum number of spores was observed during rainy season followed by summer and winter seasons respectively.

Key words **:** Fungi, endophytes, AM spores, mycorhizosphere, *Rhododendron arboreum.*

Introduction

The variety and galaxy of fungi and their natural beauty occupy prime place in the biological world and India has been hub for such fungi. Fungi play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, as biofertilizers and many other ways. The group of fungi which colonize the different parts of plant without causing any noticeable symptoms are known as endophytic fungi. They represent one of the largest reservoirs of fungal species (Dreyfuss, 1989). Endophytic fungi are recognized as a repository of different bioactive metabolites and anticancer drugs (Li *et al.*, 1998). One more group of fungi is Arbuscular Mycorrhizal (AM) fungi which forms symbiotic associations with the roots of terrestrial flowering plants (Gianinazzi and Gianinazzi-Pearson, 1986). AM fungi play a major role in uptake and translocation of phosphorus from soil beyond the root zone of absorption through proliferation of their hyphae (Lakshman *et al*., 2006). Also, the fungi present in rhizosphere of plant are useful due to their significance as phosphorus solubilizers and producer of plant growth hormones including indole acetic acid (IAA), gibberellins and cytokinins (Tien *et al*., 1979; Nietko and Frankenberg, 1989).

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A review of work on fungal associates of different plants revealed that there are many reports of work on different plants like *Azadirachta indica* (Suryanarayanan and Rajagopal, 2000; Mahesh *et al*., 2005; Sagar, 2012), *Ocimum sanctum* (Pavithra *et al*., 2012), *Picea smithiana* (Kumar and Lakhanpal, 1983; Arora, 2005), *Taxus baccata* (Thakur, 1990; Gulati, 2004). Continuing and extending these studies further, present investigations on endophytes and mycorrhizosphere fungi of *Rhododendron arboreum* were undertaken. *Rhododendron arboreum* is a flowering tree plant which is vastly known for its beauty and medicinal uses. It is an evergreen tree with a showy display of red flowers belonging to family Ericaceae and called as 'Burans, Bras, Buras or Burah ke phool' in local language.

Materials and Methods

Materials

Originally discovered in North Central India *Rhododendron arboreum* is found in the Himalyas from Kashmir to Bhutan and in the hills of Assam and Manipur. It grows at elevations of 4500 to 10500 ft. and grows

upto 40 to 50 ft. high sometimes attaining over 100 ft. Materials used in study were leaves, stem, bark, roots and soil samples from rhizosphere of this plant. The samples were collected from selected sites in District Shimla, Himachal Pradesh, India.

Methodology

Isolation of endophytic fungi

Fungal endophytes were isolated from leaf, stem, bark and root samples of *Rhododendron arboreum* following three step method of Suryanarayanan and Rajagopal (2000). Culturing was done in PDA (Rawling, 1933) and Czapek-Dox Agar medium (Raper and Thom, 1949).

Isolation of AM fungal spores

"Wet Sieving and Decanting Technique" (Gerdemann 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. AM fungal spores were identified following Trappe (1982) and Schenck and Perez (1988). AM infection in roots was assessed by following the method of Philips and Hayman (1970).

Isolation of rhizosphere fungi

Isolation of soil mycoflora was done by following dilution plate method of Wakesman (1927) and Warcup (1950). The rhizospheric fungi were identified following Nagamani *et al*. (2006).

Results and Discussion

Twelve species of endophytic fungi belonging to five genera (*Aspergillus, Penicillium, Rhizopus, Stachybotrys* and *Trichoderma*) were isolated from leaves, stem, bark and roots of *Rhododendron arboreum.* The genus *Aspergillus* was represented by four species (*i.e.* A. *flavus, A. fumigatus, A. niger* and *A. versicolor*). The genus *Penicillium* was represented by three species (*P. chrysogenum, P. citrinum* and *P. notatum*). The genus *Trichoderma* was represented by two species (*T. harzianum* and *T. viride*). The genera *Rhizopus* and *Stachybotrys* were represented by one species each (*i.e. R. oryzae* and *S. atra*). Sterile mycelium of an unidentified fungus was also isolated as an endophytic fungus (table 1).

Various workers have reported similar fungal endophytes from different plants. Sagar and Chauhan (2009) observed five species of fungal endophytes belonging to four genera (*Penicillium, Rhizopus, Gliocladium* and *Trichoderma*) from the leaves, bark and roots of *Quercus leucotrichophora*. Genus *Aspergillus* was found to be dominant with four species in the present investigation. Anitha *et al*. (2013) investigated endemic medicinal plants of Tirumala hills under the Eastern Ghats of India for the presence of endophytes and *Aspergillus* was found to be the dominant fungus. Out of the identified genera, two belonged to division Ascomycota, two to Deuteromycota and one belonged to Zygomycota in the present work.

Twenty two species of AM fungal spores belonging to five genera (*Acaulospora, Dentiscutata, Entrophospora, Gigaspora* and *Glomus*) were isolated from root adhering soil of *Rhododendron arboreum*. The genus *Acaulospora* was represented by eight species (*A. bireticulata, A. denticulata, A. foveata, A. kentinensis, A. laevis, A. longula, A. scrobiculata* and *A. tuberculata*). The genus *Glomus* was represented by eleven species (*G. clarum, G. claroideum, G, constrictum, G. fasciculatum, G. heterosporum, G. intraradices, G. microsporum, G. mosseae, G. rubiforme, G. reticulatum* and *G. spurcum*). The genera *Dentiscutata* and *Entrophospora* were represented by one species each (*i.e. D. nigra and Entrophospora* sp) (table 2). Genus *Glomus* was found to be most dominant in present investigation with eleven species. Khalil *et al*. (1992) found AM spores belonging to *Glomus, Gigaspora, Acaulospora* and *Scutellospora* associated with soybean rhizosphere soil. Among these the genus *Glomus* was most abundant. Sagar *et al*. (1993) and Kaur *et al*. (1993) studied the VAM associates of *Celtis australis* and *Grewia optiva* and reported the genus *Glomus* to be more dominant in Himachal Pradesh soils. Thomas *et al*. (2014) found *Glomus* as the predominant genus associated with Hedychium *flavescens* and *Hedychium coronarium*. Sagar *et al*. (2015) isolated 15 species of AM fungal spores belonging to six genera (*Acaulospora, Glomus, Claroideoglomus, Dentiscutata, Scutellospora* and *Gigaspora*) and found *Glomus* to be most dominant genus from the soil samples of *Triticum aestivum* from normal and disturbed fields of Darlaghat, Himachal Pradesh, India. In the present investigation, maximum number of spores was observed during rainy season followed by summer and winter seasons (fig. 1). This may be attributed to the fact that VAM colonization decreases in winter and summer and reached maximum in rainy season (Sharma *et al*., 2005)

Fifteen species of rhizospheric fungi belonging to eight genera (*Aspergillus, Cladosporium, Fusarium, Penicillium, Rhizoctonia, Rhizopus, Stachybotrys* and *Trichoderma*) were isolated from rhizosphere of *Rhododendron arboreum* in the present work. The genus *Aspergillus* was represented by five species (*A.*

Table 1 : List of endophytic fungi isolated from leaves, stem, bark and roots of *Rhododendron arboreum*.

S. no.	Division	Species
1.	Ascomycota	*Aspergillus flavus, A. fumigatus, A. niger, A. versicolor, Penicillium chrysogenum, P. citrinum, P. notatum*
2.	Deuteromycota	*Stachybotrys atra, Trichoderma harzianum, T. viride*
3.	Zygomycota	*Rhizopus oryzae*

Table 2 : List of AM fungal spores isolated from rhizosphere soil samples of *Rhododendron arboreum*.

S. no.	Genus	Species
1.	*Acaulospora*	*A. bireticulata, A. denticulata, A. foveata, A. kentinensis, A. laevis, A. longula, A. scrobiculata, A. tuberculata.*
2.	*Dentiscutata*	*D. nigra*
3.	*Entrophospora*	*Entrophospora* sp.
4.	*Gigaspora*	*G. margarita*
5.	*Glomus*	*G. clarum, G. claroideum, G. constrictum, G. fasciculatum, G. heterosporum, G. intraradices, G. microsporum, G. mosseae, G. rubiforme, G. reticulatum, G. spurcum.*

Table 3 : List of fungi isolated from rhizosphere of *Rhododendron arboreum*.

S. no.	Division	Species
1.	Ascomycota	*Aspergillus flavus, A. fumigatus, A. niger, A. versicolor, A. wentii, Cladosporium cladosporioides, Penicillium chrysogenum, P. citrinum,*
2.	Basidiomycota	*Rhizoctonia solani*
3.	Deuteromycota	*Fusarium solani, Stachybotrys atra, Trichoderma harzianum, T. viride,*
4.	Zygomycota	*Rhizopus nigricans, R. oryzae*

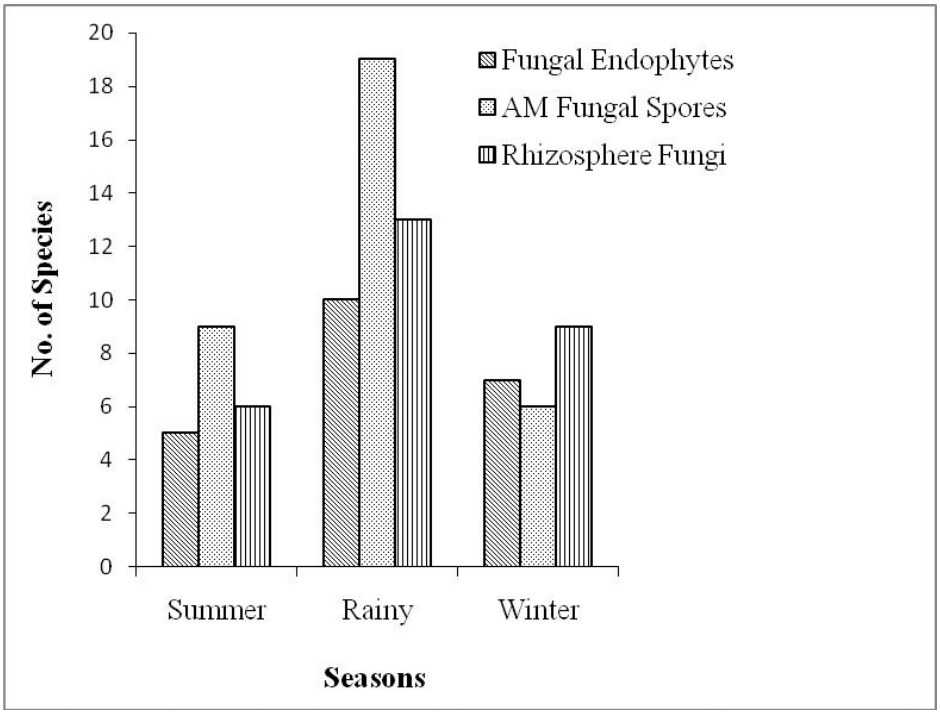

Fig. 1 : Histogram showing the seasonal distribution of fungal endophytes, AM fungal spores and rhizosphere fungi of *Rhododendron arboreum*.

flavus, A. fumigatus, A. niger, A. versicolor and *A. wentii*). The genus *Penicillium* was represented by two species (*P. chrysogenum* and *P. citrinum*). The genus *Trichoderma* was represented by two species (*T. harzianum* and *T. viride*). The genus *Rhizopus* was represented by two species (*R. oryzae and R. nigricans*). The genera *Cladosporium, Fusarium, Rhizoctonia* and *Stachybotrys* were represented by one species each (*C. cladosporioides, F. solani, R. solani* and *S. Atra*) (table 3). Genus *Aspergillus* was found to be dominant with five species. Basumatary *et al*. (2004) found *Aspergillus* as dominant species in the rhizosphere soil of Tulsi. Chandershekar *et al.* (2014) investigated 20 soil samples of different agricultural crop fields in and around Nanjangud taluk for diversity among fungi and found *Aspergillus*, *Penicillium* and *Mucor* as the dominant genera. In the present work, out of the identified genera, three belonged to division Ascomycota, one to Basidiomycota, three belonged to Deuteromycota and one belonged to Zygomycota. Sagar *et al.* (2009) conducted studies on mycorrhizosphere of *Picea smithiana*, which revealed the presence of 24 species of fungi, 18 of which belonged to subdivision Deuteromycotina, 5 belonged to Ascomycotina and 1 belonged to Zygomycotina. Further data on seasonal distribution of these fungi revealed their maximum (15 genera) presence in spring and rainy season which gradually decreased in winter (9 genera) and autumn (8 genera).

In the present work, maximum number of endophytic and rhizospheric fungi were observed during rainy season followed by winter and summer (fig. 1). Sagar and Kaur (2010) isolated the rhizosphere fungi of *Aesculus indica* and found that maximum number of fungi were recorded during rainy season (8 spp.) followed by spring (7 spp.), winter (6 spp.) and summer (5 spp.) It can be attributed to the fact that variation in individual fungal species distribution depend upon the type of soil, moisture content, depth, season of the year, concentration of organic matter. Isolation procedure employed also influences the microbial distribution around the root surface (Atkinson, 1980).

Present investigations have established a base for future exploitation of isolated fungal associates for mass multiplication of nursery seedlings of *R. arboreum* with rhizosphere and AM fungi, and commercial production of important secondary metabolites from endophytes.

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