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Biodiversity of fungi associated with rhizosphere of medicinally important plant, *Swartia petiolata* D. Don. growing in Kashmir

Mansoor Ahmad Malik*, Mohd Yaqub Bhat, Abdul Hamid Wani, Nusrat Ahmad, Latif A Peer
Department of Botany, Section of Plant Pathology, Mycology and Microbiology, University of Kashmir Srinagar, Jammu and Kashmir, India

Abstract
The study was carried out to isolate and identify the rhizospheric soil fungi associated with a medicinal plant, *Swartia petiolata* D.Don. growing in Kashmir Valley.. The results revealed that about 44 fungal strains were found associated with the test plant. These fungal strains belonged to 4 genera. The microscopic examination revealed that the fungal species isolated and identified were found as *Alternaria alternata*, *Fusarium oxysporium*, *Cladosporium* spp. and *Rhizoctonia solani.* The isolated fungi showed different frequency and also varied in their relative abundance. Among all the isolated fungi, *Alternaria alternata* was highly frequent and with highest relative abundance. However, least frequency was recorded for *Fusarium oxysporium* and *Rhizoctonia solani*, with *Fusarium oxysporium* as least abundant genera.

Keywords: fungi, frequency, medicinal plant, relative abundance, rhizosphere

Introduction

Soil contains the largest number of micro-organisms which play a crucial role in agri-ecosystem. The relationship between soil micro flora and host plant is an important practise for it growth (Buscot and Varma, 2005; Sathya *et al.*, 2016) [4, 10]. The interaction between host plant roots and micro-organisms is very strong in the rhizosphere area, the area adjacent to the plant roots which influences growth (Qiao *et al.*, 2019) [19]. The term rhizosphere was firstly described by (Hiltner 1904) [1] ass "the soil compartment influenced by plant roots". Rhizospheric soil has 10-20 times more microbial load than the rhizoplane soil. Rhizospheric fungi are an important element of the microbial population present in rhizospheric soil and play an important role in plant nutrition and productivity as well as defence against pathogens (Shaikh & Mokat, 2018) [21]. Most of the fungi have the positive effect on plant growth through their activity against pathogens, while others negatively affect plant production by infecting plant roots, (Zhang *et al.*, 2016) [25].

The Himalayas are widely known for biodiversity of medicinal plants with the potential of new compounds for drug discovery (Siddique & Jeelani, 2015) [22]. *Swartia petiolata* is restricted to the Western Himalayas, although it grows extensively in the Kashmir Valley. Because of medicinal properties, *Swertia petiolata* has been traditionally used for the treatment of various skin diseases or mental disorders, febrifuge, liver tonic, anti-inflammatory and antidiarrheal. (Bader 2014; Bader *et al.*, 2017) [2, 3]. In recent years, study on of relationship between rhizospheric fungi and medicinal plants as their hosts has been studied in superficial a way. Thus, many studies related to fungal diversity in rhizospheric soils of various medicinal plants have been previously reported however the information is scanty. As per the data available, not much work has been done on diversity of fungal flora associated with the medicinal plant, *Swartia petiolata* growing in Kashmir valley. Therefore, the study was designed to assess the diversity of the rhizospheric soil fungi associated with *Swertia petiolata*. D.Don

Materials and Methods

The present study was attempted to isolate and identify the rhizospheric soil fungal flora associated with *Swertia petiolata* D.Don. A medicinal plant growing in Kashmir Valley. The materials used and the methods used for this study are as –

Sampling Site and Sample Collection

The soil samples were collected from three different sites of Kashmir Valley, *viz.*, Gulmarg, Doodhpathri and Kashmir University Botanical Garden (KUBG) in the year 2019 to 2020 The geo-coordinates of the sampling sites are as mentioned in (Table 1). and the diagrammatically shown as in Fig.1. Soil samples from 6 to 8 spots were collected from the rhizospheric area of the test plant with the help of soil auger at two different depths of 0 to 15cm and 15 to 30 cm and collected in sterile poly bags. All the collected samples were brought to Plant Pathology, Mycology and Microbiology laboratory, Department of Botany, University of Kashmir and stored for further processing

Table 1: Sampling sites and their geographic locations.

	Sampling site	District	Elevation (m a.s.l.)	Annual rainfall (mm)	Latitude	Longitude
1	Gulmarg	Baramulla	2650	1049	34^{0}03′N	74^{0}23′E
2	Doodhpathri	Budgam	2850	669.1	33^{0}50′N	74^{0}35′E
3	Kashmir University Botanical Garden	Srinagar	1591	720	34^{0}09′N	74^{0}50′E

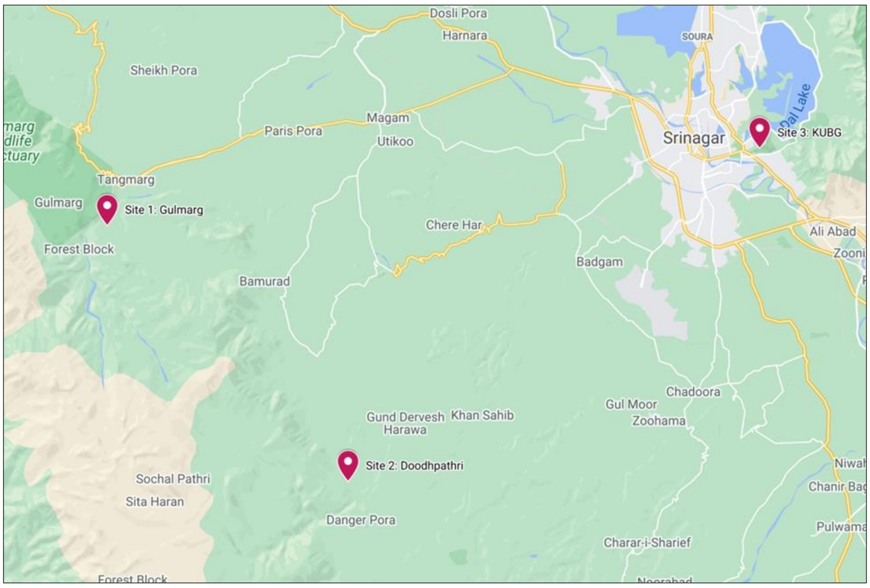

Fig 1: Sampling Sites marked in Kashmir Valley.

Isolation and Identification of Fungi

The soil samples were then processed for the isolation and culture of fungi using two different methods that is dilution plate method, in which dilution were made till 10^{-6} (Villarreal Ruiz *et al*., 2014) [23] and Warcup method (Warcup, 1950). Isolation of fungi was carried out using Potato Dextrose Agar and Richards's Liquid Medium as culture media, (Gilman, 1957) [9]. All the isolated rhizospheric soil fungi were identified and characterized based on cultural, morphological and microscopic characteristics (Gilman, 2008) [9].

Relative frequency and Abundance of soil fungi associated with *Swertia petiolata* D.Don

The frequency and relative abundance of soil fungi isolated from different sites of Kashmir were determined using formula described as (McLean and Cook, 1957)-

$$\text{Frequency} = \frac{No.\,of\ plates\ containing\ a\ particular\ fungus}{total\ no.\,of\ plates\ poured} \times 100$$

$$\text{Relative abundance} = \frac{Total\ no.\,of\ colonies\ of\ a\ fungus}{Total\ no.\,of\ colonies\ of\ all\ fungi} \times 100$$

Results

In the present study, a total of 44 fungal colonies were isolated from the rhizospheric soil associated with *Swertia petiolata* D.Don (Table 2 Fig. 2). Among the fungal colonies the major fungi identified were *Alternaria alternata*, *Fusarium oxysporium*, *Cladosporium* spp. and *Rhizoctonia solani*, However, from nineteen colonies of fungi *Alternaria alternata*, from fifteen colonies *Cladosporium* spp., from seven colonies *Rhizoctonia solani* and from three colonies *Fusarium oxysporium* was mainly identified.

Table 2: Diversity of fungal flora isolated from rhizospheric soil of *Swertia petiolata* D.Don.

Dilution series	Fungal Colonies	Total No of Colonies of particular fungus	Total No. colonies
10^{-1}	*Alternaria alternata* (2), *Fusarium oxysporium* (1)	3	44
10^{-2}	*Alternaria alternata* (4), *Fusarium oxysporium* (1)	5	
10^{-3}	*Alternaria alternata* (3), *Cladosporium* spp. (2), *Fusarium oxysporium* (1)	6	
10^{-4}	*Cladosporium* spp. (6), *Alternaria alternata* (5), *Rhizoctonia solani* (1)	12	
10^{-5}	*Rhizoctonia solani* (4), *Cladosporium* spp. (4)	8	
10^{-6}	*Alternaria alternata* (5), *Cladosporium*spp. (3), *Rhizoctonia solani*(2)	10	

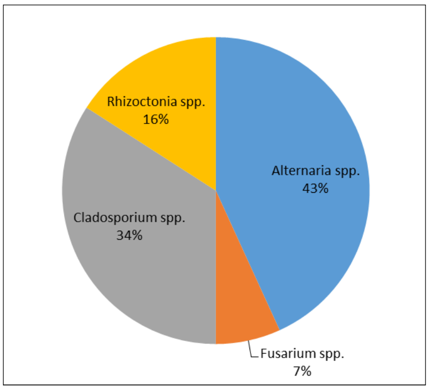

Fig 2: Graphical representation of total fungal flora isolated from rhizospheric soil of *Swertia petiolata.*

Alternaria alternata showed ample growth on PDA with the colony diameter of 5.5 to 7.5 cm after incubation at 27 ^{O}C for 6-7 days. The colonies were observed as uniform, floccose with aerial hyphae and dark brown in centre with white border (Nagrale *et al.* 2013). The mycelium was thin and greyish in the beginning which grew thicker and dark brown with longer incubation period (Fig. 3a). Microscopically, *Alternaria alternata* showed septate and brown hyphae. The multicellular conidia grew on branched and long conidiophores. Conidiophores also have beaks and are long (Fig. 3b).

Fusarium oxysporium showed rapid growth as compared to other isolates. Changes in the mycelial growth and pigmentation were observed at early growth stages. The fungus grew as woolly, white colonies with aerial mycelium. The colonies later turned to pink to purple (Fig. 3c). *Fusarium oxysporium* produced characteristic macro & micro conidia. The macro conidia were hyaline, tri/penta septate and fusiform in shaped while micro conidia were observed as small, hyaline, non-septate and were oval in shape. Conidiophores were single and short (Fig. 3d).

Cladosporium spp. showed slow to moderate growth pattern on PDA at 27^{O}C. The colonies were smooth and powdery due to profuse conidia with dark textured mycelia due to dark pigmentation (Fig. 3e). The hyphae were septate, branched and semi hyaline, which bear conidiophores having single celled oval shaped conidia in acropetal chains (Fig. 3f). *Rhizoctonia solani* showed slow growth on PDA. The colonies showed concentric rings with radial growth pattern and was light brown with cottony, smooth aerial mycelium (Fig. 3g). On microscopic examination, the hyphae were white to light brown in color, branched at vertical angle, septate like a long tube, multinucleated and absence of conidia (Fig. 3h).

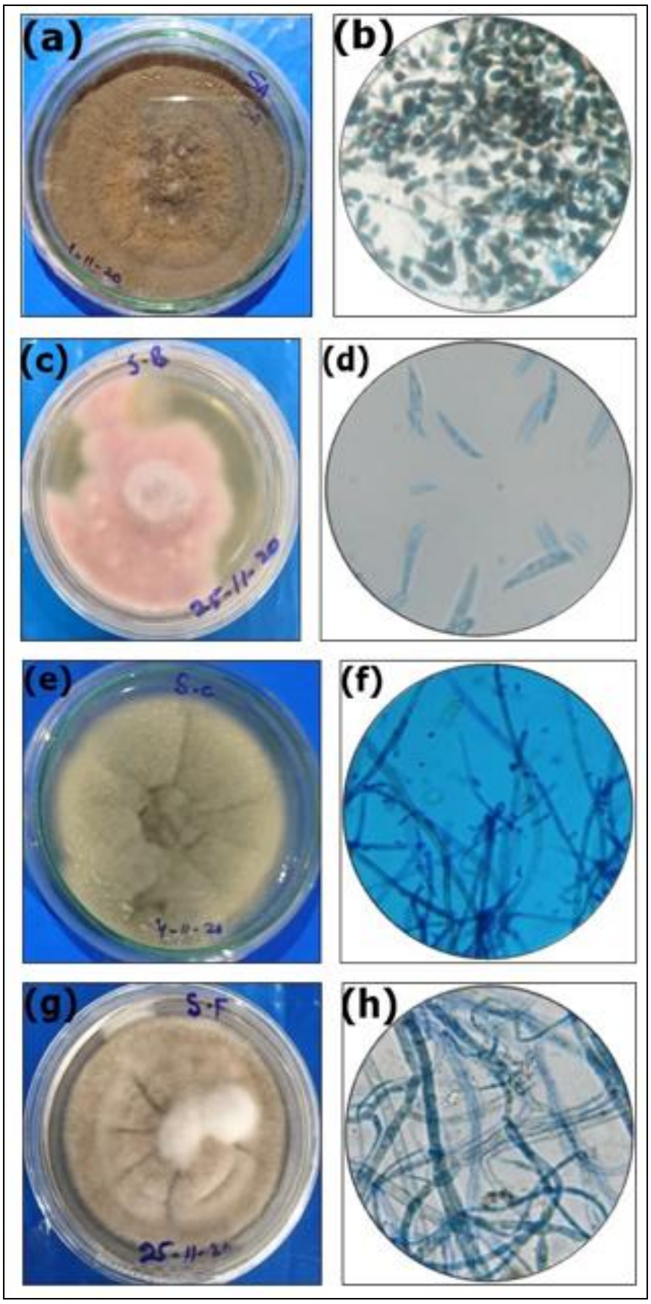

Fig 3: (a) Culture of *Alternaria alternate* (b) *Alternaria alternata* spores (c) Culture of *Fusarium oxysporium* (d) Conidia of *Fusarium oxysporium* (e) Culture of*Cladosporium* spp. (f) Hyphae and spores of *Cladosporium* spp. (g) Culture of *Rhizoctonia solani* (h) Mycellium of *Rhizoctonia solani*

Frequency and Relative abundance

Among all the fungi isolated from the rhizospheric soil of *Swertia petiolata* D.Don.

the highest frequency of 83.33% was found for *Alternaria alternata* while as lowest frequency (50%) was reported for both *Rhizoctonia solani* and *Fusarium oxysporium.* However about 66.66% frequency was reported for *Cladosporium* spp. (Fig.4). among all isolated fungi, *Alternaria alternata* showed maximum relative abundance (83.33%) followed by *Cladosporium* spp., *Rhizoctonia solani* as (34.09%) and 15.9%, respectively, whereas minimum relative abundance was reported for *Fusarium oxysporium* (Fig. 4).

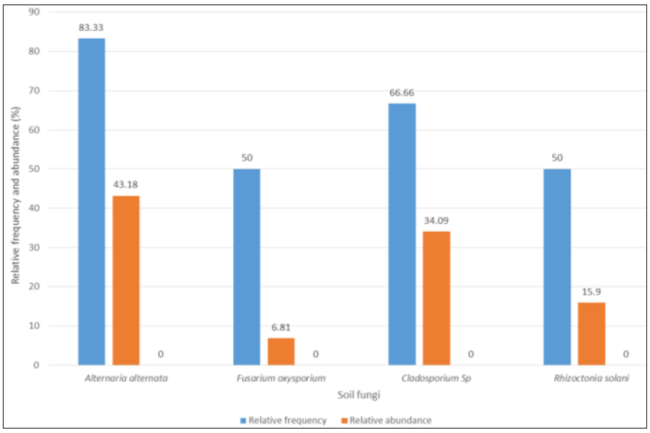

Fig 4: Relative frequency and relative abundance of fungi isolated from rhizospheric soil of *Swertia petiolata* D.Don

Discussion

Rhizospheric fungi constitute one of the most important parts of soil microbiota and play important and a critical role in maintaining soil-plant relationship and plant productivity. Also, it cannot be neglected that these fungi have both positive and negative effects on host plants. The results of the study revealed many strains of fungi associated with the rhizospherie of *Swertia petiolata*, these fungi belonged to both the saprobe and pathogenic groups. Since the plant grows at high altitudes with low mean temperature and high moisture contentwhich hindered the growth and multiplication of fungi. Smilar study was carried by many workers who have reported the occurrence of various fungi associated with different medicinal plants in different ecosystem across the globe (Jena *et al.*, 2015; Caruso *et al.*, 2020 Mir *et al* 2017 Ahmad *et al* 2021). The diversity of fungi associated with the rhizospheric soil of different host plant growing at different locations also differs (Hattori *et al.*, 2012).

In the present study it was found that *Alternaria alternata*, *Fusarium oxysporium*, *Cladosporium spp*. and *Rhizoctonia solani* fungi associated with *Swertia petiolate* L. growing in in different areas of Kashmir valley were differed in frequency and relative abundance.The findings of the present investigation are in accordance with the results of Muneer (2005), Chowdhary *etal.* (2015), Chandrakala (2018), Elgorban *et al.* (2019), Qiao *et.al.* (2019) [19], Khuseib *et al*, (2020) Ahmad *et al*, (2020) Ahmad *et al*,(2021) who have reported the occurrence similar fungal species and their abundance and frequency in different ecosystems across the world associated with different plants. The study will help in deciphering actual below ground fungal diversity associated with the test plant which will pave ways for growth and cultivation of the medicinal plant away from their natural habitat as well as will add these fugal species to global fungal data base associated with the *Swertia petiolata.*

Conclusion

It is concluded from the present study that the 44 colonies of rhizospheric soil fungi associated with *Swertia petiolata* were found. These fungi mostly belonged to 4 genera *viz Alternaria, Fusarium, Cladosporium and Rhizoctonia* Out of these fungal isolates *Alternaria alternata* was the highly frequent and both *Rhizoctonia solani* and *Fusarium oxysporium* were least frequent.

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Declarations:

Conflict of interest The authors declare that there is no conflict of interest.

Consent of Publication All authors give their consent to publish this study.

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