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Rhizospheric soil mycoflora associated with *Digitalis purpurea* L. and *Swertia petiolata* D. Don, medicinal plants Growing in Kashmir Himalaya.

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The purpose of the present study was to isolate and identify rhizospheric fungi associated with two medicinally important plants, viz. *Swertia petiolata* D. Don. and *Digitalis purpurea* L. from different sites of Kashmir valley. A total of 20 rhizospheric soil fungi representing 11 genera were obtained using a standard isolation protocol viz. *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Penicillium corylophilum*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Rhizoctonia solani*, *Trichoderma harzianum*, *Trichoderma viride*, *Rhizopus stolonifer*, *Mucor mucedo*, *Nigrosporasphaerica*, *Alternaria malorum*, *Alternaria solani*, *Penicillium nigricans*, *Nigrosporaoryzae*, *Fusarium solani*, and *Curvularia lunata*. Relative frequency and relative abundance of the fungi isolated from soil collected from different sites of Kashmir valley was also determined. Results revealed that significant variation in the relative frequency and relative abundance of rhizospheric soil fungi was found associated with *Swertia petiolata* and *Digitalis purpurea* growing in different areas of Kashmir valley.

Key words: *Digitalis purpurea*, frequency, rhizosphere, *Swertia petiolata*

INTRODUCTION

Soil is an ecosystem comprising weathered rock, organic matter, animals, plants and microorganisms that together support life on earth. The soil component of nature is known as the pedosphere besides holding the vegetation cover and diverse microorganisms associated with it, it performs different functions like storage of water, its supply and purification. Soil is an unconsolidated mineral or organic material on the surface of the earth that serves as a natural medium for the growth of land plants. The ecosystem's existence is supported by a variety of interactions among soil's physical, chemical, and biological components. (Buscot, 2005).

Soil hosts a number of biological processes that contribute to ecosystem services such as organic matter turnover, nonsymbiotic and symbiotic

nitrogen fixation, denitrification, and aggregation, among others (Chenu and Stotzky, 2002). Plant roots, soil, and soil biota all interact in the rhizosphere, which is an element of the soil ecosystem. Plants benefit from these interactions because they boost soil fertility and aid in the breakdown of harmful substances. Numerous studies show that soil-borne microorganisms interact with plant roots and soil components at the root-soil interface (Barea *et al.* 2002). The diverse interactions of root microbes result in the formation of a dynamic environment known as the rhizosphere, in which microbial populations also interact. The heterotrophic soil biota receives carbon molecules from root exudates and decaying plant material, which can be used as growth substrates, structural material, or signals by the root-associated microbiota (Werner, 2000). The quality and quantity of root exudates are affected by microbial activity in the rhizosphere, which influences rooting patterns and the delivery of accessible nutrients to plants (Barea, 2000).

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Plant species, plant developmental stage, and soil type have all been identified as key determinants of rhizosphere fungal community composition. (Broz *et al.* 2007; Singh *et al.* 2007; Broeckling *et al.* 2008). Actinomycetes are found in the plant rhizosphere soil and create active chemicals (Suzuki *et al.* 2008).

The Kashmir Valley is home to a diverse range of medicinal plants, but little research has been done on the rhizospheric soil fungi that are associated with these plants. As a result, the current investigation was carried out to isolate and identify the rhizospheric soil fungi associated with two important medicinal plants—*Digitalis purpurea* L. and *Swertia petiolata* D. Don and to understand the mechanism of native mycoflora's role which is vital in regulating the microbial host relationship and physiology of the target plant species in a specific soil environment.

Swertia petiolata D. Don. belongs to the family Gentianaceae. It is a perennial herb and grows up to 1m. It is commonly found in open alpine meadows, open dry slopes and rocky terrain at altitudes ranging from 3000-4000 m. It is used as digestive, appetizer, laxative and anti-inflammatory. It is administered for chronic fevers, anaemia and to cure gastric problems and liver disorders (Bader *et al.*, 2017).

Digitalis purpurea L. commonly known as the foxglove or common foxglove belongs to family Plantaginaceae (Olmstead *et al.* 2011). It is commonly found on sites where the ground has been disturbed, such as recently cleared woodland, or where the vegetation has been burnt. Foxglove contains chemicals from which the prescription medication digoxin (Lanoxin) is made. These chemicals can increase the strength of heart muscle contractions, change heart rate, and increase heart blood output (Sharma and Pukrait, 2012). Not much work has been carried out on the rhizosphere soil mycobiome of medicinal plants, *Digitalis purpurea* L. and *Swertia petiolata* D. Don, growing in Kashmir valley. The importance of rhizospheric microbiome for plant growth is well recognized, but information about many rhizospheric fungi is scanty. Therefore, the present study was carried out to unravel and document the rhizospheric soil fungi associated with medicinal plants, *Digitalis purpurea* and *Swertia petiolata* and to determine the relative abundance and relative

frequency of rhizospheric soil fungi associated with these two medicinal plants.

MATERIALS AND METHODS

Collection of Soil Samples

Ten soil samples were taken from the rhizospheric area of two medicinal plants, *Swertia petiolata* D. Don (at three locations, viz. Gulmarg, Doodhpathri and Kashmir University Botanical Garden (KUBG)) and *Digitalis purpurea* L., (at Gulmarg, Drung and KUBG) of Kashmir valley (Fig. 1). Details of sampling sites are given in Table 1. A soil auger was used to gather samples from each site up to a depth of 0-15 to 15-30cm near the plant base. The soil samples were gathered from each plant species and brought to the Plant Pathology and Mycology Laboratory for further analysis.

Sterilization of media and glassware

After washing the glassware, sterilization was done by physical methods via dry heat in a hot air oven. Media used (Potato Dextrose Agar and Richards Synthetic Agar) were sterilized by wet heat method in an autoclave.

Isolation of fungal flora

Isolation of the fungi associated with the rhizosphere of *Swertia petiolata* D. Don and *Digitalis purpurea* L. was done by dilution method (Waksman 1944; Ahmad *et al.* 2021). In this method, (1g) of soil was taken from the soil sample. This 1g of soil sample was dissolved in 10 ml of distilled water in sterilized test tubes to get 10^{-1} dilution. From this 1ml of soil, the suspension was taken and added to 9 ml of distilled water to get 10^{-2} . This was repeated until a final dilution of 10^{-6} was obtained. Dilutions of 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} were used to isolate fungi in order to avoid the over-crowding of fungal colonies. Inoculation was done in a laminar airflow chamber. 10 ml of media was placed in 90mm Petri plates to which 1ml of each dilution of soil suspension was poured. Plates were rotated gently to get uniform distribution of soil suspension into the medium. 1% streptomycin solution was added to the medium for preventing bacterial growth, before pouring into Petri plates. Then the Petri plates were incubated at a temperature of $25 \pm 2^\circ\text{C}$ for 5-7 days. These Petri plates were then observed for fungal growth and the number of fungal colonies in each Petri plate was recorded.

Identification of fungi

Rhizosphere mycoflora were identified based on the colony and microscopic characters. The colony growth which includes the length and width of the colony, and the presence and absence of aerial mycelium were some macro-morphological characters evaluated. The specimen was observed under the microscope for identification. Identification was done by monographs and relevant literature (Watanabe, 2002).

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

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

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

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

RESULTS

The present study was undertaken to study the rhizospheric fungi associated with *Swertia petiolata* D. Don. and *Digitalis purpurea* L. in different localities of Kashmir valley. Twenty fungi were isolated from the two selected medicinal plants. These soil fungi identified based on cultural and microscopic characters were: *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Penicillium corylophilum*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Rhizoctonia solani*, *Trichoderma harzianum*, *Trichoderma viride*, *Rhizopus stolonifer*, *Mucor mucedo*, *Nigrospora sphaerica*, *Alternaria malorum*, *Alternaria solani*, *Penicillium nigricans*, *Nigrospora oryzae*, *Fusarium solani*, and *Curvularia lunata*. Out of these 20 fungi, *Alternaria alternata*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Penicillium corylophilum*, *P. citrinum*, *P. chrysogenum* and *Rhizoctonia solani* were isolated from the rhizospheric soil of *Swertia petiolata*. *Alternaria malorum*, *A. solani*, *Penicillium nigricans*, *Nigrospora oryzae*, *Fusarium solani* and *Curvularia lunata* were isolated from the

rhizospheric soil of *Digitalis purpurea*. *Aspergillus niger*, *A. flavus*, *Trichoderma harzianum*, *T. viride*, *Rhizopus stolonifer* and *Mucor mucedo* were isolated from the rhizospheric soil of both the medicinal plants.

Alternaria alternata (Fr.) Keissl Cultural characteristics

On PDA media, *Alternaria alternata* colonies were olive green in colour, velvety in texture, and produced copious mycelium. On PDA, there is a lot of mycelial and conidial production (Fig. 2A).

Microscopic characteristics

Mycelium of *Alternaria alternata* was hyaline grey-brownish, multicelled, septate and irregularly branched. Initially hyphae of *A. alternata* are thin 2.84 µm in diameter. Conidiophores can appear individually or in groups of two to six (Fig. 3A).

Aspergillus niger Van Tieghem. Cultural characteristics

The colony of *Aspergillus niger* grow very rapidly on PDA medium. They are black in colour, irregular in outline and mostly consist of a dense tuft of erect conidiophores (Fig. 2B).

Microscopic characteristics

Mycelium is well developed, septate. Conidia are produced on conidiophores. The conidiophores are unbranched, aseptate, smoothly walled and are becoming darker at the apex and terminating in a globose vesicle. The finger-like projections called sterigmata are present over the entire surface of the vesicle. The conidia are globose, present in chains over the sterigmata (Fig. 3B).

Aspergillus flavus Link. Cultural characteristics

The colonies of *Aspergillus flavus* are yellow-green on the upper side and cream on the reverse. The growth of the fungus was rapid and colonies appeared powdery in texture (Fig. 2C).

Microscopic characteristics

Microscopic studies revealed that mycelium was septate and hyaline, conidia are smooth, globose

Table 1. Salient features of the selected sites of *Swertia petiolata* D.Don. and *Digitalis purpurea* L.

Location	District	Altitude (masl)	Latitude	Longitude	Habitat
Gulmarg,	Baramulla	2650	34°03' 141" N	74° 23' 88" E	Open sunny.
Doodhpathri	Budgam	2850	33° 50' 67" N	74° 35' 15" E	Open slopes and pastures.
Drang,	Baramulla	2200	34° 03' 15" N	74 ° 25' 10" E	Sunny and with shade areas.
*KUBG.	Srinagar	1591	34 °09' 66" N	74°50' 77" E	On the open field.

*KUBG-Kashmir University Botanical Garden.*masl-Metre above sea level



Fig.1: Sampling Sites marked (red) in Kashmir Valley.

to subglobose and produced thick mycelial mats and are 3µm - 6µm in diameter. Conidiophores are colorless. Vesicles are globose to subglobose (Fig. 3C).

***Cladosporium cladosporioides* (Fresen.) G. A. de Vries.**

Cultural characteristics

Cladosporium cladosporioides colonies on PDA were grey, olivaceous to dull green, or olivaceous to grey in colour. The colony's texture was velvety, and the colony's shape was uneven (Fig. 2D).

Microscopic characteristics

It was observed from microscopic studies that conidiophores were straight to slightly flexuose, medium brown and is up to 350µm long and 2µm - 6µm wide. Conidiophores were up to 350µm long and 2µm - 6µm wide. The conidia were aseptate, limoniform or ellipsoid, 3µm - 11µm × 2µm - 5µm

formed in long, branched chains. Conidia were spherical to subspherical, smooth walled but rough walled in some strains and pale olivaceous to brown (Fig.3D).

***Fusarium oxysporum* (Schl.) emend. Snyder and Hansen.**

Cultural characteristics

The colonies are usually fast-growing, pale, brightly coloured and covered with cottony aerial mycelium. Purple pigmentation can be seen in the fungal colony's colour. The colour changes from pale to yellow to reddish over time (Fig. 2E).

Microscopic characteristics

Slender phialides produce macro and microconidia. Macroconidia are hyaline, two to multiple celled, fusiform to sickle shaped, and have a pedicellate basal cell and an extended apical cell. The length of macroconidia varies between 19.8µm

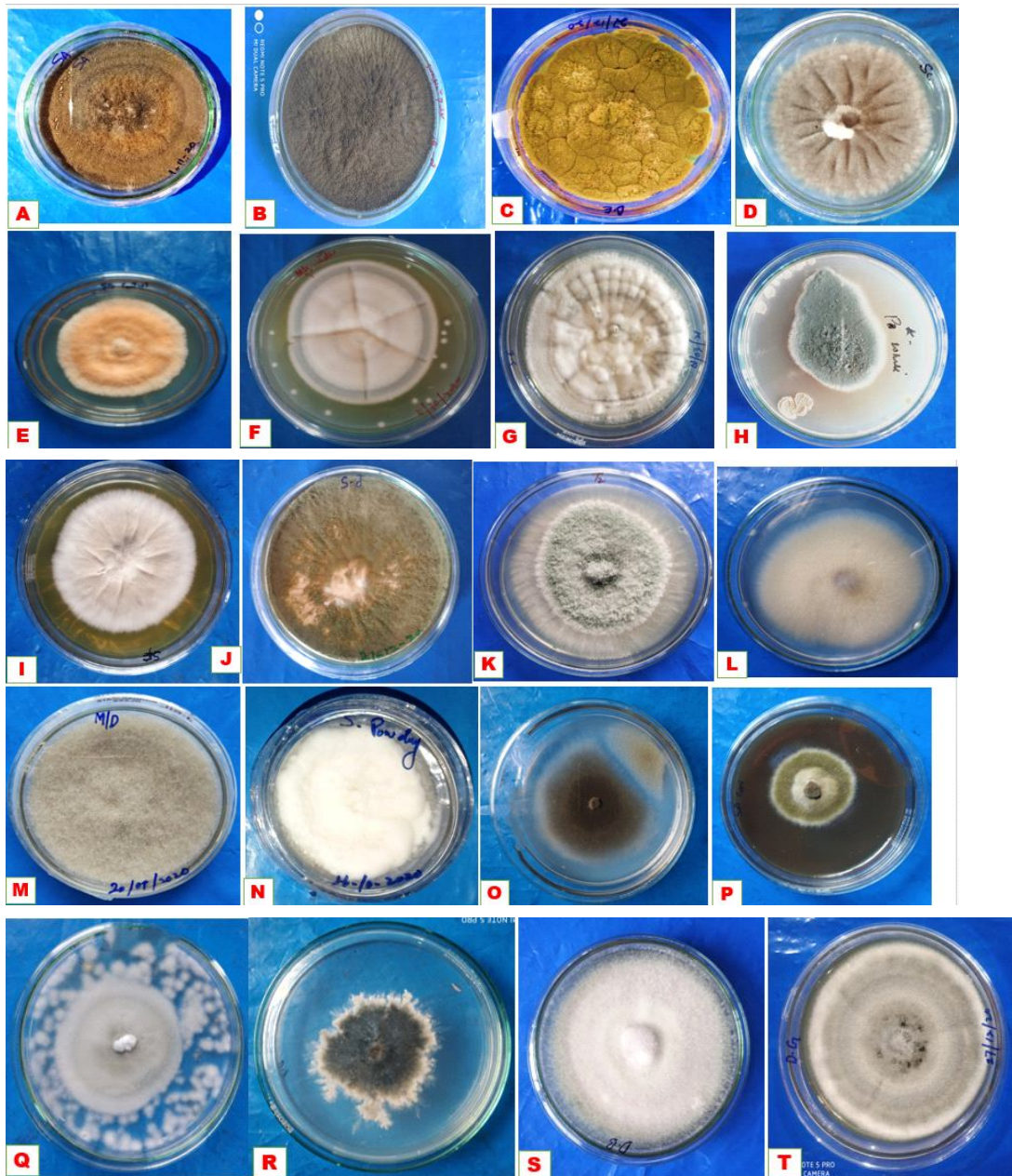


Fig.2. Cultures of fungi: A)=*Alternaria alternata*, (B)=*Aspergillus niger*, (C)=*Aspergillus flavus*, (D)= *Cladosporium cladosporioides*, (E)=*Fusarium oxysporum*, (F)=*Penicillium corylophilum*, (G)=*Penicillium citrinum*, (H)=*Penicillium chrysogenum*, (I)=*Rhizoctonia solani*, (J)=*Trichoderma harzianum*, (K)=*Trichoderma viride*, (L)=*Rhizopus stolonifer*, (M)=*Mucor mucedo*, (N)=*Nigrosporasphaerica*, (O)=*Alternaria malorum*, (P)=*Alternaria solani*, (Q)=*Penicillium nigricans*, (R)=*Nigrosporaoryzae*, (S)=*Fusarium solani* and (T) *Curvularia lunata*.

- 61.0 μ m and 3.15 μ m - 6.0 μ m. Microconidia are 1 to 2 celled, hyaline, pyriform, ovoid, straight or curved and are 5.80 μ m - 8.05 μ m in length (Fig. 3E).

***Penicillium corylophilum* Dierckx, R.p. Cultural characteristics**

Cultures on agar are lanose, white with a pinkish tint, reverse the same with a rather

intensified yellowish tint. Growth rate: under 1 cm in diameter in 10 days after incubation at 25°C (Fig. 2F).

Microscopic characteristics

Conidiophores hyaline, erect, branched penicillately at the apexes with 2–3 metula, verticillate phialides on each metula, and rather aggregated, compact, conidial heads composed of

catenulate conidia on each phialide: phialides tapering gradually or cylindrical with pointed tips. Conidiophores 120–220 μm long; phialides 10.5–12.5 \times 2.5 μm Conidia 2.7–3.5 \times 2.2–2.3 μm (Fig. 3F).

***Penicillium chrysogenum* Thom.**

Cultural characteristics

Penicillium chrysogenum colonies on PDA are initially white in colour and then turn to blue-green in colour with a velvety texture and the reverse of the colony is yellowish white (Fig. 2G).

Microscopic characteristics

Microscopically mycelium of *Penicillium chrysogenum* is composed of a densely branching network of multinucleate, septate, and typically colourless hyphae. The mycelia produce several branched conidiophores with individually constricted conidiophores. A particular conidiogenous cell termed phialide produces chains of single-celled conidia in basipetal succession. Phialides are generated singly or in groups from branched metulae, resulting in a penicillus with a brush-like appearance. Conidiophores are hyaline smooth or rough (Fig. 3G).

***Penicillium citrinum* Thom**

Cultural characteristics

Colonies on PDA grows rapidly, sometimes smaller, radially sulcate, and centrally floccose. The colony colour was white in peripheral areas, at the centers white to grayish green (Fig. 2H).

Microscopic characteristics

It is revealed from the microscopic observation that conidiophores are borne from surface to subsurface hyphae over which phialides arise in compact verticils. Conidia are globose to subglobose, 2.2 μm - 3.0 μm in diameter, smooth or finely roughened. Metulae are found in whorls. Phialides are ampulliform (Fig. 3H).

***Rhizoctonia solani* Kühn.**

Cultural characteristics

Rhizoctonia solani showed slow growth on PDA. The colonies showed concentric rings with radial growth patterns and were light brown with cottony,

smooth aerial mycelium. septated closely between main hyphae and side branches (Fig. 2I).

Microscopic characteristics

On microscopic examination, the hyphae were white to light brown in color, branched at a vertical angle, septate like a long tube, multinucleated and absence of conidia Hyphae 5–8 (-9) μm wide. Sclerotia 1–3 mm in diameter (Fig. 3I).

***Trichoderma harzianum* Kleifeld.**

Cultural characteristics

The colony colour of *Trichoderma harzianum* on PDA was white to grayish white initially and then turns to dark green. The shape of the colony was circular as a ring, aerial from the beginning and the texture of the colony is cottony. The reverse of the colony was pale yellow in colour (Fig. 2J).

Microscopic characteristics

Conidiophores were found to be hyaline, branching, erect, and contained spore masses apically at verticillate phialides, as per microscopic observation. Phialides are a type of phialide that is short and thick. Phialides are 7.2 μm - 9.8 μm \times 2.4 μm - 2.7 μm . The conidia of *Trichoderma harzianum* were globose to subglobose to ovate in shape, hyaline and are 2.1 μm - 3 μm \times 2.8 μm - 4.8 μm in diameter (Fig. 3J).

***Trichoderma viride* Rifai**

Cultural characteristics

The Colonies of *Trichoderma viride* on PDA medium appeared yellowish green, fluffy with reverse colour of the colony was brownish green (Fig. 2K).

Microscopic characteristics

The microscopic studied revealed that mycelium was septate, There are branched conidiophores with spore masses on each of the phialides. Verticillate, short, and thick phialides are common, 8.5 μm - 11 μm \times 2.4 μm - 2.7 μm in diameter. Conidia were hyaline, ovate, 1- celled, 2.4 μm - 2.7 μm \times 2.1 μm - 2.5 μm in diameter. Chlamydo spores pale brown, subglobose, granulate (Fig. 3K).

***Rhizopus stolonifer* (Ehrneb.:Fr) Vuill.**

Cultural characteristics

Colonies of *Rhizopus stolonifer* were usually fast growing, fill the petriplates and mature in 3 days.

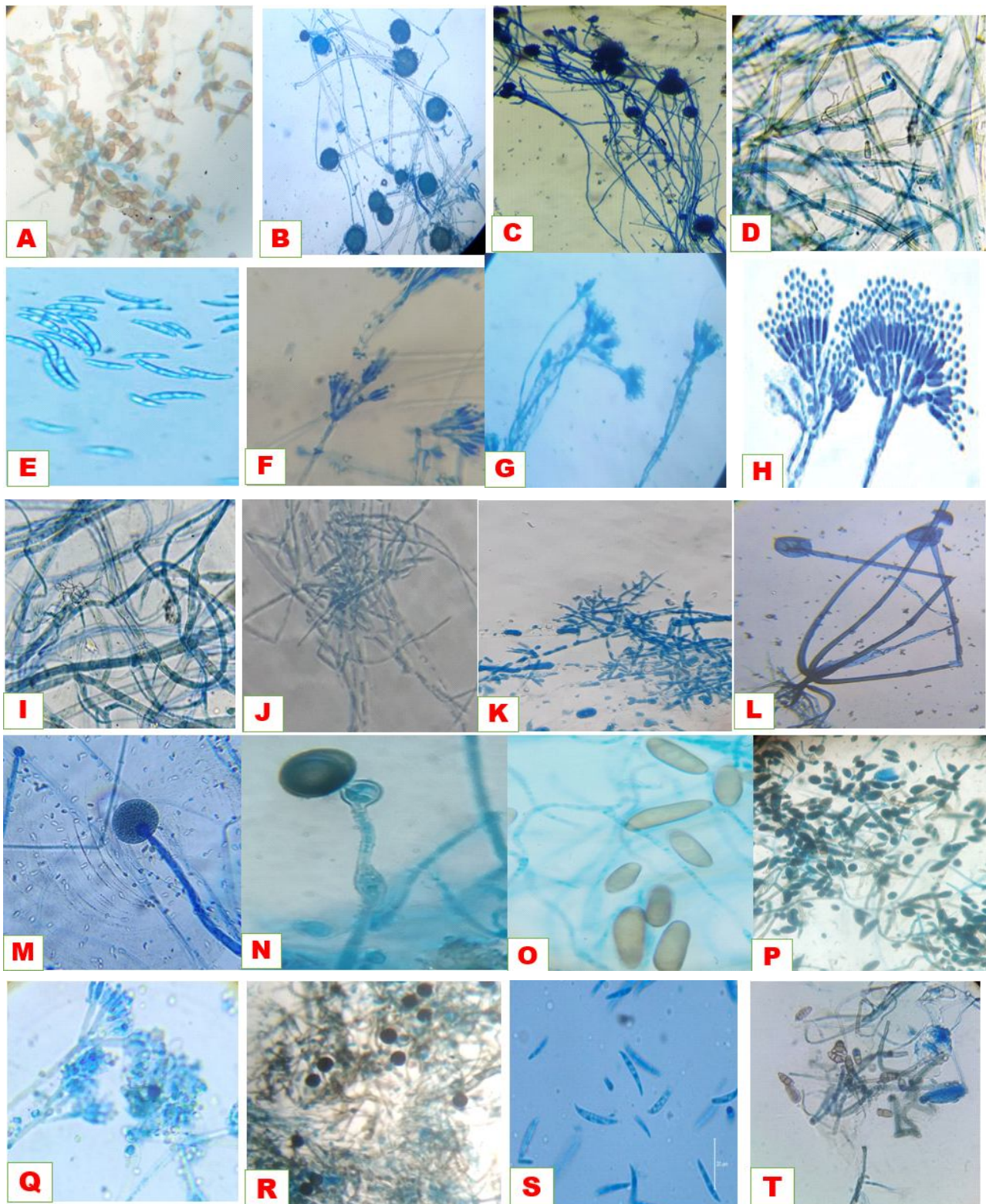


Fig.3. Microscopic features of fungi: A)= Conidia of *Alternaria alternata*, (B)=*Aspergillus niger*, (C)=*Aspergillus flavus*, (D)=Mycelium with conidia, (E)=Macro and micro conidia of *Fusarium oxysporum*, (F)=Mycelium of *Penicillium corylophilum*with conidia, (G)=Conidiophores with conidia, (H)=Branched conidiophores with conidia, (I)=branched septate mycelium, (J)=branching, erect, conidiophores, (K)=Branched conidiophores, (L)=Rhizoids and conidiophores of *Rhizopus stolonifer*, (M)=Mycelium and conidiophore of *Mucor mucedo*, (N)= Mycelium and conidiophore of *Nigrosporasphaerica*, (O)=Conidia of *Alternaria malorum*, (P)= Conidia of *Alternaria solani*, (Q)=Conidiophore of *Penicillium nigricans*, (R)= Mycelium and conidia of *Nigrosporaoryzae*, (S)=Conidia of *Fusarium solani* and (T) Conidia of *Curvularia lunata*.

From the front, the texture was typical cottony. The colony was white at first, then greyed out to a yellowish brown colour over time. The reverse is a light to medium shade of white (Fig. 2L).

Microscopic characteristics

Mycelium of *Rhizopus stolonifer* was non-septate and branched having three types of branches sporangiospores, rhizoids and stolans. Sporangiospores were brown in colour and usually unbranched and are formed in clusters. Rhizoids were found where the stolans and sporangiospores collided. Sporangia were found at the sporangiospores' tips. They were circular, with flattened bases, and hemispherical columella. Apophysis was nonexistent or only infrequently apparent. Sporangiospores were about 4µm - 11µm in diameter are unicellular, round to ovoid in shape, hyaline to brown in colour, and smooth (Fig. 3L).

***Mucor mucedo* Linnaeus**

Cultural characteristics

The colonies of *Mucor mucedo* on PDA were usually white to grey coloured and are fast growing, grow several centimeters in height. Due to the development of spores, older colonies turn grey or brown in colour (Fig. 2M).

Microscopic characteristics

It was observed from microscopic studies that mycelium of this fungus was non-septate bearing sporangiophores, sporangia, and spores. Rhizoids and stolans were absent, sporangiophores are short, erect. Sporangia are circular, grey to black in colour, and loaded with sporangiophores. They range in size from 50 µm to 300 µm in diameter (Fig. 3M).

***Nigrospora sphaerica* Mason**

Cultural characteristics

Nigrospora sphaerica produces woolly colonies on PDA medium. The colour of the colony is white initially and then turns grey to black. The reverse colony colour is black (Fig. 2N).

Microscopic characteristics

Conidiophores simple, hyaline, globose, bearing single conidia apically. Conidia aleuriosporous,

black, subglobose or disc-shaped, occasionally apiculate in the upper part. They bear a single conidium (14-20µm in diameter) at their apex. Conidia are black, solitary, unicellular, slightly flattened horizontally, and have a thin equatorial germ slit (Fig. 3N).

***Alternaria malorum* (Ruehle) U.Braun, Crous & Dugan.**

Cultural characteristics

Colonies of *Alternaria malorum* are dark brown to greyish in color initially whitish on the PDA medium (Fig. 2O).

Microscopic characteristics

In case of *Alternaria malorum*, mycelium is hyaline with thin walls to light brown or dark brown hyphae with septa. The conidia are a single cell, hyaline, aseptate, ellipsoidal to obovoid in shape (Fig. 3O).

***Alternaria solani* Sorauer**

Cultural characteristics

On PDA medium after 48 h of incubation, the fungus produced colonies with velvety texture, raised, irregular or circular in shape and greyish in colour. The colour of the colony on reverse side was blackish white (Fig. 2P).

Microscopic characteristics

The mycelium was septate and branching, as per microscopic analysis. Conidiophores were light brown in colour measuring 50µm - 60µm in size. Conidia were formed singly at the tip of conidiophores. The conidia were 16.04µm - 42.10µm long and 8.02 µm - 10.03µm in width. The conidia were septate with 1- 6 transverse and 0-2 longitudinal septa (Fig. 3P).

***Penicillium nigricans* (Bainier) Thom**

Cultural characteristics

The colonies of *Penicillium nigricans* are slightly grey to white in colour from the front. The reverse colour is white to orange (Fig. 2Q).

Microscopic characteristics

Conidia-bearing hyphae are variously short branches of aerial hyphae or whole trailing hyphae

showing thickened walls and bearing short branches with penicilli (50µm long). Conidia are 3–3.5µm in diameter and are globose and spiny (Fig. 3Q).

***Nigrospora oryzae* (Berkeley et Broome) Petch**
Cultural characteristics

Nigrospora oryzae grows rapidly and produces woolly colonies on PDA medium. The colour of the colony is white initially and then becomes grey to black. The reverse colony colour is black. (Fig. 2R).

Microscopic characteristics

It has septate and hyaline hyphae. The conidigenous cells on the conidiophores are inflated, swollen, and ampulliform in shape. They bear a single conidium (14–20µm in diameter) at their apex. Conidia are black, solitary, unicellular, slightly flattened horizontally, and have a thin equatorial germ slit (Fig. 3R)

***Fusarium solani* (Mart) Sacc.**
Cultural characteristics

The colonies of *Fusarium solani* on PDA medium were cottony white. The reverse color of the colony appears reddish pink due to the release of certain exudation by the fungus in the culture medium (Fig. 2S).

Microscopic characteristics

Microscopically, the filaments of *Fusarium solani* were hyaline, septate and branched. Conidiophores arise from the hyphae that were 16.32µm - 53.04µm × 2.04µm - 4.08µm long and produce microconidia and macroconidia. Macroconidia are long sickle shaped conidia having 3–5 septa which are 4.08µm - 20.40µm × 2.04µm - 4.08µm in diameter and microconidia are small, oval which may be smooth or curved lacking septa or may have two septa. Microconidia are 3.3 µm - 13.43 µm × 2.42 µm - 4.20 µm in diameter (Fig. 3S).

***Curvularia lunata* (Wakker) Boedijn**
Cultural characteristics

Curvularia lunata colonies develop quickly, producing a colony with a diameter of 3–9 cm on potato dextrose agar and maturing in 5 days. The

colony's texture was woolly, and the colony's surface was initially greyish before turning dark grey as it matured. The colony's backside was olive green in colour and strongly pigmented (Fig. 2T).

Microscopic characteristics

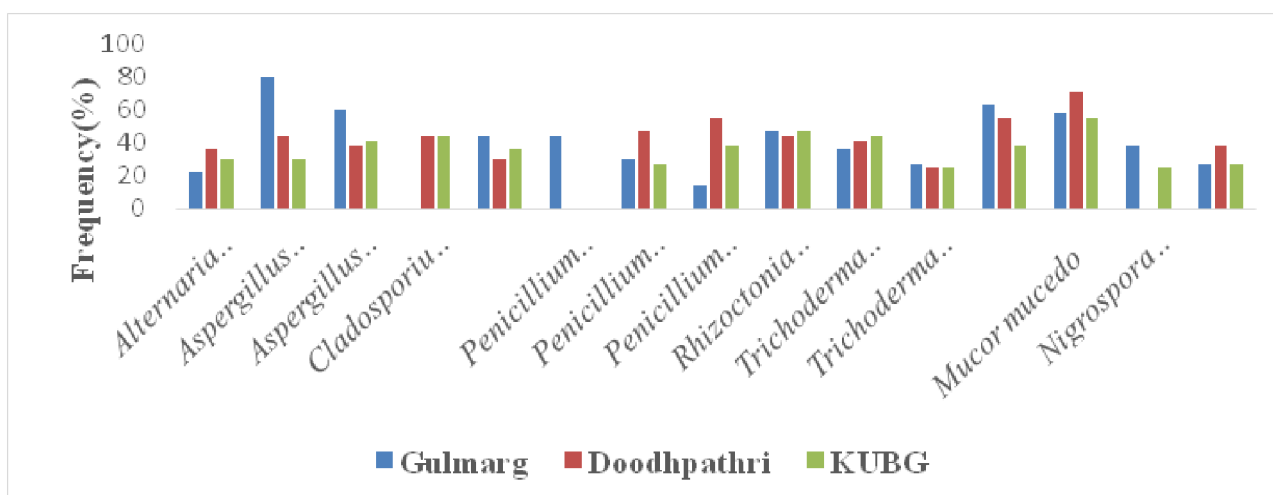
It was observed from the microscopic studies that hyphae were septate and light brown in colour. Conidiophores were 4.5µm to 6µm in diameter and were brown, simple or branched. The conidia were 2–4 celled, elliptical to cylindrical in shape, middle cells were broader than the other cells, light to dark brown in colour (Fig. 3T).

Frequency of fungi isolated from the rhizospheric area of Swertia petiolata and Digitalis purpurea

During the present study rhizospheric mycoflora of *Swertia petiolata*, important medicinal plant of Kashmir Valley was evaluated. Results (Table.2, Fig. 4) revealed significant variation in the relative frequency of rhizospheric soil fungi associated with *Swertia petiolata* growing in different areas of Kashmir valley such as Gulmarg, Doodhpathri, and KUBG. At Gulmarg, the frequency of fungi were *Alternaria alternata* (22.22%), *Aspergillus niger* (80.55%), *Aspergillus flavus* (61.11%), *Fusarium oxysporum* (44.44%), *Penicillium corylophilum* (44.44%), *Penicillium citrinum* (30.55%), *Penicillium chrysogenum* (13.88%), *Rhizoctonia solani* (47.22%), *Trichoderma harzianum* (36.11%), *Trichoderma viride* (27.77%), *Rhizopus stolonifer* (63.88%), *Mucor mucedo* (58.33%), *Nigrospora sphaerica* (38.88%), *Nigrospora oryzae* (80.55%), and *Fusarium solani* (27.77%) respectively. Similarly, at Doodhpathri, the frequency of fungi were *Alternaria alternata* (36.11%), *Aspergillus niger* (44.44%), *Aspergillus flavus* (38.88%), *Cladosporium cladosporioides* (44.44%), *Fusarium oxysporum* (30.55%), *Penicillium citrinum* (47.22%), *Penicillium chrysogenum* (55.55%), *Rhizoctonia solani* (44.44%), *Trichoderma harzianum* (41.66%), *Trichoderma viride* (25.00%), *Rhizopus stolonifer* (55.55%), *Mucor mucedo* (72.22%), *Nigrospora oryzae* (44.44%), *Fusarium solani* (38.88%) respectively. Likewise, at KUBG, the frequency of fungi were *Alternaria alternata* (30.55%), *Aspergillus niger* (30.55%), *Aspergillus flavus* (41.66%), *Cladosporium cladosporioides* (44.44%), *Fusarium oxysporum* (36.11%), *Penicillium citrinum* (27.77%), *Penicillium*

Table.2 : Frequency of fungi isolated from *Swertia petiolata* and *Digitalis purpurea*

Fungal isolates	Frequency (%) of soil fungi at different sites in Kashmir valley					
	<i>Swertia petiolata</i>			<i>Digitalis purpurea</i>		
	Gulmarg	Doodhpathri	KUBG	Gulmarg	Drung	KUBG
<i>Alternaria alternata</i>	22.22	36.11	30.55	00.00	00.00	00.00
<i>Aspergillus niger</i>	80.55	44.44	30.55	36.11	47.22	47.22
<i>Aspergillus flavus</i>	61.11	38.88	41.66	66.66	50.00	47.22
<i>Cladosporium cladosporioides</i>	00.00	44.44	44.44	00.00	00.00	00.00
<i>Fusarium oxysporum</i>	44.44	30.55	36.11	00.00	00.00	00.00
<i>Penicillium corylophilum</i>	44.44	00.00	00.00	00.00	00.00	00.00
<i>Penicillium citrinum</i>	30.55	47.22	27.77	00.00	00.00	00.00
<i>Penicillium chrysogenum</i>	13.88	55.55	38.88	00.00	00.00	00.00
<i>Rhizoctonia solani</i>	47.22	44.44	47.22	00.00	00.00	00.00
<i>Trichoderma harzianum</i>	36.11	41.66	44.44	30.55	47.22	33.33
<i>Trichoderma viride</i>	27.77	25.00	25.00	22.22	00.00	13.88
<i>Rhizopus stolonifer</i>	63.88	55.55	38.88	61.11	33.33	50.00
<i>Mucor mucedo</i>	58.33	72.22	55.55	36.11	80.55	66.66
<i>Nigrospora sphaerica</i>	38.88	00.00	25.00	16.66	00.00	33.33
<i>Alternaria malorum</i>	00.00	00.00	00.00	16.66	00.00	38.88
<i>Alternaria solani</i>	00.00	00.00	00.00	16.66	05.55	27.77
<i>Penicillium nigricans</i>	00.00	00.00	00.00	11.11	41.66	16.66
<i>Nigrospora oryzae</i>	80.55	44.44	30.55	36.11	47.22	47.22
<i>Fusarium solani</i>	27.77	38.88	27.77	27.77	33.33	13.88
<i>Curvularia lunata</i>	00.00	00.00	00.00	41.66	33.33	00.00

**Fig.4** : Frequency of soil fungi associated with *Swertia petiolata* at different sites of Kashmir Valley

chrysogenum (38.88%), *Rhizoctonia solani* (47.22%), *Trichoderma harzianum* (44.44%), *Trichoderma viride* (25.00%), *Rhizopus stolonifer* (38.88%), *Mucor mucedo* (55.55%), *Nigrospora sphaerica* (25.00%), *Nigrospora oryzae* (30.55%), *Fusarium solani* (27.77%) respectively.

Similarly, it was observed from results (Table 2, Fig. 5) revealed that significant variation in the relative frequency of rhizospheric soil fungi was found associated with *Digitalis purpurea* growing in different areas of Kashmir valley such as Gulmarg, Drung, and KUBG. At Gulmarg, the frequency of

Table3:Relative abundance of fungi isolated from *Swertia petiolata* and *Digitalis purpurea*

Fungal isolates	Relative abundance (%) of soil fungi at different sites in Kashmir valley					
	<i>Swertia petiolata</i>			<i>Digitalis purpurea</i>		
	Gulmarg	Doodhpathri	KUBG	Gulmarg	Drung	KUBG
<i>Alternaria alternata</i>	02.72	04.48	05.60	00.00	00.00	00.00
<i>Aspergillus niger</i>	17.61	10.53	06.23	08.40	09.52	09.98
<i>Aspergillus flavus</i>	13.83	07.62	11.52	24.32	15.38	14.28
<i>Cladosporium cladosporioides</i>	00.00	05.60	09.03	00.00	00.00	00.00
<i>Fusarium oxysporum</i>	05.66	06.72	04.67	00.00	00.00	00.00
<i>Penicillium corylophilum</i>	05.24	00.00	00.00	00.00	00.00	00.00
<i>Penicillium citrinum</i>	03.98	07.39	04.04	00.00	00.00	00.00
<i>Penicillium chrysogenum</i>	02.93	08.52	09.96	00.00	00.00	00.00
<i>Rhizoctonia solani</i>	05.66	07.17	07.78	00.00	00.00	00.00
<i>Trichoderma harzianum</i>	07.12	05.15	07.78	06.90	09.15	08.16
<i>Trichoderma viride</i>	05.66	03.81	04.98	04.20	00.00	03.74
<i>Rhizopus stolonifer</i>	08.80	06.95	05.91	22.52	10.98	13.26
<i>Mucor mucedo</i>	09.43	13.90	13.39	08.40	27.10	17.34
<i>Nigrospora sphaerica</i>	04.82	00.00	03.73	03.03	00.00	06.80
<i>Alternaria malorum</i>	00.00	00.00	00.00	00.00	00.00	08.84
<i>Alternaria solani</i>	00.00	00.00	00.00	02.40	00.73	04.76
<i>Penicillium nigricans</i>	00.00	00.00	00.00	02.40	08.42	03.74
<i>Nigrospora oryzae</i>	00.00	00.00	00.00	00.00	00.00	07.82
<i>Fusarium solani</i>	02.72	04.03	05.29	04.80	06.59	05.10
<i>Curvularia lunata</i>	00.00	00.00	00.00	06.30	06.59	00.00

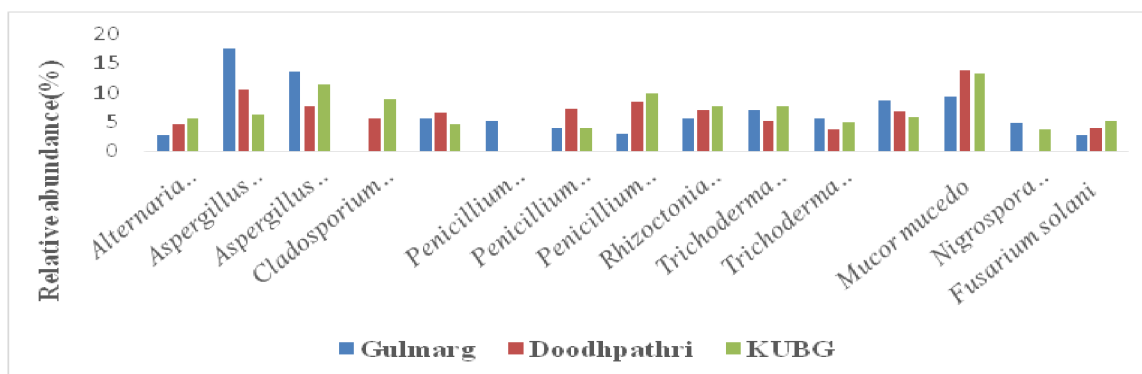


Fig. 6: Relative abundance of soil fungi associated with *Swertia petiolata* at different sites of Kashmir valley

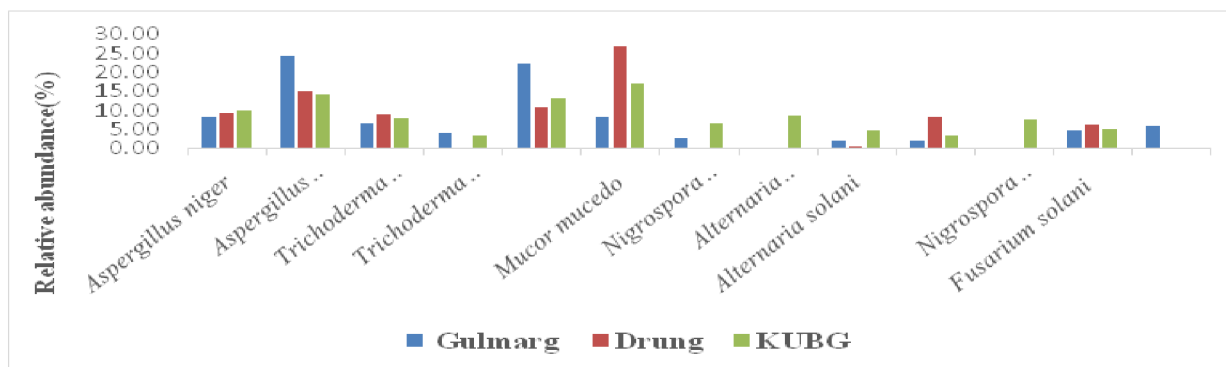


Fig. 7: Relative abundance of soil fungi associated with *Digitalis purpurea* at different sites of Kashmir Valley

fungi was *Aspergillus niger* (36.11%), *Aspergillus flavus* (66.66%), *Trichoderma harzianum* (30.55%), *Trichoderma viride* (22.22%), *Rhizopus stolonifer* (61.11%), *Mucor mucedo* (36.11%), *Nigrospora sphaerica* (16.66%), *Alternaria malorum* (16.66%), *Alternaria solani* (16.66%), *Penicillium nigricans* (11.11%), *Nigrospora oryzae* (36.11%), *Fusarium solani* (27.77%), *Curvularia lunata* (41.66%) respectively. Similarly, at Drung, the frequency of fungi was *Aspergillus niger* (47.22%), *Aspergillus flavus* (50.00%), *Trichoderma harzianum* (47.22%), *Rhizopus stolonifer* (33.33%), *Mucor mucedo* (80.55%), *Alternaria solani* (05.55%), *Penicillium nigricans* (41.66%), *Nigrospora oryzae* (47.22%), *Fusarium solani* (33.33%), *Curvularia lunata* (33.33%) respectively. Likewise, at KUBG, the frequency of fungi was *Aspergillus niger* (47.22%), *Aspergillus flavus* (47.22%), *Trichoderma harzianum* (33.33%), *Trichoderma viride* (13.88%), *Rhizopus stolonifer* (50.00%), *Mucor mucedo* (66.66%), *Nigrospora sphaerica* (33.33%), *Alternaria malorum* (38.88%), *Alternaria solani* (27.77%), *Penicillium nigricans* (16.66%), *Nigrospora oryzae* (47.22%), *Fusarium solani* (13.88%) respectively.

Relative abundance of fungi isolated from the rhizosphere of *Swertia petiolata* D. Don and *Digitalis purpurea* L.

During the present study relative abundance of rhizospheric mycoflora of *Swertia petiolata* important medicinal plant of Kashmir Valley were evaluated. Results (Table 3, Fig. 6) revealed that significant variation in the relative abundance of rhizospheric soil fungi was found associated with *Swertia petiolata* growing at different areas of Kashmir valley such as Gulmarg, Doodhpathri, and KUBG. At Gulmarg, the relative abundance of fungi were *Alternaria alternata* (02.72%), *Aspergillus niger* (17.61%), *Aspergillus flavus* (13.83%), *Fusarium oxysporum* (05.66%), *Penicillium corylophilum* (05.24%), *Penicillium citrinum* (03.98%), *Penicillium chrysogenum* (02.93%), *Rhizoctonia solani* (05.66%), *Trichoderma harzianum* (07.12%), *Trichoderma viride* (05.66%), *Rhizopus stolonifer* (08.80%), *Mucor mucedo* (09.43%), *Nigrospora sphaerica* (04.82%), *Fusarium solani* (02.72%) respectively. Likewise, at Doodhpathri, the relative abundance of fungi were *Alternaria alternata* (04.48%), *Aspergillus niger* (10.53%), *Aspergillus flavus* (07.62%), *Cladosporium cladosporioides* (05.60%), *Fusarium*

oxysporum (06.72%), *Penicillium citrinum* (07.39%), *Penicillium chrysogenum* (08.52%), *Rhizoctonia solani* (07.17%), *Trichoderma harzianum* (05.15%), *Trichoderma viride* (03.81%), *Rhizopus stolonifer* (06.95%), *Mucor mucedo* (13.90%), *Fusarium solani* (04.03%) respectively. Similarly, at KUBG, the relative abundance of fungi were *Alternaria alternata* (05.60%), *Aspergillus niger* (06.23%), *Aspergillus flavus* (11.52%), *Cladosporium cladosporioides* (09.03%), *Fusarium oxysporum* (04.67%), *Penicillium citrinum* (04.04%), *Penicillium chrysogenum* (09.96%), *Rhizoctonia solani* (07.78%), *Trichoderma harzianum* (07.78%), *Trichoderma viride* (04.98%), *Rhizopus stolonifer* (05.91%), *Mucor mucedo* (13.39%), *Nigrospora sphaerica* (03.73%), *Fusarium solani* (05.29%) respectively.

Likewise, results (Table 3, Fig. 7) revealed that significant variation in the relative abundance of rhizospheric soil fungi was found associated with *Digitalis purpurea* growing at different areas of Kashmir valley such as Gulmarg, Drung, and KUBG. At Gulmarg, the relative abundance of fungi were *Aspergillus niger* (08.40%), *Aspergillus flavus* (24.32%), *Trichoderma harzianum* (06.90%), *Trichoderma viride* (04.20%), *Rhizopus stolonifer* (22.52%), *Mucor mucedo* (08.40%), *Nigrospora sphaerica* (03.03%), *Alternaria solani* (02.40%), *Penicillium nigricans* (02.40%), *Fusarium solani* (04.80%), *Curvularia lunata* (06.30%) respectively. Similarly, at Drung, the relative abundance of fungi were *Aspergillus niger* (09.52%), *Aspergillus flavus* (15.38%), *Trichoderma harzianum* (09.15%), *Rhizopus stolonifer* (10.98%), *Mucor mucedo* (27.10%), *Alternaria solani* (00.73%), *Penicillium nigricans* (08.42%), *Fusarium solani* (06.59%), *Curvularia lunata* (06.59%) respectively. Likewise, at KUBG, the relative abundance of fungi were *Aspergillus niger* (09.98%), *Aspergillus flavus* (14.28%), *Trichoderma harzianum* (08.16%), *Trichoderma viride* (03.74%), *Rhizopus stolonifer* (13.26%), *Mucor mucedo* (17.34%), *Nigrospora sphaerica* (06.80%), *Alternaria malorum* (08.84%), *Alternaria solani* (04.76%), *Penicillium nigricans* (03.74%), *Nigrospora oryzae* (07.82%), *Fusarium solani* (05.10%) respectively.

DISCUSSION

The present study revealed the diversity of rhizospheric soil fungi associated with *Swertia petiolata* L. and *Digitalis purpurea* L. In different

localities of Kashmir valley *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Penicillium corylophilum*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Rhizoctonia solani*, *Trichoderma harzianum*, *Trichoderma viride*, *Rhizopus stolonifer*, *Mucor mucedo*, *Nigrospora sphaerica*, *Alternaria malorum*, *Alternaria solani*, *Penicillium nigricans*, *Nigrospora oryzae*, *Fusarium solani*, and *Curvularia lunata* were detected. Similar work was done by Ahmad *et al.* (2021) who also isolated ten fungal isolates from the medicinal plant, *Artemisia absinthium*. The quality of plants is largely influenced by both abiotic and biotic factors of the rhizosphere. There was found a significant variation in the frequency and relative abundance of fungi associated with *Swertia petiolata* and *Digitalis purpurea*. The highest frequency was found for *Aspergillus niger*. The relative abundance of fungi isolated from rhizosphere soil also shows significant variation. Similar studies were carried out by Motta *et al.* (2003) and isolated 49 species of filamentous fungi from the rhizospheric soil of *Helianthus annuus*. *Penicillium* and *Aspergillus* were the genera that presented the highest number of species. The findings of this investigation are similar to those of Chen *et al.* (2008) who also obtained the fungi, viz; *Penicillium chrysogenum* and *Fusarium oxysporum*, from rhizospheric soil of medicinal plants in north-eastern China. Similar study was carried out on the rhizospheric soil of other medicinal plants by different workers (Wahid *et al.* 2000; Tamilarasi *et al.* 2008). Sagar *et al.* (2009) and Sundar *et al.* (2011) also obtained several species of *Rhizobium*, *Aspergillus*, *Penicillium* and *Fusarium* and many other fungi from the rhizospheric soil samples of various medicinal plants which affected their medicinal properties and growth. Our studies are in conformity with Burni *et al.* (2011); Sagar *et al.* (2011); Shivakumar *et al.* (2012); Mir *et al.* (2017a and b) who while working on the rhizosphere soil samples of different medicinal plants reported many fungi in association with them. In the present study significant variation was observed in the frequency and relative abundance of all the rhizospheric soil fungi isolated from the rhizospheric soil of *Swertia petiolata* L. and *Digitalis purpurea* L. Among the fungi isolated from *Swertia petiolata* highest relative frequency was shown by *Nigrospora oryzae* (80.55) isolated from Gulmarg site and lowest by *Penicillium chrysogenum* (13.88) isolated from Gulmarg site. Among the fungi isolated from *Digitalis*

purpurea highest relative frequency was shown by *Mucor mucedo* (80.55) isolated from Drung site and lowest by *Fusarium solani* (13.88) isolated from KUBG site. Among the fungi isolated from *Swertia petiolata* highest relative abundance was shown by *Aspergillus niger* (17.61) isolated from Gulmarg site and lowest by *Alternaria alternata* and *Fusarium solani* (2.72 each) isolated from Gulmarg site. Among the fungi isolated from *Digitalis purpurea*, highest relative abundance was shown by *Mucormucedo* (27.10) isolated from Drung site and lowest by *Alternaria solani* and *Penicillium nigricans* (2.40 each) isolated from Gulmarg site. In similar study on other plants Dai *et al.* (2009) reported varied fungal-root association and found *Fusarium* sp. and *Verticillium* sp. in association with *Dioscorea zingiberensis*, *Atractylodes lancea*, *Euphorbia pekinensis*, *Pinellia ternate* and *Ophiopogon platyphyllum* roots. The frequency and abundance of rhizosphere fungal flora associated with some of the medicinal and aromatic plants has been reported broadly by many workers and reported significant variation with more number of fungi in the rhizosphere region as compared to the non-rhizosphere area (Srivastava and Kumar 2013; Shaikh and Nadaf 2013). Similarly, Thombre *et al.* (2016) reported *Aspergillus niger*, *Aspergillus terricola* and *Penicillium* spp. as the highly frequent and abundant fungi among isolated fungal species from rhizosphere of *Santalum album*. Similar to our results Shinkafi and Gobir (2018) reported that in the root microbiome of tomato, *Aspergillus fumigatus* and *Aspergillus niger* had the highest percentage frequency of occurrences while as *Rhizopus oryzae* and *Rhizopus stolonifer* had the lowest percentage frequency of occurrence. The variation in fungal colonization, abundance and distribution in the rhizosphere of medicinal plants varies and may depend on host plant species, growing season, soil properties, local climate and environmental factors (El-Zayat *et al.* 2008; Srivastava and Kumar, 2013). The present study carried out to characterize the rhizospheric soil fungi associated with *Swertia petiolata* and *Digitalis purpurea* will be an essential step to safeguard plant health and its productivity because below ground microbial species richness acts as predictor of aboveground plant diversity and productivity which will help in mapping the areas for cultivation of *Swertia petiolata* and *Digitalis purpurea*. This study will also help to search and isolate pathogenic fungi and beneficial fungal

bioagents for developing ecofriendly management strategies.

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