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STUDIES ON THE RHIZOSPHERIC SOIL FUNGI ASSOCIATED WITH NEPETA CATARIA L. AND RUMEX DENTATUS L. IN DISTRICT KULGAM OF KASHMIR VALLEY

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ABSTRACT

Plants get all types of nutrients from the soil either by the direct assimilation of nutrients from the soil or through various other pathways like their association with bacteria and fungi. Rhizospheric soil fungi and their association, isolation, identification and characterization with two plants viz *Nepeta cataria* L. and *Rumex dentatus L*. have been analysed. The fungi isolated from the rhizosphere soil of *Nepeta cateria* are *Rhizopus stolonifer, Pencillium notatum, Pencillium* sp. and *Aspergillus niger*. While the fungi isolated from rhizosphere of *Rumex dentatus* are *Rhizopus stolonifer, Aspergillus niger* and *Pencillium notatum.* Among these fungi the highest frequency was of *Pencillium* sp. (100%) in case of *Nepeta cateria* followed by *Rhizopus stolonifer* (80%), *Penicillium notatum* (60%) and *Aspergillus niger* (60%). While in case of *Rumex dentatus*, the highest frequency was observed in both *Rhizopus stolonifer (100%)* and *Pencillium notatum* (100%) followed by *Aspergillus niger* (80%)

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INTRODUCTION

Soil is an ecosystem comprising weathered rock, organic matter, animals, plants and micro-organisms that together support the life on earth. Soil is natural body known as 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 unconsolidated mineral or organic material on the surface of earth that serves as a natural medium for the growth of land plants (Chesworth, 2008). Soil is an oligotrophic medium for growth of fungi, and fungi strew organic matter away from the roots (Dighton *et al.,* 2005), thereby fungi are vital components of soil life. The area of the soil influenced by root varies with the type of the plant, age of the plant, soil conditions, pH of the soil, environmental conditions and moisture content of the soil, as such the diversity of the soil microflora increases including the soil fungi. It is now an established fact that the nature of the root exudates determines the fungus flora of the rhizosphere soil. Extensive studies have been made in recent years which deal with the fungus flora of the rhizosphere soil of crop plants, (Mehrotra and Kakkar 1972). The diversity and functions of microbes in the rhizosphere, a narrow region around the root, are related to the root exudates (proteins and sugars), biogeochemical reactions and respiration (Narula *et al.,* 2009). *Nepeta* is a genus of flowering plants in the family lamiaceae also known as catmints or catnip. There are about 250 Species of *Nepeta* (Nepeta flora of China). Catnip has diuretic properties and may increase amount and frequency of urination. *Rumex dentatus* is a annual medicinal herb. It is also known as toothed dock and Aegean dock. These plants contain quite high level of oxalic acid, which gives the leaves of its members of this genus an acid-lemon flavor. The oxalic acid content will be reduced if the plant is cooked. People with a tendency to rheumatism, arthritis, gout, kidney stones or hyperacidity should refrain including this plant in their diet, since it can aggravate their condition (Chopra *et al*., 1956).

EXPERIMENTAL

MATERIALS AND METHODS

All the glass wares were cleaned properly. Initial cleaning was done by laboline. After washing with laboline, glass wares were then washed with mercuric chloride followed by distilled water and allowed to air dry. After washing, glassware were thoroughly dried and sterilized properly to make them aseptic. The media used for the isolation, culturing and propagation of soil fungi in the present study was Potato Dextrose Agar, Richard's Medium.

Collection of soil samples

Various soil samples were collected from the rhizospheric area of two medicinal plants, viz. *Nepeta cataria* and *Rumex dentatus* from different sites of Yaripora area of District Kulgam of J&K. Samples were collected at random from each site with the help of soil auger up to a depth of 15cm near the plant base. Three to four samples were collected from each plant species and these soil samples were brought to laboratory, marked and packed in polythene bags. First of all we have to air dry the soil samples and then these soil samples were thoroughly mixed to make a composite soil sample.

Study of soil fungi

To study the soil fungi from the rhizosphere area of *Nepeta cataria and Rumex dentatus*, the following methods were employed.

Isolation of fungal flora

Isolation of the fungi associated with rhizospheric area *of Nepeta cataria* and *Rumex dentatus* was done by dilution plate method (Dickson and Pugh, 1965). In this method 1g of soil sample was taken from the composite soil samples of *Nepeta cataria* and *Rumex dentatus*. This 1g of soil sample is dissolved in 100ml of distilled water in sterilized test tubes and labeled as 1:100 dilution. Then serial dilutions were done from the first dilution up to 10-6. Inoculation was done in laminar air flow chamber; 10ml of the Richard's medium were transferred to each Petri plate. To the medium 1 ml of the soil suspension was added and homogenized. These Petri plates were then allowed to solidify in the laminar air flow chamber for 10-15 minutes and then incubated in BOD incubator at 25±2^{o}C for 5-7 days. These Petri plates were then observed for fungal growth and the number of fungal colonies was recorded.

Identification of fungi

The cotton blue and lectophenol wet mount preparation was used to prepare fungal slides, to observe and identify the fungi isolated from the rhizospheric area *of Nepeta cataria* and *Rumex dentatus*. Fungi are stained in order to make the microscopic characteristics clearly visible. The slides were observed under microscope and identification was done by monographs and relevant literature (Ellis, 1971; Gilman, 2008)

Raising and maintenance of pure culture of fungi

The pure culture of fungi encountered during isolation from the petri plates was maintained on Richard synthetic agar medium.

Observations and Results

The present study was undertaken to study the rhizospheric fungi associated with *Nepeta cataria* and *Rumex dentatus* in different localities of District Kulgam, J & K. The soil fungi identified on the basis of cultural and microscopic characteristics were, *viz. Rhizopus stolonifer, Pencillium* sp., *Penicillium notatum*, *Aspergillus niger.*

Characteristics of rhizospheric fungi associated with *Nepeta cataria* L. and *Rumex dentatus L.*

It was observed from the study that Rhizopus stolonifer, Pencillium sp., *Penicillium notatum* and *Aspergillus niger* were isolated from the rhizospheric soil of *Nepeta cataria* and Rhizopus stolonifer, *Penicillium notatum* and *Aspergillus niger* were islated from the rhizospheric soil of Rumex dentatus.

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

Cultural characteristics

Colonies of *Rhizopus stolonifer* grow very rapidly fill the Petri dish and mature in 4 days. The texture is typically cottony from the front; the color of the colony is white initially and turns grey to yellowish brown in time. The reverse is white to pale.

Microscopic characteristics

Mycelium is non-septate and branched having three types of branches sporangiophores, rhizoids (root like hyphae) and stolans. Sporangiophores are brown in color and usually unbranched. They can be formed in clusters. Rhizoids are located at the point where the stolons and sporangiophores meet. Sporangia are located at the tip of the sporangiophores. They are round with flattened bases. Apophysis is absent or rarely apparent and columellae are hemispherical Sporangiospores (4-11 µm in diameter) are unicellular, round to avoid in shape, hyaline to brown in color, and smooth.

***Aspergillus niger* Van Tieghem**

Cultural characteristics

Aspergillus colonies are usually fast growing, black in colour and they mostly consists of a dense felt of erect conidiophores.

Microscopic characteristics

Mycelium is septate and hyaline and heads are initially radiate. *Aspergillus niger* is biseriate wherein its vesicles form sterile cells called as metulae that support the conidiogenous phialides. Conidiophores are hyaline, smooth- walled, with length ranging from 400-300 µm long, and are becoming darker at the apex and terminating in a globose vesicles with size of 30- 75 µm in diameter. Metulae and phialides cover the entire surface of the vesicle. Conidia are globose, brown to black in colour, 4-5 µm in diameter.

***Pencillium* sp. Link**

Cultural characteristics

Colonies were fast growing, white in colour initially and the turns green, mostly consisting of a dense felt of conidiophores. Reverse colour is creamish.

Microscopic characteristics

Mycelium consists of a highly branches network of multinucleate, septate, usually colorless hyphae. Many branched conidiophores arise from the mycelia, bearing individually constricted conidiophores. The conidiophores are the main dispersal route of the fungi, and often are green in color. Microscopically, chains of single celled conidia are produced in basipetal succession from a specialized conidiogenous cell called a phialides. Phialides produce singly, groups or from branched metulae, giving a brush like appearance known as a penicillus. Conidiophores are hyaline smooth or rough.

***Penicillium notatum* Thom**

Cultural characteristics

The colonies of *Penicillium notatum* are blueish green with velvety texture. The reverse colour is yellowish white. Colonies are arranged in concentric circles alternately green and white and a significant green division. There is a heap of white mycelium at the center.

Microscopic characteristics

The thallus (mycelium) typically consists of a highly branched network of multinucleate, septate, usually colorless hyphae, many-branched conidiophores sprout on the mycelia, bearing individually constricted conidiospores. The conidiospores are the main dispersal route of the fungi and often are green in color.

Table 1. Rhizospheric soil fungi associated with *Nepeta cataria* L. in District Kulgam.

Soil fungi	Rhizospheric Fungal population				
	1	2	3	4	5
Rhizopus stolonifer	-	+	+	+	+
Pencillium sp.	+	+	+	+	+
Pencillium notatum	-	+	+	+	-
Aspergillus niger	+	-	+	+	-

Table 2. Rhizospheric soil fungi associated with *Rumex dentatus L.* in District Kulgam.

Soil fungi	Rhizospheric Fungal population				
	1	2	3	4	5
Rhizopus stolonifer	+	+	+	+	+
Aspergillus niger	+	+	+	-	+
Pencillium notatum	+	+	+	+	+

Frequency of soil fungi associated with *Nepeta cataria* L. and *Rumex dentatus L*.

The fungi isolated from the rhizosphere soil of *Nepeta cateria* are *Rhizopus stolonifer, Pencillium notatum, Pencillium* sp. and *Aspergillus niger*. While the fungi isolated from rhizosphere of *Rumex dentatus* are *Rhizopus stolonifer, Aspergillus niger* and *Pencillium notatum.* Among these fungi the highest frequency is of *Pencillium* sp. (100%) in case of *Nepeta cateria* followed by *Rhizopus stolonifer* (80%), *Penicillium notatum* (60%) and *Aspergillus niger* (60%). While as in case of *Rumex dentatus,* the highest frequency was observed in both *Rhizopus stolonifer* (100%) and *Pencillium notatum* (100%) followed by *Aspergillus niger* (80%) (Table 3).

Table 3. Frequency of Soil fungi associated with *Nepeta cataria* L. *and Rumex dentatus* L.

Soil fungi	Frequency of fungi	
	Nepeta	***Rumex***
Rhizopus stolonifer	80	100
Pencillium notatum	60	100
Pencillium sp.	100	0
Aspergillus niger	60	80

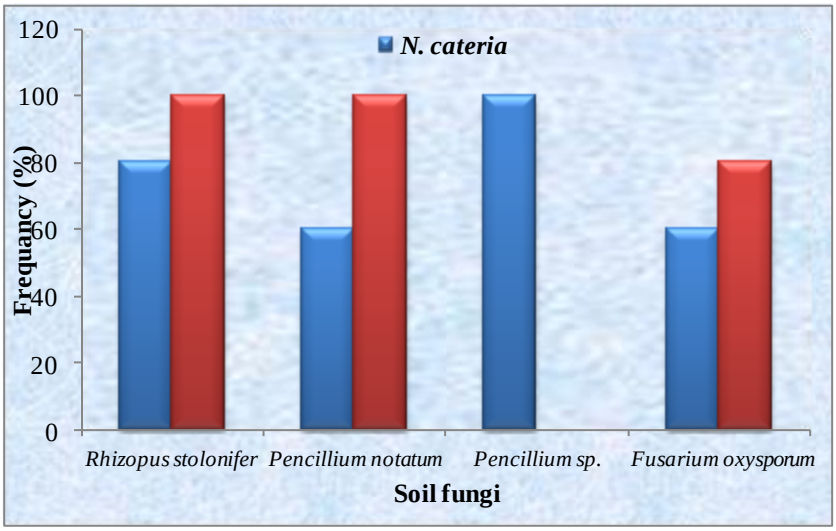


Fig 1. Frequency of soil fungi associated with *Nepeta cataria* L. *and Rumex dentatus L*.

Relative abundance of Soil fungi isolated from *Nepeta cataria* L. *and Rumex dentatus L.*

Among the fungi isolated from the rhizosphere of *Nepeta Cataria and Rumex dentatus* the highest relative abundance was found in case of *Penicillium sp.* (40.05) followed by *Pencillium notatum* (22.16) *and Aspergillus niger* (22.16) and *Rhizopus stolonifer* (13.62). In case of *Rumex dentatus* the fungi obtained from the rhizosphere soil are *Rhizopus stolonifer*, *Pencillium notatum, Penicillium* sp. and *Aspergillus niger*. The highest relative abundance is seen in both *Penicillium notatum* (43.00) followed by *Rhizopus stolonifer* (32.71) and *Aspergillus niger* (24.30).

Table 4. Number of colonies of soil fungi present in each Petri plate associated with *Nepeta cataria* L.

Soil fungi	Number of colonies				
	1	2	3	4	5
Rhizopus stolonifer	0	6	3	10	5
Pencillium sp.	25	12	11	10	16
Pencillium notatum	0	23	9	7	0
Aspergillus niger	16	0	13	10	0

Table 5. Number of colonies of soil fungi present in each Petri plate associated with *Rumex dentatus L*.

Soil Fungi	Number of colonies				
	1	**2**	**3**	**4**	**5**
Pencillium notatum	13	12	10	5	6
Rhizopus stolonifer	4	8	7	10	6
Aspergillus niger	8	2	7	0	9

Table 6. Relative abundance of soil fungi associated with *Nepeta cataria* L. and *Rumex dentatus* L.

Soil Fungi	Relative abundance (%)	
	Nepeta cataria	***Rumex dentatus***
Rhizopus stolonifer	13.62	32.71
Pencillium notatum	22.16	43.00
Pencillium sp.	40.05	0.00
Aspergillus niger	22.16	24.30

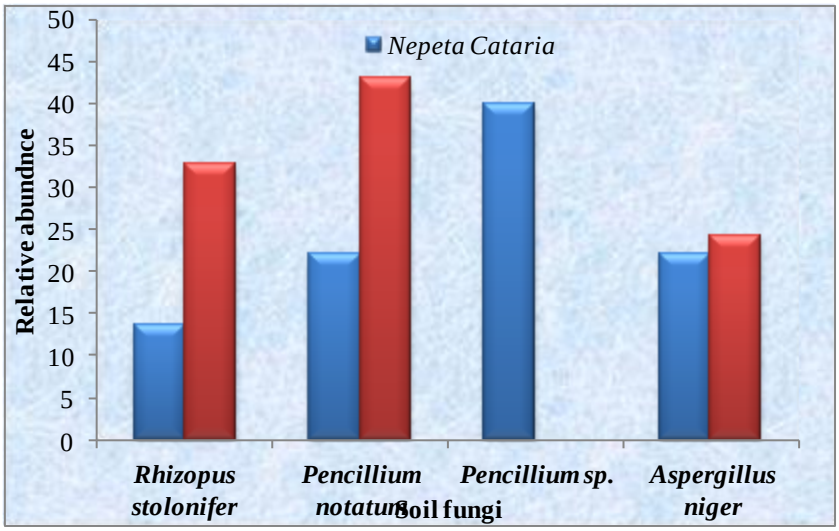
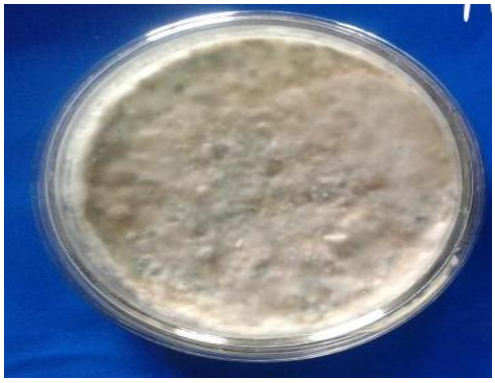


Fig 2.Relative abundance of soil fungi associated with *Nepeta cataria* L. and *Rumex dentatus L*.

Fig. a *Nepeta cataria* L.

Fig. b *Rumex dentatus* L.



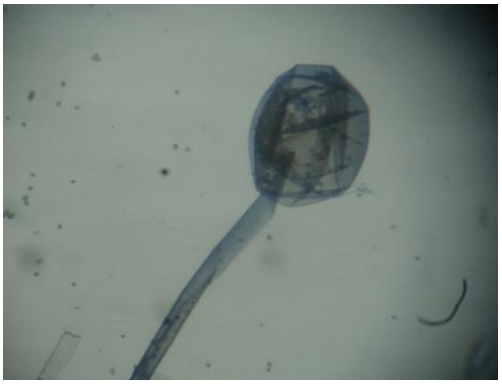


Plate 1 *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill **Showing culture of *Rhizopus stolonifer*.**

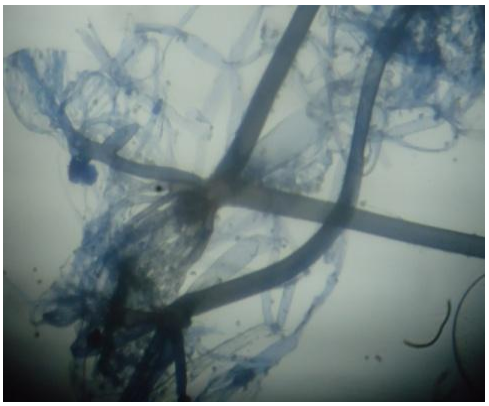
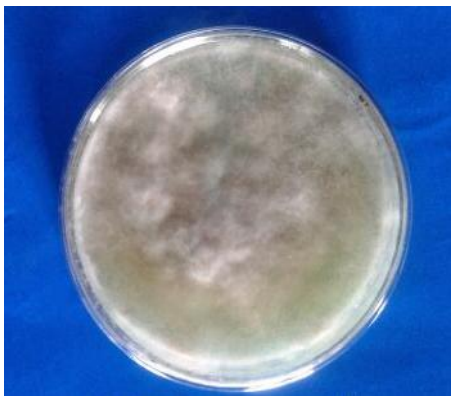
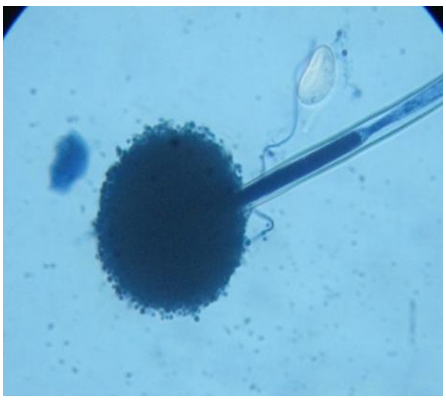


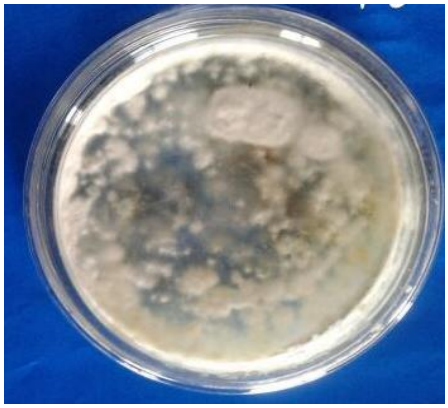
Plate 2 Showing Sporangia and Rhizoids of *Rhizopus stolonifer* (Ehrenb.: Fr.)Vuill.



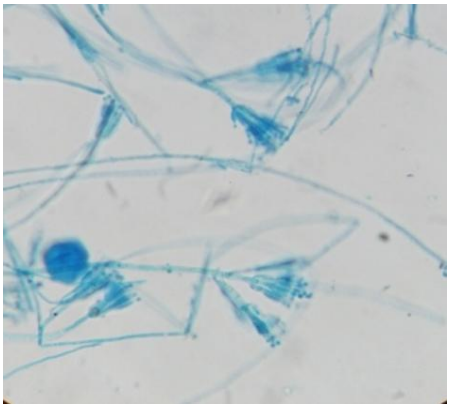


a. Showing culture of Aspergillus niger. **b. Showing Sporangiophores.**

Plate 3 *Aspergillus niger* Van Tieghem



a. showing culture of Penicillium notatum.



b. Showing Sporangiophores.

Plate 4 *Penicillium notatum* Thom

DISCUSSION

The quality of medicinal plants (active compound content) is largely influenced by abiotic and biotic factors of the rhizosphere. Present study revealed that the rhizospheric fungi associated with *Nepeta cataria* and *Rumex dentatus* in different localities of District Kulgam, of Kashmir Valley are: *Rhizopus stolonifer, Penicillium notatum, Penicillium* sp. and *Aspergillus niger*. The fungi isolated from the rhizosphere soil of *Nepeta cateria* are *Rhizopus stolonifer, Pencillium notatum, Pencillium* sp. and *Aspergillus niger*. While the fungi isolated from rhizosphere of *Rumex dentatus* are *Rhizopus stolonifer, Aspergillus niger* and *Pencillium notatum*. There was found significant variation in the frequency and relative abundance of fungi associated with *Nepeta cataria* and *Rumex dentatus*. The highest frequency was found for *Pencillium* sp. followed by other soil fungi in case of *Nepeta cataria* and *Rumex dentatus,* whereas the highest frequency was observed in both *Rhizopus stolonifer* and *Pencillium notatum* followed by other fungi. Relative abundance in fungi isolated from rhizosphere of *Nepeta cataria* and *Rumex dentatus* also showed significant variation. Our results are in accordance with the previous works done by *Mehrotra and Kakkar (1972).* Tamilarasi *et al.* (2008) reported diversity of root associated microorganisms of selected medicinal plants and influence of rhizo-microorganisms on the antimicrobial property of *Coriandrum sativum*. Muneer (2005) also observed significant variation in the rhizospheric fungal flora of some vegetable. He has reported 12 species of fungi from rhizosphere of some vegetables growing in Kashmir. Likewise, some workers have reported that several species of fungi are associated with important crop plants including medicinal plants (Berg *et al.,* 2005; Sagar and Kumari, 2009; Kumar *et al.,* 2013). Therefore, further research is recommended to better understand the diversity and function of rhizosphere fungi and their uses in the increased production of medicinal plants by identifying relationship between genetic and functional diversity of fungal species.

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