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Fungal Associates of *Centella asiatica* and *Ocimum sanctum*

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Fungal associates of two medicinal plants (*Centella asiatica* and *Ocimum sanctum*) have been isolated and identified in the present study. Rhizosphere soil samples collected from the root vicinity of these plants revealed the presence of sixteen and seventeen species of fungi respectively. Further, screening of these soil samples for isolating VAM spores revealed nine and seven species of fungi in case of *Centella asiatica* and *Ocimum sanctum* respectively. The endophytic fungi were also isolated from the roots and leaves of *Centella asiatica* (4 spp.) and *Ocimum sanctum* (3 spp.).

Key words: Rhizosphere, VAM, Endophytes, Medicinal plants.

The world of fungi provides a fascinating and almost endless source of biological diversity, which is a rich source for exploitation[1]. Fungi are ubiquitous and there is no material of biological origin that remains free of fungi. Some fungi are widely distributed in soil and some fungi live in plants as endophytes or as symbiotic partners with algae (Lichens) and plant roots (Mycorrhizae), enabling them to grow under harsh conditions which they could not do otherwise[2].

In recent years, the quest for the isolation of new compounds from medicinal plants has become a fascinating area of research. Plants with ethnopharmaceutical importance are being exploited because of there healing properties[3]. However large scale harvesting of medicinal plants has already become a major threat to biodiversity, as an alternative, microbes which live in such plants may offer tremendous potential sources of therapeutic compounds.

So far only few publications have reported the isolation of rhizosphere (root-soil interface), vesicular arbuscular mycorrhizal (fungus root) and endophytic (symptomless parasites) fungi of ethnopharmaceutically important medicinal plants. In view of the importance of these fungi (as potential biocontrol agents, source of bioactive compounds and in plant protection and industrial applications), it was considered worthwhile to study the fungal

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associates of two medicinal plants namely, *Centella asiatica* (Brahmi) and *Ocimum sanctum* (Tulsi).

These plants have been used for thousands of years in India and still have a central place in Ayurvedic medicine. While *Centella asiatica* has a longstanding reputation as a 'rejuvenator' helping in concentration and memory; *Ocimum sanctum*, the sacred herb has a wide range of uses in relieving fevers, bronchitis, asthma, stress and mouth ulcers. Because of their high medicinal value, enhancement of the growth and bioactive agents of these plants are desirable.

Materials and Methods

Root adhering soil samples of *Centella asiatica* and *Ocimum sanctum* were collected from Distt. Solan of H.P. and brought to the laboratory for further analysis. Then isolation of rhizosphere fungi was done by Dilution Plate Method[4,5]. The media used for isolating rhizosphere were Potato Dextrose Agar[6] and Czapek's Dox[7].

The spores of VAM fungi were isolated from the soil surrounding the roots of these plants by Wet Sieving and Decanting method of[8]. Fine roots of these plants were also assessed for VAM fungal infection by using method of[9].

The roots and leaves of these plants were also screened for the presence of endophytes by using Hot Water treatment (i.e. the samples were cut into small pieces and then soaked into hot water (60°C) for 15 minutes in a test tube and then inoculated on petriplates having PDA medium and incubated at 22±1°C for one week) and three step method (i.e. Firstly, the small samples pieces were washed with distilled water and then surface sterilized with 25% methanol for 5 min, followed by 50% methanol for 3 min, and 75% methanol for 2 min. and finally in sterilized water for 5 min. Then these pieces were inoculated on petriplates containing PDA medium and incubated at 22±1°C for few days). Fungi were identified following[10,11,12] and identification was further authenticated by Dr. S. K. Singh, A. R. I. Pune. Accession no. for these fungi has been taken and cultures have been deposited in A. R. I. Pune.

RESULTS AND DISCUSSION

A total number of 16 species of fungi and three non sporulating mycelia; and 17 species of fungi and one non sporulating mycelium were isolated from the rhizosphere of *Centella asiatica* and *Ocimum sanctum* respectively during different seasons (Table 1 and 2).

The study revealed the dominance of *Aspergillus* and *Penicillium* genera during different seasons. The comparison of different fungal genera in both plants revealed that *Aspergillus terreus*, *Absidia cylindrospora*, *Cladosporium oxysporum*, *Cylindrocladium* sp., *Mucor plumbeus*, *Phoma* sp. and *Syncephalastrum racimosum* were recorded in *Centella asiatica* only and *Aspergillus ustus*, *Macrophomima* sp., *Pestalotia* sp., *Rhizopus oryzae*, *Thysanophora* sp. and *Trichoderma viride* were recorded in *Ocimum sanctum* only. The

Table 1. List of Fungi Isolated from Rhizosphere Soil Samples of *Centella asiatica*

S.No.	Name of fungal genera	Name of species
1.	*Absidia*	*Absidia cylindrospora*
2.	*Aspergillus*	*Aspergillus niger*, *Aspergillus terreus*, *Aspergillus wentii*, *Aspergillus* sp.
3.	*Cladosporium*	*Cladosporium oxysporum*
4.	*Cylindrocladium*	*Cylindrocladium* sp. close to *C. scoparium*
5.	*Emericella*	*Emericella nidulans*
6.	*Fusarium*	*Fusarium moniliforme* var. *anthophilum*
7.	*Mucor*	*Mucor plumbeus*
8.	*Myrothecium*	*Myrothecium* sp. close to *M. roridum*
9.	*Paecilomyces*	*Paecilomyces* sp. close to *P. variotii*
10.	*Penicillium*	*Penicillium purpurogenum*, *Penicillium* sp.
11.	*Phoma*	*Phoma* sp.
12.	*Syncephalastrum*	*Syncephalastrum racimosum*
13.	Non sporulating mycelia	

genera which were recorded in both plants are *Emericella nidulans*, *Fusarium moniliforme* var. *anthophilum*, *Myrothecium* sp. and *Paecilomyces* sp.

In the present investigation, maximum no. of fungi was recorded during the rainy season followed by spring, summer and winter season. The variation in individual fungal species distribution depends upon the type of soil, depth, season of the year, concentration of organic matter and isolation procedure employed[13].

Both plant species show VAM colonization in the roots (fig. d and e). Nine and seven species of VAM fungi were isolated from the soil samples of *Centella asiatica* and *Ocimum sanctum* respectively (Table 3).Comparison of VAM fungi isolated from both plants revealed that *Glomus* was the most dominant genus followed by *Gigaspora*. Mohan *et al*.[14] found high soil spore population and percent colonization in their study on AM association with some important medicinal plants of Tamil Nadu.

Table 2. List of Fungi Isolated from Rhizosphere soil samples of *Ocimum sanctum*

S.No.	Name of fungal genera	Name of species
1.	Aspergillus	*Aspergillus niger, Aspergillus ustus, Aspergillus wentii, Aspergillus* sp.
2.	Emericella	*Emericella nidulans*
3.	Fusarium	*Fusarium moniliforme* var. anthophilum
4.	Macrophomina	*Macrophomina* sp.
5.	Myrothecium	*Myrothecium* sp. close to M. roridum
6.	Paecilomyces	*Paecilomyces variotii*
7.	Penicillium	*Penicillium* sp. 1, 2, 3 and 4
8.	Pestalotia	*Pestalotia* sp.
9.	Rhizopus	*Rhizopus oryzae*
10.	Thysanophora	*Thysanophora* sp. close to T. penicilloides
11.	Trichoderma	*Trichoderma viride*
12.	Non sporulating mycelium	

Table 3. Comparative List of VAM Fungal Spores Isolated from the Rhizosphere Soil Samples of *Centella asiatica* and *Ocimum sanctum*

S. No.	Name of VAM fungus isolated	*Centella asiatica*	*Ocimum sanctum*
1.	*Acaulospora laevis*	+	+
2.	*Endogone macrocarpa*	+	-
3.	*Gigaspora calospora*	-	+
4.	*Gigaspora candida*	+	-
5.	*Gigaspora gigantea*	+	+
6.	*Gigaspora* sp.	+	-
7.	*Glomus albidum*	-	+
8.	*Glomus fasciculatum*	+	+
9.	*Glomus fulvum*	+	-
10.	*Glomus macrocarpum*	-	+
11.	*Glomus maculosum*	+	-
12.	*Glomus mosseae*	-	+
13.	*Glomus reticulatum*	+	-

+ = Present – = Absent

Four endophytic fungal species; and three sporulating and one non sporulating endophytic fungal spp. were isolated from the root and leaves of *Centella asiatica* and *Ocimum sanctum* respectively (Table 4). Mahesh *et al*.[15] and Shukla *et al*.[16] also observed almost similar endophytic fungal genera in *Azadirachta indica*, *Shorea robusta*, *Pterocarpus marsupium*, *Terminalia arjuna*, *T. bellirica* and *T. chebula*.

Table 4. Comparative List of Endophytic Fungi Isolated from Roots and Leaves of *Centella asiatica* and *Ocimum sanctum*

S. No.	Name of Endophytic Fungus	*Centella asiatica*	*Ocimum sanctum*
1.	*Aspergillus niger*	+	+
2.	*Curvularia prasadii*	+	-
3.	*Fusarium* sp.	-	+
4.	*Penicillium purpurogenum*	-	+
5.	*Penicillium* sp.	+	-
6.	*Rhizopus oryzae*	+	-
7.	*Chlamydospore like bodies*	-	+

Mycoflora isolated from the rhizosphere soil samples and leaf and root of these plants did not show any uniform pattern of appearance and distribution. Such variations have also been recorded by earlier workers with different plants[17,18]. The qualitative and quantitative variations in the rhizosphere mycoflora may be due to different plant species and altered exudation pattern of root system[19,20].

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