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RESEARCH ARTICLE

FUNGAL DIVERSITY OF *SIDA ACUTA* AND *CENTELLA ASIATICA* IN LAKKAVALLI RANGE FOREST OF SHIVAMOGGA DISTRICT, KARNATAKA

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ABSTRACT

Lakkavalli range forest is dry deciduous forest comes under bhadra wild life sanctuary. Rhizosphere soil samples were collected from *Sida acuta* and *Centella asiatica* and analyzed for diversity. Fungal species were enumerated by serial dilution method. Thirty six fungal species belonging to twenty one genera were recorded. Among these, 36 fungal species belonging to 21 genera from *Sida acuta* and 29 fungal species belonging to 17 genera from *Centella asiatica* soils and two non sporulating fungi were recorded from both soils. Among these, *Aspergillus* was the dominant in both soils followed by *Penicillium* and *Trichoderma* species. Occurrence of soil fungal diversity also depends on the season due to fluctuation in climate. Maximum fungal isolate were isolated during Nov to Dec 2009 minimum in Feb 2010. Soil pH, soil moisture and acidic condition of soil and vegetation type also influence on the fungal diversity.

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INTRODUCTION

Soil microorganisms play a important role in soil processes that determine plant productivity (Tilak *et al.*, 2005). According to Waksman (1916) "without soil microbes life on this plant would come to stand still". Thousand of different species of fungi harbour the soil. Rhizosphere is an important site of microbial activity and central component of ecosystems and biogeochemical cycles (Dessaux *et al.*, 2009). Population of soil fungi ranges from 2 x 10⁴ to 10⁶ propagules per gram of dry soil (Veeresh and Rajgpal, 1988). The role of fungi in the soil is an extremely complex and is fundamental to the soil ecosystem (Warcup, 1951; Bridge and Spooner, 2001). Out of 1.5 million of fungi only 50% are characterized until now (Hawksworth, 2001). One third of fungal diversity of the globe exists in India. About 205 new genera have been described from India of which 32% were discovered by Subramanian (see in Manoharachary *et al.*, 2005). Some fungi are widely distributed in soil and other is limited to some habitat (Saravanakumar and kaviyaran, 2010). Many researches were done in agricultural soils but less attention towards natural habitat. Hence the present study was undertaken to fulfill the lacuna.

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MATERIALS AND METHODS

Study area

In Lakkavalli forest area *Sida acuta* and *Centella asiatica* were selected under dry deciduous forest. It has elevation 690 – 750 meters above MSL. Location is 13° 45.3' to North latitude and 75° 36.5' East longitude. Rainfall is 300 – 500 mm annually. Temperature is about 24.3°C average per annum. Soil is hornblende type soil and whilst chlorite schist type soil (mixture of soft ferruginous clay loam soil).

Collection of soil samples

The soil samples were collected from rhizosphere soil of selected herbs from forest in Lakkavalli range forest during 2009-2010. Soil samples were collected from the root of selected herbs at the depth of 10-15 cm was collected along with superficial ones from rhizosphere. The collected soil samples during survey are brought to the lab by cloth bags and subjected for serial dilution to screen fungi present in the soil samples.

Screening of soil samples

Serial dilution

One gram of soil sample was suspended or agitated in a known volume of sterile water blank (9 ml or so to make the total

Table 1. Percent contribution of fungal species of *Sida acuta* and *Centella asiatica*

Sl. No.	Name of the fungal species	<i>Sida acuta</i>	<i>Centella asiatica</i>	Periodicity of Occurrence
1.	<i>Absidia gluca</i>	1.21	1.22	C
2.	<i>Alternaria alternata</i>	0.49	--	O
3.	<i>Aspergillus candidus</i>	1.21	--	R
4.	<i>Aspergillus clavatus</i>	0.24	--	O
5.	<i>Aspergillus flavus</i>	6.07	6.08	F
6.	<i>Aspergillus fumigatus</i>	4.85	4.56	F
7.	<i>Aspergillus nidulans</i>	5.10	3.65	F
8.	<i>Aspergillus niger</i>	6.80	7.29	F
9.	<i>Aspergillus ochraceus</i>	2.43	2.43	C
10.	<i>Aspergillus sydowii</i>	1.94	1.82	R
11.	<i>Aspergillus terreus</i>	3.64	3.65	C
12.	<i>Aspergillus versicolor</i>	2.91	3.04	F
13.	<i>Chaetomium glabosum</i>	0.49	--	O
14.	<i>Chaetomium reflexum</i>	0.24	--	O
15.	<i>Cladosporium cladosporioides</i>	5.83	6.69	F
16.	<i>Cladosporium herbarum</i>	4.85	2.43	C
17.	<i>Cunninghamella echinulata</i>	1.94	0.91	R
18.	<i>Cylindrocladium</i> sp.	2.18	1.82	C
19.	<i>Fusarium oxysporum</i>	1.46	1.52	C
20.	<i>Gliocladium roseum</i>	7.04	8.51	F
21.	<i>Graphium penicillioides</i>	0.49	--	O
22.	<i>Mycogone</i> sp.	0.24	0.61	O
23.	<i>Nigrospora</i> sp.	1.21	1.52	R
24.	<i>Paecilomyces</i> sp.	1.70	1.82	R
25.	<i>Penicillium chrysogenum</i>	3.64	4.26	F
26.	<i>Penicillium citrinum</i>	4.61	4.56	C
27.	<i>Penicillium digitatum</i>	4.37	4.86	F
28.	<i>Penicillium</i> sp.	1.46	1.52	R
29.	<i>Pestalotiopsis</i> sp.	0.49	1.22	R
30.	<i>Phoma herberum</i>	0.24	0.30	O
31.	<i>Rhizopus</i> sp.	3.64	3.04	C
32.	<i>Robillarda sessilis</i>	--	1.52	O
33.	<i>Syncephalastrum racemosum</i>	0.49	--	O
34.	<i>Trichoderma harzianum</i>	2.43	6.38	F
35.	<i>Trichoderma koningii</i>	1.94	3.65	C
36.	<i>Trichurus spiralis</i>	1.46	--	R
37.	<i>Verticillium</i> sp.	2.67	0.91	C
38.	NSF-1 white colour	3.88	3.65	C
39.	NSF-2buff colour	4.37	4.56	C

C- Common; F- frequent; O-Occasional; R-Rare

volume to 10ml) to make suspension. This is 10^{-1} dilution. Further serial dilutions 10^{-2} to 10^{-6} are made which the suspension is in motion, by withdrawing 1ml into additional dilution blanks having 9ml sterile water in test tubes respectively. Dilution 10^{-3} plated on petri plates containing different media (Potato dextrose agar and czapek's Dox agar) and incubated in inverted position for four to seven days at $27\pm 3^{\circ}\text{C}$ and study the characterization of fungi (Aneja, 2006; Dhingra and Sinclair, 1993).

Identification of fungi

Colonies developed in plates were first observed under stereobinocular microscope for colony colour, shape of conidial head and mycelia spreading. Fungal characterization is done on the basis of structure of mycelium, conidiophore, shape of conidiophore, arrangement of conidia on conidiophore and also on the basis of spores, shape, size and structure. Identification was done by using standard literature (Domsch *et al.*, 1980; Nagamani *et al.*, 2006).

Statistical analysis

Species richness (S) = Total number of species in a given area

Simpson's diversity index, D = $1/\sum p_i^2$

Where, D= Simpson's diversity index

Pi is the proportion of the ith species to total abundance value.

Shannon-wiener's diversity index, H' = $-\sum p_i \ln p_i$

H'= Shannon-wiener's diversity index

Pi is the proportion of the ith species to total abundance value.

Shannon evenness index, J' = H'/H'_{max} , Were used for evaluation of species richness of fungal isolates (Magurran, 1988)

RESULTS AND DISCUSSION

Thirty six fungal species belonging to twenty one genera were recorded. Among these thirty six fungal species belonging to twenty one genera from *Sida acuta* and twenty nine fungal species belonging to seventeen genera from *Centella asiatica* rhizosphere soils and two non sporulating fungi were recorded from both soils. Out of Thirty eight fungal isolates thirty six were recorded from *Sida acuta* rhizosphere soil and twenty nine fungal isolates from *Centella asiatica* rhizosphere soil. Large number of species was recorded in *Aspergillus* genera followed by *Penicillium* and *Trichoderma*. *Aspergillus* and *Penicillium* were dominate in both soil (Galloway, 1936). *Gliocladium roseum* was dominant in both *Sida acuta* (7.04%)

and *Centella asiatica* (8.51%) soils. In *Sida acuta* has *Aspergillus niger* 6.80% followed *Aspergillus flavus* 6.07%, *Cladosporium cladosporioides* 5.83%, *Aspergillus fumigates* and *Cladosporium herbarum* 4.85%, *Penicillium citrinum* 4.61%, *Aspergillus versicolor* 2.91%, *Trichoderma harzianum* 2.43%, *Cunninghamella echinulata* 1.94%, *Trichurus spiralis* 1.46%, *Absidia gluca* 1.21%, *Chaetomium glabosum* 0.49% and *Mycogone* sp. 0.20%. In *Centella asiatica* *Aspergillus niger* has 7.29% followed *Cladosporium cladosporioides* 6.69%, *Trichoderma harzianum* 6.38%, *Penicillium digitatum* 4.86%, *Penicillium citrinum* 4.56%, *Penicillium chrysogenum* 4.26%, *Aspergillus versicolor* 3.04%, *Cladosporium herbarum* 2.83%, both *Cylindrocladium* sp. and *Paecilomyces* sp. has 1.82%, *Fusarium oxysporum*, *Nigrospora* sp., *Penicillium* sp., has 1.52%, *Pestalotiopsis* sp., 1.22%. *Cunninghamella echinulata* and *Verticillium* sp., 0.91%, *Mycogone* sp., 0.61% and *Phoma herberum* 0.30%.

Out of Thirty-eight fungal species, thirteen were commonly occurring *Absidia gluca*, *Aspergillus ochraceus*, *A. terreus*, *Chaetomium glabosum*, *Cladosporium herbarum*, *Cylindrocladium* sp, *Fusarium oxysporum*, *Penicillium citrinum*, *Rhizopus* sp. *Trichoderma koningii*, *Verticillium* sp. NSF-1 and NSF-2 recorded. Ten species were frequently occurring they are *Aspergillus flavus*, *A. fumigates*, *A. nidulans*, *A. niger*, *A. versicolor*, *Cladosporium cladosporioides*, *Gliocladium roseum*, *Penicillium chrysogenum*, *P. digitatum* and *Trichoderma Harzianum*. Nine occasionally recorded they are *Alternaria alternata*, *Aspergillus clavatus*, *Chaetomium glabosum*, *C. reflexum*, *Graphium penicillioides*, *Mycogone* sp., *Phoma herberum*, *Robillarda sessilis* and *Syncephalastrum racemosum*. Rarely occurring fungal species were *Aspergillus candidus*, *A. sydowii*, *Cunninghamella echinulata*, *Nigrospora* sp., *Paecilomyces* sp., *Penicillium* sp., *Pestalotiopsis* sp. and *Trichurus spiralis*.

Table 2. Various measures of diversity

Sl. No.	Diversity indices	<i>Sida acuta</i>	<i>Centella asiatica</i>
1.	Taxa_S	38	31
2.	Dominance_D	0.041	0.046
3.	Shannon_H	3.344	3.22
4.	Simpson_1-D	0.959	0.954
5.	Evenness_e ^H /S	0.7457	0.8094

Robillarda sessilis species occurs only in *Centella asiatica* rhizosphere soil. *Alternaria alternata*, *Aspergillus candidus*, *A. clavatus*, *Chaetomium glabosum*, *C. reflexum*, *Graphium penicillioides*, and *Syncephalastrum racemosum* and *Trichurus spiralis* occur only in *Sida acuta* rhizosphere soil. The diversity indices of both the sample were almost nearly same. Shannon-wiener's diversity index of *Sida acuta* rhizosphere soil was 3.344 and in *Centella asiatica* rhizosphere soil 3.222. Simpson's diversity index of *Sida acuta* rhizosphere soil was 0.959 and in *Centella asiatica* rhizosphere soil 0.954. Evenness was highest in *Centella asiatica* rhizosphere soil 0.8094 (Table-2). Richness of fungal species was thirty eight.

Conclusion

Thirty six fungal species belonging to twenty one genera were recorded. Among these thirty six fungal species belonging to twenty one genera from *Sida acuta* and twenty nine fungal species belonging to seventeen genera from *Centella asiatica* soils and two non sporulating fungi were recorded from both soils. Rhizosphere is an important site of microbial activity and central component of ecosystems and biogeochemical cycles. Soil fungi were also affected by the pH, climatic change, soil moisture and also nutrients. *Gliocladium roseum* was the dominant in both the soils. Number of taxa was high in *Sida acuta* rhizosphere soil than *Centella asiatica* rhizosphere soil.

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