INTERNATIONAL JOURNAL OF CURRENT MULTIDISCIPLINARY STUDIES
Available Online at http://www.journalijcms.com
VOL. 3, ISSUE,08, PP.794-797, AUGUST, 2017
IJCMS

RESEARCH ARTICLE

SEASONALITY OF ENDOPHYTIC FUNGI: REASONING OF MEDICINAL USE

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ARTICLE INFO

Received 18th May, 2017
Received in revised form 17th June, 2017
Accepted 5th July, 2017
Published online 28th August, 2017

Keywords:

Endophytic Fungi, Diversity Index, Colonization Rate, Seasonality, Reasoning

ABSTRACT

A total of ten medicinal herb species were selected from different sacred grooves of MadikeriTaluk, Kodagu for the present study**.** Among the identified cultures, species of *Penicillium, Cladosporium, Chaetomium, Curvularia, Aureobasidium* were found to be dominant in specific hosts. *Penicillium sp*., was found more than one host revealing its wide range of host specificity. Among the herb species, the colonization frequency (CF %) of dominant endophytic fungi ranged from a minimum (17.1100%) of *Peniciliumitalicum* in *Solanumnigrum* to a maximum (43.5533%) of *Penicilliumchrysogenum* in *Centella asiatica* during the monsoon season. The colonization frequency (CF%) of dominant endophytic fungi irrespective of host was found to be higher during monsoon (29.6633%), least in summer (12.7747%) and moderate in winter (23.3280%).The Shannon diversity index (H") showed maximum diversity during rainy season (H"2.26) and the least diversity was observed in winter (H"2.241). Maximum evenness was found in monsoon 0.9584 and least in winter. According to Simpson's diversity index, species abundance was high in monsoon (I/D=0.8912) and low in summer (0.8872).

INTRODUCTION

Biodiversity is rapidly decreasing nowadays due to habitat destruction hence various microorganisms are also disappearing. Microorganisms such as fungi are potential sources of medicines, chemicals, food etc. Food web and critical ecosystem processes are also essential for healthy ecosystem. In order to understand more about the microbial diversity and their usefulness to mankind, it is necessary to investigate untapped areas for screening the available microbial sources for bioactive molecules which are likely to yield valuable products. India is known as the emporium of medicinal plants mainly on account of its varied flora found in diversity of phyto-geographical regions. The Western Ghats comprising the Brahmagiri, Talacauvery and Pusphagiri regions of Kodagu distirct are known for their flora and fauna. Medicinal plants of Western Ghats of India are reported to have a diverse community of endophytic fungi (Raviraja 2005; Krishnamurthy *et al*., 2008). Screening of diverse groups of fungi that may produce valuable medicinal products are promising approach for obtaining traditional medicine on commercial scale. Endophytic fungi represent an important and quantifiable component of fungal biodiversity and are known to affect plant community, diversity and structure (Sanders, 2004). Sacred groves are the biological heritage and a system that has helped to preserve the representative genetic resources existing in the surrounding regions for generations. These groves are associated with beliefs, taboos and folklores which have helped in conserving the relict flora and fauna of local regions (Kushalappa and Bhagawat, 2000; Krings *et al*., 2007). Fungal endophytes, that colonize and live within healthy plant tissues without inducing symptoms of disease (Petrini, 1991), comprise a large but little explored portion of fungal diversity (Frohlich and Hyde 1999; Hawksworth, 2001). Hence, major aim of this work was to investigate qualitatively and quantitatively the seasonality of endophytic fungi and its medicinal usage.

MATERIALS AND METHODS

Collection of plant samples

Ten medicinal herb species from sacred groves were chosen. Healthy and matured leaves were collected randomly from each plant and placed in polyethene bags, sealed and brought to the laboratory within 12 hrs, stored at 4^0 C according to Kumar and Hyde (2004).

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Isolation of endophytic fungi

The endophytic fungi were isolated following the method observed by Kumaresan and Suryanarayanan, 2002. The collected leaf samples were washed thoroughly in autoclave sterilised distilled water and cut into 1cm segments using sterilized scissors. Five selected pieces were sterilized by dipping in 75% ethanol for 1 min, Sodium hypochlorite solution (3.25%) for 3 min, ethanol (75%) for 30 sec and washed thoroughly in distilled water and blot. Leaf segments were then placed on YEMA media and incubated for 7-15 days at room temperature.

Identification of the EndophyticFungi

Identification was done based on morphological characteristics such as growth pattern, hyphal characters, colour of colony and medium, surface texture, margin character, aerial mycelium, mechanism of spore production and characteristics of the spores (Barnett and Hunter, 1972; Subramanian, 1983; Von Arx, 1981; Nagamani *et al*., 2006).

EndophyticColonization Rate

Isolated endophytic fungi were assessed for colonization rate, total colonization frequency and distribution of species in different seasons using appropriate statistical packages. Percentage of colonization rate (%) was calculated as the number of segments colonized by any fungus divided by total number of segments incubated, multiplied by 100 (Devarajan *et al*., 2002).

Distribution and Seasonality of species diversity of fungal Endophytes

The data were subjected to a software program PAST (Hammer *et al*., 2001) which generates nine diversity indices. Shannon diversity indexes (H0), Shannon evenness index (J0) and Simpson diversity index (1/D) were used for the evaluation of fungal species richness.

RESULTS AND DISCUSSION

Collection of plant samples

A total of ten medicinal herb species from different families were selected from the study area for the isolation of endophytic fungi. Taxonomy of the selected plants has been tabled in the table no. 1. (Shetty *et al.,* 2002; Keshavamurthy and Yoganarasimhan, 1990; Subbaiah, 1978; CEE, 2004)

Table 1 Taxonomy of selected medicinal herb species

Host	Family	Local name
Achyranthe saspera Linn	*Amaranthaceae*	Uttarani
Colocasia esculenta	*Aracaceae*	Kesu
*Centella asiatica (*Linn.) Urban	*Apiaceae*	Ondelaga
Eclipta alba (Linn.) Linn.	*Asteraceae*	Bhringaraja
Leucas aspera(Willd) Link	*Lamiaceae*	Thumbe
Ocimum sanctum Linn	*Labiatae*	Kari tulasi
Phyllanthus amarus Schum and Thonn.	*Euphorbiaceae*	Nelanelli
Solanum xanthocarpum L. Schrad. &Wendl.	*Solanaceae*	Chunde
Sidar hombifolia Linn	*Malvaceae*	Kadiraberu
Solanum nigrum L.,	*Solanaceae*	Kakkehannu

Colonization Frequency of dominant endophytic fungi

A total of ten medicinal herb species were selected in the present study. Among the identified cultures, species of *Penicillium, Cladosporium, Chaetomium, Curvularia, Aureobasidium* were found to be dominant in specific host. *Penicillium* sp., was found in more than one host revealing its wide range of host specificity. Among the herb species, the colonization frequency (CF %) of dominant endophytic fungi ranged from a minimum (17.1100%) of *Penicilium italicum* in *Solanum nigrum* to a maximum (43.5533%) of *Penicillium chrysogenum* in *Centella asiatica* during monsoon season. It ranged between a minimum (14.2200%) of *Penicilium italicum* in *Solanum nigrum* and a maximum (31.5533%) of *Penicilliumcitrinum* in *Phyllanthus amarus* during winter season. Similarly, the CF (%) ranged from a minimum (7.9967%) of *Pyllosticta minima* in *Sidarhombifolia* to a maximum (18.6633%) of *Penicillium chrysogenum* in *Centella asiatica* during the summer season (table 2. and 3)

Table 2 Colonization Rate of endophytic fungi among the herb species

Host	Colonization Rate (CR %)			
	Monsoon (June-Sept)	Winter (Oct-Jan)	Summer (Feb-May)	Mean Total
Achyranthes aspera (Aa)	46.2200	45.3300	17.3300	36.2933
Centella asiatica (Ca)	58.2167	54.6633	19.1100	43.9967
Colocasia esculenta (Ce)	19.1100	14.2200	7.5533	13.6278
Eclipta alba (Ea)	39.5533	31.8867	27.1067	32.8489
Leucas aspera (La)	29.7733	23.1100	8.8833	20.5889
Ocimum sanctum (Os)	36.8833	32.4400	15.1100	28.1444
Phyllanthus amarus (Pa)	32.8867	29.7733	15.1067	25.9222
Solanum xanthocarpum (Sx)	32.8833	24.4400	14.2200	23.8478
Sidar hombifolia (Sr)	26.6633	23.1067	10.6633	20.1444
Solanum nigrum (Sn)	28.7767	30.2200	16.4400	25.1456

Table 3 Total Colonization Frequency of dominant endophytic fungi

Dominant endophytic fungi	Colonization Frequency (CF%)			
	Monsoon (June-Sept)	Winter (Oct-Jan)	Summer (Feb-May)	Mean Total
Alternaria alternata	0.1773	0.2217	0.0443	0.1478
Aspergillusflavus	0.2660	0.2217	0.0887	0.01921
Chaetomiumglobosum	0.2660	0.0443	0.1330	0.1478
Curvularialunata	0.2217	0.2217	0.0887	0.1773
Cladosporium cladosporioides	0.2217	0.2217	0.0000	0.1478
Fusariumoxysporum	0.2663	0.2217	0.0887	0.1922
Penicillium chrysogenum	0.2663	0.1773	0.0887	0.1774
Penicilliumadametzi	0.2660	0.2217	0.2217	0.2364
Penicilliumcitrinum	0.2217	0.0887	0.1330	0.1478
Penicilliumaurantiogriseum	0.3110	0.2217	0.1777	0.2368
Penicilliumitalicum	0.2217	0.0887	0.1330	0.1478

The colonization frequency (CF%) of dominant endophytic fungi irrespective of host was found to be higher during monsoon (29.6633%), least in summer (12.7747%) and moderate in winter (23.3280%), found significant seasonally. The Scheffe Post hoc test revealed that, the colonization frequency of *Penicilium italicum (*13.2567%) in *Solanumnigrum* was found to be least and *Penicilliumchrysogenum (*30.3678%) in *Centella asiatica* was found to be high irrespective of the season depicts their dominance through the year. Rajgopal and his coworkers (2010), isolated a total of eighteen species of endophytic fungi from five different species of medicinal herbs. In their study,

ascomycetes were the most dominant one. Of the five medicinal herbs studied, more number of endophytes could be seen in *Ocimum basilicum* and *O. sanctum, Coleus aromaticus* and *Tridax procumbens* had the least number of endophytic fungi. They isolated *Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Phoma* spp., *Phyllosticta* spp., *Phomopsis* spp., *Pestalotiopsis* spp., *Chaetomium indicum, Sporormiella minima, Xylaria* sp, *Chaetomium globosum, Talaromyces* sp. and sterile forms as dominant endophytic fungi in the medicinal herbs they studied. Krishnamurthy *et al.,* (2008) also isolated endophytic fungi from the medicinal herbs from Malnad region.

Measurement of Diversity

Though ecologists have designed a large range of indices and models for measuring the diversity still it is so hard to define (Magurran, 1983). This is because; diversity consists of two components, namely the variety and the relative abundance of species. Investigations on ecological diversity are often restricted to species richness, which is the direct count of the number of species present (Pianka, 1983).

Table 4 Diversity Indices for the endophytic fungi in medicinal herbs

Diversity indices	Diversity of endophytic fungi in herbs		
	Monsoon	Winter	Summer
Dominance_D	0.1088	0.1128	0.1123
Simpson_1-D	0.8912	0.8872	0.8877
Shannon_H	2.26	2.241	2.242
Evenness_e^H/S	0.9584	0.9405	0.9415

Colonization did not differ significantly among the medicinal herb species. Shannon diversity index (H") showed maximum diversity during rainy season (H"=2.26) and the least diversity was observed in winter (H"=2.241). Maximum eveness was found in monsoon 0.9584 and least was found in winter. According to Simpson's diversity index, species abundance was high in monsoon (I/D=0.8912) and low in summer (0.8872). In the present study it was observed from the diversity index that, the number and colonization rate of endophytes were more during monsoon followed by summer and the least in winter season. This may be due to the fact that greater rainfall in the monsoon season could promote the dispersal of fungal spores. In addition, moderate temperature would allow a higher viability of the fungal propagules and therefore, their success in colonizing plant tissues. A moderate diversity of fungi was seen among the host plants studied, which included some ubiquitous forms along with some rare species. Variation in colonization pattern of endophytic fungi strengthens the view that, endophytic microbial populations positively correlated with various environmental factors.

In Kodagu specially Madikeri receive high rainfall and has moist weather almost in all the months except February to April. May be due to this condition during monsoon and winter highest endophytic fungi were present.

CONCLUSION

In the present investigation, a significant variation was detected in the colonization frequency of endophytic species at different seasons of the year, indicating the environmental factors such as rainfall and atmospheric humidity and their effect on host plant. Therefore, survey of endophytic fungal communities at different seasons of the year might favor a higher recovery of particular species. Furthermore, our result also showed that the samples collected in monsoon harbored abundant endophytic populations than in summer, implying a seasonal fluctuation of endophytes.

Medicinal plants have been used for medication usually during monsoon, post monsoon and in winter season some times in summer also for different ailments. Therefore, seasonality of medicinal use of plants is in turn seasonality of endophytic fungi for their antibiotic or therapeutic properties through their host plant specificity.

Hence, it is presumed in this study that, endophytes within their host plants have therapeutic values, and practice of ancient medicine must have come into existence according to the availability of medicinal plants within which presence of endophytic fungi as well.

Acknowledgement

The authors are thankful to Mangalore University for providing fellowship and facilities to carry out the research work.

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