



Original Research Article

Seasonal diversity of endophytic fungi from ten medicinal plants

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A B S T R A C T

Keywords

Seasonal variation,
Colonization frequency,
Amravati

In the present investigation ten medicinal plants were analyzed for the presence of endophytic fungi during three different seasons (winter, summer, and rainy). A total of 20 fungal species were isolated from 630 segments of stem and leaves and identified based on the morphology of fungal culture and spores. Colonization frequency was greater during rainy season than winter and summer. Some endophytic fungi were common to more than one host and few shows host-specificity.

Introduction

Endophytic fungi are defined as the fungi which spend the whole or part of their lifecycle colonizing inter- and/or intracellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease (Petrini, 1991; Carroll, 1988). Medicinal plants are reported to harbour endophytes (Strobel, 2002), and have a capacity to protect their host from infectious agents and also provide adaptability to survive in adverse conditions.

Endophytes are the potential producers of novel biologically active compounds of immense value in agriculture, medicine and industry (Tan *et al.*, 2000; Tan and Zou, 2001; Aly *et al.*, 2010; Shankar and Krishnamurthy, 2010). Many studies have

shown that some medicinal properties of plants may be due to endophytic fungi living inside the plants (Azevedo *et al.*, 2002).

Therefore it is important to determine the endophytic fungal diversity of medicinal plants.

Medicinal plants investigated in this studies i.e. *Murraya koenigii* (L.) Spreng., *Helicteres isora* L., *Ocimum sanctum* L., *Cissus quadrangularis* L., *Calotropis procera* (Aiton) W.T.Aiton, *Dioscorea bulbifera* L. *Withania somnifera* (L.) Dunal, *Coffea arabica* L., *Adathoda vasica* L., *Vitex negundo* L. are widely use for their medicinal properties to treat various diseases. Therefore present study was

carried out to determine the diversity of endophytic fungi in three different seasons.

Materials and Methods

Sample Collection

Medicinal plants were collected from various parts of Amravati district in three different seasons. The samples were brought to laboratory in sterile paper bags and stored at 4°C till further use.

Isolation of endophytic fungi

Collected samples were rinsed gently in running water to remove adhered dust and debris and cut into 1–2 mm segments. Surface sterilization was done according to the method described by (Suryanarayanan *et al.*, 2011).

The sterilized samples were placed in Petri dishes containing potato dextrose agar (PDA). Petri dishes were sealed with parafilm and incubated at room temperature (25±2°C) for one week. The fungi growing out from the samples were subcultured on fresh (PDA) medium to get pure culture.

Identification of endophytic fungi

The endophytic fungi were identified based on morphological characters of fungal culture and spores by standard mycological manuals (Barnett and Hunter 1998, Sutton 1980). The colonization frequency (CF) expressed as percentage was calculated according to (Kumareson and Suryanarayanan, 1998) as follows

$$\%CF = \frac{\text{Number of tissue segments colonized by a fungus}}{\text{Total number of tissue segments plated}} \times 100$$

Results and Discussion

The plant tissues, specially leaves and stems are excellent reservoirs for endophytic fungi (Petrini, 1991; Bokhary *et al.*, 2000). In the tropical region only a few studies have been carried out on endophytes (Fröhlich and Hyde, 2000). Environmental factors such as temperature, rainfall and atmospheric humidity and their effect on host plant made the variations in occurrence of endophytic fungi and their colonization frequency (Selvanathan *et al.*, 2011). Therefore, in present survey of endophytic fungi isolated from ten medicinal plants in three seasons have the different colonization frequency.

Twenty species of endophytic fungi belonging to 17 genera and 1 sterile mycelia were recovered from green leaves and stems of ten medicinal plants during three different seasons. *Arthrimum sp.*, *Fusarium oxysporum*, *Cladosporium cladosporioides*, *Phoma sp.*, *Verticillium albo-atrum*, *Alternaria alternata*, *Pithomyces sp.*, *Stachybotrys microspora*, *Acremonium kiliense*, are some of the endophytes recovered with high colonization frequency. Colonization frequency was greater during rainy season than winter and summer (Table 1, 2, 3).

Some of the fungal endophytes show host-specificity. Host specificity observed in occurrence of *Phoma crysanthemicola*, *Curvularia lunata*, *Fusarium oxysporum*, *Acremonium fusidioides*, *Stachybotrys microspora*, *Colletotrichum gloeosporioides*. Whereas *Pithomyces sp.*, *Verticillium albo-atrum*, *Alternaria alternata*, *Pestalotia macrotricha*, *Cladosporium cladosporioides* did not shows host specificity.

Table.1 Colonization frequency of endophytic fungi isolated from ten medicinal plants during winter season

Plant species Plant Parts	AV		MK		CQ		DB		HI		CA		WS		OS		CP		VN	
	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L
ENDOPHYTES	COLONISING FREQUENCY(%)																			
<i>Pithomyces sp.</i>	—	—	—	—	—	—	—	—	—	—	3.17	—	—	—	—	—	—	—	—	—
<i>Verticillium albo-atrum</i>	—	—	—	0.79	—	—	—	—	1.58	—	—	—	—	—	—	—	—	—	—	—
<i>Phoma sp.</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Phoma crysanthemicola</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Curvularia lunata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Pestalotia macrotricha</i>	—	—	—	—	—	—	—	—	—	—	—	—	2.85	—	—	—	—	—	—	1.74
<i>Alternaria alternata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.22
<i>Fusarium oxysporum</i>	—	—	1.11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Penicillium sp.,</i>	—	—	—	—	0.79	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Arthrinium sp.</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Cladosporium cladosporioides</i>	—	—	—	—	—	—	—	—	3.33	—	—	—	—	3.80	—	1.42	3.01	—	—	—
<i>Aspergillus nidulans</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Xylaria sp.</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Epicoccum nigrum</i>	—	—	—	—	—	1.11	—	—	—	—	—	11.11	—	—	—	—	—	—	—	—
<i>Acremonium fusidioides</i>	—	—	—	—	—	—	—	—	—	—	—	2.53	—	—	—	—	—	—	—	—
<i>Acremonium kiliense</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Stachybotrys microspora</i>	—	—	—	—	—	—	—	1.26	—	—	—	—	—	—	—	—	—	—	—	—
<i>Trimastroma sp.</i>	—	—	—	—	—	—	—	—	—	2.22	—	—	—	—	—	—	—	—	—	—
<i>Colletotrichum gloeosporioides</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.12	—	—	—	—	—
<i>Colletotrichum acutatum</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sterile mycelia	2.22	1.44	—	—	—	—	0.95	—	—	—	—	—	—	—	—	—	—	—	—	—

MK: *Murraya koenigii* (L.) Spreng., HI: *Helicteres isora* L., OS: *Ocimum sanctum* L., CQ: *Cissus quadrangularis* L., CP: *Calotropis procera* (Aiton) W.T.Aiton, DB: *Dioscorea bulbifera* L., WS: *Withania somnifera* (L.) Dunal, CA: *Coffea arabica* L., AV: *Adhatoda vasica* L., VN: *Vitex negundo* L., S: Stem, L: Leaf

Table.2 Colonization frequency of endophytic fungi isolated from ten medicinal plant during summer season

Plant species Plant Parts	AV		MK		CQ		DB		HI		CA		WS		OS		CP		VN	
	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L
ENDOPHYTES	COLONISING FREQUENCY (%)																			
<i>Pithomyces sp.</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Verticillium albo-atrum</i>	—	—	—	—	1.90	—	—	—	1.74	—	—	—	2.22	1.90	1.74	1.74	—	—	—	—
<i>Phoma sp.</i>	—	—	—	1.90	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Phoma crysanthemicola</i>	—	—	2.53	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Curvularia lunata</i>	—	—	—	—	—	—	—	—	2.06	—	—	—	—	—	—	—	—	—	—	—
<i>Pestalotia macrotricha</i>	1.42	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.33
<i>Alternaria alternata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.80
<i>Fusarium oxysporum</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Penicillium sp.,</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Arthrinium sp.</i>	—	1.42	—	—	—	—	—	—	—	—	—	—	—	—	—	2.69	—	—	—	—
<i>Cladosporium cladosporioides</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.22	—	—	—
<i>Aspergillus nidulans</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.06	2.69	—	—
<i>Xylaria sp.</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Epicoccum nigrum</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Acremonium fusidioides</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Acremonium kiliense</i>	—	—	—	—	—	—	2.69	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Stachybotrys microspora</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Trimastroma sp.</i>	—	—	—	—	—	—	—	—	—	1.74	—	—	—	—	—	—	—	—	—	—
<i>Colletotrichum gloeosporioides</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Colletotrichum acutatum</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sterile mycelia	—	—	—	—	—	—	—	—	—	—	1.58	—	—	—	—	—	—	—	—	—

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Table.3 Colonization frequency of endophytic fungi isolated from ten medicinal plant during rainy season

Plant species Plant Parts	AV		MK		CQ		DB		HI		CA		WS		OS		CP		VN	
	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L
ENDOPHYTES	COLONISING FREQUENCY (%)																			
<i>Pithomyces sp.</i>	20.2	25.3	—	—	—	—	—	—	—	—	—	2.38	—	—	—	—	—	—	—	—
<i>Verticillium albo-atrum</i>	—	—	—	—	—	—	—	—	—	—	—	—	3.49	3.80	—	2.22	—	—	—	—
<i>Phoma sp.</i>	—	—	—	4.44	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Phoma crysanthemicola</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Curvularia lunata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Pestalotia macrotricha</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Alternaria alternata</i>	—	—	—	—	—	—	—	—	4.60	—	—	—	—	—	—	—	—	—	—	3.65 4.44
<i>Fusarium oxysporum</i>	—	—	4.12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Penicillium sp.,</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Arthrinium sp.</i>	—	3.96	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Cladosporium cladosporioides</i>	—	—	—	—	4.28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Aspergillus nidulans</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.69	3.01	—
<i>Xylaria sp.</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Epicoccum nigrum</i>	—	—	—	—	—	3.80	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Acremonium fusidioides</i>	—	—	—	—	—	—	—	—	—	—	—	3.17	—	—	—	—	—	—	—	—
<i>Acremonium kiliense</i>	—	—	—	—	—	—	4.44	—	4.76	—	—	—	—	—	—	—	—	—	—	—
<i>Stachybotrys microspora</i>	—	—	—	—	—	—	—	4.60	—	—	—	—	—	—	—	—	—	—	—	—
<i>Trimastroma sp.</i>	—	—	—	—	—	—	—	—	—	3.80	—	—	—	—	—	—	—	—	—	—
<i>Colletotrichum gloeosporioides</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Colletotrichum acutatum</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.53	—	—	—	—
Sterile mycelia	3.65	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

MK: *Murraya koenigii* (L.) Spreng., HI: *Helicteres isora* L., OS: *Ocimum sanctum* L., CQ: *Cissus quadrangularis* L., CP: *Calotropis procera* (Aiton) W.T.Aiton, DB: *Dioscorea bulbifera* L., WS: *Withania somnifera* (L.) Dunal, CA: *Coffea arabica* L., AV: *Adhatoda vasica* L., VN: *Vitex negundo* L., S: Stem, L: Leaf

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