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Diversity of endophytic fungi associated with some medicinal herbs and shrubs

Deepika Mahobiya* and Ashwini Kumar Gupta
SoS in Life Science, Pt. Ravishankar Shukla University, Raipur, 492010, Chhattisgarh, India
**Corresponding author Email: deepikamahobiya@gmail.com*
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ABSTRACT

In the present investigation, 46 endophytic fungi were isolated from surface sterilized plant parts (root, stem, leaf and petiole) of six medicinal herbs *viz*., *Aloe vera* (L.) Burm. f., *Abrus precatorius* L., *Asparagus racemosus* Willd., *Catharanthus roseus* (L.) G. Don., *Hemidesmus indicus* (L.) R. Br. & *Plumbago zeylanica* L. and 34 endophytic fungi were isolated from three medicinal shrubs *viz*., *Abutilon indicum* (L.) Sweet, *Adhatoda vasica* Nees and *Ocimum sanctum* L. A total of 12 genera of fungi including *Acremonium*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Bipolaris*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Neocosmospora* and seven sterile forms were identified. *Colletotrichum gloeosporioides* was dominant in *Asparagus racemosus*, *Catharanthus roseus* and *Plumbago zeylanica*. Maximum similarity of endophytic assemblages was observed between *P. zeylanica* and *Asparagus racemosus*. Notably, the fungi identified as *Fusarium proliferatum* KY853412 and *Neocosmospora falciformis* KY853414 based on ITS-rDNA and LSU (D1D2) rDNA sequence analysis, were found to be host specific. Maximum species richness and diversity of endophytic fungi was recorded in *Aaparagus racemosus*. Highest species evenness was recorded in *Hemidesmus indicus* and it was minimum in *Catharanthus roseus*.

Key words: Fungal endophytes, medicinal plants, endophyte diversity.

INTRODUCTION

Endophytic microorganisms including fungi are non-pathogenic and live inside plant tissue either for a short or prolonged period. The occurrence of fungal endophytes in a plant species may be influenced by the type of the plant host, the host tissue and nature of phytochemicals present in the plant (Sun *et al.,* 2008; Jia *et al.,* 2016). The endophytic fungi promote plant growth, enhance disease resistance and therefore play an important role in ecological process of plant succession (Singh *et al*., 2011). Medicinal plants are reported to harbor endophytes, which provide protection to their host from infectious diseases and also provide adaptability to survive in adverse environmental conditions (Strobel, 2002; Faeth and Fagan, 2002; Bailey *et al.*, 2006). Endophytic fungi mainly belong to *Ascomycota*. Some taxa of *Basidiomycota*, *Zygomycota* and *Oomycota* are also found as endophytes (Sun and Guo, 2012).We studied the diversity, distribution and tissue preference of endophytic fungi of the six medicinal herbs and three shrubs found in Chhattisgarh state of northern India.

MATERIALS AND METHODS

Sample collection: Leaves, petioles, stems and roots of apparently healthy medicinal herbs, viz., *Aloe vera* (L.) Burm. f., *Abrus precatorius* L., *Asparagus racemosus* Willd., *Catharanthus roseus* (L.) G. Don., *Hemidesmus indicus* (L.) R. Br. and *Plumbago zeylanica* L. and medicinal shrubs, *viz*. *Abutilon indicum* (L.) Sweet, *Adhatoda vasica* Nees and *Ocimum sanctum* L. were collected in poly bags and stored at 4°C till use (Hazalin *et al.*, 2009). The plants were collected from the premises of S.o.S. in Life Science, Pt. R.S.U Raipur, Chhattisgarh (22° 33' N to 21° 14'N Lat., 82° 6' to 81° 38'E Long.) and identified using e-Herbarium of Chhattisgarh State Medicinal Plant Board.

Isolation and characterization of endophytic fungi: The plant parts were surface sterilized by triple surface sterilization technique (Mahobiya and Gupta, 2014). Samples were cleaned under running tap water. For this purpose 1cm sized plant segments were washed in 0.01 % tween 20, 70% ethanol for 60 seconds, 2-4% aqueous solution of sodium hypochlorite (2% for leaves and 4% for root, stem and petiole) for 60 seconds, 70% ethanol for 60 seconds and three rinses with sterile distilled water. Surface sterilized samples were further peeled and cut into 5mm pieces. The sterilized tissue segments were inoculated on water agar, 2% malt extract agar or potato dextrose agar medium containing 100 µg per ml streptomycin and incubated for three to four weeks at 28±2°C. The efficiency of surface sterilization procedure was checked by two methods - first by rolling sterilized plant parts (for stem and root) on isolation media and second by inoculating last washing of above sterilization step on isolation media. Isolated endophytic fungi were identified on the basis of morphological characteristics using manuals of Ellis (1976), Barnett and Hunter (1998), Hanlin (1998), Watanabe (2002), Nagamani *et al.* (2006) and Seifert *et al.* (2011).

Molecular characterization of host specific isolates: Genomic DNA was isolated from the 8 days old cultures. The ITS region and LSU (D1D2) of rDNA were amplified using fungal universal primers ITS 4 & ITS 5 and LROR & LR7, respectively. The sequencing polymerase chain reaction (PCR) was set up with ABI-BigDye® Terminatorv3.1 Cycle Sequencing Kit. The raw sequence obtained from ABI 3100 automated DNA sequencer was manually edited for inconsistency. The sequence data was aligned and compared with publically available NCBI (National Center for Biotechnology Information) GenBank nucleotide database using mega BLAST algorithm. The amplified sequences of endophytic fungi were submitted in GenBank (http://www.ncbi.nlm.nih.gov/genbank) for accession purpose.

Construction of phylogenetic tree of isolate CrR3 and ApR6: A phylogenetic tree of host specific isolate CrR3 (NFCCI 3300) and ApR6 (NFCCI 3301) based on ITS rDNA and LSU rDNA sequence was constructed using MEGA v.6.0.6 (Molecular Evolutionary Genetics Analyses). The reference sequences were retrieved from the GenBank in NCBI. The phylogenetic tree was constructed by neighbor-joining (NJ) method with bootstrap (500 replications)

sampling using aligned nucleotide sequences of ITS-rDNA and LSU rDNA.

Statistical analysis: The colonization frequency and percentage of infection rate (EIR) of the endophytes were calculated following Suryanarayanan and Thennarasan (2004). Endophytic infection rate (EIR) was determined by dividing total number of infected segment by total number of segments incubated. Colonization frequency was calculated as number of segments colonized by an endophyte divided by the total number of segments screened. Both are expressed in percentage.

A coefficient of similarity was calculated to compare the endophyte assemblage of different plant parts of a host and between different host plants following Carroll and Carroll (1978) and Suryanarayanan and Vijaykrishna (2001).

Similarity coefficient (SC) in percentage was determined using the formula 2w/(a+b) x100; where 'a' is the sum of colonization frequency for all fungal species in a tissue or host and 'b' is the similar sum of another tissue or host, 'w' is the sum of lower colonization frequencies for fungal endophytes common between the tissues or host. Three indices were used to estimate species diversity, species richness and species evenness following Ludwick and Reynold (1998).

1. Shannon and Weaver (1949) and Simpson diversity index values were obtained by following equation:

$H = -\sum [(p_i) \times \ln (p_i)]$ (Shannon's index)

$= -\sum [n_i (n_i-1)] / N (N-1)$ (Simpson's index)

p_i is proportion of the a particular species (p_i=ni/N), n_i is the number of individuals of each individual species, N is the total number of all species in a plant host

2. Species richness (R_1 and R_2) was obtained by using the following equations:

$R_1 = (S-1)/\ln (N)$ (Magalef, 1958)

$R_2 = S/\sqrt{N}$ (Menhinick, 1964)

3. Species evenness (Shannon equitability) was determined by using the following equation:

$(E_H) = H/H_{max} = H/\ln(S)$

H_{max} [here H_{max} = In(S)] is maximum diversity possible, 'S' is number of species in a plant

RESULTS

A total of eighty endophytic fungi were isolated from surface sterilized plant parts (root, stem, leaf and petiole) of nine medicinal herbs and shrubs (*Aloe vera* , *Abrus precatorius*, *Asparagus racemosus*, *Catharanthus roseus*, *Hemidesmus indicus*, *Plumbago zeylanica*, *Abutilon indicum, Adhatoda vasica* and *Ocimum sanctum* (**Fig. 1**). Maximum endophytic infection rate (EIR) was observed for *A. vasica* and *A. racemosus*. Only *O. sanctum* and *A. indicum* showed presence of endophytic fungi in petiole. *O. sanctum* showed highest EIR in petiole followed by stem and root (**Fig. 2**). *A. vasica*, *H. indicus*, *A. precatorius*, *A. indicum* and *A. vera* showed high rate of EIR in roots. *P. zeylanica*, *C. roseus* and *A. racemosus* showed highest EIR in stem.

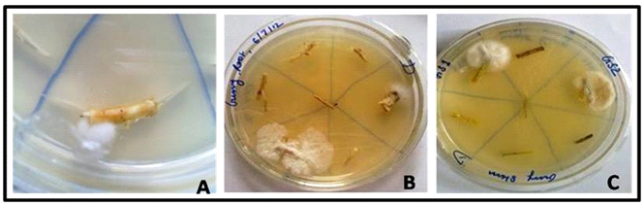

Fig. 1: Isolation of endophytic fungi on different isolation media (A. Water agar; B. 2% Malt extract agar; C. Potato dextrose agar)

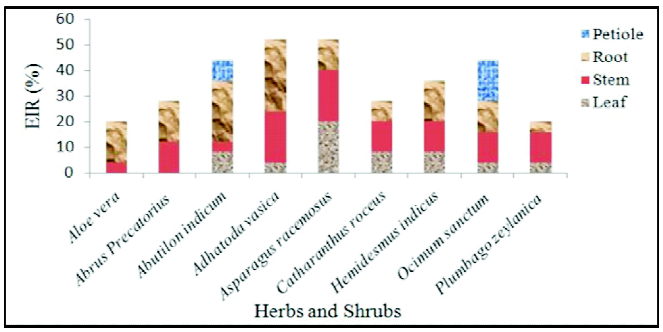

Fig. 2: Percentage of the endophytic infection rate (EIR) of different parts of selected medicinal herbs and shrubs

A total of twelve genera of fungi including *Acremonium*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Bipolaris*, *Colletotrichum*, *Curvularia*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Fusarium*, *Neocosmospora* and seven sterile forms were isolated from all the medicinal plants screened (**Figs. 3 & 4**). Isolate CrR3 and ApR6 could be isolated only from *Catharanthus roseus* and *Abrus precatorius*, respectively. CrR3 showed 100% sequence similarity with

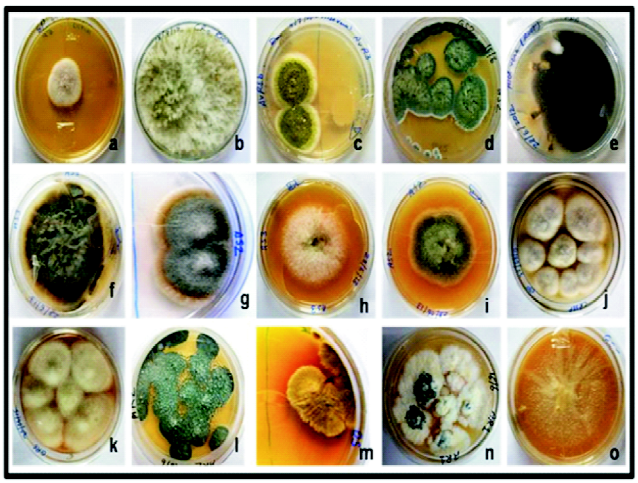

Fig. 3: Isolated endophytic fungi from herbs and shrubs [a-*Acremonium* sp., B-*Alternaria alternata*, c-*Aspergillus flavus*, d-*Aspergillus fumigatus*, e-*Aspergillus niger*, f-*Aureobasidium pullulans*, g-*Bipolaris* sp., h-*Colletotrichum gloeosporioides*, i-*Curvularia lunata*, j-*Fusarium proliferatum*, k-*Neocosmospora falciformis*, l-*Paecilomyces* sp., m-*Penicillium purpurogenum*, n-*Pestalotiopsis* sp., o-Sterile form 1]

Table 1: BLAST analysis of amplified sequence from isolate CrR3 and ApR6

Isolate (Sequence analyzed)	NCBI Gene Bank accession No.	Description	Max score	Query cover	Query coverage	E value	Identity (%)
CrR3 (ITS)	KJ439117.1	*Fusarium proliferatum* isolate P9-94	933	933	100%	0.0	100%
CrR3 (D1D2)	KT462721.1	*Fusarium proliferatum* voucher CulTenn SPH6	1092	1092	100%	0.0	100%
ApR6 (ITS)	KM235740.1	*Fusarium solani* isolate ZK004	973	973	100%	0.0	99%
ApR6 (D1D2)	KJ126455.1	*Fusarium falciforme* culture-collection BCCM/IHEM:15570	1447	1447	100%	0.0	100%

Fusarium proliferatum **(Table 1)**. The Phylogenetic analysis using ITS rDNA and LSU rDNA sequence indicated that isolate CrR3 and reference sequence from *Fusarium proliferatum* were clustered into one group with 93 and 100 bootstraps, respectively (**Figs. 5** & **6**). The NCBI GenBank accession number for the CrR3, LSU rDNA sequence is KY853412 (https://www.ncbi.nlm.nih.gov/ nuccore/ KY853412). The BLAST analysis of ITS sequence of tested fungal strain ApR6 (NFCCI 3301) showed 99% sequence similarity with *Fusarium solani*. The LSU (D1D2) sequence analysis of ApR6 (NFCCI 3301) showed 100% sequence similarity with *Neocosmospora falciforme* (*Fusarium falciformis*). The phylogenetic analysis using ITS rDNA sequence of ApR6 and reference sequence from *Fusarium solani* clustered into one group with 55 bootstraps value and

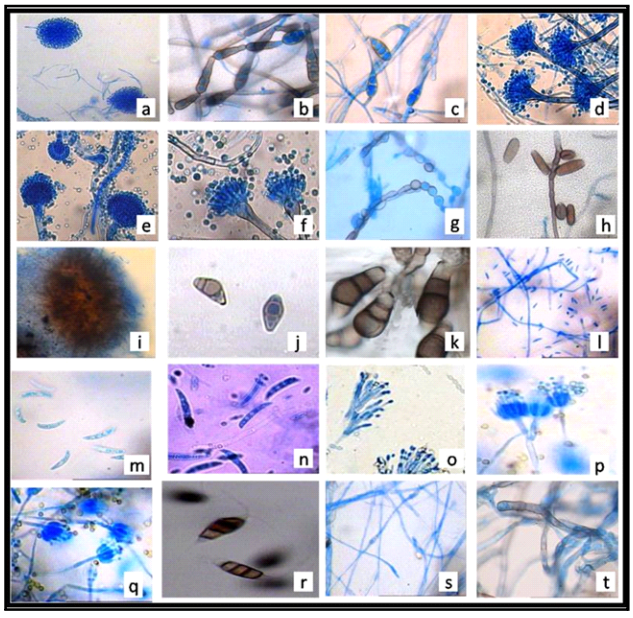

Fig. 4: Microscopic view of isolated endophytic fungi from herbs and shrubs [a-*Acremonium* sp., b-*Alternaria alternata*, c-*Alternaria* sp.2, d-*Aspergillus flavus,* e-*Aspergillus fumigatus*, f-*Aspergillus niger,* g-*Aureobasidium pullulans*, h-*Bipolaris* sp., i-*Colletotrichum gloeosporioides,* j-*Curvularia lunata*, k-*Curvularia* sp.2, l-*Fusarium proliferatum,* m-*Fusarium solani,* n-*Neocosmospora falciformis* o-*Paecilomyces* sp., p-*Penicillium purpurogenum, q-Penicillium* sp. 2, r-*Pestalotiopsis* sp., s-Sterile form 1, t- Sterile form 2

Table 2: Colonization frequency of endophytic fungi isolated from different plant parts of medicinal herbs

MH	PP	Isolated endophytic fungi																													
		A	b	c	d	e	f	g	H	i	J	k	l	m	n	o	p	q	r	s	t	u	V	w	x	y	z
Av	R	-	-	-	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-
	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ap	R	4	-	-	-	-	-	-	-	-	-	4	-	-	-	-	4	-	-	8	-	-	-	4	-	-	-
	S	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ar	R	-	-	-	-	4	-	-	-	-	4	-	-	-	-	4	4	-	-	-	-	-	-	-	-	-	-	-
	S	-	-	-	-	-	-	-	-	4	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	4	-	4
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	L	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hi	R	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	8	-	-	-	-	-	-
	S	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-
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	L	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-
Pz	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
	S	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-
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MH- Medicinal herb; PP -Plant parts; R -Root, S-Stem, P-Petiole, L-Leaf ; Av - *Aloe vera,* Ap-*Abrus precatorius,* Ar-*Asparagus racemosus,* Cr-*Catharanthus roseus,* Hi-*Hemidesmus indicus,* Pz-*Plumbago zeylanica*; a-*Acremonium* sp., b-*Alternaria alternata*, c-*Alternaria* sp.2, d -*Aspergillus flavus,* e-*Aspergillus fumigatus*, f -*Aspergillus niger,* g-*Aspergillus* sp.4, h-*Aureobasidium pullulans*, i-*Bipolaris* sp., j-*Colletotrichum gloeosporioides,* k-*Curvularia lunata*, l-*Curvularia* sp.2, m -*Paecilomyces* sp., n -*Penicillium purpurogenum,* o-*Penicillium* sp. 2, p -*Pestalotiopsis* sp., q -*Fusarium proliferatum,* r-*Fusarium solani*, s-*Neocosmospora falciformis,* t-Sterile form 1, u -Sterile form 2, v -Sterile form 3, w -Sterile form 4, x - Sterile form 5, y -Sterile form 6, z -Sterile form 7

Table 3: Colonization frequency of endophytic fungi isolated from different plant parts of medicinal shrubs

MS	PP	Isolated endophytic fungi																										
		a	b	c	d	e	f	g	h	I	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z
Ai	R	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-
	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-
	P	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-
	L	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-
Ad v	R	-	-	-	-	4	-	-	-	-	-	-	8	-	-	4	4	-	8	-	-	-	-	-	-	-	-
	S	-	-	-	-	-	-	-	-	-	-	-	8	-	4	-	4	-	-	-	-	-	4	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-
Os	R	-	-	-	4	-	-	4	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	S	-	-	-	4	-	-	-	-	-	-	-	4	-	-	4	-	-	-	-	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	4	-	-	-
													2														
	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-

MS- Medicinal shrub; PP -Plant parts; R -Root, S -Stem, P -Petiole, L -Leaf; Ai - *Abutilon indicum,* Hi-*Adhatoda vasica*, Os-*Ocimum sanctum* ; a -*Acremonium* sp., b-*Alternaria alternata*, c -*Alternaria* sp.2, d -*Aspergillus flavus,* e- *Aspergillus fumigatus*, f -*Aspergillus niger,* g-*Aspergillus* sp.4, h-*Aureobasidium pullulans*, i-*Bipolaris* sp., j-*Colletotrichum gloeosporioides,* k-*Curvularia* sp.1, l-*Curvularia* sp.2, m -*Paecilomyces* sp., n -*Penicillium purpurogenum,* o-*Penicillium* sp. 2, p -*Pestalotiopsis* sp., q -*Fusarium proliferatum,* r-*Neocosmospora falciformis,* s-*Fusarium solani* 3, t-Sterile form 1, u-Sterile form 2, v -Sterile form 3, w-Sterile form 4, x- Sterile form 5, y-Sterile form 6, z-Sterile form 7

LSU rDNA sequence clustered into one group with reference sequence of *Fusarium falciforme* with 100 bootstraps (**Figs. 7 & 8**). The NCBI GenBank accession number for the ApR6, LSU rDNA sequence is KY853414 (https://www.ncbi.nlm.nih.gov/nuccore/KY853414).

Colletotrichum gloeosporioides was the dominant species in *A. racemosus*, *C. roseus* and *P. zeylanica* (**Table 2**). Sterile forms of endophytic fungi were observed in all plants except *C. roseus. Paecilomyces* sp. was isolated only from *O. sanctum* and it was found to be dominant (**Table 3**). *Alternaria alternata* was dominant in *A. indicum*. *Curvularia* was isolated from six plants except *A. vera, A. indicum* and *P. zeylanica*. Sterile form 1, 3 and 7 could not be detected from herbs whereas sterile form 2 and 6 were not noticed in shrubs.

Table 4: Similarity coefficient (SC) of endophytic fungi between plant tissues of individual plant

Medicinal Herbs	A	B	C	D	E	F
Aloe vera (L.) Burm.	-	NA	NA	NA	NA	NA
Abrus precatorius L.	44.44	-	-	-	-	-
Asparagus racemosus Willd.	-	-	22.22	-	44.44	-
Catharanthus roseus (L.) G. Don.	-	-	-	-	-	-
Hemidesmus indicus (L.) R. Br.	57.14	-	-	-	-	-
Plumbago zeylanica L.	-	-	-	-	-	-
Medicinal Shrubs						
Abutilon indicum (L.) Sweet	-	57.14	28.57	-	66.66	50.00
Adhatoda vasica Nees	50.00	-	25.00	-	-	-
Ocimum sanctum L.	33.33	-	-	28.57	-	-

A= Root-Stem; B = Root -Petiole; C = Root -Leaf; D =Stem -Petiole; E = Stem -Leaf; F = Petiole-Leaf; NA = Not applicable

Table 6: Species richness and diversity indices of endophytic fungi in medicinal herbs and shrubs

Indices	Index	A	B	C	D	E	F	G	H	I
Species Richness	R_1	1.86	1.92	**3.13**	1.54	2.27	1.44	1.30	2.34	2.50
	R_2	1.79	1.77	**2.49**	1.51	2.00	1.50	1.27	1.94	2.11
Species Diversity	λ	0.10	0.11	**0.06**	0.29	0.17	0.17	0.20	0.13	0.13
	H'	1.33	1.58	**2.12**	1.15	1.77	1.04	1.33	1.80	1.76
Species Evenness	E	0.96	0.98	0.96	0.83	**0.99**	0.95	0.96	0.92	0.90

(R_1): Margalef's index; (R_2): Menhinick index; λ: Simpson's index; H': Shannon -Weiner index; E: Evenness index; A-*Aloe vera* (L.) Burm. f. , B-*Abrus precatorius* L., C-*Asparagus racemosus* Willd., D -*Catharanthus roseus* (L.) G. Don ., E-*Hemidesmus indicus* (L.) R. Br. , F-*Plumbago zeylanica* L., G-*Abutilon indicum* (L.) Sweet , H-*Adhatoda vasica* Nees, I-*Ocimum sanctum* L.

Acremonium sp., *Fusarium proliferatum, Aspergillus fumigatus, Aspergillus niger* and *Penicillium* sp. 2 were isolated only from roots.

A. vera, *C. roseus* and *P. zeylanica* harbors uncommon endophytic fungi between their tissues (**Table 4**). *Abrus precatorius*, *H. indicus*, *A. vasica*, *O. sanctum* showed 44.44%, 57.14%, 50.00% and 33.33% similarity between root and stem endophytes, respectively. Stem and leaf of *A. racemosus* and *A. indicum* was found to contain 44.44% and 66.66% overlapping endophytes assemblages, respectively. Only *A. indicum* showed 57.14% similar endophytic fungi between root and petiole.

Table 5: Similarity coefficient (SC) of endophytic fungi between six herbs and between three shrubs

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
SC	17.0	0	0	0	0	10.0	0	0	18.18	40.0	18.18	47.06	25.00	36.36	0	0	9.52	8.33

1. *Aloe vera* (L.) Burm. f.-*Abrus precatorius* L. 2. *Aloe vera* (L.) Burm. f. - *Asparagus racemosus* Willd., 3.*Aloe vera* (L.) Burm. f.-*Catharanthus roseus* (L.) G. Don ., 4. .*Aloe vera* (L.) Burm. f.-*Hemidesmus indicus* (L.) R. Br., 5. *Aloe vera* (L.) Burm.f.-*Plumbago zeylanica* L., 6. *Abrus precatorius* L.- *Asparagus racemosus* Willd., 7. *Abrus precatorius* L. –*Catharanthus roseus* (L.) G. Don., 8. *Abrus precatorius* L. -*Hemidesmus indicus* (L.) R. Br. 9. *Abrus precatorius* L.-*Plumbago zeylanica* L. 10. *Asparagus racemosus* Willd.-*Catharanthus roseus* (L.) G. Don ., 11. *Asparagus racemosus* Willd.-*Hemidesmus indicus* (L.) R. Br. , 12. *Asparagus racemosus* Willd.-*Plumbago zeylanica* L. 13. *Catharanthus roseus* (L.) G. Don .-*Hemidesmus indicus* (L.) R. Br ., 14. *Catharanthus roseus* (L.) G. Don.-*Plumbago zeylanica* L., 15. *Hemidesmus indicus* (L.) R. Br. -*Plumbago zeylanica* L., 16. *Abutilon indicum* (L.) Sweet- *Adhatoda vasica* Nees , 17.*Abutilon indicum* (L.) Sweet-*Adhatoda vasica* Nees, 18.*Adhatoda vasica* Nees-*Ocimum sanctum* L.

Similarity coefficient (SC) of endophytic fungi between host plants were also studied (**Table 5**). Maximum similarity of endophytic fungi (47.06%) was observed between two plants viz., *A. racemosus* and *P. zeylanica*. Endophytic assemblages of four herbs i.e., *P. zeylanica* (47.06%), *C. roseus* (40 %), *H. indicus* (18.18) and *A. precatorius* (10%) showed similarity with *A. racemosus. A. precatorius* showed 17% common endophytic fungi of *A. vera* and 18.18% of P. zeylanica. The endophytic fungi of *A. vasica* showed similarity with *A. indicum* and *O. sanctum*.

Species richness and diversity indices are presented in **Table 6**. Maximum species richness of endophytic fungi was recorded in *A. racemosus* with 3.13 Margalef's index and 2.49 Menhinick index followed by *O. sanctum, A. vasica* and *H. indicus.* Minimum species richness was observed in *A. indicum* with 1.30 Margalef's index (R_1) and 1.27 Menhinick index (R_2). The highest Shannon-Weiner index (2.12) and minimum Simpson's index (0.06) was noticed in *A. racemosus. P. zeylanica* exhibited minimum Shannon-Weiner index. *C. roseus* showed maximum Simpson's index (0.29). In all cases, species evenness values for all plants were between 0.90 to 0.99 except *C. roseus.*

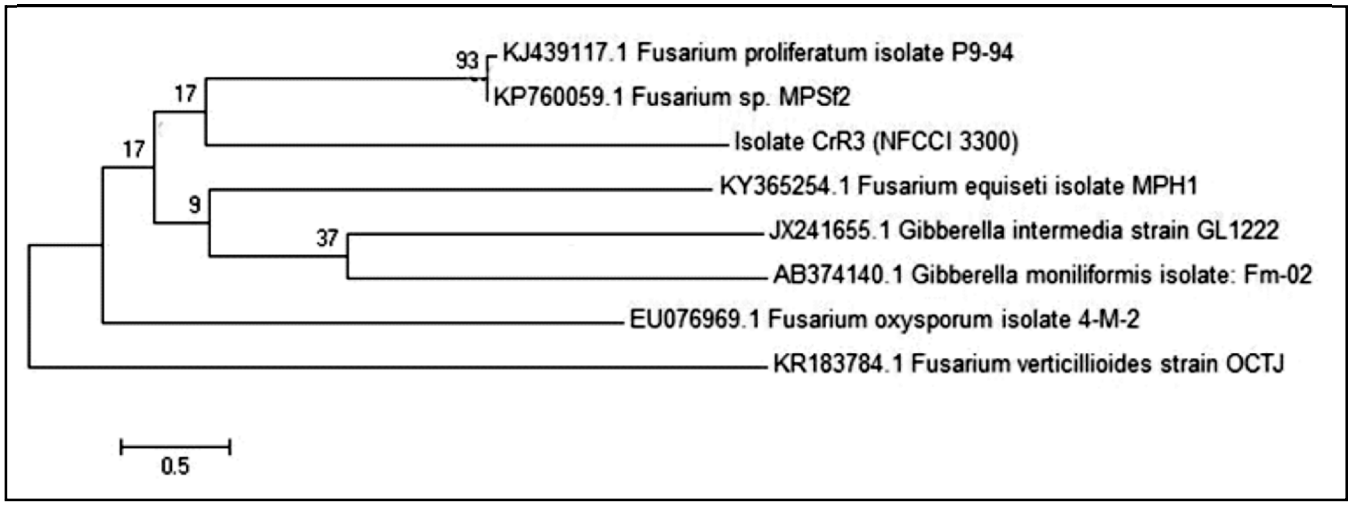

Fig. 5: Phylogenetic tree (rectangular) of isolate CrR3 (NFCCI 3300) based on ITS-rDNA sequence amplified by fungal-conserved ITS4 and ITS5.The numbers at branch node indicate the confidence value of bootstrap replication.

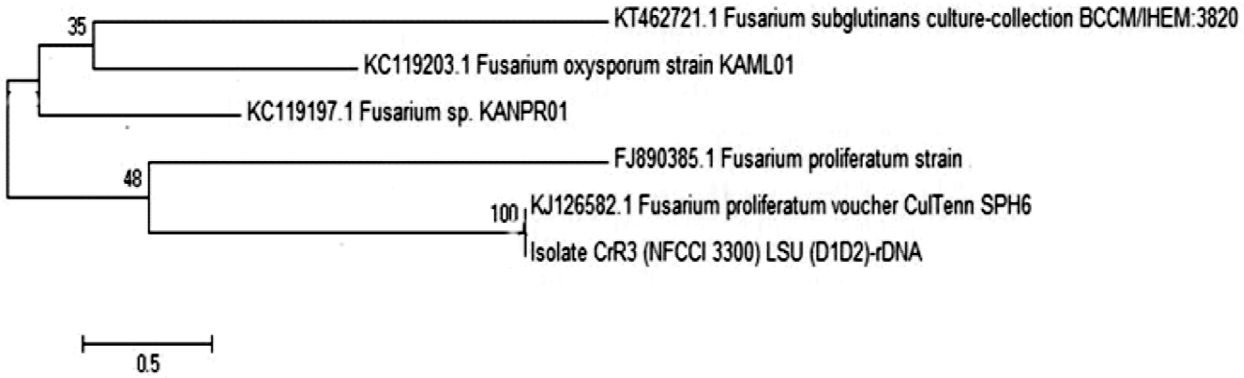

Fig. 6: Phylogenetic tree (rectangular) of isolate CrR3 (NFCCI 3300) based on LSU (D1D2)-rDNA sequence. The numbers at branch node indicate the confidence value of bootstrap replication.

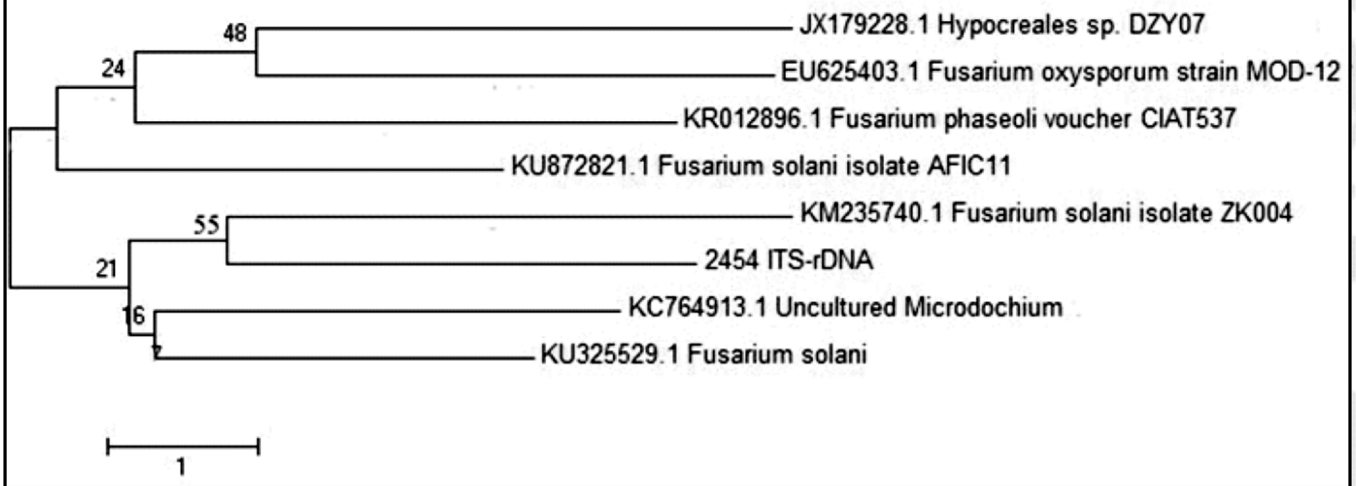

Fig. 7: Phylogenetic tree (rectangular) of isolate ApR6 (NFCCI 3301) based on ITS-rDNA sequence amplified by fungal-conserved ITS4 and ITS5.The numbers at branch node indicate the confidence value of bootstrap replication.

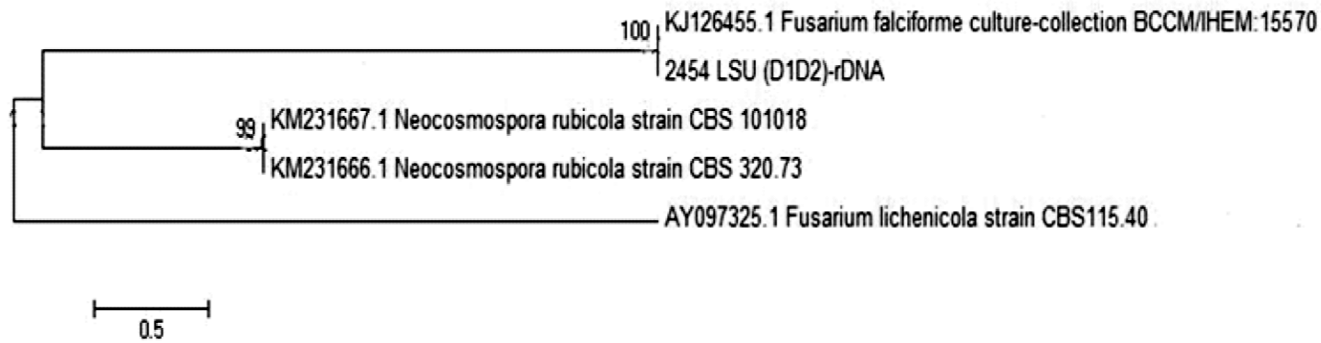

Fig. 8: Phylogenetic tree (rectangular) of isolate ApR6 (NFCCI 3300) based on LSU (D1D2)-rDNA sequence. The numbers at branch node indicate the confidence value of bootstrap replication.

DISCUSSION

The occurrence of endophytic fungi varied with the plant species studied. The isolated fungal genera including *Acremonium, Alternaria, Aspergillus, Aureobasidium, Bipolaris, Colletotrichum, Curvularia, Paecilomyces, Penicillium, Pestalotiopsis, Fusarium*, *Neocosmospora* and sterile forms were reported earlier in tropical host plants (Petrini *et al.*,1992; Naik *et al.*,2008; Krishnamurthy *et al.*,2008 and Naik *et al.*, 2014). *Ocimum sanctum* was reported to harbor maximum endophytic fungi in petiole. Rajagopal *et al.* (2010) also reported the presence of endophytic fungi in the petiole of *O. sanctum* and suggested that the movement of water and food through the petiole could be favorable for fungal growth. In the study, the results suggested that *Colletotrichum gloeosporioides* and *Curvularia* sp. was non-host specific as they are dominantly isolated from different host plants. In contrast, *Fusarium proliferatum* and *Neocosmospora falciformis* were identified by Internal Transcribed Spacer (ITS) and D1D2 sequence analysis from the roots of *C. roseus* and *A. precatorius*, respectively. These results strongly suggested that the fungi are host specific. Some of the fungal species such as *Acremonium* sp., *F. proliferatum, Aspergillus fumigatus, A. niger* and *Penicillium* sp. 2 were found to be specific towards root tissues as they were isolated only from roots of different plants. In the present study, some endophytic fungi were common in more than one tissue of the host plant. This indicates that the endophytes may have the ability to penetrate from one part to another part of the host tissues (Kumar and Hyde, 2004). In contrast, endophytic fungi isolated from *A. vera, C. roseus* and *P. zeylanica* was uncommon between their different plant tissues, due to the lack of systemic growth by endophytic fungi from one tissue to another. The species composition was reported to vary with different tissues of plant and between host plants (Rodriguez, 1996; Suryanarayanan and Vijaykrishna, 2001; Rodriguez *et al.*, 2009 and Wearn *et al.*, 2012). Notably, the percentage of common endophytic fungi differ between medicinal herbs i.e. some plants shared 50% common endophytic fungi, some showed less than 20% common fungi and some plant hosts did not show similar endophyte between each other in the study.

CONCLUSION

These results of the study suggested that the occurrence of fungal endophytes is influenced by the genetic background of the host, type of plant host, host tissue and nature of phytochemicals present in the host plant (Sun *et al*., 2008; Rajagopal *et al*., 2010 and Jia *et al*., 2016). Shannon-Weiner and Simpson's indices indicates the degree of diversity and dominance. In our result, higher value of Shannon-Weiner index and lower value of Simpson's index by *A. racemosus* indicates high species diversity and low degree of dominance by isolated species. The higher value of Simpson's index is represented by *C. roseus* as this host plant was dominated by *Colletotrichum gloeosporioides*. Evenness indices indicate that all species are equally abundant in most of the host plants.

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REFERENCES

Bailey, B.A., Bae, H., Strem, M.D., Roberts, D.P., Thomas, S.E., Crozier, J., Samuels, G.J., Choi, I.Y., and Holmes, K.A.2006. Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma sp*. *Planta* **224**:1449-1464. doi: 10.1007/s00425-006-0314-0.

Barnett, H.L. and Hunter, B.B. 1998. *Illustrated Genera of Imperfect Fungi*. APS Press Burgess, St. Paul, Minnesota USA.

Carroll, G.C. and Carroll, F.E. 1978. Studies on the incidence of conifereous needle endophytes in the Pacific northwest. *Can. J.Bot.* **56**: 3034-3048.

Ellis, M.B. 1976. *Dematiaceous Hyphomycetes*. CAB, Commonwealth Mycological Institute, Kew, Surrey, England.doi: 10.1139/b78-367.

Faeth S.H. and Fagan,W.F. 2002.Fungal endophytes: common host plant symbionts but uncommon mutualists. *Integ. Comp. Biol.* **42**:360-368.doi: 10.1093/icb/42.2.360.

Hanlin, R.T. 1998. *Combined keys to illustrated genera of Ascomycetes*, vols.I & II, 1st edn. APS Press, Saint Paul MN.

Hazalin,N., Ramasamy, K., Lim, S.M., Wahab, I.A., Cole, A.L.J. and Majeed, A.B. 2009. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. *BMC Complement Altern. Med.* **9**:46.doi: 10.1186/1472-6882-9-46.

Jia, M., Chen, L., Xin, H.L., Zheng,C.J., Rahman, K., Han, T. and Qin, L.P. 2016. A friendly relationship between endophytic fungi and medicinal plants: A Systematic review. *Front. Microbiol.* **7**:1-14.doi: 10.3389/fmicb.2016.00906.

Krishnamurthy, Y.L., Shankar, N.B. and Shashikala, J. 2008 Fungal communities in herbaceous medicinal plants, Malnad region, Southern India. *Microbes. Environ.* **23:**24-28. doi:10.1264/jsme2.23.24

Kumar, D.S.S. and Hyde, K.D. 2004. Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*. *Fungal Divers.* **17**: 69-90.

Ludwick, J.A. and Reynold, J.F. 1998. *Stastistical ecology: A primer on Method and Computing*. A Wiley-Interscience publication, New York.

Mahobiya, D. and Gupta, A.K. 2014. Endophytic fungi from selected herbs & shrubs of Raipur and their L-asparaginase producing potentials. *Journal of Ravishankar University*-B, **28**:51-59.

Magalef, R. 1958. Information theory in ecology. *Gen. System.* **3**: 36-71.

Menhinick, E.P. 1964. A comparison of some species-individual diversity indices applied to samples of field insects. *Ecology* **45**:849-881.**doi:**10.2307/1934933.

Nagamani, A., Kunwar, I.K. and Manoharachary, C. 2006. *Handbook of Soil Fungi*. I.K. International Pvt. Ltd., New Delhi, India.

Naik, B.S., Shashikala, J. and Krishnamurthy, Y.L. 2008. Diversity of fungal endophytes in shrubby medicinal plants of Malnad region, Western Ghats, Southern India. *Fungal Ecol.* I.89-93. Doi:10.1016/j.funeco.2008.05.001.

Naik,B.S., Krishnappa, M. and Krishnamurthy, Y.L. 2014. Biodiversity of endophytic fungi from seven herbaceous medicinal plants of Malnad region, Western Ghats, Southern India. *J. For. Res*.**25**:707-711. **doi**:10.1007/s11676-014-0511-9.

Petrini, O., Sieber, T.N., Toti, L. and Viret, O. 1992. Ecology, metabolite production and substrate utilization in endophytic fungi. *Nat. Toxins* **1**:185-196. doi: 10.1002/nt.2620010306.

Rodriguez, K.F. 1996. Fungal endophytes of palms. In: *Endophytic fungi in grasses and woody plants: Systematics, ecology and evaluation*. (Eds.: Redlin,S.C. and Carris, L.M.). APS Press, St. Paul, Minnesota, USA, 121-132 pp.

Rodriguez, R.J., White, J.F., Arnold, A.E. and Redman, R.S. 2009. Fungal endophytes: diversity and functional roles. *New Phytol.* **182**: 314-330. **doi**:10.1111/j.1469-8137.2009.02773.x

Rajagopal, K., Kalavathy, S., Kokila, S., Kathiravan, G., Prasad, R. and Balasubramanian, P. 2010. Diversity of fungal endophytes in few medicinal herbs of south India. *Asian J. Exp. Biol. Sci.* **1**: 415-418.

Seifert, K.A., Morgan-Jones, G., Gams, W. and Kendrick, B. 2011. *The genera of Hyphomycetes*, 2nd edn. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherland.

Singh, L.P., Gill, S.S. and Tuteja, N. 2011. Unraveling the role of fungal symbionts in plant abiotic stress tolerance. *Plant Signal. Behav.* **6**:175-191. doi: 10.4161/psb.6.2.14146

Strobel, G.A. 2002. Mirobial gift from rain forests. *Can. J. Plant Pathol.***24**:14-20.**doi**: 10.1080/07060660109506965

Shannon, C.E. and Weaver, W. 1949. *The mathematical theory of communication*. University of Illinois press, Urbana.

Sun. J., Guo, L., Zang, W., Ping, W. and Chi, D. 2008. Diversity and ecological distribution of endophytic fungi associated with medicinal plants. *Sci. China. Ser.* C51:751-759. **doi**: 10.1007/s11427-008-0091-z

Sun, X. and Guo, L.D. 2012. Endophytic fungal diversity: Review of traditional and molecular techniques. *Mycology* **3**: 65-76. doi: 10.1080/21501203.2012.656724.

Suryanarayanan, T.S. and Vijaykrishna, D. 2001. Fungal endophytes of aerial roots of *Ficus benghalensis*. *Fungal Divers*. **8**:155-161. doi:10.1.1.566.42

Suryanarayanan, T.S. and Thennarasan, S. 2004. Temporal variation in endophyte assemblages of *Plumeria rubra* leaves. *Fungal Divers*.**16**:131-140. Doi:10.1.1.459.66.

Watanabe, T. 2002. *Pictorial atlas of Soil and Seed Fungi*, 2nd edn., CRC Press, Boca Raton Landon, New York, Washington, D.C.

Wearn, J.A., Sutton, B.C., Morley, N.J. and Gange, A.C. 2012. Species and organ specificity of fungal endophytes in herbaceous grassland plants. *J. Ecol.* **100**:1085-1092.doi: 10.2307/23257530.