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Diversity of Endophytic fungi in liana, *Celastrus paniculatus* collected from few sites of Jhargram and Paschim Medinipur districts, West Bengal, India

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Abstract

To determine the identity and diversity of endophytic fungi associated with the liana from five different forest localities of Jhargram and West Medinipur districts of West Bengal. On the basis of differentiation of weather and microclimate, I have to select the regions. Between the two regions, the distance is at least 25-30 km, the microclimate and moisture under the canopy will differ, it affects mainly on the presence of endophytes. Leaf, fruit and stem segments were collected randomly in summer, winter and monsoon in 2018. It is impossible to take all leaves and other organs of a plant because plant parts should be collected sustainably so that minimum damage of stock occurs. Surfaces of all samples were sterilised just before putting on pot5ato dextrose agar (PDA) media for the growth of endophytic fungal mycelia and their isolation. Fungi were isolated and identified based on the morphology of its colony, and mycelial form and morphology, sexual and asexual reproductive structures and their characters, spore-form and nature of attachment, cultural conditions etc. were taken in consideration to identify them. Total 1125 samples were used for endophytic growth. The total of 1558 endophytic fungi were isolated from 797 sample segments of *Celastrus paniculatus*. The dominant endophytic fungi belong to genera *Fusarium* sp., *Aspergillus* sp., *Chaetomium* sp., *Beltrania* sp., *Pestalotiopsis* sp., *Verticillium* sp., *Arthrinium* sp, *Penicillium* sp., *Podospora* sp., *Alternarium* sp., *Acrocylindrium* sp. etc. Maximum endophytic isolates were obtained from leaf segments followed by fruit and then stem. In monsoon, colonization frequency shows highest (80.53%) and in summer, it is lowest (61.87%) from the plant samples of all locations. The examples from Chilkigarh shows the highest colonization frequency (90.22%) and from Nayagram, it is the lowest (61.78%). The leaf's colonization frequency is maximum (84.53%) and the stem is minimum (62.4%). Most of the isolated endophytic fungi were found under the group Deuteromycetes. Endophytic fungi show a wide range of Shannon-Weiner and Simpson's indices. These indicators point to an equal and throughout distribution of different species. The findings add to our knowledge of the identity and diversity of endophytic fungi, which are expected to have a variety of interactions with their host plants.

Keywords: Celastrus sp., diversity, endophytic fungi, lianas, sacred grooves.

Introduction

Endophytes are the microbial organisms (fungi & bacteria) that take accomodations within tissue of various organs of higher plants without causing any disease or disease symptoms. They may be pathogens or symbionts, or mutualistic. They may influence the structure and community of plants. Fungal endophytes may provide protection to their host. They are under-explored groups of microorganisms. They are found to protect their host against insect pests, pathogens and even domestic herbivores (Weber, 1981). Some of the microorganisms produce novel secondary metabolites that are useful in medicine, agriculture, and industries. Endophytic diversity is more significant in tropical and subtropical forests than in other climatic zones. Endophytes are essential components of plant symbiosis, influencing the tolerance of host to stress, plant defence system, and bringing diversity in plant community. Types of tissue, age of host, season, topography may influence their prevalence. Isolation of a potent anticancer drug –taxol from the yew plant *Taxus baccata* produced by *Pestalotiopsis* sp., an endophytic fungus, and phytohrome-producing fungi *Gibberella* sp. from the rice plant suggests that endophytes have immense power. Lianas are a special group of plants which are woody climbers in the forest and climb up tall staff trees.

Anton De Bary was the pioneer to use the word ‘endophyte’ in 1866, and it was used for microorganisms within plant i.e., 'organisms that exist inside plant tissues'. Endophytic fungi are microorganisms that reside in the interior tissue of plants and cause no visible symptoms (Wilson, 1995). Even though endophytic fungi are mainly mutualistic, they may not remain endophytes throughout their lives (Porras-Alfaro and Bayman, 2011). Endophytic fungi were found in all environmental conditions investigated so far, except for the aquatic state (Caroll, 1988; Petrini, 1991). Endophytes are microorganisms remain in plant tissues without causing illness or causing damage to the plant (Bacon et al., 2000). Although fungi are the most common, endophytes may be bacteria or even viruses (Strobel et al., 2003). Endophytes exist without causing any symptoms inside plant tissues, according to Carrol and Carrol (1988). Novel bioactive compounds are produced by endophytic microbes (Daisy et al., 2003). Endophytes may persist in intercellular spaces of leaf, petioles, fruits, flowers, roots, and stems (Van Wyk et al., 1990; Verstraete et al., 2011). Various lianas plants have various fungal endophytes that are thought to generate antibacterial compounds (Banerjee et al., 2006).

Fungal endophytes in *Theobroma cacao* and *Solanum melongena* reduced foliar and root diseases, respectively, and treatment of *Glycine max* with culture filtrate of endophyte- *Cladosporium sphaerospermum* increased plant height (Mejia et al., 2008; Narisawa et al., 2002; Hamayun et al., 2009). Duong et al., (1994) studied fungal endophytic diversity and community patterns in healthy and yellowing leaves of *Citrus lemon*. Gilbert et al., (2007) reported fungal symbionts in tropical trees. Some researchers isolated very diverse group of endophytic fungi from plant tissues of tropical and subtropical rain forests (Arnold et al., 2001). Endophytes may vary over seasons (Maheswari et al., 2013). Endophytic microbes are the normal microflora within plant tissues (Ganley et al., 2004). They protect the plants against pests. They also

enhance the defence mechanisms of host plants against unfavourable environments. Endophytic fungi show considerable antibacterial and antifungal activity (Jena et al., 2013). Various antifungal agents have been explored, but the control of many fungal diseases has not been achieved.

The aim of the study was

(i)To isolate endophytic fungi from the selected liana of various regions of the two districts.

(ii)To identify the endophytic fungi.

(iii) To determine the diversity of endophytic fungi

(iv)To determinate the host & organ specificity in fungal endophytes.

(v)To study the impact of seasons and regions over the rate & frequency of fungal endophytes.

Methodology

Study site and sampling strategy

Authors collected leaves, fruits and stem pieces randomly during winter in 2018. Between the two areas, the distance is at least 25-30 km. The study was conducted in the West Medinipur and Jhargram districts of West Bengal, India. The districts are situated in between the latitude of $22^{o}25'$ to $22^{o}57'$ North and the longitude of $87^{o}11'$ East. The altitude is 23M above sea level. The climate is tropical, warm and humid, with a mean temperature of $33^{o}C$ and an average rainfall of 120cm. One liana (*Celastrus paniculatus*, family- Celastraceae) was selected from five different localities of forest for the study. We have selected 5 major forest regions at 4 corners based on separate microclimate. The specific locations are few forest sites of Jhargram (Belpahari and Chikigarh) and West Medinipur (Arabari, Khajra and Nayagram) district, West Bengal. India. The forests of these locations possess various types of lianas plants concerned with the work. Few disease-free stems, leaves with fruits were collected in ziplock packets from five different places. It is carried in the laboratory and preserved at $4^{0}C$ in refrigeration. Samples should be managed sustainably so that minimum damage of stock occurs. It is convenient to handle 25 samples and would be suitable for manipulating the data obtained. Each smaller sample is surface sterilized by submerging in 70% ethanol, then drying in flame & then it is put on the agar medium in plate. All the fungal micelia are picked up separately and put in potato dextrose agar media in petri dishes.

Sampling procedure

Plant samples (leaves, fruits and stems) were randomly selected from mature, diseases less plants from each site in three seasons. The samples were kept in zipper-lock plastic bags, carried to the laboratory, and stored at 4°C for until the isolation process was started.

Surface disinfection

All samples were washed under running tap water and surface sterilised by submerging in 70% ethanol for 1 minute, 1% sodium hypochlorite (NaOcl) for 4 minutes, again 70% ethanol for 20 seconds. Finally, samples were washed three times with sterile distilled water and drying on the surface in sterile conditions.

Placing the samples in media

Using a sterile scissor, samples were cut into 1 sq cm pieces and five samples of each were put on separate plate of water agar medium(WA). For fruit and stem removing peels, inner tissues were cut with a sterile knife and sequentially sterilized, then placed

on medium. As a result, 5 duplicate plates were produced for each sample from a single locality's plant.

Isolation of endophytic fungi

After few days, fungal growth was found as hyphae. Fungal hyphae were picked up from each sample and transferred to PDA medium by cutting a square block of water agar. The plates were incubated at 23°C in light. In most cases, huge mycelial and occasionally reproductive growth occurred after 10-15 days. Culture slants were made and stored at 4°C for future work.

Identification of endophytes

Making sketches of mycelia and spores, taking photographs of all endophytic fungal isolates, fungi were compared with standard identification keys. All fungi were examined under an optical compound microscope. The fungal isolates were identified using conventional identification guides based on their physical and reproductive characteristics.

Data analysis

The colonization frequency was determined using Hata and Futai's formula: CF = (N_{col}/N_t x100, where N_{col} is the no. of segments colonised by at least one fungus and N_t is the total no. of segments plated. The percentage of colony frequency divided by the total percentage of colony frequency of all endophytes x100 yielded dominant endophytes. N_i/N_s x100, where N_i is the percentage of colony frequency of individual endophytes and N_s is the colony frequency of all endophytes. The following diversity indices were computed using the Palaeontological Statistics software programme (PAST):

(a) The Simpson's Diversity Index (1-Dominance) was determined using the formula 1-D, where D = n(n-1)/N. (N-1). Here, n denotes the total no. of creatures in a specific species, whereas N is the total no. of organisms in all species.

(b) The Shannon-Wiener diversity index was determined using the formula: H' = Symbol for variety in a sample of species or kinds, s = number of species in the sample, P_i = relative abundance of ith species or kinds and measured by = n/N, N = total number of individuals of all kinds, n_i = number of individuals of ith species in = base 2 logarithm

(c) The following formula was used to determine evenness: H'max is the most significant value of diversity for the number of species, thus evenness (E) = H'/H'max.

Result and discussion

A total of 1558 endophytic fungal isolates were found from 797 various sample segments of *Celastrus* sp. However, 1125 samples were introduced to grow endophytes. In the winter study of *Celustrus* sp. total 375 plant segments were plated, out of which 363 were infested with endophytic fungi and 546 fungi were isolated from them. A total of 232 segments infested by fungi and 383 isolates, 302 segments infected and 629 fungal isolates were found from 375 sample segments each from summer and monsoon, respectively. Maximum endophytic isolates were obtained from leaf segments followed by fruit and then stem. In monsoon, colonization frequency shows highest (80.53%) and in summer, it is lowest (61.87%) from the plant samples of all locations. The samples from Chilkigarh shows the highest colonization frequency (90.22%) and from Nayagram, it is the lowest 61.78%). The leaf's colonisation frequency is maximum (84.53%) and of stem is minimum (62.4%).

Table 1. Colonization frequency of endophytes in various organs of *Celastrus paniculatus* from different sites in summer.

Place	Total no. of segments plated	No. of segments infested with fungi	No. of Fungi isolated from the segments	Colonization frequency (CF%)	CF in leaf (%)	CF in fruit (%)	CF in stem (%)
Belpahari	75	38	55	50.67	80	28	44
Chilkigarh	75	53	155	70.67	100	64	48
Arabari	75	48	64	64	80	84	68
Khajra	75	51	67	68	64	80	60
Nayagram	75	32	42	42.67	68	32	28
Total	375	232	383	61.87	78.4	57.6	49.6

Table 2. Endophytic fungi isolated from leaf (L), fruit (F) and stem (S) segments of *Celastrus paniculatus* from five different locations in summer.

Endophytic isolation	Total	Belpahari			Chilkigarh			Arabari			Khajra			Nayagram		
		L	F	S	L	F	S	L	F	S	L	F	S	L	F	S
Acremonium vitis	5	1	0	0	2	2	0	0	0	0	0	0	0	0	0	0
Alternaria sp.	14	1	0	1	4	4	0	0	0	0	1	0	0	2	1	0
Arthrinium sp.	17	2	1	1	5	2	0	0	0	3	2	0	0	1	0	0
Arthrobotrys sp.	17	1	0	0	4	0	0	0	1	2	3	0	2	3	0	1
Beltrania sp.	18	5	1	1	2	3	0	2	0	1	1	0	0	0	1	1
Blastomyces sp.	12	0	0	0	5	0	1	0	1	0	2	0	0	2	1	0
Curvularia sp.	23	2	0	1	4	4	0	1	0	1	5	2	0	1	1	1
Diplodia sp.	3	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0
Fusarium sp.	23	0	0	2	7	1	1	4	1	6	1	0	0	0	0	0
Lasiodiplodia sp.	19	2	0	1	6	2	0	1	1	5	1	0	0	0	0	0
Mucor sp.	22	5	0	0	7	2	1	0	0	0	5	0	0	1	0	1
Mycelia sterilia	15	0	0	5	1	0	0	5	2	0	1	0	0	0	0	1
Nigrospora sp.	23	4	0	0	10	0	1	1	0	2	0	0	0	2	0	3
Papularia sp.	10	1	0	0	4	1	0	0	0	0	4	0	0	0	0	0
Papulospora sp.	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium sp.	22	1	0	3	15	0	0	0	0	0	0	0	1	2	0	0
Peronospora sp.	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Pestalotiopsis sp.	34	0	3	3	9	4	2	4	0	0	3	0	3	2	0	1
Podospora sp.	9	0	0	0	3	0	0	4	0	0	0	0	0	1	0	1
Scopulariopsis sp.	4	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0
Tolyposporium sp.	10	0	0	0	6	0	1	0	0	0	0	0	3	0	0	0
Torula sp.	3	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Unidentified genera	38	0	0	1	0	0	0	5	5	1	9	7	8	0	1	1
Verticillium sp.	36	2	0	0	16	3	7	2	0	1	2	0	0	3	0	0
Zygorhynchus sp.	2	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Total	383	29	7	19	113	28	14	29	13	22	41	9	17	24	6	12

Table 3. Diversity indices and species richness of endophytic fungi from different locations in summer.

Parameter	Belpahari	Chikigarh	Arabari	Khajra	Nayagram
Taxa_S	18	20	14	17	17
Individuals	55	155	64	67	42
Dominance_D	0.07438	0.07704	0.104	0.1682	0.06916
Simpson_1-D	0.9256	0.923	0.896	0.8318	0.9308
Shannon_H	2.723	2.754	2.435	2.268	2.745
Evenness_e^H/S	0.8461	0.7851	0.8153	0.5684	0.9157
Fisher_alpha	9.317	6.113	5.53	7.344	10.63

Table 4. Colonization frequency of endophytes in various organs of *Celastrus paniculatus* from different sites in monsoon.

Place	Total no. of segments plated	No. of segments infested with fungi	No. of Fungi isolated from the segments	Colonization frequency (CF%)	CF in leaf (%)	CF in fruit (%)	CF in stem (%)
Belpahari	75	51	71	68	80	88	36
Chilkigarh	75	75	262	100	100	100	100
Arabari	75	44	82	58.67	72	52	52
Khajra	75	62	108	82.67	100	68	80
Nayagram	75	70	106	93.33	100	92	88
Total	375	302	629	80.53	90.4	80	71.2

Table 5. Endophytic fungi isolated from leaf (L), fruit (F) and stem (S) segments of *Celastrus paniculatus* from five different locations in monsoon.

Endophytic fungi	Total	Belpahari			Chilkigarh			Arabari			Khajra			Nayagram		
		L	**F**	**S**	**L**	**F**	**S**	**L**	**F**	**S**	**L**	**F**	**S**	**L**	**F**	**S**
Achlya sp.	22	2	0	2	8	3	0	0	0	0	0	0	0	5	2	0
Acrocylindrium sp.	61	14	1	10	18	4	2	3	0	0	2	2	0	4	0	1
Alternaria sp.	44	2	0	0	15	5	5	3	1	0	3	0	4	5	0	1
Apophysomyces sp.	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Arthrinium sp.	41	1	0	1	11	2	5	2	3	0	5	0	0	4	5	2
Arthrobotrys sp.	10	0	0	0	0	1	0	0	0	0	4	0	0	4	0	1
Aspergillus sp.	19	4	0	1	5	0	0	2	2	1	1	0	2	0	0	1
Blastomyces sp.	3	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
Botryotrichum sp.	3	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1
Chaetomium sp.	51	4	1	1	18	4	7	1	3	2	5	1	1	3	0	0
Chromelosporium sp.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Chrysosporium sp.	11	1	0	0	1	0	0	0	1	2	2	1	2	0	0	1
Colletotrichum sp.	5	0	0	0	2	0	1	0	0	0	1	0	0	0	0	1
Curvularia sp.	24	0	0	1	8	0	3	0	0	0	6	1	2	0	2	1
Diplodia sp.	16	2	0	0	5	1	0	0	1	0	3	0	0	0	3	1

Drepanopeziza sp.	2	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Eutypella sp.	4	0	1	0	2	0	0	0	0	0	0	0	0	0	1	0
Fusarium sp.	82	3	4	0	19	2	11	7	5	1	9	1	8	11	1	0
Lasiodiplodia sp.	45	0	2	0	10	5	5	0	0	2	7	0	6	6	1	1
Mucor sp.	12	0	0	0	3	0	1	0	0	4	0	0	0	2	0	2
Mycelia sterilia	48	0	3	0	5	0	0	0	0	5	2	3	12	3	3	12
Nigrospora sp.	20	0	0	0	7	4	0	2	3	1	2	1	0	0	0	0
Penicillium sp.	13	1	0	0	0	0	2	1	0	0	1	0	2	0	0	6
Pestalotiopsis sp.	5	0	0	0	0	0	1	4	0	0	0	0	0	0	0	0
Pyrenochaeta sp.	2	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Stachybotrys sp.	4	0	1	0	2	0	1	0	0	0	0	0	0	0	0	0
Unidentified genera	44	2	2	0	12	1	6	5	2	7	2	2	0	0	0	3
Uromyces sp.	6	0	0	0	4	1	1	0	0	0	0	0	0	0	0	0
Verticillium sp.	30	0	3	0	16	2	1	2	1	0	0	0	0	1	4	0
Total	629	36	19	16	173	35	54	32	23	27	55	14	39	48	22	36

Table 6. Diversity indices and species richness of endophytic fungi from different locations in monsoon.

Parameter	Belpahari	Chikigarh	Arabari	Khajra	Nayagram
Taxa_S	18	25	18	17	21
Individuals	71	262	82	108	106
Dominance_D	0.1601	0.0736	0.08894	0.09431	0.08135
Simpson_1-D	0.8399	0.9264	0.9111	0.9057	0.9187
Shannon_H	2.346	2.805	2.636	2.572	2.729
Evenness_e^H/S	0.5799	0.6609	0.7751	0.7704	0.7291
Fisher_alpha	7.772	6.799	7.124	5.67	7.854

Table 7. Colonization frequency of endophytes in various organs of *Celastrus paniculatus* from different sites in winter.

Place	Total no. of segments plated	No. of segments infested with fungi	No. of Fungi isolated from the segments	Colonization frequency (CF%)	CF in leaf (%)	CF in fruit (%)	CF in stem (%)
Belpahari	75	52	79	69.33	100	48	60
Chilkigarh	75	75	238	100	100	100	100
Arabari	75	51	65	68	64	60	60
Khajra	75	48	95	64	68	56	68
Nayagram	75	37	69	49.33	72	32	44
Total	375	263	546	70.13	84.8	59.2	66.4

Table 8. Endophytic fungi isolated from leaf (L), fruit (F) and stem (S) segments of *Celastrus paniculatus* from five different locations in winter.

Endophytic isolation	Total	Belpahari			Chilkigarh			Arabari			Khajra			Nayagram		
		L	F	S	L	F	S	L	F	S	L	F	S	L	F	S
Acremoniella sp.	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Arthrinium sp.	25	2	1	0	6	6	0	0	0	1	1	0	0	5	1	2
Arthrobotrys sp.	9	0	0	0	3	0	1	1	0	0	2	1	0	1	0	0
Aspergillus sp.	58	4	1	2	21	5	4	3	1	3	8	0	2	3	0	1
Beltrania sp.	31	6	1	1	13	1	6	0	0	0	1	0	0	0	1	1
Blastomyces sp.	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Botryotrichum sp.	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Chaetomium sp.	76	7	4	4	29	0	11	8	3	0	4	0	3	2	1	0
Chromelosporium sp.	10	5	0	0	0	0	0	0	0	0	0	0	0	2	0	3
Chrysosporium sp.	5	0	0	1	0	0	0	0	0	0	4	0	0	0	0	0
Diplodia sp.	7	0	0	0	2	0	0	1	0	1	0	0	1	2	0	0
Fusarium sp.	54	0	6	0	26	0	9	0	0	0	5	2	0	4	1	1
Gymnoascus sp.	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Lasiodiplodia sp.	3	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Mucor sp.	30	5	5	0	7	2	1	0	2	0	5	0	0	2	0	1
Mycelia sterilia	22	0	0	10	1	0	0	5	4	0	1	0	0	0	0	1
Nigrospora sp.	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium sp.	27	2	0	0	8	0	2	0	0	0	1	0	4	9	1	0
Peronospora sp.	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Pestalotiopsis sp.	45	0	2	5	17	0	2	4	0	0	7	0	3	1	1	3
Podospora sp.	10	0	0	0	3	0	0	4	0	0	0	0	0	2	0	1
Scopulariopsis sp.	3	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0
Tolyposporium sp.	10	0	0	0	6	0	1	0	0	0	0	0	3	0	0	0
Unidentified genera	42	0	0	1	0	0	0	5	1	1	9	12	8	0	2	3
Verticillium sp.	68	2	0	0	25	12	5	15	0	1	5	0	0	3	0	0
Zygorhynchus sp.	2	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Total	546	33	22	24	170	26	42	46	12	7	56	15	24	41	8	20

Table 9. Diversity indices and species richness of endophytic fungi from different locations in winter.

Parameter	Belpahari	Chilkigarh	Arabari	Khajra	Nayagram
Taxa_S	14	16	12	18	20
Individuals	79	238	65	95	71
Dominance_D	0.1075	0.1179	0.1418	0.1391	0.07122
Simpson_1-D	0.8925	0.8821	0.8582	0.8609	0.9288
Shannon_H	2.385	2.33	2.149	2.373	2.805
Evenness_e^H/S	0.7753	0.6421	0.715	0.5958	0.8262
Fisher_alpha	4.943	3.869	4.325	6.575	9.262

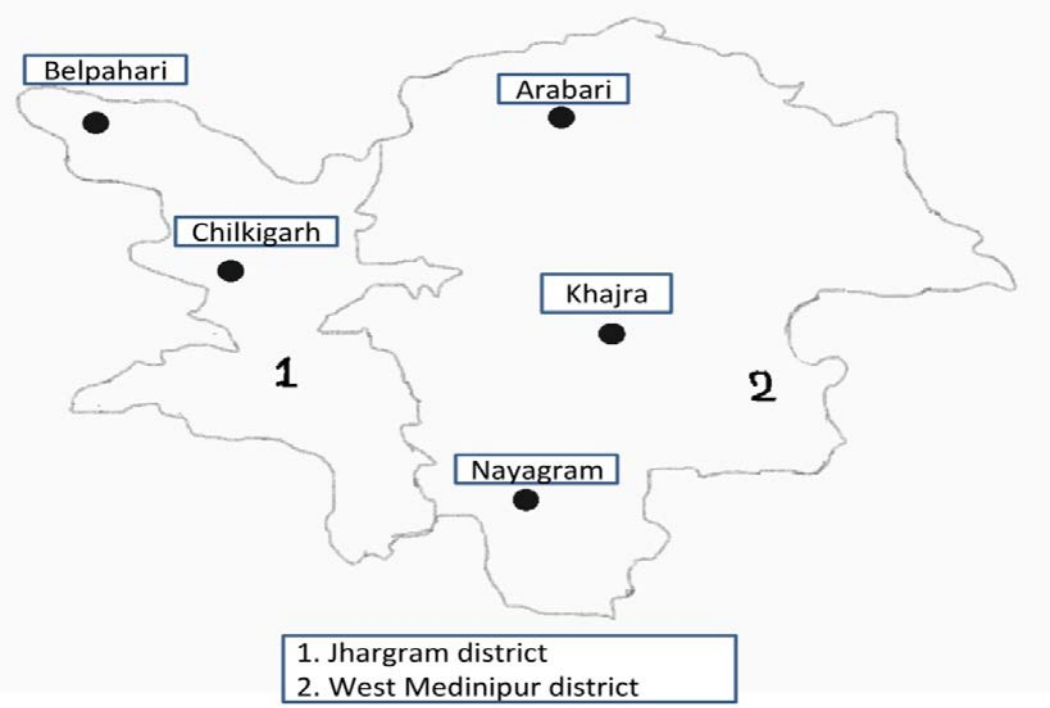

Figure 1. Locations from where plant samples were collected, distance between the sites is more than 30 km.

Figure 2. Liana plant- *Celastrus paniculatus* (whole plant), flowering plant, fruiting plant.

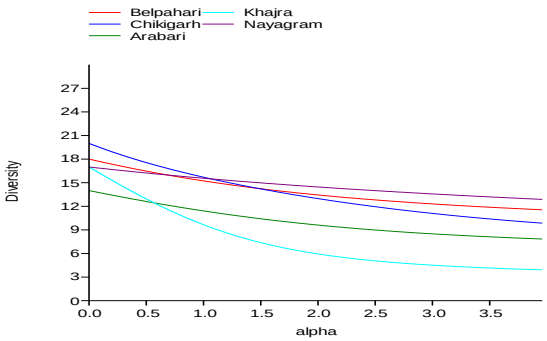

Figure 3. Line graph showing alpha diversity of endophytes in five different locations in

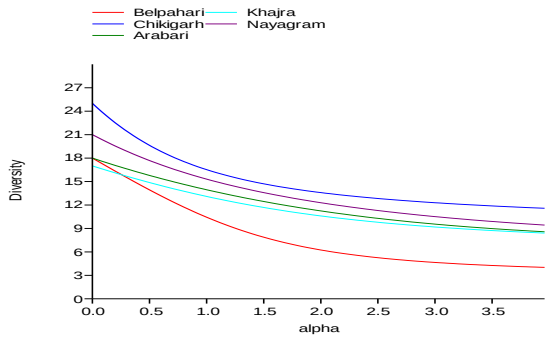

Figure 4. Line graph showing alpha diversity of endophytes in five different locations in

summer.

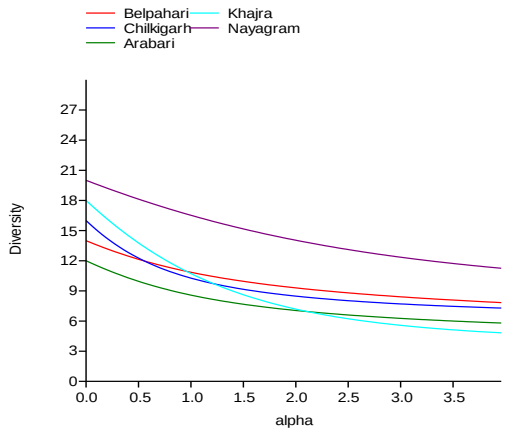

Figure 5. Line graph showing alpha diversity of endophytes in five different locations in winter.

monsoon.

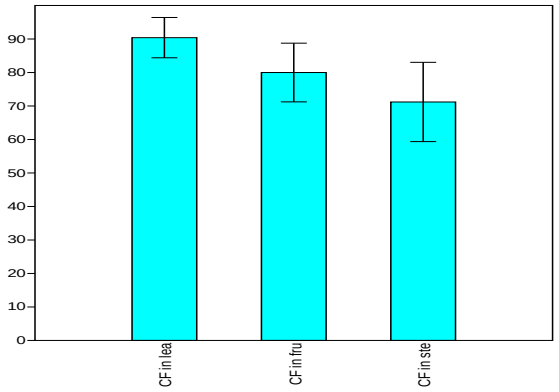

Figure 6. Comparison of colonization frequency (CF) of isolated endophytic fungi in tissues of three different organs (Leaf, fruit and stem) in monsoon.

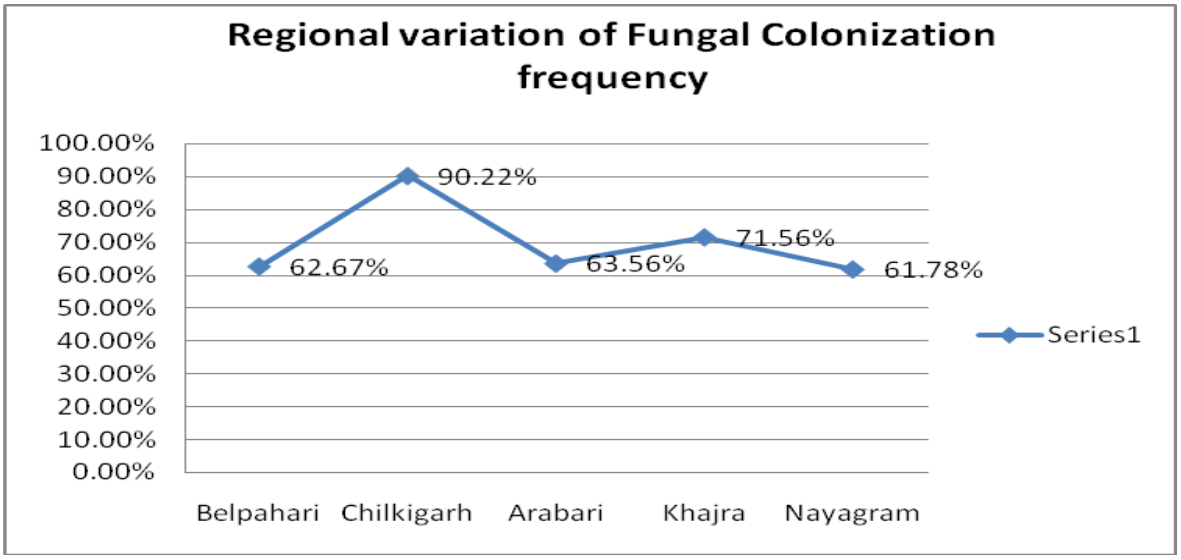

Figure 7. Comparison of colonization frequency (CF) of isolated endophytic fungi from various locations.

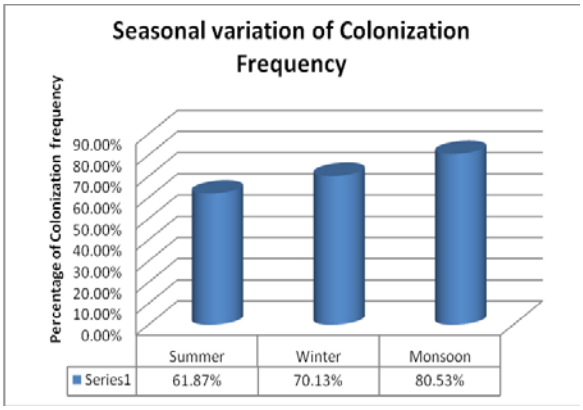

Figure 8. Comparison of colonization frequency (CF) of isolated endophytic fungi in three

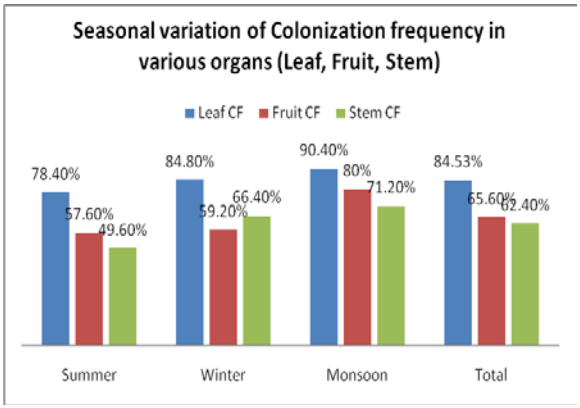

Figure 9. Variation of colonization frequency (CF) of isolated endophytic fungi in various

different seasons.

organs in different seasons.

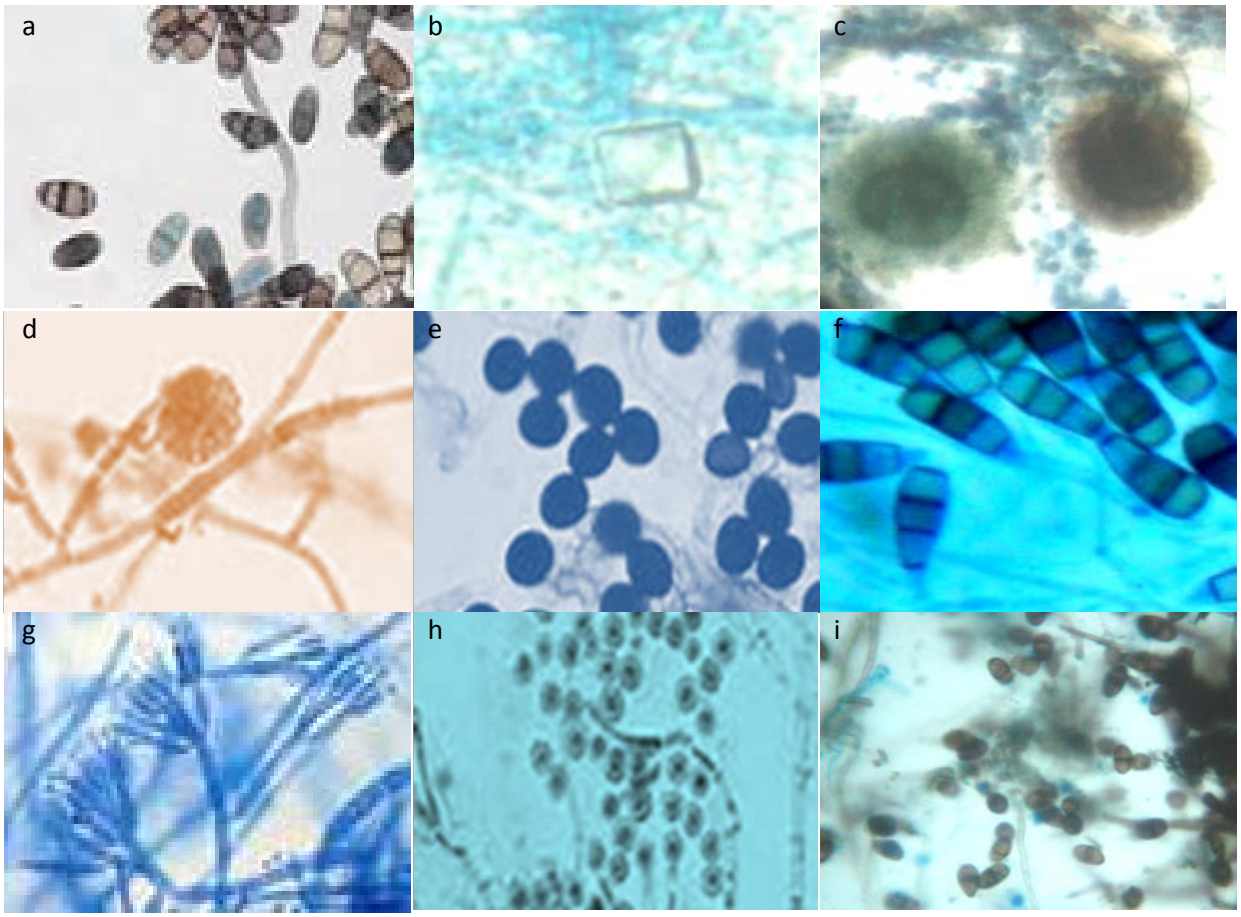

Figure 10. Pictures of few isolated endophytic fungi (a) *Curvularia* sp. (b) *Beltrania* sp. (c) *Aspergillus* sp. (d) *Papulospora* sp. (e) *Nigrospora* sp. (f) *Pestalotiopsis* sp. (g) *Penicillium* sp. (h) *Scopulariopsis* sp. (i) *Diplodia* sp.

All these results correlate with the given tables and figures. Suryanarayanan and Rajagopal (2000) isolated 963 fungi from bark of 10 tropical tree species in southern India. Banerjee et al., (2009a) reported 14 endophytic fungal genera in 3 medicinal herbs. Many different fungal genera and few unidentified with sterile mycelia were found. Most endophytic fungi belong to genera *Fusarium* sp., *Aspergillus* sp., *Chaetomium* sp., *Beltrania* sp., *Pestalotiopsis* sp., *Verticillium* sp., *Arthrinium* sp, *Penicillium* sp., *Podospora* sp., *Alternarium* sp., *Acrocylindrium* sp. etc.

Maximum isolated fungi fall under the group Deuteromycetes. Endophytic fungi exibit wide range of Shannon-Weiner and Simpson's indices. These indicators point to an equal distribution of different species. The findings increase our knowledge on how endophytes make variety of interactions with their host. Geographic and seasonal effects on the spread of fungal endophytes were discovered by Collado et al., (1999).

The graph observed that the highest diversity of isolated endophytic fungi was found in plants in the monsoon. Endophyte

diversity is much lower in various kinds of tropical forests in southern India than in the neotropics, perhaps due to low floristic diversity, presence of relatively open canopies, and highly variable annual rainfall, dry-season ground fires (Murali et al., 2007). Monsoon colonization frequency (CF) is highest because moisture favours the growth and mutualistic association of endophytes. Chilkigarh shows maximum CF because the forest has a dense shady canopy with variable microclimate and high moisture content within that canopy.

Conclusion

Deuteromycetes were the most common endophytic fungus among all groups. Endophyte colonisation has been shown to be unique to the organ and tissue of the host. In the research, a variety of fungal endophytes were discovered in several lianas plants. The majority have been recognised, with a few genera still unknown. During the winter, summer, and monsoon, the total isolated fungus in all chosen plants of the study were 546, 383, and 629, respectively. Samples were taken in April, August, and December of each year. Summer, rainy season in August and winter in December are all represented by April. Summer displays a low frequency of endophyte colonisation because high temperatures reduce the presence and rate of endophytic fungi because it is more reliant on the environment's moisture. The microclimate and moisture under the canopy differs, it affects mainly on the presence of rate and frequency of endophytes. Forest vegetation of Chilkigarh has variable microclimate under forest canopy, shady, moistened air and floor, wet leaf-litter that favour well growth band development of fungal endophytes in that location, so it shows highest rate and frequency of fungi. Huge precipitation favours the growth of endophytes, so monsoon shows maximum growth and colonization frequency.

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