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Endophytic Fungal Diversity and Their Comparison in Two Woody Climbers, from Few Regions of West Medinipur District, West Bengal, India

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Abstract

To determine the identity and diversity of endophytic fungi associated with two lianas (woody climber) from five different forest localities of Paschim Medinipur district of West Bengal.We collected leaves, petioles and stem pieces randomly during spring season in 2013. Samples were surface sterilized , then placed on water agar and PDA media for growth and isolation of endophytic fungi, respectively. Fungi were isolated and grouped based on colony morphology and identified based on mycelia shape and structure; sexual and asexual reproductive characters; attachment of spores; cultural conditions and so on. A total of endophytic isolates were obtained from different sample segments of *Celastrus* sp. and *Combretum* sp. The dominant endophytic fungi belong to genera. Maximum endophytic isolates were obtained from stem segments followed by leaves and petioles. Among all endophytic fungi class deuteromycetes were dominant over other fungal classes. Shannon-Weiner and Simpson's indices showed rich diversity of endophytic fungi. This indices suggest even and uniform occurrence of various species. The results improve our understanding of the identity and diversity of endophytic fungi that likely have different kinds of interactions with the host plants.

Key words: *Endophytic fungi, lianas, diversity, sacred grooves.*

1. Introduction

Endophytic fungi are microorganisms that live within the inner tissue of plants without causing apparent symptoms [1]. Although endophytic fungi are primarily mutualistic and commensalistic symbionts, they may not continue as endophytes throughout their life cycles [2]. Endophytes are ubiquitous in idistribution. Endophytic fungi that infest plants were found in all environments studied [3, 4]. Microorganisms that colonize internal plant tissues without causing any diseases symptoms or apparent injury are called endophytes [5]. Many fungal, bacterial, actinomycetean members are endophytes but most frequently isolated endophytes are fungi [6]. Carrol and Carrol (1988) reported that endophytes live without any symptoms and sometimes systematically within the plant tissues. They have been found infested with every plant species investigated so far. It is believed that plants from unique environmental settings and which are endemic are likely to accommodate distinct endophytic microorganisms as well as microorganisms making novel bioactive products [6]. Many endophytic fungi present in the root tissues forming mycorrhizal symbionts play a vital role on phosphate uptake in plant nutition. Others are present in the intercellular- spaces of leaves, petioles and inner tissues of stems [7, 8]. Lianas plants are woody climbers which grow supporting another straight and strong long trees and cover the topmost canopy of it. Sacred grooves are those holy plants which are used to worship in situ condition by races. Different lianas plants harbour some distinct fungal endophytes that are believed to be associated with the production of antimicrobial substances [9] and pharmaceutical products [10]. Huge research work is going on and it has expanded in recent years on isolation of endophytes, its antimicrobial activity and production of novel pharmaceutical products at every corner of the world.

Some endophytes can reportedly reduce plant diseases and enhance plant growth and products [11, 12, 13]. For example, 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 [12, 14, 15, 16].

Inspite of the omnipresence of endophytic fungi in plants, the extent of their contribution to fungal biodiversity and their interaction among themselves and with host still remain unclear. Few studies have been conducted with regards to the diversity and colonization of the endophytes.

Despite the largest diversity of endophytic species in tropical and subtropical rainforests, their biodiversity in tropical country is still poorly studied. It has been speculated that the bioactivity

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of various plants may be due to its associated endophytes. Some researchers isolated very diverse groups of endophytic fungi from plant tissues [17].

Endophytes are the normal microflora of the plant tissues [18]. They protect the plants against pests. Other fungal pathogens help in protection of host plants against grazing animals . They also enhance the defense mechanisms of host plant against unfavourable environments.

There is a very limited knowledge on fungal endophytic communities of Celastrus sp. and Combretum sp. In the western part of Paschim Medinipur district there are some forest areas with some unique environmental settings and huge plant resources with many lianas in patches. In this area, the study of endophytes in these two plants has not been covered till now. The optimul method to identify fungal endophytes is largely based on the research questions to be answered. It is known that endophytic fungi existing in the plant are potential sources of antimicrobial substance [9, 19, 20, 21]. Endophytic fungi show considerable antibacterial and antifungal activity [22]. Various antifungal agents have been explored, but the control of many of the fungal diseases has not been achieved.

The goal of the study was to identify the fungal endophytic communities in leaves, petioles and stems of Celastrus sp. and Combretum sp.. The objectives were to: (i) isolate the endophytic fungi (ii) determine the identity and diversity of endophytic fungi (iii) compare the endophytic fungal isolation between two plants and compare the endophytic diversity pattern in the two plants of five different regions.

2. Materials and methods

2.1 Study sites and collection of samples

The study was conducted in Paschim Medinipur district of West Bengal, India. The district is situated in between the latitude of 22°25′ to 22o57′North and longitude of 87°11′East. The altitude is 23M above from the sea level. The climate is tropical, warm and humid with a mean temperature of 33°C and an average rainfall of 120cm. Two lianas plants (*Celastrus paniculatus*, family- Celastraceae and *ombreCtum* sp., family- Combretaceae) were selected from five different localities of forest for present study.

Map:-Paschim Medinipur district showing five localities, from where samples were collected.

2.2 Sampling procedure

Plant samples (leaves, stems, petioles) were collected randomly from mature, healthy, disease-free plants from each location during spring (February). The samples immediately after collection were kept in zipper-lock plastic bags, brought to the laboratory and stored at 4°C within 2-3 hours of collection until isolation procedure was accomplished.

2.3 Surface disinfection

Samples collected from different localities were thoroughly washed under running tap water before processing and following sequences were followed: leaf, petiole and stem samples were surface sterilized by sequentially dipping into 80% ethanol for 1 min, 1% sodium hypochlorite (NaOcl)(4% available chlorine) for 4 min, 90% ethanol for 20sec. Finally, samples were rinsed with sterile distilled water for 3 times, then allowed to surface dry under sterile condition.

2.4 Placing the samples in media

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Sterile leaves were cut into pieces of about 1 square cm size by sterile scizor and placed in plate of water agar (WA), 5 samples in each, equidistant from each other. Similarly 5 sterile petioles of 0.5-1cm long were placed in another WA plate. Stem tissues were cut into short pieces of 4-5 cm long and after sequential sterilization, the outré layer was removed and inner tissues were peeled with sterile scalpel. Thin peels from various depth were placed on another WA plate. Thus, 5 replica plates for each sample from the plant of one locality were made.

2.5 Isolation of endophytic fungi

After placing the samples fungal growth was observed each and every day. Within 2-3 days fungal hyphae were in appearance. Some samples show more than one hyphal growth. From each sample fungal hypha was isolated and transferred to PDA media by cutting a square block of water agar. The plates were incubated in light chamber at 24^{o}C. After 10-15 days huge mycelial and sometimes reproductive growth was occurred in most cases. Culture slants were made and preserved for identification at 4^{o}C and also for further work in future.

2.6 Identification of endophytes

The endophytic fungal organisms were studied under optical compound microscope. The fungal isolates were identified based on their morphological and reproductive characters using the standard identification manuals [26, 29, 30, 33].

2.7 Data analysis

The relative colonization frequency (CF%) was calculated as the number of sample segments colonized by at least a fungus divided by total number of segments plated x100 using the formula outlined by Hata and Futai: CF = (N_{col}/N_t x100, where N_{col} = number of segments colonized by at least a fungus, N_t = total number of segments plated. Dominant endophytes were calculated as percentage of colony frequency divided by sum of percentage of colony frequency of all endophytes x100 [21]. Dominant endophyte percentage (D) = N_i/N_s x100, where N_i = percentage of colony frequency of individual endophytes, N_s = percentage of colony frequency of all endophytes. Using PAlaeontological STatistics software package (PAST) [31], following diversity indices were calculated:-

(a) Simpson's Diversity Index (1-Dominance) was calculated using the formula 1-D, where D = ∑n(n-1)/N(N-1). Here, n = the total number of organisms of a particular species, N = the total number of organisms of all species.

(b) Shannon-Wiener diversity index was calculated using the following formula: Shannon-Wiener index (H′) = -∑s(Pi)(In Pi), where H′ = Symbol for the diversity in a sample of species or kinds, s = the number of species in the sample, Pi = 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 = log to the base 2.

(c) Evenness was calculated using the following formula: Evenness (E) = H′/H′max, where H′max is the maximum value of diversity for the number of species.

3. Result and discussion

The two lianas plants had a huge number of endophytic fungal association. It has been found that a single plant species may harbour hundreds of endophytes and they may assemble in all available plant tissues like leaf, petiole, stem, twig, bark, xylem, root, fruit, flower and seed [27, 35, 39]. Banerjee et al. and Raviraja et al. reported earlier that endophytic colonization is higher in leaf segments than in stem segments of some tropical medicinal plants. In the present study also high diversity of endophytic fungi was obtained from three organ tissues (leaf, petiole and stem) of the two plant species. A total of 328 and 387 endophytic fungi were isolated from 3 different tissue segments of *Celastrus paniculatus* (Family-Celastraceae) and *Combretum roxburghii* (Family-Combretaceae) respectively. The endophytic fungi isolated from *Celastrus* sp. belong to genera *Acrocylindrium* sp., *Chrysosporium* sp., *Arthrinium* sp., *Fusarium* sp., *Lasiodiplodia* sp., *Eutypella* sp., *Aspergillus* sp., *Nigrospora* sp., Arthrobotrys sp., *Achlya* sp., *Diplodia* sp., *Uromyces* sp., *Pestalotiopsis* sp., *Pyrenochaeta* sp., *Blastomyces* sp., *Colletotrichum* sp., *Penicillium* sp., *Stachybotrys* sp., *Botryotrichum* sp., *Mucor* sp., *Apophysomyces* sp., *Drepanopeziza* sp., *Curvularia* sp., *Alternaria* sp., *Verticillium* sp. and

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Chromelosporium sp. with a number of unidentified genera and sterile mycelia (table-1). The tissues of *Combretum* sp. were to colonize with following endophytic fungal genera- *Verticillium* sp., *Podospora* sp., *Gymnoascus* sp., *Lasiodiplodia* sp., *Pestalotiopsis* sp., *Chromelosporium* sp., *Mucor* sp., *Peronospora* sp., *Chaetomium* sp., *Fusarium* sp., *Penicillium* sp., *Acromoniella* sp., *Aspergillus* sp., *Nigrospora* sp., *Scopulariopsis* sp., *Chrysosporium* sp., *Zygorhynchus* sp., *Botryotrichum* sp., *Blastomyces* sp., *Tolyposporium* sp. with few unidentified genera and mycelia sterilia. Both the plant species were found to accomodate many common fungal genera but genera of *Podospora* sp., *Gymnoascus* sp., *Peronospora* sp., *Acremoniella* sp., *Scopulariopsis* sp., *Zygorhynchus* sp., *Blastomyces* sp and *Tolyposporium* sp. were found only in *Combretum* sp. Among the genera *Verticillium* sp. showed the highest colonization frequency (19.4%), then *Chaetomium* sp.(15.25%), *Aspergillus* sp.(10.1%) and so on in *Combretum* sp., but in *Celastrus* sp. highest CF was for *Acrocylindrium* sp. (18.9%), then *Lasiodiplodia* (11.3%), *Achlya* sp.(5.8%), *Arthrinium* sp. (5.2%) and so on. In *Celastrus* sp. maximum endophytic isolates were obtained from stem segments (61.3%), but in *Combretum* sp. it was from leaf segments (41.8%). Bar diagram (Figure 3) shows the comparison of various classes of isolated fungi in two plants. Out of 500 tissue segments, 297 segments were colonized with fungi i.e. CF=59.4%. In *Celastrus* sp., in respect of total tissue segments plated only in Belpahari, CF was 67%, in Chilkigarh-41%, in Godapiasal-63%, in Malabati-57% and in Nayagram CF was 69%. But in respect of 5 regions, CF in Belpahari-22.55%, Chilkigarh-13.8%, Godapiasal-21.2%, Malabati-19.2% and Nayagram-23.2%. Again in respect of tissue segments of the plant collected from 5 regions, CF in leaves was 30.56%, in petioles-35.65% and in stems-33.78%. Similarly in *Combretum* sp. only for Belpahari, CF was 81.33%, for Chilkigarh-73.33%, for Godapiasal-69.33%, for Malabati-98.66% and for Nayagram-70.66%. In respect of 5 regions, CF of Belpahari was 20.67%, Chilkigarh-18.64%, Godapiasal-17.63%, Malabati-25.1% and Nayagram-17.97%. Similarly, in respect of different tissues, CF in leaves was 32.2%, in petioles-32.2% and in stems-35.6%.

Endophytic fungi	Malabati			Belpahari			Chilkigarh			Godapiasal			Nayagram		
	L	**P**	**S**	**L**	**P**	**S**	**L**	**P**	**S**	**L**	**P**	**S**	**L**	**P**	**S**
Acrocylindrium sp.	1	9	37	0	4	11	0	0	0	0	0	0	0	0	0
Arthrinium sp.	1	0	0	4	0	5	2	2	0	1	0	0	0	0	2
Fusarium sp.	1	1	0	4	0	2	0	0	1	1	0	0	4	1	0
Lasiodiplodia sp.	0	2	0	2	0	0	0	0	2	0	2	10	2	7	10
Eutypella sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus sp.	0	0	1	2	0	0	2	2	1	1	0	2	0	0	1
Nigrospora sp.	0	0	0	0	4	0	0	3	1	0	1	0	0	0	0
Arthrobotrys sp.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Achlya sp.	0	0	0	0	3	0	0	0	0	0	0	0	5	2	9
Diplodia sp.	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Uromyces sp.	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Pestalotiopsis sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pyrenochaeta sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Blastomyces sp.	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Colletotrichum sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Penicillium sp.	0	0	0	0	0	2	1	0	0	1	0	2	0	0	6
Stachybotrys sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Botryotrichum sp.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1
Mucor sp.	0	0	0	0	0	1	0	0	4	0	0	0	2	0	2
Apophysomyces sp.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Drepanopeziza sp.	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Curvularia sp.	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1
Alternaria sp.	0	0	0	0	0	0	0	0	0	0	0	4	0	0	1
Verticillium sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Chromelosporium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Chrysosporium sp.	1	0	0	1	0	0	0	1	2	2	1	2	0	0	1
Unidentified genera	2	0	0	6	1	12	5	2	7	2	2	0	0	0	3
Mycelia sterilia	0	0	0	0	0	0	0	0	5	2	3	25	3	3	12

Table-1:-Endophytes isolated from *Celastrus* sp. of five regions

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Diversity and species richness of endophytic fungi were studied in different tissues of the two plant species. Endophytes of each species were used to calculate diversity indices using PAST (PAleontological STatistics) software packages, ver.-1.89 [31]. The result showed that both the plant species were rich in endophytic fungi. Various diversity indices in respect of regions and different tissues (leaves, petioles and stems) of two plant species (Table-5&6). Jaccard's similarity coefficient index was calculated to determine the colonization similarity of fungal endophytes in two host plants, based on the colonized endophytic fungi using the following formula:

Similarity Coeficient = C/(A+B+C). where, A and B are the total number of endophytic fungal genera isolated from two host plants and C is the number of common genera isolated from plants. In respect of endophytic isolation between two host plants, if 100% similarity is there, the similarity coefficient will be 0.33. In our finding, the similarity coefficient was = 11/(27+20+11) = 11/58 = 0.189 i.e. 56.76%.

Similarity of endophytic communities across sites was compared using the SØrensen coefficient of similarity [32]. The formula is: C=2w/(a+b); where w= the number of common species between two areas being compared, a= number of species in one area, b=number of species of the other area (Table-7).

Table-2:-Endophytic isolation from *Combretum* sp. of five regions

Endophytic isolation	Malabati			Belpahari			Chilkigarh			Godapiasal			Nayagram		
	L	P	S	L	P	S	L	P	S	L	P	S	L	P	S
Verticillium sp.	0	0	0	12	24	30	0	0	6	0	0	0	3	0	0
Podospora sp.	0	0	0	0	0	0	4	0	0	0	0	0	5	6	1
Gymnoascus sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Lasiodiplodia sp.	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0
Pestalotiopsis sp.	0	6	11	0	0	0	0	0	0	0	0	0	1	1	3
Chromelosporium sp.	5	0	0	0	0	0	0	0	0	0	0	0	2	0	3
Mucor sp.	5	5	5	0	0	0	0	2	0	0	0	0	1	2	14
Peronospora sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Chaetomium sp.	38	6	0	0	0	0	8	0	0	1	0	3	2	1	0
Fusarium sp.	0	6	0	0	0	0	0	0	0	0	0	0	0	1	0
Penicillium sp.	7	0	0	0	0	0	0	1	0	11	0	4	0	1	0
Acremoniella sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Aspergillus sp.	21	5	3	0	0	0	3	1	3	0	0	2	0	0	1
Nigrospora sp.	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Scopulariopsis sp.	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Chrysosporium sp.	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
Zygorhynchus sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Botryotrichum sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Blastomyces sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Tolyposporium sp.	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Unidentified genera	0	0	1	0	0	0	5	14	1	9	12	8	0	2	3
Mycelia sterilia	0	0	10	1	0	0	5	4	0	1	0	0	0	0	1

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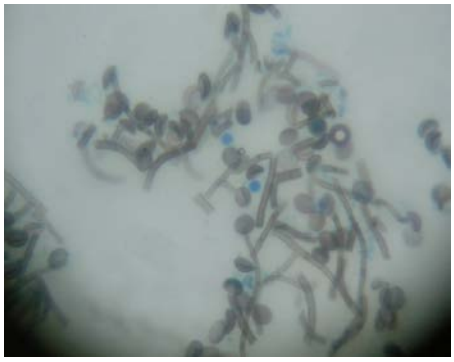

Figure-1: *Chaetomium* sp.

Figure-2: *Alternaria* sp.

Table-3:-Isolated endophytic fungal genera in leaves, petioles and stems of *Celastrus* sp. and their colonization frequency

Isolated fungi	Number of isolates				Colonization frequency(CF%)			
	Leaves	Petioles	Stems	Total	Leaves	Petioles	Stems	Total
***Acrocylindrium* sp.**	1	13	48	62	0.3	3.9	14.6	18.9
***Arthrinium* sp.**	8	2	7	17	2.4	0.6	2.1	5.2
***Fusarium* sp.**	10	2	3	15	3.0	0.6	0.9	4.5
***Lasiodiplodia* sp.**	4	11	22	37	1.2	3.3	6.7	11.3
***Eutypella* sp.**	0	1	0	01	-	0.3	-	0.3
***Aspergillus* sp.**	5	2	5	12	1.5	0.6	1.5	3.6
***Nigrospora* sp.**	0	8	1	09	-	2.4	0.3	2.7
***Arthrobotrys* sp.**	0	1	0	01	-	0.3	-	0.3
***Achlya* sp.**	5	5	9	19	1.5	1.5	2.7	5.8
***Diplodia* sp.**	0	2	0	02	-	0.6	-	0.6
***Uromyces* sp.**	0	1	1	02	-	0.3	0.3	0.6
***Pestalotiopsis* sp.**	0	0	1	01	-	-	0.3	0.3
***Pyrenochaeta* sp.**	0	0	1	01	-	-	0.3	0.3
***Blastomyces* sp.**	0	0	2	02	-	-	0.6	0.6
***Colletotrichum* sp.**	0	0	2	02	-	-	0.6	0.6
***Penicillium* sp.**	2	0	10	12	0.6	-	3.0	3.6
***Stachybotrys* sp.**	0	0	1	01	-	-	0.3	0.3
***Botryotrichum* sp.**	0	1	2	03	-	0.3	0.6	0.9
***Mucor* sp.**	2	0	7	09	0.6	-	2.5	3.1
***Apophysomyces* sp.**	0	0	1	01	-	-	0.3	0.3
***Drepanopeziza* sp.**	0	2	0	02	-	0.6	-	0.6
***Curvularia* sp.**	0	1	3	04	-	0.6	0.9	1.2
Alternaria sp	0	0	5	05	-	-	1.5	1.5
***Verticillium* sp.**	1	0	0	01	0.3	-	-	0.3
***Chromelosporium* sp.**	0	0	1	01	-	-	0.3	0.3
***Chrysosporium* sp.**	4	2	5	11	1.2	0.6	1.5	3.3
Unidentified genera	15	5	22	42	4.5	1.5	6.7	12.8
Mycelia sterilia	5	6	42	53	1.5	1.8	12.8	16.2
Total	62	65	201	328	18.9	19.8	61.3	100

Table-4:-Isolated endophytic fungi from leaves, petioles and stems of *Combretum* sp. and their colonization frequency

Endophytic isolates	Number of isolates				Colonization frequency(CF%)			
	Leaves	Petioles	Stems	Total	Leaves	Petioles	Stems	Total
Verticillium sp.	15	24	36	75	3.88	6.0	9.2	19.4
Podospora sp.	9	6	1	16	2.3	1.5	0.3	4.8
Gymnoascus sp.	1	0	0	01	0.3	-	-	0.3
Lasiodiplodia sp.	3	2	0	05	0.8	0.6	-	1.29
Pestalotiopsis sp.	1	7	14	22	0.3	1.8	3.6	5.6
Chromelosporium sp.	7	0	3	10	1.8	-	0.8	2.58
Mucor sp.	6	9	19	34	1.5	2.3	4.9	8.8
Peronospora sp.	1	0	1	02	0.3	-	0.3	0.6
Chaetomium sp.	49	7	3	59	12.7	1.8	0.8	15.2
Fusarium sp.	0	7	0	07	-	1.8	-	1.8
Penicillium sp.	18	2	4	24	4.6	0.6	1.2	6.4

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Acremoniella sp.	0	0	2	02	-	-	0.6	0.6
Aspergillus sp.	24	6	9	39	6.0	1.5	2.3	10.1
Nigrospora sp.	0	2	0	02	-	0.6	-	0.6
Scopulariopsis sp.	0	2	0	02	-	0.6	-	0.6
Tolyposporium sp.	0	0	3	03	-	-	0.8	0.8
Chrysosporium sp.	4	0	0	04	1.2	-	-	1.2
Zygorhynchus sp.	1	0	0	01	0.3	-	-	0.3
Botryotrichum sp.	1	0	0	01	0.3	-	-	0.3
Blastomyces sp.	1	0	0	01	0.3	-	-	0.3
Unidentified genera	14	28	13	55	3.6	7.2	3.4	14.2
Mycelia sterilia	7	4	11	22	1.8	1.2	2.8	5.6
Total	162	106	119	387	41.8	27.4	30.7	100

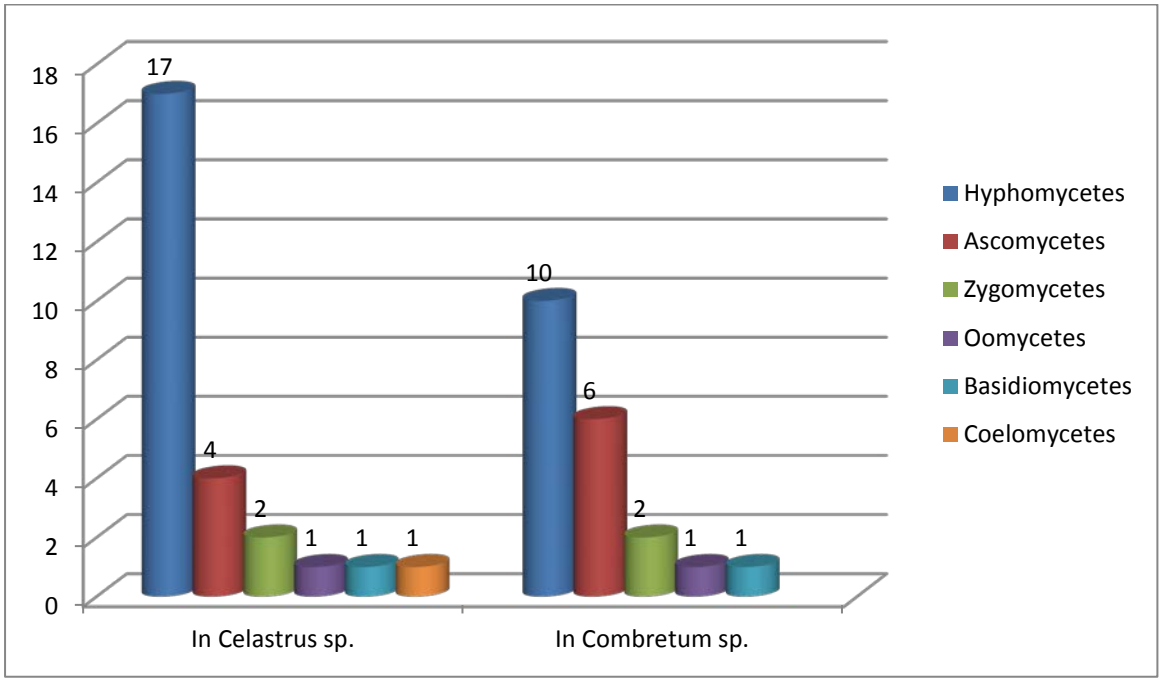

Figure 3:-Bar diagram showing the comparison of various classes of isolated endophytes in two plants- *Celastrus* sp. and *Combretum* sp. (Bar indicates from left to right- Hyphomycetes, Ascomycetes, Zygomycetes, Oomycetes, Basidiomycetes and Coelomycetes respectively).

Table-5:-Diversity indices of endophytes in *Celastrus* sp. and *Combretum* sp. of five regions (using PAST software)

Parameters	***Celastrus* sp.**					***Combretum* sp.**				
Regions--->	Malabati	Belpahari	Chilkigarh	Godapiasal	Nayagram	Malabati	Belpahari	Chilkigarh	Godapiasal	Nayagram
Taxa_S	8	19	13	12	16	10	2	9	10	15
Individuals	57	74	47	69	81	136	67	59	61	64
Dominance_D	0.6848	0.1381	0.1426	0.2384	0.1584	0.1896	0.9706	0.1882	0.2997	0.1377
Simpson_1-D	0.3152	0.8619	0.8574	0.7616	0.8416	0.8104	0.02941	0.8118	0.7003	0.8623
Shannon_H	0.7954	2.373	2.239	1.882	2.155	1.9	0.07757	1.891	1.586	2.291
Evenness_e^H/S	0.2769	0.565	0.7215	0.5475	0.5395	0.6683	0.5403	0.7361	0.4882	0.6591
Brillouin	0.6581	2.061	1.901	1.657	1.909	1.778	0.06276	1.68	1.389	1.993
Menhinick	1.06	2.209	1.896	1.445	1.778	0.8575	0.2443	1.172	1.28	1.875
Margalef	1.731	4.182	3.117	2.598	3.413	1.832	0.2378	1.962	2.189	3.366
Equitability_J	0.3825	0.8061	0.8727	0.7575	0.7774	0.825	0.1119	0.8606	0.6886	0.846
Fisher_alpha	2.534	8.271	5.945	4.198	5.974	2.488	0.3877	2.959	3.4	6.17
Berger-Parker	0.8246	0.2568	0.2979	0.4348	0.2346	0.3235	0.9851	0.339	0.4754	0.2656
Chao-1	9.5	23.67	15	13.5	30	10	2	9	13	18.33

Table-7:- Similarity coefficient index in respect of endophytes between two sites for *Celastrus* sp. and *Combretum* sp. using Sorensen coefficient of similarity.

Two sites compared	Similarity coefficient index

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		Celastrus sp.	*Combretum* sp.
Malabati	Belpahari	0.518	0.166
Malabati	Chilkigarh	0.545	0.631
Malabati	Godapiasal	0.601	0.500
Malabati	Nayagram	0.500	0.720
Belpahari	Chilkigarh	0.606	0.363
Belpahari	Godapiasal	0.516	0.166
Belpahari	Nayagram	0.514	0.235
Chilkigarh	Godapiasal	0.692	0.526
Chilkigarh	Nayagram	0.666	0.666
Godapiasal	Nayagram	0.710	0.400

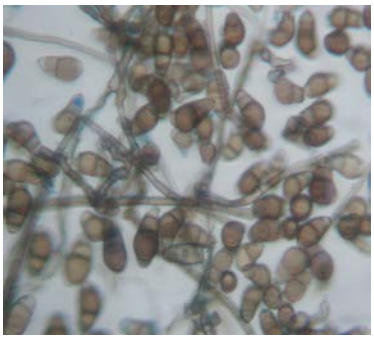

Figure-4: *Curvularia* sp.

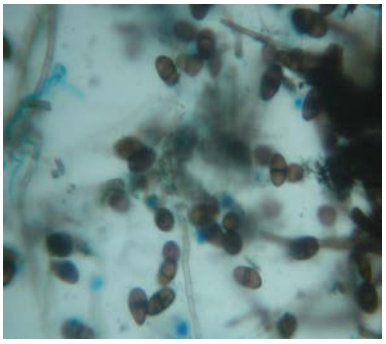

Figure-5: *Lasiodiplodia* sp.

Table-6:-Comparison of various diversity indices among leaves, petioles and stems of *Celastrus* sp. and *Combretum* sp. respectively and between *Celastrus* sp. & *Combretum* sp.

	Celastrus sp.			*Combretum* sp.			*Celastrus* sp.	*Combretum* sp.
Parameter	Leaves	Petioles	Stems	Leaves	Petioles	Stems		
Taxa_S	12	17	23	17	13	13	20	19
Individuals	62	65	201	162	106	119	282	324
Dominance_D	0.1316	0.111	0.1337	0.1512	0.1506	0.1605	0.1347	0.1412
Simpson_1-D	0.8684	0.889	0.8663	0.8488	0.8494	0.8395	0.8653	0.8588
Shannon_H	2.219	2.459	2.423	2.228	2.184	2.105	2.299	2.228
Evenness_e^H/S	0.7667	0.6876	0.4699	0.5462	0.6833	0.6314	0.4745	0.4886
Brillouin	1.954	2.129	2.247	2.07	1.994	1.938	2.181	2.13
Menhinick	1.524	2.109	1.693	1.336	1.263	1.192	1.251	1.056
Margalef	2.665	3.833	4.337	3.145	2.573	2.511	3.545	3.114
Equitability_J	0.8931	0.8678	0.7624	0.7866	0.8515	0.8207	0.7551	0.7568
Fisher_alpha	4.433	7.489	7.107	4.788	3.891	3.718	5.246	4.407
Berger-Parker	0.2419	0.2	0.2388	0.3025	0.2642	0.3025	0.2199	0.2315
Chao-1	12.33	18.43	29.25	32	13	13.5	24.75	20.5

4. Conclusions

There is a diverse groups of endophytes in lianas plants found from my study. Majority has been identified with some unknown genera and some mycelia sterilata. We may draw conclusion that there is a host specificity by endophytes and also they have organ and tissue specificity. Both the plant species are more or less similar in having endophytic fungi.

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