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RESEARCH ARTICLE

SCREENING OF ENDOPHYTIC FUNGI FROM A LIANAS OF THREE LOCALITIES

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ABSTRACT

An woody lianas- *Bauhinia vahlii*, collected from 3 localities during rainy season was studied for screening of endophytic fungal diversity. Aerial tissues (leaf, petiole and stem) were selected for endophytic isolation. A total of 150 tissue segments out of 225 were infested with fungi and 153endophytic fungi were isolated. Average colonization frequency (CF%) was 66.66% and petioles of the climber were colonized by a great number of endophytic fungi i.e. 82.66%. CF% is maximum in the plant collected from Belpahari, it is 78.66% and minimum in the plant of Godapiasal, it was 54.66%. A total of 10 fungal genera with few unknown genera and few sterile fungi were isolated. Highest Shannon-Wiener index (2.067) was shown in the plant of Godapiasaland with highest Simpson's diversity (0.85). It indicates great species specificity. Fungi of Dothidiomycetes were maximum (38.81%).

INTRODUCTION

The term endophytes include all the organisms that live symtomlessly within various plant tissues (Petrini 1986). They are classified into two distinct groups- bacteria and fungi (Wilson 2007). But the most frequently isolated endophytes are fungi (Strobel *et al.,* 2000). Huge number of endophytes remain in association with shade and moisture-loving woody climbers in stand forest (Banerjee and Bagchi 2013). Carroll and Carroll (1988) reported that endophytes live asymptomatically and sometimes systematically within the plant tissues. Endophytic fungi are very important in the biodiversity since they have an effect on structure and defence mechanism of plants and ultimately in the ecosystem (Wilson 2000). Arnold *et al.* (2000) isolated extremely abundant and very diverse group of endophytic fungi from plant tissues. Endophytic fungi are ubiquitous in distribution found within the tissues of plants, residing intercellular or intracellular, at least for a portion of their life cycle without causing apparent symptoms of diseases. Dreyfuss and Chapela (1994) estimated that there may be one million species of endophytic fungi in the world and only a few metabolites have been discovered from very few group of endophytes for pharmaceuticals. Ganley *et al.* (2004) reported that endophytes are the normal inhabitants of the plant tissues. They protect plants against pests (Azevedo *et al.,* 2000) fungal pathogens (Arnold *et al.,* 2003) even enhance the defense mechanism of host plants against grazing animals (Clay 1998) or increase the tolerance power of host against harsh environment (Redman *et al.,* 1999). Recent work has been started to explore and isolate the endophytic fungal symbionts and their diversity in a woody lianas- *Bauhinia vahlii* (Caesalpiniaceae),collected from three distant localities of West Medinipur district. Fungal endophytes of woody lianas represent one class of microbial symbionts that have so far been neglected in diversity studies. These endophytes are estimated to occur in 25% to 60% of plant segments and can play important ecological roles in plant communities (Clay *et al.,* 1999). Endophytic fungi can also increase drought resistance (Elmi *et al.,* 1995) and enhance drought resistance (Malinowski *et al.,* 2000). Many symbiotic endophytic fungi have been isolated from three plants of Lamiaceae family Banerjee *et al.,* 2009).

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MATERIALS AND METHODS

Study sites and collection of samples

The study was conducted in WestMedinipur district of West Bengal, India. (latitude 22°25′ to 22°57′North, longitude 87°11′East, altitude 2meters from the sea level) during rainy season, 2013. The climate is tropical, warm and humid with a mean temperature of 33°C and an average annual rainfall of 120cm. The lianas plant *Bauhiniavahlii* was selected from Belpahari, Chilkigarh and Godapiasal of the district for endophytic fungal screening. All sample types were collected from mature, healthy, disease free plants.

Sampling procedure

Plant samples (leaves, stems, petioles) were collected randomly from all the locations during rainy season. 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 started.

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.

Placing the samples in media

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 outer layer was removed and inner tissues were peeled with sterile scalpel. Thin peels from various depthwere placed on another WA plate. Thus, at least 5 replica plates for each sample from the plant were made.

Isolation of endophytic fungi

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 23°C. After 10-15 days huge mycelial and in some cases reproductive growth was observed. Culture slants were made and preserved for identification at 4°C and also for further work in future.

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 (Barnett and Hunter: Illustrated genera of Imperfect fungi, Gilman: A Manual of soil fungi).

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 = (Ncol/Nt x100, where Ncol = number of segments colonized by at least a fungus, Nt = 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. Dominant endophyte percentage (D) = Ni/Ns x100, where Ni = percentage of colony frequency of individual endophytes, Ns = percentage of colony frequency of all endophytes. Using PAlaeontological S Tatistics software package (PAST) (Hammer *et al.,* 2001), following diversity indices were calculated- (a) Simpson's Diversity Index (1-Dominance) was calculated using the formula 1-D, where D = $\sum n(n-1)/N(N-1)$. 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′) = - $\sum s(Pi)(In\ Pi)$, 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, ni = number of individuals of ith species, In = log to the base 2.

RESULTS

A total of 150 tissue segments out of 225 were infested with fungi and 153 endophytic fungi were isolated. Average colonization frequency (CF%) was 66.66% and petioles of the climber were colonized by a great number of endophytic fungi i.e. 82.66%.

Table 1. Endophytic fungi isolated from leaf, petiole and stem segments of *Bauhinia vahlii* from different localities of West Medinipur during rainy season

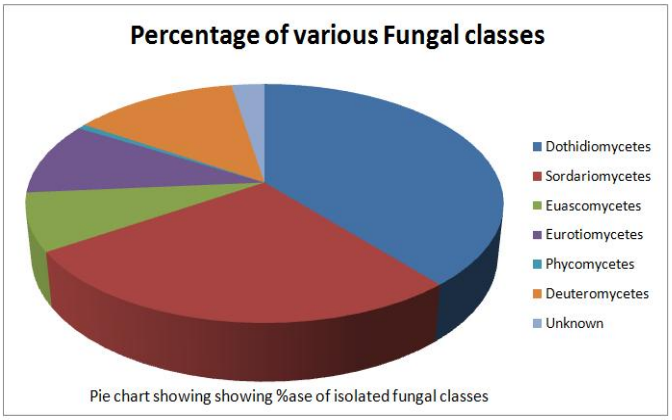
Endophytic fungi	Total Isolates	Belpahari			Chilkigarh			Godapiasal		
		L	P	S	L	P	S	L	P	S
Arthrinium sp.	02	1	0	0	0	1	0	0	0	0
Aspergillussp	05	0	1	0	3	0	0	0	0	1
Beltrania sp.	26	5	9	1	4	5	2	0	0	0
Chaetomium sp.	10	0	0	3	0	3	0	0	4	0
Curvularia sp.	02	0	0	0	0	0	0	0	0	2
Diplodia sp.	03	1	2	0	0	0	0	0	0	0
Fusariumsp	15	1	1	0	2	5	1	2	1	2
Lasiodiplodia sp.	56	11	9	9	3	8	6	2	5	3
Mucorsp	01	0	0	0	0	1	0	0	0	0
Mycelia sterilia	13	1	2	1	1	2	2	1	3	0
Nigrospora sp.	02	0	0	0	0	0	0	0	1	1
Penicillium sp.	10	1	0	0	0	0	1	6	0	2
Unknown genera	04	0	1	0	1	1	0	0	0	1
Verticillium sp.	03	0	0	0	0	0	0	0	0	3
Total	152	21	25	14	14	26	12	11	14	15

CF% is maximum in the plant collected from Belpahari, it is 78.66% and minimum in the plant of Godapiasal, it is 54.66%. A total of 10 fungal genera with few unknown genera and few sterile fungi were isolated (Table 1). Highest Shannon-Wiener index (2.067) was shown in the plant of Godapiasal and with highest Simpson's diversity (0.85) (Table 2). It indicates great species specificity. Fungi of Dothidiomycetes are maximum (38.81%) and Phycomycetes is minimum (0.66%) (Pie diagram).

Table 2. Diversity indices and species richness of endophytic fungi from *Bauhinia vahlii* in 3 localities drng rainy season

Parameters	Belpahari	Chilkigarh	Godapiasal
Taxa_S	10	10	10
Individuals	60	52	40
Dominance_D	0.3078	0.1938	0.15
Simpson_1-D	0.6922	0.8062	0.85
Shannon_H	1.564	1.89	2.067
Evenness_e^H/S	0.478	0.6617	0.7903
Brillouin	1.367	1.648	1.757
Menhinick	1.291	1.387	1.581
Margalef	2.198	2.278	2.44
Equitability_J	0.6794	0.8207	0.8978
Fisher_alpha	3.427	3.681	4.28
Berger-Parker	0.4833	0.3269	0.25
Chao-1	13	11.5	10.33

Figure 1 shows growth of mycelium of Fusarium sp. in petri plate. Figures 2-9 show compound microscopic pictures of asexual spores of few isolated fungi. Figures 10-11 show sterio microscopic pictures of *Aspergillus* sp. and *Curvularia* sp.



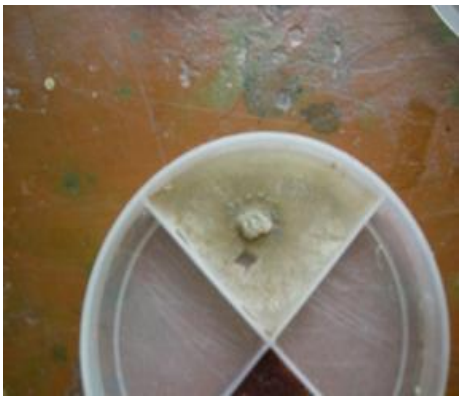
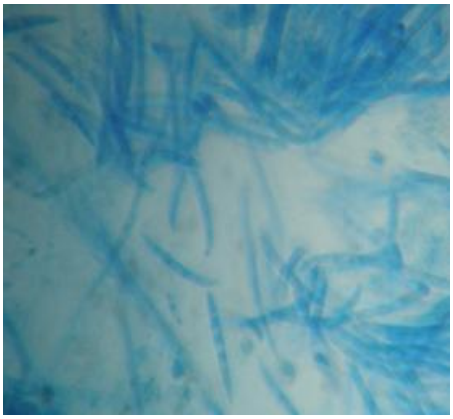
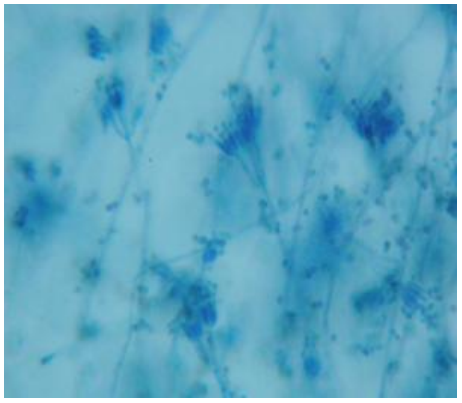


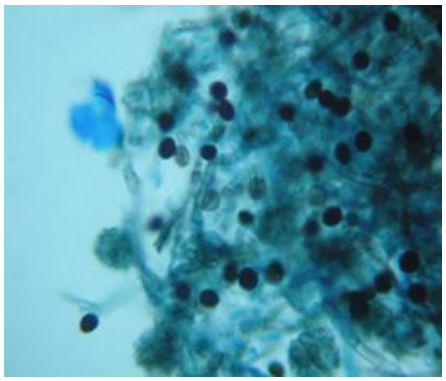
Plate pic. of *Fusarium* sp.



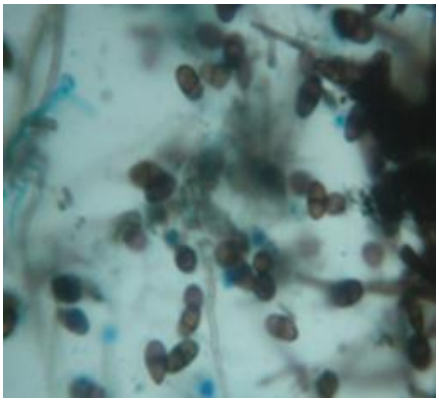
Fusarium sp.



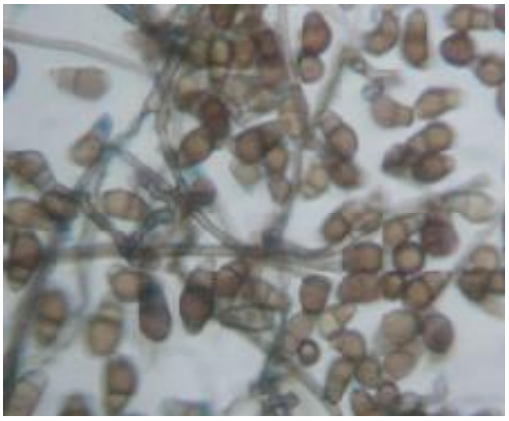
Penicillium sp.



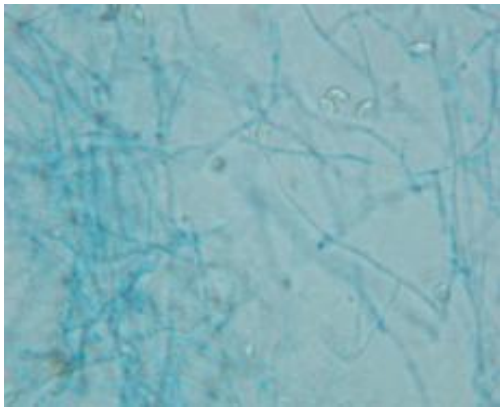
Nigrospora sp.



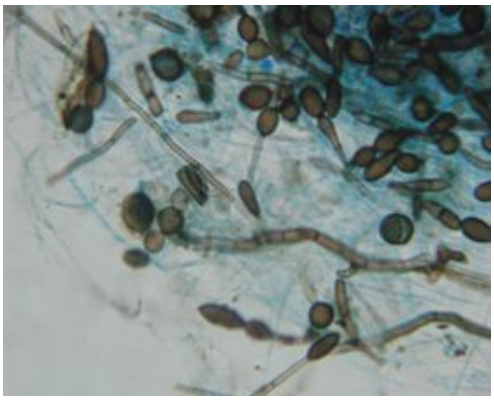
Lasiodiplodia sp.



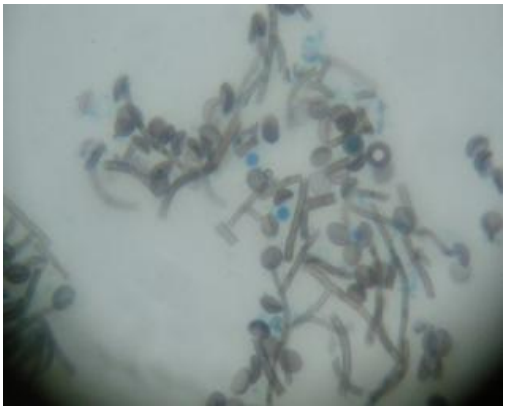
***Curvularia* sp.**



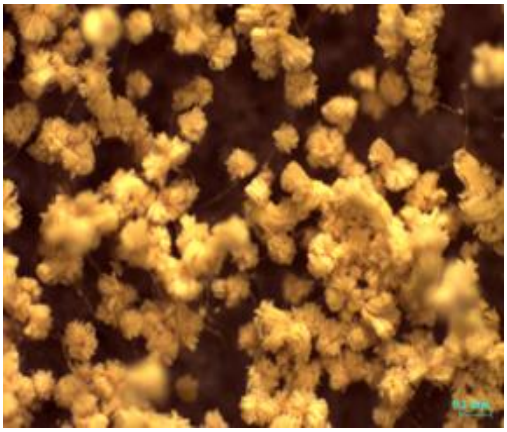
***Beltrania* sp.**



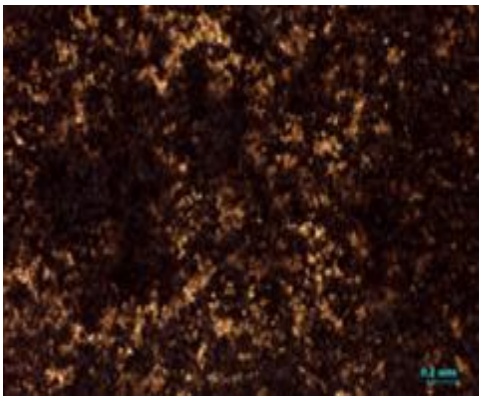
***Arthrinium* sp.**



***Chaetomium* sp.**



Sterio pic. of Aspergillus sp.



Sterio picture of Curvularia sp.

DISCUSSION

The woody lianas plant of all places was infested with huge number of endophytic fungi forming an symbiotic association. Altogether 153 fungal endophytes were isolated from 225 segments of leaf, petiole and stem from the lianas plant. The endophytes belong to 10 genera, few unknown and few sterile mycelia. Previous studies also showed that diverse types of endophytic fungi were isolated from other woody lianas plants of different locations. The highest number of fungal endophytes was isolated from the plant of Belpahari.It was 60 out of 75(78.66%). Most of the endophyticfungi were colonized in petioles (CF=82.66%). Leaf shows intermediate colonization frequency (62.66%) and stem showed minimum (54.66%). It is an evidence for the tissue specificity of endophytes. Previous researchers also observed tissue specificity of endophytes in their studies (Banerjee et al, 2009 and Raviraja, 2005).

All the statistical analyses were made using the formulae stated by Raviraja (2005) with the statistical software PAST. Species diversity of endophytes was determined using the Simpson's diversity index, Shannon-Wiener index, Fisher alfa index, Manhinif index etc. Bauhinia of Godapiasal showed the highest Simpson's diversity (0.85) with maximum Shannon-Weiner index (2.067). Highest Fisher alfa index (3.681) was in plant of Chilkigarh. All these indices indicate great species specificity of endophytes. In the present study *Lasiodiplodia sp., Beltrania sp., Fusarium sp., Chaetomium sp., Penicillium*

sp. were the dominant fungal genera of the lianas plant of all three localities.

Conclusion

There is a diverse groups of endophytes in the lianas plant of all localities found from my study. Majority has been identified with some unknown genera and some mycelia sterilia. We may draw conclusion that there is a host specificity by endophytes and also they have organ and tissue specificity. The plant of *Bauhinia vahlii* shows maximum number of endophytesin Belpahari region. Maximum fungal genera are *Lasiodiplodia* sp. and *Beltrania* sp. Endophytic fungi may give a permanent structure of plant community by giving some facility to the plant.

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