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Research Paper

A RELATIVE STUDY OF ENDOPHYTIC FUNGI DURING WINTER AND MONSOON IN *Bauhinia vahlii* FROM CHILKIGARH

Biplab Bagchi

Department of Botany,
Bangabasi College,19-Rajkumar Chakraborty Sarani, Kolkata-700009,
India.

Abstract

Endophytes are the normal microflora of the plant tissues. They are most important components of plant microbiomes. Endophytes may be various types*, but most frequently isolated endophytes are fungi.* Endophytes are more in the plants of tropical regions. Lianas are the special group of plants which are woody climbers in the forest and climb up tall tree. Fungal endophytes from *Bauhinia vahlii* were studied collecting from Chilkigarh of Jhargram district of West Bengal during winter season and monsoon. A total of 108 plant tissue segments were resided by endophytic fungi among 150 tissue segments and endophytic fungi of the number of 119 were isolated from 108 various tissue specimens such as leaf, petiole and stem. The fungal isolates belong to the genera as many as 17, along with few unidentified fungi and few sterile mycelia. *Aspergillus sp.*, *Beltrania* spp., *Fusarium* spp., *Lasiodiplodia* spp., *Pestalotiopsis* spp., *Cylindrocladium* sp., *Chaetomium* sp. etc., under the class Sordariomycetes and Deuteromycetes, were found most commonly out of all isolated fungal endophytes. 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 sterilia. We may draw conclusion that there is organ and tissue specificity by endophytes. The plant of *Bauhinia vahlii* shows huge assemblage of endophytes in both seasons but more isolated in winter season. Colonization frequency is more in winter than in monsoon.

Key words: Endophytes, diversity, lianas, fungi, indices.

INTRODUCTION

The term endophyte is most commonly used for those micro organisms which infect and colonize internally and here the tainted tissues in host plant will not show any instant symptoms [1]. Fungal endophytes in aerial tissues of host are culturable on synthetic media.

Endophytic fungi are very important in the biodiversity [2]. Arnold et al., 2000 [3] 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. Gilbert *et al*., 2007 [4] reported fungal symbionts in tropical trees. Bissegger *et al*., 1989 [5] reported endophytic fungal assemblages in coppice shoots of *Castanea sativa*. Various diversity indexes were measured search associative and mutualistic relations between endophytic microbes and host plants. Very often investigator uses the magnitudes having in one or more diversity indexes, like species richness (richness is how many species cannot consider the rarity of individuals, on the other hand biodiversity can) [6-8]. Raviraja, 2005 [9] analysed all the findings with the help of PAST statistical software. Species diversity of all isolated endophytic fungi was measured by the formulae, Simpson's diversity index, Shannon-Wiener index, Fisher alfa index, Manhinif index. All these indices indicate great species specificity of endophytes.

Lianas are a group of plants which are woody climbers in the forest and climb up tall tree. So, present research was focused on a relative study of endophytic diversity of a lianas plant at Chilkigarh of Jhargram district. Endophytic fungi have been kept as a seldomly studied group of microorganisms in microbiology, although they have potential to yield varieties of bioactive novel secondary metabolic compounds. *Bauhinia vahlii* is such a plant which is important for few applications pharmaceutically and ethnobotanically, and the plant is an important component of sacred groves to the native races.

MATERIALS AND METHODS

Sampling

The study was conducted in Jhargram district of West Bengal, the lianas plant-*Bauhinia vahlii* (Caesalpiniaceae), was selected from Chilkigarh for endophytic fungal screening. Samples from healthy, disease free mature plants were collected in winter. The samples in zipper-lock plastic packets, brought to the laboratory and were processed within a few hours.

Surface sterilization

Samples were thoroughly washed under tap water, then were immersed in 70% ethanol for 1 minute, immersed in sodium hypochlorite (NaOCl) solution (3% available chlorine) for 3 minutes (5 minutes for bark segment), again immersed in 70% ethanol solution for 1 minute, finally rinsed with sterile distilled water surface dried.

Placement of samples

Samples were cut into pieces and placed into water agar (WA) .Fungal hyphae appeared in almost every sample of water agar plate.

Isolation of fungi

Each hypha was transferred to a plate of potato dextrose agar (PDA) media and was incubated in light chamber of incubator at 23^{o}C. Huge mycelial growth was observed after 7/8 days of incubation.

Identification

The fungi were studied under optical compound microscope. Identification of the isolated fungi was madeusing cultural and reproductive characteristics and done with the help of standards manuals [10-13].

Statical calculation

The relative colonization frequency (CF%) was calculated using the formula outlined by Hata and Futai (1996) [14]: CF = (N_{col}/N_t ×100, where N_{col}= no. of segments colonized by at least a fungus, N_t = total number of segments plated. Dominant endophyte percentage (D)=N_i/N_s×100, 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) (Hammer et al.,-2001 [15]), (a) Simpson's Diversity Index (1-Dominance) using the formula 1-D, where D = $\sum n(n-1)/N(N-1)$. Here, n = the total no. of organisms of a particular species, N = the total no. of organisms of all species.(b) Shannon-Wiener index (H′) = -$\sum s(P_i)(\text{In } P_i)$, where H′ = Symbol for the diversity in a sample of species or kinds, s = the number of species in the sample, P_i = relative abundance of i^{th} species or kinds and measured by = n/N, N = total no. of individuals of all kinds, n_i = number of individuals of i^{th} species, In = log to the

base 2. Evenness (E) = H′/H′max, where H′max is the maximum value of diversity for the no. of species.

RESULTS AND DISCUSSION

In winter study of *Bauhinia vahlii*, it was observed that the entire isolated and screened fungal endophytes demonstrated their fidality for particular organs and tissues. Out of entire parts of host organs, petiole occupied the utmost figure of fungal endophytes (CF=96%). Leaf and stem contain 64% and 72% respectively. Here, leaf lamina, petiolar stalk and stem were fantastic residence of fungal endophytes. Colonization frequency was found as following order–petiole (96%)> stem (72%)> leaf (64%). 79 fungal isolates under 21 genera from bark, foliar leaf and roots of *Aegle marmelos* was described and reportd [16] from Varanasi of India. Sum figure of 15 genera of isolated fungi with few unidentified genera and few imperfecti fungi were screened. Fungi in the class Sordariomycetes were maximal. Present investigation also shows the greatest assemblage of fungal endophytes in foliar leaf parts of the host. Earlier it has been reported that colonization fungal endophytes was much greater in foliar leaf parts rather than stem parts of few medicinally important plant host in tropical regions [9,17]. Sum total of 66 fungal endophytes were screened from 3 separate tissue parts of *Bauhinia vahlii* (Family-Caesalpiniaceae). The principal genera of fungal endophytes screened from the host with few unidentified genera and fungi imperfecti are as following- *Beltrania* spp., *Fusarium* spp., *Lasiodiplodia* spp., *Pestalotiopsis* spp., *Cylindrocladium* sp., *Chaetomium* sp. etc. Maximum were under the class Sordariomycetes and Deuteromycetes. Diversity richness and species richness of fungal isolates were investigated in various tissues of the host species. Endophytic populations residing in a specific host plant may be universal that is very often regarded as host specificity [18-20]. The result showed the richness of plant species in endophytic fungi. Banerjee *et al*. [17] isolated different endophytic fungi from *Vitex negundo* in Midnapur, West Bengal, India.

In monsoon study of *Bauhinia vahlii*, a sum of 50 pieces of tissues among 75 was assemblaged with fungal endophytes and 53 were isolated and screened for study of fungal biodiversity. Mean colonization frequency (CF) was 66.66% and innumerable endophytic fungal members inhabited on petiolar stalk i.e. 96%. A sum of 10 genera of fungi with few unidentified ones and few imperfcti fungi were screened. It indicates

great species specificity. Fungi are of Dothidiomycetes and Phycomycetes. The woody lianas plant was associated with numerous fungal endophytes making a mutualistic assemblage.

As a whole, 119 endophytic fungi were screened from 150 pieces of foliar leaf, petiolar part and stem from the host lianas and they include 17 genera. Earlier investigation also demonstrated that vast diverse groups of fungal endophytes were screened from other woody host lianas in various regions and seasons. Most of the endophytic fungi were colonized in petioles (CF=96%). Leaf and stem show same colonization frequency (60%). It is a proof of tissue specificity by endophytic fungi. Earlier workers also noted and reported the tissue specificity of fungal endophytes [9,21]. In the present study *Lasiodiplodia sp., Beltrania sp., Fusarium sp., Chaetomium sp., Penicillium* sp. were the dominant fungal genera of the lianas plant.

Table1 Colonization frequency of endophytes in various organs of *Bauhinia vahlii* in 2 seasons

Seasons	Segments plated	Segment infested by fungi	Fungi isolated from segments	Colonization frequency, CF%	CF% in leaf	CF% in petiole	CF% in stem
Winter	75	58	66	77.33%	64%	96%	72%
Monsoon	75	50	53	66.66%	56%	96%	48%
Total/ Mean	150	108	119	71.99%	60%	96%	60%

Table 2 Endophytic fungi isolated from leaf (L), petiole (P) and stem (S) segments of *Bauhinia vahlii* from Chilkigarh in winter and monsoon

Endophytic fungi	Total isolates	Winter			Monsoon		
		L	P	S	L	P	S
Arthrinium sp.	01	0	0	0	0	1	0
Aspergillus versicolor	03	0	0	0	3	0	0
Beltrania sp.	15	2	0	2	4	5	2
Bispora sp.	01	0	1	0	0	0	0
Chaetomium sp.	05	0	1	1	0	3	0
Curvularia sp.	01	0	0	1	0	0	0
Cylindrocladium sp.	10	7	3	0	0	0	0
Fusarium sp.	09	0	0	1	2	5	1
Fusidium viride	01	0	1	0	0	0	0
Geotrichum candidum	02	1	1	0	0	0	0
Lasiodiplodia sp.	21	1	3	0	3	8	6

Mucor sp.	05	1	0	3	0	1	0
Mycellia sterilia	08	0	2	0	1	3	2
Nigrospora sphaerica	01	0	0	1	0	0	0
Papulospora sp.	05	2	3	0	0	0	0
Penicillium sp.	04	0	0	3	0	0	1
Pestalotiopsis sp.	17	2	6	9	0	0	0
Torula sp.	03	2	1	0	0	0	0
Unidentified	07	0	4	1	1	1	0
Total	119	18	26	22	14	27	12

Table 3 Diversity indices and species richness of endophytic fungi in *Bauhinia vahlii* from Chilkigarh during winter and monsoon

Parameter	Winter	Monsoon
Sp. richness	17	10
Individuals	66	52
Simpson diversity	0.8802	0.8062
Shannon-Wiener index	2.452	1.89
Evenness	0.6832	0.6617
Fisher-alpha diversity	7.415	3.681

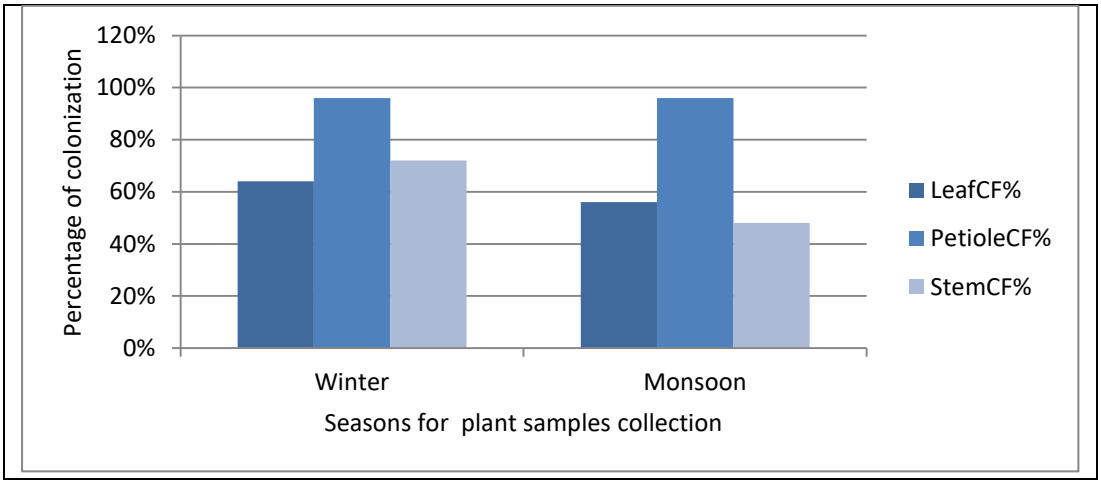
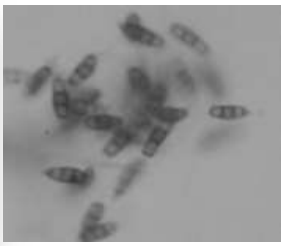
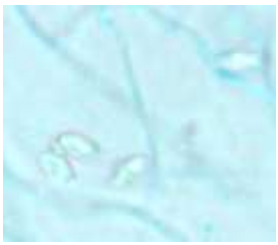


Figure 1 Graph showing comparison of colonization frequency in various organs in winter and monsoon


2Nigrospora


3Pestalotiopsis


4Beltrania


5Nigrospora


6Chaetomium


7Lasiosiplodia


8Fusarium

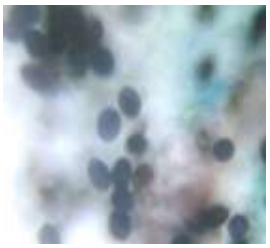

9Chaetomium

Fig.2&5*Nigrospora*, Fig.3*Pestalotiopsis*, Fig.4*Beltrania*, Fig.6&9*Chaetomium*, Fig.7*Lasiodiplodia*, Fig.8*Fusarium*

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 sterilia. We may draw conclusion that there is organ and tissue specificity by endophytes. The plant of *Bauhinia vahlii* shows huge assemblage of endophytes in both seasons but more isolated in winter season. Colonization frequency is more in winter than in monsoon.

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