

## **DIVERSITY OF FUNGAL ENDOPHYTES IN *BAUHINIA VAHLII* (A LIANAS) FROM DIFFERENT REGIONS OF PASCHIM MEDINIPUR DISTRICT OF WEST BENGAL**

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**Abstract:** Fungal endophytes are microfungi that live within various tissues of plants without causing apparent symptoms of infection. This study was conducted to identify the endophytic fungi isolated from leaf, petiole and stem of *Bauhinia vahlii* from three different localities of West Medinipur district. A total 300 isolates were obtained from 375 sample segments. The isolated fungi belong to 38 genera, with a few unknown genera and sterile mycelial fungi. Among all fungi *Penicillium* sp. (19.57%), *Pestalotiopsis* sp. (12.17%) and *Aspergillus* sp.(10.43%) were most commonly found. *Phialophora* sp., *Nigrospora* sp., *Torula* sp., *Bispora* sp., *Curvularia* sp. were also isolated. The colonization frequency (CF) is much higher in petiole (86.67%) in comparison to stem (77.33%) and leaf (70.67%). This study showed the tissue specificity of endophytic symbiosis. There was a variation of CF in respect of localities. Endophytic fungal diversity was higher in plants of Chilkigarh.

**Keywords:** *Bauhinia vahlii*, endophyte, diversity, forest, lianas, fungi.

### **Introduction**

The microorganisms that reside in the tissues of living plants at any stage of their life cycle are known as endophytes [1]. The term includes all the organisms that live symptomlessly within plant tissues [2]. Generally they are symbionts [3,4] and are described as mutualistics, sometimes pathogen also. They are classified in distinct two groups- bacteria and fungi [5]. Endophytic fungi are mostly important in the biodiversity since they have an effect on structure and defense mechanism of plants and ultimately in the ecosystem [6]. Endophytic fungi are also used to study the host-parasite relationship in natural ecosystem [6,7].

Lianas are those woody climber plants [6,7] that climb up supporting another strong and tall tree by a crawling movement. Fungal endophytes present in the root system of various plants have been thoroughly studied as mycorrhiza, but least studied in other tissue system of plants. Only few herbaceous plants, shrubs and very few tree members have yet been studied for endophytes. Dreyfuss and Chapela estimated that there may be one million

species of endophytic fungi in the world [8]. Only a few metabolites have been discovered till now from this group of fungi [9]. Above all, endophytic fungi are under-explored group of microorganisms and there may have a bright future for discovery of various drugs [8,9,10] as like as taxol from endophytic fungi. The fungal isolates have also some antimicrobial activity [11]. The beneficial role of endophytes were established from the study of grasses, conifers and woody perennials [12,13,14].

One of the important roles of endophytic fungi is to initiate the biological degradation of dead or dying host plant, which is essential for nutrient recycling. Endophytes also provide adaptability to the plant community to survive in adverse environmental condition. In Indian subcontinent, very little work has been done on endophytes [15,16,17]. The occurrence of endophytic fungi, hosted by living tissues of forest trees seems to be a common phenomenon [34]. Endophytic fungi are ubiquitous organisms found in the plants, residing intercellular or intracellular, at least for a portion of their lives without causing apparent symptoms of infection [39]. Almost all parts of plant are known to harbour endophytes [39]. No work has been done on endophytes of *Bauhinia vahlii* at Medinipur district. In this communication, we have reported endophytic diversity of *Bauhinia vahlii* collected from different sites of Paschim Medinipur district of West Bengal.

## **Materials and Methods**

### **(i) Study site and sampling strategy and plant material**

Plants were collected in zipper-lock polythene pack from three distinct locations of Paschim Medinipur district (latitude 22°25' to 22°57' North; longitude 87°11' East; altitude 23M), of West Bengal, India, having a tropical warm & humid climate with a mean temperature of 34°C. Average annual rainfall is 120cm. Samples were brought to the laboratory within 5-6 hours of collection and preserved at 4°C temperature in refrigerator. Belpahari forest range, Chilkigarh of Jhargram forest range and Godapiasal of Arabari forest range were selected for sample collection.

*Bauhinia vahlii* (Wt. & Arn.) Benth. (Family- Caesalpinaceae, a gregarious and gigantic woody climber) is an evergreen liana of tropical region, under forest cover, native of Indian subcontinent. It is a very long liana tree, more or less 150 M long, forming a thick canopy over the host tree, found in some restricted areas of Paschim Medinipur, Bankura and Purulia districts of West Bengal. For the 1<sup>st</sup> time, we isolated the endophytic fungi of the plant from different forest regions of Paschim Medinipur, West Bengal, India.

**(ii) Sampling procedure:** Samples from mature, healthy and disease free plants were collected randomly from each location during late winter season. Few leaves with petioles and few pieces of stems with barks were chosen for isolation of endophytic fungi.

**(iii) Surface sterilization:** Samples collected from three localities were thoroughly washed under running tap water before processing. Following immersion sequences had been performed during surface sterilization following the method of Banerjee et al, 2010. Firstly, selected and cleaned plant parts were immersed in 90% ethanol for 20 seconds and then allowed for surface drying under sterile condition or near the flame of spirit burner.

**(iv) Isolation of endophytic fungi:** Thin peels of sterile segments were placed on water agar plate. Each and every day plates were observed for hyphal growth. Within 2-3 days, fungal hyphae from each sample were appeared. Hyphal tip of each fungus was transferred to petridish of potato dextrose agar (PDA) medium. The petridishes were then incubated at 24°C. After few days of growth, the organisms were preserved for identification.

**(v) Identification of isolated endophytes:** The isolated fungi were observed under light microscope. Each isolate was identified after studying cultural, morphological and reproductive characters. The isolated fungi were identified using appropriate manuals [18,19,20,21].

**(vi) Statistical analysis:-**The colonization frequency (CF%) of endophytic fungi was calculated by the formula outlined by Hata and Futai [12,27] using PAST software (Palaeontological Statistics Software Package).  $CF = (N_{col} / N_t) * 100$ ; where  $N_{col}$  = Number of segments colonized by each fungus,  $N_t$  = Total number of segments. Different diversity parameters were calculated using PAST – Palaeontological Statistics Software Packages, ver. 1.89 available free from the internet. Simpson's diversity (1-dominance); Shannon-Wiener index and Evenness were calculated by using the following formulae- Simpson's diversity =  $1 - \sum (pi)^2$ , where pi = Proportion of frequency of colonization of  $i^{th}$  species in a plant; Shannon Wiener index ( $H'$ ) =  $-\sum (pi \log_2 pi)$ ; Evenness ( $E_H$ ) =  $H' / H'_{max}$ ; where  $H'_{max}$  is the maximum value of diversity for the number of species. Evenness expresses how evenly the individuals in a community are distributed among the different species. Species richness S is the simplest measure of biodiversity and is simply a count of the number of different species in a given area. These indices take into account the relative abundance of the taxa. Generally, the Shannon & Fisher's Alpha indices emphasize rate of species, whereas the Simpson index is strongly influenced by the most abundant species in a community. Berger Parker index is the simplest and most easily understood diversity index, since it only calculates the proportion of

the commonest species in a sample. Berger Parker index(d)=  $N_{\max}/N$ . where N= total number of individuals in the sample.  $N_{\max}$ = The maximum number of individual in a taxon.

## Results and Discussion

Endophytic fungi from *Bauhinia vahlii* collected from three different localities of Paschim Medinipur district, West Bengal during winter season were studied. A total number of 238 plant segments were inhabited by fungi out of 375 segments and 300 endophytic fungi were isolated from 238 different sample segments (leaf, petiole, stem). The isolated fungi belong to 29 genera, with 41 unidentified fungal genera. 23 are sterile mycelia. Among all isolated endophytes, *Penicillium* sp. (19.57%), *Pestalotiopsis* sp. (12.17%), *Aspergillus* sp. (10.43%) were most commonly found; whereas *Curvularia* sp., *Botrytis* sp., *Phialomyces* sp., *Arthrinum* sp., *Fusidium* sp. are least abundant (each 0.43%). *Phialophora* sp., *Torula* sp., *Nigrospora* sp., *Cylindrocladium* sp., *Cladosporium* sp., *Bispora* sp., were moderately found. Maximum number of endophytes were isolated from plant samples of Chilkigarh forest (CF=67.2%), then Godapiasal forest (CF=64%), and lowest at Belpahari forest (59.2%). This difference may be due to deepness of the forest, variation in moisture content inside the forest canopy and soil nutrition of forest floor. It was found that soil environment and microclimate were responsible for endophytic diversity. Among all the isolates *Aspergillus* sp., *Pestalotiopsis* sp., *Penicillium* sp., *Bispora* sp. and *Phialophora* sp. were found in all types of plant samples i.e. leaf, petiole, stem; but *Chaetomium* sp., *Periconia* sp., *Oidium* sp., *Mucor* sp., *Humicola* sp., *Aphanomyces* sp., *Candida* sp., *Phialophora* sp., *Cladosporium* sp. were mostly found in stem. *Aspergillus* sp., *Penicillium* sp. were mostly found in petiole and *Nigrospora* sp. was found in leaf and petiole. All the isolated endophytic fungi showed their preference for specific tissues. Among all organs of plant, petiole possessed the maximum number of endophytic fungi (86.67%). Leaf and bark contains 70.67% and 77.33% endophytic fungi respectively. Above all, leaf, petiole and stem are very good habitat for endophytic fungi. The highest species richness of endophytes was found from plant samples from Chilkigarh. Fungal endophytes remain as a poorly investigated microbial group representing a huge source of bioactive compounds. *Bauhinia vahlii* has several pharmaceutical and ethnobotanical uses and it is a member of sacred grooves to the tribal people dominated three districts of Paschim Medinipur, Purulia and Bankura. Colonization frequency was found in following order: petiole (86.67%)> stem(77.33%)> leaf(70.67%) (table 1). Earlier, Raviraja [10] and Banerjee et al [11] reported that endophytic fungal

colonization is higher in leaf segments rather than stem segments of some tropical medicinal plants. To find out the relationships among the endophytes and the plants, different diversity indices were calculated. The Shannon-Wiener index and the Simpson's diversity index were used to determine the species diversity of fungal endophytes from *Bauhinia vahlii* in different localities. Shannon-Wiener index and the Simpson's diversity index were found highest in Chilkigarh (table 2). Taxa are maximum at Belpahari. Individuals are also maximum at Belpahari. Evenness index is maximum at Chilkigarh. Fisher alpha is also maximum at Chilkigarh. Only Dominance is maximum at Godapiasal. Berger Parker index is also maximum at Belpahari (table 2).

**Table 1:** Endophytic fungi isolated from leaf(L), petiole(P), stem(S) segments of *Bauhinia vahlii* in three different localities of Paschim Medinipur

Endophytic fungi	Total Isolates	Belpahari			Chilkigarh			Godapiasal		
		L	P	S	L	P	S	L	P	S
<i>Aspergillus</i> sp.	24	1	2	2	0	0	4	1	5	9
<i>Torula</i> sp.	11	2	1	4	1	1	2	0	0	0
<i>Cladosporium</i> sp.	11	2	0	8	0	0	1	0	0	0
<i>Geotrichum</i> sp.	06	2	0	2	0	0	2	0	0	0
<i>Scopulariopsis</i> sp.	02	1	0	1	0	0	0	0	0	0
<i>Curvularia</i> sp.	01	1	0	0	0	0	0	0	0	0
<i>Botrytis</i> sp.	01	1	0	0	0	0	0	0	0	0
<i>Phialophora</i> sp.	12	1	1	8	0	0	1	1	0	0
<i>Penicillium</i> sp.	45	1	12	7	0	0	10	0	4	11
<i>Bispora</i> sp.	08	0	1	0	0	1	0	3	1	2
<i>Monilia</i> sp.	02	0	0	2	0	0	0	0	0	0
<i>Chaetomium</i> sp.	02	0	0	2	0	0	0	0	0	0
<i>Periconia</i> sp.	01	0	0	1	0	0	0	0	0	0
<i>Oidium</i> sp.	02	0	0	1	0	0	0	0	0	1
<i>Nigrospora</i> sp.	09	0	0	1	0	0	0	6	2	0
<i>Rhizopus</i> sp.	03	0	0	3	0	0	0	0	0	0
<i>Pestalotiopsis</i> sp.	28	0	0	1	2	2	10	11	1	1
<i>Cylindrocladium</i> sp.	16	0	0	0	7	4	0	0	0	5

<i>Papulospora</i> sp.	06	0	0	0	2	3	1	0	0	0
<i>Hymenella</i> sp.	02	0	0	0	1	0	0	1	0	0
<i>Fusidium</i> sp.	01	0	0	0	0	1	0	0	0	0
<i>Mucor</i> sp.	04	0	0	0	0	1	3	0	0	0
<i>Humicola</i> sp.	03	0	0	0	0	0	2	0	0	1
<i>Dicoccum</i> sp.	01	0	0	0	0	0	1	0	0	0
<i>Perisporium</i> sp.	01	0	0	0	0	1	0	0	0	0
<i>Acrocylindrium</i> sp.	01	0	0	0	0	0	1	0	0	0
<i>Arthrinum</i> sp.	01	0	0	0	0	0	0	0	0	1
<i>Phialomyces</i> sp.	01	0	0	0	0	0	0	0	0	1
<i>Beltrania</i> sp.	02	2	0	0	0	0	0	0	0	0
Sterile mycelia	23	8	2	6	2	1	1	2	0	1
Unidentified genera	41	4	4	6	4	6	5	2	6	4

**Table 2:** Diversity indices and species richness of endophytic fungi from *Bauhinia vahlii* in three different localities of India

Parameter	Chilkgarh	Godapiasal	Belpahari
Taxa_S	20	19	15
Individuals	103	84	84
Dominance_D	0.1104	0.1074	0.1281
Shannon_H	2.48	2.494	2.251
Simpson_1-D	0.8896	0.8926	0.8719
Evenness_e^H/S	0.597	0.6375	0.6329
Menhinick	1.971	2.073	1.637
Fisher_alpha	7.4	7.652	5.317
Berger-Parker	0.1942	0.1786	0.1786

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