Aayushi International Interdisciplinary Research Journal (AIIRJ)
UGC Approved Sr.No.64259
Vol - V Issue-III MARCH 2018 ISSN 2349-638x Impact Factor 4.574

Diversity of Fungal Endophytes Isolated from *Adathoda vasica* from Melghat Forest

K. P. Suradkar
Department of Botany,
Indira Mahavidyalaya, Kalamb (MS)

D. V. Hande
Department of Botany,
Shri Shivaji Science College,Amravati (MS)
*Corresponding author: kishorsu5@gmail.com

Abstract:

Adathoda vasica Linn was investigated to study the diversity of endophytic fungi in different seasons. Plant parts were collected in three different seasons for isolation of fungi. Variation in colonization frequency and occurrence was observed. Highest colonization rate was recorded in monsoon as compare to other season. Some endophytes colonized the host in particular season only and disappeared in other season.

Key words: Diversity, fungal endophytes, *Adathoda vasica*

Introduction:

A unique group of individuals is indicating that, in order to servive, they have to be, in one way or the other, dependent on plants or animals directly or indirectly. This attitude of a large group of unit of life fingers towards a fact that "Evolution is not only progressive but can also be retrogressive."This large group of individuals known as "microbes" is playing a 'key-role' in many different ways. These "tiny lives" have proved to be useful in multifold ways, not only to mankind but also to other organisms in remaining "fit for survival."In the struggle for existence, these tiny lives adopted for heterotrophic habits.Naturally, they have developed, exophytic as well as entophytic habits. Some of these "tiny and innovative lives" are known as "Fungi."

Endophytic fungi are microbes that colonize living internal tissues of plants without causing any harm to host (Brown *et al*. 1998, Hyde and Soytong 2008).Endophytic fungi are ubiquitous in most plant species growing in natural environment. The association between fungal endophytes and host plants may be symbiotic or antagonistic or slightly pathogenic (Arnold *et al*. 2007, Schulz and Boyle 2005) in nature. Major impact of endophytes is observed on ecology, distribution and physiology along with immunity of plants. One to several endophytes can be isolated from a single plant by selecting suitable isolation protocol. (Tintjer and Rudger 2006, Albrectsen *et al*. 2010). Endophytes play significant role to establish fungal diversity. The research attention has been rising in biology, ecology and importance of endophytes. It is believed that endophytic fungi are diverse in those areas where diversity of plants are diverse and certainly India is one among these areas which has near about 17527 Angiosperm and 67 Gymnosperm species but studies on endophytic fungal diversity from India is extremely inadequate (Singh, 2012).

To date, only a few plants have been extensively investigated for their endophytic biodiversity and their potential to produce bioactive secondary metabolites. It is, therefore, important to determine endophytic fungal diversity of medicinal plants. In present study medicinal plant *Adathoda vasica* was investigated in different season to study the colonization variations and tissue specificity of fungal endophytes.

Materials and Methods:

Study Area

Plant samples from *Adathoda vasica* Linn. Were collected from several locations of Melghat forest. Melghat is the north western block of forest which spreads over 3,075 sq. km. in Amravati region. It lies between latitude 21° 46' and 20°11'north and longitude77° 34' and 76° 38' east (Amravati District Gazetteer).

Collection of Plant Samples

The plant samples were collected in three different seasons: monsoon, winter and summer for two consecutive years i.e. 2012–13 & 2013-14. The samples were collected in sterilized polythene bags. The collected samples were brought to the laboratory and processed within 24 hrs of collection.

Isolation of the endophytic fungi

The collected plant samples were washed under running tap water to remove surface adherents. Surface sterilization were done according to the method described by Suryanarayanan *et al.*(2001)(Table No.1),to remove epiphytic microbes. The surface sterilized explants then inoculated at 26 ± 2°C into the Petri dishes containing water agar. The plates were periodically observed for fungal growth. The fungi obtained on water agar plates were further sub cultured on fresh PDA plates to obtain pure isolates. The pure endophytic fungal cultures were transferred on PDA slant and stored as stock culture for further studies. All procedures were carried out aseptically under laminar air flow hood.

Microscopic Observation

Permanent slides were prepared from pure colonies of isolated endophytic fungi. Morphological characters such as pycnidia, conidia and condiogenous cells (Coelomycetes); conidia and conidiophores (Hyphomycetes); ascocarp, ascospores and asci (Ascomycetes) were studied under Carl Zeiss, Trinocular Research Microscope (Axioscope-A-1) with magnification of 5x, 10x, 40x and 100x.Microphotography was done using same research microscope.

Mountants and Stain

In the present study, microscopic observations of isolated endophytic fungi were initially done in water mountant. Various fruiting structures were observed by mounting in lactophenol-cotton blue.

Identification of Endophytic Fungi

All the endophytic isolates were identified morphologically and placed in appropriate genera and species of fungi using standard taxonomic keys and monographs. Barnett and Hunter (1972) ,Ellis (1971, 1976), Subramanian (1971) and Sutton (1980) were referred for identification of endophytes.In addition, other papers relating taxonomy of endophytes were also referred.

Data analysis

The Colonization Frequency (CF %) of fungal endophytes were calculated by using the following formula (Kumaresan and Suryanarayanan 2001).

$$\text{Colonization Frequency (CF \%)} = \frac{\text{Total Number of segments colonized by Fungi}}{\text{Total Number of segments studied}} \text{X } 100$$

Results:

A total of 13 endophytic fungi were isolated from different plant samples collected in the 2012-13. Out of the total endophytic fungi obtained in the present study, *Alternaria alternata* showed higher colonization frequency in petiole region during all the seasons (Fig.1).*Arthrinium hydei* and *Arthrinium phaeospermum* colonized present host only in monsoon with higher colonization frequency in leaf region. Both the species of *Stachybotrys*i.e. *S. chartarum* and *S. nilgirica* were present in monsoon and winter but disappeared in summer (Table 1).*Epicoccum nigrum, Nigrospora oryzae, Pestalotiopsis funerea, Virgaria nigra* and *Xylaria* sp. were present during winter only.

During the season 2013-14 total fifteen fungal endophytes were isolated. All the isolates exhibited seasonal variations in occurrence. *Arthrinium phaeospermum* showed higher colonization rate in leaf tissue in monsoon whereas in winter it exhibited high colonization frequency in stem tissue. *Aspergillus stellatus* was present throughout the study but in winter season it showed colonization frequency high as compared to other seasons. Very less number of fungi that is *Alternaria alternata, Aspergillus nidulans, Curvularia lunata, Penicillium chrysogenum* was recovered during summer season with lower colonizing frequency (Fig.2). *Fusarium oxysporum* showed high colonization frequency in petiole as compared to stem and leaf tissues during monsoon and summer (Table 2).*Arthrinium hydei*, *Phoma crysanthemicola, Stachybotrys chartarum* and *Stachybotrys nilgirica* recovered only from rainy season while *Colletotrichum gloeosporiodes* and *Pithomyces chartarum* were obtained only in winter season.

Table. 1. Colonization frequency (%) of endophytic fungi isolated from *Adathoda vasica* during 2012-13.

Sr.No.	Endophytes	Monsoon			Winter			Summer		
		Stem	Leaf	Petiole	Stem	Leaf	Petiole	Stem	Leaf	Petiole
1.	*Alternaria alternate*	22.85	27.58	29.30	12.08	15.62	23.47	2.06	4.95	6.20
2.	*Arthrinium hydei*	16.59	34.48	22.10	_	_	_	_	_	_
3.	*Arthrinium phaeospermum*	19.47	25.04	17.65	_	_	_	_	_	_
4.	*Curvularia lunata*	12.05	24.66	18.44	16.60	13.64	11.09	5.05	4.22	3.99
5.	*Epicoccum nigrum*	_	_	_	17.61	19.22	25.16	_	_	_
6.	*Fusarium oxysporum*	14.52	12.55	19.47	_	_	_	3.25	1.88	4.23
7.	*Nigrospora oryzae*	_	_	_	26.84	20.56	29.02	_	_	_
8.	*Pestalotiopsis funereal*	_	_	_	18.97	23.50	20.90	_	_	_
9.	*Pithomyces chartarum*	10.30	24.24	17.99	_	_	_	6.01	8.66	3.77
10.	*Stachybotrys chartarum*	13.46	19.22	15.84	14.22	15.33	21.04	_	_	_
11.	*Stachybotrys nilgirica*	22.33	25.87	19.55	16.32	25.82	19.60	_	_	_
12.	*Virgaria nigra*	_	_	_	19.34	17.54	22.57	_	_	_
13.	*Xylaria* sp.	_	_	_	22.44	14.89	27.34	_	_	_

–absent

Table.2. Colonization frequency (%) of endophytic fungi isolated from Adathoda *vasica* during 2013-14.

Sr.No.	Endophytes	Monsoon			Winter			Summer		
		Stem	Leaf	Petiole	Stem	Leaf	Petiole	Stem	Leaf	Petiole
1.	*Alternaria alternata*	_	_	_	_	_	_	7.78	9.70	3.58
2.	*Arthrinium hydei*	19.75	35.29	51.61	_	_	_	_	_	_
3.	*Arthrinium phaeospermum*	25.98	35.59	30.09	39.45	32.65	21.21	_	_	_
4.	*Aspergillus nidulans*	_	_	_	_	_	_	8.90	10.62	7.12
5.	*Aspergillus stellatus*	14.81	23.53	18.39	38.28	24.89	27.45	5.55	4.98	8.04
6.	*Cladosporium cladosporioides*	_	_	_	25.97	36.66	30.84	4.50	8.23	6.96
7.	*Colletotrichum gloeosporiodes*	_	_	_	18.88	22.90	28.47	_	_	_
8.	*Curvularia lunata*	_	_	_	_	_	_	9.73	5.82	6.96
9.	*Fusarium oxysporum*	16.44	21.88	28.31	_	_	_	10.25	8.44	5.89
10.	*Penicillium chrysogenum*	_	_	_	_	_	_	10.20	2.66	4.54
11.	*Phoma crysanthemicola*	16.25	19.99	17.42	_	_	_	_	_	_
12.	*Pithomyces chartarum*	_	_	_	14.78	19.45	23.40	_	_	_

13.	*Stachybotrys chartarum*	20.80	16.35	24.66	_	_	_	_	_	_
14.	*Stachybotrys nilgirica*	26.09	30.22	18.74	_	_	_	_	_	_
15.	*Xylaria* sp.	22.72	19.33	40.32	40.75	50.44	32.07	_	_	_

–absent

Discussions:

Diversity of endophytic fungi was studied from medicinal plants collected from different localities of Amravati district. Some endophytes enjoy growing in winter ie. at low temperature and moderate to high humidity while some relish growing in less humidity and high temperature. This indicates that colonization of endophytic fungi in host depends on several environmental factors like temperature, rain fall, humidity which make them sensitive to host response. Owing to sensitivity, the endophytes adjust themselves to the reactions of and/or responses of the hosts. This sensitive nature of fungi is a result of survival urge. Such survival urge finally leads to diversified survival attitudes of fungi. Thus exhibiting diversity of extreme propensity.

During the present study of *Adathoda vasica* in both the year of investigation, it showed great variations in isolation and colonization of endophytic fungi. In first year of investigation total thirteen fungi were isolated and in the next year fifteen fungi were isolated. Among these isolates two species of *Stachybotrys* i.e. *S. chartarum* and *S. nilgirica* were observed in monsoon and winter season of first year and disappeared in summer of same year (Table 1**)** but they reappeared during monsoon season of the next year (Table 2**)**. It indicated that humidity was important in colonization of endophytic fungi.

Conclusions:

Association of endophytic fungi with host may affect by many environmental factors like temperature, humidity, and rainfall and forest type. In present work variations are observed incolonizations of endophytes during different seasons. Endophytes showed higher colonization frequency in monsoon as well as in winter as compared to summer. Conclusively it suggested that environmental factors play an important role in establishment of endophytic fungal diversity.

References:

1) Albrectsen BR, Bjorken L, Varad A, Hagner A, Wedin M, Karlsson J, Jansson S. 2010. Endophytic fungi in European aspen (Populus tremula) leave diversity, detection, and a suggested correlation with herbivory resistance. Fungal Diversity 41,17-28.
2) Arnold AE, Henk DA, Eells RA, Lutzoni F, Vilgalys R. 2007. Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental pcr. Mycologia 99, 185–206.
3) Barnett HL, Hunter BB. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company. p. 240.
4) Brown KB, Hyde KD, Guest DI 1998. Preliminary studies on endophytic fungal communities of Musa acuminate species complex in Hong Kong and Australia. Fungal Diversity 1, 27–51.
5) Ellis MB. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew.p. 595.
6) Ellis MB 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew. p. 494.
7) Halmschlager E, Butin H, Donaubauer H. 1993. EndophytischePilze in Blttern und Zweigen von Quercuspetraea. Europian Journal of Pathology 23,51-63.
8) Hyde KD, Soytong K. 2008.The fungal endophyte dilemma. Fungal Diversity 33, 163-173.
9) Petrini O, Fisher PJ. 1988. A comparative study of fungal endophytes in xylem and whole stem of Pinussylvestrisand Fagussylvatica Trans. British Mycological Society 91**,** 233–238.
10) Rodrigues KF. 1994. The foliar fungal endophytes of the Amazonian palm Euterpeoleracea. Mycologia 86, 376-85.
11) Schulz B, Boyle C. 2005. The endophytic continuum, Mycological Research 109, 661-686.

12) Singh SK 2012. Endophytic Fungi:Diversity.Ex situ Conservation and Biotech Potential. National Workshop on Taxonomy,Ex Situ Conservation and Bioprospecting of Fungi, Agarkar Research Institute, Pune. p.46-51.
13) Sutton BC 1980. The Coelomycetes, Fungi Imperfecti with pycnidiaAcervuli and Stromata. Robert Mac Lechose and Co. Ltd. University of Glasgow. England. p. 696.
14) Suryanrayanan TS, Kumaresan V, Johnson JA 2001. Fungal endophytes: The tropical dimension. In Trichomycetes and other fungal groups. Edited by J K Misra and B W Horn. Science Publishers, Inc, Enfield, N. H. p. 197–207.
15) Subramanian CV. 1971. Hyphomycetes, Indian Council of Agricultural Research, New Delhi. p. 930.
16) Suryanarayanan TS, Kumarsan V, Jonson JA. 1998. Foliar fungal endophytes from two species of the mangrove *Rhizophora*. Canadian Journal of Microbiology. 44**,**1003–1006.
17) Suryanarayanan TS, Vijaykrishna D. 2001. Fungal endophytes of aerial roots of *Ficus benghalensis*. Fungal Diversity 8,155–161.
18)Tintjer T, Rudger AJ. 2006. Grassherbivore interaction altered by strains of a native endophyte, New Phytology 170, 513-521.
19) Wang Y, Guo LD. 2007. A comparative study of endophytic fungi in needles, bark, and
20) xylem of *Pinus tabulaeformis*. Canadian Journal of Botany 85**,** 911–917.