Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2013 Vol. 3 (3) July-September, pp.76-80/Das et al.
Research Article

ENDOPHYTIC FUNGAL ASSEMBLAGES IN AN AQUATIC WEED: *EICHHORNIA CRASSIPES* (MART.) SOLMS

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ABSTRACT
We have investigated endophytic fungi in leaves, petiole and root of an aquatic weed *Eichhornia crassipes.* A total of 33 fungi and sterile hypha were identified from 146 isolates. Sterile hypha was isolated from all the plant parts. *Mucor racemosus* and *Cladosporium cladosporioides* possess the high isolation rate isolated from root and petiole, respectively in the month of February.

Key Words: *Endophytes, Fungi, Eichhornia crassipes, Isolates and Month*

INTRODUCTION
Endophytes reside asymptomatically with in most living plant tissues examined to date (Schulz *et al.*, 2002; Li *et al.*, 2007 and Tao *et al.*, 2008) and are found in diverse habitats ranging from coastal mangroves (Kumaresan and Suryanarayanan, 2001) to temperate and alpine areas (Espinosa-Garcia and Langenheim, 1990). Endophytic fungi have been recovered from healthy tissues of plant species growing in different biomes such as tundra, dry deserts, and tropical rain- forests from the Arctic to Antarctica (Luiz *et al.*, 2012). Individual plants may be the host to one or more endophytes and many endophytes may colonize certain hosts, suggesting that there may be many undiscovered endophyte species (Petrini, 1991; Strobel and Daisy, 2003; Huang *et al.*, 2007). Endophytes play a major role in plant community health by providing resistance to hosts against different biotic and abiotic stresses (Kharwar *et al*., 2008; Gond *et al.*, 2010). Fungal species that establish an endophytic role may contribute to the well-being of the host plant by producing bioactive secondary metabolites (Gao *et al.*, 2010; Schulz *et al*., 2002). Different works carried out so far regarding the role of endophytes in host plants indicate that they can stimulate plants growth, increase disease resistance, improve plant's ability to withstand environmental stresses and recycle nutrients (Tayung and Jha, 2008).
The water hyacinth, *Eichhornia crassipes* (Mart.) Solms is an invasive plant which is native of the Amazon basin and whose capacity for growth and propagation causes major conservation problems with considerable socioeconomic repercussions (Téllez *et al.*, 2006).
In the present scenario, we have undertaken this work to study the endophytic fungal assemblages in different parts (leaf, petiole and root) of *E. crassipes.*

MATERIALS AND METHODS
Site Description and Collection
To analyse the diversity of endophytes; plant samples of *E. crassipes* were collected from a pond in Durjoynagar, Tripura, northeast India. Samples were kept in closed sterile polythene bags and processed within 24 hrs of collection.
Surface Sterilization and Preparation of Plant Material
Segments of lamina were taken from the middle portion of fresh healthy leaves, segments from the basal part of the petiole and segments from the fresh root were taken with the help of sterile scissor. The segments were about 0.5 cm in length. All segments were dipped in 70% ethanol for 5 seconds and rinsed in sterile distilled water for 10 seconds.

Preparation of Media

Two types of culture media used for the analysis: Rose Bengal Agar (RBA) media and Potato Dextrose Agar (PDA) media. After preparing the media they were then sterilized in an autoclave and then 10 mg /litre of antibiotic was added for preventing them from the contamination of bacteria. After the media are prepared, they were then poured separately into the sterile plates. Then they were allowed to cool at room temperature.

Inoculation

Twenty segments of leaf, petiole and root each were teased with the help of sterile needle and forcep. Five segments of each placed on 25 ml PDA and RBA media in each sterile petri dish with the help of sterile forcep. Then it was incubated at 25^0C for one week till the colonies grow well. Inoculation was done under laminar air flow.

Identification of Endophytic Fungi

The colonies were then identified by staining with Lactophenol cotton blue with the help of a compound microscope (Olympus 107263). The fungal species were identified on the basis of cultural characteristics and morphology of fruiting bodies and spores by using standard texts and keys. The species was then identified by using the identification manual (Ellis, 1993; Domsch *et al.*, 1980; Watanabe, 2002). Isolates which did not sporulate were placed under near UV light (black light for 12h dark: 12h light) in an attempt to stimulate sporulation. Isolates which did not produce spores were treated as sterile mycelium (Lacap *et al.*, 2003).

Data Analysis

Colonisation rate and isolation rate were calculated by the following formula:

Colonisation rate = $N_c / N_t \times 100$

Isolation rate = $N_i / N_t \times 100$

Where, N_c is the total no. of segments from which fungi were isolated in a sample, N_i is the no. of segments from which a given species was isolated and Nt is the total no. of segments used for isolation. Diversity index analysis was conducted using the software PAST (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

The incubation of fungal inoculum from the tissues of *E. crassipes* yielded 146 isolates. The month of January, February and March reveals 40, 53 and 53 isolates, respectively (Table 1). The lowest number of morphotypes was isolated from roots in the month of January and highest from leaf in the month of March. A total of 33 morphotypes were isolated and a sterile mycelium. Sterile mycelium was isolated from all the parts of plants and in all the months. Sterile mycelium has the highest isolation rate. *Nigrospora sphaerica* also possesses the highest isolation rate in the month of January isolated from petiole. *Mucor racemosus* and *Cladosporium cladosporioides* also possess the high isolation rate isolated from root and petiole, respectively in the month of February. The highest colonization rate was noted in the month of March i.e., 95% in leaf (Table 2). Genera of *Alternaria alternata* and *Cladosporium cladosporioides are* common epiphytes but can also occur as endophytes which were isolated in this study; this result supports many earlier works (Bacon and White, 2000; Kharwar *et al.*, 2010) and surprisingly, these two genera also dominated over the most cosmopolitan species of *Aspergillus* which suggests that it may be due to substrate specificity (Gond *et al.*, 2012).

Table 1: Details of fungal isolate and morphotypes isolated from *Eichhornia crassipes*

	January		February		March	
Samples	**Isolates**	**Morphotypes**	**Isolates**	**Morphotypes**	**Isolates**	**Morphotypes**
Leaf	15	8	17	9	20	11
Petiole	18	10	19	5	14	7
Root	7	4	17	7	19	9

Table 2: Isolation and colonization rate of endophytic fungi isolated from *Eichhornia crassipes*

	January			February			March		
Fungi	**Leaf**	**Petiole**	**Root**	**Leaf**	**Petiole**	**root**	**leaf**	**petiole**	**Root**
Alternaria longipes	5	-	-	-	-	-	-	-	-
Alternaria alternata	-	5	-	-	5	-	10	15	-
Alternaria sonchi	-	-	-	5	-	-	-	-	-
Alternaria tenuissima	-	-	-	-	-	-	15	-	-
Arthrobotrys oligospora	-	5	-	-	-	-	-	-	-
Aspergillus flavus	10	-	-	-	-	-	-	-	10
Aspergillus niger	-	5	-	-	-	-	5	-	-
Aspergillus japonicus	-	-	5	-	-	-	-	-	-
Bactrodesmium traversianum	-	-	-	-	-	-	5	-	-
Cladosporium cladosporioides	-	-	-	15	25	5	10	10	5
Cladosporium macrocarpum	5	-	-	-	-	-	5	5	-
Cladosporium sphaerospermum	-	5	-	-	-	-	-	-	-
Curvularia brachyspora	5	-	-	-	-	-	-	-	-
Curvularia ovoidea	-	-	-	5	-	-	-	-	-
Dendryphion nanum	5	-	-	-	-	-	-	-	-
Dendryphion comosum	-	5	-	-	-	-	-	-	-
Fusarium oxysporum	10	-	-	-	-	-	-	-	-
Fusarium redolens	-	-	-	-	-	-	5	-	-
Mucor hiemalis	-	-	-	10	5	10	10	10	10
Mucor racemosus	-	-	-	5	-	20	-	-	-
Mucor circinelloides	-	-	-	5	-	-	-	-	10
Mortierella parvispora	-	-	10	-	-	5	-	-	15
Mortierella gemmifera	-	-	-	-	-	5	-	5	-
Mortierella tsukubaensis	-	-	-	-	-	-	5	-	5
Nigrospora sphaerica	-	20	-	-	-	-	-	-	-
Phoma eupyrena	-	5	-	-	-	-	-	-	-
Pestalotia sp	-	5	-	-	-	-	-	-	-
Periconia byssoides	-	5	-	-	-	-	-	-	-
Pythium ultimum	-	-	-	5	-	-	-	-	-
Pythium elongatum	-	-	-	-	-	-	-	-	5
Pythium angustatum	-	-	-	-	-	-	-	5	-
Rhizopus oryzae	-	-	5	5	10	5	5	-	15
Scolecobasidium humicola	10	-	-	-	-	-	-	-	-
Sterile hypha	25	30	15	25	25	25	20	20	10
Colonization rate	75	90	35	80	70	75	95	70	85

Table 3: Diversity indices of fungi isolated from *Eichhornia crassipes*

	January			February			March		
	Leaf	**Petiole**	**Root**	**Leaf**	**Petiole**	**Root**	**Leaf**	**Petiole**	**Root**
Dominance	0.18	0.19	0.31	0.17	0.29	0.22	0.12	0.18	0.13
Shannon	1.89	1.99	1.28	1.98	1.39	1.71	2.26	1.81	2.12
Simpson	0.82	0.81	0.69	0.83	0.71	0.78	0.88	0.82	0.87
Evenness	0.83	0.73	0.90	0.80	0.80	0.79	0.87	0.87	0.93

According to Zalar *et al.,* (2007) members of the *Cladosporium* genus are distributed worldwide and *Cladosporium cladosporioides* is one of the most common endophytic species found in association with various plant species. Rajgopal *et al.,* (2010) have isolated endophytes in five medicinal plants. They have reported the presence of 13 endophytic species and five sterile forms of hyphae. Among them *Alternaria alternata, Fusarium oxysporum, Curvularia sp, Phoma sp* and sterile form of hyphae also present in the present work.

The dominance was least in the month of March in the fungi isolated from leaf and evenness was high in the month of March in the fungi isolated from root. The Shannon diversity index was maximum in the leaf in the month of March and least in root in January. Simpson index of diversity was maximum in the month of March in leaf and less in root in the month of January (Table 3).

ACKNOWLEDGEMENT

The authors are thankful to the Head, Department of Botany for providing facilities in the laboratory.

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