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Endophytes Fungi Associated With a Water Hyacinth of *Eichhornia Crassipes* (Mart.) Solms

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***Abstract*:** Nineteen endophytes fungal species were isolated from leave and petiole of an aquatic hyacinth weed plant of Eichhornia crassipes. These plants were collected from a freshwater pond in different months at Mannargudi in Tamil Nadu. Among these two belonging to Ascomycetes, four fungal species belonging to Coelomycetes and eleven Hyphomycetes beside to two sterile forms. These endophytic fungi were studied shows fewest colonies that some of the distinct occupying the leaf and petiole tissue. Commonly isolated genera from both month and tissue were *Alternaria alternata*, *Aspergillus niger*, *Colletotrichum cloeosporioides*, *Curvularia lunata*, *Fusarium oxysporum*, *Penicillium* sp. *Phomopsis* sp., and Yeast form. This study revealed that fungal diversity varied with tissue types and by host plant in representative two months. This diversity of fungi has been assessed to vary depending on the specificity of the tissue. According to the RPO statistical analysis, Hyphomycetes fungi have been dominated by both tissues. Similarly, according to the CF% calculation, more no. of fungal colonies has been isolated from leaf tissue. Assumed, that these distributed fungi have been dependent on tissues specificity.

Keywords: *Eichhornia crassipes*, fungal diversity, endophytes, hydrophyte, tissus specificity.

I. INTRODUCTION

Eichornia crassipes is an invasive perennial aquatic macrophyte belonging to the Pontederiaceae family. Pontederiaceae considered native in South America and widespread in tropical and subtropical as North America, Asia, Australia, Africa and New Zealand [1]. Solms or water hyacinth as common names is aquatic flowering plants and its name *Eichhornia* was derived from documented 19th-century Prussian politician J.A.F. Eichhorn [2]. *Eichhornia crassipes* was regarded as a hydrophytic plant that can be improved effluent quality from oxidization ponds and as a main part de integrated advanced system for the treatment of municipal, agricultural, and industrial wastewaters [3, 4, 5, and 6]. Water hyacinth (*Eichhornia crassipes*) is an aquatic vascular plant with rounded, upright, and glossy green leaves and lavender flowers almost like orchids [7]. An individual basal cluster is erect and free-floating with assorted stolen. Petioles are bulbous and spongy with large with much air spaces, which allows plants to float on a water surface. However, floating leaves will vary in size and morphology according to growth conditions, and also the stage of community development. Leaves with bulbous petioles are dominant in open water whereas elongated petioles predominate in dense colonies [8]. Aquatic treatment; a variety of the special character of constructed wetlands that treat industrial, municipal, and agricultural wastewater (Pollution Indicator) by floating and submerged aquatic plants [7]. *Eichhornia crassipes* is regarded as an environmental weed in many parts of the world and has been widely classified as the "world's worst aquatic weed" and also water hyacinth has a number of other uses. Phytoremediation used for removing heavy metals and other pollutants is a newly developed environmental protection technique. Water hyacinths are potentially an excellent source of biomass. These are processed an anaerobic fermentation, polluted hyacinths are converted to the natural gas-methane. These are dried and cleansed plants can be used as fertilizer and plant mulch. The plant can be cultivated for use in wastewater treatment because it is growing on pollution water.

Endophyte was coined by de Bary [9] to define, “Organisms that colonize internal plant tissues.” Microorganism live-in association with the plant (endophytic microbes) is commonly observed in nature [10]. They are commonly found in coniferaceae, Graminosperm, [11]. Fungal endophytes have also been reported from marine algae [12], mosses and ferns [13], mangroves [14] and angiosperm trees [15, 16]. Seaweeds host a number of endophytic organisms, including bacteria [17], algae [18]. The freshwater marsh always undergoes biological or microbial succession, which aids in the maintenance of biodiversity [19]. In the course of time, the definition of endophytes has evolved to embrace those fungi which have lengthy epiphytic phases and latent endophytic phase

(latent pathogens), that may live symptomlessly in their hosts for some time in their life [20]. Species richness and distribution of fungal endophytes vary in relation to the collection site [21, 22]. Fungi, which exist as a unique kingdom amongst all living things in the universe, are cosmopolitan and fascinatingly occupy various ecological niches. A general discussion of fungal adaptations to freshwater existence is given by Thomas [23]. Endophytic fungi could be favorable to their host plants throughout reciprocal life, but they can also become latent pathogens during a certain period of the hosts' life pattern or under certain environmental conditions [24]. The aim of this study was to identify the distribution and diversity of fungi from adapted to tissues in *Eichhornia crassipes* aquatic plant species.

II. MATERIALS AND METHODS

Taxonomy and Collection of plant materials *Eichhornia crassipes* is a hydrophyte plant taxonomically known as Pontederiaceae family. Hyacinth weed grows floating in freshwater ponds, lakes, river especially abundant growing in pollution water. The host plant studied was collected from Mannargudi in Tamil Nadu, South India.

The blades of the top leaves are oval, orbicular in shape and the blade surfaces are conspicuously swollen, bulbous in the middle. It is occurred Petioles or bulbous and spongy with large and much air spaces. This variation of plant parts was collected to the investigation (fig.1a). Leaf samples were collected from healthy plants. Randomly select fifty plants and their one hundred and fifty tissue segments were cut from fifty plants. However, sterilization techniques were followed in before cutting these segments. They were processed within 24 hours of collection [25]. The plant is washed thoroughly with running water and then the whole plant has sterilized as followed. Surfaces sterilization of leaves One hundred filaments whorled branched leaves bits of 0.5 cm^2 each were cut and surfaces sterilized by the method of Suryanarayanan et al [14]. The samples were washed in running water, dipped in 70% ethanol for five seconds, immersed in 4% NaoC1 for 90 seconds, and then washed in sterile water for 10 seconds or three times. The sterilized samples were placed on PDA medium amended with an antibiotic contained in Petri dishes. Incubation, isolation, and identification of endophytes ten segments were placed on PDA medium contained in a Petri dish. The Petri dish was sealed with Parafilm™ and incubated in a light chamber at 26+1°C for 7 to 21 days [26, 27]. The light regimen given was 12 hours light followed by 12 hours darknesses. Fungi that grew from the segments were periodically observed and the endophytes were identified.

Statistical Analysis:

i. Colonization frequency (CF %) of an endophyte species was calculated by the method of Hata and Futai [37].

$$CF\% = \frac{N_{colonies}}{N_{total}} \times 100$$

Where, $N_{colonies}$ and N_{total} are the number of segments colonized by each endophyte and the total number of segments observed respectively.

ii. Relative Percentage of Occurrence (RPO) of each group (*viz.* Ascomycetes, Coelomycetes, Hyphomycetes and sterile forms) of fungal species in each plant species was calculated as follows:

$$RPO = \frac{\text{Total colonization frequency of one group}}{\text{Total colonization frequency for all the Groups of fungi}} X100$$

III. RESULTS AND DISCUSSION

Microorganisms associated with each of the plant tissues, including fungi are widely distributed in Universal habitats. The present investigation of endophytes fungi in *Eichhornia crassipes* plant species, we personally obligate recorded 18 endophytes were isolates during two (November 2019 and January 2020) months from leaf and petiole segments. In most of the cases, each tissue segment was infected by more than one fungal species [28]. Several ecological groups of plants such as mangroves [14], halophytes [29], and trees of the tropical forest [15] have been studied for their endophyte assemblages. Based on their studies on two neotropical tree hosts, Arnold et al [28], concluded that tropical endophytes are hyperdiverse and the figure of 1.5 million, "may markedly underestimate fungal diversity." However, several aquatic plants have not been studied for their endophyte association.

Hence, this study was carried out *E. crassipes* species of aquatic plants screened for the presence of fungal endophytes harboured in their leaves and petiole (Table. 1). A total of 19 fungal species was obtained during study periods. The endophytes included two Ascomycetes, four Coeleomycetes, eleven Hyphomycetes and two sterile forms (fig.1-2). A few ascomycetes such as *Chaetomium globosum*, *Sporormiella* were isolated. Intriguingly, a few coprophilous genera (*Chaetomium*, *Sporormiella* etc) have been reported as endophytes from several hosts [13].

A Coeleomycete member in *Colletotrichum gloeosporioides*, *Phoma* sp., *Phomopsis* sp., *and Phyllosticta capitalensis* species was abundance isolated and also dominated the endophyte assemblages in leaves in during two months of the study (Fig.1). This fungus is ubiquitous forms and contributes substantially to the endophyte assemblage of the leaves of many plants [15, 16]. In addition, some hyphomycete fungi including *Alternaria alternate, Aspergillus flavus, Aspergillus niger, Aspergillus* sp. *Aureobasidium pullulans, Cladosporium cladosporioides, Curvularia lunata, Drechslera hawaiiensi, Fusarium oxysporum, Penicillium* spp. were frequently isolated as leaf and petiole endophytes (fig.1). Similar reports have been made by some other mycologists on the endophytic aquatic hyphomycetes [30, 31, and 32] on different hosts. No basidiomycete member was present as an endophyte. Members of this class are rarely encountered as endophytes [13], and no recovery in and chytridiomycetes, these taxa not be reported to isolate in growth media. The procedure for the inoculation of endophytes from leaves and petiole of these portions was exactly more densely colonized by leaves endophytes when compared with petiole in both months. (November 2019 and January 2020). Similarly, the number of endophyte species also increased during the study periods (Table1 and Fig.1-2). This study shows that hydrophyte plants harbour least numbers of endophytic fungi in their leaves followed by the petiole are very low richness; these endophytes are different from those that occur on the leaf and petiole of these hydrophytes. Many reported freshwater ascomycetes are an ecological assemblage of fungi that occur on submerged or partially submerged substrates in aquatic habitats [33, 34]. This plant is a floating aquatic plant. Similar information for most of the fungi has been reported [35].

Anatomical of leaf: A transverse section of lamina has a very thin cuticle on the epidermal cells, which are rectangular in outline and form a single layer. The mesophyll is differentiated into a palisade and spongy mesophyll. Palisade layer is present on both the upper and lower side beneath the epidermis. The upper epidermis has a few layers of cells, and the lower epidermis has Inside the palisade layer are densely staining material which may be supportive in nature. The spongy mesophyll consists of a large number of air spaces surrounded by thin walls full of the chloroplast. Sclereids are observed in cells facing air spaces [36].

Anatomical of Petiole: This floating-leaved characteristic attributes to the bizarre structure of its petiole. The middle part of its petiole swells to a sphere. The anatomy shows the inside looks like intense bubbles filled with air. To be precise, its petiole is like polyfoam. The epidermis of the petiole is also single-layered and composed of parenchyma cells. The cuticle is absent. Vascular bundles are embedded in outer parenchyma cells. Each vascular bundle has a bundle cap of sclerenchyma cells making up the petiole. The hexagonal air spaces are surrounded by bands of single-layered parenchyma cells. Vascular bundles are immersed in aerenchyma [36].

The richness and density of fungi vary according to the structure of Eichhornia plant tissues. When looking at the above-mentioned the structure of anatomy in *Eichhornia* plants' leaf and petiole (fig.1b,c), and also, these endophytic fungi have been occurring to vary from plant tissues of the plant. Thus, we find this study that the presence of endophytic fungi was dissimilar (fig 2 -3).

The present study suggested that the least endophyte diversity in *E. crossipes*, also these fungi dissimilar in petioles than leaves. For the described in tissues fungi, an aquatic hyacinth weed plant of *Eichhornia crassipes* was screened for endophytes. Coelomycetes, hyphomycetes, ascomycetes and sterile forms were present as endophytes. Coelomycete members contributed more to the endophyte assemblage of the plant. The diversity and density of fungi are diverse in form of tissues build in the plant portion. Further studies are warranted can throw more light on climatic and nutrient factors and endophytic fungi would elucidate their importance in the freshwater plant.

IV. CONCLUSION

In the study of an aquatic process, plant-microbe interaction in *Eichhornia crassipes* has been isolated the microfungi, especially on the leaf and petiole; to plant inner tissues has made up of parenchyma cells tissues containing much air chamber. The colonization of fungi has been varied depending on the structure of these plant tissues. Although the basidiomycetes, chytridiomycetes fungi not present, the hyphomycetes fungi are slightly increased but these endophytic fungi have occurred is not much as they may be found in hypodermis areas. This investigation was done in the purpose of tissues basis, although these results show that fungi differ in the structure of the tissues.

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Authors Profile

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Arun Gunasekaran obtained his biology bachelor's and master's degrees at the M.R. Government Arts College, Mannargudi, Tamil Nadu, and India. His is studying as fresher water fungal diversity and their metabolites products.

Table 1. Fungal endophytes isolated from the leaves and petiole of *Eichhornia crassipes* during different months.

S.No.	Name of endophytes	Leaf-Nov-2019	Leaf- Jan- 2020	Petiole-Nov-2019	Petiole – Jan-2020
	Ascomycetes				
1	*Chaetomium globosum*	0.0	0.7	0.7	0.0
2	*Sporormiella* species 1	0.7	0.7	0.0	0.7
	Ceolomycetes				
3	*Colletotrichum gloeosporioides*	12.0	14.7	1.3	2.0
4	*Phoma* species 1	8.0	4.0	0.0	0.0

5	*Phomopsis* species 1	13.3	12.7	1.3	0.7
6	*Phyllosticta capitalensis*	15.3	17.3	0.0	0.0
	Hyphomycetes				
7	*Alternaria alternata*	5.3	4.0	0.7	1.3
8	*Aspergillus flavus*	1.3	2.0	0.0	0.0
9	*Aspergillus niger*	2.0	1.3	2.0	0.7
10	*Aspergillus* species 1	0.7	1.3	0.7	0.0
11	*Aureobasidium pullulans*	5.3	4.0	0.0	0.7
12	*Cladosporium cladosporioides*	0.0	2.0	0.7	1.3
13	*Curvularia lunata*	1.3	0.7	0.7	0.7
14	*Drechslera hawaiiensi*	2.7	4.0	0.0	0.0
15	*Fusarium oxysporum*	2.0	1.3	1.3	0.7
16	*Penicillium* species 1	1.3	0.7	0.7	0.7
17	*Penicillium* species 2	2.0	0.7	0.0	0.0
	Yeast Forms				
18	Yeast form 1	0.7	1.3	0.7	0.7
19	Yeast form 2	0.0	0.7	0.0	0.7
	Total No. of Species CF%	**74.0**	**74.0**	**10.7**	**10.7**
	Total No. of Species	**16**	**19**	**11**	**12**
	Total No. of Colonies	**111**	**109**	**16**	**15**

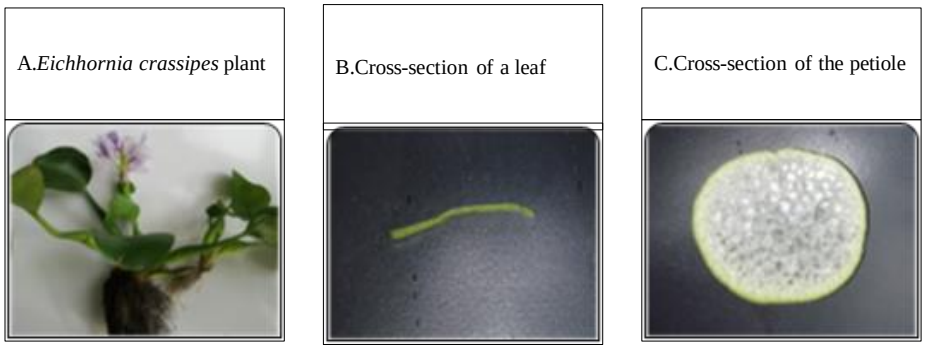

Figure 1. A. An *Eichhornia crassipes* plant. B. Cross-section of a leaf. C. Cross-section of the petiole of a plant.

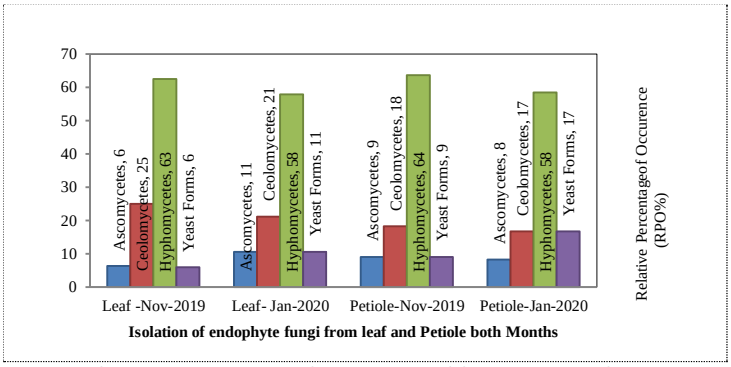

Figure 2. Relative Percentage of Occurrence (RPO) of each group of fungal species from *Eichhornia crassipes* Plant.

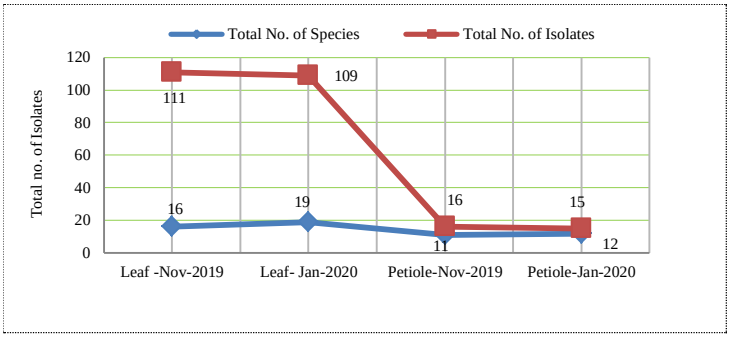

Figure 3. No. of fungal species and colonies isolated from *Eichhornia crassipes* plant during both months.

