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Endophytic Fungi as latent Pathogens in *Eichhornia Crassipes* (Mart.) Solms

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Abstract

Eichhornia crassipes (water hyacinth) are one of the most invasive aquatic weed. Endophytic fungi and pathogenic fungi were isolated from *E. crassipes.* Fungi isolated as endophytes were tested to find if they are capable of causing disease symptoms on healthy leaves of *E. crassipes.* Endophytes, *Aspergillus flavus, A. fumigatus, A. glaucus, Curvularia lunata, Eurotium repens, Oidium* sp., *Chaetomium globosum* and sterile mycelia showed disease symptoms on healthy leaves. The results confirmed that endophytic fungi may act latent pathogen in their host plant under certain stress conditions.

Key words: *Eichhornia crassipes,* endophytic fungi, pathogenic fungi, latent pathogens.

1. Introduction

In India, many rivers, irrigation canals, lakes both natural and man - made, are choked by the explosive growth of aquatic weeds, resulting in enormous direct losses. Besides different types of algae, the most important representatives of aquatic weeds in India are: *Eichhornia crassipes* (free floating), *Nymphaea stellata* (rooted floating), *Nelumbo nucifera* (rooted floating), *Hydrilla verticillata* (root submerged), *Typha angusta* (emergent), etc. (Varshney *et al.,* 2008).

Eichhornia crassipes (Mart.) Solms, commonly known as water hyacinth is considered as the most predominant, troublesome aquatic weed (Pathak and Kannan, 2015) and listed as one of the most productive plant on the earth (Kayathri *et al.,* 2015). The plant is otherwise called as "Blue Devil" or "Bengal terror" in India (Bhattacharya *et al.,* 2015). It has been proven to be a persistent and expensive weed problem all over the world (Sotolu, 2012).

Plants serve as reservoirs of large numbers of microorganisms known as endophytes (Bacon and White, 2000); that colonize the living tissues of plants without causing any negative effect on the host plant (Hirch and Broun, 1992). They are the best example of positive plant - microbe interaction and association of different plant species with fungi and bacteria (Hallmann, 1997). The term "endophyte" was coined by German phytopathologist Heinrich Anton De Bary in 1884, and is used to define fungi or bacteria living inside plant tissues without showing any disease symptoms in the host plant (Wilson, 1995, Azevedo, 1998). The word endophyte has come from two Greek words, "endon" means within and "phyton" means plant. They play a vital role and constitute an important component of plant micro-ecosystems (Tan and Zhou, 2001; Rodriguez *et al.,* 2009).

Endophytic fungi play a role in plant protection from pests and insects, but at the same time they can be pathogenic (Weber, 1981; Malinowski and Belesky, 2006). Under certain conditions endophytes may become parasitic, causing symptomatic infection in their host (Brown *et al.,* 1998). The disease symptoms of host plant can be caused by endophytes under stress conditions (Clay and Schardl, 2002; Schulz and Boyle, 2005). It can be regarded as an unbalanced status of a symbiosis when the host is stressed and physiological or ecological conditions favors virulence (Muller *et al.*, 2005; Schulz and Boyle, 2005; Kogel *et al.*, 2006). Endophytes of certain plant could be a pathogen of other plants, depending on the balance between pathogenicity and endophytism of the microorganism in the different hosts (Saikkonen *et al.*, 2004). Among all aquatic weeds, fungal pathogens associated with water hyacinth have been extensively studied (Tessmann *et al.*, 2001).

Endophytes may turn into a pathogen in response to some environmental cue (Hendry *et al.*, 2002); such a shift in the nature of the endophyte also result in a change in its metabolite profile. The environmental conditions which affect host plant growth, influence the number and variety of endophytic populations, and also affect metabolites production in endophytes. The purpose of this study was to identify endophytic fungal taxa that might exist as latent pathogens in *E. crassipes*.

2. Materials and Methods

2.1 Isolation of endophytic fungi

Endophytic fungi were isolated from leaves, petioles and roots of healthy and asymptomatic tissues of *E. crassipes* from Singanallur and Ukkadam wetlands, Coimbatore. The collected plant samples were surface sterilized, cut into 1 cm long and 3-4 mm broad pieces and aseptically transferred to PDA medium. The plates were incubated at 25^0C for 7 days and observed regularly for fungal growth. The mycelial outgrowth from each segment was separated and subcultured for further study.

2.2 Isolation of pathogenic fungi

Pathogenic fungi were isolated from diseased and infected leaves of *E. crassipes* from Singanallur Lake. The collected plant samples were surface sterilized, cut into 1×1 cm pieces and aseptically transferred to PDA medium. The plates were incubated at 27^0C for 7 days and observed regularly for fungal growth.

2.3 Pathogenecity tests of endophytic fungi

The isolated endophytes were subjected to pathogenecity test to check if they act as latent pathogens on leaves of *E. crassipes.* Fungal isolates were grown on potato dextrose agar medium for one week. Healthy leaves of *E. crassipes* were washed under running tap water, surface sterilized, placed in sterile petriplates layered with sterilized moist tissue paper. Endophytic fungi were reinoculated into a healthy leaves by wounding technique and incubated for 1week under room temperature.

3. Results

Thirteen endophytic fungi were isolated from leaves, petioles and roots of *E. crassipes* and 5 pathogenic fungi were isolated from infected leaves of *E. crassipes* (Table 1).

Aspegillus flavus and Curvularia lunata were isolated in both healthy and infected leaves of *E. crassipes.*

Thirteen endophytic fungi were tested for their pathogenecity on leaves of *E. crassipes.* The fungi were inoculated into the leaves by artificial wounding method. After one week, *Aspergillus flavus, A. fumigatus, A. glaucus, Curvularia lunata, Eurotium repens, Oidium* sp., *Chaetomium globosum* and sterile mycelia showed disease symptoms on leaves of *E. crassipes*. The other five species and control did not produce any disease symptoms (Figure 1 & 2).

Table 1: Endophytic and pathogenic fungi isolated from *E. crassipes*

Endophytic fungi	Pathogenic fungi
Acremonium bolchii	*Acremonium strictum*
Acremonium strictum	*Aspegillus flavus*
Aspergillus flavus	*Curvularia lunata*
Aspergillus fumigatus	*Penicillium digitatum*
Aspergillus glaucus	*Penicillium restrictum*
Aspergillus japonicus	
Chaetomium globosum	
Curvularia lunata	
Eurotium repens	
Oidium sp.	
Penicillium digitatum	
Penicillium restrictum	
Sterile mycelia	

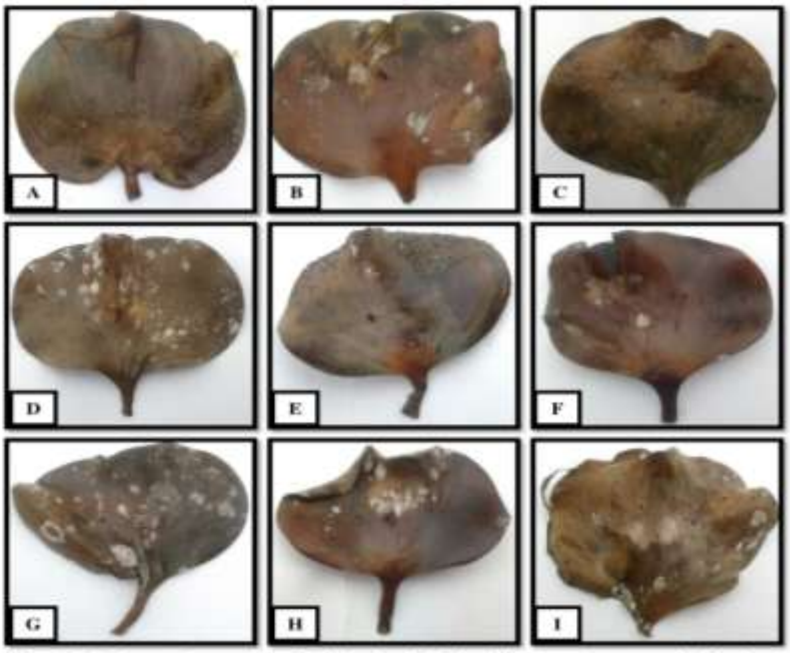

Figure:-1. Pathogenecity test of endophytic fungi: Typical disease symptoms on healthy leaves of *E. crassipes* when endophytic isolates were inoculated by wounding method.
A. Control **B** *Aspergillis flavus* **C.** *Aspergillus fumigatus* **D.** *Aspergillus glaucus* **E.** *Chaetomium globosum* **F.** *Curvularia lunata* **G.** *Eurotium repens* **H.** *Oidium* sp. **I.** Sterile mycelia.

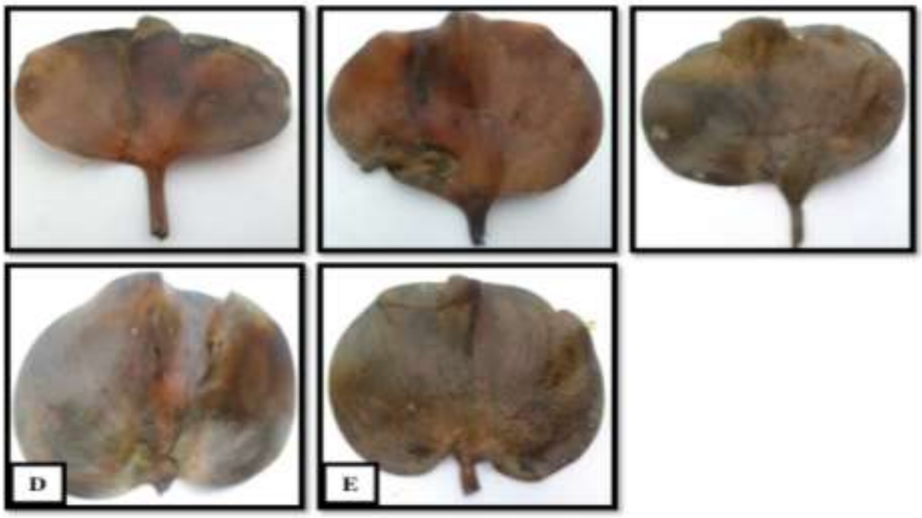

Figure 2:- Pathogenecity test of endophytic fungi: Without showing disease symptoms on healthy leaves of *E. crassipes* when endophytic isolates were inoculated by wounding method.
A. *Acermonium bolchii* **B** *Acremonium strictum* **C.** *Aspergillus japonicus* **D.** *Penicillium digitatum* **E.** *Penicillium restrictum*

4. Discussion

Eichhornia crassipes is considered one of the world's worst aquatic plants (Toft, 2000). Out of the 160 aquatic weeds, *Eichhornia crassipes* is of primary concern in India (Sushilkumar, 2011). The endophytic fungi spend the whole or part of their life cycle in healthy living tissues of host plant by colonizing inter or intra- cellularly without causing any apparent symptoms of diseases (Azevedo, 1998). Evidences that an endophytic fungi showed disease symptoms in host plants under certain conditions has been discussed by several authors (Clay and Schardl, 2002; Schulz and Boyle, 2005). Thus the purpose of the study was establish if any fungi isolated as endophytes from *E. crassipes* have the ability to be latent pathogens.

Thirteen endophytic fungi and 5 pathogenic fungi were isolated. Genera that include common endophytes and isolated as pathogens from *E. crassipes* were *Acremonium, Aspergillus, Curvularia and Penicillim. Curvularia lunata* was isolated in both healthy and infected leaves of *E. crassipes*. Fungal pathogens associated with water hyacinth have been extensively studied (Tessmann *et al.*, 2001). *Curvularia lunata* is already reported as serious pathogen of water hyacinth *(*Pathak and Kannan, 2015) and number of Pathogens had previously recorded from *E. crassipes* (Table 2).

Table 2: Fungi previously recorded as pathogens of *E.crassipes*

Fungi	Reference
Alternaria eichhorniae, *Myrothecium roridum* and Visarathanonth, 1975	Rakvidhasatra and *Rhizoctonia solani*
Myrithecium spp., *Rhizoctonia* spp. and *Pestalosia* sp.	Syed, *et al.*, 1978
Helminthosporium, and *Chaetomella*	Caunter, 1982
*Alternaria alternate, *Aspergillus niger, **Curvularia lunata, Fusarium oxysporum, *Penicillium chrysogenum, Pythium* sp., *Trichoderma viride, Trichothecium* sp.	Pathak and Kannan, 2011

Note: *Genus isolated as endophytes **Species isolated as endophytes

Some pathogens have a latent phase within the host tissue and some saprobes can also be facultative parasites (Millar, 1980; Andrews *et al*., 1985). Endophytes and pathogens both possess many of the same virulence factors. Therefore, they can produce similar symptoms (Schulz *et al*., 2002; Seier and Romero, 1996, 1997). However, fungal endophytes are closely related to plant pathogens, the fungi can either biotrophic fungi or necrotrophic fungi in their host plant (Delaye *et al*., 2013).

Endophytes have isolated by plant pathogologists and described as weak pathogens under physiological stress conditions (Kulik, 1984). Brown *et al* (1998) suggested that endophytes are also thought to be latent pathogens and were slightly pathogenic to the host plant (Padhi *et al*., 2013). The endophytic fungi *Acremonium strictum, A. bolchii, A. flavus, A. fumigatus, A. glaucus, A. japonicus, Curvularia lunata, Eurotium repens, Chaetomium globosm, Oidium sp., P. digitatum, P. restrictum* and sterile mycelia were tested for their pathogenecity on leaves of *E. crassipes.* Among the thirteen endophytes, *A. flavus, A. fumigatus, A. glaucus, Curvularia lunata, Eurotium repens, Oidium* sp., *Chaetomium globosum* and sterile mycelia showed disease symptoms on leaves. These results were similar to earlier works of Wright, 1998. Endophytic fungi act latent pathogens were previously reported in plants (Table 3).

A latent phase represents a specific condition where the fungus can either develop symptoms or cause change in their physiology of host plant (Romero *et al*., 2001). Several researchers reported that endophytes may become latent pathogen due to changes in environmental conditions such as CO_2 accumulation or O_2 depletion (Lund and Wyatt, 1972). They have evolved directly from plant pathogeneic fungi (Caroll, 1988; Isaac, 1992).

Acremonium strictum, P. digitatum and P. restrictum were did not produce any symptoms of disease in *E. crassipes*. Praveena and Naseema (2004) reported that, *Penicillium* sp. and *Aspergillus* sp. were non pathogenic. True endophytic fungal colonization never showed any visible disease symptoms in their host plant (Mostert *et al.,* 2000). Endophytic fungi are capable of living in host plants without causing any symptoms (Petrini *et al.,* 1992).

Table 3: Previous study showed endophytic fungi act latent pathogens in plants

Endophytic fungi	Name of plant	Disease
Phomopsis citri	*Citrus* spp.	Stem end rot (Wright, 1998)
Fusicoccum aesculi	*Citrus* spp.	Stem end rot (Wright, 1998)
Lasiodiplodia theobromae	*Citrus* spp.	Stem end rot (Wright, 1998)
Phomopsis viticola	*Vitis vinifere*	Leaf lesions (Mostert *et al*., 2000)
Deightoniella torulosa	*Musa acuminate*	Leaf spots (Photita *et al.*, 2004)

5. Conclusion

Endophytic fungi and pathogenic fungi were isolated from healthy and infected tissues of *E. crassipes* respectively. Fungi isolated as endophytes were tested for their pathogenecity on healthy leaves *of E. crassipes*. Thus present investigation revealed that endophytic fungi acted latent pathogens in healthy leaves of *E. crassipes*.

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