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Diversity of endophytic fungal assemblages in an aquatic eel weed plant of *Vallisneria spiralis* L.

Venkatesan Govindan[1] and Ramesh Kumar Jalaintharajan

P.G. and Research Department of Botany, Mannai Rajagopalaswami Government Arts College, Mannargudi-614001, Thiruvarur - District, Tamil Nadu, India.
*Corresponding author (e-mail: gv2032@gmail.com[1]) https://orcid.org/0000-0002-3395-1692

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ABSTRACT

Twenty-two endophytic fungal species were isolated from three hundred segments of eel weed aquatic plant of Vallisneria spiralis. These plants were collected from freshwater ditches in winter season at Mannargudi in Tamil Nadu. These studies have demonstrated that plants indoor and outdoor environments and leaf age influence the density of endophytes present in leaves of the freshwater. The endophytic fungi have been found to study in different leaf portion (lower, middle, top region). Among these 2 (9%) fungal species belonging to ascomycetes, 4 (18%) fungal species belonging to coelomycetes and 14 (64%) species from hyphomycetes beside to two (9%) sterile forms. Hyphomycetes fungi are frequently isolated; however, chytridiomycetes fungus was not present in this result. Top leaf portion was abundance fungal species followed by the middle and lower portion were isolated. Some endophytic fungi were examined in a few enzyme (Amylolytic, Lipolytic, Laccase production) activities. The enzymes were tested three coelomycetes fungi are showing maximum and good activity.

Keywords: Aquatic plants, biologically active, lentic ecosystem, diversity of fungi, *Vallisneria spiralis*.

INTRODUCTION

Microorganism live in association with the plant is commonly observed in nature (Bacon, 2000). Fungi are ubiquitous in worldly, marine, saline and freshwater environments, where they play diverse and important ecological roles that usually are frequently used for applications in agriculture, medicine, and industry (Arnold *et al.* 2003; Hawksworth, 2001; Shearer *et al*. 2007). According to Dreyfuss and Chapela (1994), tropical endophytes are among the groups of fungi that are to be studied to realize this predicted figure of 1.5 million. Fungal habitats include plants, soil, water, and organisms that may harbour large numbers of understudied fungi, estimated to outnumber plants by at least 6 to 1. More recent estimates based on high-throughput sequencing methods recommend that as many as 5.1 million fungal species exist (Hawksworth, 2001; Blackwell, 2011).

Many undiscovered fungi likely occur in symbiosis with other organisms, such as plants, and in previously un- or underexplored environments (Arnold and Lutzoni, 2007; Wurzbacher *et al*. 2010). Even though roughly about 70 % of the Earth's surface is covered by water (Polunin, 2008; Wetzel, 2001) relatively very few aquatic ecosystems have been examined thoroughly for fungal biodiversity (Wurzbacher *et al*. 2010). 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 (1996). On the continental scale, freshwater environments are very heterogeneous. These include waterfalls, lakes, dams, ditches, swamps, ponds, rivers, streams and creeks. Most fungi found in freshwater, however, must be able to cope with drought. There are different ways in which water-borne fungi survive during droughts; e.g. the zoosporic fungi form encysted spores in mud (Fuller and Jaworski, 1987). Fungal conidia are the means of asexual reproduction, spore produce, dispersal, survival and their habitats, interactions have great importance in the lifecycle of fungi (Brown and Hovmoller, 2002). Hyphomycetes fungi may be considered as common airborne fungi occurring in both indoor and outdoor environments (Shelton *et al*. 2002; de Ana *et al*. 2006). *Aspergillus* and *Penicillium* spores have been shown to occur commonly in air samples (Fogelmark *et al*. 1994). Hyphomycetes fungi have been known to disperse their hydrophobic conidia spores under desiccated environmental conditions, and also with the aid of raindrops by means of splash dispersal (Fitt and Nijman, 1983; Tadych *et al.* 2007). It was also suggested that the most effective means of spore dissemination among soil hyphomycetes is through the movement of rainwater (Sutton *et al*. 1976; Horn *et al*. 2001).

The presence of microbe's cells in the plant tissue was first observed by De Bary (1866), who coined the term 'endophytes'. De Bary defined endophytes as "any organism that grows within plant tissue." Later on, the description had been moderated as the studies on endophytes progressed. Endophytic or endophytes are a group of highly diverse fungi that live inside plant tissues including leaves, petioles, stems, twigs, bark, root, fruit, flower and seeds for at least part of their life cycle without causing any immediate overt disease symptoms in their host (Hyde and Soytong, 2008). Endophytic fungi have been reported mostly from conifers (Petrini and Corroll, 1981) and grasses (Bacon *et al*. 1977; Clay, 1988), mosses and ferns (Petrini, 1986). A few tropical plants including palms (Rodrigues, 1994; Southcott and Johnson, 1997), banana (Petrini, 1986) and mangroves (Suryanarayanan *et al*. 1998; Rajamani *et al*. 2018) have also been reported to have endophytes in their tissues. Although many studies have examined the relationships of terrestrial plants with fungi (Suryanarayanan and Kumaresan, 2000; Suryanarayanan *et al*. 2002; 2011), associations between freshwater aquatic plants and fungal symbionts are not as well characterized (Kandalepas *et al*. 2010; Li *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. (Schulz *et al*. 2002; Gao *et al*. 2010). The present study, *Vallisneria spiralis* aquatic plants were screened for endophytic fungi. For the first time, *Vallisneria spiralis* species various leaf portion (Base, Middle, top) was isolated for endophytic fungi diversity and these fungal bioactive compound.

MATERIALS AND METHODS

Study area

The *Vallisneria spiralis* L., the aquatic plant was collected from village Mannargudi (10.6649° N, 79.4507° E), District Thiruvarur, Tamil Nadu, and India. The *Vallisneria spiralis* plant belonging to Hydrocharitaceae family. Hydrocharitaceae family of monocotyledonous flowering plants genera of rooted submerged freshwater aquatic herbs. The *Vallisneria spiralis* is considered to be native to southern Europe, northern Africa, the Middle East, and southwest. The *V. spiralis* species vernacular names are called the eel-grass, Jungle Val, Tape-grass in English, Calanili, Jalanili, Valukkuppaci in Tamil. This plant is found worldwide in tropical Fungal and subtropical regions. It is mostly found in river, lakes, ponds, streams, swamps, ditches and estuaries (Plate 1). In these plants were collected from freshwater ditches in winter season at Mannargudi in Tamil Nadu, during 2019-2020.

Sample Collection**:** Leaf samples were collected from healthy *V. spiralis* (hydrophytes) plant and transported to the lab in closed sterile (suitable) bags. They were processed within 24 hours of collection.

Surface Sterilization and Culture Protocols

One hundred of ribbon-like leaves segments of 0.5 cm^2 (Cutting the leaf – top (aerial), middle, sheath (Lower) position) each was cut and surface sterilized by the method of Suryanarayanan (Suryanarayanan *et al*. 1998). The samples were washed in running water, dipped in 70% ethanol for 5 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 antibiotic contained in Petri dishes. 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 5 -21 days (Bills and Polishook, 1992; Suryanarayanan, 1992). The light regimen given was 12 hours light followed by 12 hours darkness. The fungi that grew from the segments were periodically observed and the endophytes were identified.

Statistical Analysis

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.

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

Detection of Extracellular Enzymes Production

The isolated endophytic fungi from these aquatic plants of *Vallisneria spiralis* and selected dominant and common Coleomycetes group of endophytes were screened extracellular enzyme activity. The enzyme examine was made on prepared by both a spread-plate technique with spores and mycelium (Hankin and Anagnostakis, 1975).

Amylolytic Activity

GYP medium (1g glucose +0.1g Yeast extract + 0.5g peptone, 16g agar in 1000ml distilled water) plus 0.2% soluble starch, pH 6, was used. After 7 days of mycelium growth, the plates were poured with an iodine solution. A yellow area surrounded the fungal colony in an otherwise blue medium referred amylolytic activity.

Lipolytic Activity

Tween 20 was sterilized by autoclaving for 15min at 103kPa pressure and 1ml of it was added to 100 ml of sterile, cooled agar medium (Peptone10g, NaCl 5g, $CaCl2.2H_2O$ 0.1g, Agar 20g in 1000 ml distilled water, pH6. Clear precipitation around the fungal colony referred lipolytic activity.

Laccase Activity

GYP medium with 0.05g 1- naphthol 1^{-1}, pH6 was used. As the fungus grows, the colourless medium turns blue colour due to the oxidation of 1- naphthol by laccase.

RESULTS AND DISCUSSION

Fungal endophytes were isolated from the tissues of *Vallisneria spiralis*. These tissues have found out 22 species. The tissues of the top, middle and lower portion reveal 8, 19 and 22 isolates, respectively (Table 1). The least number of the fungal species was isolated from the lower portion of the leaf and highest or nearly equals number of the endophytes isolated from the leaf of the middle and top portion. A total of 22 species of fungi were isolated and were two sterile mycelia. Sterile mycelium was isolated from all the parts of the leaf portion. Sterile mycelium has the least isolation rate. Hyphomycetes were the abundant group followed by Coelomycetes, Ascomycetes and sterile forms (Table 1 and Fig 2). Basidiomycetes, Chytridiomycetes were the absent (Table 1). Basidiomycetes are usually isolated in deficient numbers as endophytic fungi (Petrini, 1986; Suryanarayanan *et al*. 1998). In the present investigation, the study of three Coleomycetes endophytic fungi isolated from *V. spiralis* aquatic plant was tested for three (Amylolytic, Lipolytic, Laccase) enzymes tested. The endophytes used in the present studies are *Colletotrichum gloeosporiodes*, *Phomopsis* sp. and *Phoma* sp. were produce all bioactive metabolites sources (Plate 1).

In this study, *Vallisneria* plant species of host plant were found to be least colonized by endophytes. These are usually isolated in very low numbers as endophytic fungi (Petrini, 1986; Suryanarayanan *et al*. 1998). To our knowledge, not much reported have been studied on the isolation of fungal endophytes from *Vallisneria Spiralis*, Although, many endophytic fungi isolated were common endophytic fungi already been reported in other studies. However, in our study from 22 different endophytic fungal species were yielded in the hosts of *V. Spiralis* (Table 1 and Fig 2), eight fungal species showed considerable colonization frequency,

above 64% hyphomycetes, 18% of coleomycetes, 9% of ascomyctes and sterile forms (Fig. 3). *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* spp., *Fusarium* sp., *Penicillium* sp. and sterile forms have been isolated from entire leaf portion studied. *Alternaria* sp., *Aspergillus* spp., *Curvularia* sp., *Drechslera* sp., *Chaetomium indicum*, *Colletotrichum gleosporioides*, *Drechslera* sp., *Geotrichum* sp., *Nigrospora oryzae*, *Penicillium* sp., *Pestalotiopsis* sp., *Phoma* sp., *Phomopsis* sp. and sterile forms have been isolated from the middle portion studied. Some endophytic fungi were specifically isolated from the upper portion leaf namely, *Lasiodiplodia* sp., *Colletotrichum gleosporioides*, *Chaetomium indicum*, *Phoma* sp., *Phomopsis* sp. and sterile mycelia were present, and it may be yielded nearly in the middle portion (Table 1, Fig 2 and 3).

Table 1. Fungal endophytes in the leaves of *Vallisneria spirials* L.

Fungus	No. of colonies from leaf		
	I Top portion	**II** Middle portion	**III** Lower portion
Ascomycetes			
Chaetomium indicum	**-**	2	3
Pestalotiopsis sp. 1	**-**	1	4
Coelomycetes			
Lasiodiplodia sp. 1	-	-	3
Colletotrichum gleosporioides	-	2	4
Phoma sp. 1	-	**3**	**5**
Phomopsis sp. 1	-	1	2
Hyphomycetes			
Alternaria sp. 1	-	1	3
Aspergillus flavus	2	2	4
Aspergillus niger	**4**	**7**	**6**
Aspergillus sp. 1	1	1	1
Aspergillus sp. 2	1	1	2
Curvularia sp. 1	-	2	3
Curvularia sp. 2	-	1	2
Drechslera sp. 1	-	1	1
Drechslera sp. 2	-	-	1
Fusarium sp. 1	1	3	2
Geotrichum sp. 1	-	1	1
Nigrospora oryzae	-	1	3
Penicillium sp. 1	**2**	**3**	**5**
Phaeotrichoconis sp. 1	-	-	1
Sterile forms			
Sterile form 1	1	1	2
Sterile form 2	1	2	1
No. of Endophytes	**13**	**36**	**59**
Total No. of species	**8**	**19**	**22**

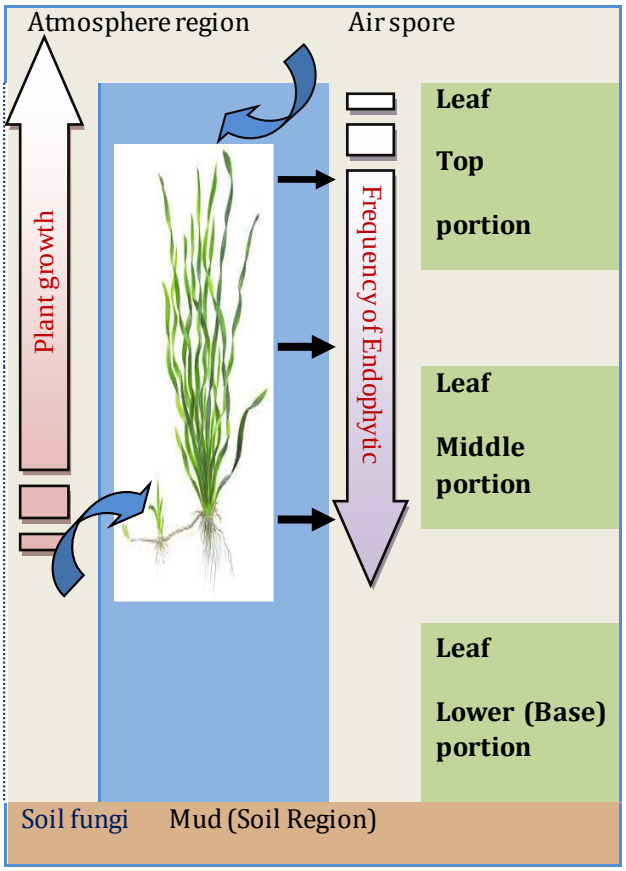

Figure 1. An imagery of plant-microbe interactions; assemblages of fungal spores from air and soil; isolation of endophytic fungi from the various portion of the leaf (*Vallisneria spirials* L.)

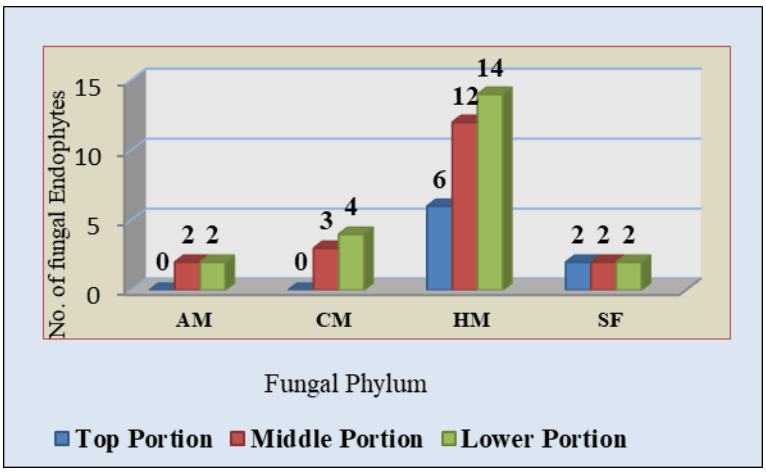

Figure 2. Relative Percentage Occurrence (RPO) of endophytes belonging to different groups of fungi from *Vallisneria spirials* L

AM- Ascomycetes; CM- Coleomycetes; HM- Hyphomycetes; SF- Sterile Forms

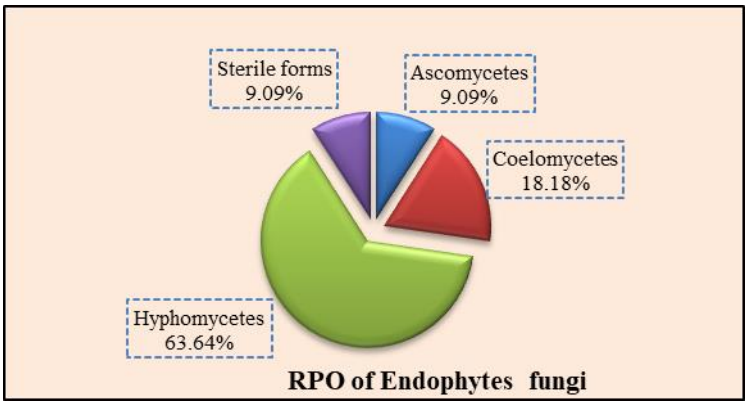

Figure. 3. Relative Percentage Occurrence (RPO) of endophytes belonging to different groups of fungi from *Vallisneria spirials* L

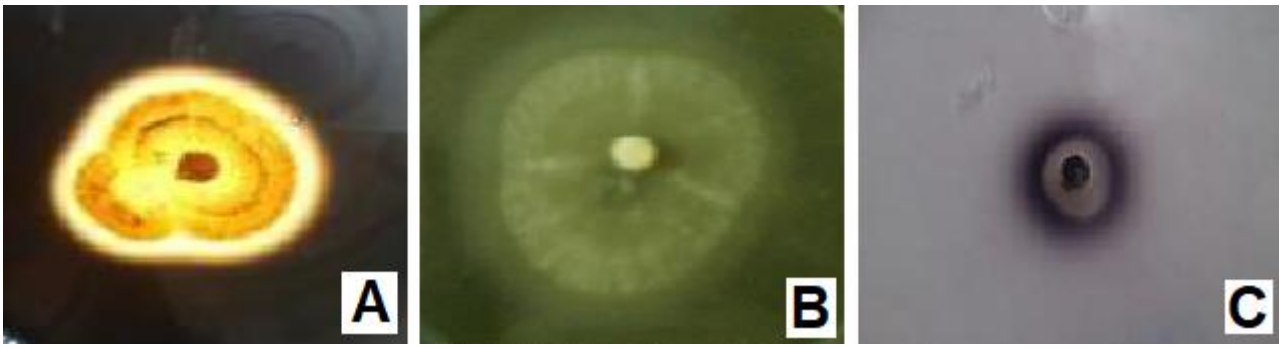

Plate 1. Extracellular Enzymes tested of Endophytic fungi.

A. *Phomopsis* sp. 1 showing amylase activity. Note yellow zone around the colony margin indicating the production of amylase.

B. *Colletotrichum gleosporioides* showing lipase activity. Note precipitate around the colony.

C. *Phoma* sp. 1 showing laccase activity. Note the medium turns blue due to oxidation of 1- napthol by laccase.

Endophytic studies on true hydrophytic hosts are very limited; however, a few studies have been carried out on the distribution of endophytic fungi in aquatic plants (Tejesvi *et al*. 2006; Das *et al* 2013; Young *et al*. 2015). Rajagopal *et al*. (2018) isolated 18 various fungal species from the *Nymphaea nouchali* while *Eichhornia crassipes* 12 endophytes species are reported. They were identified as *Bipolaris* spp., *Cercospora* sp., *Chaetomium* sp., *Curvularia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Phoma* sp., this report from designate that endophyte distribution differs due to local environmental and climate change. Similarly, Ranga *et al*. (2016) reported 8 dissimilar endophytic isolates from a host of *Nymphaea nouchali*. These eight fungal endophytes isolated, *Chaetomium* sp., *Colletotrichum* sp. and *Fusarium* sp. was found to be dominant in leaves. In our study also comparable endophytic fungi were isolated. Certain hyphomycetes have been found in aquatic habitats other than streams, lakes or ponds. Fungal species such as *Geotrichum candidum, Fusarium aquaeductuum, Aspergillus* spp. and *Penicillium* spp. have been recorded worldwide in water treatment plants (Cooke, 1970; Jones, 1976; Subramanian, 1983).

Some authors have demonstrated that fungal endophyte community may be influenced by diverse biotic and abiotic factors, such as the type of plant tissues; a heterogeneous profile of microhabitats; and different substrates, climate and vegetation changes (Koide *et al.* 2017; Shen *et al*. 2007). Airborne fungal spore concentrations and their diversity vary with the season of the year, geographical region, air, meteorological parameters, presence of local resources, and vegetation. In our investigation was based on isolated freshwater plant-fungal endophytes has been done location around agricultural lands, during a few months of the winter season of the

region. Thus, we found that the most widespread airborne fungi were also the most frequent viz., *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium* were isolated (Table 1). This suggests that in distress of the above season of variation, these fungal species are ubiquitous in the Earth's atmosphere. *Aspergillus* and *Penicillium* spores have been shown to occur commonly in dry air samples (Shen *et al*. 2007).

Plant endophytic fungi produce extracellular enzyme such as cellulose and pectinases, which enable them to penetrate and colonize host tissues (Collmer and Keen, 1986; Schulz *et al*. 2002). The endophytic fungi, which mimic these activities of endophytic fungi to profit entry into host tissue, are known to produce such enzymes (Petrini and Corroll, 1981). We study the endophytic fungi such as *Colletotrichum gloeosporiodes*, *Phomopsis* sp., and *Phoma* sp. the all Coleomycetes group of endophytes fungi were produces Amylolytic, Lipolytic, Laccase enzymes activity (Plate 1).

Based on this study, that rooted submerged freshwater aquatic plant is found indoor and outdoor of the water, although, these plants part associated with the atmospheric region are closely related to the fungi found in the air. Similarly, the parts found inside the water may be related to aquatic fungi and these fungi may also change during the growth of aquatic plants. When the seeds germinate, the water is a wet or very low or reduced condition, when the plants grow on freshwater ecosystem adaptation, this plant height (growth change) has been depended on the water level, these plant has been growing between inside and outside surfaces of water and also, the presence of endophytes species may be assembled depending on an ecosystem (Fig 1).

Fungal spore or mycelia have been germinated with into seed or plant tissues when a suitable environmental condition; then mycelia has spread on through tissues. The endophytic fungi are varying from the inner part to the outer part of the plant leaf portion (Fig 1). These analyses provide a first estimation of endophytic fungal distributions in the aquatic plants and lentic waters (ditches, Channel) of Tamilnadu, southern India. Our work shows that despite the least isolation frequency of endophytes fungi associated with leaf of *Vallisneria Spiralis*.

CONCLUSION

In this study, *Vallisneria* plant species of host plant were found to be least colonized by endophytes. These aquatic plants were isolated 22 endophytes species and for the first time, endophytic distribution in the leaf various leaf portion of *Vallisneria spiralis* was studied. Also, it was finding shown that the few endophytes produce bioactive compounds. The least number of fungal species were isolated in hydrophytic plants. Because these plants might be the presence of Aerenchyma cells, large air cavities are present in the Parenchyma tissues.

In contrast, submerged plants occur beneath the water surface, with occasional floating leaves or flower stalks that protrude only a small distance from the water. Many aquatic plants life cycles die back in winter in strongly seasonal sites, with new plant growth initiated in spring from overwintering shoots and roots.

We assumed that they grow only in low-water conditions, short periods of life; although the freshwater plant may present of least endophytic fungi; but not found true fungi and much endophytes. Whatever, these endophyte fungi were isolated in the freshwater plant tissues are abnormal present. Endophytic fungi are rich sources of bioactive natural products that can be used to gratify of Pharmaceutical, medicinal, agriculture and industries.

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Supplementary data

Figure 1: An imagery of plant-microbe interactions; assemblages of fungal spores from Air and soil; isolation of endophytic fungi from the various portion of the leaf. Figure 2: Relative Percentage Occurrence (RPO) of endophytes belonging to different groups of fungi from *Vallisneria spirials* L. Figure 3: Relative Percentage Occurrence (RPO) of endophytes belonging to different groups of fungi from *Vallisneria spirials* L. Plate 1: Extracellular Enzymes tested of Endophytic fungi.

Conflict of Interest

The author declares that there is no conflict of interest.

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