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Research Article

FUNGAL ENDOPHYTES FROM *Phyllanthus acidus* (L.) AND *Catharanthus roseus* (L.)

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ABSTRACT

Due to the importance of bio molecules research from the ubiquitous endophytic fungi, there is a huge need for the study of biodiversity in endophytic fungi. Endophytic fungi, are the microorganisms that reside inside the tissues of healthy plants. In the present investigation, medicinal plants such as *Phyllanthus acidus* (L.) and *Catharanthus roseus* (L.) were collected from in and around of Western Ghats of Sathyamangalam region. For the isolation of endophytic fungi and to study the biodiversity within the tissues of selected plants, the selected explant tissues were surface sterilized using standard surface sterilization techniques. Grown endophytic fungal propagules were keenly isolated and identified using standard manuals. Biodiversity of endophytic fungi in different segments of the two plants were identified through statistical analysis by means of Endophytic fungi Infection Rate (EIR %) and Colonization Frequency (CF%). The occurrence of endophytic fungi was highly dominated by *Phyllosticta* Sp, *Phomopsis* Sp, *Curvularia lunata*, *Pestalotiopsis* Sp and eight non sporulating species.

Keywords: Endophytic fungi, Surface sterilization, explant tissues, statistical analysis.

INTRODUCTION

The term “endophytes” indicates the microorganism that grow intracellularly in the plant tissues and they all are significant source for biological natural product production.[1] Endophytes are microbes that colonize the internal plant tissues beneath the epidermal cell layers without causing any apparent harm or symptomatic infection to their host.[2] The major metabolic compounds like terpenoid, alkaloid and steroid derivatives are isolated from several endophytic fungi. In the present investigations two medicinal plants such as *P.acidus* (L)and *C.roseus* (L) were selected. A brief description of the medicinal plants plants are : *P. acidus* (L) is a small, glabrous tree up to 10 m tall with phyllanthoid branching, bark rough, grey, with prominent lenticels; cataphylls not persistent, blackish-brown, their stipules triangular-ovate; deciduous branchlets ascending with 25-40 leaves. It belongs to phyllanthaceae family.[3] *C. roseus* is widely used medicinal plant which comes under Apocynaceae family, contains several alkaloid metabolites cures the malignant diseases like lymphoma, myeloma etc., Even though the roots and shoots extracts of this plant is poisonous, yet they are used for tackling several diseases especially in the Ayurvedic system of medicine.[4]

MATERIAL AND METHODS

Collection of sample

P. acidus (L.) and *C. roseus* (L.) were collected from in and around Sathyamangalam, Tamil Nadu (India). The disease free parts of the plants were collected and were brought to the laboratory in sterile bags, and were processed under sterile condition within 24 hours.

Isolation of endophytic fungi

The endophytic fungi were isolated by surface sterilization of leave, stem and root samples by the modified procedure[9]. All the parts of leaves, stem and root samples of plant species were first washed thoroughly under running tap water to remove dust and debris. The surface sterilization was carried out in a clean airflow bench system. The samples were immersed in 75% ethanol (v/v) for one minute followed by 4% sodium hypochlorite (NaOCl) (v/v) for three minutes and then immersed in 70% ethanol. The samples were rinsed three times in changes of sterile distilled water and dried on sterile tissue papers. The surface sterilized plant tissues were cut into 1.0 cm x 0.1cm using sterile scalpel. The plant segments were placed equidistantly on the potato dextrose agar (PDA) medium supplemented with the antibiotic streptomycin. The petriplates inoculated with the plant segments were incubated at 28 ± 2°C for 2 to 4 weeks. Fungi growing out of the plant explants were sub cultured on separate PDA plates were incubated at 28°C for 3 weeks. Pure cultures were then transferred to potato dextrose agar (PDA) slants and cultivated for 14 days at 28°C.

Identification of fungal endophytes

The endophytic fungi were observed morphologically and microscopically. For this purpose, a small bit of fungal mycelia was taken from pure culture grown on PDA and stained with lactophenol cotton blue for visual observation. Sporulating isolates were identified down to species level with the help of standard manuals[10]. Sterile isolates could not be assigned to any taxonomic group and were sorted into morpho-species on the basis of colony surface texture, hyphal pigmentation, exudates, and growth rates, as described[11]. Such sterile forms were included as ‘species’ for the analysis of the results. Sporulating isolates were identified down to species level with the help of standard manuals. Photomicrographs were taken with the help of CX31 Olympus Trinocular Research microscope and DSLR Canon EOS 700D were used[11]

Statistical Analysis

Colonization frequency

The percentage of colonization frequency (CF%) was performed as follows [11]:

$$CF\ (\%) = \frac{\text{Number of species isolated}}{\text{Number segments screened}} \times 100$$

Endophytic Infection Rate

The EIR percentage was calculated as follows [11]:

$$EIR\ (\%) = \frac{\text{Number of infected segments}}{\text{Total number of segments screened}} \times 100$$

RESULTS AND DISCUSSION

Altogether seventy segments were screened from the *P. acidus* (L.) and *C. roseus* (L.). A total of 15 isolates were obtained in which 7 were sporulating and 8 were non sporulating species (Figure 1- 4). Endophytic fungi isolated from the leaves of two plants were listed as *Phyllosticta* sp.1, *Phyllosticta* sp.2, *Phyllosticta* Sp.3, *Phomopsis Sp.*, *Curvularia lunata*, *Pestolotiopsis* Sp. and *Drechslera* Sp (Table 1).

The overall percentage of endophytic infection rate (EIR %) in *C. roseus (L.)* was high (96.67 %) compared to *P. acidus* (L.) (87.5%). The overall percentage of colony frequency of *P. acidus* (L.) (17.5%) and low in case of *C. roseus* (L.) (16.7%) represented (Figure 5).

Figure 1: Habitat of *Phyllanthus acidus* (L.)

Figure 2: Habitat of *Catharanthus roseus* (L.)

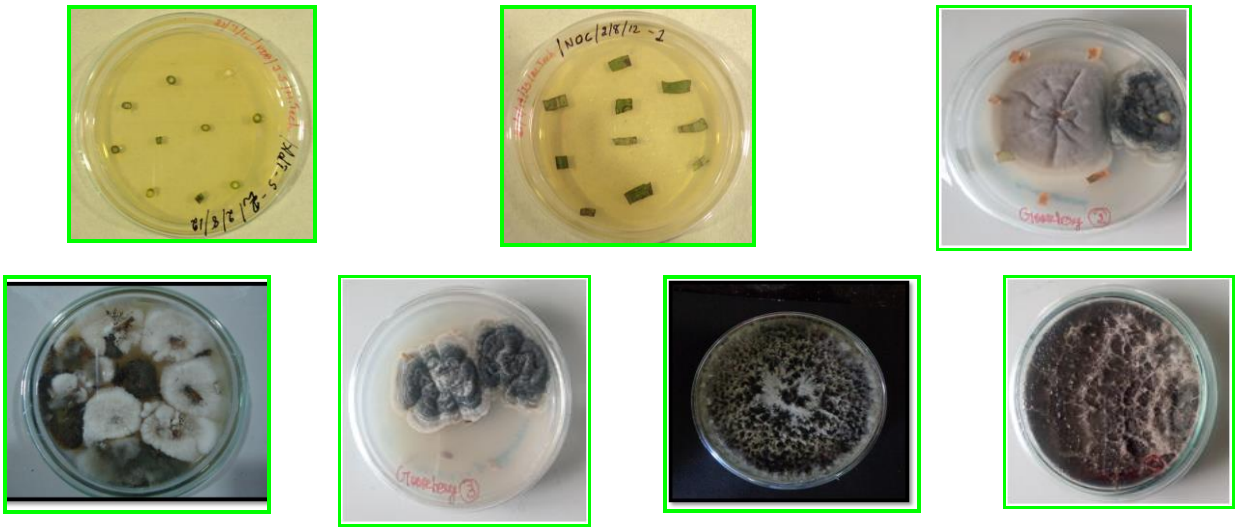
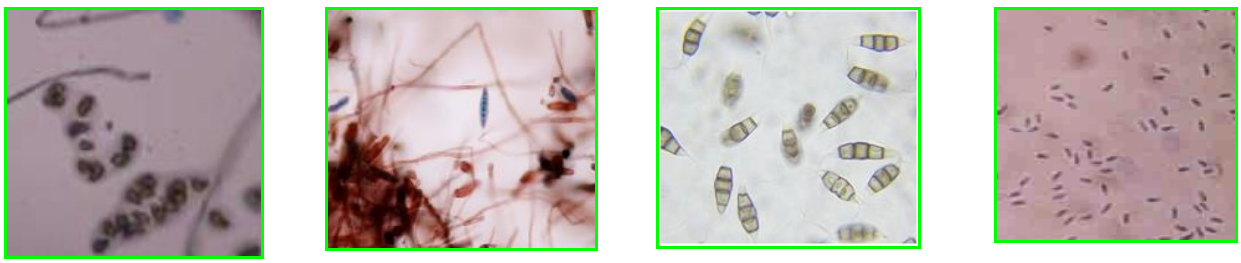


Figure 3: Endophytic fungal propagules emerging from tissues of *Phyllanthus acidus* (L.) and *Catharanthus roseus* (L.)



Curvularia lunata ***Drechslera sp*** ***Pestolotiopsis sp*** ***Phomopsis* sp**

Figure 4: Microscopical view of selected endophytic fungi reported from *Phyllanthus acidus* (L.) and *Catharanthus roseus* (L.)

Table 1: Statistics of endophytic fungi from *Phyllanthus acidus* (L.) and *Catharanthus roseus* (L.).

S.No	Name of the Plant	EIR (%)	CF (%)	Total number of segments	Number of sporulative species	Endophytic fungi isolated	Number of Non sporulative species
1.	*Phyllanthus acidus* (L.)	87.5	17.5	35	5	*Phyllosticta* sp (5 species)	5
2.	*Catharanthus roseus* (L.)	96.67	16.7	30	2	*Curvularia lunata* *Pestalotiopsis* sp	3

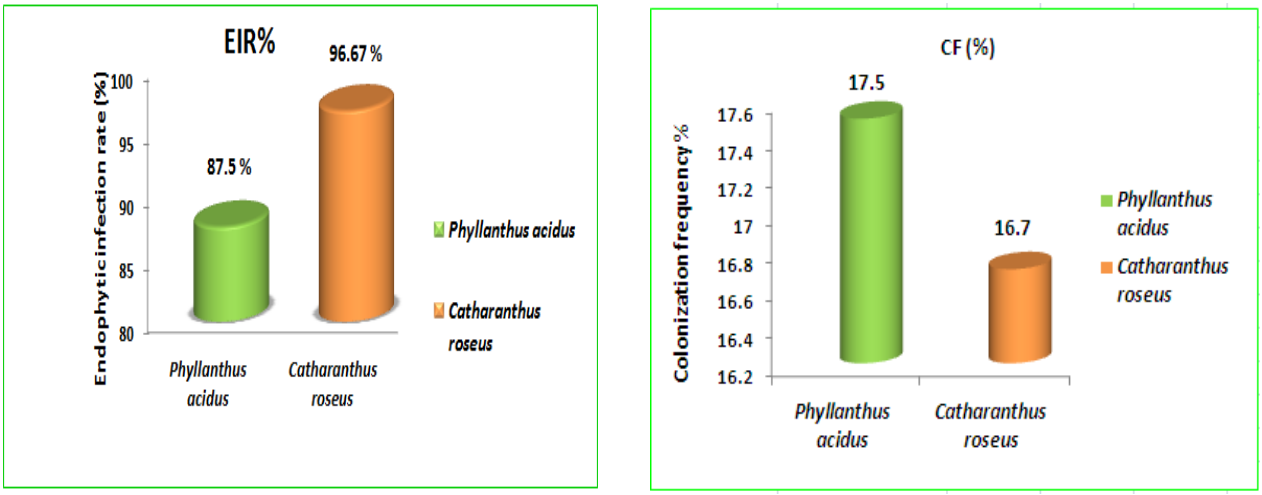


Figure 5: EIR % & CF% in *Phyllanthus acidus* (L.) and *Catharanthus roseus* (L.) leaves.

DISCUSSION

Earlier from the medicinal plants *Catharanthus roseus* (L.), following endophytes viz: *Colletotrichum* Sp., *Macrophomina phaseolina*, *Nigrospora sphaerica* and *Fusarium solan* were recordedi.[12] and similarly from *Emblica officinalis* (L.), *Phomopsis sp*. was isolated and identified.[13] In the tropical area, fungal endophytes investigations have recently gained much importance. Fungal endophytes isolated from the plants have been screened for their potential to produce specific metabolites of agricultural and pharmaceutical importance.[14] The several functional metabolites screened by the fungal endophytes are recently reviewed.[15]

CONCLUSION

Endophytic fungi in the medicinal plants have recently gained significance, even though they have occurred in a wide variety of plants in temperate parts of the world. This study revealed that endophytic fungi isolated from the selected plants were rich in diversity. Hence, this research finding can be further developed by performing molecular level of gene identification and utilizing these species to produce secondary metabolites for various applications.

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