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Isolation and Characterization of Endophytic Fungi from the Parasitic Plant *Dendrophthoe falcata*

Research Article

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Abstract

The present study aimed to identify, characterize and diversity in two season of endophytic fungal species from parasitic plant, *Dendrophthoe falcata* grows on *Lagerstromia speciosa*. The mass cultured pure endophytic fungal species identified using molecular technique 18S rRNA and studied the percentage frequency studies. Based on the results identified endophytic fungal species are *Diaporthe pterocarp, Diaporthe pseudophenicola, Colletotrichum cobbittiense, Astromella pistachiarum* and *Colletotrichum siamensis*. These endophytes noticed from two different parts (leaves and stem) in two different season (wet and dry). The percentage frequency studies of each endophytes were different and deposited endophyte sequences in NCBI. These endophytes used for isolation of bioactive compounds and their biological studies..

Keywords: *Dendrophthoe falcate*; endophytic fungi; 18S rRNA; colony frequency

Introduction

Dendrophthoe falcata is a hemiparasitic plant belongs to Loranthaceae and it is most common in India. The *D. falcata* reported on many host plants viz., Sugar Apple, Sapota, Guava, Pomegranate, Mango, Myristica, Artocarpus, Neem and many plants. The *D. Falcata* possessing properties of wound healing, antimicrobial, antioxidant and antinociceptive [1,2]. The plant is also using to treat tuberculosis, asthma, menstrual disorders, wounds, ulcers, renal problems[3,4], anticancer activity[5]. Many biologically important bioactive compounds were identified they are steroids, sesquiterpenoids, pentacyclic triterpenoids, flavonoids, hydrocarbons, carboxylic acids, phenols and tannins, esters, alcohols, glycosides, triglycerides, enzymes and they exhibited antitumor, hepatoprotective, antihyperlipidimic, thrombolytic, antioxidant, antidiabetic, antibiofilm, cytotoxic, anxiolytic, antifungal, immunostimulatory activities[6]. *Nigrospora sphaerica* [7], *Pestalotiopsis* species [8], *Alternaria alternata* [9] are reported as endophytic fungi of *D. falcata*. Based on the literature, the present research was aimed to survey for *D. Falcate* on different host in identified area and identification of endophytic fungi from *D. Falcate* was carried out in two season collected samples. Endophytic fungi possessan wide variety of applications include, protection against diseases caused by pathogenic microorganisms, protection against herbivory, plant growth promotion, production of secondary metabolites, secondary metabolites of biotechnological interest, endophytic fungi as biological control agents of phytopathogens etc [10].

Significance of endophytic fungi of parasitic plants includes, Increase the nutrition absorption from soil [11], enhances the abiotic stress tolerance [12], increases the production of secondary metabolites and plant hormones[13], endophytic fungi protect the host plant from biotic stress by opposing the pest and pathogen which disturb the plant defense mechanisms[14].

Materials and Methods

Collection of plant: To collect the parasitic plant parts two different seasons was selected (wet and dry season) and collected

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plant parts viz., leaves and stem of *Dendrophthoe falcata*. The plant was collected from the host *Lagerstromia speciosa* was used for the present study. *Dendrophthoe falcata* is a common parasitic plant found on a variety of hosts including native and introduced species. *Dendrophthoe falcata* is known to parasitize a wide range of host plants. While studies have focused on its primary hosts, such as *Mangifera indica* (mango) and various timber trees [15], there is limited evidence specifically addressing its interactions with *Lagerstroemia* species as a host. Prior research typically identifies other common hosts and documents the ecological and economic impacts of *D. Falcate* on those species [16]. Given the lack of extensive research explicitly linking *Dendrophthoe falcata* to *Lagerstroemia* species, this host plant which was available in the Devarayanadurga area was chosen. An understanding of how *D. Falcate* interacts with Lagerstroemia not only contributes to knowledge in Botany but could also reveal insights into the endophytic community that resides within hemiparasitic plants like *Dendrophthoe* [15,16]. The plant was dried and made herbarium and identified the plant *Dendrophthoe falcata* by Dr Haleshi C, Taxonomist (Voucher no.HDUD304) by making herbarium. The separated stem and leaves were thoroughly washed with running tap water, and then the stem and leaves were surface sterilized with 70% ethanol by dipping for 30 seconds followed by 2% sodium hypochlorite for 30 seconds later rinsed in sterilized distilled water to remove excess of ethanol and sodium hypochlorite. The stem and leaves were dried using sterilized filter paper on a Petri plates. Using sterilized surgical blade, the leaves and stem were cut into 1 to 1.5 cm pieces and carefully placed it on Potato Dextrose Agar (PDA) media containing Petri plates aseptically. The plates were incubated at room temperature (26±2^{0}C) for 8-10 days and plates were routinely observed for the development of hyphae from incubated parts. The grown fungi from incubated parts were isolated and subjected to mass culture and stored at 4^{0}C.

Mass culture of endophytic fungal species

Each endophytic fungal species were grown from incubated parts were carefully transferred to PDA containing Petri plates and Potato Dextrose Broth (PDB) containing conical flasks to obtain pure culture by incubating at room temperature (26±2^{0}C) for 8-10 days.

Identification of endophytic fungi

Each endophytic fungal species were identified based morphological characters such as colony morphology, pigmentation, growth pattern, hyphal characters and spore morphology using with the help of standard fungal manuals[17-18].

Molecular identification of isolated endophytic fungal species

Totally 05 endophytic fungal species were selected for molecular identification. The molecular identificationwas done using ITS1 and ITS 4 primers. 100 mg DNA was isolated from the culture. Its quality was evaluated on 1.0% Agarose Gel and a single band of high-molecular weight DNA was observed. A fragment of ITS region was amplified by PCR. A single discrete PCR amplicon band of 700 bp was observed when resolved on agarose. The PCR amplicon was purified to remove contaminants. The forward and reverse DNA sequencing reaction of PCR amplicon was carried out with ITS1(5'-TCCGTAGGTGAACCTGCGG) and ITS4(5'-TCCTCCGCTTATTGATATGC) primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. A consensus sequence of the PCR amplicon was generated from forward and reverse sequence data using aligner software. The ITS region sequence was used to carry out BLAST with the database of NCBI Genbank. Based on maximum identity score, first ten sequences were selected and aligned using multiple alignment software program Clustal W. The distance matrix was generated and the phylogenetic tree was constructed using MEGA 7 [19].

Percentage Frequency Studies

Colonization frequency was calculated by dividing the number of individual fungal endophytes recorded by the total number of segments screened and then multiplying the result by 100. This formula helps determine the percentage of plant tissue segments colonized by fungal endophytes [20].

$$CF(\%) = \frac{\text{No of individuals recorded}}{\text{Total number of segments screened}} X100$$

Results and Discussion

Hemiparasitic plants were observed on several host trees including *Pongamia pinnata, Albizzia lebbeck, Lagerstroemia speciosa, Nyctanthes arbor-tristis* and *Mangifera indica.* The most prominent hemiparasitic species were *Scurrula* sps, *Dendrophthoe gamblei*, *Viscum album* and *Dendrophthoe falcata* during the survey at Devarayana Durga region of Tumakuru (13.3379^{0}N, 77.1173^{0}E, temperature range from 17^{0}C to 28^{0}C), Karnataka, India and survey was conducted in January-March 2021 and September-December 2022. *Dendrophthoe falcata* was a common hemiparasite seen and was chosen for isolation of endophytic fungi. The *D. falcata* grows on *Lagerstroemia speciosa* was selected for the present study.

Leaf part of *D. Falcate* collected in wetter season yielded four different endophytic fungal species viz., *Diaporthepterocarp, Diaporthepseudophenicola, Colletotrichum cobbittiense* and *Astromella pistachiarum* whereas stem part yielded one fungus *Colletotrichum siamensis.* In drier season, the stem part exhibited the presence of *Colletotrichum cobbittiense* whereas leaf part also showed the presence of *Colletotrichum cobbittiense* and *Diaporthepterocarp.* The *Colletotrichum cobbittiense* and *Diaporthepterocarp* were noticed to presence in two seasons and present in both leaf and stem parts (Table 1).

Table 1: List of endophytic fungal species were identified from different parts of *D. falcata*

Parts used	Identified fungi
Wetter Season	
Leaf	*Diaporthe pterocarp* (July)
	Diaporthe pseudophenicola (July)
	Colletotrichum cobbittiense (July)
	Astromella pistachiarum (July)
Stem	*Colletotrichum siamensis* (October)
Drier Season	
Stem	*Colletotrichum cobbittiense* (December)
Leaf	*Colletotrichum cobbittiense* (March)
	Diaporthe pterocarp (March)

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Isolated the endophytic fungi *Alternariaalternata* from *Dendrophthoe falcata* and synthesized the silver nanoparticles from *Alternariaalternata* extracts showed strong antibacterial and antioxidant activity [9]. Seven different species of *Diaporthe* were reported as endophytic fungi in Citrus [21].

Colletotrichum cobbittiense was reported as pathogen from *Cyanthilliumcinereum* [22]. *Colletotrichumcobbittiense* was reported as endophytic fungi from *Pteridiumacquilinium* and *Newbouldialaevis* leaves [23]. The *Colletotrichum siamensis* reported as endophytic fungi from *Lycopersiconesculantum* (Mill.) and exhibited the plant growth promoting activities [24].

All the endophytic fungal species mass cultured on PDA media and used for molecular identification studies (Figure 1). Based the sequence draw the phylogenetic tree using Mega 7 (Figure 2 to Figure 6). All the five different endophytic fungal species sequences (*Colletotrichum cobbittiense* (Accession number: PP159096), *Asteromellapistachiarum* (Accession number: PP159099), *Diaporthepseudophenicola* (Accession number: OR858835), *Colletotrichum siamensis* (Accession number: OR827685) are deposited in NCBI and expecting to submit *Diaporthepterocarp*.

Percentage Frequency Studies

The diversity of endophytic fungal community was analysed from two different parts tissues. The diversity of isolated endophytic microbes on different culture media can be examined through non-(CF) for endophytic fungi can be determined manually using the formula provided by Hata and Futai (1995) [20]: % CF= (Ncol/ Nt) x 100, where molecular methods. Various approaches can be employed to assess this diversity, including colonization frequency, isolation frequency, and alpha diversity indices such as Simpson's and Shannon-Wiener diversity indices, as well as measures of species richness and evenness[20,25,26]. Colonization frequency Ncol represents the number of segments colonized by each fungus and Nt denotes the total number of segments studied. Based on the colonization frequency data, leaf samples showed greater colonization for all the endophytic fungi isolated compared to stem samples (Figure 7) (Figure 8). *Colletotrichumsiamense* and *Colletotrichum cobbittiense* were the two fungi isolated in the dry season from the stem (Figure 7). This signifies that the above two species of endophytic fungi are common to both stem and leaf. Further studies are suggested to confirm the same.Wetter seasons favoured more diverse and abundant growth of fungal endophytes. Similar results were observed from the earlier reports[27]. The reason could be attributed to increased moisture, availability of nutrients in the host plant, increased germination and growth rates. Rainwater washes nutrients from the atmosphere and soil, enriching plant tissues. These nutrients support both plant growth and endophyte

Figure 1: Pure culture of endophytic fungi isolated from leaves and stem parts of *Dendrophthoe falcata*, A- *Diaporthe pterocarpi*, B-*Astromella pistachiarum*, C-*Diaporthe pseudophoenicola*, D- *Colletotrichum siamense*, E-*Colletotrichum cobbittiense*

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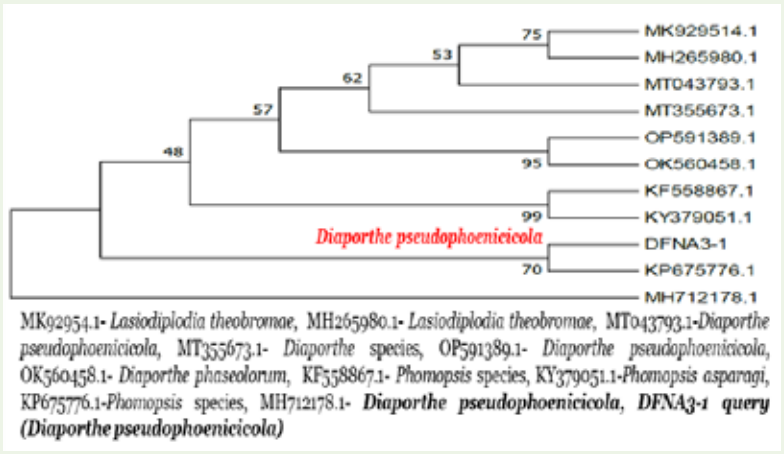
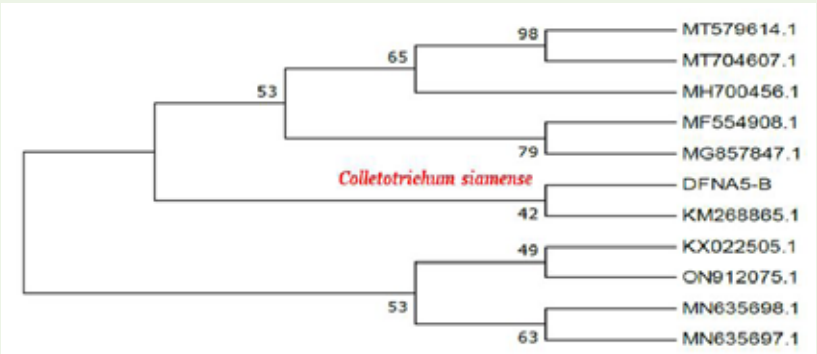


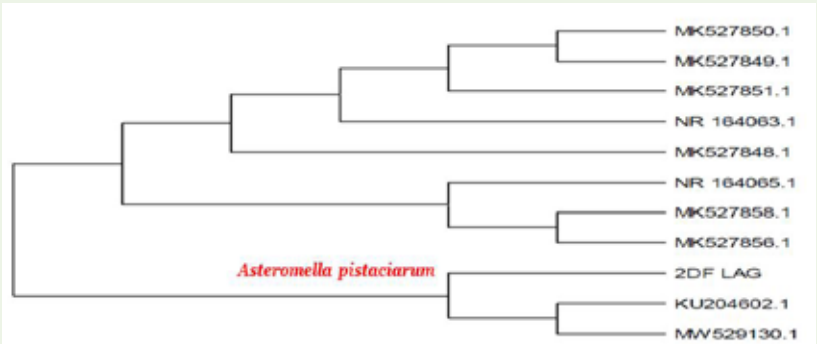
MK92954.1- *Lasiodiplodia theobromae*, MH265980.1- *Lasiodiplodia theobromae*, MT043793.1-*Diaporthe pseudophoenicicola*, MT355673.1- *Diaporthe* species, OP591389.1- *Diaporthe pseudophoenicicola*, OK560458.1- *Diaporthe phaseolorum*, KF558867.1- *Phomopsis* species, KY379051.1-*Phomopsis asparagi*, KP675776.1-*Phomopsis* species, MH712178.1- ***Diaporthe pseudophoenicicola, DFNA3-1 query (Diaporthe pseudophoenicicola)***

Figure 2: Molecular identification of endophytic fungal species, *Diaporthe pseudophoenicola* of *D. falcata* with phylogenetic analysis



MT579614.1- *Colletotrichum fructicola*, MT704607.1- *Colletotrichum fructicola*, MH700456.1- *Colletotrichum gloeosporiodes*, MF554908.1- *Colletotrichum siamense*, MG857847.1- *Colletotrichum siamense*, KM268865.1- *Colletotrichum siamense*, KX022505.1- *Colletotrichum gloeosporiodes*, ON912075.1- *Colletotrichum siamense*, MN635698.1- *Colletotrichum siamense*, MN635697.1- *Colletotrichum siamense*, **DFNA5-B query (*Colletotrichum siamense*)**

Figure 3: Molecular identification of endophytic fungal species, *Colletotrichum siamense* of *D.falcata* with phylogenetic analysis.



MK527850.1- *Leptosillia acerina*, MK527849.1- *Leptosillia acerina*, MK527851.1, NR164063.1-*Leptosillia acerina*, MK-527848.1- *Leptosillia acerina*, NR164065.1-*Leptosillia muelleri*, MK527858.1-*Leptosillia muelleri*, MK527856.1-*Leptosillia muelleri*, KU204602.1-*Asteromella pistaciarum*, MW529130.1-*Sordariomycetes* species, 2DF LAG query -***Asteromella pistaciarum***

Figure 4: Molecular identification of endophytic fungal species, *Astromella pistachiarum* of *D. falcata* with phylogenetic analysis.

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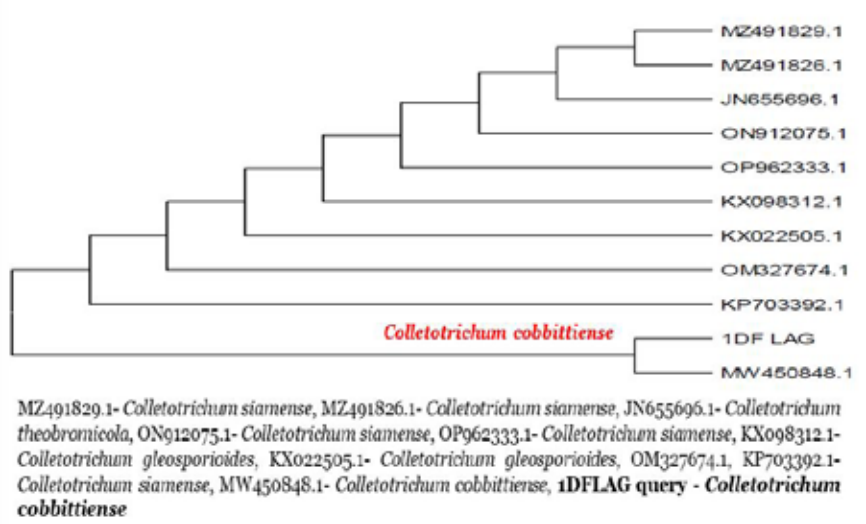

Figure 5: Molecular identification of endophytic fungal species, *Colletotrichum cobbittiense* of *D. falcata* with phylogenetic analysis.

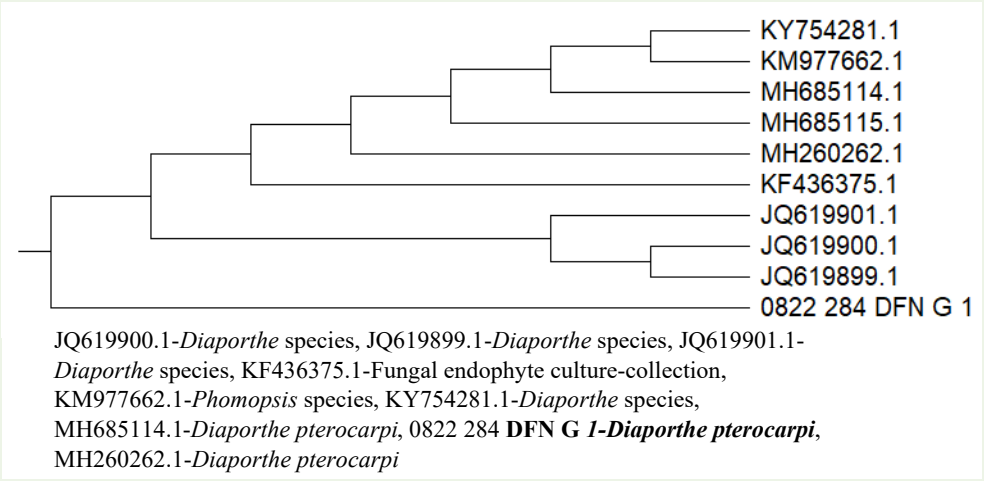

Figure 6: Molecular identification of endophytic fungal species, *Diaporthe pterocarpi* of *D. falcata* with phylogenetic analysis.

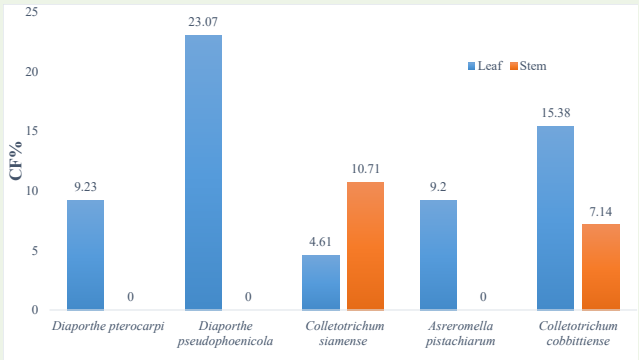

Figure 7: Colonization frequency of endophytic fungal species in dry season in two different parts of *D. falcata*.

proliferation[28]. Among all the endophytic fungal species, a total number of 05 species obtained from the leaf and stem segments are *Diaporthepterocarpi* (18.95%) (18.60%), *Diaporthepseudophoenicicola* (17.24%) (11.62%), *Colletotrichumsiamense* (11.20%) (11.62%), *Asteromellapistaciarum* (12.06%) (4.65%), *Colletotrichum cobbittiense* (23.27%) (9.30%) respectively, and form the dry season a total number of 05 endophytic fungal species obtained from leaf segment are *Diaporthepterocarpi* (9.23%), *Diaporthepseudophoenicicola* (23.07%), *Colletotrichumsiamense* (4.61%), *Asteromellapistaciarum* (9.20%), *Coletotrichum cobbittiense* (15.38%) and from the stem segment of 02 endophytic fungal species obtained are *Colletotrichumsiamense* (10.71%) and *Colletotrichum cobbittiense* (7.14%) (Figure 7) and (Figure 8). Our results are confirmation with the results of Verma et al. (2007) [25] and Mitchell et al. (2020) [26].

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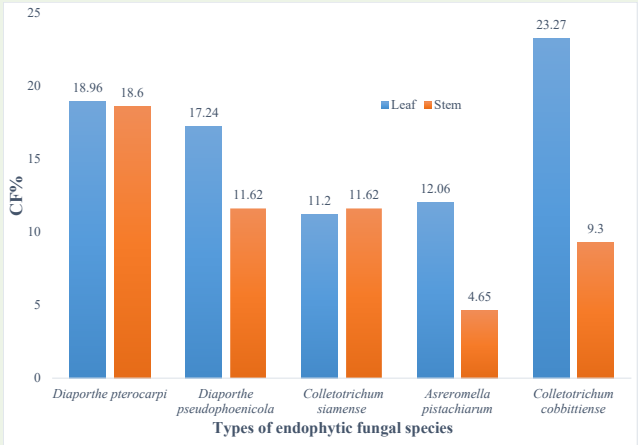

Figure 8: Colonization frequency of endophytic fungal species in wet season in two different parts of *D. falcata*.

Conclusions

The present study provides idea to know diversity of endophytic fungi associated with hemiparasitic parasitic plant *Dendrophthoe falcata*. Five different endophytic fungi were isolated and identified by molecular techniques and they were isolated from two parts (leaves and stem) in two seasons (wet and dry) separately. The literature on endophytic fungi reveals that many endophytic fungi are producing many secondary metabolites, which are biologically potent. Such biologically important potent bioactive compounds can be produced in high amount within short duration.

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