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RESEARCH PAPER

Isolation and identification fungal endophytes from the leaves and fruits of two therapeutically important *Ficus* species

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ABSTRACT

The constant demand for new biochemicals to advance human life necessitates the study of organisms from novel biotopes. The scientific community is paying more attention to endophytes, one of the inhabitants of novel biotopes, as they have already gained recognition for their production of numerous novel biochemicals and for their enormous potential to contribute to the development of effective solutions to the world's most pressing problems. The aim of the current study is to discover, compare, and isolate the endophytic community of *Ficus exasperata* Vahl. and *Ficus drupacea* Thunb. The presence of endophytes was discovered in the leaves and fruits of both plant species. A greater number of organisms were found in the leaves, while only a few were found in the fruits. All of the isolates were from the Ascomycota and Basidiomycota divisions. The majority of the isolates belonged to the Ascomycota division, with only a few belongings to the Basidiomycota division.

Keywords: endophyte, isolation, *Ficus*, fungal endophytes, identification

РЕЗЮМЕ

Нушиба Насер П.Т., Топпил Дж.Э. Изоляция и идентификация грибковых эндофитов из листьев и плодов двух терапевтически важных видов *Ficus*. Постоянный спрос на новые биохимические препараты для улучшения жизни человека обуславливает необходимость изучения организмов из новых биотопов. Все большее внимания уделяется эндофитам, обитателей новых биотопов, поскольку уже доказана выработка ими многочисленных новых биохимических веществ и установлен их огромный потенциал, способствующий разработке эффективных решений наиболее актуальных проблем поиска новых лекарств. Целью настоящего исследования является обнаружение, сравнение и выделение эндофитного сообщества *Ficus exasperata* Vahl. и *Ficus drupacea* Thunb. Присутствие эндофитов было обнаружено в листьях и плодах обоих видов растений. В листьях было обнаружено большее число организмов, в то время как в плодах лишь несколько. Все изоляты принадлежали к отделам Ascomycota и Basidiomycota. Большинство изолятов относилось к отделу Ascomycota, и только несколько – к отделу Basidiomycota.

Ключевые слова: эндофит, изоляция, фикус, грибковые эндофиты, идентификация

Переведено редколлегией

The challenges that humanity is currently confronting are connected to the fields of medicine and world food production. sporadic pandemic outbreaks, an increase in bacteria with medication resistance, a growth in the population's immunocompromised individuals, and disease-causing organisms that wreak havoc on enormous food fields, Despite the success of scientific research, problems like this still exist. All of this results in a constant demand for new agrochemicals, antibiotics, and chemotherapeutics. This reveals the significance of microbes. When comparing the number of microorganisms to their potential, they are underutilised. Regardless of their contribution to science, only 5 % of fungi have been studied to date (Hawksworth 2001). These microorganisms have the potential to act as novel compound introducers, bio pesticides, bio fertilisers, nutrient supplements, and so on. Finding the most powerful organisms to improve various fields through research is an important topic to investigate

Plant endophytes are endosymbiotic microorganisms that live inside plants without causing visible disease symptoms. They are kept in a finely tuned balance of virulence and defence (Schulz et al. 2002). Searching for new molecules inside the same framework as the past won't lead to anything innovative. For this reason, more attention should be paid to novel and different biotopes. Endophytes will frequently interact with different bio components and physiochemical factors within the host while living inside the plant tissue. Endophytes become occupants of unique biotopes as a result, and they may create novel biochemicals (Schulz et al. 2002). Almost all plants on the planet will be associated with one or more endophytes at some point in their life cycle (Govindappa et al. 2014). There are surprisingly few plants when compared to the plants that have already had their endophytic components investigated. Endophytes are microorganisms that may mimic and produce host biochemicals after spending a long period inside the host (Zhou et al. 2010). This ability can be

used to produce necessary biochemicals without harming the plants in an eco-friendly friendly manner.

Endophytes should be studied more thoroughly in a variety of fields, including endophytes of therapeutically important plants for biomedical applications, endophytes of plants that live in stressful environments (e.g., halophytes), and endophytes of disease resistant wild varieties of crop plants for crop improvement, etc. Concentrating on different fields can eventually use the capacity of these microorganisms as a holistic solution to all of the human problems. Despite the fact that the world has shifted to chemistry and automation, the importance of natural bioactive compounds has not diminished, as 40 % of the prescribed drugs that we use today are solely based on natural compounds (Strobel & Daisy 2003). It is remarkable how well natural molecules can serve as a guide for many other compounds. whereas screening one million chemical structures each day using combinatorial chemistry, results in the production of a single commercial product per year (Schulz et al. 2002). In this study, we attempted to separate and identify endophytic fungi from the fruits and leaves of two understudied *Ficus* species for their endophytes: *Ficus drupace* and *Ficus exasperata*. The genus *Ficus* is well known for its medicinal and nutraceutical potentials, including its anticancer, anti-diabetic, antibacterial, and antihelminthic properties (Odunbaku et al. 2008, Salem et al. 2013, Yessoufou et al. 2015, Mbosso Teinkela et al. 2018). There are only a few reports on endophytes from the genus, and it appears that neither the endophytic community of the species *F. drupacea* nor the fruit endophytes of *F. exasperata* have been studied despite the plants' reputedly enormous importance in the medical and nutritional fields. Through this study, 12 species of fungus were successfully isolated and identified, 3 of which were from fruits.

MATERIAL AND METHODS

Purified agar, Potato sucrose agar (PDSA), KH_2PO_4, $MgSO_4$, and Peptone, were obtained from Hi Media Laboratories Pvt Ltd (Mumbai, Maharashtra). Leaves and fruits were collected from the Calicut University Botanical Garden. Plant parts collected were washed surface sterilised and processed within 24 hours after collection.

Media preparation. Media were specially prepared for both fruit, leave mediated isolation. PDSA (0.04 mg/ml), KH_2PO_4 (0.0018 mg/ml), $MgSO_4$ (0.0001 mg/ml), Peptone (0.005 mg/ml) Purified agar (0.02 mg/ml), dried fruit powder (0.02 mg/ml) for fruit mediated endophyte isolation, and dried leaf powder (0.02 mg/ml) for leaf mediated endophyte isolation. All these components were mixed well in distilled water and autoclaved at 121ºC and 15 Lbs pressure for 15 minutes.

Leaves. Collected leaves were washed thoroughly in running tap water and surface sterilized using 3 % sodium hypochlorite (3 minutes) and washed in sterile distilled water followed by 75 % ethyl alcohol for 5 minutes. Again washed in sterile distilled water and taken in to the sterilised LAF. Sterilized leaves were cut in to 2 cm square pieces and inoculated to potato dextrose sucrose agar plates.

Fruits. Fully ripened fruits of *F. drupaceae*, *F. exasperata* were handpicked from the tree, and collected in to sterile plastic bags, which were thoroughly washed in running tap water, and soaked in 3 % sodium hypochlorite for 5 minutes. The fruits were then cut open and again soaked in 3 % sodium hypochlorite for 3 minutes. Which were washed with sterilised water and soaked in 75 % ethyl alcohol for 4 minutes and again washed in sterile distilled water. Sterilized fruits are taken in to sterile LAF. Sterilized blotting paper is used to remove the water droplets, skin and exposed parts were removed by using sterile scalpel, and they were made in to 2 cm square pieces and inoculated in to potato dextrose sucrose agar medium under sterile conditions.

Sterilisation was strictly followed to avoid any sort of contamination throughout the process.

RESULTS

Extensive fungal growths were observed from the cut ends of the plant parts after 3 days of incubation at room temperature (Fig. 1). Based on external morphology, 22 endophytes were isolated and pure cultured. Streak plate method were used to isolate pure colonies. These isolated pure fungal cultures were used for DNA isolation, followed by PCR amplification and sequencing. Among this only 3 of them were isolated from the fruits. Using DNA sequencing of ITS, LSU region could reveal only 12 out of the 22 isolates to the genus level. So in this report, only 12 of them are included. Among this 3 of them were fruit endophytes and the rest of them are leaf endophytes.

Pure isolates of identified fungal samples were represented as follows. Isolates from leaves of *F. drupaceae* and *F. exasperata* were DRPL I, DRPL II, DRPL III, DRPL IV, DRPL V, EXPL I, EXPLII, EXPL IV, EXPL V respectively.

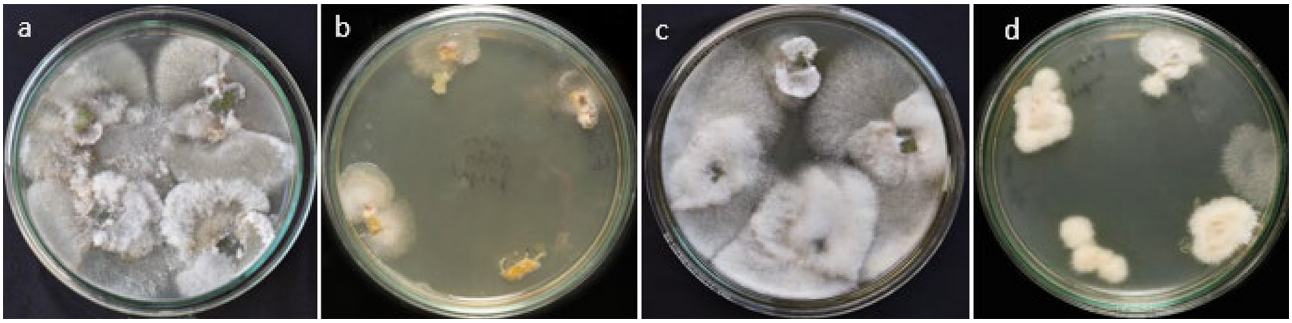

Figure 1 73 hrs. of endophytic growth from fruits and leaves of both the plants on nutrient medium: a – endophytic growth from the leaves of *Ficus drupacea*, b – endophytic growth from the fruits of *F. drupacea*, c – endophytic growth from the leaves of *F. exasperata*, d – endophytic growth from the fruits of *F. exasperata*

Fruit isolates were named as DRPFT I, DRPFT II and EXPFT I respectively.

All the 12 organism identified based on the sequencing of ITS, LSU region confirmed they were basically from two divisions of the fungi which are Ascomycota and Basidiomycota. Majority of the endophytes were found to be from Ascomycota (Table 1).

Looking in to the total number of isolates from both the plants, higher number of colonisation were found in the leaves rather than in the fruits. More than one organism were found to be same in the leaves, and one of the fruit endophyte isolated were also same for both the plants studied (Fig. 2).

DISCUSSION

Twelve distinct fungal species were found when endophytes from *F. exasperata* and *F. drupacea* are studied. The majority of these species had already been described from other plants and had significant bioactivities. Among

Table 1. Results of isolated fungi identification

Sl No	Name of the isolate	Similar organism	Percentage of similarity (%)	Division
1	DRPL I	*Phanerochaete* sp.	99.62	Basidiomycota
2	DRPL II	*Aspergillus nomiae*	87.29	Ascomycota
3	DRP L III	*Daldinia eschscholtzii*	99.80	Ascomycota
4	DRPL IV	*Endomelanconiopsis endophytica*	99.77	Ascomycota
5	DRPL V	*Alternaria alternata*	90.06	Ascomycota
6	DRPFT I	*Trichoderma asperellum*	97.44	Ascomycota
7	DRPFT II	*Phlebia* sp.	99.82	Basidiomycota
8	EXPL I	*Endomelanconiopsis endophytica*	99.77	Ascomycota
9	EXPL II	*Alternaria alternata*	90.06.	Ascomycota
10	EXPL IV	*Lasiodiploida psuedotheobromae*	100	Ascomycota
11	EXPL V	*Malassezia restricta*	98.71	Basidiomycota
12	EXPFT I	*Phlebia* spc	99.82	Basidiomycota

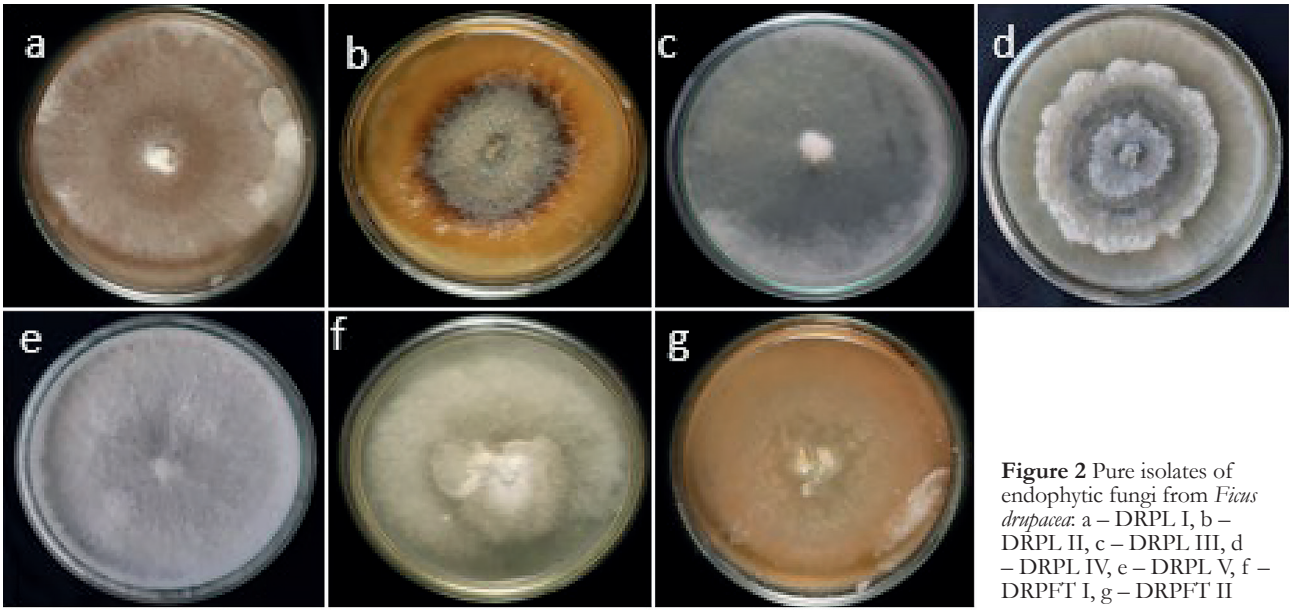

Figure 2 Pure isolates of endophytic fungi from *Ficus drupacea*: a – DRPL I, b – DRPL II, c – DRPL III, d – DRPL IV, e – DRPL V, f – DRPFT I, g – DRPFT II

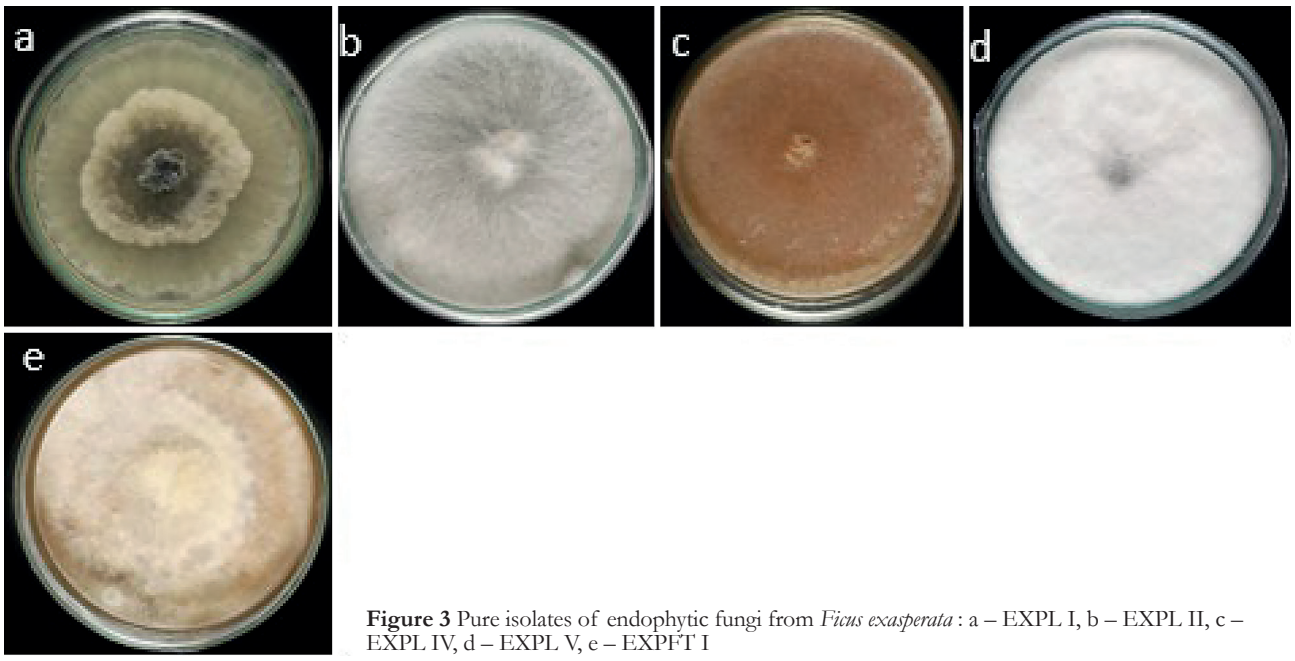

Figure 3 Pure isolates of endophytic fungi from *Ficus exasperata* : a – EXPL I, b – EXPL II, c – EXPL IV, d – EXPL V, e – EXPFT I

the isolated species *Phlebia* and *Lasiodiploida psuedotheobromae* confirms the thought of Schulz et al. (2002). That is, the emergence of illness may result from a disruption in the balance between plant defence and fungal virulence. Most of the previous studies supports our research findings on the endophytes of the selected species. Do Nascimento et al. (2020) identified *Phanerocheate* species from *Androanthus impetigenosis* with high trypanocydal activity. Endophytc *Aspergillus nomiae* from *Aloe vera* was found to have anticancerous activity against breast cancer cell lines (Mane & Vedamurthy 2020). *Daldinia eschscholtzii* were also isolated and identified with potential herbicidal property on monocotyledonous and dicotyledonous weeds (Flores-Reséndiz et al. 2021). In a study on *Ficus hirta* researchers found *Endomelanconiopsis endophytica* which was able to contribute two new xyloketals to the scientific world (Sun et al. 2016). *Alternaria alternata* isolated from *Camellia sinensis* yielded three new compounds .This fungus was also found to have antimicrobial as well as cytotoxic potentials (Wang et al. 2014). Endophytic *Trichoderma asperellum* was found to be very effective against the vascular streak dieback disease of *Cacao* by scientists in 2015 (Rosmana et al. 2015). A study on endophytic *Lasiodiploida psuedotheobromae* revealed its therapeutic ability in xanthine oxidase inhibitory activity (Kapoor & Saxena 2014). *Malassezia restricta* was also isolated as an endophyte in a previous study by (Harrison et al. 2016). Endophytic *Phlebia* species were found to be useful in textile dye decolourisation and degradation (Bulla et al. 2017).

All the previous reports on this endophytic species revealed the importance of this species in various fields from therapeutics to biopesticides and as environmental protectors. So all the isolated endophytes of both the plants are extremely important to in order to revealing their abilities. As the host plants differ in their biological activities, so will their biochemical status. Among the isolates, two of the leaf endophytes and one of the fruit endophyte were found to be common in both the species which supports the previous research finding of Solis et al. (2016) from the genus *Ficus*. Phylogenetic distance has an inverse relationship with the number of similar endophytic organisms within the host species(Solis et al. 2016). Representation of higher number of ascomycetes and few basidiomycetes in the present study is also in accordance with the research findings of the same team.

CONCLUSION

The present study was to isolate and identify the endophytic community from the fruits and leaves of the two therapeutically important *Ficus* species. All the isolates fall under two divisions, Ascomycota and Basidiomycota. Most of the members were under Ascomycota. Among the total 12 isolates, 3 of them were similar for both the plant species. This may be due to the similarity in the phylogeny of the two plant species which might have contributed to the similarity in their endophytic population. Almost all the isolates were already reported as endophytes with various benefits to human life. Further studies are inevitable for making use of these microorganisms as a wholesome solution for mankind.

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Ethics approval and Consent to participate. Not applicable.

Consent for publication. Both authors here by agree that there is no problem with publishing the data and work with mycological progress.

Data availability. The datasets generated during the current study are available from the corresponding author on reasonable request.

Competing interests. The authors have no relevant financial or non-financial interests to disclose, also there is no conflict of interest.

Author contributions. Both the authors contributed to the manuscript, First author or the corresponding author (Nushiba Naser P.T.) designed and developed the work plan collected needed materials for the work and done all the manual work, second author (John E. Thoppil) contributed to result analysis and verification along with making necessary corrections in the written manuscript. Both the authors read and approved the final manuscript.

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