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STUDIES ON BIODIVERSITY OF PHYLLOPLANE AND ENDOPHYTIC FUNGI FROM MATURE LEAVES OF MEDICINAL PLANT, *CARICA PAPAYA* L. WITH ANTIMICROBIAL POTENTIAL

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ABSTRACT

Variety of phylloplane and endophytic fungi are used to consider as one of the rich source of novel compounds of biological activities and have a high level of structural diversity on the leaf surfaces. Bioactive compounds produced by these phylloplane and endophytes have shown promising potential towards human health, for which, it is necessary to apprehend and operate this vital microbial resource and make it more helpful for the welfare of mankind. In our present study, isolation and enumeration of ectophytic (phylloplane) and endophytic fungal species was carried out from one medicinal plant, *Carica papaya* L. with the host relationship based on the methodology i.e., agar plate method. Altogether, 18 phylloplane and endophytic fungal species of 9 genera were isolated from the medicinal plant, *Carica papaya* L. In distribution of leaf surface fungi, phylloplane contributed the maximum (62%) followed by the endophytes (38%) in *Carica papaya* plant. In the phylloplane and endophytic fungal population, *Alternaria raphani, Aspergillus awamori, Aspergillus nidulans, Aspergillus niger*, *Curvularia lunata*, *Curvularia ovoidea*, *Curvularia robusta*, *Penicillium citrinum, Moniliella ruber*, *Penicillium chrysogenum*, Gray sterile mycelia, Pink sterile mycelia and White sterile mycelia were the most dominant fungal species in the agar plate method. Fungi isolated from both the top and sub-surface of leaf were more or less similar to each other. Antimicrobial potential of the leaf surface fungal extracts was very effective against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Shewanella putrefaciens* and *Candida albicans*. The host relative favorite and tissue description sign was established between the phylloplane and endophytes based on the fungal community distribution and composition.

KEYWORDS: Phylloplane and Endophytic fungi, Biodiversity, Medicinal plant, Mature leaves, *Carica papaya* L.

INTRODUCTION

Phylloplane defines the upper surface of leaves which is inhabited by numerous fungi and bacteria those who resides on the vicinity and get the nutrients available on it, but Endophytic means the inner parts of the plants where fungi spend the whole or part of their life cycle colonizing inter or intracellular way inside the healthy tissues of the host plants, typically triggering no outward disease signs.[1-3] Leaf surface fungi have been found in every plants examined and it is projected that there are over one million phylloplane and endophytic fungi are in the nature.[4] Leaf surface fungi have been recognized as an important and novel resource of natural bioactive products with potential application in food, agriculture and medicine industry.[5-7] Different bioactive compound were discovered from the endophytic fungi,[8] many of the scientists have been growing their benefits in studying fungal endophytes as potential producers of novel and biologically active compounds. During last few decades, many cherished bioactive compounds with insecticidal, antimicrobial, anticancer and cytotoxic activities have been positively discovered from the leaf surface fungi. These bioactive compounds are classified as terpenoids, steroids, alkaloids, quinones, lactones, phenols, and lignans.[2,9] There is a co-evolution and friendly relationship was gradually set up between ectophytic and endophytic fungi with their host plant. The host plant is used to supply required nutriments and easeful habitation for the survival of its ectophytes and endophytes.[6,7] By means of which, the leaf surface fungi produce a number of bioactive compounds for helping the host plants to resist external biotic and abiotic stresses and benefiting for the host growth in return.[3,10] Few of the fungi have developed the ability to produce

the same or similar bioactive substances as those originated from the host plants. It is always beneficial for us to analyze the relations between the endophytes and ectophytes with their host plants and to develop an substitutable approach for competently producing these unusual and valuable bioactive compounds.[6, 11] In our present study, isolation and identification of phylloplane and endophytic fungi were carried out from one medicinal plant species viz., *Carica papaya* L. collected from KMGIPSR campus, Pondicherry- 605008, India employing agar plate methods. The potential relationships of the ectophytes and endophytes with the host plants were also discussed with their antimicrobial potential.

MATERIALS AND METHODS

Collection of sample

Different leaves of medicinal plant, *Carica papaya* L. were collected in fresh condition from our KMGIPSR campus, Puducherry- 605008, India. Mature leaves were carefully segregated and brought to the Microbiology Laboratory, Department of Botany with utmost care and kept in room temperature for the isolation of leaf surface fungi by agar plate method.

Description of the plant

Binomial name **: *Carica papaya* L.**
Family : Caricaceae
Common name : Pawpaw
Vernacular name : Papaw

Plate I: *Carica papaya* L.

Carica papaya (Plate I) is an herbaceous succulent plant popularly known as pawpaw. It belongs to the Caricaceae family. It is mostly native to the tropical regions of the Americas but now is extensively cultivated in other tropical regions of the world for its edible melon-like fruit that is available all over the year. Diverse parts of the plant are engaged in the treatment of dissimilar human and veterinary diseases in various parts of the world. For example, in Asian folk medicine, the latex is employed as an antiseptic for wound dressing, abortificient and as a cure for dyspepsia, whereas in Africa, the root infusion is presumed for treating venereal diseases, yaws and piles. In Cuba, the latex is used in the treatment of ringworm, psoriasis and cancerous growth. Its' fruit and seed extracts are used to possess distinct bactericidal activity against pathogenic bacteria.

Isolation of Phylloplane and Endophytic fungi

For the isolation of endophytic fungi, the collected healthy leaves were thoroughly washed in running tap water. Then the leaves were cut into small segments (about $1cm^2$) including midrib portion. The leaf samples were surface sterilized by 0.1 % mercuric chloride for 60 seconds and then rinsed in sterile distilled water for 10 seconds (three times). For phylloplane mycoflora study, the leaf segments were not surface sterilized since phylloplane fungi grown on the surface of the leaves. Without washing the segments, they were placed on the PDA plates equidistantly.

Culture of leaf samples on agar plates

After sterilization, the excess water was blotted out by sterile filter paper from the leaf segments and kept separately. Then the surface sterilized segments were placed in petridishes containing PDA supplemented with streptomycin. Five (5) leaf such as Young, Mature, Yellow and Litter segments of a centimeter square, both sterile and unsterile were placed separately on the PDA media plates equidistantly by the help of sterile forceps and pressed later on followed by incubation for 3 to 7 days.

Incubation for the growth of fungi

All the plates were incubated at 25±3ºC temperature in the incubation chamber. Incubation time was maintained differently since, 7-8 days is meant for the fungal growth of fungi in agar plate method, but in moist chamber method, 1 to 3 weeks are required for the growth of fungi. Every day watch of the petriplates and check the growth of fungi was almost necessary in our present study after 3rd day of incubation.

Identification of fungi

After three days of incubation, the fungal colonies were counted for individual species and the total number was enumerated. Microscopic slides stained with lacto phenol cotton blue were prepared from each colony of the fungus and observed microscopically under the trinacular digital photography microscope to identify up to species level. The colony which was not been identified directly from plates was sub cultured in SDA/PDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal CFUs up to species level.[11-15] The presence and absence based on the occurrence of individual fungus in the phylloplane and endophytic were determined and plotted in the form of tables and figures.

Antibacterial activity of the active agar plugs of dominant fungi

The active agar plugs of 6mm size were taken carefully from the three-day pure culture plates of the leaf surface

fungi and were studied for antimicrobial activity against pathogenic bacteria and one fungus using agar plug assay method. The test organisms used were from MTCC cultures i.e., *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Shewanella putrefaciens* and *Candida albicans*. The microbes were grown in nutrient broth for 12 h. Lawns of pathogenic bacteria and *Candida albicans* were prepared on nutrient agar plates using sterile cotton swabs. Active agar plugs were placed on nutrient agar plates and each plug was placed inside the wells prepared earlier by cork borer. The plates containing bacteria and active plugs of endophytic fungi discs with extracts were incubated at 37^{o}c for 24 to 48 hours in the BOD incubator. The plates were examined for the zone of inhibition after 24 hrs, which appeared as clear area around the wells. Inhibition zone diameter was measured in mm by the HI-Media scale.

RESULTS AND DISCUSSION

During the present study, altogether a total of 18 leaf surface fungal species under 9 genera were isolated and identified from mature leaf samples of the medicinal plant, *Carica papaya* L. by agar plate method. The phylloplane fungi were recorded under 16 species and 8 genera whereas endophytic fungi were of 8 species under 5 species. Abundance and percentage occurrence of phylloplane and endophytic fungi isolated by agar plate method from the medicinal plant, *Carica papaya* is given in Table 1. Distribution and frequency of phylloplane and endophytic fungi are given in Fig 1 & 2. In fungal distribution, phylloplane supported the growth of the maximum fungi (62%) in comparison to endophyte (38%) (Fig 1). Leaf surface fungi like, *Alternaria raphani, Aspergillus awamori, Aspergillus flavipes, Aspergillus flavus, Aspergillus nidulans, Aspergillus niger, Curvularia lunata, Curvularia ovoidea, Curvularia robusta, Drechslera* sp., *Moniliella ruber, Penicillium citrinum, P. notatum, P. oxalicum, Penicillium chrysogenum,* Gray sterile mycelia, Pink sterile mycelia and White sterile mycelia were isolated from the mature leaves of *Carica papaya. Alternaria raphani, Aspergillus awamori, A. niger, Curvularia robusta, C. lunata, Penicillium citrinum* were recorded as dominant phylloplane fungi. *Aspergillus awamori, Aspergillus niger, Curvularia ovoidea, Penicillium citrinum,* Gray sterile mycelia and White sterile mycelia were documented as dominant endophytes. Two distinct fungi, *Drechslera* sp. and *Penicillium oxalicum* were recognized as special endophytes but not recorded as phylloplane (Table 1). Six fungi of the endophytes were also included with phylloplane fungi but ten of phylloplane were not recorded as endophytes, they remained as phylloplane only (Table 1, Fig 2). Bharathidasan and Panneerselvam[10] recorded a total 10 fungal species viz. *Aspergillus flavus, A. niger, Aspergillus sp., Penicillium sublateritium, Phoma chrysanthemicola, P. hedericola, Phoma sp.* and *Candida albicans* from *Avicennia marina*. Among the endophytic flora, *Phoma* was the most prominent genus. Interestingly no endophytes were isolated from 110 leaves samples and overall colonization frequency from surface in their work.

Table 1: Abundance and percentage occurrence of leaf surface fungi of *Carica papaya* L.

Sl. No.	Leaf surface fungi	Phylloplane	Endophyte
1	*Alternaria raphani*	2/40	-
2	*Aspergillus awamori*	3.5/70	2/40
3	*Aspergillus flavipes*	1/20	-
4	*A. flavus*	1.5/30	-
5	*A. niger*	4/80	4/80
6	*A. nidulans*	1.5/30	-
7	*Curvularia lunata*	2/40	-
8	*C. ovoidea*	1.5/30	2/40
9	*C. robusta*	2.5/50	-
10	*Drechslera* sp.	-	1/20
11	*Moniliella ruber*	1.5/30	-
12	*Penicillium chrysogenum*	0.5/10	-
13	*P. citrinum*	4.5/90	3/60
14	*P. notatum*	0.5/10	-
15	*P. oxalicum*	-	1/20
16	*Grey sterile mycelia*	0.5/10	2/40
17	Pink sterile mycelia	1/20	-
18	White sterile mycelia	1.5/30	4/80

Fig 1: Distribution of leaf surface fungi in *Carica pappaya* L.

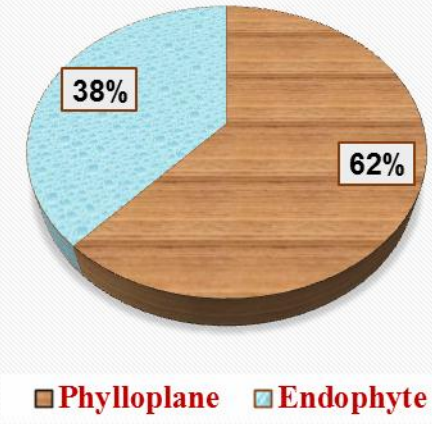
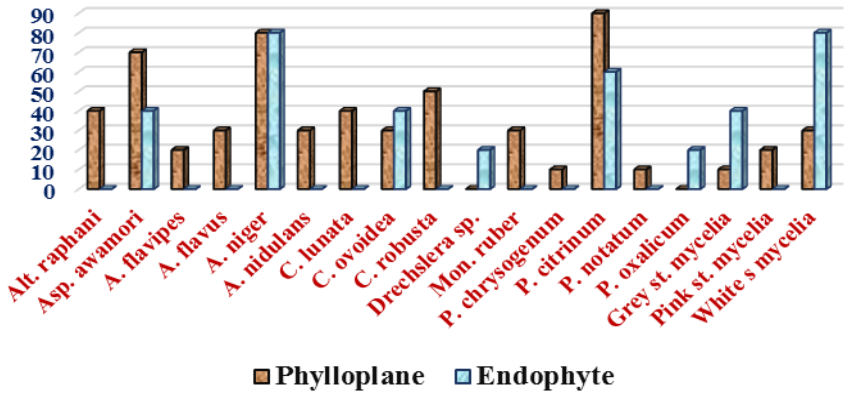
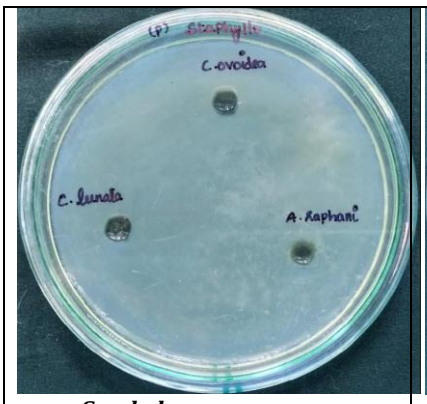


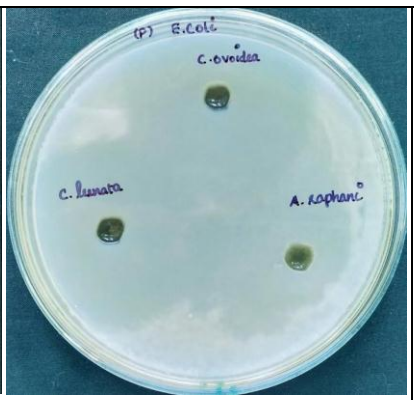
Fig 2: Percentage occurrence of leaf surface fungi on *Carica papaya* L.



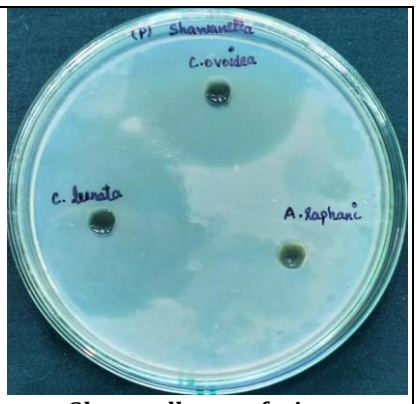
Ectophytic and endophytic organisms have received considerable attention as they are found to protect their host against pest pathogens and even domestic herbivorous.[1] Most of the isolated fungi belonged to anamorphic fungi in particular to Deuteromycetes and Zygomycetes.[3] The isolated fungi of the endophytic and phylloplane origin may lead to the production of special compound within the host, medicinal plant, *Carica papaya*.[7,9] Fungi have been widely known as a source of bioactive compounds, an excellent example for the anti-cancer drug taxol, which was previously supported to occur only in the plant.[9] *Carica papaya* is a plant having a broad spectrum of medicinal properties.



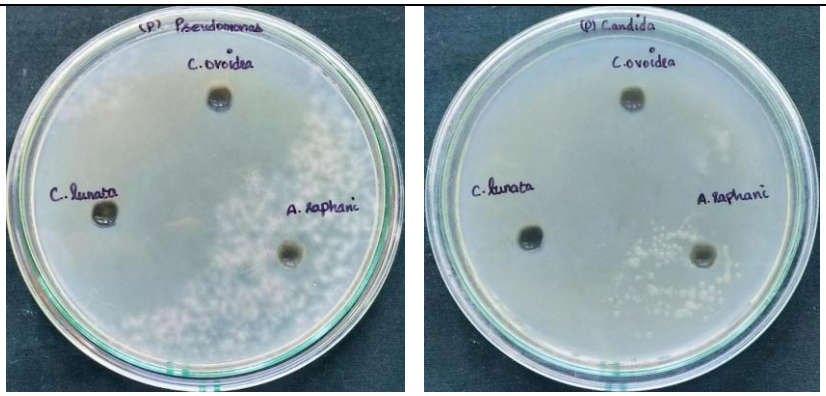
Staphylococcus aureus



Escherichia coli

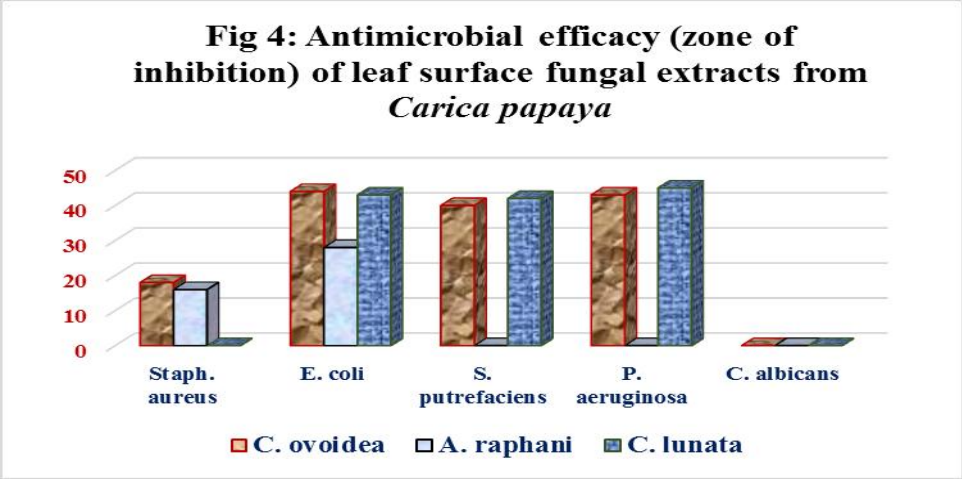


Shewanella putrefaciens



Pseudomonas aeruginosa ***Candida albicans***

Fig 3: Antimicrobial potential of leaf surface fungal extracts based on their active agar plugs.



Antimicrobial potential of the leaf surface fungal extracts and their active plugs were found to be good against the MTCC bacteria but not against *Candida albicans* (Fig 3 and 4). Extracts of *Curvularia lunata and Curvularia ovoidea* showed good efficiency against the pathogens in comparison to *Alternaria raphani*. *E. coli, Pseudomonas aeruginosa* and *Shewanella putrefaciens* were found to be more susceptible towards the fungal plugs and extracts compared to other pathogenic microbes (Fig 4).

Every part of the plant is used in one or the other types of medicine. Isolation of only 18 taxa of phylloplane and endophytic fungi showed that the medicinal property of the plant has some role to play in the colonization of fungi.[11] Recently studies have been carried out about the endophytic biodiversity, taxonomy, reproduction, host ecology and their effort on host.[1,2,11,18] Endophytes, are now considered as an outstanding source of bioactive natural products, because they occupy unique biological niches as they grow in so many unusual environments.[10,11] A study of endophyte biodiversity of medicinal plant in Puducherry, India was conducted by the Nayak et al.[17] They have reported diversity of fungal species ranging within 30 and they documented the common endophytic fungal isolates and similarity coefficient studies on different medicinal plants by agar plate method. In the present study 18 different species with high frequency were isolated from *Carica papaya* which is slightly less than the above cited study.[18]

CONCLUSION

Fungi are used as medicinal agents with natural products once serving as the basic source of most of the sicknesses. *Carica papaya* is one of the medicinal plants which have broad spectrum medicinal properties. In the present study, a total of 18 phylloplane and endophytic fungal species under 9 genera were recorded by agar plate method. The most of the fungi isolated belonged to the class Deuteromycetes and Ascomycetes. Among the phylloplane and endophytic fungal population, *Aspergillus awamori, Aspergillus nidulans, Aspergillus niger, Curvularia lunata, Curvularia ovoidea, Curvularia robusta, Penicillium citrinum, Moniliella ruber, Penicillium chrysogenum,* Gray sterile mycelia, Pink sterile mycelia and White sterile mycelia were the most dominant fungal species. It was confirmed that the leaf surface fungi of the medicinal plant, *Carica papaya* L. have antimicrobial potential to prevent the growth of *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Shewanella putrefaciens* and *Candida albicans* in our present study.

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