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Diversity and distribution of microfungi in foliage leaf, bark and leaf litter of *Couroupita guianensis*

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ABSTRACT

The diversity and distribution of micro fungal assemblages in the foliage leaf, bark and leaf litter tissues of the sacred plant, *Couroupita guianensis*. For the host, we recorded 1005 fungal species isolates from 900 tissue segments. A total of 62 fungal species were isolated from foliar endophytes, phelloglyphytes and leaf litter. The colonization frequency of the fungi species has been recorded as 111.66% from the endophytes, phelloglyphytes and leaf tissues. The fungi species recovered in various groups included ascomycetes, coelomycetes, hyphomycetes, zygomycetes and sterile fungi. The number of fungal species is increased for the plant tissues, it showed that the species diversity is increasing. A correspondence analysis also showed that the fungal species assemblages were different from each type of tissue.

KEYWORDS: *Couroupita guianensis*, endophytes, phelloglyphytes, litter fungi, sacred, tissue specificity

INTRODUCTION

Endophytes are microorganisms that live within plant tissues without causing symptoms of the disease. Plant-associated fungi are usually divided into a different ecological group based on mycorrhizal, pathogenic, epiphytic, endophytic, and saprotrophic fungi. Most studies focus on only one of these groups, and very few consider interactions among them or between fungi and bacteria. In the investigation, *Couroupita guianensis* is being investigated for the approaches to study plant-associated fungal microbes' communities. Endophytes are microorganism live in within plants without causing any harm to their growth and development. Unlike disease-causing microorganisms, endophytes are non-pat pathogenic and many of them are known to enhance their plant host's fitness [1]. Fungal endophytes colonize living plant tissues without producing any apparent symptoms or obvious negative effects [2]. The term endophyte was coined by de Bary [3] to denote organisms that colonize internal plant tissues. The distribution pattern of endophytes is influenced by various factors. Among the endophytic fungi are well-known in tree leaves [4] geographically varied. Fungi play key roles in ecosystems as decomposers, mutualists and pathogens, while in most cases, the role of individual fungus in nature is still unknown [5]. Phelloglyphytes of some temperate trees [6] and a few tropical angiosperms [7,8] have been studied. In bark tissues, however, whether these factors affect the

community of endophytic fungi is unknown. Fungi, as associates of higher plants in the form of mycorrhizae, endophytes and pathogens, and as decomposers of plant litter, play a crucial role in structuring and maintaining plant communities [9,10]. The fungal kingdom is species diversity, and fungi perform an abundance of functions in ecosystems, yet the extent of fungal diversity is faulty known. Fungi play an indispensable role in the decomposition of plant substrates in various ecosystems [11,12]. The fungal diversity in decaying plant matter has previously been studied in tropical and temperate regions [13]. The tropical and sub-tropical rainforest is most rich in plant diversity, so the endophytic diversity in this region has been studied [14,15]. Each plant harboured at least one or more fungal endophytes, thus fungal endophytes have high biodiversity. It has been estimated that all the plant, animal or microbial species would be described in about 30–90 years before they became extinct, considering that there are probably 1.5–3 million undescribed species on the earth with an extinction rate of 0.01–1% (at most 5%) every decade [16]. In contrast, an updated estimate of fungal diversity showed that the fungal species ranged from 2.2 to 3.8 million worldwide [17]. Although there are reports from the tropics regarding the fungal diversity, distribution, and contribution to the global fungal taxa of a few ecological groups of fungi such as leaf litter fungi [18], endophytes [19,20]. A few studies in the tropics that compare these aspects of different ecological groups of fungi associated with plant communities [21]. The aim of our

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study was to compare a single host of the fungal species diversity of endophytes(leaf), phellophytes(bark) and litter(leaf litter) ecology niche groups of fungi and to illuminate their possible association in fungal diversity records.

MATERIALS AND METHODS

Plant Description and Sample Collection

Cannonball tree is one of the most striking examples of cauliflory is *Couroupita guianensis*. It belongs to the family Lecythidaceae. The species finds the habitat in almost all tropical and sub-tropical regions of the world with the greatest abundance in Amazonian. In India, it is also grown as an ornamental and sacred species (Fig.1). The plant was collected from Mannargudi, Tamil Nadu; South India was studied for the fungal association. It's located at 10.67°N 79.43°E in the Delta district from collected for studying. Three trees species from Mannargudi area in Tamilnadu state were collected. Sixty-one mature, healthy leaves were collected from three individuals of each host species (20 ± 25 from each individual) and screened for endophytes during winter (raining season - June to October 2019) season. The leaf and bark samples were brought to the laboratory in sterile polythene bags and processed within 24 hours of collection. Litters sample was collected after the raining season (October to November 2019), when the leaves fall down. This continued to investigate the autumn season at the start of decomposition of the leaf.

Sterilization of Plant Materials

Healthy foliar leaves from selected plant material were washed thoroughly in running water and three segments of 0.5cm² were cut from the midrib/lamina portion of each leaf. All segments were surface sterilized in 70% ethanol for 5 secs, followed by 4% NaOCI for 90 secs then they were washed in sterile water for 10 secs [22]. For bark cut into 0.5 cm² segments and surface sterilized by dipping in 75% ethanol for the 60s, immersed in 4% NaOCl for 180s, and finally dipped in 75% ethanol for 30s [23]. A total of 150 leaves and bark segments were screened for the presence of leaves and bark fungi.

For litter, mature brown leaves without any symptoms of decay were collected from the plant floor beneath the tree, brought to the laboratory in sterile polythene bags, and processed within 24 h of collection. From each leaf lamina, 5 segments (approx. 0.5 cm² each) were cut. These segments were washed in sterile distilled water, and 150 segments were randomly selected and incubated in sterile Petri dishes lined with sterile moist filter papers. Ten segments each were placed in a Petri dish. These segments were observed by direct microscopic observation, and the fruiting structures and spores of fungi were isolated and identified [24].

Statistical Analyses

The colonization frequency (CF%) of an endophyte/phellophyte fungal species was calculated [25]. Fisher's α was used to estimate species diversity since it is a better discriminator and is not unduly affected by the abundance of the most common species [26]. The Correspondence Analysis and the Bray-Curtis measure of distance were calculated [27]. The density of colonization and isolation frequency was calculated as follows. Isolation frequency (IF%) = Total number of isolates in particular species/Total number of segments x 100. Relative Percentage of Occurrence (RPO) of each group (viz. Ascomycetes, Coelomycetes, Hyphomycetes, Zygomycetes and sterile forms) of fungal species in each plant species was calculated as follows. RPO = Total colonization frequency of one group /Total colonization frequency for all the Groups of fungi x100.

RESULTS AND DISCUSSION

We had an investigation of the *Couroupita guianensis* plant as a precursor of the diversity of fungi in various tissues, the diversity of fungi in endophytes in leaves, phellophytes in the bark and leaf-litter saprophytic fungi. Although, based on the results of the recovered fungal species of this plant, it is described as follows. A total of 467 isolates belonging to 22 fungal species were isolated as endophytes and 346 isolates belonging to 29 fungal species were isolated from bark tissues (phellophytes) and 192 isolates belonging to 52 fungal species were isolated as leaf litter from this plant of *C. guianensis*,

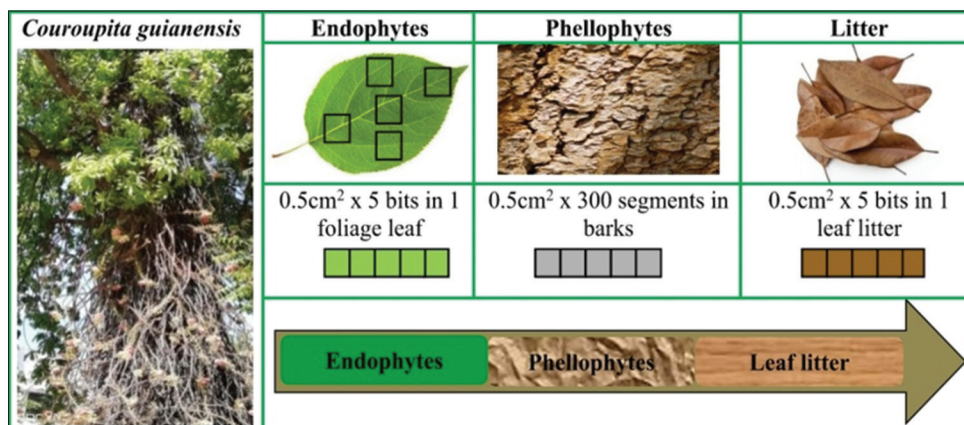


Figure 1: A view of *Couroupita guianensis* plant and schematic strategy for isolating fungi

respectively. For the plant, we recorded 1005 fungal species from 900 segments (leaf, bark, and leaf litter/300 segments each) (Table 1). A total of 22 fungal species were isolated from 300 leaf segments incubated from *C. guianensis* plant. These fungi belonged to Ascomycetes (18.18%), Coelomycetes (27.2%), Hyphomycetes (40.9%), and sterile forms (13.6%) (Table 1). *Phomopsis* species is dominant and *Colletotrichum* sp. *Sporormiella* sp., *Phyllosticta capitalists*, and Xylariaceae form were the most frequently isolated. Twenty-nine fungal species were isolated from 300 bark segments. These bark fungi were dissimilar from leaf tissues. A total of 29 species among 17.24% in Ascomycetes, 20.68% in Coelomycetes, 62.06% in Hyphomycetes fungal group were isolated from bark tissue. *Lasiodiplodia theobromae* is a species dominated in the bark and *Phomopsis* sp., *Colletotrichum* sp., *Sporormiella* sp., *Phyllosticta capitalists*, Xylariaceae form, *Chaetomella* sp., *Alternaria* sp., *Aspergillus* sp., *Fusarium* spp., *Nigrospora* sp., had most frequently isolated. A few species similar to the leaf endophytes were frequently isolated. A total of 52 fungal species were recovered from 300 leaf litters. These saprophytic fungal species belonging to 9.61% ascomycetes, 9.61% coelomycetes, 75.0% hyphomycetes, 5.76% zygomycetes are recorded (Table 1 and Fig 2). Most of the species were isolated in fewer colonies or spores only. It may not be colonized in

decomposed tissues, hence it will not be isolated colonies. It has occurred only in spore stages. Table 1 are described the details of each isolated and identified taxon of the fungal isolates in various tissues from *C. guianensis* (Table 1). A total colonization frequency (CF %) has been dissimilar significantly among the leaf endophytes, bark fungi, and leaf litter fungi in *C. guianensis* plant, these fungal ranged from 64% in the leaves tissue, 87% in the bark tissues, and 64% leaf litter fungi. Though, as 90% of CF (%) was recorded in high diversity in a total of 900 segments. This fungal diversity is isolated in live and dead tissues of a single host although considerable has high diversity (Table 1). A few fungi species which failed to sporulate were designated as “mycelia sterile”. They can be identified later with different incubation such as sporulation in UV, so for colony characteristics, the mycelia were transferred into PDA agar media.

This enlists some genera *Colletotrichum*, *Phomopsis* and *Phyllosticta* are ubiquitous endophytes and have been reported from most plant hosts [28,29] and they are globally dominant in which *Colletotrichum* sp., *Phyllosticta* sp. is the most frequently encountered endophytic fungi [30] that may be either host-specific or generalist [31] and the remaining genera such as *Cladosporium* in *Azadirachta*

Table 1: Number of species, isolates, and diversity of the endophytes, phellophytes and leaf litter fungi from the *Couroupita guianensis*

Sl.No.		Endophytes	Phellophytes	Litter	Total
1	Total no. of fungal species	22	29	52	62
2	Total no. of colonies isolates	467	346	192	1005
3	Total no. of segments	300	300	300	900
4	Colonization Frequency (CF %)	155.66	115.33	64.0	111.66
5	R1(Margalef's)	3.41	4.78	9.70	8.82
6	H' (Shannon Index)	1.8	3.08	3.56	3.03
7	Fisher's alpha	4.79	7.53	23.44	14.60
8	Relative Percentage of Occurrence (RPO) of each group of fungal species				
	Ascomycetes	18.18	17.24	9.61	11.29
	Coelomycetes	27.2	20.68	9.61	9.67
	Hyphomycetes	40.9	62.06	75.0	69.35
	Zygomycetes	0.0	0.0	5.76	4.8
	Sterile forms	13.6	0.0	0.0	4.8

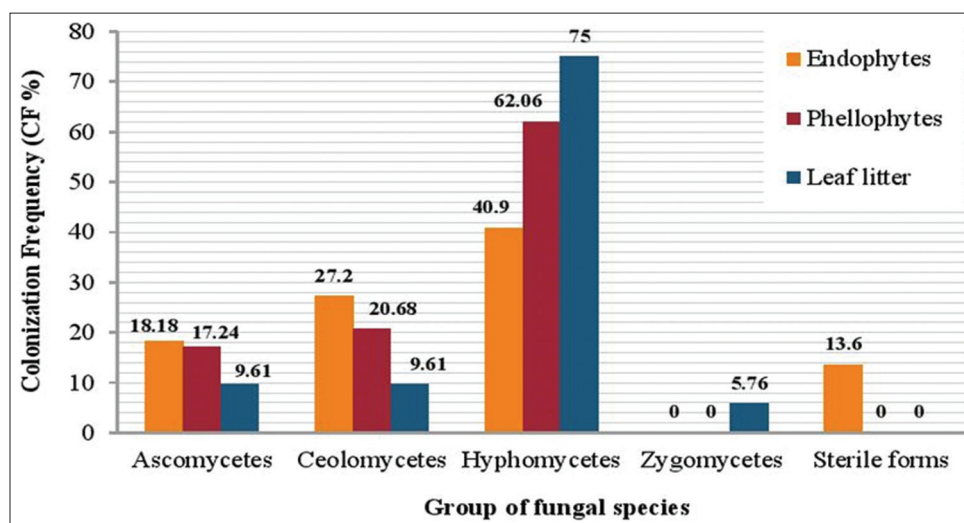


Figure 2: Relative Percentage Occurrence (RPO) of the endophytes, phellophytes and leaf litter fungi from the *Couroupita guianensis*

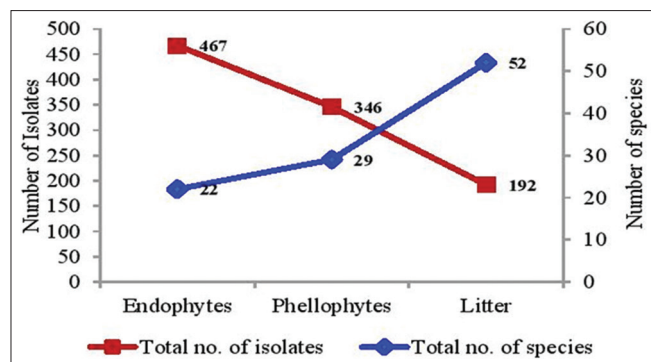


Figure 3: No. of fungal species and colonies isolated for the endophytes, phellyphytes and leaf litter fungi from the *Couroupita guianensis*

indica [32], *Fusarium* in *Murraya koenigii* [33], *Ocimum sanctum* [34,35] was also reported as ubiquitous endophytes. Some coelomycetes such as *Colletotrichum* sp, *Pestalotiopsis* sp. *Phomopsis* sp and *Phyllosticta* sp. are routinely isolated as endophytes [36]. In addition, a few phylloplane fungi including *Alternaria*, *Aureobasidium*, and *Cladosporium* are regularly isolated as endophytes. Intriguingly, some coprophilous genera (*Chaetomium*, *Sordaria*, *Sporormiella*, etc.,) have been reported as endophytes from several hosts [37]. Fungal species could grow to continue from alive tissues to dead tissues, so fungi could be occupied in the bark tissues. Although these mycelia or spores had such fungi a structure, they had assemblages alive in dead tissues' structure and other dormant structures not clear of such fungi. An early study for bark fungi has been reported in a tropical forest [38]. In the study of fungal diversity in *Couroupita guianensis*, 52 saprophytic fungi in leaf litters, 29 phellyphytes in the bark tissues and 22 endophytes in foliar tissues were recovered (Table 1); While, dissimilar fungal colonization had occurred from these kinds of tissues, as 467 colonizes in leaf tissues, 346 colonizes in bark tissues, and 192 fungi colonize in leaf litter (Table 1). Hyphomycetes were the abundant group of fungi followed by Coelomycetes Ascomycetes and sterile forms in all kind of tissues (Table 1 and Fig. 2). The species richness or the abundance of the species diversity index (Fisher's alpha) of the entire fungal assemblage of these plant tissues was 14.60. The species richness of fungal assemblage of endophytes in 4.79, phellyphytes assemblage was 7.53 and leaf litters in 23.44 were found (Table 1). Although, as the results have shown, the assemblage of the (richness) fungal species might diversity in leaf litter than the endophyte, phellyphyte (Table 1 and Fig. 3). Moreover, the richness index (R 1) and evenness index (H 1) has been similar to the results (Table 1). Correspondence analysis revealed that endophytes, phellyphytes and leaf litter fungi assemblages the tree's tissues into three distinct clusters (Fig. 4). The study in various ecological groups of fungi brings to being associated with different tissues of a single tree. Also, such a survey would identify degrees of host restriction, a critical factor in the evaluation of biodiversity. Tissue specificity fungi among endophytes, phellyphytes and leaf litter have been consequently diverse to increase.

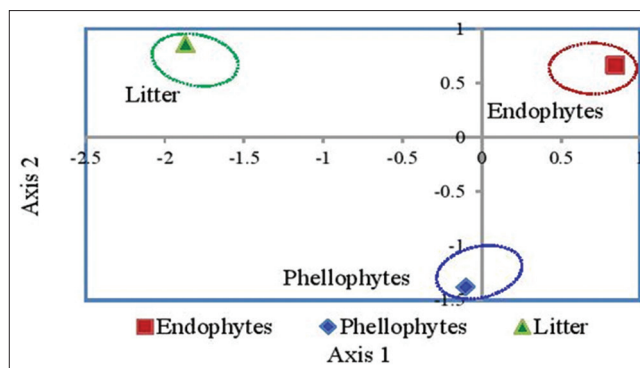


Figure 4: Correspondence analyses for the endophytes, phellyphytes and leaf litter fungi assemblages of the *Couroupita guianensis*

CONCLUSIONS

In the endophyte, phellyphyte and leaf litter fungal species belonging to various genera were isolated. A significant variation in microbial number was recorded in different tissues of the plant. Our study revealed that the highest microbial population in these samples was recorded in the litter fungi and the second-highest number of fungal in the bark was recorded beside the leaves tissues in the study periods. Found in living tissues such as *Colletotrichum*, *Phomopsis*, *Phyllosticta*, etc. fungal species. But, species richness is high, especially in the case of leaf litter fungi that the number of species is high here and dominant fungi are low due to nutrient deficiency. The nutrient has been in abundance in living tissues; so, widespread in fungal mycelium, the maximum part may be occupied and a few fungi were dominated in their tissues parts. On the contrary, the bark tissues may be less nutrient-rich than the dead tissues, and because the number of microbes within a given range, a few species were more difficult to dominate. But the number of fungal species was higher. Nevertheless, we assume that leaf litter can interact with soil, and saprophytic fungi may be involved in the decomposing process; so, there have been diverse taxa to increase the number of fungi.

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