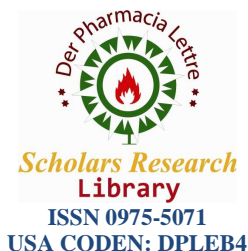




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Endophytic fungal community study of varied aging leaves of *Acalypha indica*

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ABSTRACT

Endophytic fungal isolation and enumeration was carried out from different aged leaf varieties viz., young, mature, yellow, dry and infected of *Acalypha indica* in the Microbiology Laboratory, Department of Biomedical Engineering, Sathyabama University, Chennai-600119. Two methods viz., moist chamber and agar plate were used to isolate the endophytic fungi. Altogether 20 fungal species were isolated under 15 genera from both agar plate and moist chamber method from the aged leaves of *Acalypha indica*. Of which 14 species of 11 genera and 13 species of 10 genera were recorded from Agar plate and moist chamber respectively. Endophytic fungi isolated from *Acalypha indica* both in Agar plate and Moist chamber method were *Alternaria alternata*, *Alternaria geophila*, *Botrytis cinerea*, *Wallemia sebi* and white sterile mycelia. In Agar plate method, infected and dry leaf samples harboured more number of endophytic fungi followed by yellow. The dry and infected leaf samples contained the maximum number of fungal species. The fungus *Geotrichum* sp. and *Helminthosporium* sp. were predominant in moist chamber method.

Keywords: Fungal community, *Acalypha indica*, Aging leaves; Yellow, Infected & Dry

INTRODUCTION

Fungi those who live endophytically inside the tissues of living plants are the group of microorganism generally involve in the production of noble metabolites. Endophytes are mostly unexplored group of microorganisms, but a few studies show them as a huge source of medicinal compounds. It was estimation by Dreyfuss & Chapela [1] that there may be one million species of endophytic fungi alone in the world. All vascular plants harbour endophytic organisms [2]. There is lack of information about endophytic fungal diversity in most part of the world. During the recent days they have received considerable attention in order to find out the involvement of these endophytic fungi to produce noble compounds. Medicinal plants are reported to harbour endophytes [3], which in turn provide protection to their host from infectious agents and also provide adaptability to survive in adverse environmental conditions. To date, only a few plants have been extensively investigated for their endophytic biodiversity. A number of works pertaining to endophytic fungi of different medicinal plants in and around of India were carried out by various workers [4,5], but few works are available in Tamilnadu. It is necessary to establish the patterns of distribution of endophytic fungi from different aged leaves as well as the succession of endophytic fungi adhered to the leaves based on the ageing of the plant as well as to recognise the fungi related to the metabolites produced from this medicinal plant. In the present study, it was an attempt to isolate widespread endophytic fungi that may be specific to the medicinal plant, *Acalypha indica* and further to screen out and identify the endophytic fungi by employing two techniques to find out the method specificity.

MATERIALS AND METHODS

Acalypha indica, Family: Euphorbiaceae

Acalypha indica (English: Indian acalypha, Indian nettle, Three-seeded mercury French: Ricinelle des Indes, Oreille de chatte, Herbe chatte, Tamil: Kuppamaimeni) is a plant species. It occurs throughout tropical Africa, South Africa, India and Sri Lanka as well as in Yemen and Pakistan. It may be introduced elsewhere as a weed. In west and east Africa the plant is used as a medicinal plant. In West Africa the leaves are cooked and eaten as a vegetable. It is also browsed by cattle. This plant is held in high esteem in traditional Tamil siddha medicine as it is believed to rejuvenate the body

Medicinal uses

The leaf juice or decoction of leaves is prepared and given in dose of 1-2 tablespoon in case of small children and 15-30ml in case of adults to induce emesis or purgation and hence expel the intestinal worms and mucus from intestine. The juice of leaves is boiled along with gingelly and this is applied externally over painful areas of body. The leaves are ground with salt and externally applied over skin infections like scabies. The leaves are ground along with manjal and applied externally over ulcers, poisonous bites. The paste of leaves along with lime or chunambu is externally applied overhead ache. The dried leaf powder is bandaged over the bed-sore areas and hence produces an anthelmintic action. The decoction of roots is also used to induce purgation. The leaf juice along with neem oil is applied over uvula of small children. This will induce emesis and mucus from intestine. Siddha treatment is based on complete physical examination of the patient, Naadi diagnosis and other diagnostic criteria of the disease.

Collection of plant samples

The leaf samples viz., young, mature, yellow, dry and infected of the medicinal plant, *Acalypha indica* were collected from Shollinganallur area, Chennai and brought to the Microbiology Laboratory, Department of Biomedical Engineering, Sathyabama University, Chennai-600119 in aseptic condition and kept in the refrigerator at 4-8°C up to the completion of the experiment (Plate I).

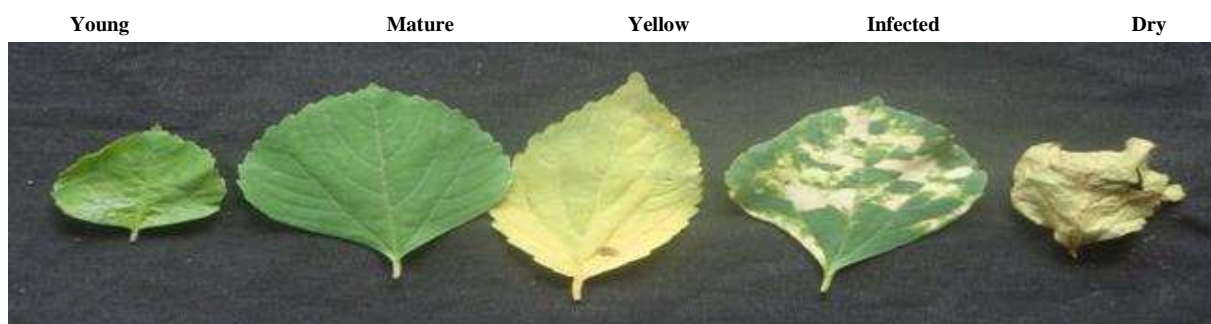


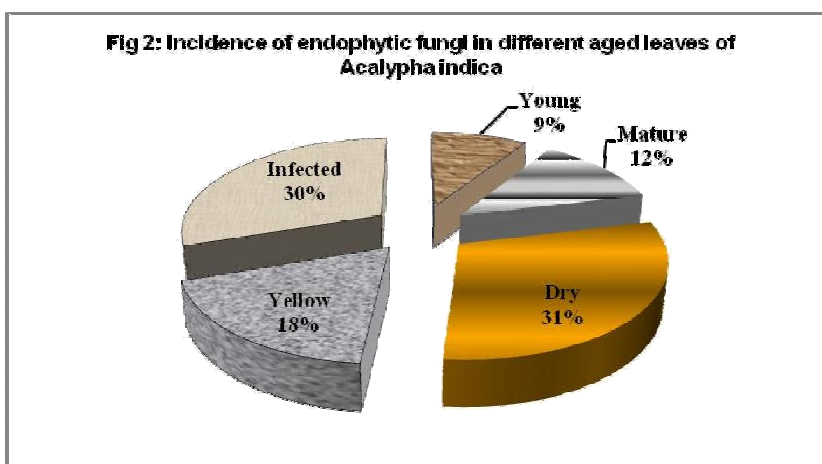
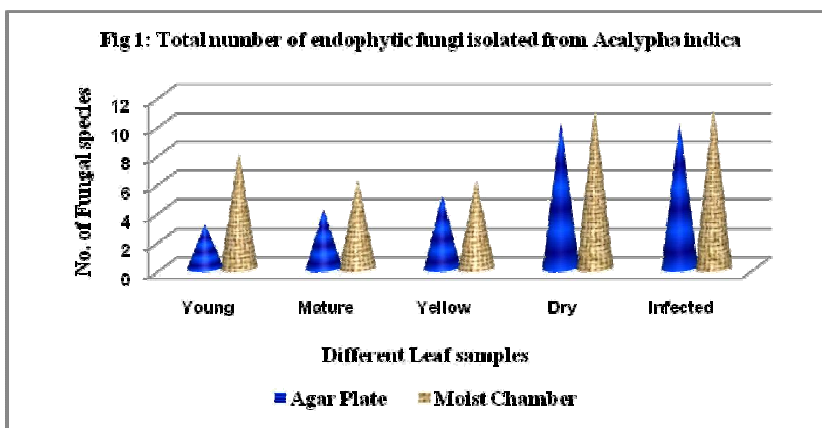
Plate I: Aged leaf samples of *Acalypha indica* collected for isolation of endophytic fungi

Isolation of endophytic fungi

For the present study, different aged leaf samples were rinsed gently in running tap water to remove dusts and debris. The leaves were cut into segments (0.5 – 1cm). The samples were immersed in 70% ethanol for 5 seconds followed by 4% sodium hypochlorite for 90 seconds and then rinsed in sterile distilled water for 10 seconds/ three times in a way. The excess moisture was blotted in a sterile filter paper. The surface sterilized segments were placed in petridishes containing PDA medium as well as in moist chamber plates. The petridishes were sealed using para film and incubated at $25 \pm 3^\circ\text{C}$ at 12-h light/dark cycle. After incubation of three day, the petridishes were monitored every day to check the growth of endophytic fungal colonies from the segments and were identified separately based on the availability of Laboratory manuals and references [6,7]. The sterile endophytes i.e., the non-sporulating sterile forms that could not be assigned to any taxonomic group were given separate numbers and maintained in pure cultures. They were distinguished from each other by their cultural characteristics such as colony morphology, growth rates, hyphal mat characteristics and pigmentation of the fungal colony and medium. All the endophytic isolates were documented and maintained in PDA slants. The tables and figures were prepared on the basis of presence and absence of endophytic fungi on the leaf samples.

RESULTS AND DISCUSSION

In *Acalypha indica*, altogether 20 fungal species were isolated under 15 genera from both agar plate and moist chamber method. Of which 14 species of 11 genera and 13 species of 10 genera were recorded from Agar plate and Moist chamber respectively. Incidence of endophytic fungi isolated from *Acalypha indica* is given in Table 1 & 2, which showed that five fungi were recorded both from Agar plate and moist chamber methods, i.e., *Alternaria alternata*, *Alternaria geophila*, *Botrytis cinerea*, *Walleimia sebi* and white sterile mycelium. Few fungi were recorded in all the leaf samples started from young to mature. In both the methods, infected and dry leaf samples harboured more number of endophytic fungi followed by yellow. The rest young leaf samples contain less number of fungi. The fungus *Geotrichum* sp. and *Helminthosporium* sp. were predominant in moist chamber method. Total number of fungi isolated from different leaf samples of *Acalypha indica* by both agar and moist chamber method is given in Fig. 1, which showed that infected and dry leaves of the plant harboured maximum number of endophytic fungi. As taking in the case of agar plate method infected and dry contain the maximum number of fungi followed by yellow and mature. The young leaf contains the less number of fungal species. But in the case of Moist chamber method infected and dry showed the maximum fungi followed by young and yellow leaves. Mature leaf contains less number of fungal species. The incidence of endophytic fungi in different aged leaves of the medicinal plant (Fig 2) showed that dry leaves carried 31% of total endophytic fungi in comparison to infected (30%) and yellow (18%). The result showed that moist chamber method was suitable for the isolation of fungal species in the leaves of *Acalypha indica*.



Moist chamber method was suitable to isolate and record the endophytic fungi correctly in comparison to agar plate method. White sterile mycelia and *Curvularia* were predominant in the agar plate method. But in moist chamber technique, *Colletotrichum* sp., *Curvularia*, *Penicillium citrinum* and White sterile mycelia were predominant.

Table 1: Occurrence of endophytic fungi isolated from *Acalypha indica* by Agar Plate method

Sl. No.	Endophytic fungi	Leaf samples				
		Young	Mature	Dry	Yellow	Infected
1	<i>Alternaria alternata</i>	-	+	+	+	+
2	<i>Alternaria geophila</i>	-	-	+	-	+
3	<i>Botrytis cinerea</i>	+	+	+	-	-
4	<i>Cladosporium</i> sp.	-	-	-	-	+
5	<i>Cladosporium</i> sp.	-	-	+	-	+
6	<i>Curvularia lunata</i>	-	-	+	+	+
7	<i>Curvularia geniculata</i>	-	-	+	+	+
8	<i>Fusarium oxysporum</i>	-	+	+	-	+
9	Green sterile mycelia	-	+	+	+	+
10	<i>Mortierella</i>	-	-	-	-	+
11	<i>Penicillium funiculosum</i>	+	-	+	+	-
12	<i>Wallemia sebi</i>	-	-	+	-	-
13	White sterile mycelia	+	-	-	+	+
14	<i>Ulocladium langinosum</i>	-	-	-	-	+

In this study, various leaf samples from the medicinal plant, *Acalypha indica* of Chennai region was screened for diversity and composition of endophytic fungal communities is equivalent to the previous work made by Goveas et al [8]. *Alternaria alternata*, *Aspergillus* sp., *Cladosporium* sp. etc isolated from *Acalypha indica* is agreed with the previous workers who had also reported the same endophytic fungi in their study [4,9]. These common endophytes were isolated frequently from the leaves of medicinal plants. Petrini [10] reported that *Alternaria* sp., *Cladosporium* sp. were not host specific fungi, but they used be recorded from tissues of different host plants. Few endophytic fungi may be highly host specific while others are generally distributed [10,11]. Petrini and Carroll [9] contended that fungal endophytes exhibit some degrees of host specificity at least for families of host plant and that this specificity determines endophytic distribution more than the geographic location of the host plant. The occurrence of the endophytes is influenced by the age of leaf tissues [9]. Generally their colonization frequency and species richness increase with the age of leaf tissue [2,3], which was proved in our study since the endophytic flora generally increased according to the ageing of the leaves. Studies on endophytic microbes over the past two decade indicate that they occupy a unique ecological niche and are thought to influence the plant distribution, their ecology, physiology and biochemistry [12]. Raviraja [13] isolated 18 species of endophytic fungi from bark stem and leaf segments of 5 medicinal plants species growing with the Western Ghats of India was mostly similar to our present work.

Table 2: Occurrence of endophytic fungi isolated from *Acalypha indica* by Moist chamber

Sl. No.	Endophytic fungus	Leaf samples				
		Young	Mature	Dry	Yellow	Infected
1	<i>Alternaria alternata</i>	+	-	+	-	+
2	<i>Alternaria geophila</i>	+	-	+	-	+
3	<i>Alternaria tenuis</i>	+	-	+	+	-
4	<i>Botrytis cinerea</i>	+	-	+	-	+
5	Brown sterile mycelia	-	+	-	+	+
6	<i>Cladosporium herbarum</i>	-	+	+	-	-
7	<i>Cladosporium</i> sp.	+	-	-	+	+
8	<i>Colletotrichum falcatum</i>	+	-	+	-	+
9	<i>Dreschleria</i> sp.	+	-	+	-	+
10	<i>Geotrichum</i> sp.	-	+	+	+	+
11	<i>Helminthosporium</i> sp.	+	+	+	+	-
12	<i>Wallemia sebi</i>	-	+	+	+	+
13	White sterile mycelia	-	+	+	-	+

CONCLUSION

Endophytic fungal community found more in the aged leaves of the medicinal plant, *Acalypha indica* plant in comparison to the young ones. Moist chamber was found to be the suitable method for isolation and enumeration of endophytic fungi correctly with regards to agar plates. The similarity coefficient was 44.4% between the endophytic fungi isolated by moist chamber method and the agar plate method from *Acalypha indica*.

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