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Analysis of similarity coefficient and enumeration of common endophytic fungi of five medicinal plants by moist chamber method

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ABSTRACT

Studies on similarity coefficient and enumeration of common endophytic fungi of young, mature, yellow, dry and infected leaf samples of five medicinal plants, viz., Acalypha indica, Andrographis paniculata, Cucumis maderaspatnus, Pongamia pinnata and Zizyphus mauritiana was carried out by moist chamber method in the Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies (Autonomous), Pondicherry, India. Altogether 40 endophytic fungi were isolated under 26 genera from various leaf samples of five medicinal plants. Of which, 12 species of 10 genera were isolated from Acalypha indica,12 species under 11 genera from Andrographis paniculata, 13 species of 10 genera from Cucumis maderaspatanus, 14 species of 12 genera from Pongamia pinnata and 14 species of 10 genera from Zizyphus mauritiana. Similarity coefficient of endophytic fungi isolated from medicinal plants showed that Andrographis paniculata and Cucumis maderaspatnus were more similar (38.5%) in the distribution of endophytic fungi followed by Acalypha indica and Pongamia pinnata (37.03%). The least number of similarity coefficients was found between Pongamia pinnata and Zizhyphus mauritiana (7.69%) followed by Acalypha indica and Zizhyphus mauritiana (8.0%). Common trend of fungal endophytes was not found among different leaves of the medicinal plants but few fungi showed their common occurrence among the medicinal plants. Pongamia pinnata and Zizyphus mauritiana were found to harbor maximum fungi in comparison to other plants. More endophytic fungi were recorded from young leaves of A. Paniculata and Z. maurtiana but least number were from C. maderaspatnus and Pongamia pinnata. Moreover, during the enumeration of endophytic fungi from leaf samples of medicinal plants, infected leaves were more prone to harbor more endophytes than dry, yellow and mature leaves of the plants.

Keywords: Similarity coefficient, Endophytic fungi, Medicinal plants, Moist chamber method

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INTRODUCTION

Endophytic fungi are found in all kinds of plants viz., trees, grasses and herbaceous plants. They live within the cortical region of the plant leaf without causing any symptoms or apparent dysfunction to the host [1]. The colonization of endophytic fungi in the leaves of plant is mora or less equivalent to those of plant mycorrhizae colonization comprises a sequence of steps involving host recognition by the fungus, spore germination, penetration of the epidermis and tissue colonization [2]. During the work of Clay [3], he described that natural substrates are infested by fungi without any external manifestation of disease other than endophytic ones. The term, endophytes itself has been contentious since its occurrence [4] and there are diverse reports that endophytes may amend to pathogen under few environment [5]. A number of unrefined plant communities whose endophytic fungal

biodiversity was not studied, especially in the tropical countries, basically in India. During modern time, many antimicrobial compounds are being produced by endophytic fungi in culture and they are proven as efficient drugs against pathogenic microbes. Research works on endophytic fungi of diverse medicinal plants in and around India were carried out by different workers, but there is no works in the equivalent field in Puducherry. It is obligatory to find out the patterns of allocation of endophytic fungi from different curative plants as well as their sequence on the leaves based on the ageing of the plant and to recognise the fungi related to the metabolites twisted from these medicinal plants. During the present study, it is an effort to isolate endophytic fungi from different age group leaves of *Acalypha indica, Andrographis paniculata, Cucumis maderaspatnus, Pongamia pinnata* and *Zizyphus mauritiana* collected from Kanchi Mamunivar Centre for Post Graduate Studies (KMCPGS) campus, Puducherry, India.

MATERIALS AND METHODS

Collection of plant samples

Leaf types based on different age group viz., young, mature, yellow, infected and dry of the medicinal plants of *Acalypha indica, Andrographis paniculata, Pongamia pinnata, Cucumis maderaspatnus* and *Zizyphus mauritiana* were carefully chosen, collected from Kanchi Mamunivar Centre for Post Graduate Studies (KMCPGS) campus, Pondicherry and brought to the Microbiology Laboratory, Department of Botany in aseptic condition and kept in the refrigerator at 4-8^{o}C up to the completion of the experiment.

Isolation of endophytic fungi

The 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 equidistantly in moist chamber Petridishes. The Petridishes were incubated at 25 ± 3^{o}C at 12-h light/dark cycle. After incubation of three to seven 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 [6,7,8]. 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. All the endophytic isolates were documented and maintained in PDA slants after their identification.

RESULTS AND DISCUSSION

During our study, altogether 40 endophytic fungi were isolated under 26 genera by moist chamber method from various leaf samples of five medicinal plants. Of which, 12 species of 10 genera were isolated from *Acalypha indica,*12 species under 11 genera were from *Andrographis paniculata,* likewise, 13 species of 10 genera from *Cucumis maderaspatanus,* 14 species of 12 genera from *Pongamia pinnata* and 14 species of 10 genera were from *Zizyphus mauritiana*. Incidence of endophytic fungi recorded from five medicinal plants by moist chamber method is given in Table 1. Table 2 explained about the similarity coefficient of endophytic fungal species isolated from the medicinal plants. Similarity coefficient value among the common endophytic fungi isolated from the leaf samples of *Andrographis paniculata* and *Cucumis maderaspatnus* were more similar (38.5%) in the distribution of endophytic fungi followed by *Acalypha indica* and *Pongamia pinnata* (37.03%). The least number of similarity coefficients was found between *Pongamia pinnata* and *Zizhyphus mauritiana* (7.69%) followed by *Acalypha indica* and *Zizyphus mauritiana* (8.0%). Total number of fungal endophytes isolated from different leaves of medicinal plants is plotted on Fig 1, which defined the pattern of incident of endophytes among the leaf samples. White sterile mycelia were recorded in maximum numbers in all the leaf samples. Some of the fungal species were found in all the leaf samples viz., *Alternaria alternata, Aspergillus niger, Penicillium citrinum, Colletotrichum falcatum, Botrytis cinerea* and white sterile mycelia. *Aureobasidium pullulans, C. Palescens* and *Nigrospora* sp. were isolated from *Pongamia pinnata. Aspergillus awamori, Fusarium moniliforme,* Green sterile mycelia, *Curvularia geniculata* and *Volutella buxi* were isolated from *Cucumis maderaspatinum* only. Likewise, *Alternaria geophila, Alternaria tenuis,* Brown sterile mycelia, *Geotrichum* sp. were restricted to *Acalypha indica. Aspergillus flavipes,* Grey sterile mycelia, *F. oxysporum, Humicola* sp., *Penicillium italicum* and *Saccharomyces* sp. were isolated from *Zyzphus mauritiana* (Table 1). *Pongamia pinnata* and *Zizyphus mauritiana* were found to harbor maximum fungi in comparison to other plants. Infected leaves were more flat to gather endophytes and it was followed by dry, yellow and mature leaves of

the plants. The moist chamber method was found suitable for isolation and enumeration of endophytic fungi and to identify fungi more accurately.

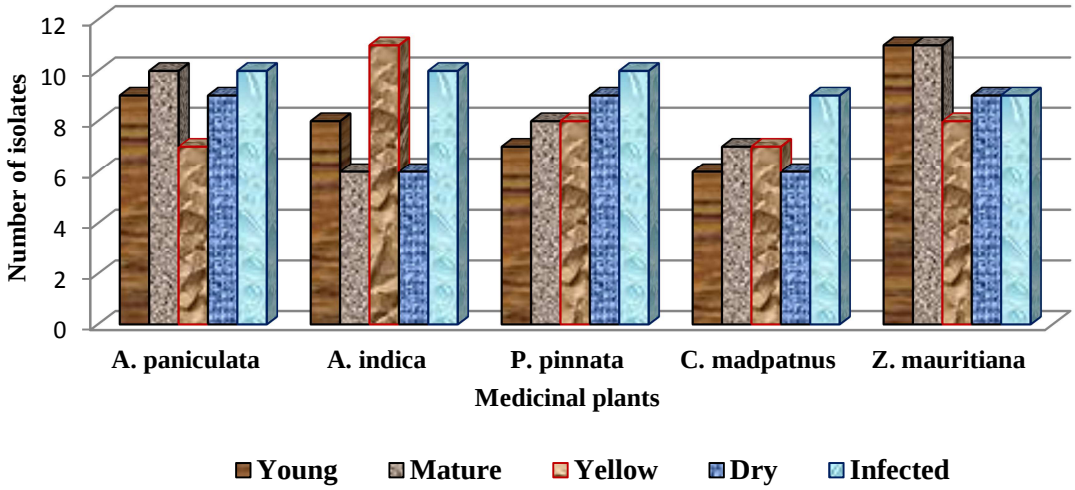
Table 1: Incidence of endophytic fungi recorded from five medicinal plants by moist chamber method.

Sl. No.	Endophytic fungi	Medicinal plants				
		Acalypha indica	*Andrographis paniculata*	*Cucumis maderaspatinum*	*Pongamia pinnata*	*Zizyphus mauritiana*
1	*Alternaria alternata*	+	+	+	+	+
2	*Alternaria geophila*	+	-	-	-	-
3	*Alternaria tenuis*	+	-	-	-	-
4	*Aspergillus awamorii*	-	-	+	-	-
5	*A. flavipes*	-	-	-	-	+
6	*A. fumigatus*	-	-	-	+	+
7	*A. niger*	-	+	+	-	+
8	*Aspergillus sp.*	-	+	+	-	-
9	*Aureobasidium pullulans*	-	-	-	+	-
10	*Botrytis cinerea*	+	+	+	-	-
11	Brown sterile mycelia	+	-	-	-	-
12	*Cercospora heteromella*	-	+	-	-	-
13	*Cladosporium cladosporioides*	-	-	-	+	+
14	*C. herbarum*	+	+	-	-	-
15	*Cladosporium* sp.	+	-	-	-	-
16	*Colletotrichum falcatum*	+	+	-	+	-
17	*Curvularia catenulata*	-	-	-	+	-
18	*Curvularia lunata*	-	-	+	-	+
19	*C. geniculata*	-	-	+	-	-
20	*C. pallescens*	-	-	-	+	-
21	*Dreshlerea* sp.	+	+	-	-	-
22	*Gliocladium* sp.	-	+	-	-	+
23	Grey sterile mycelia	-	-	-	-	+
24	Green sterile mycelia	-	-	+	-	-
25	*Fusarium moniliforme*	-	-	+	-	-
26	*F. oxysporum*	-	-	-	-	+
27	*Geotrichum* sp.	+	-	-	-	-
28	*Helminthosporium* sp.	+	-	-	+	-
29	*Humicola* sp.	-	-	-	-	+
30	*Nigrospora* sp.	-	-	-	+	-
31	*Penicillium citrinum*	-	+	+	-	+
32	*P. italicum*	-	-	-	-	+
33	Pink sterile mycelium	-	+	-	+	-
34	*Saccharomyces* sp.	-	-	-	-	+
35	*Trichoderma* sp.	-	+	-	+	-
36	*Ulocladium* sp.	-	-	+	+	-
37	*Volutella buxi*	-	-	+	-	-
39	*Wallemia sebi*	+	-	-	+	-
40	White sterile mycelia	+	-	+	+	+

Table 2: Similarity coefficient of endophytic fungal species isolated from five medicinal plants by agar plate method

Medicinal plants	*Andrographis paniculata*	*Acalypha indica*	*Pongamia pinnata*	*Cucumis maderaspatnus*	*Zizyphus mauritiana*
Andrographis paniculata	**0**	30.8	29.6	**38.5**	24.0
Acalypha indica	30.8	**0**	37.03	30.76	8.0
Pongamia pinnata	29.6	37.03	**0**	23.07	**7.69**
Cucumis maderaspatnus	**38.5**	30.76	23.07	**0**	30.8
Zizyphus mauritiana	24.0	8.0	**7.69**	30.8	**0**

Fig 1: Number of endophytic fungi isolated from different leaves of medicianl plants



The existence of maximum number of endophytic fungi in infected leaves compare to mature and yellow leaves may be due to the availability of more nutrients in the concerned leaves of the plants [9]. Moist chamber method was found suitable to isolate and to count fungal numbers may be due to its suitability to prevail humidity condition inside the chamber [9]. White sterile mycelia and *Alternaria* were predominant in the study and diverse fungi emerged from the leaf segments signifying that segments may be occupied by more than one fungus. The variety of endophytic fungi and their composition was found equivalent to the previous works made by others [9,10]. Our work suggested that the smaller and scattered plant fragments have more probability of approaching the real diversity of endophytic fungal communities. The recorded endophytes was agreed with the previous workers who had also reported the same endophytic fungi in their studies [1,9]. Nayak [9] and Petrini and Carroll [10] reported that *Alternaria* spp and *Cladosporium* spp were not host specific fungi, but they used to be recorded from most of the plants. a small amount of endophytic fungi may be highly host specific while others are generally distributed [9,11,12]. Nayak [13] also contended that endophytic fungi exhibit different degrees of host specificity including families of the host plant and this specificity determines endophytic distribution beyond the geographic location of the host plant. Availability of the endophytic fungi is influenced by the age of leaves and their colonization frequency and species abundance based on the age of the plant [9,13], which was proved in the study since the endophytic flora generally increased according to the senescence of the leaves [13,14].

CONCLUSION

Enumeration and identification of endophytic fungi was done from different leaf samples of five medicinal plants by moist chamber method. *Zyzyphus mauritiana* and *Pongamia pinnata* were found to harbor the maximum fungi in comparison to other plants. Infected leaves were more prone to harbor endophytes and it was followed by dry, yellow and mature leaves of the plants. Based on similarity coefficient analysis, *Andrographis paniculata* and *Cucumis maderaspatnus* were found more similar (38.5%) in the distribution of endophytic fungi followed by *Acalypha indica* and *Pongamia pinnata* (37.03%). The least number of similarity coefficients was found between *Pongamia pinnata* and *Zizyphus mauritiana* (7.69%) followed by *Acalypha indica* and *Zizhyphus mauritiana*

(8.0%). The moist chamber method was found suitable for isolation and enumeration of endophytic fungi in our present study.

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