



Common endophytic fungal isolates and similarity coefficient studies on different medicinal plants by agar plate method

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ABSTRACT

A comparative study of isolation and enumeration of endophytic fungi from young, mature, yellow, dry and infected leaf samples of five medicinal plants, viz., *Andrographis paniculata*, *Acalypha indica*, *Pongamia pinnata*, *Cucumis maderaspatnus* and *Zizyphus mauritiana* was carried out by agar plate methods in the Microbiology Laboratory, Department of Botany, K. M. Centre for P. G. Studies (Autonomous), Pondicherry, India. A number of fungi isolated from the medicinal plants were found common to each other. Similarity coefficient of endophytic fungi isolated by Agar plates showed that *Andrographis paniculata* and *Acalypha indica* were more similar (41.37%) in the distribution of endophytic fungi followed by *Zizyphus mauritiana* and *Andrographis paniculata* (35.71%). The least number of similarity coefficient was found between *Acalypha indica* and *Zizyphus mauritiana* (7.40%). Common number of species and similarity coefficient were (35.71%) found within the medicinal plants of *Andrographis* and *Zizyphus mauritiana*. No common trend of fungal endophytes found among different leaves of the medicinal plants. Maximum endophytes were isolated from *A. Paniculata* and least number from *Zizyphus mauritiana*. More endophytic fungi were recorded from young leaves of *A. Paniculata* but least number were from *A. indica* and *Z. mauritiana*, likewise more numbers were isolated from dry and infected leaves of *A. Indica* and the least were found in *C. maderaspatnus* than other leaves of medicinal plants. During the enumeration of endophytic fungi isolated from different leaf samples of five medicinal plants, *Andrographis paniculata* and *Pongamia pinnata* were found to harbor maximum fungi in comparison to other plants. Infected leaves were more prone to accumulate endophytes and it was followed by dry, yellow and mature leaves of the plants.

Keywords: Similarity coefficient, Endophytic fungal isolates, Medicinal plants

INTRODUCTION

Endophytic fungi that reside inside plant tissues without making any symptoms of disease to the host plant [1]. During the work, Clay [2] described that natural substrates are infested by fungi without any external manifestation of disease other than endophytic fungi. Endophyte term itself has been controversial since its occurrence [3] and there are various reports that endophytes may alter to parasites under few conditions [4]. The colonization of plant tissues by endophytic fungi occurs in a similar manner to those of plant pathogens and mycorrhizae colonization in sequence of steps involving host recognition by the fungus, spore germination, penetration of the epidermis and tissue colonization [5]. Still today there are a large number of unexplored plant communities whose endophytic fungal biodiversity was not known, especially in the tropical countries. Now a day, many antimicrobial compounds are being produced by endophytic fungi in culture and they are proven as effective drugs against pathogenic microorganisms. Works related to endophytic fungi of different medicinal plants in and around India were carried out by different workers, but there is little work in the same field in Puducherry State. It is necessary to find out the patterns of distribution of endophytic fungi from different medicinal plants as well as their succession on the leaves based on the ageing of the plant and to recognise the fungi related to the metabolites produced from these medicinal

plants. In the present study, it is an attempt to isolate endophytic fungi from the leaves of *Andrographis paniculata*, *Acalypha indica*, *Pongamia pinnata*, *Cucumis maderaspatnus* and *Zizyphus mauritiana* collected from K. M. Centre for Post Graduate Studies (KMCPGS) campus, Puducherry, India.

EXPERIMENTAL SECTION

Collection of plant samples

Various leaf types based on age viz., young, mature, yellow, infected and dry of the medicinal plants of *Andrographis paniculata*, *Acalypha indica*, *Pongamia pinnata*, *Cucumis maderaspatnus* and *Zizyphus mauritiana* were carefully chosen, collected from K. M. 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°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 in Petridishes containing PDA mediated 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 [6,7,8]. 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 and medium. All the endophytic isolates were documented and maintained in PDA slants.

RESULTS AND DISCUSSION

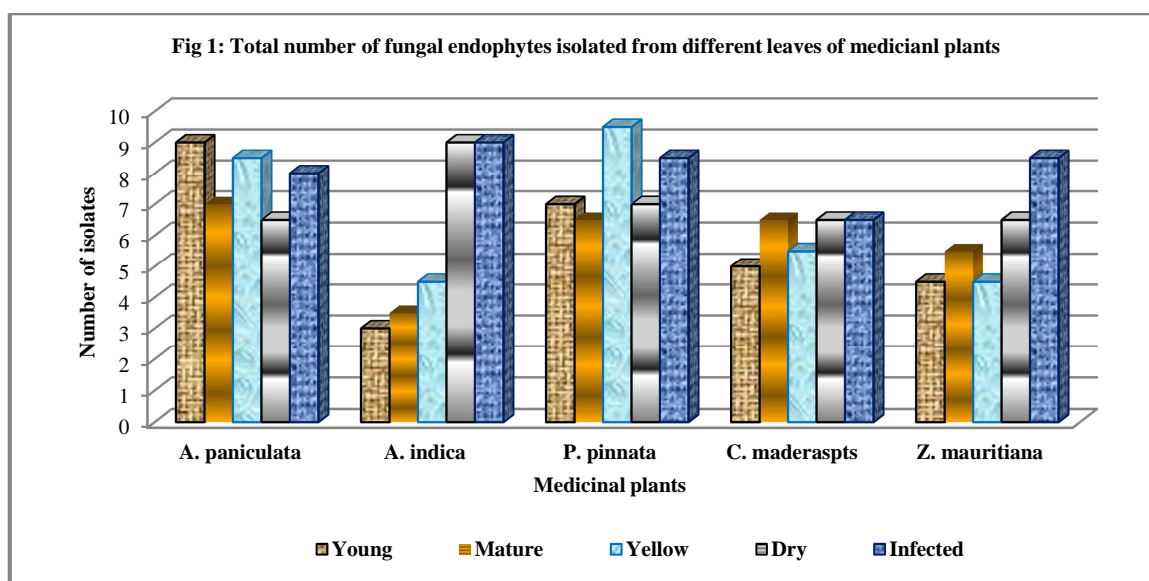
During our study, altogether 39 endophytic fungi were isolated under 24 genera from the Agar plate method from the varietal leaves of five medicinal plants. Of which, 15 species under 14 genera were recorded from *Andrographis paniculata*, likewise, 14 species of 11 genera from *Acalypha indica*, 13 species of 11 genera from *Pongamia pinnata*, 13 species of 12 genera from *Cucumis maderaspatanus* and 13 species of 10 genera from *Zizyphus mauritiana* were recorded. Incidence of endophytic fungi recorded from five medicinal plants by agar plate method is given in Table 1. Table 2 explained about the similarity coefficient of endophytic fungal species isolated from the medicinal plants. Total number of fungal endophytes isolated from different leaves of medicinal plants is plotted on Fig 1. White sterile mycelia were recorded maximum number in all the leaf samples. Most of the fungal species were found in all the leaf samples viz., *Alternaria alternata*, *Curvularia lunata*, *C. Geniculata*, *Botrytis cinerea* and white sterile mycelia were isolated from all leaf samples. *Nigrospora* sp. was only isolated from *Pongamia pinnata*. *Andrographis paniculata* and *Pongamia pinnata* were found to harbor maximum fungi in comparison to other plants. Infected leaves were more prone to accumulate endophytes and it was followed by dry, yellow and mature leaves of the plants. Similarity coefficient of endophytic fungi isolated by Agar plates showed that *Andrographis paniculata* and *Acalypha indica* were more similar (41.37%) in the distribution of endophytic fungi followed by *Zizyphus mauritiana* and *Andrographis paniculata* (35.71%). The least number of similarity coefficient was found between *Acalypha indica* and *Zizyphus mauritiana* (7.40%). Agar plate 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 agar plate method

Sl. No.	Endophytic fungi	Medicinal plants				
		<i>Andrographis paniculata</i>	<i>Acalypha indica</i>	<i>Pongamia pinnata</i>	<i>Cucumis maderaspatinum</i>	<i>Zizyphus mauritiana</i>
1	<i>Alternaria alternata</i>	+	+	+	+	+
2	<i>Alternaria geophila</i>		+			
3	<i>Aspergillus awanorii</i>				+	
4	<i>A. flavipes</i>					+
5	<i>Aspergillus flavus</i>					+
6	<i>A. fumigatus</i>			+		
7	<i>A. niger</i>				+	+
8	<i>A. penicilloides</i>			+		
9	<i>Aureobasidium pullulans</i>			+		
10	<i>Botrytis cinerea</i>	+	+	+	+	+
11	<i>Cercospora heteromella</i>	+		+		
12	<i>Cladosporium cladosporioides</i>		+	+		
13	<i>C. herbarum</i>	+				
14	<i>Cladosporium sp.</i>		+			
15	<i>Colletotrichum sp.</i>	+				
16	<i>Curvularia lunata</i>	+	+	+	+	+
17	<i>C. geniculata</i>	+	+	+	+	+
18	<i>C. palescens</i>			+		
19	<i>Gliocladium sp.</i>	+				+
20	<i>Grey sterile mycelia</i>					+
21	<i>Green sterile mycelia</i>	+	+		+	
22	<i>Fusarium moniliforme</i>				+	
23	<i>F. oxysporum</i>		+			+
24	<i>Helminthosporium sp.</i>			+		
25	<i>Humicola sp.</i>					+
26	<i>Mortierella sp.</i>		+			
27	<i>Neurospora sitophila</i>			+		
28	<i>Neurospora sp.</i>			+		
29	<i>Nigrospora sp.</i>			+		
30	<i>Penicillium citrinum</i>	+			+	+
31	<i>P. fellutanum</i>					+
32	<i>P. funiculosum</i>		+			
33	<i>Pink sterile mycelium</i>	+		+		
34	<i>Saccharomyces sp.</i>					+
35	<i>Ulocladium languginosum</i>				+	
36	<i>Ulocladium sp.</i>	+	+			
37	<i>Volutella buxi</i>	+			+	
38	<i>Wallemia sebi</i>	+	+		+	
39	<i>White sterile mycelia</i>	+	+	+	+	+

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

Medicinal plants	<i>Andrographis paniculata</i>	<i>Acalypha indica</i>	<i>Pongamia pinnata</i>	<i>Cucumis maderaspatanus</i>	<i>Zizyphus mauritiana</i>
<i>Andrographis paniculata</i>	0	41.37	21.4	21.42	35.71
<i>Acalypha indica</i>	41.37	0	29.6	22.2	7.40
<i>Pongamia pinnata</i>	21.4	29.6	0	7.69	15.38
<i>Cucumis maderaspatanus</i>	21.42	22.2	22.2	0	30.76
<i>Zizyphus mauritiana</i>	35.71	7.40	15.38	30.76	0



The 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 host plant [9]. Agar plate method was suitable to isolate and count numbers may be due to its host specific nature and suitable composition of media for growth of fungi. White sterile mycelia and *Curvularia* were predominant in the agar plate method and different fungi emerged from the leaf segments indicating that segments may be occupied by more than one fungus. In our study, leaf samples from five medicinal plants of Pondicherry U.T. region were screened for diversity and composition of endophytic fungal communities is equivalent to the previous works made by others [9,10]. The present data suggested that the smaller and the more scattered the plant fragments sampled, the higher the probability of approaching real diversity of endophytic fungal communities. The endophytic fungi isolated was agreed with the previous workers who had also reported the same endophytic fungi in their study [1,9]. Petrini and Carroll [10] reported that *Alternaria* spp, *Cladosporium* spp were not host specific fungi, but they used to be recorded from most of the plants. Few endophytic fungi may be highly host specific while others are generally distributed [11]. They [12] contended that fungal endophytes exhibit various 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 endophytic fungi is influenced by the age of leaf tissues and their colonization frequency and species richness increase with the age of the plant [9,10], which was proved in our study since the endophytic flora generally increased according to the aging of the leaves [9].

CONCLUSION

During the enumeration of endophytic fungi isolated from different leaf samples of five medicinal plants, *Andrographis paniculata* and *Pongamia pinnata* were found to harbor maximum fungi in comparison to other plants. Infected leaves were more prone to accumulate endophytes and it was followed by dry, yellow and mature leaves of the plants. Similarity coefficient of endophytic fungi isolated by Agar plates showed that *Andrographis paniculata* and *Acalypha indica* were more similar (41.37%) in the distribution of endophytic fungi followed by *Zizyphus mauritiana* and *Andrographis paniculata* (35.71%). The least number of similarity coefficient was found between *Acalypha indica* and *Zizyphus mauritiana* (7.40%). Agar plate method was found suitable for isolation and enumeration of endophytic fungi and to identify fungi more accurately.

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