

## Distribution of endophytic fungi in *Areca catechu* growing in two different habitats

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### ABSTRACT

Endophytic fungi were isolated from leaf and bark (phellophyte- endophytic fungi of bark) tissues of the monocotyledon tree, *Areca catechu* L. growing in two different habitats. Five hundred leaf and bark segments were screened for endophytic fungi. A total of 305 endophytic fungal isolates representing 27 different fungal taxa were isolated. Leaf tissue had more endophytic fungi than the bark tissue. *Hyphomycetes* were the most dominant group in this study followed by *Coelomycetes*, *Ascomycetes*, *Zygomycetes* and sterile mycelia. Both the habitats had few common endophytic fungi and some appeared to be host and habitat specific.

**Key words:** *Areca catechu*, leaf, bark, endophytic fungi, *Hyphomycetes*, habitat

### INTRODUCTION

Endophytic fungi live inside healthy tissues of plants as dormant microthalli and form symbiotic associations with them. Researches done in the last 30 years have indicated that the presence of endophytic fungi is ubiquitous. Almost 400,000 types of plants that exist on earth associate with one or several types of endophytes (Gary and Bryn, 2003). However, only a few have been explored for their fungal endophytic diversity including crop plants (Denise *et al.*, 2002), cacao (Hanada *et al.*, 2010), tropical palm (Azevedo *et al.*, 2000), oil palm (Pinruan *et al.*, 2010), medicinal plants (Rezwana *et al.*, 2010), Orchids (Bayman *et al.*, 1997) and forest plants (Suryanarayanan *et al.*, 2003). Structure and diversity of endophytic fungi were found to be different in each plant due to canopy cover, leaf age, location of plant growth (Debdulal, 2011), plant health (Rodriguez *et al.*, 2009) and climate (Shankar and Shashikala, 2010). Tropical plants are expected to support a high diversity of endophytes and only a few monocotyledon trees have been screened so far for endophytic fungi distribution (Rajagopal, 2004). There have been more studies on the endophytic fungi in temperate regions than in the tropical regions, but interest in these fungi in the tropics is escalating (Rodrigues and Petrini, 1997). Suryanarayanan (2017) reported that the horizontally transmitted endophytic fungi establish endosymbiotic relationship with host plants of all lineages. The global presence of endophytic fungi has been reported from plants of variable ecological niches. Further, these groups of fungi have the ability to produce an array of secondary metabolites with different bioactivity. This has led to the use of these fungi for biotechnological applications. Wang *et al.* (2016) revealed the chemical interactions of endophytic fungi and showed that they can be exploited as microbial factories and their sustainable applications in biotechnology.

*Areca catechu* L. often called the betel tree, is a handsome tree cultivated in warmer parts of Asia, and belongs to the family *Arecaeae* (commonly referred as Palm family). It has been used for a long time as a source of herbal medicine in many countries. Many studies have documented that *A. catechu* has

potential pharmacological effects because of being associated with antinematodal, antibacterial, anti-venom, antioxidant and molluscicidal activities. *A. catechu*, deserves more focus from the scientific community and public health specialists and there is plenty to be explored to reap its full range of benefits for the welfare of the society. Considering the above facts in the present investigation the leaf and bark of *A. catechu* are screened for endophytic assemblage.

### MATERIALS AND METHODS

The leaf and bark samples of host plant were collected from Yercaud Hill, (11°46'46" N 78°12'12" E) (Habitat 1) located in Salem and *Chengalpattu Reserve Forest* (12°41'60" N 79.98°E) (Habitat 2), 50 km south of Chennai. at an elevation of 1515 meters above sea level with a moderate climate (Habitat 1). During summer the temperature ranges from 16°C to 30 °C and in winters from 12 °C to 25 °C. The rainfall ranges between 1500-2000 mm. Chengalpattu reserve forest is situated near the coastal region with dry climate. Temperature during summer ranges from 21 °C to 45 °C and during winter from 14 °C to 28 °C. The rainfall ranges from 250 to 850 mm.

Leaf and bark segments with no visible symptoms of disease were carefully selected after physical examination. The bark tissues were collected randomly from ten trees from a distance of about 2 m above the ground. The collected samples were transported to the laboratory in a closed sterile polythene bags and processed within 24 hr of collection (Fisher and Petrini, 1987; Suryanarayanan *et al.*, 1998). About 500 segments, around 5 × 5 mm<sup>2</sup> were removed from the midrib region of healthy leaves and correspondingly 5 × 5 mm<sup>2</sup> were removed from the bark. To begin with the segments were surface sterilized by dipping in 70% ethanol (Merck, German) (for 30 Sec), immersed in 4% sodium hypochlorite (Sigma, St. Louis, MO, USA) (for 90 Sec) and rinsed in autoclaved double distilled water for 5 Sec (Dobranic *et al.*, 1995). As many as 5-6 segments per Petri dish were placed in Potato Dextrose Agar medium (PDA) with 10mg/L of chloramphenicol in order to inhibit the growth of bacteria.

Inoculated Petri dishes were incubated at 27 °C for 12 hrs under white fluorescent light: 12 hrs dark cycles (Bills and Polishook, 1992). Petri dishes were observed daily for up to 3-4 weeks. Endophytic fungi that grew from sterilized leaf segments were transferred to fresh slants. Colony characters such as growth rate, colony surface, texture, colony margin and pigmentation that are used to determine the specific sterile endophytic fungus were documented (Bills and Polishook, 1992; Rajagopal and Suryanarayanan, 2000).

**Data analysis:** Colonization Frequency (CF) was calculated as the number of plant segments colonized by single endophyte species divided by the total number of segments observed  $\times 100$  (Photitaet *et al.*, 2001). Isolation frequency (IF) was calculated as the total no. of isolates of one species divided by total no. of isolates in that sample  $\times 100$ . For calculating dominance and diversity of endophytic fungi Simpson dominance index and Shannon-Wiener's diversity index were used (Poole, 1974; Groth and Roelfs, 1987).

## RESULTS

Several trees and shrubs of tropical region have been extensively screened for the presence of endophytic fungi in their leaf and bark tissues however, in comparison very less attention has been paid to investigate the endophytic fungal distribution in palm family particularly in the tropics (Rajagopal, 2004; Frohlich *et al.*, 2000; Song *et al.*, 2015). In the present investigation *A. catechu*, a tropical palm was screened for endophytic fungi.

**Endophytic fungi distribution in leaf and bark tissues of Habitat I:** Although 22 different endophytic fungi were isolated from leaf and bark tissues of habitat I, only 11 endophytic fungi from leaf showed CF above 5% and in the bark tissue only 7 endophytic fungi showed CF above 5% (Table 1). In Habitat I, leaf of *A. catechu* yielded a total of 23 taxa and 106 isolates and bark yielded 20 taxa and 72 isolates (Table 1).

**Endophytic fungi distribution in leaf and bark tissues of Habitat II:** In habitat II leaf tissue had 20 endophytic fungi of which only 9 showed CF above 5%. In bark tissue a total 16 endophytic fungi were isolated and only 3 endophytic fungi showed CF above 5% (Table 1). In habitat II from leaf tissue 75 endophytic fungal isolates and from bark tissue in comparison only 52 endophytic fungal isolates were documented.

**Tissue specific distribution of endophytic fungi:** Although several endophytic fungi were present in both leaf and bark tissues, only few of them showed tissue specificity. *Curvularia geniculata*, *Drechslera*, *Penicillium sp.*, *Rhizopus oryzae* and sterile form 2 were present only in leaf tissue, whereas, *Acremonium cereals* was documented only in the bark tissue (Table 1).

**Habitat specific distribution of endophytic fungi:** Endophytic fungi like *Guignardia bidwellii*, *Acremonium cereals*, *Drechslera sp.*, *Penicillium sp.* 2, 3 and sterile form 2 were present only in habitat I (Table 1). Similarly, *Curvularia geniculata* and *Rhizopus oryzae* were isolated only from the habitat II (Table 1).

## DISCUSSION

The study shows that endophytic fungi colonizes both leaf and bark tissues of palm growing in two different habitats. In both the habitats leaf tissues had more endophytic fungi than bark tissue (Table 1). These findings were in consonance with findings of endophytic fungi in leaf tissue of *Azadirachta indica* and tropical forest trees which had more endophytic fungi in the leaf tissue in comparison to the bark tissue (Maheswari and Rajagopal, 2011; Suryanarayanan and Rajagopal, 2000; Kumaresan and Suryanarayanan, 2002). Both habitat I and II showed domination of Hypomycetous fungi followed by Coelomycetous fungi and Ascomycetous fungi. However the sterile forms and Zygomycetous fungi were less in number (Table 1, 2).

Endophytic fungi including *Chaetomium indicum*, *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Pestalotiopsis sp.*, *Phoma chrysanthemicola*, *Phomopsis sp.*, *Phyllosticta sp.*, *Htarraria alternata*, *Alternaria tenuissima*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus stellatum*, *Cladosporium sphaerospermum*, *Curvularia lunata*, *Drechslera hawaiiensis*, *Fusarium solani*, *Penicillium sp.*, *Trichoderma atroviride* and sterile form 1 were present in both the habitats investigated. In case of bark, more endophytic fungi could be isolated from higher altitude (Habitat I) and less endophytic fungi from plains (Habitat 2) (Table 1).

As per the studies undertaken by Petrini (1986) and Suryanarayanan and Rajagopal (1997), host plant can be dominated by only one or few endophyte species. *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Pestalotiopsis sp.*, *Phoma chrysanthemicola*, *Phomopsis sp.*, *Phyllosticta sp.*, *Alternaria alternata*, *Alternaria tenuissima*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus stellatum*,

**Table 1.** Distribution of endophytic fungi in leaf and bark tissues of *Areca catechu* growing in two different habitats

Name of the Endophyte	Habitat I (Yercaud Hill)						Habitat II (Jungle Forest)					
	Leaves			Bark			Leaves			Bark		
	<sup>o</sup> NE	<sup>o</sup> CF%	<sup>o</sup> IF	<sup>o</sup> NE	<sup>o</sup> CF%	<sup>o</sup> IF	<sup>o</sup> NE	<sup>o</sup> CF%	<sup>o</sup> IF	<sup>o</sup> NE	<sup>o</sup> CF%	<sup>o</sup> IF
<b>ASCOMYCETES</b>												
<i>Chaetomium indicum</i>	7	7.7	6.6	3	3.3	4.1	2	2.2	2.6	-	-	-
<i>Guignardia bidwellii</i>	5	5.5	4.7	5	5.5	6.9	-	-	-	-	-	-
<b>COELOMYCETES</b>												
<i>Botryodiplodia theobromae</i>	9	9.9	8.4	7	7.7	9.7	7	7.7	9.3	4	4.4	7.6
<i>Colletotrichum gloeosporioides</i>	4	4.4	3.7	2	2.2	2.7	3	3.3	4.0	1	1.1	1.9
<i>Pestalotiopsis sp.</i>	6	6.6	5.6	3	3.3	4.1	5	5.5	6.6	4	4.4	7.6
<i>Phoma chrysanthemicola</i>	4	4.4	3.7	3	3.3	4.1	5	5.5	6.6	2	2.2	3.3
<i>Phomopsis sp.1</i>	7	7.7	6.6	4	4.4	5.5	4	4.4	5.3	1	1.1	1.9
<i>Phyllosticta sp.</i>	4	4.4	3.7	4	4.4	5.5	1	1.1	1.3	4	4.4	7.6
<b>HYPHOMYCETES</b>												
<i>Acremonium cereals</i>	-	-	-	2	2.2	2.7	-	-	-	-	-	-
<i>Alternaria alternata</i>	7	7.7	6.6	5	5.5	6.9	4	4.4	5.3	6	6.6	11.5
<i>A. tenuissima</i>	5	5.5	4.7	1	1.1	1.3	3	3.3	4.0	2	2.2	3.3
<i>Aspergillus flavus</i>	7	7.7	6.6	-	-	-	5	5.5	6.6	2	2.2	3.3
<i>A. niger</i>	10	11.1	9.4	9	9.9	12.5	5	5.5	6.6	5	5.5	9.6
<i>A. stellanum</i>	3	3.3	2.8	5	5.5	6.94	1	1.1	1.3	6	6.6	11.5
<i>Cladosporium sphaerospermum</i>	5	5.5	4.7	-	-	-	-	-	-	1	1.1	1.9
<i>Curvularia lunata</i>	3	3.3	2.8	1	1.1	1.3	6	6.6	8.0	4	4.4	7.6
<i>C. geniculata</i>	-	-	-	-	-	-	3	3.3	4	-	-	-
<i>Drechslera hawaiiensis</i>	3	3.3	2.8	2	2.2	2.7	5	5.5	6.6	3	3.3	5.7
<i>Drechslera sp.3</i>	2	2.2	1.8	-	-	-	-	-	-	-	-	-
<i>Fusarium solani</i>	-	-	-	2	2.2	2.7	3	3.3	4.0	-	-	-
<i>Penicillium sp.1</i>	3	3.3	2.8	6	6.6	8.3	1	1.1	1.3	4	4.4	7.6
<i>Penicillium sp.2</i>	2	2.2	1.8	1	1.1	1.3	-	-	-	-	-	-
<i>Penicillium sp.3</i>	1	1.1	0.9	-	-	-	-	-	-	-	-	-
<i>Trichoderma atroviridae</i>	8	8.8	7.5	6	6.6	8.3	6	6.6	8.0	3	3.3	5.7
<b>ZYCOMYCETES</b>												
<i>Rhizopus oryzae</i>	-	-	-	-	-	-	5	5.5	6.6	-	-	-
<b>STERILE MYCELIUM</b>												
Sterile mycelia 1	-	-	-	1	1.1	1.0	1	1.1	1.3	-	-	-
Sterile mycelia II	1	1.1	1.0	-	-	-	-	-	-	-	-	-

<sup>o</sup>NE- Number of Endophytes; <sup>o</sup>CF- Colonization Frequency; <sup>o</sup>IF- Isolation Frequency

*Cladosporium sphaerospermum*, *Curvularia lunata*, *Drechslera hawasiensis*, *Fusarium solani*, *Penicillium* sp., *Trichoderma atroviride* were reported from both the tissues (leaf and bark) of two habitats studied. The endophyte assemblages of both the habitats were dominated by *Aspergillus niger*, *Alternaria alternata*, *Pestalotiopsis* sp., *Botryodiplodia theobromae* and *Trichoderma atroviride* (**Table 1**). This difference between the habitats and host tissue of endophytic taxa composition and their colonization frequency indicated that few endophytes were tissue specific and also selective in their distribution. Rajagopal (1998), Maheswari and Rajagopal (2011) and Suryanarayana (1998) reported that only one or few endophytic fungi dominate a single species.

During the present investigation sterile form I was documented from both the habitats while sterile form 2 was present only in the leaf of habitat I. This clearly indicates that these endophytic fungi are tissue and habitat specific (**Table 1**). These results support the findings of Bills and Polishook (1992), Kowalski and Kher (1992) and Suryanarayanan and Rajagopal (2000) who described the endophytic fungi colonizing bark tissues as Phellomyces. This study also indicated the presence of few phellomyces which are tissue specific such as *Acremonium cereals* that could only be found in the bark (**Table 1**). In a similar study only the bark of *Azadirachta indica* has been reported to show the presence of a dark septate endophyte (Suryanarayanan and Rajagopal, 2000). One fascinating thing is that the endophytic fungi occupying such niche as bark of *A. catechu* are rich in tannins - a class of antifungal compounds (Punnawich *et al.*, 2010). During the present study 21 of the 27 phellomyces species were also isolated from the leaf tissue of the *A. catechu*, which suggests that some of the phellomyces might have switched to endophytic form of life.

In addition to the ecological factors, there might be another factor that affected the endophyte diversity and dominance. The highest Simpson dominance indices of leaves of habitat I and habitat II was 0.9425 and 0.9383, respectively (**Table 2**). This did not show any discrepancies in their endophytic fungal distribution. These collective values are considerably on the higher side compared to the individual values that ranged from 0.9223 to 0.9425. This explains that individual tissues show less number of rare species than the entire endophytic community. The Shannon-Wiener diversity index showed that endophytic fungal inhabitants are distributed in an uneven manner in the tissues of both the habitats (**Table 2**).

*Areca catechu* growing in habitat I showed more diversity in leaf (2.952) as compared to (2.811) the bark and a similar pattern was observed in habitat II (2.863) (**Table 2**). The study indicates that habitat I shows comparatively higher diversity and distribution of endophytic fungi than habitat II. This could be due to habitat I environment being more conducive for endophytic mode of life. This might be due to more rainfall and humidity which favour the growth of endophytic fungi as compared to habitat II which is in plains with low rainfall and less humidity. These conditions serve as limiting factors fungal growth particularly during endophytic mode of life. Further studies on these endophytes need to be initiated

**Table 2.** Diversity, Dominance and Evenness indices of endophytic fungi isolated from leaf and bark tissues of *Areca catechu*

Location	Name of the Tissue	Total Number of Isolates	Simpson Index	Shannon Wiener Index	Evenness	Dominance
Habitat I (Vercaud Hill)	Leaf	106	0.9425	2.952	0.8702	0.05749
	Bark	72	0.9313	2.811	0.8309	0.06867
Habitat II (Jungle Forest)	Leaf	75	0.9383	2.863	0.8759	0.06169
	Bark	52	0.9223	2.643	0.8785	0.07766

for the production of bioactive compounds as they are a promising source in this regard. Because endophytes are known to produce bioactive compounds in culture and they are active against human and plant pathogens as well. (Pettrini *et al.*, 2001; Tan and Zou, 2001).

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