

Original Research Article

Diversity of Endophytic Mycobiota colonizing the Aerial tissues of *Thuja plicata* (Donn ex. D. Don.)

S.Chandrasekar¹, S.Thiyagarajan^{2*}, R.Sridhar¹ and B.Ambethkar¹

¹CAS in Botany, University of Madras, Maraimalai (Guindy) Campus,
Chennai-600 025, India

²P.G and Research Department of Microbiology, Asan Memorial College
of Arts and Science, Chennai-600 100, India

*Corresponding author e-mail: drestear@gmail.com

A B S T R A C T

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Endophytic fungi are generally recognized as one of the important sources of novel bioactive compounds and secondary metabolites for various biological purposes. The present investigation was undertaken to document the diversity of endophytic fungi of the Gymnospermous plant, *Thuja plicata* (Donn ex. D. Don.). The endophytic fungi which are found colonizing the tissues of the host plant are biologically significant ones. A total of 100 segments of aerial parts (leaf and stem) of *Thuja plicata* (Donn ex. D. Don.) plant were collected from Chennai district of Tamil Nadu, India and processed for the isolation of endophytic fungi. The endophytic fungi thus isolated were identified and grouped based on their macro and micro morphological traits adopting standard monographs into 14 fungal taxa. The result revealed the preponderance of *Phomopsis* sp. and *Colletotrichum* sp. in the aerial parts of the *Thuja plicata* (Donn ex. D. Don.).

Introduction

Endophytes, the microorganisms that reside in the internal tissues of living plants without causing any immediate overt adverse effects, have been reported to occur in almost all the plant species examined till date. These endophytic microorganisms are recognized as the potential sources of many bioactive natural products for their exploitation in agriculture, medicine and food industry (Bacon and White, 2000; Strobel and Daisy, 2003).

Plant endophytic fungi are defined as those which spend the whole or part of their lifecycle colonizing inter-cellularly or intra-cellularly inside the healthy tissues of the host plants, typically causing no apparent symptoms of disease. They play a vital role and constitute an important component of plant micro-ecosystems (Tan and Zhou, 2001; Rodriguez *et al.*, 2009). Plant endophytic fungi are found in each plant species examined so far, and it is estimated that there exist over one

million fungal endophytes in nature (Petrini, 1991). Endophytic fungi have been isolated from all parts of the plant including leaves, twigs, barks and roots of several angiosperms and gymnosperm members (Stone and Becon, 2000). Fungal endophytes belong to Ascomycetes and Deuteromycetes (Mitosporic fungi) and very few members of Basidiomycetes family generally occur as endophytes (Petrini, 1986). The fungi of the Ascomycetes genera such as *Chaetomium*, *Glomerella* and *Guignardia* are commonly encountered as endophytes (Suryanarayanan *et al.*, 1998). Among these endophytic Ascomycetes, the xylariaceous forms are often isolated from tropical hosts (Dreyfuss and Petrini, 1984). Some Phylloplane fungi such as *Alternaria*, *Aureobasidium* and *Cladosporium* are routinely isolated as endophytes from a wide range of plant species growing in different habitats (Petrini, 1986).

Plants with ethnobotanical and pharmaceutical potential are often exploited for the purpose of extraction of bioactive compounds, thus posing a great threat to sustenance of biodiversity in a given geographical area. Instead, the endophytic mycobiota which colonize such plants considered to be the potential sources of therapeutic compounds may be explored for desirable applications. In view of this, the present research work was designed to investigate the distribution, prevalence of endophytic fungi and their host affinity pertaining to the plant *Thuja plicata* (Donn ex. D. Don.).

Thuja plicata (Donn ex. D. Don.), commonly known as Western or Pacific red cedar, is an evergreen coniferous tree of the cypress family Cupressaceae and of

the native of Western North America (Figure.1). It is generally grown as an ornamental plant throughout the world especially in tropical countries. In the present study, the aerial parts (leaf and stem) of Gymnospermous plant *Thuja plicata* (Donn ex. D. Don.) were examined to explore the endophytic mycobiota colonizing the plant.

Figure.1 *Thuja plicata*-A twig showing aerial parts



Materials and Methods

Healthy leaf and stem parts of *Thuja plicata* (Donn ex. D. Don.) were collected from various places including domestic gardens, public parks, plant nurseries, etc. located in and around of Chennai city of Tamil Nadu, India. The samples (5 – 10 leaves and stem of 8 – 10 cm. length) were collected by aseptic procedures and brought to the laboratory of Center for Advances Studies in Botany, University of Madras, Chennai, India and processed within 24 hours of collection.

Isolation of Endophytic fungi

The samples were surface sterilized by following the modified protocol of

Dobranic *et al.*, (1995). The samples were washed in running tap water and cut into pieces of 0.5 cm diameter. These segments were then surface sterilized by immersing them in 70% ethanol for 5 seconds, followed by treatment in 4% sodium hypochlorite (NaOCl) for 90 seconds and then rinsing in sterile distilled water for 10 seconds. The excess moisture adhering to the plant materials was removed by blotting.

The samples thus prepared were then individually inoculated on to a petridish containing PDA (Potato Dextrose Agar) medium amended with Chloramphenicol (150mg/l). The petridishes were sealed using Parafilm™ and incubated at 25 ± 1°C in a light chamber [Bills and Polishook, 1992] and were monitored every day for the growth of endophytic fungi. Further to the development of colonial growth on the media, the fungi were isolated from the main culture plate and made into pure cultures in test tubes containing sterile SDA slopes.

The pure cultures were continuously monitored for spore formation. The isolated endophytic fungi were identified based on their macro and microscopic morphology and by using standard monographs. The colonization frequency (CF %) of each endophytic fungi was calculated and determined using the following formula (Hata and Futtai, 1995):

$$CF \% = \frac{\text{Number of segments colonized by an endophyte}}{\text{Total number of segments}} \times 100$$

Result and Discussion

The leaf and stem segments (each 50 samples) of the gymnospermous plant

Thuja plicata (Donn ex. D. Don.) were processed by standard mycological procedures for the isolation and identification of endophytic mycoflora. A total of 14 fungal species belonging to the families Hypomycetes and Coelomycetes were obtained (Figures. 2- 4; Table 1). Marginal differences with respect to their colonization frequencies among these fungi isolated from leaf and stem samples were recorded (Tables 2, 3). The number of endophytic fungal taxa occurring in the stem samples was similar to that of leaf samples. Among the fourteen identified fungal species, four species such as *Penicillium oxalicum*, *Colletotrichum gloeosporioides*, *Phomopsis* sp. and *Alternaria* sp. were found in both stem and leaf segments. The remaining nine species were specifically and individually associated with stem and leaf segments.

The fungi *Chaetomium spirale*, *Dothiorella* sp., *Aspergillus fumigatus* and *Camarosporium* sp. were found only in the stem samples, while *Aspergillus flavus*, *Pestalotiopsis* sp., *Phoma* sp., *Fusarium* sp. and *Phyllosticta* sp. were recovered only from the leaf samples. One isolate of unknown taxa (sterile form) was found associate with the stem sample with the colonization frequency of 10. The endophytic fungal taxa *Phomopsis* sp. and *Colletotrichum gloeosporioides* were the most predominant fungi isolated from leaf samples and the colonization of *Alternaria* sp. was low in leaf samples (Table 2). The examination of fungal isolates of stem segments indicated there was a preponderance of *Phomopsis* sp. while the *Camarosporium* sp. was found to be least occurring fungi (Table 3).

The survey of endophytic fungi occurring in tropical trees constitutes a very

Table.1 Isolation of Endophytic fungi from *Thuja plicata* (Donn ex. D. Don.)

S.No	Endophytic Fungi Isolated	No. of isolates	
		Leaf segments (n=50)	Stem segments (n=50)
1	<i>Aspergillus flavus</i>	6	-
2	<i>Chaetomium spirale</i>	-	2
3	<i>Penicillium oxalicum</i>	9	3
4	<i>Colletotrichum gloeosporioides</i>	12	7
5	<i>Pestalotiopsis sp.1</i>	5	-
6	<i>Phomopsis sp.</i>	14	10
7	<i>Phoma sp.</i>	7	-
8	<i>Alternaria sp.</i>	4	3
9	<i>Fusarium sp.</i>	6	-
10	<i>Phyllosticta sp.</i>	10	-
11	<i>Dothiorella sp.</i>	-	2
12	<i>Aspergillus fumigatus</i>	-	9
13	Unknown taxa 1(sterile form)	-	5
14	<i>Camarosporium sp.</i>	-	1
	Total	73	42

Figure.2 Development of mycelium from mature stem segments of *Thuja plicata*



Figure.3 Colony morphology of Endophytic mycoflora



Figure.4 Microphotographs of Endophytic fungi isolated from *Thuja plicata*

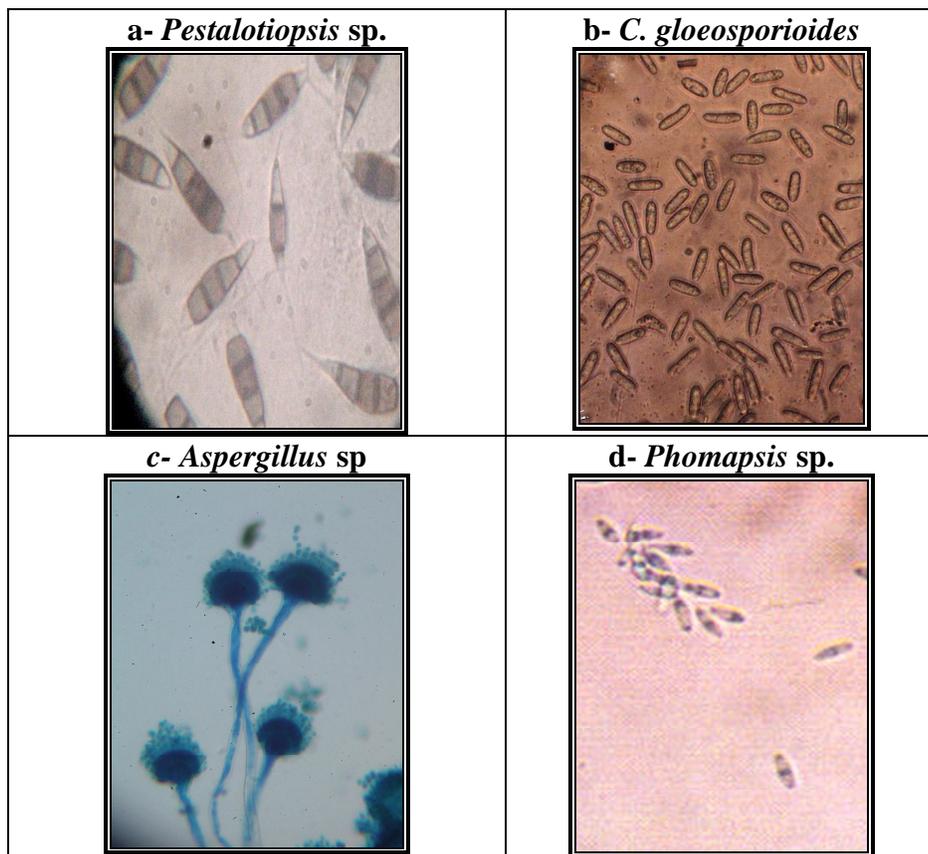


Table.2 Colonization frequency of endophytic fungi isolated from Leaf segments of *Thuja plicata* (Donn ex. D. Don.)

S.No	Endophytic Fungi Isolated	No. of colonies grown	% of Colonization Frequency
1	<i>Aspergillus flavus</i>	6	12
2	<i>Chaetomium spirale</i>	-	-
3	<i>Penicillium oxalicum</i>	9	18
4	<i>Colletotrichum gloeosporioides</i>	12	24
5	<i>Pestalotiopsis sp.1</i>	5	10
6	<i>Phomopsis sp.</i>	14	28
7	<i>Phoma sp.</i>	7	14
8	<i>Alternaria sp.</i>	4	8
9	<i>Fusarium sp.</i>	6	12
10	<i>Phyllosticta sp.</i>	10	20
11	<i>Dothiorella sp.</i>	-	-
12	<i>Aspergillus fumigatus</i>	-	-
13	Unknown taxa 1(sterile form)	-	-
14	<i>Camarosporium sp.</i>	-	-

Table.3 Colonization frequency of endophytic fungi isolated from Stem segments of *Thuja plicata* (Donn ex. D. Don.)

S.No	Endophytic Fungi Isolated	No. of colonies grown	% of Colonization Frequency
1	<i>Aspergillus flavus</i>	-	-
2	<i>Chaetomium spirale</i>	2	4
3	<i>Penicillium oxalicum</i>	3	6
4	<i>Colletotrichum gloeosporioides</i>	7	14
5	<i>Pestalotiopsis sp.1</i>	-	-
6	<i>Phomopsis sp.</i>	10	20
7	<i>Phoma sp.</i>	-	-
8	<i>Alternaria sp.</i>	3	6
9	<i>Fusarium sp.</i>	-	-
10	<i>Phyllosticta sp.</i>	-	-
11	<i>Dothiorella sp.</i>	2	4
12	<i>Aspergillus fumigatus</i>	9	18
13	Unknown taxa 1(sterile form)	5	10
14	<i>Camarosporium sp.</i>	1	2

important component in congregating the quantifiable data of fungal diversity. Owing to their high abundance and close association with numerous host species, the fungal endophytes appear to be vastly diverse among tropical gymnosperms.

With a view of investigating the diversity of endophytic fungi occurring in the plant tissues a total of one hundred samples of stem and leaf segments of the tropical gymnosperm *Thuja plicata* (Donn ex. D. Don.) were subjected to standard procedures for isolation of fungi. A total no. of 115 isolates identified to be of 14 fungal species were isolated from the samples. Petrini (1986) from their study on the isolation of endophytic fungi from several plant species had reported that the fungi *Phomopsis* sp. were the common endophytic fungi of gymnosperm trees. In accordance with their finding the present study has witnessed that the colony frequency of *Phomopsis* sp. were substantially higher compared to the other fungi. Fungal endophytes such as *Colletotrichum* sp. and *Phomopsis* sp., although reported to be not host specific, have been isolated from tissue of different host plants. The occurrence of endophytic fungi, *Phomopsis* sp. in the aerial roots of *Ficus bengalensis* had been well documented by Suryanarayanan and Vijaya Krishnan (2001).

Pestalotiopsis sp., which is generally regarded as microbial factory of evergreen forest with wide genetic and biological activity (Li *et al.*, 1996) has been isolated as an endophyte in the present study with a significant colonization frequency.

The occurrence endophytes like *Chloroscypha* sp., *Cylindrosporella* sp., *Geniculosporium* sp., *Microsporella* sp., etc. in *Thuja plicata* (Donn ex. D. Don.) of Oregon had been reported by Petrini and

Carroll (1981). However none of these fungi have been isolated in the present study which investigated *Thuja* of Chennai region. Earlier studies on *Colletotrichum gloeosporioides* isolated from the Angiospermic plant *Plumeria acutifolia* had reported the production of an effective taxol (an anticancer drug) by this endophytic fungus (Nithya and Muthumary, 2009). The *Colletotrichum gloeosporioides* has been isolated in this study with a colonizing frequency of 24% and 14% respectively from leaf and stem of *Thuja plicata*. This finding advocates the possible exploration of many such bioactive compounds from this potential fungi.

The occurrence of fungal endophytes such as *Aspergillus* sp., *Phoma* sp. and *Penicillium* sp. in mangrove tree *Avicennia marina* had been reported by many earlier researchers (Bharathidasan and Panneer selvam, 2011). Although these endophytes had been reported to occur in halophytic plants, the present study gains its significance by isolating them from the tropical terrestrial plant *Thuja plicata*. The *Dothiorella* sp. isolated from mangrove plant *Avicennia* had been proved to possess antimicrobial activity and cytotoxicity effect against human epidermal carcinoma of oral cavity (Xu *et al.*, 2005). The present study has documented the isolation of this endophytic fungi in low colonization frequency in the stem of *Thuja plicata* (Donn ex. D. Don.), which could be explored for more biological activities.

Another interesting observation made in this study is that most of the fungal endophytes colonizing the aerial tissues of *Thuja plicata* (Donn ex. D. Don.) were of economically significant taxa. Although the isolates obtained in the present study

are common endophytes of most plants, further insight investigation of these strains might pave way for their uniqueness in producing compounds of biological importance.

Thus the present study could be considered as an earnest attempt in exploring the common ornamental tropical plant *Thuja plicata* (Donn ex. D. Don.) for the isolation of endophytic fungi distributed in South India. The study also advocates the need for preserving the diversity of both the tropical plants and their endophytic fungi, thereby researchers could make noble attempt in extracting precious bioactive compounds endowed with various pharmacological properties.

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