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Research Article

BUTEA MONOSPERMA ROXB. EX WILLD ASSOCIATED WITH ENDOPHYTIC FUNGAL

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ABSTRACT

Endophytic fungi are one of the most unexplored and diverse group of organisms that make symbiotic associations with higher life forms and are said to produce beneficial metabolites for host. So for only few plants have been screened for their endophyte biodiversity. *Butea monosperma* Roxb. ex willd. known as "*Flame of forest*" possess medicinal properties. A total of 200 segments from 20 different plant parts were screened for their endophytic mycoflora. Twenty one fungal species of *Aspergillus* (11%), *Alternaria* (9%), *Chaetomiun* (13%), *Colletotrichium* (8%), *Fusarium* (12%), *Mucor* (12%), *Micrococus* (10%), *Penicillium* (12%), *Rhizopus* (9%) and *Verticillium* (5%) were isolated. In this study the most dominant endophytic flora was found to be *Chaetomium globosum* (13%).

KEY WORDS

Biodiversity, Endophytic fungi, *Butea monosperma, Chetomium globosum,* Medicinal plant.

INTRODUCTION

Endophytic fungi represent an important and quantifiable component of biodiversity. This group colonizes living, internal tissue of plants without causing any immediate, overt negative effects (Krings *et al.,* 2007). Dreyfuss & Chapela (1994) estimated that there may be at least one million species of endophytic fungi alone. Recent studies of endophytic fungi from tropical and temperate forest support the high estimates of species diversity (Sanchez Marquez *et al.,* 2007).

A variety of relationships exist between fungal endophytes and their host plants, ranging from mutualistic or symbiotic to antagonistic or slightly pathogenic (Arnold, 2007). They may produce a plethora of substances of potential use to modern medicine, agriculture, and industry, such as novel antibiotics, antimycotics, immuno-suppressants, and anticancer compounds (Mitchell *et al.,* 2008). There is great potential of finding new drugs from endophytes for treating new disease in animals (Kumar *et al.,* 2005), in addition the studies of endophytes fungi and their relationship with host plants will shed light on the ecology and evolution of both the endophytes and their hosts: the evolution of endophyte-plant symbioses; the ecological factors that influence the direction and strength of the endophyte-host plant interaction (Saikkonen *et al.,* 2004).

The relationships of endophytes with single or multiple plant hosts can be described in terms of host-specificity, host-recurrence, host selectivity, or host-preference (Cohen, 2006). Host-specificity is the relationship in which a fungus is restricted to a single host or a group of related species, but does not occur in other unrelated plants in the same habitat. The frequent or predominant occurrence of an endophytic fungus on a particular host or a range of plant hosts is often defined as host-recurrence, but the fungus can also occur infrequently on other host plants in the same habitat (Zhou and Hyde, 2001). A single endophytic fungal species may form relationships with two related plant species but demonstrate a preference for one particular host, and this phenomenon is categorized as host-selectivity (Cohen, 2004,). The term 'host-pre-ference', however, is more frequently used by mycologists to indicate a common occurrence or uniqueness of the occurrence of a fungus on a particular host, and the term is also used to indicate the differences in fungal community compositions and isolation frequencies from different host plants (Bettucci *et al.,* 2004). The differences in endophyte assemblages from different hosts might be related to the chemical differences of the hosts (Paulus *et al.,* 2006). Medicinal plants are known to harbour endophytic fungi that are believed to be associated with the production of pharmaceutical products (Zhang *et al.*, 2006). It is believed that medicinal plants and their endophytic flora produce similar pharmaceutical products. The use of endophytic fungus for the production of pharmacologically active metabolites has been on rise. Taxol, a potent anticancer drug, is one such example, produced by *Taxus brevifolia* plant and endophytic fungi, including *Taxomyces andreanae* and several other fungi (Gangadevi & Muthumary, 2007).

Butea monosperma (Palas) is a medium-sized deciduous tree belongs to family Leguminosae-Papilioneae. This tree is also called 'Flame of the Forest' and Bastard Teak. It is found in grater parts of India, Burma and Sri Lanka. It is capable of growing in water logged situation, black cotton soils, saline, alkaline, swampy badly drained soils and on barren lands except in arid regions. It is erect, medium sized tree of 12-15m high, with a crooked trunk and irregular branches, the shoots are clothed with gray or brown silky pubescence. The bark is ash coloured. The leaves 3 foliate, large and stipulate,. Petiole is 10-15 cm long. Leaflets are obtuse, glabrous above, finely silky and conspicuously reticulate veined beneath with connate or deltoid base. From January to March the plants is bald, flower in rigid racemes of 15 cm long, densely brown velvety on bare branches. Calyx is dark, olive green to brown in colour and densely velvety outside. The corolla is long with silky silvery hairs outside and bright orange red; stamens are diadelpous, anthers uniforms. Ovary with 2 ovule, style filiform, curved and stigma capitate. Pods argenteo-cansent, narrowed, thickened at the sutures, splitting round the single apical seed, lowest part indehiscent; the seeds are flat, reniform, curved.

The present study was conducted to determine the Biodiversity of the Endophytic fungi isolated from *Butea monosperma.*

MATERIAL AND METHODS

Isolation of endophytic fungi from plants

Stems and leaves of *Butea monosperma* were sampled for the investigation of endophytic fungal communities. Healthy and mature plants were carefully chosen for sampling. Samples from different sites of each plant were randomly collected and brought to the laboratory in sterile bags and processed within a few hours after sampling to reduce the chances of contamination.

Surface sterilization and incubation

Isolation of endophytic fungi was done according to the method described by Petrini (1986). The plants samples were rinsed gently in running water to remove dust and debris. Leaf samples were cut into 3-4 x 0.5-1 cm pieces with and without midrib; stems and roots samples were cut into 0.5-1.0 cm long pieces. Each sample was disinfected with 75% ethanol for 1 min followed by immersion in Sodium hypochlorite (NaOCl 1-13% for 3-10 minutes, depending on the type of samples) and then once again in 75% ethanol for 30 seconds. The segments were then rinsed three times in sterile distilled water and the pieces were blotted-dry on sterile blotting paper. The efficiency of surface sterilization procedure was ascertained for every segment of tissues as per method of Schulz *et al.*, (1993). Isolation of fungi from leaves, stems, and roots pieces were made separately. About 5-6 segments were placed on Potato dextrose agar (PDA) supplemented with penicillin G (100 units ml-1) and streptomycin (100 µg ml-1). The dishes were sealed with parafilm and incubated at 27±2^{o}C for 4-6 days. Most of the fungal growth was initiated within 3 days of inoculation. The fungi that grew out from the segments were periodically isolated and identified by transferring the hyphal tips to fresh PDA plates without antibiotics.

Colonization Frequency (CF) was calculated as described by Suryanarayanan *et al.,* (2003). Briefly, proper time of incubation was given for CF counting.

Colonization frequency (%) = (Number of segment colonized by fungi X 100) / Total no of segment observed

Fungi were grown on specified media under specified culture condition, for identification. The fungi were identified on the basis of their morphological and cultural characteristics (Sutton, 1980). This experiment was repeated three times with similar pattern and showed all the result of one experiment.

RESULT

The plant materials were collected from different places of India. Two hundred- (116 leaf samples and 84 stem samples) segments from 20 plants of *Butea monosperma* were processed for the isolation of endophytic fungi. Twenty five morphological funguses are *Alternaria alternate; Alternaria mali; Alternaria carthami; Aspergillus flavus; Aspergillus sp.; Aspergilus niger; Chetomium globosum; Chetomium indicum; Chetomium subterraneum; Colletotrichum capsici; Colletotrichum musae; Fusarium udum; Fusarium moniliformi; Fusarium roseum; Micrococus manginii; Mucor spp.; Penicillium digitatum; Penicillium expansum; Penicillium itaclicum; Rhizopus arrhizus ; Verticillium alvo atrum* isolated. (Table 1) These fungi belong to Ascomycotina, Deuteromycotina, and Zygomycetes. Most fungi isolated from this plant produced only sterile mycelium, while a few fungi produced pycnidia. In this study the most dominant endophytic fungus in *Butea monosperma* flora was found to be *Chaetomium globosum*. It has been isolated from leaf. It was isolated three times from three different plants at different times (i.e. February, July, and November). 121 segments have endophytic fungus but 79 segments have no fungus. Overall colonization frequency was measured as 10-13% of surface sterilized tissues Table 2. Experiment was repeated 3 times with similar pattern and showed no variation in isolation of endophytic fungus, but only the difference in unidentified sterile mycelia isolation. Many of the pharmaceutical compounds produced by medicinal plants are reportedly produced by their endophytic fungi.

Figure 1: Butea monosperma

Table 1: Endophytic fungi isolated from different parts of Butea monosperma

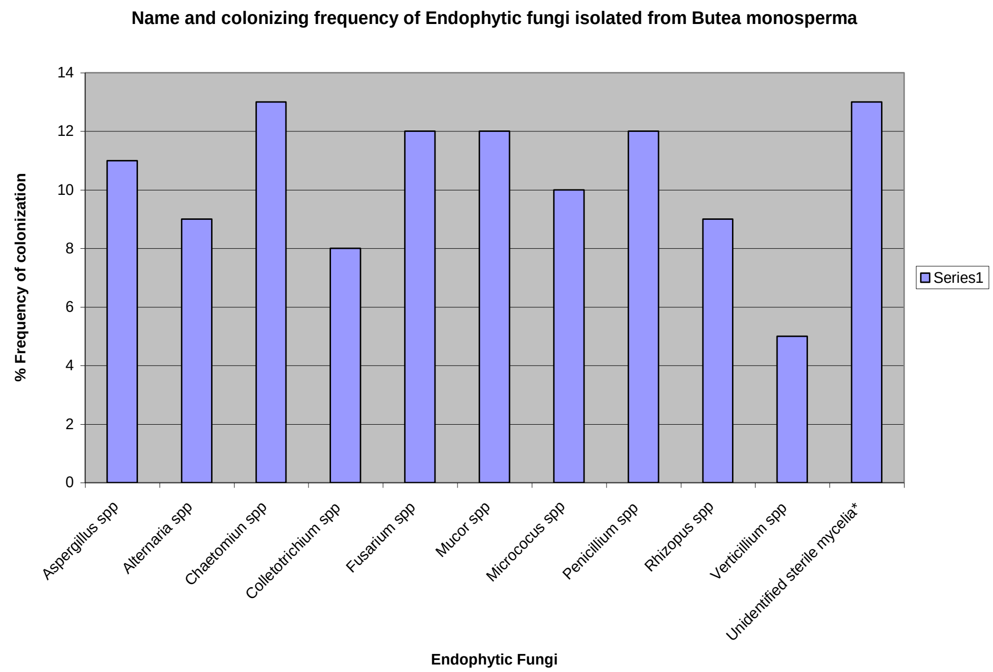
Site of isolation	(Number of samples)	Number of fungi isolated
Leaves	(116)	17
Stems	(84)	8
Total number of isolates	200	25

Table 2: Name and colonizing frequency of Endophytic fungi isolated from Butea monosperma

S. No	Endophytic Fungi	LUL No.	% frequency of colonization		Number of Spp.
			Leaves	stem	
1	*Aspergillus spp*	56	11.00	-	3
2	*Alternaria spp*	32	9.00	-	3
3	*Chaetomiun spp*	29	13.00	-	3
4	*Colletotrichium spp*	82	-	8.00	2
5	*Fusarium spp*	48	-	12.00	3
6	*Mucor spp*	69	12.00	-	1
7	*Micrococus spp*	34	10.00	-	1
8	*Penicillium spp*	24	-	12.00	3
9	*Rhizopus spp*	29	-	9.00	1
10	*Verticillium spp*	25	-	5.00	1
11	Unidentified sterile mycelia*	05	-	13.00	4

LUL No, Lucknow university Lucknow specimen no.

*No perfect stage was seen in this fungus



DISCUSSION

Endophytic fungi are one of the most unexplored and diverse group of organisms that make symbiotic associations with higher life forms and may produce beneficial substances for host (Shiomi *et al.*, 2006). Fungi have been widely investigated as a source of bioactive compounds. An excellent example of this is the anticancer drug, taxol, which had been previously supposed to occur only in the plants (Strobel & Daisy, 2003). Endophytic fungi from medicinal plants can therefore be used for the development of drugs. The endophytic flora, both numbers and types, differ in their host and depends on host geographical position (Gange *et al.*, 2007).

Butea monosperma has been used traditionally for many of the diseases like anti-inflammatory, antimicrobial, anthelmintic, antidiabetic, diuretic, analgesic, antitumor, anticancer, astringent etc. The leaves and seeds are useful as, in hemorrhage, astringent, diuretic and have anti-implantation and anti-ovulatory properties (Kiritikar, K, R., and Basu, B.D., 1996). The bright colour of the flower is attributed to the presence of chakones and auron. Flowers have aphrodisiac and tonic properties. Bark are used in tumors, bleeding piles, ulcers and have inhibitory action against *E.coli* and *Micrococcus pyrogens*. Roots are used to cure night blindness. Chemical component of *Butea monosperma* are alkaloids and recently reported Euphane triterpenoid ester and pterocarpan. Seed contains palasonin,-d-methyl cantharidin, α-amyrin, and β-sitosterol and alkaloid-monospermine. Glycerides of palmitic, stearic, linoceric, oleic and linoleic acids, proteolytic and lipolytic enzymes. While bark contains tannins and gum (Butea gum), leucocyanidin and its tetramer procyanidin, gallic acid and mucilaginous material. Its flowers contain isobutin, coreoopsin, monospermoside and their derivatives sulphurein, palastrin.

Therefore, the present work was initiated to find out endophytic flora associated with in this widely used medicinal plant. Diverse endophytic population was detected to colonize this plant. Almost all the isolates were recovered from older plant samples than younger ones. The highest species richness, as well as frequency of colonization was found in stem. In this study the most dominant endophytic in *Butea monosperma* flora was found to be *Chaetomium globosum*. In most of the cases, Ascomycetes, Deuteromycetes and Basidiomycetes are reported as endophytic fungi (Dayle *et al.*, 2001). A large number of genera and species of fungi, belonging to first two classes, are able to live endophytically in plants. In the present study, the isolated fungi belonged to the class Ascomycetes Deuteromycetes *and* Zycomycotina. Previously, this fungus was reported as a common endophyte to other plant species like *Triticum aestivum, Zea mays* (Zhang *et al.*, 2006).

There is sufficient evidence that endophytic fungi play an important role in host plant physiology. They receive nutrition, protection and propagation opportunities from their hosts (Clay & Schardl, 2002), while host plants are also benefited from this symbiosis. Endophytes provide protection to their hosts from insects, pests, and herbivore, and help their hosts to adapt in different stress conditions (Knop *et al.*, 2007; Malinowski *et al.*, 2006). However, endophytes also act as opportunistic microorganisms under some conditions. Attempts are being made to isolate and identify bioactive metabolites from endophytic fungi (Strobel *et al.*, 2004). Studies were also carried out on endophytic fungi to screen them for antibiotics, antiviral and anticancer, antioxidants, insecticidal and immunomodulatory compounds (Tan & Zou, 2001).In the present study, 25 different fungal species were isolated from *Butea monosperma.* Hence, it is important to study medicinal plants for their endophytic mycoflora for biodiversity and then to determine their medicinal properties. The present work was therefore initiated to study the endophytic fungal population in *Butea monosperma* a commonly used medicinal plant in the subcontinent

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