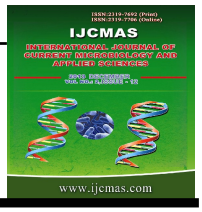
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 12 (2013) pp. 615-627**
http://www.ijcmas.com



Original Research Article

Endophytic mycobiota of medicinal plant *Butea monosperma*

Darshan S. Tuppad and S. Shishupala*

Department of Microbiology, Kuvempu University (Davangere University), Shivagangotri, Davangere-577002, Karnataka, India
**Corresponding author*

A B S T R A C T

Keywords

Endophytes; *Butea monosperma*; fungal diversity; species distribution index.

First complete information on occurrence, distribution and diversity of endophytic fungi associated with organs of *Butea monosperma* is presented. Seventy three endophytic fungal isolates belonging to genera *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Pithomyces*, *Scopulariopsis*, *Colletotrichum*, *Chaetomium*, *Papulaspora*, *Sclerotium* and three different morphotypes were found in different tissues. *Colletotrichum* sp. was dominant in most of the plant parts with relative frequency of 21.9%. Isolates belonging to *Sclerotium* sp. had relative frequency of 13.6%. Endophytic fungal diversity appeared maximum in stem and lamina samples. Frequency of occurrence of endophytic fungi differed greatly in different plant parts. Extent of similarity in endophytic fungal colonization was maximum between stem and lamina as indicated by Jaccard's coefficient. Differential distribution of fungi in various tissues of *B. monosperma* was evident.

Introduction

Endophytic fungi are an important group of microorganisms associated with many of the plant species (Hyde and Soytong, 2008; Rodriguez *et al*., 2009). Understanding the extent of diversity in this group of unique fungi is essential to assess fungal diversity. A wide range of fungi have been reported as endophytes of several plants (Hyde and Soytong, 2008; Rodriguez *et al*., 2009). Many of the plant characters like tolerance to biotic and abiotic stress, chemical constituents of plant parts, growth rate and extent of reproduction are attributed to associated endophytic fungi (Schulz *et al*., 1993; Bayman *et al*., 1997; Schardl and Phillip, 1997; Ananda and Sridhar, 2002; Suryanarayanan *et al*., 2003; Ganley *et al*., 2004; Li *et al*., 2007; Marquez *et al*., 2008; Bezerra *et al*., 2012; Sun *et al*., 2012). Little is known about diversity and abundance of endophytic fungi in tropical and subtropical trees (Arnold *et al*., 2001; Bezerra *et al*., 2013). Endophytic fungal diversity is not well documented in woody plants (Clay and Schardl, 2002; Arnold *et al*., 2003; Strobel and Daisy, 2003). Pattern of abundance, diversity and taxonomic components of tropical endophytes appears to be distinct (Arnold *et al*., 2001). Species rich ecological assemblages of fungi were found as endophytes in tropical trees (Ganley *et al*., 2004; Tejasvi *et al*., 2005). There is a

need to study endophyte diversity of different tropical plant species (Arnold and Lutzoni, 2007). Medicinal plants have been recognized as great deposits of endophytic fungi (Huang *et al*., 2008; Kumar and Hyde, 2004; Strobel *et al*., 2004; Kumar *et al*., 2005; Tejasvi *et al*., 2007). These fungi have been recorded to produce bioactive compounds which originally thought to be of plant origin (Stierle *et al*., 1993; Strobel *et al*., 2004). *Butea monosperma* (Lam.) Taub. is a medicinal tree, renowned for its various therapeutic value (Rao, 2000). Different parts of *B. monosperma* are used to treat flatulence, diarrhea, dysentery, rectal diseases, wounds, skin diseases, boils and tumors (Prajapathi *et al*., 2003; Sumitra *et al*., 2005; Chokchaisiri *et al*., 2009; Sharma and Deshwal, 2011). In spite of wide medicinal uses of *B. monosperma*, no extensive attempts have been made to characterize its fungal endophytes. The medicinal value of such plants may be due to endophytic fungi. Detection of endophytes in such plants is a prerequisite to explore for possible bioactive compound producer. Hence, a detailed investigation was carried out in order to isolate and identify endophytic fungi from *B. monosperma*. This paper reports occurrence of endophytic fungi in different parts of the plant.

Materials and Methods

Plant material

Moderately grown (3 – 5.5 m height) *Butea monosperma* trees were used to assess endophytic mycobiota. The sampled trees were present in dry and shrubby regions of Davangere University campus (14° 23′ 32″ N; 75° 57′ 46″ E), Channagiri (14° 00′ 49″ N; 75° 54′ 19″ E) and Bhadravathi (13° 56′ 18″ N; 75° 47′ 30″ E), Karnataka, India. Representative plant parts were obtained from apparently healthy trees without any observable disease symptoms and brought to laboratory in polythene bags. The plant samples were separately washed in running tap water and air dried. The samples were processed for isolation of endophytic fungi within 10 hrs of collection.

Isolation of endophytic fungi

From the sampled plant material, different parts like root, stem, petiole, lamina, flower and fruit were separated. The plant parts were cut into small pieces of about 10 mm. For isolation of endophytic fungi, one of the recommended procedures was followed (Schulz *et al*., 1993). Briefly, all the parts were separately treated with 70% ethanol (v/v) for one minute and rinsed in sterile distilled water. They were treated with sodium hypochlorite (4% available chlorine) for three minutes, rinsed in sterile distilled water followed by 70% ethanol treatment for 30 sec. Further, they were repeatedly rinsed in sterile distilled water.

Effectiveness of surface sterilization protocol was determined by placing an imprint of the surface sterilized plant part on sterile potato dextrose agar (PDA) medium (Schulz *et al*., 1998; Marquez *et al*., 2008). The plant parts were then placed separately in Petri plates with PDA or malt extract agar (MEA) media containing chloramphenicol (200 mg/l). For each of the plant part a minimum of 75 pieces were placed. All the plates were incubated at 25-30°C for 15-20 days and observed periodically for the growth of fungal colonies. The fungal hyphae emerging exclusively from the plant parts were sub-cultured onto PDA slants for establishing pure cultures.

Identification of endophytic fungi

Endophytic fungal isolates from *Butea monosperma* were inoculated on potato dextrose agar medium separately and incubated for 8-10 days. The plates were observed for fungal growth and colony morphology. Slide culture technique was followed to prepare microscopic slides of fungi using potato carrot agar medium (Benson, 1994). In some cases, to induce sporulation of fungi, grass leaves on water agar, potato agar, potato carrot agar and modified Czapek Dox agar (CZA) medium with cellulose and pectin separately as sole carbon source were used (Srinivasan *et al*., 1971; Kumar and Hyde, 2004). The slides were observed for microscopic features of mycelia, shape of conidia and arrangement of conidia on conidiophore using a binocular compound microscope (Olympus CH20 i). Endophytic fungal isolates were identified by comparing their cultural characteristics and microscopic features (Thom and Raper, 1945; Benoit and Mathur, 1970; Ellis, 1971; Barnett and Hunter, 1972; Booth, 1977; Domsch *et al*., 1980; Sutton, 1980; Hawksworth *et al*., 1995; Larone, 1995; Leslie and Summerell, 2006; http://www.index fungorum.org/names/names.asp).

Data analysis

The extent of colonization of various endophytic fungi in different parts of *Butea monosperma* was assessed by considering different parameters. Relative abundance (%) of each endophytic fungal species was calculated as number of isolates of each endophytic fungal species divided by total number of endophytic fungal isolates x 100 (Kharwar *et al*., 2008). For assessment of tissue specificity, association of each isolate with respect to total number of sampled plant parts was considered. The frequency of occurrence indicates extent of occurrence for a specific fungus with respect to particular plant part. The frequency of occurrence (%) was calculated as number of each plant part colonized by specific fungus divided by total number of each plant part plated x 100. Paired t test was performed to compare frequency of occurrence of endophytic fungi in different parts of *B. monosperma* (Ananda and Sridhar, 2002).

Endophytic infection rate signifies percentage of plant segments infected by endophytic fungi. Endophytic infection rate (%) was calculated as total number of segments colonized by endophytic fungi divided by total number of segments screened x 100 (Suryanarayanan *et al*., 2000). Isolation rate indicates the percent of fungal isolates obtained from total plant parts plated. It is calculated as total number of endophytic fungal isolates obtained divided by total number of segments screened x 100 (Kumar and Hyde, 2004).

Simpson and Shannon diversity indices were calculated using the formula, Simpson diversity index=$1-\sum (ni/n)^2$, Shannon diversity index= $-\sum \{(ni/n) \times \ln(ni/n)\}$, where "n" is the total number of isolates in particular part of the plant and "ni" is the number of individuals of the taxon 'i' (Ananda and Sridhar, 2002; Sun *et al*., 2012). Species distribution index denotes percentage association of endophytic fungal species with respect to each plant part screened. It is calculated as total number of species isolated divided by total number of segments screened x 100.

Individual plant parts were compared with each other to establish tissue affinity of endophytic fungi in *B. monosperma*. Comparison between different parts of the

plant was made by calculating Jaccard's coefficient (S_j) using the formula Sj=a/(a+b+c), where, 'a' is the number of species present in both plant parts. 'b' is the number of species restricted to one plant part alone. 'c' is the number of species restricted to other plant part alone. This was based on presence or absence of specific fungal species in both the plant parts compared (Kumar and Hyde, 2004). A dendrogram was constructed with the Jaccard's coefficients, using Unweighted Pair-Group Method with Arithmetic mean (UPGMA) software, *ver*. 4.0 (http://www.genomes.uvr.cat/UPGMA).

Results and Discussion

Fungal growth was not observed on PDA medium with imprints of plant parts indicating effective surface sterilization protocol. Fungi started emerging from different plant parts plated on both PDA and MEA media. Some of the plant parts were able to provide more than one fungal isolates. A total of 73 endophytic fungal isolates were obtained from 506 plant parts of *Butea monosperma*. Occurrence of endophytic fungi in different plant parts and their relative abundance are shown in Table 1. Among the endophytic fungal isolates obtained, 10 isolates (13.7%) belonged to Basidiomycota (anamorphic *Sclerotium* sp. Tode) and 63 isolates (86.3%) belonged to Ascomycota (two isolates of Sordariomycetes – *Chaetomium crispatum* Fuckel and *Papulaspora immersa* Hotson, 39 of anamorphic Hyphomycetes – *Aspergillus fumigatus* Fresen, *A. sydowii* Bainier and Sartory, *Cladosporium* sp., *Curvularia lunata* (Wakker) Boedijn, *Fusarium solani* (Mart.) Sacc., *F. sterilihyphosum* Britz, Marasas and M.J. Wingf., *F. verticillioides* (Sacc.) Nirenberg, *Pithomyces chartarum* (Berk. and M.A. Curtis) M.B. Ellis, *Scopulariopsis canadensis* F.J. Morton and G. Sm., Morphotype 2 and Morphotype 3, 16 isolates of anamorphic Coelomycetes – *Colletotrichum* sp. Corda and six isolates of Morphotype 1. *Curvularia lunata* isolates showed copious conidial production on modified CZA containing pectin/cellulose as sole carbon source but not on PDA. *Pithomyces chartarum* sporulated well on grass leaves and potato agar.

Thirteen isolates belonging to three different morphotypes are shown in figure 1. Morphotype 1 showed initially grey colony and profuse growth on PDA medium. Thin, brown, septate, branched hyphae showed dark brown thick walled structures (Fig. 1a) which hardly stained with cotton blue. Morphotype 2 had initially off-white coloured colony later turned to creamish-pale brown with puffy growth on PDA medium. Hyphae were thick, pale brown coloured, branched, septate with thick wall layer. It sporulated on PDA medium after incubating the plates at 27° C in dark for 30 days. Conidiophore was short, hyaline, unbranched, bearing single, round, aseptate, black coloured conidia of 5-6 micrometers in length (Fig 1b). The colony of Morphotype 3 on PDA medium was white, wrinkled and verruculose. Hyphae were thin, hyaline, septate and branched. Conidiophores were thin, hyaline, aseptate, unbranched and phialidic. Conidia were symbiform, holoblastic, hyaline, aseptate, chain of conidia in basipetal succession. Size of a conidium was 3-4 micrometers in length (Fig. 1c).

Maximum number of isolates (16) belonged to *Colletotrichum* sp. showing highest relative frequency of 21.9%. This fungus was found to be distributed in all

the plant parts, tested except flower and fruit. Eight isolates of *Colletotrichum*, were obtained from stem only. Ten isolates of *Sclerotium* sp. were found distributed between stem, petiole and lamina samples showing the relative frequency of 13.6%. Differences in relative frequencies among 16 species of endophytic fungi were also noticed between the plant parts tested (Table 1).

Among the different endophytic fungi, *Colletotrichum* sp. and *Curvularia* sp. were found to be associated with most of the trees sampled. Maximum number of endophytic fungal isolates were obtained from stem (27 isolates), followed by lamina (25 isolates). The flower segments yielded only three isolates. Two isolates among them belonged to *Pithomyces chartarum* and the other isolate was Morphotype- 2. Interestingly, fungi like *A. fumigatus, Cladosporium* sp., *Fusarium solani* and *Papulaspora immersa* were singletons, found to be associated exclusively with roots of *B. monosperma*. Likewise, *Chaetomium crispatum* was exclusively associated with petiole sample only. The fruit samples did not yield any endophytic fungi.

Frequency of occurrence of each endophytic fungal species in different parts of *B. monosperma* is shown in Table 2. Total frequency of occurrence ranged between 0.0 - 3.1%. However, the values were different for various plant parts studied. In stem, highest frequency of occurrence 9.3% was shown by *Colletotrichum* sp. The same fungus also showed highest total frequency of occurrence (3.1%) even when all the plant parts were considered. In case of root, maximum frequency of occurrence (3.9%) was noticed with respect to Morphotype 1 isolate. All the fungal isolates found in petiole showed 1.2% frequency of occurrence. In case of lamina, the frequency of occurrence of individual fungal species ranged between 1.1 - 5.8%.

The other fungal isolates showed moderate frequency of occurrence in different parts of *B. monosperma*. Significant difference in frequency of occurrence of endophytic fungi was noticed in case of stem-petiole ($p=0.0249$), stem-flower ($p=0.0186$), lamina-petiole ($p=0.0234$) and lamina-flower ($p=0.0117$). No significant difference was observed in frequency of occurrence of endophytic fungi in stem-lamina ($p=0.7588$), root-stem ($p=0.0866$), root-petiole ($p=0.6051$), root-lamina ($p=0.1313$) and root-flower ($p=0.1166$).

Relative distribution and diversity of endophytic fungi in different plant parts was assessed (Table 3). In the present investigation, fungal endophytes were obtained from only 65 segments out of 506 plant segments of *B. monosperma* screened with a total endophytic infection rate of 12.8% and total isolation rate of 14.4%. Endophytic infection rate (26.7%) and Isolation rate (31.3%) were found to be highest in stem samples. The highest Simpson diversity index was noticed in petiole (0.87). Maximum Shannon diversity index of 2.08 was observed in lamina. The total Simpson and Shannon diversity indices of *B. monosperma* was 0.88 and 2.42 respectively.

Distribution of endophytic fungal species with respect to each plant part is shown as species distribution index (Fig. 2). The stem and lamina showed highest species distribution index. Least number of endophytic fungal species was found in flower (2.2%).

Table.1 Occurrence and relative abundance of endophytic fungi in different parts of *Butea monosperma*.

Classification		Fungi	Plant part					Total number of fungal isolates	Relative abundance (%)
			Root	Stem	Petiole	Lamina	Flower		
			Number of isolates						
Basidiomycota		*Sclerotium* sp.	0	4	1	5	0	10	13.6
Ascomycota	Sordariomycetes	*Chaetomium crispatum*	0	0	1	0	0	1	1.3
		Papulaspora immersa	1	0	0	0	0	1	1.3
	Hyphomycetes	*Aspergillus fumigatus*	1	0	0	0	0	1	1.3
		Aspergillus sydowi	0	1	0	2	0	3	4.1
		Cladosporium sp.	1	0	0	0	0	1	1.3
		Curvularia lunata	0	3	1	5	0	9	12.3
		Fusarium solani	1	0	0	0	0	1	1.3
		Fusarium sterilihyphosum	1	1	0	0	0	2	2.7
		Fusarium verticillioides	0	3	1	1	0	5	6.8
		Pithomyces chartarum	0	1	1	2	2	6	8.2
		Scopulariopsis canadensis	0	2	1	1	0	4	5.4
		Morphotype- 2	0	0	0	1	1	2	2.7
		Morphotype- 3	0	2	1	2	0	5	6.8
	Coelomycetes	*Colletotrichum* sp.	2	8	1	5	0	16	21.9
Morphotype- 1			3	2	0	1	0	6	8.2
Total number of fungal isolates			10	27	8	25	3	73	

Table.2 Frequency of occurrence of each endophytic fungal species in different parts of *Butea*

	Plant part					
	Root	**Stem**	**Petiole**	**Lamina**	**Flower**	**Total**
Total number of plant parts plated	76	86	81	85	87	506
	Frequency of occurrence (%)					
Aspergillus fumigates	1.3	0.0	0.0	0.0	0.0	0.1
Aspergillus sydowi	0.0	1.1	0.0	2.3	0.0	0.5
Chaetomium crispatum	0.0	0.0	1.2	0.0	0.0	0.1
Cladosporium sp.	1.3	0.0	0.0	0.0	0.0	0.1
Colletotrichum sp.	2.6	9.3	1.2	5.8	0.0	3.1
Curvularia sp.	0.0	3.4	1.2	5.8	0.0	1.7
Fusarium solani	1.3	0.0	0.0	0.0	0.0	0.1
Fusarium verticilloides	0.0	3.4	1.2	0.0	0.0	0.7
Fusarium sterilihyphosum	1.3	1.1	0.0	1.1	0.0	0.5
Papulaspora immerse	1.3	0.0	0.0	0.0	0.0	0.1
Pithomyces chartarum	0.0	1.1	1.2	2.3	2.2	1.1
Sclerotium sp.	0.0	4.6	1.2	5.8	0.0	1.9
Scopulariopsis canadensis	0.0	2.3	1.2	1.1	0.0	0.7
Morphotype- 1	3.9	2.3	0.0	1.1	0.0	1.1
Morphotype- 2	0.0	0.0	0.0	1.1	1.1	0.3
Morphotype- 3	0.0	2.3	1.2	2.3	0.0	0.9

Sampling efficiency for species diversity has a profound impact on infection domain of endophytes (Stone *et al*., 2004). In the present study, more than 75 samples for each plant part was used providing substantial sample size for diversity assessment. Host specificity and tissue recurrence are important considerations during fungal diversity estimates (Hyde, 2001). Majority of the fungal endophytes were found to be associated with stem and lamina. Such tissue specific occurrence has been reported in other plants (Arnold *et al*., 2001; Ananda and Sridhar, 2002; Kumar and Hyde, 2004; Marquez *et al*., 2008; Sun *et al*., 2012).

The results also indicated that maximum number of fungal isolates belonged to *Colletotrichum* sp. capable of colonizing in most of the plant parts studied. Isolates belonging to *Curvularia* sp. and *Sclerotium* sp. were distributed among stem, petiole and lamina samples. Few of the fungal isolates were confined to specific plant part. This highlights the importance of assessing endophytic fungal diversity with respect to plant parts. Many of the reports concerning endophytic fungi use one or two plant parts for isolation (Bayman *et al*., 1997; Arnold *et al*., 2001; Ananda and Sridhar, 2002; Marquez *et al*., 2008; Zhang *et al*., 2009; Sun *et al*., 2012).

Table.3 Distribution of endophytic fungi in different parts of *Butea monosperma*

Activity	Root	Stem	Petiole	Lamina	Flower	Fruit	Total
Number of segments screened	76	86	81	85	87	91	506
Number of segments colonized by fungi	9	23	8	22	3	0	65
Total number of fungal species obtained	7	10	8	10	2	0	16
Total number of fungal isolates obtained	10	27	8	25	3	0	73
Endophytic Infection rate (%)	11.8	26.7	9.8	25.8	3.4	0.0	12.8
Isolation rate (%)	13.1	31.3	9.8	29.4	3.4	0.0	14.4
Simpson diversity index	0.82	0.84	0.87	0.85	0.44	0.0	0.88
Shannon diversity index	1.83	2.07	2.07	2.08	0.63	0.0	2.42

However, tissue specific distribution of endophytic fungi is also evident (Suryanarayanan and Vijaykrishna, 2001; Kumar and Hyde, 2004; Rosa *et al*., 2012). Differential distribution of endophytic fungi in different parts of *Lepenthes* sp. has been noticed (Bayman *et al*., 1997). Endophytic fungal diversity and tissue specific distribution in *B. monosperma* was established in this study. Frequency of occurrence (%) provides information on extent of occurrence of a specific fungus associated with plant part, in spite of sample size being different in each part. It is an excellent parameter for understanding distribution and diversity of endophytic fungi (Ananda and Sridhar, 2002).

Higher percentage of endophytic infection rate and isolation rate in stem and lamina samples indicated the maximum colonization of endophytic fungi in them. Though various parameters are used for assessing endophytic association with plants, the species distribution index calculated here show relative distribution of fungal species with respect to plant parts screened. Species composition and frequency of occurrence depends on tissue type. Some endophytic fungi show host or tissue specificity (Rivera-Orduna *et al*., 2011; Sun *et al*., 2011a; Sawmya *et al*., 2013). Some fungi are found to be associated with stem, petiole and lamina owing to continual colonization (*Curvularia* sp., *F. verticillioides*, *P. chartarum*, *S. canadensis*, *Colletotrichum* sp., *Sclerotium* and Morphotype-2). There are isolates exclusively growing in root (*A. fumigatus*, *P. immersa* and *Cladosporium* sp.) and petiole (*C. crispatum*). Occurrence of some fungi exclusively in stem and lamina of *Suaeda* species and few overlapping fungal species present in both the parts have been reported (Sawmya *et al*., 2013; Sun *et al*., 2011b).

Figure.1 Endophytic fungal morphotypes associated with *Butea monosperma*. a) Morphotype–1, b) Morphotype–2, c) Morphotype–3. Bar=50µm

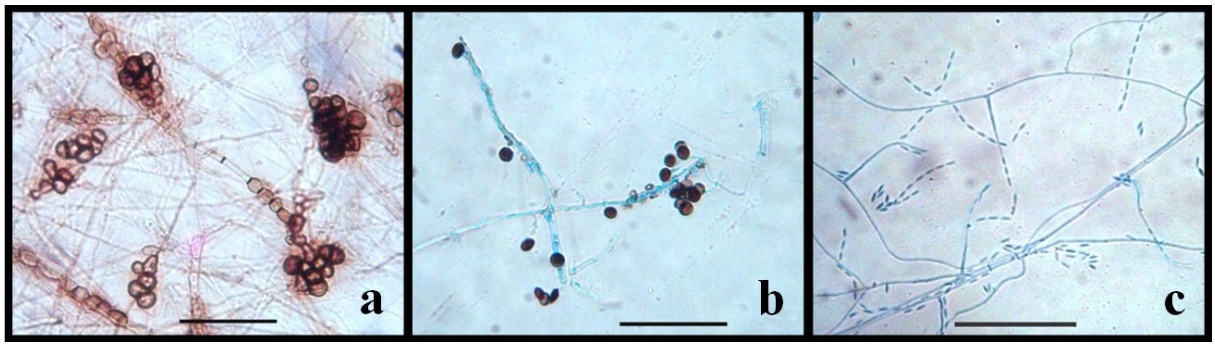


Figure.2 Species distribution index (%) of endophytic fungi in different parts of *Butea monosperma*.

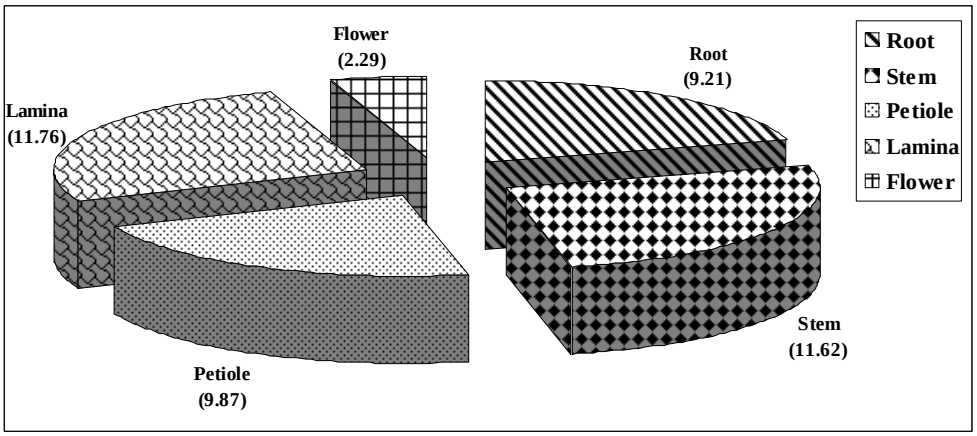
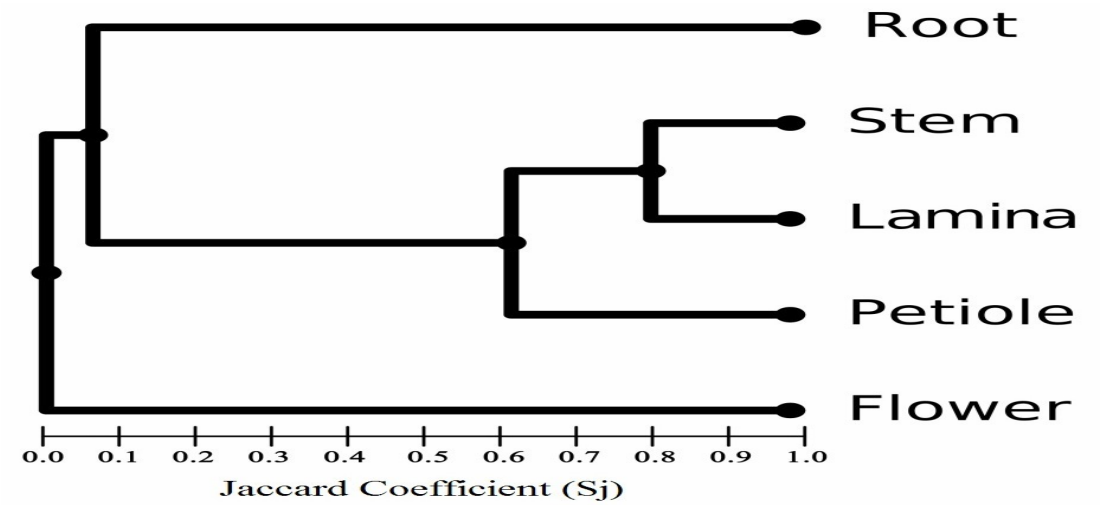


Figure.3 Dendrogram showing relationship among different parts of *Butea monosperma* with respect to endophytic fungal colonization based on Jaccard's coefficients (S_j).



Preferential colonization of each of this depends on fungal isolate and the tissue growth. In case of downy mildew fungi actively dividing cells at the shoot tip and differentiated tissues are not colonized (Safeeulla, 1976). Low number of fungi were found in flower, may be due to non availability of sufficient nutrients in comparison with leaves. No fungi were found associated with fruit which may be due to the presence of inhibitors or differential growth of fungi. The plant is mainly propagated through seeds and hence vertical transmission of endophytic fungi needs further investigation. Simpson and Shannon diversity indices obtained in the present study appears to be significant in comparison with other endophytic fungal diversity of tropical plants (Sun *et al*., 2012; Bezerra *et al*., 2013).

Calculation of Jaccard's coefficient provided information on relationship of plant parts with respect to colonization by endophytic fungi. Higher Jaccard's coefficient between stem and lamina probably indicate extended colonization of endophytic fungi between the parts. Endophytic fungi present in stem, petiole and lamina samples were *Colletotrichum* sp., *Fusarium verticilloides*, *Pithomyces chartarum*, *Scopulariopsis canadensis*, *Curvularia* sp. and Morphotype-2. Hence, it is essential to follow component plating for isolation of endophytic fungi. Even the medicinal value of plants is dependent on the chemistry of the plant part used. Further, it will be prudent to test the influence of endophytic fungi on medicinal value of the plant.

Acknowledgement

We sincerely express our gratitude to Kuvempu University and Davangere University for providing the laboratory facilities. First author is grateful to OBC cell, Kuvempu University and Indian Institute of Science, Bangalore for financial assistance.

References

Ananda, K., and Sridhar, K.R., 2002. Diversity of endophytic fungi in the roots of mangrove species on the west coast of India. Can. J. Microbiol. 48: 871-878.

Arnold, A.E., and Lutzoni, F., 2007. Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hot spots. Ecology. 88: 541-549.

Arnold, A.E., Maynard, Z., and Gilbert, G.S., 2001. Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycol. Res. 12: 1502-1507.

Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D., and Kursar, T.A., 2000. Are tropical endophytes hyperdiverse? Ecol. Lett. 3: 267-274.

Arnold, E.A., Mejia, L.C., Kyllo, D., Rojas, E., Maynard, Z., Robbins, N., and Herre, E.A., 2003. Fungal endophytes limit pathogen damage in a tropical tree. Proc. Nat. Acad. Sci. USA 100: 15649–15654.

Barnett, H.L., and Hunter, B.B., 1972. Illustrated genera of imperfect fungi, 3rd ed. Burgess publishing company, USA. pp. 218.

Bayman, P., Lebron, L.L., Tremblay, R.L., and Lodge, D.J., 1997. Variation in endophytic fungi from roots and leaves of *Lepenthes* (Orchidaceae). New. Phytol. 135: 143-149.

Beniot, M.A., and Mathur, S.B., 1970. Identification of species of *Curvularia* on rice seed. Proc. Int. Seed Testing Assoc. **35**, 99-119.

Benson, H.J., 1994. Microbiological applications, 6th ed. Wm.C. Brown Publishers, Dubuque. pp. 447.

Bezerra, J.D.P., Santos, M.G.S., Barbosa, R.N., and Svedese, V.M., 2013. Fungal endophytes from cactus *Cereus jamacaru*

in Brazilian tropical dry forest: a first study. Symbiosis 60, 53-63.

Bezerra, J.D.P., Santos, M.G.S., Svedese, V.M., Lima, D.M.M., Fernandes, M.J.S., Paiva, L.M., and Souza-Motto, C.M., 2012. Richness of endophytic fungi from *Opuntia fiscus-indica* Mill. (Cactaceae) and preliminary screening for enzyme production. World J. Microbiol. Biotechnol. 28: 1989-1995.

Bhuvaneshwari, V., and Muthumary, J., 2005. Diversity and seasonal influence of fungal endophytes in five medicinal plant species. Kavaka. 33: 39-55.

Chokchaisiri, R., Suaisom, C., Riphota, S., and Chindaduang, A., 2009. Bioactive flavonoids of the flowers of *Butea monosperma*. Chem. Pharm. Bull. 57: 428-432.

Clay, K., and Schardl, C.L., 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. American Naturalist 160, 99–127.

Domsch, K.H., Gams, W., and Anderson, T.H., 1980. Compendium of soil fungi, Vol.1. Academic press Ltd. Great Britain. pp. 859.

Ellis, E.B., 1971. Dematiaceous Hyphomycetes. Common wealth mycological institute Kew, England. pp. 608.

Ganley, R.J., Brunsfeld, S.J., and Newcombe, G., 2004. A community of unknown endophytic fungi in western white pine. Proc. Nat. Acad. Sci. USA 101: 10107-10112.

Guo, L.D., Huang, G.R., and Wang, Y., 2008. Seasonal and tissue age influences on endophytic fungi of *Pinus tabulaeformis* (Pinaceae) in the Dongling Mountains, Beijing. J. Integra. Plant Biol. 50: 997–1003.

Hawksworth, D.L., Kirk, P.M., Sutton, B.C., and Pegler, D.N., 1995. Ainsworth and Bisby's Dictionary of the Fungi. 8th ed. CAB International, Kew, United Kingdom. pp. 616.

Huang, W.Y., Cai, Y.Z., Hyde, K.D., Corke, H., and Sun, M., 2008. Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Divers. **33**, 61-75.

Hyde, K.D., 2001. Where are the missing fungi? Does Hong Kong have any answers? Mycol. Res. 105: 1514-1518.

Hyde, K.D., and Soytong, K., 2008. The fungal endophyte dilemma. Fungal Divers. 33: 163-173.

Kharwar, R.N., Verma, V.C., Strobel, G., and Ezra, D., 2008. The endophytic fungal complex of *Catharanthus roseus* (L.) G. Don. Curr. Sci. 95: 228-233.

Kumar, D.S.S., and Hyde, K.D., 2004. Biodiversity and tissue reoccurrence of endophytic fungi from *Tripterygium wilfordii*. Fungal Divers. 17: 69-90.

Kumar, D.S.S., Lau, C.S., Wan, J.M.F., Yang, D., and Hyde, K.D., 2005. Immunomodulatory compounds from *Pestalotiopsis leucothes* (HKUCC 10197), an endophytic fungus of *Tripterygium wilfordii*. Life Sciences 78: 147-156.

Larone, D.H., 1995. Medically important fungi. 3rd ed. ASM Press, Washington DC, USA. pp. 274.

Leslie, J.F., and Summerell, B.A., 2006. The *Fusarium* laboratory manual. Blackwell Publishing, Iowa, USA. pp. 388.

Li, W.C., Zhou, J., Guo, S.Y., and Guo, L.D., 2007. Endophytic fungi associated with lichens in Baihua mountain of Beijing, China. Fungal Divers. 25: 69–80.

Mahesh, B., Tejasvi, M.V., Nalini, M.S., Prakash, H.S., and Shetty, H.S., 2005. Endophytic microflora of inner bark of *Azadirachta indica* A. Juss. Curr. Sci. 88: 218-219.

Maheswari, S., and Rajagopal, K., 2013. Biodiversity of endophytic fungi in *Kigelia pinnata* during two different seasons. Curr. Sci. 104: 515-518.

Marquez, S.S., Bills, G.F., and Zabalgogeazcoa, I., 2008. Diversity and structure of fungal endophytic assemblage from two sympatric coastal grasses. Fungal Divers. **33**, 87-100.

Prajapathi, N.P., Purohit, S.S., Sharma A.K., and Kumar, T., 2003. A hand book of medicinal plants. Agribios, Jodhpur, India. pp. 545.

Rao, C.K., 2000. Material for the database of

medicinal plants. Karnataka State Council for Science and Technology, Karnataka, India. pp. 230.

Rivera-Orduna, F.N., Suarez-Sanchez, R.A., Flores-Bustamante, Z.R., Gracida-Rodriguez, J.N., and Flores-Cotera, L.B., 2011. Diversity of endophytic fungi of *Taxus globosa* (Mexican yew). Fungal Divers. 47: 65-74.

Rocha, A.C.S., Garcia, D., Uetanabaro, A.P.T., and Carneiro, R.T.O., 2011. Foliar endophytic fungi from *Hevea bresiliensis* and their antagonism on *Microcyclus ulei*. Fungal Divers**. 47:** 75-84.

Rodriguez, R.J., White, J.F., Arnold, A.E., and Redman, R.S., 2009. Fungal endophytes: diversity and functional roles. New Phytol. 182: 314-330.

Rosa, L.H., Tabanca, N., Techen, N., Wedge, D.E., Pan, Z., Bernier, U.R., Becnel, J.J., Agramonte, N.M., Walker, L.A., Moraes, R.M., 2012. Diversity and biological activities of endophytic fungi associated with micropropogated medicinal plant *Echinacea purpurea* (L.) Moench. American J. Plant Sci. 3: 1105-1114.

Safeeulla, K.M., 1976. Biology and control of the downy mildews of pearl millet, sorghum and finger millet. Wesley press, Mysore, India. pp. 304.

Sawmya, K., Vasudevan, T.G., and Murali, T.S., 2013. Fungal endophytes from two orchid species – pointer towards organ specificity. Czech Mycol. 65: 89–101.

Schardl, C.L., and Phillips, T.D., 1997. Protective grass endophytes. Plant Diseases. 81: 430-438.

Schulz, B., Guske, S., Dammann, U., and Boyle, C., 1998. Endophyte-host interactions II. Defining symbiosis of the endophyte-host interaction. Symbiosis. 25: 213-227.

Schulz, B., Wanke, U., Draeger, S., and Aust, H.J., 1993. Endophytes from herbaceous plants: effectiveness of surface sterilization methods. Mycol. Res. 97: 1447-1450.

Sharma, A. K., and Deshwal, N., 2011. An overview: on phytochemical and pharmacological studies of *Butea monosperma*. Intern. J. Pharm. Tech. Res. 3: 864-871.

Srinivasan, M.C., Chidambaram, P., Mathur, S.B., and Neergaard, P., 1971. A simple method for inducing sporulation in seed-borne fungi. Trans. Br. Mycol. Soc. 56: 31-35.

Stierle, A., Strobel, G., and Stierle, D., 1993. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260: 214-216.

Stone, J.K., Polishook, J.D., and White Jr., J.F., 2004. Endophytic fungi. In: Mueller, G.M., Bills, G.F., Foster, M.S., (Eds.) Biodiversity of fungi. Elsevier Academic Press, Burlington. pp. 241-270.

Strobel, G., Daisy, B., Castillo, U., and Harper, J., 2004. Natural products from endophytic microorganisms. J. Nat. Prod. **67:** 257-268.

Strobel, G.A., and Daisy, B., 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol. Mol. Biol. Rev. 67: 491-502.

Sumitra, M., Manikandan, P., and Suguna, L., 2005. Efficacy of *Butea monosperma* on dermal wound healing in rats. The Intern. J. Biochem. Cell Biol. 37: 566-573.

Sun, X., Ding, Q., Hyde, K.D., and Guo, L.D., 2012. Community structure and preference of endophytic fungi of three woody plants in a mixed forest. Fungal Ecol. 5: 624-632.

Sun, X., Guo, L., and Hyde, K.D., 2011a. Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. Fungal Divers. 47: 85-95.

Sun, Y., Wang, Q., Lu, X.D., Okane, I., and Kakishima, M., 2011b. Endophytic fungi associated with two *Suaeda* species growing in alkaline soil in China. Mycosphere 2: 239-248.

Suryanarayanan, T.S., Murali, T.S., Thirunavukkarasu, N., and Rajulu, M.B.G., 2011. Endophytic fungal communities in woody perinnials of three tropical forest types of the Western Ghats, southern India. Biodivers. Conserv. 20: 913-928.

Suryanarayanan, T.S., Senthilarasu, G., and

Muruganandam, V., 2000. Endophytic fungi from *Cuscuta reflexa* and its host. Fungal divers. 4: 117-123.

Suryanarayanan, T.S., Venkatesan, G., and Murali, T.S., 2003. Endophytic fungal communities in leaves of tropical forest trees: Diversity and distribution patterns. Curr. Sci. **85**, 489-493.

Suryanarayanan, T.S., Vijaykrishna, D. 2001. Fungal endophytes of aerial roots of *Ficus benghalensis*. Fungal Divers**. 8:** 155-161.

Sutton, B.C., 1980. The Coelomycetes: Fungi imperfecti with pycnidia, acervuli and stromata. Common Mycological Institute, Kew, England. pp. 537.

Tejasvi, M.V., Kini, K.R., Prakash, H.S., Subbiah, V., and Shetty, H.S., 2007. Genetic diversity and antifungal activity of species of *Pestalotiopsis* isolated as endophytes from medicinal plants. Fungal Divers. 24: 37-54.

Tejasvi, M.V., Mahesh, B., Nalini, M.S., Prakash, H.S., Kini K.R., Subbiah V., and Shetty H.S. 2005. Endophytic fungal assemblages from inner bark and twig of *Terminalia arjuna* W and A (Combretaceae). World J. Microbiol. Biotechnol. 21 1535-1540.

Thom, C., and Raper, K.B., 1945. A manual of *Aspergilli*. The Williams and Wilkins Company. Baltimore, USA. pp. 273.

Xu, L., Zhou, L., Li, J., Li, X., and Wang, J. 2007. Fungal endophytes from *Dioscrorea zingiberensis* rhizomes and their antibacterial activity. Lett. Appl. Microbiol. 46**:** 68-72.

Zhang, C., Yin, L., and Dia, S., 2009. Diversity of root-associated fungal endophytes in *Rhododendron fortune* in subtropical forests of China. Mycorrhiza 19: 417-423.