Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 10	No. 3	pp. 459-461	2011

Original Research Paper

Isolation and Identification of Seasonal Endomycophytes of Inner Bark of *Pachira insignis*

T. G. Nagaraja
Department of Botany, The New College, Kolhapur-416 012, Maharashtra, India

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 8/2/2011
Accepted: 1/4/2011

Key Words:
Endomycophytes
Pachira insignis
Inner bark

ABSTRACT

Endophytic fungi from inner bark of *Pachira insignis* were studied in three different seasons during 2009-2010. A total of 25 endophytes were recorded during rainy season followed by 36 in winter season and 19 in summer season. *Aspergillus niger, A. flavus* and *Verticillium* sp. were found to be dominant endophytes followed by *Biospora punctata, Rhizophus stolonifer* and *Cladosporium* species.

INTRODUCTION

Endophytic microorganisms are those that inhabit interior of plants, especially in branches, bark and stems showing no apparent harm to host (Azevedo 1998). As they live inside the plant tissues they utilize the nutrients and play important roll in protecting the host plant from the insects and pathogens and have potential to produce novel antimicrobial secondary metabolites. Plants get benefits extensively by harbouring such endophytes as they promote plant growth and confer enhanced resistant to various pathogens by producing antibiotics along with secondary metabolites. Endophytic organisms stimulate greater resistance to stress condition, alteration in physiological properties, production of phytohormones and other compounds of biotechnological interests (Daniella et al. 2004). Thus, endophytes advocate a good tool for the protection of host by various pathways. Therefore, an attempt was made to screen out endophytic fungi in bark of *Pachira insignis*, a tribal medicinal plant.

Pachira insignis commonly called Malabar chestnut or Guiana chestnut, a native of central and northern part of South America, growing to a height of up to 60 feet and cultivated in gardens in India. It produces white fragrant flowers with brown pod fruit one foot long, containing several large seeds. The raw seeds are supposed to taste like peanuts, and its oil is used for several skin diseases by tribal people in South America.

MATERIAL AND METHODS

The bark pieces of *Pachira insignis* (Bambaceae) were collected from Town Hall garden, Kolhapur periodically in three different seasons. The bark pieces were cut at 1-2 meters above the ground level and to the depth of 1-1.5 cm in the trunk. The collected bark samples were brought to the laboratory and surface sterilized by 70 percent ethanol (v/v) for one minute followed by 1-2 minutes in 3.5 percent sodium hypochlorite solution (v/v) in a beaker. They were later rinsed three times in distilled water for one minute to remove traces of sodium hypochlorite (Petrini 1986). The outer skin was removed slowly with sterilized knife and inner portion containing cortex was cut into small pieces of 0.2 × 0.8 mm in dimension (Mahesh et al. 2005). Approximately 100 segments were cut and plated on nutrient agar and PDA media mixed with septran (100 mg/L) and incubated in a chamber for 21 days at 12 hours light/dark cycles at 28 ± °C. The Petri plates were allowed to grow endophytic fungi and monitored regularly. Isolation was done for pure culture of the fungi from each Petri plate after 18th to 20th day by sub-culturing onto appropriate media. The seasonal endomycophytic flora were identified based on morphological characters using standard identification manual during.

The number of endophytes were calculated in all Petri plate. Percentage of colonizing frequencies were calculated according to the method prescribed by Fisher & Petrini (1987). The dominant fungi in all the three seasons were estimated by the method of Kumaresan & Suryanarayan (2002).

RESULTS AND DISCUSSION

Seasonal distribution of endophytes from the inner bark of *Pachira insignis* is depicted in Tables 1, 2 and 3. A few studies have been carried out on endophytic mycoflora of tropical trees (Frohlich & Hyde 1999, Nagaraja & Devakar 2010,

Table 1: Endophytic fungi isolated from inner bark of *Pachira insignis* during rainy season.

Sr. No.	Endophytic Fungi	Number of Endophytes	Colonization Frequency	Dominant Fungi (%)
1.	*Aerobasidium* sp.	3	3	12
2.	*Aspergillus niger*	4	4	16
3.	*Aspergillus flavus*	3	3	12
4.	*Bisopora punctata*	3	3	12
5.	*Fusarium oxysporum*	2	2	08
6.	*Mucor* sp.	1	1	04
7.	*Geotrichum* sp.	1	1	04
8.	*Rhizopus stolonifera*	3	3	12
9.	*Verticillium albo-arum*	1	1	04
10.	*Verticilllim* sp.	2	2	08
11.	Sterile mycelia	2	2	08
	Total Isolation	25	25	

Total segments: 100; Total endophytes: 25

Table 2: Endophytic fungi isolated from inner bark of *Pachira insignis* during winter season.

Sr. No	Endophytic Fungi	Number of Endophytes	Colonization Frequency	Dominant Fungi (%)
1.	*Aspergillus niger*	3	3	8.3
2.	*Aspergillus flavus*	3	3	8.3
3.	*Bispora punctata*	1	1	2.7
4.	*Cephalosporium* sp.	2	2	5.55
5.	*Choanospora* sp.	1	1	2.7
6.	*Cladosporium acacola*	2	2	5.55
7.	*Curvularia lanata*	2	2	5.55
8.	*Geotrichum albidium*	1	1	2.7
9.	*Humicola fuscoatra*	2	2	5.55
10.	*Fusarium oxysporum*	3	3	8.3
11.	*Penicillium expansum*	3	3	8.3
12.	*Papularia* sp.	1	1	2.7
13.	*Sphaerosporium acacia*	1	1	2.7
14.	*Trichoderma viridae*	2	2	5.55
15.	*Trichothecium* sp.	1	1	2.7
16.	*Torula* sp.	1	1	2.7
17.	*Verticillium acasia*	2	2	5.55
18.	*Verticilllium* sp.	3	3	8.3
19.	Sterile mycelia	2	2	5.55
	Total isolation	36	36	

Total segments: 100; Total endophytes: 36

Nagaraja & Shinde 2010). A total of 80 fungal species have been recorded in the inner bark of this tree during 2009-2010. The genera like *Aspergillus* sp. *Aerobasidium* sp., *Bispora* sp., *Geotrichum* sp., *Fusarium* sp., *Mucor* sp., *Rhizopus* sp. and *Verticillium* sp. were dominant fungi during rainy season.

A total of 36 species of hypomycetous fungi were recorded in the inner bark of *Pachira insignis* during winter season from November 2009 to January 2010 (Table 2). *Aspergillus niger, A. flavus, Cladosporium* sp., *Curvularia* sp., *Cephalosporium* sp., *Trichoderma* sp. and *Verticillium* sp. were dominant fungi followed by *Trichothecium* sp., *Bispora* sp., *Torula* sp., *Choanospora* sp. and *Sphaerosporium acacia*. Meanwhile a few endophytes were recorded in summer period from February 2010 to May 2010 (Table 3). *Aspergillus* sp. and *Curvularia* sp. were dominant followed by *Rhizopus stolonifer.*

Endophytic fungi associated with grass have been shown to protect grasses against pests and diseases (Clay 1989). The endophytic fungi show mutualistic association with grasses benefiting the host plant (Clay 1996). In mutualisitic association, endophyte infected plants are protected from

Table 3: Endophytic fungi isolated from inner bark of *Pachira insignis* during summer season.

Sr. No	Endophytic Fungi	Number of Endophytes	Colonization Frequency	Dominant Fungi (%)
1.	*Aspergillus flavidus*	2	2	10.52
2.	*Aspergillus niger*	3	3	15.78
3.	*Aspergillus pachirae*	1	1	5.26
4.	*Aspergillus flavus*	1	1	5.26
5.	*Bispora* sp.	1	1	5.26
6.	*Curvularia* sp.	2	2	10.52
7.	*Fusarium* sp.	1	1	5.26
8.	*Monilia* sp.	2	2	10.52
9.	*Mucor* sp.	2	2	10.52
10.	*Rhizopus stolonifer*	2	2	10.52
11.	Sterile mycelia	2	2	10.52
	Total isolations	19	19	

Total segments: 100; Total Endophytes: 19

attack by some species of insects, nematodes and fungi, while in return, the endophyte is provided with shelter and nutrition by the host plant (Saikkoneu et al. 1998, Schardl et al. 2004). The endophytic fungi like *Fusarium* sp. and *Trichoderma* sp. are basically pathogenic to crop, but sometimes they get modified by mutation and grow into non-pathogenic endophytes (Freeman & Rodriquez 1993). Some root colonizing plant beneficial fungi such as *Fusarium* sp. and *Trichoderma* sp. have developed symbiotic relationship with host plant (Haas & Defego 2005). So these results coincide with their findings.

The toxic products have been synthesized by endophytes in woody plants that were able to modify growth and death rates in larvae of the spruce bud worm *C. fumiferanna* feeding on balsam fir (Calhoun et al. 1992). The endophytes were identified as *Phyllosticata* sp. and *Hormonema dematioides* and the toxic compounds were mainly heptelidic acid and regulosine, even tremorgenic toxin in tropical woody plant infected with an endophytic fungus from the *Phomopsis* (Billis et al. 1992) was recorded. Antibiotic phomol was isolated from fermentation by *Phomopsis* sp., the endophytic fungus from *Erythrina cristagalli* (Webber 1981). Thus, endophytes provide protection against pathogens and are potential biocontrol agents, and could be utilized to protect tissue culture plants before they are transplanted in the field.

ACKNOWLEDGEMENT

The author is highly thankful to Co-ordinator, Department of Agrochemicals and Pest Management, Shivaji University, Kolhapur for providing laboratory facilities.

REFERENCES

Azevedo, J.L. 1998. Microorganismos endofiticos. In: Ecologia Microbiana, Melao I.S and Azevedo, J.L. (Eds.) Editora EMBRAPA Jaguariuna, Sao Paulo, Brazil. pp. 117-137.

Bills, G.F., Giacobbe, R.A., Lee, S.H., Pelez, F. and Tkacz, J.S. 1992. Tremorgenic Mycotoxin Paspallitrem A and C from tropical *Phomopsis.* Mycological Research, 96: 977-983.

Calhoun, L.A., Findrlay, J.A., Miller, J.D. and Whitney, N.J. 1992. Metabolite toxic to spruce bud worm from Balsam fir Needle endophytes. Mycological Research, 96: 281-286.

Clay, K. 1989. Clavicipitaceous endophytes of grasses: Their potential as biocontrol Agents. Mycologial Research, 92:1-12.

Clay, K. 1996. Interactions among fungal endophytes, grasses and herbivores. Researches on Population Ecology, 38: 191-201.

Daniella, W., Olov, S., Timm, A., Susanna, C., Virgina, M. and Christina, A. 2004. Phomol, a new anti inflammatory metabolite from endophyte of the medicinal plant *Erythrina crista-galli.* J. Antibiotics., 57(9): 559-563.

Fisher, R.S. and Petrini, O. 1987. Trans, Br. Mycol. Soc., 89: 246-249.

Freeman, S. and Rodriquez, R. J. 1993. Science., 260: 75-78.

Frohlich, J. and Hyde, K.D. 1999. Biodiversity of palm fungi in the tropics. Are global fungal diversity estimates realistic? Biodiversity and Conservation, 8: 977-1004.

Haas, D. and Defago, G. 2005. Biological control of soil-borne pathogens by fluorescent Pseudomonads. Natural Reviews in Microbiology, 12: 1-13.

Kumaresen, V. and Suryanarayan, T.S. 2002. Fungal Diversity, 9: 81-91.

Mahesh, B., Tejesvi, M.V., Nalini, M.S., Prakash, H.S., Kini, K.R., Subbiah, V. and Shetty, H.S. 2005. Endophytic mycoflora of inner bark of *Azadirachta India.* Curr Sci., 88(2): 218-219.

Nagaraja, T. G. and Devkar, P.G. 2010. Seasonal occurrence of endophytic mycoflora of inner bark of medicinal plant *Acacia catechu* Wild. The Bioscan, 5(2): 243-245.

Nagaraja, T.G. and Manisha D. Shinde 2010. Seasonal occurrence of endomycophytes from inner bark of *Barringtonia acutangula* (L.) Gaertn. Nature Environment and Pollution Technology, 9(1): 141-144.

Petrini, O. 1986. Microbiology of the Phyllosphere (Eds., Fokkema, N.J. and Van Den Hueval, J.) Cambridge University Press, Cambridge, pp. 175-187.

Saikkonen, K., Faeth, S.H., Helander, M. and Sullivan, T.J. 1998. Fungal endophytes: A continuum of interactions with host plants. Annual Review of Ecology and Systematics, 23: 319-343.

Schardl, C.L., Leuchtmann, A. and Spiering, M.J. 2004. Symbioses of grasses with seedborne fungal endophytes. Annual Review of Plant Biology, 55: 315-340.

Webber, T. 1981. A natural control of Dutch Elm disease: Nature, London, 292: 449-451.