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# SEASONAL OCCURRENCE OF ENDOPHYTIC MYCOFLORA OF INNER BARK OF MEDICINAL PLANT *ACACIA CATECHU* WILLD

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**ABSTRACT**
Endophytic fungi from inner bark of medicinal plant *Acacia catechu* Willd were studied in different seasons during 2007-2008. A total of 32 endophytes were recorded during rainy season followed by 29 endophytes in winter season and 19 endophytes in summer season. *Aspergillus niger, A. flavus, Trichoderma viridi and Penicillium* sp., *were* found to be dominant endophytes followed by *Fusarium oxysporum, Chaetomium globossum and Verticillium* sp.

## INTRODUCTION

Endophytic microorganisms are those that inhibit interior of plants especially in branches, bark and stems showing no apparently harm to host (Azevedo, 1998). Endophytes include fungi that have one or more of a variety of interaction with their host plant, some fungi are wide spread and are found on many different plant species and others are highly specific to single hosts in single environment.

*Acacia catechu* Willd (Mimosaceae) is moderate sized deciduous tree growing naturally in Konkan and western ghats. The bark of this medicinal plant is used in infusions or decoctions as astringent, antidysenteric and anti pyretic. The most important product is catechu or katha, useful in melancholia, conjunctivitis, haemoptysis and skin diseases. Various plant parts are used in sore mouth, pain in chest, asthma, coliky pain, gravel phthisis etc., gum resin used in masticatories has been reported from this plant (Joshi, 2000). Since *Acacia catechu* Willd like all other plants harbours endophytic fungi. We become interested in a possible contribution of fungal diversity in the bark. Endophytic fungi have been isolated from leaves, stem and roots of many woody plants in the tropics (Bettucci *et al.,* 1999). Endophytes have a capacity to protect their hosts against insect pests, pathogens and even domestic herbivores, animals and production of phytohormones (Ma *et al.,* 2004) and other compounds of biotechnological interest (Strobel and Daisy, 2003). Taxol, an potent anti cancer agent was isolated from an endophyte *Pestalotiopsis microspora* in a Yew tree and Gibberllic acid an growth hormone from an endophyte *Gibberella fujkuroi* harbouring rice plant advocates endophytes are potential source of useful metabolites.

The present study was carried out to isolate and identify fungal endophytes in the bark of *Acacia catechu* Willd or katha, which is indigenous medicinal plant in India.

## MATERIALS AND METHODS

Fresh bark samples were harvested from tree growing in Shivaji University, campus, Kolhapur on morning hours, in three different seasons viz., rainy, winter and summer seasons with the help of ethanol disinfected knife, bark pieces were cut at 1-2 meter above the ground level and the depth of 1-1.5 cm in the trunk region. The collected bark pieces were brought to the laboratory and surface sterilized by dipping the sample pieces in 70 percent alcohol (v/v) for 1 minute followed by 1-2 minutes in 3-5 % sodium hypochlorite solution (v/v) in a 500 mL beaker. Soon after bark pieces were cut in to the size of 4 X 4 cm for experimental study, later bark pieces were washed thoroughly with distilled water with several times. The excess of water was blotted to dry with blotting paper. The outer skin was removed slowly with ethanolic disinfected knife and inner portion containing cortex segments were cut and are plated on water agar medium (20 g $L^{-1}$) mixed with septran (100 mg $L^{-1}$) and incubated in a chamber for 21 days at 12 hrs light/dark cycles at 24ºC. The petriplates were allowed to grow endophytic fungi monitored regularly. From the sterilized bark pieces of hyphal tips started growing slowly, such hyphal

masses were transferred to PDA. Each fungus was assigned a number and stored properly. The endophytic mycoflora were identified based on morphological characters using standard manual, some isolates were documented at the Department of Botany, Shivaji University, Kolhapur.

The percent frequency of occurrence of endophyic mycoflora was calculated by the number of bark segments of *Acacia catechu* Willd colonizing a specific fungus divided by total number of segments plated on agar multiplied by 100 (Fisher and Petrini, 1987). The dominant endophytes were measured as percentage colony frequency divided by sum total percentage of colony frequency of all endophytes multiplied by 100.

## RESULTS AND DISCUSSION

The results have been depicted in Table 1, 2 and 3. only a few studies have been carried out endophytic mycoflora of tropical trees (Mahesh *et al.,* 2005). Genera like *Bispora punctuata, Geotrichum acaciae*, *Cephalosporium* sp.*, Trichothecium* sp., *Torula* sp., *Verticillium* sp., *etc.,*have been recorded for the first time as endophytes (Table 1, 2 and 3). During rainy season (Jun 2007 to Oct 2007) a total of 32 species belonging to Ascomycotina and Hypomycotina fungi were recorded in the bark of *Acacia catechu* Willd (Table 1). During winter *Trichoderma viridi* was found to be dominant species, followed by *Aspergillus flavus*. Whereas in summer season (Feb 2008 to Mar 2008) *Penicillium* sp., were found to be dominant. Thus *Aspergillus flavus, A. niger, Fusarium oxysporum, Drechslera sp., Chaetomium globossum and Trichothecium roseum were dominant endophytes isolated from inner bar of Acacia catechu Willd.* A similar mycoflora was documented by Mahesh *et al.,* (2005) in inner bark *of Azadirachta indica.*

The endophytes such as *Acremonium* sp., *Phialocephala* sp., commonly have been isolated from tropical and sub tropical plants (Koga *et al.,* 1997). Whereas *Fusarium oxysporum, Drechslera* sp., *are pathogenic to crops, bust some* times, they may get modified by mutation and grow into useful non pathogenic endophytes.

*Aerobasidium* sp., *Populari* sp., *Thielopsis* sp., was found during rainy season. *Cladosporium* sp., *Cephelosporium* sp., *Choannephora* sp., *was isolated during winter season. Whereas Phoma* sp., *Penicillium* sp., *Trichothecium* sp., was recorded in summer season. This seems occurrences of these endophytes are influenced by several environmental variation (Ahlholm *et al.,* 2002).

The toxic products synthesized by endophytic fungi in woody plants and that were able to modify growth and death rates in larvae of the spruce bud worm *C. fumiferanna* feeding on balsam fire (Calhoun *et al.,* 1992). The endophytes in this case were identified as *Phyllosticta and Hormonema dematioides* and the toxic compounds were mainly heptelidic acid and regulosine, even tremorgenic toxins in tropical woody plant infected with an endophytic fungus from the genus *Phomopsis* (Bills *et al.,* 1992). *Penicillium* sp., have been found to produce important antibiotics, which kill bacteria and other microorganism. Antibiotic Phomol was isolated from fermentation *of Phomopsis* sp., endophytic fungus from *Erythrina cristagalli* (Weber *et al.,* 2004). Thus endophytes providing protection against pathogens as well as they are potential biocontrol agents and could be utilized to protect tissue culture plants before they are transplanted to the field. Again we are currently studying the secondary metabolites sysnthesized by endophytes, which may capable of protecting plants from pest and disease attack.

**Table 1: Endophytic fungi isolated from inner bark of *acacia catechu* willd during winter season**

| Endophytic Fungi | Number of Endophytes | Colonization Frequency | Dominant Fungi |
|---|---|---|---|
| *Hypomycetes Fungi Aspergillus awamori* | 1 | 0.66 | 3.42 |
| *A. flavus* | 3 | 2.00 | 10.40 |
| *A. niger* | 3 | 2.00 | 10.40 |
| *A. japonicus* | 1 | 0.66 | 3.42 |
| *Bispora punctata* | 1 | 0.66 | 3.42 |
| *Cladosporium acacicola* | 2 | 1.33 | 6.90 |
| *Choanephora sp.,* | 1 | 0.66 | 3.42 |
| *Geotrichum albidium* | 1 | 0.66 | 3.42 |
| *Fusarium oxysporum* | 2 | 1.33 | 6.90 |
| *F. moniliformis* | 1 | 0.66 | 3.42 |
| *Verticillium alboatrum* | 1 | 0.66 | 3.42 |
| *Verticillium sp.,* | 1 | 0.66 | 3.42 |
| *Trichoderma viridi* | 7 | 4.66 | 24.20 |
| *Torula herbarum* | 1 | 0.66 | 3.42 |
| *Trichothecium roseum* | 1 | 0.66 | 3.42 |
| *Sterile mycelia* | 2 | 1.33 | 6.90 |
| Total endophytes | 29 | 19.25 | |

Total segments: 150

**Table 2: Endophytic fungi isolated from inner bark of acacia catechu willd during summer season**

| Endophytic Fungi | Number of Endophytes | Colonization Frequency | Dominant Fungi |
|---|---|---|---|
| *Coelomycetes FungiPhoma eupyrema* | 1 | 0.66 | 5.23 |
| *Hypomycetes Fungi Acremonium sp.,* | 1 | 0.66 | 5.23 |
| *Diplodia sp.,* | 1 | 0.66 | 5.23 |
| *Fusarium moniliformis* | 2 | 1.33 | 10.55 |
| *F. oxysporum* | 1 | 0.66 | 5.23 |
| *Drechslera avenacea* | 2 | 1.33 | 10.55 |
| *Aspergillus niger* | 2 | 1.33 | 10.55 |
| *Gilomastrix sp.,* | 1 | 0.66 | 5.23 |
| *Penicilllium sp.,* | 3 | 2 | 15.87 |
| *Monilia acaciae* | 1 | 0.66 | 5.23 |
| *Torula herbarum* | 1 | 0.66 | 5.23 |
| *Trichothecium roseum* | 1 | 0.66 | 5.23 |
| *Sterile mycelia* | 2 | 1.33 | 10.55 |
| Total endophytes | 19 | 12.6 | |

Total segments: 150

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## REFERENCES

**Ahlholm, J. U., Helander, M., Lehtimaki, S., Wali, P. and Saikkonean, 2002.** Vertically transmitted fungal endophytes: different responses of Host-Parasite systems to environmental condition. *Oikos.* **99:** 173-183.

**Azevedo, J. L. 1998.** Microorganismos endofiticos In: Ecologia Microbiana Melo., I.S and Azevedo., J. L (Edt) Editor EMBRAPA, jaguariuna Sao Paulo, Brazil. pp. 117-137.

**Bills, G. F., Giacobbe, R. A., Lee, S. H., Pelaez, F. and Tkacz. 1992.** Tremogenic mycotoxins paspalitrem A and C from a tropical *Phomopsis., Mycological Research.* **96:** 977-983.

**Bettucci, L., Alons, R. and Tiscornia, S. 1999.** Endophytic mycobiota of Healthy twigs and the assemblage of species associated with twig Lesions *of Eucalyptus globules and E. grandis in* Uruguay., *Mycological Research.* **103:** 468-472.

**Calhoun, L. A., Findrlay, J. A., Miller, J. D and Whitney, N. J. 1992.** Metabolites toxic to spruce bud worm from balsam fir needle Endophytes. *Mycological Research.* **96:** 281-286.

**Fisher, P. J. and Petrini, O. 1987.** *Trans, Br Mycol, Soc.* **89:** 246-249.

**Joshi, S. G. 2000.** Medicinal Plants. Oxford & IBH Pub Co Pvt New Delhi, pp 271.

**Koga, H., Hirai, Y., Kanda, K., Tsukiboshi, T. and Uematsu, T. 1997.** Successive transmission of resistance to bluegrass webworm to perennial ryegrass and tall fescue plants by artificial Inoculation with *Acromonium* endophytes., *Japan Agricultural Research Quaterly* **31:** 109-115.

**Mahesh, B., Tejesvi, M. V., Nalini, M. S., Prakash, H. S., Kini, K. R. Ven Subbiah and H. S. Shetty, 2005.** Endophytic mycolfora of inner bark *of Azadirachta indica., Curr, Sci.* **88:** 218-219.

**Ma, Y. M., Li, Y., Liu, J. Y., Song, Y. C. and Tan, R. X. 2004.** Anti-Helicobacter pylori metabolites from *Rhizoctonia* sp., Cyo64, an endophytic fungus *in Cynodon dactylon Fitoterapia.* **75:** 45-456.

**Strobel, G. and Daisy, B. 2003.** Bioprospecting for microbial endophyte and their natural products. *Microbiology and Molecular Biology reviews.* **67:** 491-502.

**Weber, D., Olov Sterner, Timm Anke., S. Gorzalczancy., V. Martino And C. Acevedo 2004. Phomol, a** New Anti inflammatory Metabolite from an Endophyte of the medicinal plant *crista-galli., J. Antibiotics.* **9:** 559-563.