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ISOLATION AND IDENTIFICATION OF SEASONAL ENDOMYCOPHYTES OF INNER BARK OF *CASTANOSPERMUM AUSTRALE* A. CUNN AND C

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ABSTRACT
Endophytic fungi from inner bark of *Castanospermum australae* A.Cunn and C were studied in three different season during 2009-2010. A total of 29 endophytes were recorded during rainy season, followed by 28 in winter season and 20 fungi in summer season. *Aspergillus niger, A. flavus, Mucor* sp., *Fusarium oxysporum and Verticillium* sp., were found to be dominant endophytes followed by *Biospora punctata, Drechsclera* sp., *Nigrospora* sp., *Rhizophus stolonifer* and *Cladosporium species.*

INTRODUCTION

Endophytes may be defined as the fungi which can be live as a part of their life cycle, or invade and live inside the tissues of the living host plant without causing any disease symptom in or any apparent injury to host plant (Wilson, 1995; Saikkonen *et al.,* 1998). As they live inside the plant tissue they utilize the nutrients and play important role in protecting the host plant from the insects and pathogens, endophytes have potential to produce novel anti microbial secondary metabolites, therefore, confer enhanced resistant to various pathogens. Endophytic organisms stimulates greater resistance to stress condition, alteration in physiological properties, production of phytohormones and other compounds of biotechnological interests (Daniella *et al.,* 2004). Therefore, an attempt was made to screen out endophytic fungi in the bark of *Castanospermum australae A. Cunn and C. Castanospermum australae A. Cunn* and *C*, a tall evergreen tree (Family: Fabaceae) native of Australia, commonly called Astralian chestnut, growing to the height of more than 40 meters tall with pinnate leaves. The fruits are cylindrical pod 12-20 cm long with 3-5 seeds. The seeds are used for several skin diseases by tribal people in Australia.

MATERIALS AND METHODS

The bark pieces of *Castanospermum australae* A. Cunn and C (Family: Fabaceae) 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 1minute followed by 1-2 minutes in 3.5 percent sodium hypochlorite solution (v/v) in a beaker, later rinsed three times in distilled water for 1 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 x 0.8 mm in dimensions (Mahesh *et al.,* 2005). Approximately 100 segments were cut and are plated on Nutrient Agar and PDA media mixed with septran (100 mg/L) and incubated in a chamber for 21 days at 12 hr light/dark cycles at 28 $\pm$°C. The petriplates are allowed to grow endophytic fungi and monitored regularly. Isolation was done for pure culture of the fungi from each petriplates after 18th to 20th day by subculturing on to appropriate media (PDA and CDA). The seasonal endomycophytic floras were identified based on morphological characters using standard identification manual during 2009-2010. The number of endophytes are calculated among all petriplats. Percentage of colonizing frequencies were calculated according to the method prescribed by Fisher and Petrini (1987). The dominant fungi in all three seasons were estimated by the method of Kumaresen and Suryanarayan (2002).

RESULTS AND DISCUSSION

Seasonal distribution of endophytes from the inner bark of *Castonospermum australae* A. Cunn and C. are depicted in Tables (1, 2 and 3). A few studies have been carried out in endophytic mycoflora of tropical trees (Frohlich and Hyde, 1999; Nagaraja and Devkar, 2010; Nagaraja and Shinde, 2010). A total of 77 fungal species have been recorded in the inner bark of this tree during 2009-2010. The genera like

Aspergillus sp., *Alternaria altermata Bispora* sp., *Drechsclera avenaceus., Fusarium oxysporum, Mimnoniella* sp., *Rhizopus* sp., *Stachylidium* sp., *Thielopsis basicola* and *Verticillium* sp., *were dominant fungi during rainy season.*

A total of 28 species of hypomyceteous fungi were recorded in the inner bark of *Castanospermum australae* A. Cunn and C. during winter seasion from Nov, 2009 to Jan, 2010 (Table 2). *Aspergillus niger, A. flavus, Cladosporium* sp., *Curvularia* sp., *Humicola fuscoatra, Penicillium luteum* and *Verticillium sp* were dominant fungi followed by *Bisopora punctata,., Choanosporium sp.and sterile mycelia*. Mean while a few endophytes were recorded in summer period from Feb 2010 - May 2010 (Table 3). *Aspergillus* sp. and *Curvularia lunata, Nigrospora spherica* and *Cladosporum* sp., were dominant followed by *Rhizopus stolonifer.*

Table 1: Endophytic fungi isolated from inner bark of *Castanospermum australae* A.Cunn and C. during rainy season 2009-2010

Sr. No.	*Endophytes*	Colonization Frequency	Dominant Fungi
1.	*Aspergillus niger*	2	6.90
2.	*Alternaria alternata*	2	6.90
3.	*Drechslera avenaceum*	2	6.90
4.	*Bispora punctata*	1	3.45
5.	*Fusarium Oxysporum*	4	13.79
6.	*Memnoniella*Sp.	2	6.90
7	*Mucor* sp.,	3	10.34
8.	*Nigrospora sphaerica*	1	3.45
9.	*Verticilllium Albo-atrum*	3	10.34
10.	*Stachylidium* Sp.,	1	3.45
11.	*Thielaviopsis basicola*	2	6.90
12.	*Rhizopus stolonifera*	4	13.79
13.	*Sterile mycelia*		6.90
	Total isolation	29	

Total segments; 100; Total endophytes; 29

Table 2: Endophytic fungi isolated from inner bark of *Castanospermum Australae* A. Cunn and C during winter season 2009-2010

Sr. No	*Endophytes*	Colonization Frequency	Dominant Fungi
1.	*Aspergillus flavus*	1	3.57
2.	*Aspergillusluteola*	2	7.14
3.	*Aspergillusniger*	4	14.28
4.	*Bisporapunctata*	2	7.14
5.	*Chladosporiumfulvum*	3	10.71
6.	*ChoanephoraSp.,*	2	7.14
7.	*Curvularialunata*	2	7.14
8.	*Fusariumoxysporum*	3	10.71
9.	*Humicolafuscoatra*	1	3.57
10.	*Penicilliumluteum*	2	7.14
11.	*VerticilliumAlbo-atrum*	4	14.28
12.	*Sterile mycelia*	2	7.14
	Total isolation	28	

Total segments; 100; Total endophytes; 28

Table 3: Endophytic fungi isolated from inner bark of *Castanospermum australae* A. Cunn and C during summer season 2009-2010

Sr. No.	Endophytes	Colonization Frequency	Dominant Fungi
1.	*Aspergillus flavidus*	2	10
2.	*Aspergillus gravius*	4	20
3.	*Cladosporium harbarum*	2	10
4.	*Curvularia lunata*	2	10
5.	*Drechslera* Sp.,	1	5
6.	*Rhizopus stolonifera*	1	5
7.	*Rhizoctonia* Sp.,	2	10
8.	*Nigrospora sphaerica*	3	15
9.	*Verticillium Albo-atrum*	3	15
	Total isolation	20	

Total segments: 100; Total endophytes: 20

Endophytes have also been shown to influence photosynthesis rates in host plants tall fescue plants infected by *N. coenophialum*, photosynthesized faster and flowered earlier than uninfected plants (Newman *et al.,* 2003). Again endophyte-infected tall fescue plant exhibited higher survival and flowering frequency (Hill *et al.,* 1991). Control of insect pests by using endophytic fungi was reported by Funk *et al.,* (1983) showing protection of the perennial ryegrass *Lolium perenne* L against sod web worm. Mean while Wilson and Carroll (1997) investigated a systerm where endophytic fungus provokes mortality of the gall forming insect *Besbicus mirabilis.*

Endophytic fungi associated with grass have been shown to protect grasses against pests and diseases (Clay, 1989). 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 and Rodriquez, 1993). Meanwhile some root colonizing plant beneficial fungi such as *Fusarium* sp. and *Trichoderma* sp. which have developed symbiotic relationship with host plant (Haas and Defego, 2005).So our results coincides with their findings.

The toxic products synthesized by endophytes 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 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* (Bills *et al.,* 1992) recorded. Antibiotic phomol was isolated from fermentation by *Phomopsis* sp., endophytic fungus from *Erythrina cristagalli* (Webber, 1981). 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.

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