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Original Research Paper

Seasonal Occurrence of Endomycophytes in Inner Bark of *Kleinhovia hospita* Linn.

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

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ABSTRACT

Endophytic fungi from inner bark of *Kleinhovia hospita* Linn. were studied in three different seasons during 2009-2010. A total of 24 endophytes were recorded during rainy season, followed by 33 endomycophytes in winter and 18 in summer season. *Aspergillus niger, A. flavus, Rhizopus stolonifer, Choanephora* sp. and *Mucor racemosus* were found to be dominant endophytes followed by *Bispora punctata, Cladosporium herbarum, Curvularia lunata, Fusarium oxysporum* and *Nigrospora* sp.

INTRODUCTION

The term endophyte has been defined as an organism growing within the plant, without harming the host plant. Bacon et al. (1977) reported the close association of an endophyte (*Sphacelia typhina*) in infected tall fescue (*Festuca arundinacea*). Similarly Latch et al. (1984) have described two seedborne endophytes in perennial ryegrass and tall fescue with *Gliocladium* sp., and *Phialophora* sp. As they live inside the plant tissue they utilize the nutrients and play important role in protecting the host plant from insects and pathogens, and have potential to produce novel anti microbial secondary metabolites. The 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 conditions, 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 different seasons in the bark of *Kleinhovia hospita* Linn.

Kleinhovia hispita Linn., a tall tree, commonly called Queen's flower tree belongs to family Sterculiaceae, native of China, Malaysia and tropical Asia, is medium sized evergreen tree with simple leaves, ovate and cordate bushy, with rosy pink flowers. This tree is often cultivated as ornamental shade tree in gardens and national parks in India. The decoction of the leaves of this tree is used for skin eruption and scabies, while bark is used as hair wash for lice as a traditional medicine in Malaya.

MATERIALS AND METHODS

The bark pieces of *Kleinhovia hospita* 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 1 minute 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 0.2×0.8 mm in dimensions (Mahesh et al. 2005). Approximately 100 segments were cut and plated on Nutrient Agar and PDA media mixed with Septran (100 mg per litre) 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 18^{th} to 20^{th} day by sub-culturing on to appropriate media. The seasonal endomycophytic flora were identified based on morphological characters using standard identification manual during 2009-2010.

The number of endophytes were calculated in all the Petri plates. Percentage of colonizing frequencies was 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 *Kleinhovia hospita* is depicted in Tables 1 and 2. A few studies have been carried out in endophytic mycoflora of tropical trees (Frohlich & Hyde 1999, Nagaraja & Devakar 2010, Nagaraja & Shinde 2010). A total of 75 fungal species have been recorded in the inner bark of this tree during 2009-2010. The genera like *Aspergillus niger, Bispora punctata, Fusarium oxysporum, Curvularia lunata, Mucor racemosus* and *Verticillium sp,* were dominant fungi during rainy season.

A total of 33 species of hypomyceteous fungi were recorded in the inner bark of *Kleinhovia hospita* during winter season from November 2009 to January 2010 (Table 2). *Aspergillus niger, A. flavus, Cladosporium* sp.*, Nigrospora sphaerica,* and *Verticillium albo-atrum* were dominant fungi followed by *Fusarium oxysporum*, *Cladosporium* sp., and sterile mycelia. Meanwhile a few (18 endomycophytes) endophytes were recorded in summer period from February 2010 to May 2010 (Table 3). *Aspergillus sp.* and *Bispora punctata* and *Cladosporum* sp., were dominant followed by *Nigrospora* sp.

Table 1: Endophytic fungi isolated from inner bark of *Kleinhovia hospita* during rainy season 2009-2010.

Sr. No.	*Endophytes*	Number of Endophytes	Colonization Frequency	Dominant Fungi
1.	*Aspergillus niger*	4	4	16.66
2.	*Aspergillus flavus*	3	3	12.50
3.	*Bispora punctata*	2	2	8.33
4.	*Aspergillus gravius*	2	2	8.33
5.	*Curvula rialunata*	1	1	4.16
6.	*Geotrichum* sp.	1	1	4.16
7.	*Monilia* sp.	1	1	4.16
8.	*Mucor racemosus*	2	2	8.33
9.	*Rhizopus stolonifer*	4	4	16.66
10.	*Trichoderma viridae*	2	2	8.33
11.	*Sterile mycelia*	2	2	8.33
12.	Total isolates	24	24	
13.	Total segments	100		
14.	Total endophytes	24		

Table 2: Endophytic fungi isolated from inner bark *Kleinhovia hospita* during winter season, 2009-2010.

Sr. No.	*Endophytes*	Number of Endophytes	Colonization Frequency	Dominant Fungi
1.	*Aspergillus flavus*	3	3	9.09
2.	*Aspergillus gravius*	2	2	6.06
3.	*Aspergillus niger*	4	4	12.12
4.	*Aspergillus fulvus*	1	1	3.03
5.	*Aspergillus viresus*	1	1	3.03
6.	*Bispora punctata*	2	2	6.06
7.	*Choanephora* sp.	3	3	9.09
8.	*Fusarium oxysporum*	2	2	6.06
9.	*Drechslera* sp.	2	2	6.06
10.	*Nigrospora sphaeria*	1	1	3.03
11.	*Curvularia lunata*	2	2	6.06
12.	*Verticillium albo-atrum*	1	1	3.03
13.	*Rhizoctonia* sp.	1	1	3.03
14.	*Rhizopus stolonifer*	2	2	6.06
15.	*Sterile mycelia*	2	2	6.06
16.	*Verticillium albo-atrum*	1	1	3.03
17.	*Cladosporium harbarum*	2	2	6.06
18.	Total isolates	33	33	
19.	Total segments	100		
20.	Total endophytes	33		

Table 3: Endophytic fungi isolated from inner bark of *Kleinhovia hospita* during summer season, 2009-2010.

Sr. No.	Endophytes	Number of Endophytes	Colonization Frequency	Dominant Fungi
1.	*Aspergillus flavus*	2	2	11.11
2.	*Aspergillus niger*	3	3	16.66
3.	*Bispora punctata*	1	1	5.55
4.	*Cladosporium* sp.	1	1	5.55
5.	*Fusarium oxysporum*	2	2	11.11
6.	*Mucor racemosus*	3	3	16.66
7.	*Nigrospora sphaerica*	1	1	0.55
8.	*Monilia* sp.	1	1	5.55
9.	*Rhizopus stolonifer*	2	2	11.11
10.	*Sterile mycelia*	2	2	11.11
11	Total isolation	18	18	
12.	Total segments	100		
13.	Total endophytes	18		

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* against sod web worm. Meanwhile Wilson & Carroll (1997) investigated a system 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 & Rodriquez 1993). Meanwhile 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 synthesized by endophytes in woody plants 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) was also recorded. Antibiotic phomol was isolated from fermentation by *Phomopsis* sp., endophytic fungus from *Erythrina crista-galli* (Webber 1981). Thus, endophytes provide 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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