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Seed mycoflora of *Dendrocalamus brandisii* (Munro.) and disinfection of the seeds using fungicides and botanical extracts

P. Sharada, O. K. Remadevi* and H. C. Nagaveni
Institute of Wood Science and Technology, Malleswaram-18th cross, Bangalore-560003. Karnataka, India.

ABSTRACT: *Dendrocalamus brandisii* (Munro.) is one of the economically important bamboo species, the culms of which are used for building house, baskets and decorative articles. The edible young shoots are used in food items. The germination percentage is reduced due to the problem of fungal infection in seed stages which can further cause diseases in the nurseries and sometimes at plantation stages. Adopting standard techniques, fungal species associated with the seeds were evaluated. Total 38 species of seed borne fungi were encountered, of them the major species were *Alternaria, Aspergillus, Chaetomium, Colletotrichum, Cladosporium, Curvularia, Fusarium, Mucor, Penicillium* and *Rhizopus* along with few non-sporulating fungi. Of the total isolates, species of *Alternaria* were the common fungal isolates in both blotters and agar plate techniques. Agar plate method showed more fungi than blotter method. *In vitro* control studies were conducted by disinfecting the seeds using fungicides (0.12% of Dithane M-45, Captaf, Blitox, Bavistin and Emisan) and leaf and bark extracts of *Prosopis juliflora* (0.1, 0.5 and 1%). Dithane M-45 and Captaf were most effective and 1% leaf extract of *P. juliflora* showed complete inhibition of seed borne fungi.

Keywords: *D. brandisii*, disinfection,seed mycoflora,*Prosopis juliflora* extracts and fungicides

INTRODUCTION

Dendrocalamus brandisii (Munro.) is a tropical giant bamboo, which is similar to the genus *Bambusa*. Also known under the names sweet dragon bamboo or teddy bear bamboo, it is one of the tallest bamboos in the world reaching a height up to 15-20 m with 8-15 cm culm diameter. Plant is suitable for sub- tropical and tropical climates found from the Indian subcontinent throughout South-east Asia. *Dendrocalamus brandisii* were introduced to Karnataka at Coorg and Kerala in 1913-24 and plantation was raised during 1971-73. This bamboo is used for house building, making baskets and decorative items. Young shoots are edible and they are even eaten raw. In North-east India, bamboo comes up naturally. Of late, it is cultivated in different southern states of India like Karnataka, Kerala, and Andhra Pradesh. But they are facing the problems in nursery establishment and plantation work because of various fungal disease problems, both from seed source and seedling stage.

The reduction in viability of seeds during storage has been a major constraint in success of several plantation programmes. During flowering, seeds formation and storage of seeds, they are attacked by pathogens which will lead to the loss of quality seeds. All fruits and seeds support a variety of micro-organisms as their natural flora and fungi were predominant. The selected bamboo species is also having the problems of viability, seed decay and seedling diseases. It is recognized that seed diseases are important and inflict a significant amount of damage to forests and their development, and the forest environment (Singh, 1996). The most commonly reported negative impacts of seed borne fungi include reduction in life span of seeds in storage, seed rotting, reduction in seed germination and vigour and damping-off in the nurseries (Dhingra *et al*., 2002). Several fungal species that are generally considered to be saprophytes, behave as pathogens under certain conditions. Such conditions include injury to the seed or

*To whom correspondance should be addressed: sharada1031@gmail.com

the seed or seed coat, moisture and temperature conditions which favour fungal growth and increase physiological and physical vulnerability of tree cone/fruit, seed, or seedling to infection (Singh and Mathur, 1993).

Hence to assess the problems, the study of the seed mycoflora was taken up. The term seed mycoflora or seed borne fungi is used for both qualitative as well as quantitative analysis of fungi occurring on or in the seeds (Neergard, 1977 and Paul *et al*., 1987). Seed borne fungi can weaken and predispose seeds and seedlings to a variety of diseases. Other harmful effects of seed borne fungi include diseases of reproductive structures resulting in reduced seed production, twig dieback and stem cankers (Mamatha *et al.*, 2000).

There are some reports on seed mycoflora available on few species of Bamboo. *Dendrocalamus strictus, B. bambos, B. tulda and B. nutans* encountered *Acremonium* sp., *Arthrinium sphaerospermum, Beltraniopsis* sp., *Bipolaris* sp., *Cercospora* sp., *Cladosporium cladosporioides, Cladosporium* sp., *Curvularia borreriae, C. brachyspora, C. eragrostidis, C. lunata, C. oryzae, C. palescens, C. senegalensis, C. stapeliae, Curvularia* sp., *Dinemasporium* sp., *Dreschlera halodes, D. hawaiiensis, D. tetramera, Epicoccum purpurascens, E. purpureum, Fusarium semitectum, Fusarium sp., Memnoniella echinata, Myrothecium roridum, Nigrospora oryzae, Nodulisporium sp., Penicillium sp., Periconia sp., Phaeotrichonis sp., Phoma sp., Phomopsis sp., Pithomyces sp., Pseudomonas sp., Rhizopus sp., Stachybotrys atra, Stachybotrys sp., Torula sp., Trichoconis padwickii., Trichoderma harzianum, Xanthomonas sp.* (Jamaluddin, 1992, Tewari, 1992 and Mohanan, 2002). *Alternaria sp. and Arthrinium sphaerospermum* was reported on *Bambusa* sp. by Rangaswami *et al.*, 1970. There are no reports available on seed mycoflora of *D. brandisii.*

Due to commercial importance of freeing seeds from moulds and other fungi during germination, sufficient work has been carried out on chemical control of seed borne fungi. Singh and Chohan (1975) reported the efficacy of Dithane and Captaf in eliminating seed borne fungi caused by *Fusarium equiseti* and *Cladosporium cladosporoides* in gram seeds. Mittal and Sharma (1981) reported the commonly occurring seed borne fungi by use of Bavistan, Brassicol and Dithane. Seed dipping in Topsin or Bavistin for 5 minutes was effective in minimizing damage to Eucalyptus seedlings by *Verticillium* sp., which was associated with seeds (Harsh *et al*., 1992).

Many plant products (botanicals) possess antimicrobial activities which are mammalian non-toxic and eco-friendly (Dwivedi and Dubey, 1986; Singh and Agarwal, 1988). Several reports are available on efficacy of different plant extracts on seed borne fungi (Srivatsava *et al.*, 1997; Hur *et al.,* 2000). The main intention of seed health testing is related to the actual policy towards seed improvement, seed trade and plant protection (Anon, 1993).However, no reports are available on seed mycoflora and their control measures for selected species. Hence, the present study, seed mycoflora and their control measures both by fungicides and plant extracts was taken up.

MATERIALS AND METHODS

Study of seed mycoflora

The seeds of *D. brandisii* were collected from Karke, Bagamandala of Coorg district of Karnataka, India. Seed associated mycoflora were studied using blotters and Agar plate (Anon, 1993) method. For both the methods, surface sterilized as well as non-surface sterilized seeds were used. Surface sterilization was done with 0.25% Sodium hypochlorite (NaOCl) solutions for 2-3 minutes and washed repeatedly with sterilized distilled water to remove traces of NaOCl and transferred to Petri dish. In blotters

test, seeds were placed on 3-4 layers of moistened sterilized blotter papers in Petri dish with the help of a sterilized forceps. In agar plate method, seeds were placed on the sterilized potato dextrose agar (PDA) in Petri dish. 50 seeds were placed equidistantly for each replicate and 3 replications were kept for each treatment. Seeded Petri dishes were incubated at 25°C. Fungi growing on the seeds in both the methods were studied from the fourth day onwards and then on alternate days up to the 15th day (Doyer, 1938). Isolates were maintained on potato dextrose agar and also on selective and differential media for observing morphological characters. Fungi isolated were identified by studying microscopic characters of colony, hyphae, fruiting bodies, spore colour, shape and size using identification keys (Barnett & Hunter, 1998) and species identity was confirmed with Agarkhar Research Institute, Pune.

Plant material and extraction

Prosopis juliflora leaf and bark material was collected from Ghati, Doddaballapur and Gulbarga area of Karnataka state, India. The collected bark and leaf materials were processed, shade dried and powdered.

The powdered material was subjected to soxhlets extraction with petroleum ether 60-80 grade to remove the fatty materials for 18 hrs, further extracted with methanol for 18hrs. The solvent fraction thus obtained was cooled and distilled in rotary vacuum evaporator to recover the solvent and extract separately. The extract was dried off completely without any traces of the solvent. The extract thus obtained was weighed and stored in sample bottles for further use. The yield of leaf extract was 5.3% and bark extract was 15.5%. Methanol extract of bark (BE) and leaf (LE) were taken for its evaluation of antifungal activity. The extracts were dissolved in dimethyl sulfoxide (DMSO) solvent and three different concentrations (0.1%, 0.5 % and 1%) were taken for further studies.

Seed treatment

The seeds were treated with commercially available chemical fungicides, Bavistin- Triveni chemicals Ltd, India (Methyl N-(1H-benzo imidazol-2-yl) carbamate), Blitox - Rallis India Ltd. (Copper Oxychloride), Captan - Rallis India Ltd (cis-5- hydroxyl-1,2,3,6-tetrahydrophthalimide chloride), Emisan - Excel crop care Ltd, India (2-Methoxy ethyl mercury chloride) and Dithane M-45 - Indofil company, Bombay (Manganese ethylene bis-dithio-carbamate polymeric) at 0.2% concentration of active ingredients by seed dressing method where moistened seeds were dressed with fungicide powder by mixing with sterilized talcum powder to obtain required concentration (Anon, 1993; Mathur and Kongsdal, 2003) and the plant extracts (0.1, 0.5 and 1% leaf & bark extract of P. *juliflora*) by soaking the seeds for an hour (Mathur and Kongsdal, 2003). Seeds without treatment served as control. Treated and untreated seeds were placed in Agar plate and incubated at room temperature for 9 days and observed for fungal colonies (Anon, 1993). 50 seeds were placed equidistantly for each replicate and 3 replications were kept for each treatment.

Data analysis

The fungi found in seeds are presented in terms of percentage occurrence and abundance by using the formula (Anon, 1993).

$$\text{Occurrence \%} = \frac{\text{No. of samples which the fungus is detected}}{\text{Total number of samples examined}} \times 100$$

$$\text{Abundance \%} = \frac{\text{Abundance of taxon A}}{\text{Abundance of all taxa}} \times 100$$

RESULTS

Seed borne fungi

Seed borne fungi from agar-plate and blotter methods of both sterilized and non-sterilized seeds (table 1) showed that fungal species and their per cent of occurrence vary with the method. Some fungal species are common between methods and percentage of occurrence varies with methods.

Table 1: Percentage occurrence of individual fungal species and their % of abundance

		% of occurrence of individual species					
		Agar plate-		Blotter-			
No.	Name of fungi	Surface sterilized	Surface non-sterilized	Surface sterilized	Surface non-sterilized	Total	% of abundance
1	*Alternaria sp. I*	-	0.66	-	2.66	3.32	1.78
2	*Alternaria sp. II*	-	-	-	3.33	3.33	1.79
3	*Alternaria sp. III*	-	-	3.33	0.66	3.99	2.14
4	*Alternaria sp. IV*	-	-	4.05	-	4.05	2.17
5	*Aspergillus niger*	-	2.66	-	-	2.66	1.43
6	*Aspergillus flavus.*	-	0.66	-	-	0.66	0.35
7	*Aspergillus sp. I*	-	0.66	-	-	0.66	0.35
8	*Aspergillus sp. II*	-	2.66	-	-	2.66	1.43
9	*Aspergillus sp. III*	-	0.66	-	-	0.66	0.35
10	*Aspergillus sp. IV*	-	17.99	7.33	0.66	25.98	13.95
11	*Aspergillus sp. V*	-	0.66	-	0.66	1.32	0.71
12	*Aspergillus sp. VI*	-	0.66	-	-	0.66	0.35
13	*Aspergillus sp. VII*	-	2.05	-	2.0	4.05	2.17
14	*Aspergillus sp. VIII*	-	-	-	1.33	1.33	0.71
16	*Chaetomium sp.*	-	-	-	9.33	9.33	5.01
17	*Cladosporium oxysporum*	15.99	0.66	-	0.66	17.31	9.29
18	*Cladosporium sp. I*	-	0.66	-	-	0.66	0.35
19	*Cladosporium sp. II*	-	-	-	0.36	0.36	0.19
20	*Colletotrichum sp.*	-	7.33	-	-	7.33	3.94
21	*Curvularia sp.*	-	5.33	-	-	5.33	2.86
22	*Fusarium sp.*	-	-	-	8.66	8.66	4.65
23	*Mucor sp.*	10.66	3.99	-	-	14.65	7.87
24	*Non sporulating fungi*	6.32	6.64	26.66	4.66	44.28	23.77
25	*Penicillium sp.*	13.32	-	-	-	13.32	7.15
26	*Penicillium sp. I*	0.66	-	-	-	0.66	0.35
27	*Penicillium sp. II*	1.33	-	-	-	1.33	0.71
28	*Rhizopus sp.*	-	6.6	-	-	6.6	3.54
		Total	48.28	61.63	41.37	34.97	186.25

The non-surface sterilized seeds of agar plate technique yielded the maximum isolates of about 20 different species, followed by non-surface sterilized seeds by blotters methods with 11 species and surface sterilized seeds of agar plate and blotters technique showed about 7 and 8 species respectively. Abundance of *Aspergillus* species were more followed by *Cladosporium oxysporum*, *Mucor* and *Chaetomium sp*.. Non-sporulating fungi represented in surface sterilised and un-sterilised of both agar and blotter method. *Alternaria* sp. and *Aspergillus* sp. were observed commonly on Agar plate and Blotters method.

Mohanan (1988) reported *Alternaria sp., Aspergillus sp., Beltraniopsis sp., Cercospora sp., Chaetomium sp., Cladosporium sp., Curvularia sp., Drechslera sp., Dactylaria sp., Epicoccum sp., Fusarium sp., Memnoniella sp., Mucor sp., Nigrospora sp., Penicillium sp., Periconia sp., Phoma sp., Phomopsis sp., and Pithomyces sp. fungi on stored seeds of Bambusa arundinacea and Dendrocalamus strictus. Aspergillus niger, Alternaria alternata, Cladosporium oxysporum, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium oxysporum, Penicillium sp*., are commonly isolated fungal species on seeds (Swapna Priya and Nagaveni, 2011a).

Though fungi are detected from the present study are of saprophytic nature, they may become pathogenic in storage or seedling development stage under certain conditions. Such conditions include injury to the seed or seed coat, moisture and temperature conditions which favor fungal growth and increase physiological and physical vulnerability of tree cone/fruit, seed, or seedling to infection (Singh and Mathur, 1993). The most commonly reported negative impacts of seed borne fungi include reduction of seed viability in storage, seed rotting, reduce seed vigor and germination and damping-off in the nurseries (Dhingra *et al.,* 2002).

Control measures with fungicides and Botanical extracts

The efficacy of seed treatment with fungicide is given in figure 1. Result showed complete inhibition of fungal growth with Dithane M-45 and Captaf, followed by Emisan with 97.78%, Bavistin (91.12%) and Blitox (85.57%).

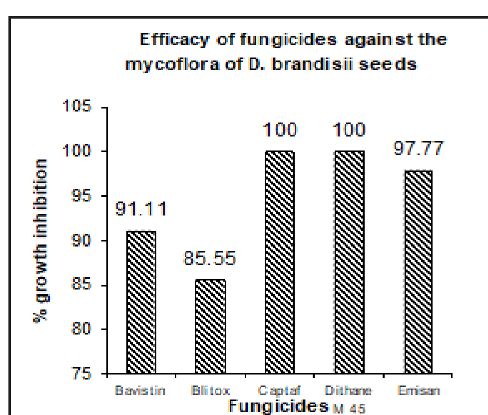

Figure 1. Efficacy of fungicides

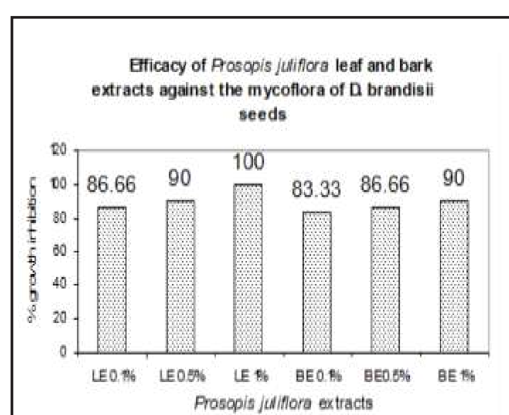

Figure 2. Efficacy of *P. juliflora* extracts

Similar type of results are given by Swapna Priya and Nagaveni (2011b), where they have studied the efficacy of fungicides viz., Emisan, Captaf, Dithane M - 45, Bavistin and Blitox in controlling seed borne fungi of *Garcinia gummi-gatta*.

The efficiency in bringing down the seed mycoflora was noteworthy with *P. juliflora* extract (fig. 2). The 1% leaf extract showed complete inhibition, followed by 0.5% and 0.1%. Bark extract showed considerably good effect in all 3 concentrations used. There was not much difference between the concentrations and it was between 83.33% - 90.01%. It is reported that *P. juliflora* plant contains many alkaloids such as julifloricine and julifloridine (Ahmad *et al*., 1978), juliprosine (Daetwyler *et al*., 1981) which may be the reason to get the anti- mycoflora effect. Saravanan and Valluparidasan (2001) reported the efficacy of *P. juliflora* at 20% concentration to inhibit the growth of *Acremonium strictum, Alternaria tenuis, Helminthosporium halodes* and *H. tetramera*, seed borne fungi of Sorghum seeds. Raghavendra *et al*., 2009 reported that Alternaria alternata species was controlled by alkaloids of *P. juliflora.*

Prosopis juliflora is widely distributed and available in all places in India and leaf and bark can be taken by pruning the side branches without destroying the plant. Comparison of *P.juliflora* with other commercially available fungicides may help to practice the use of its extracts for fungal disease management. Further work is needed for exploring the possibilities of controlling both seed borne and seedling disease in field conditions.

CONCLUSION

The seed borne fungi obtained were *Alternaria* sp., *Aspergillus* sp., *Chaetomium* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Mucor* sp., *Penicilliium* sp., and *Rhizopus* sp., along with few non-sporulating species. These fungal isolates were common fungi which reduces the health of seeds (Jamaluddin, 1999), and were also responsible for various nursery and plantation diseases. The fungicides and plants extracts selected showed that Dithane M-45, Captaf and leaf extract of *P. juliflora* (1%) were most effective and shows complete inhibition of fungal flora.

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