

## Seed Mycoflora of Some Important Forest Tree Species

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Good quality seeds lead to the production of healthy and vigorous seedlings, prerequisite for reforestation programme and development of social forestry. The seeds of forest trees are reported to harbour a number of mycoflora associated with different parts of seeds [1]. Detection of fungal inoculum on a seed lot can be important in order to avoid or reduce the risk of diseases in forest nurseries [2]. Even slightly infected seeds will germinate to serve as infection source from where the disease spreads in the field causing significant damage [3]. Hence, with an increasing demand for large quantities of seeds to meet the growing demand of seedlings for afforestation programme, it is essential to screen and evaluate more and more species for their potential of germination and viability [4]. Very little is known about various types of pathogens being associated with tree species [5]. Hence investigation was carried out to detect the fungi associated with seeds of forest tree species namely *Acacia nilotica*, *Albizia lebbek*, *Bixa orellana*, *Cassia siamea*, *Holoptelia integrifolia*, *Leucaena leucocephala*, *Peltaphorum*, *Prosopis juliflora*, *Samanea saman* and *Thespesia populnea* valued for timber and medicine collected from Mysore and Shimoga districts of Karnataka.

**Collection of Seeds** - Fruits of ten different forest tree species namely *Acacia nilotica*, *Albizia lebbek*, *Bixa orellana*, *Cassia siamea*, *Holoptelia integrifolia*, *Leucaena leucocephala*, *Peltaphorum*, *Prosopis juliflora*, *Samanea saman* and *Thespesia populnea* were collected from Mysore and Shimoga districts of Karnataka, India. Seeds from the fruits were

removed manually and packed in polythene bags. The association of mycoflora with the seeds of above tree species was studied adopting ISTA Rules [6] in which one set of the sampled seeds were surface sterilized by treating them with 2 per cent sodium hypochlorite solution for a duration of 2-6 mins whereas the other set of seeds were used without surface sterilization. In both the treatments, the seeds were placed in 10 or 25 numbers on three layers of wet blotters at a fixed distance according to the size of the seed. In each treatment 4 replicates of 100 seeds were used. Care was taken to keep the blotters moist for the entire period of experimentation. The plates were incubated at  $22\pm 2^\circ\text{C}$  under 12h/12h cycles of light and darkness for 7 days. On 8<sup>th</sup> day the mycoflora associated was observed under stereo binocular microscope and compound microscope. The pathogens were identified based on the structure of fruiting bodies and shape of the conidia. The percentage association of each mycoflora was calculated based on the number of seeds showing particular mycoflora out of the total number of seeds under study.

The per cent incidence of seed mycoflora tested in the present study is depicted in Table 1 and represented tree-wise. From Table 1, it is clear that a large number of fungi are associated with seeds of *Acacia nilotica*, *Albizia lebbek*, *Bixa orellana*, *Cassia siamea*, *Holoptelia integrifolia*, *Leucaena leucocephala*, *Peltaphorum*, *Prosopis juliflora*, *Samanea saman* and *Thespesia populnea*. Amongst these, *Aspergillus niger* was the most common fungi appearing on almost

all the seed samples studied irrespective of surface sterilized and non-surface sterilized seeds followed by *Aspergillus flavus* which appeared on all the seeds except *Peltophorum pterocarpum* and *Bixa orellana*. Similarly, *Penicillium* sp. also occurred on all except *Leucaena leucocephala* and *Holoptelia integrifolia*.

*Alternaria zinniae*, *Fusarium oxysporum* and *Phoma sorghina* were seen only on surface sterilized seeds of *Bixa orellana* whereas presence of *Verticillium* sp. was restricted to surface sterilized seeds of *Albizia lebbek* and *Holoptelia integrifolia*. Similarly, *Myrothecium verrucaria*, *Pestotatia*, *Aspergillus ochraceus* and *Fusarium oxysporum* were recorded only on the surface sterilized seeds of *Leucaena leucocephala*. Occurrence of *Botryodiplodia theobromae* on both surface sterilized and non-surface sterilized seeds of *Thespesia populnea* represents its external and internal seed-borne nature. However, presence of *Colletotrichum lindemuthianum* and *Curvularia lunata* on surface sterilized seeds of *Cassia siamea* exhibits its internal seed-borne nature.

In the present investigation, it was also found that pretreatment using 2 per cent sodium hypochlorite solution reduces the incidence of most of the externally seed-borne fungi. For instance, presence of *Cladosporium cladosporioides* on non-surface sterilized seeds of *Thespesia populnea*, *Holoptelia integrifolia* and *Bixa orellana* was completely eliminated after the treatment of 2 per cent sodium hypochlorite solution. Similarly, *Aspergillus flavus* on *Cassia siamea*; *Aspergillus versicolor* on *Prosopis juliflora* and *Albizia lebbek*; *Aspergillus glaucus* on *Holoptelia integrifolia*; *Aspergillus flavipes* and *Aspergillus nidulans* on *Albizia lebbek*; *Chaetomium crispatum* on *Samanea saman*; *Myrothecium roridum* on *Leucaena leucocephala* were also eliminated completely after the treatment. Pretreatment of seeds is advisable as it not only reduces the counts of fast growing microorganisms but also facilitates the growth of certain slow growing fungi in blotter test [7]. Apart from this, it was also observed that certain fungi occurred only on surface sterilized seeds like *Fusarium verticilloides* and *Fusarium oxysporum* on *Acacia nilotica*; *Aspergillus nidulans* and *Aspergillus sydowi* on *Holoptelia integrifolia* and *Fusarium solani* on *Thespesia populnea*. This may be due to the

suppression of these fungi on non-surface sterilized seeds due to the presence of storage fungi and saprophytic fungi; it may also be due to its slow growing nature.

During the course of investigation it was also observed that incidence of storage fungi viz., *Aspergillus* spp., *Penicillium* sp. and *Rhizopus* was high compared to field fungi viz., *Fusarium* spp., *Botryodiplodia theobromae*, *Colletotrichum* spp. and *Alternaria alternata*. This may be attributed to the ability of saprophytic fungi to occur in high percentage due to the rapid germination of spores, quick hyphal invasion, high competitive nature and their ability to utilize wide variety of substrate [8].

Occurrence of *Aspergillus niger*, *Penicillium* sp. and *Rhizopus* on *Peltaphorum pterocarpum* in the present investigation coincides with the results of *Peltaphorum ferrugineum* [9]. Similarly, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* and *Penicillium* sp. observed on *Acacia nilotica* is in line with the study of Mehrotra and Singh [10]. *Aspergillus flavus* and *Aspergillus niger* reported on *Leucaena leucocephala* [11] is similar to the result obtained in the present study. Presence of *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium digitatum* shows inhibitory effect on germination of *Leucaena leucocephala* [12].

Majority of the *Aspergillus* sp., detected in seeds of various forest tree species inhibited seed germination and causing seed rot [13]. Hence, the reduction in germination among the forest seeds studied may be attributed to the presence of *Aspergillus* sp.

Occurrence of *Alternaria*, *Aspergillus* and *Curvularia* lead to the cause of 30-60 per cent rotting of seeds and seedlings [14]. The presence of *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata* and *Fusarium moniliforme* on *Cassia siamea* reported by Ghyare [15], is similar to the results obtained in the present study.

Presence of *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger* and *Curvularia lunata* on *Prosopis juliflora* [15] coincides with the present results on *Prosopis juliflora*. However, the information generated on mycoflora of *Thespesia populnea*, *Samanea saman*, *Holoptelia integrifolia* and *Bixa orellana* is a new document.

Table 1. Per cent incidence of mycoflora associated with all the ten tree species in different categories of seeds

Tree Name	Acacia nilotica		Albizia lebbek		Bixa orellana		Cassia siamea		Holoptelia integrifolia		Leucaena leucocephala		Peltaphorum juliflora		Samanea saman		Thespesia populnea			
	Shimoga	NS	Shimoga	NS	Mysore	NS	Shimoga	NS	Shimoga	NS	Shimoga	NS	Shimoga	NS	Shimoga	NS	Shimoga	NS		
<i>Alternaria alternata</i>	-	3	-	-	12	9	-	48	5	-	-	4	4	2	1	-	-	31	10	2
<i>Alternaria zinniae</i>	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus flavipes</i>	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus flavus</i>	6	2	53	8	-	-	4	1	7	2	1	7	4	9	13	5	5	-	-	2
<i>Aspergillus flavus-oryzae</i>	-	-	5	4	-	-	-	-	-	13	3	-	-	1	1	-	-	-	-	-
<i>Aspergillus glaucus</i>	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus nidulans</i>	-	-	1	-	-	-	-	-	-	1	2	1	-	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	100	10	68	3	-	14	1	1	1	1	7	1	4	19	4	54	9	-	-	4
<i>Aspergillus ochraceus</i>	-	-	2	-	-	-	1	1	-	1	3	-	-	2	1	1	1	-	-	2
<i>Aspergillus sydowi</i>	-	-	6	3	-	-	-	-	2	2	2	-	-	2	1	-	-	-	-	-
<i>Aspergillus versicolor</i>	-	-	6	-	-	-	1	1	1	-	-	-	-	1	-	-	-	-	-	-
<i>Botryodiplodia theobromae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26	8	-	-
<i>Chaetomium crispotum</i>	-	-	-	-	-	-	-	26	-	-	-	-	-	-	-	10	2	-	-	-
<i>Chaetomium globosum</i>	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium indicum</i>	-	-	-	-	-	-	2	6	-	-	-	-	-	6	1	1	3	-	-	-
<i>Cladosporium cladosporioides</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colletotrichum lindemuthianum</i>	-	-	-	-	-	-	4	-	1	-	-	-	-	-	-	-	-	-	-	-
<i>Curvularia lunata</i>	-	2	-	-	-	-	1	1	-	-	-	-	-	1	1	-	-	-	-	-
<i>Fusarium verticilloides</i>	-	2	-	1	-	-	14	2	-	-	-	-	-	-	-	-	-	30	1	2
<i>Fusarium oxysporum</i>	-	-	-	1	-	1	-	-	-	-	-	1	-	-	-	-	5	7	13	7
<i>Fusarium solani</i>	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	6	-	-	-
<i>Macrophomina phaseolina</i>	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	21	10	-	-
<i>Myrothecium roridum</i>	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
<i>Myrothecium verrucaria</i>	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
<i>Penicillium sp.</i>	20	3	1	-	8	4	3	6	4	-	-	2	12	7	1	14	2	-	-	3
<i>Pestalotia sp.</i>	-	-	-	-	9	7	-	-	-	-	-	-	-	-	-	-	2	-	-	-
<i>Phoma sorghina</i>	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Phomopsis sp.</i>	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	24	7	-	-
<i>Rhizopus sp.</i>	13	3	12	-	-	-	-	-	-	-	-	4	1	5	6	13	2	-	-	62
<i>Verticillium sp.</i>	-	-	-	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	24

Note: POC - Place of Collection; NS - Non-surface sterilized seeds; SS - Surface sterilized seeds; Data obtained from 4 replicates of 100 seeds each

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