ABSTRACT

The present study was carried out to investigate the mycoflora associated with different varieties of shisham (Dalbergia sissoo Roxb.). Pods of 12 varieties of shisham namely S-4, R-1, R-2, US-1, US-2, US-5, US-6, US-7, US-9, US-10, US-11, US-12, were collected during October 2007 from Lahore. Pods were sun-dried and stored in open polythene bags at room temperature till October 2009. Ten seeds of each variety of shisham were incubated on a moist sterilized filter paper bed in Petri plates for seven days to isolated the mycoflora. A total of four fungal species all belonging to genus Aspergillus viz. A. flavus, A. fumigatus, A. japonicus and A. teerius were isolated from seeds of 13 varieties of shisham. In general, A. flavus (15–48%) and A. fumigatus (15–53%) were found to be the most frequently occurring fungal species followed by A. japonicus (0–33%). A. teerius was the least frequently occurring species (0–10%). None of the seeds of any variety germinated up to 20 days. The present study concludes that seeds of all the 12 shisham varieties are equally susceptible to fungal attack during storage.

Key words: Dalbergia sissoo, mycoflora, seeds, shisham varieties.

INTRODUCTION

Seeds are regarded as a highly effective means for transporting plant pathogens over long distances. Compared to seed-borne disease problems of agricultural crops, research has been limited on seed-borne pathogens of forest tree species and in the development of disease prevention and control practices. Seeds of soft wood and hardwood trees are attacked by fungi and other microbes (Ivory, 1987). Various fungal species are commonly associated with seeds of many tree species including both pathogens and saprophytes. Several species of fungi which are generally considered as saprophytes behave as pathogens under certain circumstances including injury to the seed or seed coat, and conducive moisture and temperatures which favour fungus growth and increase the physiological and physical vulnerability of tree cones and fruits, seeds, and seedlings to infection (Mittal et al., 1990). Seeds may be infected internally, often resulting in the destruction of endosperm and embryo, or simply contaminated, whereby the pathogen is associated primarily with the seed coat. Fungi that are inside the seed most often occur as mycelium. Seed-borne pathogens of trees affect nursery seedlings and reduce seed germination and seedling vigour. Several fungi cause deformation, decay, and reduce germination of seeds or destroy seeds. They also decrease the longevity of stored seeds. Certain seed-borne fungal pathogens appear to have minor effect on other developmental stages of trees. Examples include Caloscypha fulgens, which affects seed quality of spruce (Picea spp.), pines (Pinus spp.) and fir (Abies spp.) in the northern United States and Canada, and Lasiodiplodia theobromae, which destroys slash pine (Pinus elliottii var. elliottii) seeds in the southern United States and South Africa (Anderson, 1986; Sutherland et al., 1987). In contrast to that, there are several distinguished pathogens of conifers that can be seed-borne and could have severe economic and ecological consequences if they are introduced and become established in areas where they are not native. These pathogens include Sirococcus conigenus - responsible for a blight of pine, spruce, hemlock (Tsuga spp.) and fir in Canada, Europe, and the northern and western regions of the United States, Sphaeropsis sapinea – cause of blight of pines grown outside their natural range, and Fusarium circinatum - the pitch canker fungus of pines (Sutherland, 1987; Dwinell et al., 2000; Munck and Stanosz, 2009).

Shisham (Dalbergia sissoo Roxb.), a native of Tarai situated in the subtropical and dry temperate foothills in Nepal, was introduced in Pakistan in mid-1800s. At present shisham is the major tree species in the irrigated plantation of Punjab. These plantations were established at various sites in Punjab mainly for production of fuel wood for steam engines. Shisham has been extensively planted along roadsides, canal...
bears and sometimes on the private vacant and agricultural lands. Shisham occupies more than 80% forest area of the Punjab (Gill et al., 2001). In the recent past this valuable species has been infected with two types of diseases viz. dieback and wilt (Bajwa et al., 2003). Eighteen varieties of shisham, including both susceptible and resistant, have been reported by Javaid et al. (2003, 2004). The present study was undertaken to investigate the mycoflora associated with stored seeds of different varieties of shisham.

**MATERIALS AND METHODS**


**RESULTS AND DISCUSSION**

Analysis of variance revealed that there was insignificant difference for stored seed mycoflora among the shisham varieties (V). However, there was significant difference (P≤0.001) among the isolated stored seed fungal species (S). The interactive effect of V×S was also significant (Table 1). A total of four fungal species all belonging to genus Aspergillus namely A. flavus, A. fumigatus, A. japonicus and A. teeriu were isolated from seeds of 12 varieties of shisham. In general, A. flavus and A. fumigatus were found to be the most frequently occurring fungal species followed by A. japonicus. A. teeriu was the least frequently occurring species (Fig. 1). Earlier, investigations on seed-borne fungi associated with forest and shade trees in Islamabad was carried out by Ahmad and Bhutta (1993) reported 13 fungi (both storage and field fungi) associated with stored seeds of different varieties of shisham.

**Isolation and identification of mycoflora:** Stored pods of 13 varieties of shisham were carefully broken and to collect the seeds. Seeds were surface sterilized with 1% sodium hypochlorite solution for two minutes followed by several washings with sterilized water. Ten seeds of each variety of shisham were placed on a sterilized filter paper bed in sterilized Petri plates. Three milliliter of distilled water was added to each Petri plate. Each treatment was replicated three times. Plates were incubated at room temperature for seven days. Fungi appearing on seeds were isolated, purified and identified. The occurrence of various isolated fungal species was calculated as follows:

\[
\text{Occurrence} \% = \frac{\text{No. of seed on which a fungal species appear}}{\text{Total No. of seeds}} \times 100
\]

Germination of the seeds was recorded up to 20 days.

**Statistical analysis:** Standard errors of means of three replicates were calculated using computer software MS Excel. All the data were analyzed by ANOVA followed by Duncan’s Multiple Range Test to separate the treatment means at P≤0.05.

<table>
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<th>Sources of variation</th>
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<td>26634</td>
<td>807</td>
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<tr>
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<td>49875</td>
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</tr>
<tr>
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<td>161500</td>
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</table>

*, significant at P≤0.001. ns: non-significant

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Table 1: ANOVA for the association of four fungal species with stored seeds of 12 *Dalbergia sissoo* varieties.
Occurrence of various fungal species was highly variable in different varieties of shisham. 

Fig. 1: Mycoflora associated with stored seeds of different varieties of *Dalbergia sissoo*. Vertical bars show standard errors of means of three replicates. Bars with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.
frequency of occurrence of A. flavus was recorded in variety US-12 (75%) followed by US-1 (48%), US-2 (35%) and S-4 (30%). In other varieties the occurrence of A. niger ranged from 15–25%. Percentage occurrence of A. flavus in US-12 was significantly higher as compared to all the other shisham varieties (Fig. 1A). Highest occurrence of A. fumigatus was recorded in variety US-11 (53%) followed by US-6 (43%), S-4 (43%) and US-5 (40%). In other varieties the occurrence of A. fumigatus ranged from 15–30% (Fig. 1B). The frequency of occurrence of A. japonicus was lower than that of A. flavus and A. fumigatus. Highest frequency of 40% of this fungal species was recorded in US-6 followed by 33% in US-10. In rest of the shisham varieties, the occurrence of this fungal species ranged from 0–25% (Fig. 1C). The frequency of occurrence of A. teeriis was 10% in US-11 and 2.5% in S-4, R-1, US-2 and US-11. In case of other shisham varieties, A. teeriis was absent (Fig. 1D). The variation in occurrence of different fungal species on different varieties of shisham may be attributed to different genetic makeup of the various shisham varieties (Bajwa and Javaid, 2007). None of the seeds of any variety germinated. It could be attributed to the damage of embryos due to fungal attack during storage (Chisholm and Coates-Beckford, 1997).

The present study concludes that seeds of all the 12 shisham varieties are equally susceptible to fungal attack during storage. So for little work has been done regarding seed-borne mycoflora of forest trees in Pakistan. Much more work needs to be carried out in the field of forest seed pathology in Pakistan.

LITERATURE CITED


