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ISOLATION OF FUNGI ASSOCIATED WITH SHISHAM TREES AND THEIR EFFECT ON SEED GERMINATION AND SEEDLING MORTALITY

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Abstract

A total of 10 fungi *viz*., *Fusarium solani*, *F. moniliforme*, *F. equiseti*, *F. oxysporum*. *F. semitectum*, *Rhizoctonia solani*, *Alternaria alternata*, *Curvularia lunata*, *Aspergillus niger* and *Penicillium* sp. were isolated from infected roots, bark, seed and stem of shisham. *F. solani* was most predominant followed by *R. solani* and *C. lunata*. Maximum infection frequency of 75.00% was exhibited by *F. solani* colonizing stem tissues followed by 47.39% from bark tissues, 29.83% from seeds and 15.62% from roots. The colonization percent of *F. solani* was highest in stem tissues collected from Ghotki (85.50%) as compared to Pano Akil (70.00%), Mirpur Mathelo (65.50%), Daharki (56.50%) and Hala (34.60%) followed by *F. moniliforme* ranging from 3.00-18.50% as compared to other isolated fungi. Seed germination percentage was also reduced (50.00%) in soil infested with *F. solani* and seedling mortality was 93.33% followed by soil infested with *R. solani* (60.00%) with mortality rate (66.66%) and *C. lunata* (70.00%), seedling mortality rate of 42.85% as compared to *F. moniliforme* and *F. oxysporum*, respectively.

Introduction

Shisham (*Dalbergia sissoo* Roxb.), native to Haryana and other parts of India, Pakistan and Nepal is of great importance because of its multiple uses, such as furniture wood, building timber, plywood and fuel. It is medium to large sized deciduous tree with a light crown and is perpetuated by seeds and suckers (Hassan, 2005). It is cultivated in forest plantation, along water channel and canal banks, road sides and railway lines. Unfortunately shisham is susceptible to dieback, wilt and several other soilborne pathogens (Sah *et al.,* 2003). Previously Bakshi (1974) isolated *Phellinus gilvus* from roots of trees affected by dieback. Richardson (1990) reported several species of *Aspergillus, Penicillium, Rhizopus, Alternaria, Fusarium, Chaetomium, Drechslera* and *Curvularia* from forest tree seeds. Parajuli *et al.,* (1999) reported *Fusarium oxysporum* from *Dalbergia sissoo* on water-logged soils in Nepal. Manadhar & Shrestha (2000) isolated *Botryodiplodia* sp., and *Fusarium solani* from five diseased samples of *D. sissoo.* Khan *et al.,* (2001b) detected *Aspergillus niger, A. flavus, A. terreus, Aspergillus* sp., *Alternaria alternata, Chaetomium* sp., *Drechslera australiensis, Fusarium pallidoroseum, F. solani, Fusarium* sp., *Penicillium* sp., *Rhizopus* sp., *and Geotrichum* sp. from seeds of shisham trees. Rajput *et al.,* (2008) isolated *F. solani, Rhizoctonia solani* and *Curvularia lunata* as predominant fungi from shisham die-back trees.

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The insufficient production of shisham is due to dieback and decline and high mortality of seeds and seedlings (Khan & Khan, 2000). The pathogens penetrate into the seed coat and embryo during storage and are responsible for poor germination as well as seedling mortality (Bhansli & Jindal, 1997). Mortality as high as 20-28% has been obtained in Nepal (Joshi & Baral, 2000). Bakhsa & Bask (2000) reported that shisham decline resulted in sudden death of 10% plantation within 6 months after crowns exhibited symptoms of the disease. Bajwa *et al.,* (2003) found 20-28% shisham trees affected by decline during a survey of different areas of Punjab. The present studies were taken up for the first time in Sindh to isolate and identify fungi associated with shisham dieback and their impact on seed germination and seedling mortality.

Materials and Methods

Isolation from root and stem: A survey of shisham growing areas of Sindh *viz*., Ghotki, Pano Akil, Mirpur Mathelo, Dahrki, Hyderabad, Hala, Tandojam and Dadu was carried out. From these localities the samples were collected from shisham trees showing infected branches. Diseased samples including root bark from collar portion, and stem, collected in sterilized polythene bags and brought to the laboratory for the isolations of the associated pathogens. At least 50 samples were taken from five different sites of each locality.

The infected roots, bark, and stem were used for isolation as described by Pathak (1987). Isolations were made on three layers of moistened blotter papers and potato dextrose agar medium (Saleem & Nasir, 1991). Samples were surface sterilized with 0.01% $HgCl_2$ for two minutes, rinsed with sterile distilled water for 2-3 minutes and then placed on blotter papers and PDA under aseptic conditions. Plates were incubated at 25ºC for 7 days.

The fungi growing from infected tissues were identified on the basis of colony characteristics and conidial morphology using keys of Barnett & Hunter (1972), Booth (1977), Neergaard (1979) and Hawksworth *et al.,* (1995).

Isolation from seed: Samples of shisham seed were collected from various areas of Sindh including Ghotki, Pano Akil, Mirpur Mathelo, Daharki, Dadu, Hyderabad, Tandojam and Hala. Seeds were assayed for the presence of seed-borne fungi by standard blotter method (Anon., 1996) on PDA, 100 seeds from each seed lot were placed on moistened blotter papers and freshly prepared PDA medium with five seeds per plate. Plates were incubated at 25ºC for 7 days and observed for fungal growth. The frequency of fungi was estimated using the following formula:

$$\text{Colonization (\%)} = \frac{\text{Number of root pieces/ seeds colonized by a pathogen}}{\text{Total number of pieces/seeds studied}} \times 100$$

Seed germination and seedling mortality: Thirty shisham seeds were grown in earthen pots containing 2kg steam sterilized soil previously infested with either *Fusarium solani*, *F. moniliforme, F. oxysporum*. *Rhizoctonia solani* or *Curvularia lunata* separately. Seeds were allowed to germinate under natural day light conditions and watered when ever needed.

Germination percent was recorded as under:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds used}} \times 100$$

The seedling mortality was also estimated using the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead seedlings}}{\text{Total number of seeds germinated}} \times 100$$

Results and Discussion

Isolation of fungi: *Fusarium solani* (Mart.) Sacc. was isolated most abundantly from all plant parts. Mycelial growth was sparse to dense and grayish to white. Micro-conidia formed abundantly on the aerial mycelium, may be single septate, 9-16 x 2-4 u. Macro-conidia 4-5 septate were well developed and were 40-100 x 5-7.5 u.

Fusarium semitectum var. *majus* Wollenw; showed cottony white with abundant conidiophores in aerial mycelium.

Fusarium oxysporum Schlecht; showed white but usually with light purple colour mycelium. Micro and macro-conidia formed frequently.

Fusarium equiseti (Corda) Sacc., showed first white but later deep olive in colour mycelium.

Fusarium moniliforme Sheld., produces extensive cottony mycelial growth. Conidia are multi-septate and larger formed on typical conidiophores.

Alternaria alternata Auct; develops typical mycelial growth with small conidia but in long chains.

Curvularia lunata (Walker) Boedjin; produces septate mycelium, at first hyaline which later becomes brown.

Rhizoctonia solani Kuhn; produces a dense pale to dark brown mycelium which becomes darker with age.

F. solani was isolated predominantly from stem tissues collected from all locations (34.6-85.50%) as compared to root pieces followed by *F. moniliforme* (3.00-18.50%), *Rhizoctonia solani* (7.50-15.50%) and *Curvularia lunata* (5.50-14.50%), respectively, than all other fungi which were isolated with low frequency (Table 1).

Maximum infection frequency (75.00%) was exhibited by *F. solani* stem tissues followed by 47.39% bark tissues, 29.83% seeds and 15.62% root tissues (Table 2) as compared to *F. moniliforme* 20.00% from bark tissues, 15.00% stem tissues, 11.66% seeds and 9.37% root tissues, *Rhizoctonia solani* (25.00%) from bark, 19.37% stem tissues, 15.00% root tissues and *Curvularia lunata* 25.50% from seeds, 23.75% bark tissues, 17.50% stem tissues and 14.75% root tissues (Table 2). The other fungi were isolated with very low frequency from all parts of shisham tree (Table 2). Ahmad & Bhutta (1993) isolated *F. solani,* and *F. pallidoroseum* from seeds of *Dalbergia sissoo* and *Leucaena leucocephala.* Shakir *et al.,* (1999) reported the association of *F. solani* with roots of affected shisham trees alongwith speices of *Aspergillus, Cladosporium, Fusarium, Verticillium* and *Tylenchorhyncus, Helicotylenchus, Hoplolaimus, Dorylaimus* and *Xiphinema* nematodes. Manadhar & Shrestha (2000) found *Botryodiplodia* sp., and *Fusarium solani* associated with 5 diseased samples of *D. sissoo.* The species of *Alternaria, Aspergillus* and *Fusarium* were also detected from seeds of *D. sissoo* (Manadhar *et al*., 2000). Results of our studies also agreed with those reported by Khalid *et al.,* (2002). Mustafa *et al.,* (2004) isolated *Rhizoctonia solani, Fusarium solani, F. oxysporum, F. moniliforme, Aspergillus niger, Alternaria alternata* and *Helminthosporium* sp., as seed-borne fungi from seed samples of shisham.

Table 3. Effect of predominant fungi on seed germination and seedling mortality.

Fungi	Inoculated germination (%)	No. of dead seedlings	Mortality (%)
Fusarium solani	50	14	93.33
Rhizoctonia solani	60	12	66.66
Curvularia lunata	70	9	42.85
Fusarium moniliforme	80	6	25.00
Fusarium oxysporum	90	3	11.11

Seed germination percentage was also affected in soil infested with *Fusarium solani* (50.00%) followed by *Rhizoctonia solani* (60.00%), *Curvularia lunata* (70.00%), *F. moniliforme* (80.00%) and *F. oxysporum* (90.00%) respectively, as compared to uninfested soil (Table 3). Seedling mortality rate was maximum (93.33%) for *F. solani* followed by *R. solani* (66.66%), *Curvularia lunata* (42.85%), and *F. moniliforme* (25.00%). The least mortality rate of 11.11% was obtained in pots infested with *F. oxysporum* (Table 3). Vigayan & Rehill (1990) and Pathan *et al*., (2007) found reduction in germination of shisham seeds infected with *Aspergillus flavus, A. niger, F. oxysporum* and *F. solani.* Saleem (1999) observed that disease progressed in mango downward and bark was discolored some distance from the tip. Khan *et al.,* (2004) observed mortality ranging from 25.00-30.00% and a disease incidence of 20.50-40.00% in shisham trees in various districts of Punjab including Kasur, Toba Tek Singh, Hafizabad and Gujranwala. Our results also agreed with Shailendra *et al.,* (2004), who recorded highest mortality in shisham plantation in India due to *Fusarium solani*.

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