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Incidence of Shisham (*Dalbergia sissoo* Roxb.) Decline and *In Vitro* Response of Isolated Fungus Spp. to Various Fungicides

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ABSTRACT

A thorough survey of eleven districts of Punjab was carried out to study the present scenario of shisham (*Dalbergia sissoo* Roxb.) decline in these areas. Maximum mortality of 25-30% was observed in Kasur and T.T. Singh. Maximum disease incidence of 20.5 to 40.4% was recorded in Hafizabad and Gujrawnwala. There was an invariable association of *Botryodiplodia theobromae* with aerial as well as under ground parts. *Fusarium solani* was mostly isolated from roots and stem while, *Colletotrichum* sp. was only isolated from stem and branches. Inoculation of healthy plants with *B. theobromae* either alone or in combination with *F. solani* and *Colletotrichum* sp. produced typical symptoms; whereas, *F. solani* and *Colletotrichum* sp. failed to produce these symptoms. *In vitro* effect of fungicides on the mycelial growth rate of *B. theobromae* revealed Topsin-M and Score to be effective at 100 ppm, respectively. Trimiltox was the least effective fungicide against the fungus at all concentrations.

Key Words: Shisham; *Dalbergia sissoo*; *Botryodiplodia theobromae; Fusarium solani; Colletotrichum* sp.; Fungicide

INTRODUCTION

Shisham (*Dalbergia sissoo* Roxb), a deciduous tree of family Papilionaceae, is an important plant of great economic importance. It is cultivated in forest plantations as well as along the canals, roadsides, railway lines, water channels and boarders of the agricultural fields. Shisham wood is used in furniture, construction work, agricultural implements, plywood industries and fuel purposes. According to Greeks, shisham plant has some medicinal properties. It provides financial support to the farmers as it is considered as cash plant. The area under such plantation in Punjab is 154,886 ha, with an average annual production of 28,000 m^3 (Khan & Khan, 2000). A number of diseases like powdery mildew, leaf rust, leaf blight, collar rot, wilt, die-back and *Ganoderma* root rot are reported by various research workers like, Khan *et al.* (1956), Khan (1960, 1961), Khan and Bokhari (1970), Bagchee (1952), Bakshi (1954) and Zakaullah (1999) to occur on this plant in Indo-Pakistan. No record of bacterial or viral diseases has so for been made.

Shisham has been inflicted with decline or dieback few years ago and the incidence is also reported in the Tarai tract of Nepal, believed to be its home. The decline has been reported as early as 1900 but has never assumed an alarming proportion. It was in 1998 that decline or dieback was reported as an epidemic in central tract of Punjab Province (Naz, 2002; Bajwa *et al*., 2003). The present studies were undertaken to investigate the cause of this disease and evaluate some toxicants against the pathogen under *in vitro* conditions.

MATERIALS AND METHODS

Survey of infected field. A survey of shisham growing areas of Punjab *viz*., Sahiwal, Sheikhupura, Narowal, T. T. Singh, Faisalabad, Vehari, Kasur, Jhang, Hafizabad, Sargodha and Gujrawnwala was carried out to study the symptoms, spectrum and severity of shisham decline in these localities. Diseased samples including roots bark from collar portion, stem and branches were collected for the isolations of the associated pathogen (s). Isolations were made on Potato Dextrose Agar medium (PDA) and filter paper method. Samples collected from different locations were processed separately. The fungi isolated were identified with the help of keys (Booth, 1977; Neergaard, 1979). All the organisms were maintained on PDA for further studies.

Isolations. The infected roots, bark, stem and branches were passed through the process of isolation as described by Pathak (1987). Three layers of well moisture filter papers in plastic petri dishes and PDA medium (Saleem & Nasir, 1991) were used during this process. All the petri-plates were incubated at 25±1ºC for seven days for the isolation of fungi. The frequency of the fungi in the collected specimens was recorded using the following formula:

$$\text{Colonization \%} = \frac{\text{Number of pieces colonized by a pathogen}}{\text{Total number of pieces}} \times 100$$

Pathogenicity. Pathogenicity tests of the most frequent fungi viz., *Botryodiplodia theobromae, Fusarium solani* and *Colletotrichum* sp. were carried out in screen house of Plant Pathology Section, Ayub Agricultural Research Institute, Faisalabad. In each experiment, apparently healthy looking shisham seedling were selected and specimens were taken

from their roots and branches to confirm the absence of the test pathogens. Seedlings found infected with the test pathogen (s) were not used in the study. Healthy seedling (6-8″) transplanted in 12″ earthen pots. When the seedling reached about one-foot height; roots, stem and branches of separate plants were inoculated with the test organisms either alone or in combination.

A cut in the stem or root was made using a sterilized knife. A 1x 2 cm inoculum block from the 7-8 days old culture of test fungus on PDA was placed in the gap and the inoculated portion was wrapped with Para film. A 1x2 cm PDA block without fungus was placed in the control plants. Plants were irrigated after inoculations and the wrapping material was removed after 2 weeks of inoculation. Plants were monitored for the development of the disease symptoms and isolations were made from the roots, stem and branches of the test plants to confirm the pathogenicity. The experiments were carried out in screen house, and treatments were arranged in randomised complete block design (RCBD) replicated by tetra-fold. Data on development of disease symptoms were recorded and statistically analysed. The key described by Khanzada *et al* (2004) was used for the assessment of disease symptoms.

Key Scale	Description of symptoms
0	No symptoms
1	Very light
2	Moderate
3	Severe symptoms

Screening of fungicides. To test the *in vitro* efficacy of fungicides; Topsin M-70 WP (Thiophanate methyl), Score 250 E.C (Difenoconazole), Trimiltox forte (Copper+mancozeb) and Dithane M-45 (Mancozeb) at 10, 20, 50 and 100 ppm concentrations were added to the autoclaved PDA medium. A 4-mm-diameter disk was removed with a cork borer from the growing margin of a 7-8-days-old *B. theobromae* colony and transferred to the centre of new PDA plate into which the chemical to be tested was incorporated. Test plates were incubated at 25ºC. Colony diameter was measured in two directions on a daily basis for a maximum of 14 days. Growth rates (mm day^{-1}) were determined by plotting mean colony diameter against time and calculating the slope of the regression line for the linear growth phase. The experiment was conducted in completely randomised design (CRD) and there were four replications for each treatment.

RESULTS AND DISCUSSION

Survey of infected field. Shisham decline was frequent in all the areas visited during the present studies. Initially the top leaves of infected plants turn light yellow. The withering of individual branches from top to downward takes place. Bark at the collar region becomes rotted; turn brown and often splits, and roots become dark brown to black. Bark of trunk splits, gets separated from cambium and can be peeled off easily. There are holes in the bark and galleries in the cambium with dark brown powder like material due to insects that may contribute in the disease spread. The whole plant dries up gradually and takes several months to dry completely. The disease attacks the plant regardless of age and reduces the quality of wood and price value. The symptoms were more severe in areas under water stress as compared to regularly watered plants.

The highest percentage of disease incidence of shisham decline, ranging from 35.0-40.4% was recorded in Sahiwal and Gujrawnwala. The percent disease incidence in Faisalabad, Sheikhupura, Kasur, T.T.Singh, Sargodha, Jhang and Norowal was also fairly high ranging from 25-30.5%. The least percent disease incidence 20.5 and 24.7% was recorded in Hafizabad and Vehari. In Kasur and T.T. Singh, the highest percentage of mortality (25-30%) of shisham trees was recorded. The data collected from other nine Districts of the Punjab revealed that the disease has caused great damage, as 8.0–20.5% trees have been dried (Table I). There are only a small number of reports in the literature regarding shisham decline or dieback of shisham. The roadside disease severity data collected from eight districts of the Punjab revealed that the disease has caused great damage, as 20.6–43.2% trees have been dried on public land (Gill *et al.,* 2001). The mortality rate of shisham decline along the roadsides especially along highways was also fairly high ranging from 20–40%. The least mortality and disease incidence of 10% or below was recorded on shisham trees growing on Agricultural lands either in linear rows along the boundaries of the field or scattered plants (Bajwa *et al.,* 2003).

A total of 10 fungi viz., *B. theobromae*, *F. solani*, *F. dimarium*, *F. semitectum*, *Pestalotia* sp., *Curvularia* sp., *Drechslera* sp., *Chaetomium* sp., *Colletotrichum* sp. and *Ganoderma* sp. were isolated from roots, stem and branches of shisham plants. Of these, *B. theobromae* was the most abundant fungus that was isolated from all locations as well as from all the plant parts. *F. solani* was the second most frequent fungus isolated from the roots. *Colletotrichum* sp. was third fungus that was isolated from stem and branches. All other fungi were isolated occasionally with very low

Table I. Shisham decline disease incidence and plant mortality in different districts of the Punjab

Name of District	No. of sites	Disease incidence (%)	Plant mortality (%)
Faisalabad	10	30.5	20.0
Gujrawnwala	5	40.4	18.0
Hafizabad	4	20.5	15.0
Jhang	7	25.5	16.5
Kasur	5	30.0	25.0
Narowal	4	25.0	8.0
Sahiwal	7	35.0	14.0
Sargodha	3	28.0	15.5
Sheikhupura	16	30.0	17.0
T.T. Singh	13	28.5	30.0
Vehari	6	24.7	20.5

frequencies (Table II). Manandhar and Shrestha (2000) examined five diseased samples of *D. sisso* and *Botryodiplodia* sp. and *F. solani* were found associated with the samples.

Pathogenicity. *B. theobromae* alone or with *F. solani* and *Colletotrichum* sp. showed typical symptoms of the disease, whereas, no such symptoms were observed on plants inoculated with *F. solani* and *Colletotrichum* sp. alone. Un-inoculated plants showed no gummosis. Plants inoculated with *B. theobromae* either alone or in combination with *F. solani* and *Colletotrichum* sp. showed gummosis; whereas, no gummosis, was observed in plants inoculated with *F. solani* and *Colletotrichum* sp. alone. In control plants, no vascular browning was observed whereas in plants inoculated with *B. theobromae* either alone or with *F. solani* and *Colletotrichum* sp., browning of vascular tissues was prominent. Similarly, decline or death of branches and leaves was only observed where plants were inoculated with *B. theobromae* either alone or in combination with *F. solani* and *Colletotrichum* sp. (Table III). It is clearly indicated that the shisham decline is caused by *B. theobromae* and *F. solani* and *Colletotrichum* sp. play no significant role in disease development. Re-isolation from the dead and green branches of *B. theobromae* inoculated plants showed up to 95% recovery of the fungus.

The results of the present studies are in close confirmation to those of Manandhar and Shrestha, (2000) who recorded similar symptoms of dieback (shisham decline) and found that after artificial inoculations with *B. theobromae*, *Cladosporium* sp., *Aspergillus* sp., *Fusarium spp*. and *Alternaria* sp. only *B. theobromae* caused the disease.

Screening of fungicides. The effectiveness of the test fungicides in reducing the mycelial growth of *B. theobromae* varied greatly (Table IV). Topsin M at 100 ppm completely suppressed mycelial growth of *B. theobromae*. At 10 ppm and 100 ppm, score reduced the growth rate of the test fungus by 34.2 and 83.7%, respectively. Dithane M-45 at 50 ppm and 100 ppm reduced the growth rate by 48.4 and 65.2%, respectively. However, *B. theobromae* was tolerant to trimiltox, showing faster mycelial growth rate (13.8 & 10.0 mm day^{-1}) at 50 and 100 ppm respectively. Shelar *et al.* (1997) Studied *in vitro* effectiveness of fungicides against *B. theobromae* and reported that thiophanate-methyl (0.1%) and benomyl (0.1%) could completely suppress the growth of fungus. Bank *et al.*

Table II. Fungi isolated from root and stem of shisham decline plants in different location of Punjab, Pakistan

Fungi isolated	Colonization % at different Districts																					
	F. Abad		Gujrawn-wala		Hafizabad		Jang		Kasur		Norowal		Sahiwal		Sargodha		Shiekhu-pura		T.T. Singh		Vehari	
	R*	S*	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Botryodiplodia theobromae	22.2	41.2	40.5	45.5	33.5	45.5	34.9	45.8	50.0	60.0	5.0	90.6	33.9	41.5	35.5	50.0	55.5	70.5	20.5	45.5	15.5	60.5
Fusarium solani	12.5	1.5	10.5	2.8	9.5	2.7	16.3	2.2	10.5	3.5	9.5	3.0	15.5	2.5	13.2	3.9	15.5	5.5	5.4	0.0	12.5	2.5
Fusarium dimarium	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	3.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0
Fusarium semitectum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	3.0	0.0	2.5	0.0
Pestalotia sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	0.0	2.5	0.0	0.0	0.0	0.0	0.0	3.5	0.0	3.5	0.0	0.0	0.0	0.0
Curvularia sp.	0.0	1.5	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0	3.5	0.0	1.8	0.0	2.5	0.0	3.5	0.0	2.0
Drechserla sp.	0.0	2.5	0.0	0.0	0.0	3.0	0.0	0.0	0.0	1.5	0.0	3.5	0.0	4.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0
Chaetomium sp.	0.0	3.5	0.0	2.5	0.0	0.0	3.5	2.5	0.0	0.0	2.5	0.0	6.5	3.5	0.0	0.0	0.0	3.5	0.0	0.0	10.0	5.0
Colletotrichum sp.	0.0	10.5	0.0	8.5	0.0	14.0	0.0	8.5	0.0	3.5	0.0	5.0	0.0	9.5	0.0	8.5	0.0	5.5	0.0	3.5	0.0	3.5
Ganodema sp.	0.0	2.5	0.0	1.5	0.0	2.5	0.0	1.4	0.0	2.5	0.0	2.5	0.0	2.0	1.5	0.0	0.0	0.0	0.0	1.5	0.0	2.5

R* = Roots, S* = Stem & branches

Table III. Severity of symptoms on shisham plants inoculated with *Botryodiplodia theobromae, Fusarium solani* and *Colletotrichum* sp.

Pathogen	Symptoms produced on shisham plants inoculated in stem and roots					
	Drying of tips		Gum exudation		Internal browning	
	Stem	Roots	Stem	Roots	Stem	Roots
Botryodiplodia theobromae	3 a*	3 a	3 a	2.8 a	3 a	3 a
Fusarium solani	0 c	0 c	0 c	0 c	0 c	0 c
B. theobromae + F. solani	3 a	2.5 b	3 a	2.8 a	3 a	2.6 b
Colletotrichum sp.	0 c	0 c	0 c	0 c	0 c	0 c
B. theobromae + Colletotrichum sp.	2.5 b	2.5 b	2.5 b	2.3 b	2.5 b	3.0 a
Control (with injury + PDA)	0 c	0 c	0 c	0 c	0 c	0 c
Control (Healthy plants)	0 c	0 c	0 c	0 c	0 c	0 c
Lsd (p = 0.05)	0.4	0.4	0.4	0.5	0.4	0.3
CV%	18.0	24.7	18.0	26.7	18.0	14.7

Means sharing similar letters do not differ from each other at P = 0.05

Table IV. Effect of fungicides at different concentrations on the mycelial growth (mm day^{-1}) of B. theobromae on potato dextrose agar at 25 ±1 °C

Fungicides	10 ppm		20 ppm		50 ppm		100 ppm	
Topsin M	**Mycelial growth (mm day^{-1})**	**Percent decrease over control**	**Mycelial growth (mm day^{-1})**	**Percent decrease over control**	**Mycelial growth (mm day^{-1})**	**Percent decrease over control**	**Mycelial growth (mm day^{-1})**	**Percent decrease over control**
	10.8 e	48.1	7.5 e	63.9	4.0 e	81.3	0.0 a	100
Score	13.7 d	34.2	11.5 d	44.7	8.3 d	61.1	3.5 d	83.7
Dithane	15.4 c	26.0	13.8 c	33.7	11.0 c	48.4	7.5 c	65.2
Trimiltox	17.8 b	14.5	16.3 b	21.6	13.8 b	35.2	10.0 b	53.5
Control	20.8 a		20.8 a		21.3 a		21.5 a	
Lsd	1.08	0.97	0.97	0.87				
CV%	4.6	4.7	5.6	6.79				

Means sharing similar letters do not differ from each other at P = 0.05

(1998) found that carbendazim at 400 ppm completely inhibited the linear growth of *B. theobromae* followed by thiophanate-methyl at 450 ppm. Mahmood *et al*; (2002) reported that benlate @ 100 ppm while Topsin-M @ 50 ppm completely inhibited the colony growth of *B. theobromae* whereas Benlate @ 100 ppm proved effective against *Colletotrichum gloeosporioides*.

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