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PATHOGENICITY AND HOST RANGE OF *FUSARIUM SOLANI* (MART.) SACC. CAUSING DIEBACK OF SHISHAM *(DALBERGIA SISSOO* ROXB.*)*

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Abstract

Pathogenicity test of predominantly isolated *Fusarium solani* from shisham dieback trees was conducted on shisham seedlings by inoculating either alone or in combination with less frequently isolated *Rhizoctonia solani* and *Curvularia lunata.* Internal browning of stem and roots were rated on 0-5 scale. Shisham plants inoculated with *F. solani* alone produced maximum disease incidence, showed prominent typical symptoms of the disease with internal browning of stem and roots. However, *R. solani* and *C. lunata* either completly failed or caused very rare infection on test plants. *F. solani* produced moderate infection on shisham seedlings when inoculated with either *R. solani* or *C. lunata*. Maximum reduction in root and shoot length was observed in plants inoculated by injecting spore suspension of *F. solani* as compared to soil amended with spore suspension of the fungus or plants sprayed with spore suspension. Root and shoot weight was also decreased when spore suspension of the *F. solani* was injected into stem followed by soil amended with spore suspension and plants sprayed with spore suspension. Similar trends were also observed in reduction percentage in whole plant growth and weight of plants. Host range studies were coducted by inoculating seedlings of 10 different trees with *F. solani* indicated that the test fungus was moderate to highly pathogenic to shisham, Indian laburnum, siris and gold mohar.

Introduction

Shisham (*Dalbergia sissoo* Roxb.) is one of the most important trees cultivated in forest plantations alongwith water canals, road sides, irrigation channels and railway lines. Shisham attacked by the disease has been so epidemic in the central irrigated Punjab during 1998 (Naz, 2002) and caused great damage, almost 70% of shisham trees were affected by dieback disease (Khan *et al.,* 1999). Bakshi *et al.,* (1976) found that the primary causal agent involved in the root infection was *Fusarium* sp. Sah *et al.,* (2003) observed that this important tree species is attacked by dieback and wilt diseases in plain land and natural forest sites of Nepal. Sharma *et al.,* (2000) found that *Ganoderma lucidium* may be the cause of shisham dieback. Gill *et al.,* (2001) reported *Phytophthora cinnamomi* as the only cause of shisham trees cultivated in moist conditions or alongwith canal sides. Bajwa *et al.,* (2003a) reported the occurrence of sudden decline in shisham trees of the Punjab. *Fusarium solani* has been found to be the cause of wilt (Bajwa *et al.,* 2003b), while there are controversial reports regarding the causal agent of dieback (Bakshi, 1974; Sharma *et al*., 2000). Dayaram *et al.,* (2003) estimated the damage of *Dalbergia sissoo* due to shisham dieback caused by *Fusarium solani* f. sp. *dalbergiae*. In our previous study *F. solani* was predominantly associated with shisham trees causing dieback disease, although other fungi *viz., R. solani* and C. *lunata* were also associated with diseased trees less frerquently (Pathan *et al*., 2007). This is the first study so far conducted on etiology of the casual agent of disease in Sindh.

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Materials and Methods

Pathogenicity test of most frequent fungi: Pathogenicity test of the most frequent fungi *F. solani* was undertaken either alone or in combination with *R. solani* and *C. lunata.* In this experiment, apparently healthy looking shisham seedlings of uniform size (about 2 foot height) with obtained from Divisional Forest, Hyderabad. The plants were transferred in sterilized earthen pots containing 2kg steam sterilized soil.

A cut in the stem or root was made by a sharpe sterilized knife. Two ml spore suspension (10^6 cfu/ml) of fungi prepared from 7-8 days old culture on potato-dextrose agar medium was injected in the cut and the inoculated portion was wrapped with Para film. Two ml distilled sterile water was injected in the cut of plants treated as control. Plants were watered after inoculation and Para film was removed after two weeks of inoculation. Data on the symptoms development was recorded using 0-5 scale (Fig. 1) described as under:

0= No infection, 1= Slight infection, 2= Moderate infection, 3= Slightly severe infection, 4= Severe infection, 5= Dead leaves / shisham plants.

The observations were also recorded on internal browning of infected stem and roots selected randomly. The experiment was arranged in randomized complete block design (RCBD) with four replications.

In second experiment, healthy shisham seedlings of uniform size were transplanted in sterilized earthen pots containing 2kg steam sterilized soil inoculated with 5ml spore suspension (10^6 cfu/ml) of *Fusarium solani* using different treatments. The uninoculated seedlings transplanted were treated as control. After 45 days, seedlings were depotted and the data was recorded on root length and weight, shoot length and weight.

In another experiment, earthen pots containing 2kg sterilized soil were inoculated with predominantly isolated fungi. After 5 days, 25 seeds were grown in infested soil. The experiment was depoted after 45 days, and data was recorded on germination and seedling mortality.

Host range studies: Healthy seedlings of uniform size (about 2 foot height) of 10 different trees (Table 1) i.e., shisham, mango (desi), eucalyptus, siris, acacia, guava, banyan, Indian laburnum, gold mohar and neem were transfered in sterilized earthen pots containing 2 kg steam sterilized soil. The stem and roots were inoculated with 2ml spore suspension (10^6 cfu/ml) of *F. solani* as described previously. After 45 days of inoculation, the observations were taken on root length and weight, shoot length and weight respectively.

Results and Discussion

Plants inoculated with *F. solani* either alone or in combination with *R. solani* or *C. lunata* showed typical dieback symptoms with yellowing of leaves (Fig. 2), and internal browning of stem and roots (Table 2) as compared to control plants in which 100% green leaves were noticed (Fig. 2). It is clear indication that *F. solani* was the casual agent of shisham dieback disease, whereas *R. solani* and *C. lunata* did not have significant role in development of the disease.

Fig. 1. Shisham plants showing variation in yellowing of leaves after inoculation with *Fusarium solani*.

Fig. 2. Pathogenicity test of *Fusarium solani* showing healthy and infected shisham plants.

Table 1. List of trees used in host range study.

S. No.	Local name	English name	Botanical name
1.	Talli	Shisham (Sissoo)	*Dalbergia sissoo* Roxb.
2.	Neem	Aza	*Azadirachta indica* L.
3.	Sufedo	Eucalyptus	*Eucalyptus alba* L.
4.	Amaltas	Indian laburnum	*Cassia fistula* L.
5.	Aumb	Mango	*Mangifera indica* L.
6.	Sirianh	Siris	*Albizia lebbeck* L.
7.	Bubar	Acacia	*Acacia nilotica* Lam.
8.	Amrood	Guava	*Pisidium guajava* L.
9.	Bar	Banyan	*Ficus bengalansis* L.
10.	Gul Mohar	Gold Mohar	*Delonix regia* L.

Table 2. Disease severity of shisham plants inoculated with *F. solani* either alone or in combination with *R. solani* and *C. lunata*.

Treatment	Stem	Internal browning	Roots	Internal browning
F. solani	5.000 a	5.000 a	3.500 a	3.500 a
R. solani	0.000 d	0.250 d	0.750 c	0.250 c
C. lunata	0.000 d	0.000 d	0.000 d	0.000 c
F. solani + *R. solani*	4.250 b	3.500 b	2.500 b	2.500 b
F. solani + *C. lunata*	2.500 c	2.250 c	2.500 b	2.250 b
Control (PDA Suspension)	0.000 d	0.000 d	0.000 d	0.000 c
Control (without PDA Suspension)	0.000 d	0.000 d	0.000 d	0.000 c
LSD (P = 0.05)	0.532	0.507	0.600	0.600

Root (11.00 cm) and shoot length (19.00 cm) were significantly reduced in shisham plant when spore suspension of the fungus was injected (Table 3) as compared to soil amended with inoculam (12.75 and 27.00 cm) or plants sprayed with spore suspension of *F. solani* (15.25 and 28.00 cm), respectively (Table 3). The overall reduction in growth of plants inoculated by injecting spore suspension was 45.67% as compared to inoculum applied in the soil (18.04%) and plants sprayed with spore suspension of the fungus (10.82%) respectively (Table 3). The significant reduction in root and shoot weight was also observed in plants inoculated by injecting the spore suspension (140.00 and 300.00 mg) than the soil amended with spore suspension (235.00 and 370.00 mg) or plants sprayed with sproe suspension of the fungus (275.00 and 437.00 mg) as compared to control plants (465.00 and 612.00 mg) respectively (Table 3). Seed germination recorded in shisham plants inoculated with *F. solani* alone was (52.00%) followed by *R. solani* (64.00%) and *C. lunata* (88.00%) as compared to plants inoculated with *F. solani* in combination with *R. solani* (56.00%) and *C. lunata* (72.00%) and in control in which 100.00% germination was observed (Table 4). The seedling mortality rate was increased when plants were inoculated with *F. solani* alone (92.30%) followed by *F. solani* inoculated in combination with *R. solani* (78.57%) or plants inoculated with *R. solani* alone (56.25%) (Table 4).

Table 4. Effect of with *F. solani* either alone or in combination with *R. solani* and *C. lunata* on seed germination and seedling mortality of shisham plants.

Treatment	Seeds germination (out of 25 seeds)	Germination (%)	Number of dead seedlings	Mortality (%)
Fusarium solani	13	52.00	12	92.30
Rhizoctonia solani	16	64.00	09	56.25
Curvularia lunata	22	88.00	03	13.63
F. solani + *R. solani*	14	56.00	11	78.57
F. solani + *C. lunata*	18	72.00	07	38.88
Control (-)	25	100.00	0	0

Host range studies: Seedlings of 10 different trees were inoculated with spore suspension of *F. solani.* Root and shoot length were significantly reduced in shisham seedlings (10.50 and 25.50 cm) by stem inoculation followed by Indian laburnum (12.75 and 27.25 cm), siris (14.25 and 30.00 cm), as compared to acacia (16.75 and 32.25 cm), eucalyptus (17.25 and 32.25 cm) and that was increased in mango (20.00 and 34.25 cm) and banyan (21.25 and 36.25 cm) respectively (Table 5). Maximum reduction in root and shoot weight was also observed in shisham (317.75 and 295.00 mg) followed by Indian laburnum (362.50 and 380.00 mg), siris (362.50 and 387.50 mg) and guava (390.00 and 450.00 mg) as compared to gold mohar (467.50 and 1062.50 mg), mango (1012.50 and 1362.50 mg) and banyan (1282.50 and 1522.50 mg), respectively (Table 5).

Growth of shisham seedlings also decreased by root inoculation (13.00 and 27.50 cm) followed by Indian laburnum (15.75 and 29.50 cm) as compared to banyan (25.50 and 43.75 cm), respectively (Table 6). Significant reduction in root and shoot weight was also found in shisham (315.00 and 295.00 mg), Indian laburnum (402.50 and 472.50 mg) and Siris (487.50 and 475.00 mg) as compared to gold mohar (875.00 and 1095.00 mg) and bar (1385.00 and 2017.50 mg) (Table 6).

Bakshi (1974) reported *Phellinus gilvus,* the cause of shisham dieback infecting roots. Parajuli *et al.,* (1999) found that sissoo was infected by a fungus, *Fusarium oxysporum* on water logged soil in Nepal. The results so obtained on causal agent of the disease are in accordance with Khan & Khan (2000) who also found that *F. solani* was the only cause of the disease in different districts of the Punjab. Manandhar & Shrestha (2000) and Manandhar *et al.,* (2000) found that *Alternaria* spp., *Aspergillus* spp. and *Fusarium* spp., were associated with seed of *Dalbergia sissoo.* Aslam (2004) reported that there are number of causes of the disease which may vary in intensity from region to region in Punjab. Nizamani *et al.,* (2005) observed the symptoms caused by *F. equiseti* by injecting spore suspension in stem of different mango varieties as compared to tooth pick and other inoculation methods.

Vigayan & Rehill (1990) obtained significant reduction in germination of *Dalbergia sissoo* seeds infested with *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* and *Fusarium solani*. Khan *et al.,* (2004) recorded maximum mortality of 25-30% in Kasur and T.T. Singh with 20.5–40.4% disease incidence in Hafizabad and Gujranwala districts. The highest mortality and sudden wilting of *Dalbergia sissoo* plantations in Himachal Pardesh were recorded as a result of *F. solani* infestation (Shailendra *et al.,* 2004). Javaid *et al.,* (2004) while surveying 10 districts of Punjab found that *Dalbergia sissoo* Roxb. and *Acacia nilotica* (Lam.) Willd. ex Delile., were most affected with die-back disease.

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