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Original article

Fungi species causing dieback and wilt diseases in shisham [*Dalbergia sissoo* (Roxb)] and impact of various fungicides on their management

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ABSTRACT

Objectives: *Dalbergia sissoo* commonly known as 'shisham' is a multipurpose tree commonly used to produce high-quality timber wood. However, it is under extinction threat due to several biotic and abiotic factors. The fungi species causing dieback and wilt diseases are poorly studied and efficacy of different fungicides for their management has been rarely tested. Therefore, infected root and shoot samples of shisham trees were collected from different localities in Bahawalpur through this study.

Methods: Samples were analyzed for pathological observations which revealed that *Fusarium*, *Alternaria*, *Curvularia*, *Dreschlera*, *Phoma*, *Aspergillus*, *Paecilomyces*, *Haphlographium*, *Steganosporium*, *Ostracoderma*, *Cephalospora*, *Penicillium*, and *Briosia* spp were the fungi species infesting the collected samples. Four fungicides were tested to manage/suppress the mycelial growth of identified fungi species.

Results: Mycelial growth of *Fusarium*, *Alternaria*, *Phoma*, *Lichenocomium*, *Penicillium* and *Aspergillus* was significantly inhibited by the application of Chlorothalonil and Procymidone fungicides, while Sulphur proved least effective by inhibiting the mycelial growth of *Fusarium* spp.

Conclusions: The results revealed that various fungi species infest shisham trees and available fungicides can suppress the growth of these fungi species. Therefore, application of fungicides at appropriate time could lower shisham decline in the country. Nevertheless, field studies are required for further recommendations.

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1. Introduction

Shisham [*Dalbergia sissoo* (Roxb)] is a deciduous tree in *Papilionaceae* family, which has great economic importance due to its fine quality timber wood (Parveen et al., 2009, Naqvi et al., 2019). Although, shisham is widely accepted common name for the species, it is also known as Indian Rosewood (Kumar 2013, Timilsina et al., 2020). Shisham is abundantly grown in India, Pakistan, Nepal, Bhutan, Bangladesh, Malaysia and Afghanistan (Ashraf et al., 2010). Similarly, shisham forests are profusely found in foothills of Himalayan tract either solely or in combination with other

species (Devagiri et al., 2007). Shisham plantations is spread over 10,000 ha producing 28000 m³ timber wood annually (Ashraf et al., 2010; Naqvi et al., 2019).

Shisham is a multipurpose tree, well-known for its rapid growth and production (Kumar et al., 2011). Commonly it is used as fuel wood and good quality timber wood, while other uses of the species include nitrogen fixation, shade, and shelter. Various parts of shisham have high medicinal value (Lal and Sanjay 2012).

Unfortunately, shisham is vulnerable to extinction because of various complex diseases, i.e., wilt, dieback caused by several soil borne fungi (Shah et al., 2010). Dieback disease causes extensive damages to shisham throughout South-Asia and trees of all ages are highly susceptible (Ahmad et al., 2015; Rehman et al., 2012b). Dieback started as an epidemic in central Punjab, Pakistan during 1998 (Mukhtar et al., 2012). Shisham is infested by dieback due to several biotic and abiotic factors in several countries around the world (Bajwa and Javaid 2007). However, the severity of symptoms depends on variety and varies greatly among countries (Webb and Hossain 2005, Shukla 2008). Dieback causes massive damage to plantations and individual trees growing along water channels and borders of agricultural lands (Mukhtar et al., 2015; Rehman et al., 2012b; Shakya and Lakhey, 2007).

Various fungi species are responsible for dieback of shisham trees including *Fusarium solani* (Mustafa et al., 2004), *Cercospora sissoo*, *Phyllactinia dalbergiae*, *Phellinus dalbergiae*, *Ganoderma lucidum* and *Phytophthora cinnamomi* (Dayaram et al., 2003, Harsh et al., 2011, Naqvi et al., 2019). The characteristic symptoms include initial dieback of branches, bark splitting, and gummosis from main stems and consequently death of the whole tree. Likewise, wilting, cankers, internal chlorosis and necrosis are also disease symptoms after severe attack (Javaid 2008, Rehman et al., 2008).

Soil-borne fungi are only managed through the application of highly effective fungicides. These fungicides have significant potential to inhibit the mycelial growth of these fungi (Arif et al., 2021). The application of two fungicides (Captan, Carbendazim) has been found effective against *F. solani*, which was responsible for shisham dieback. The application of Benomyl fungicide significantly inhibited the mycelial growth of *F. solani*. The Dithane M-45 and Vitavax also suppressed mycelial growth of *F. solani* (Mukhtar et al., 2015). Keeping in view the importance of this disease, this study was planned to isolate fungi species infesting shisham trees. Evaluating the efficacy of four fungicides against identified fungi species was the second major objective of the study.

2. Materials and methods

2.1. Sample collection

The infested plant samples of shisham were collected from two different areas (i.e., Dera Nawab and Bahawalpur). Root samples were collected from 30 to 40 cm soil depth. The infected samples were taken from vascular bundles (xylem and phloem) portion. Samples were taken into polythene bags (17 × 13 cm), labeled, and brought in Plant Pathology Lab. Regional Agricultural Research Institute, Bahawalpur, Pakistan. The samples were stored in a refrigerator at 4 °C until use. The trees with varying disease infestation level are given in Fig. 1.

2.2. Cutting, blending and incubation of infested root samples

Root samples were washed with running tap water for 2–3 min and left for 12 h. The shade-dried root samples were chopped and rinsed with HgCl₂ for surface sterilization/disinfestation. These

samples were placed on 9 cm sterilized Petri plates containing moistened blotting paper at the bottom. The samples were incubated at 25 ± 2 °C for fungal growth. The samples were incubated until clear colonies of fungi were developed.

2.3. Isolation of pathogens from different plant parts

The isolates were taken from different plant parts, i.e., root, xylem, and phloem for the isolation of pathogens, and transferred to freshly prepared PDA medium in which 0.25 mL streptomycin per Petri plate was added to prevent the growth of bacterial colonies. The Petri plates were sealed with scotch tape and incubated at 25 ± 2 °C.

2.4. Identification of fungal species

The fungi growing from infected root tissues and plant parts were identified on the basis of colony characteristics and conidial morphology using keys, books and already published literature (Ahmad et al., 2015; Ahmadi and Arzanluo, 2019; Bajwa and Javaid, 2007; Javaid et al., 2010; Rehman et al., 2012b; Barnett and Hunter, 1972). Different pathogens identified during the study are given in Fig. 2.

2.5. In-vitro evaluation of fungicides against various isolates of shisham decline

In-vitro efficacy of four fungicides, i.e., Matiram (Cabreotop), Sulphur (Cumulus), Mancozeb (Acrobat) and Chlorothalonil + Pro cymidone (Protocol) was evaluated by adding calculated quantity of each into autoclaved PDA medium. The 20mL of poisoned melted PDA medium was poured into each sterilized Petri plate and allowed to solidify. The PDA medium without fungicides was regarded as control. After solidification of medium, 6 mm agar plugs of the fungal colonies were transferred to Petri plates containing PDA under Laminar Flow Chamber. Each treatment had three replications. The experiment was laid out according to Completely Randomized Design (CRD). All the plates were incubated at 25 ± 2 °C for 7–12 days.

2.6. Data collection

The growth inhibition rate by each fungicide was recorded after 3 days of incubation. Percent inhibition rate for each fungal colony was calculated by following the methods of (Bajwa and Javaid 2007, Vogel et al., 2011, Rajput et al., 2012).

2.7. Statistical analysis

The collected data were statistically analyzed by Analysis of Variance technique (ANOVA). Normality in the data was tested prior to ANOVA and data having non-normal distribution were transformed by Arcsine transformation technique to meet the normality assumption of ANOVA. Least significant difference post hoc test was used to observe the differences among means at 5% probability where ANOVA indicated significant differences.

3. Results

Various physical and chemical soil properties were determined for the soil samples collected from Dera Nawab (Table 1) and Bahawalpur (Table 2). The soil properties were determined to check the involvement of abiotic factors in disease development. Several abiotic factors including soil type, pH, fertilizer concentration, organic matter content, irrigation source, temperature and relative



Fig. 1. Shisham plants with varying disease severity, slight infection (a), moderate infection (b), slightly severe infection (c), severe infection (d) and dead plant (e).

humidity are responsible for dieback disease in shisham. Shisham decline or dieback has become a threatening disease in shisham growing areas of the world, particularly in Pakistan. The characteristic symptom of disease includes initial dieback of branches, bark splitting, gummosis and consequently death of the whole tree.

There was slight variation in the percentage infestation of different plant parts. The variation in fungal species may be due to different climatic conditions, isolation periods and different storage containers. The highest infestation percentage was recorded for phloem, xylem, and roots. Fungi species found in the root portion were soil-borne fungi and responsible dieback, while fungi prevailing in the xylem and phloem were aftereffects of disease attack. The highest fungi percentage was in roots; hence, it is predicted that any of the fungi found in the roots is the major cause of shisham decline. *Botrytis* and *Alternaria* spp. were recorded in roots and can be considered as the major cause of dieback. The highest percentage of *Alternaria* in xylem and phloem suggested that it played an important role as saprobic fungus as well as main cause of the disease. Presence of *Paecilomyces* in same proportion of xylem and roots and slight appearance in phloem confirmed that it acted as a pathogenic fungus in the spread of the disease. *Cephalospora* was only observed in phloem indicating that it acted as saprobic fungi. *Ostracoderma* acted as soil-borne fungi rather than

saprobic. Slight variations were noted in the distribution of *Penicillium*, *Aspergillus*, *Phoma*, *Curvularia*, *Fusarium* sp., *Lichenomium*, *Haphlographium*, *Briosia*, and *Steganosporium* in all parts of the tree. Fungi species that were suspected to be the cause of shisham decline were grown on agar medium to observe their colonial growth. *Alternaria* and *Fusarium* showed maximum radial growth than others when grown on agar plates indicating that these could be the prominent cause of shisham dieback. However, the colonial growth of *Drehsclera* was suppressed in agar medium.

The results indicated that all the fungicides significantly altered mycelial growth of different fungi (Table 3). The lowest diameter of fungal colony was noted for *Drechslera* on Potato Dextrose Agar (PDA) medium as 0.100 mm/day, while other fungi species had higher diameter, i.e., *Haphlographium* (1.40 mm/day), *Paecilomyces* (1.60 mm/day), *Curvularia* (3.30 mm/day), *Aspergillus* (4.10 mm/day), *Penicillium* (4.60 mm/day), *Fusarium* spp (5.30 mm/day) and *Alternaria* spp. (5.70 mm/day). It was observed that *Alternaria* spp had bigger fungal colony as compared to all other fungi (Table 4). The *Phoma* spp. expressed minimum fungal mycelial growth (1.00 mm/day) after the application of Chlorothalonil + Pro cymidone as compared to control (10.0 mm/day), whereas Mancozeb and Metiram totally inhibited the fungal mycelial growth (0 mm/day). *Aspergillus* exhibited the highest colony growth on

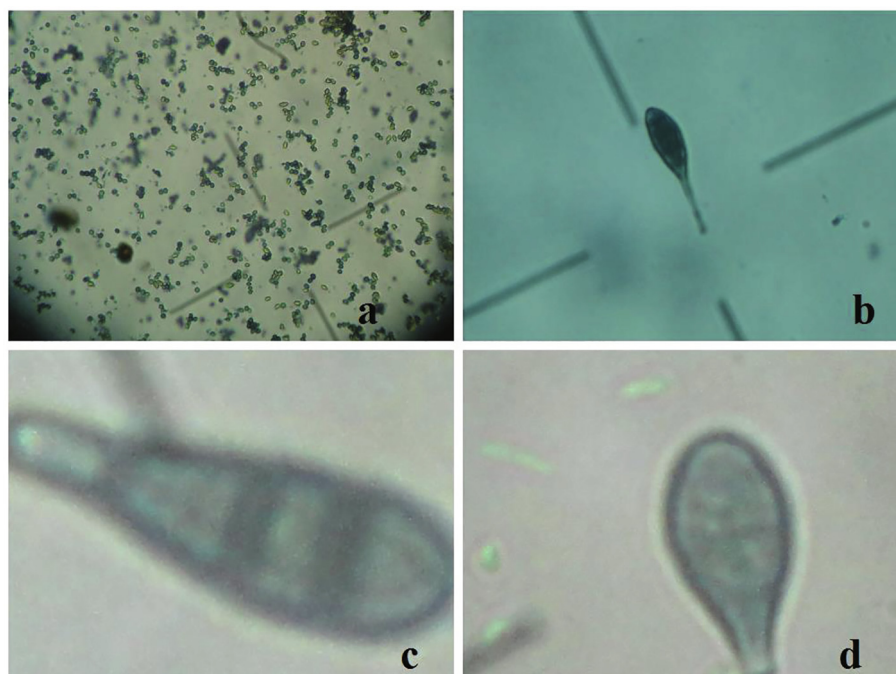


Fig. 2. Different pathogens detected during the study, *Aspergillus* spp. (a), *Alternaria alternata* (b), *Alternaria tenuis* (c), and *Alternaria sesame* (d).

Table 1

Soil characteristics of the sample collection sites in Dera Nawab, Pakistan.

Sr. No.	Depth (cm)	Electrical Conductivity (EC) (dS/m)	pH	O.M %	Phosphorus (ppm)	Saturated Hydraulic Conductivity (Ks) (mm/h)	Potassium (ppm)	Saturation (%)
1.	0–6	5.7	7.7	0.69	8.8	44	174	38
2.	6–12	5.3	7.7	0.45	–	–	–	36
3.	0–6	4.8	7.8	0.62	5	48	190	38
4.	6–12	4.7	7.7	0.45	–	–	–	36

Table 2

Soil characteristics of the sample collection sites in Bahawalpur, Pakistan.

Sr. No.	Depth (cm)	Electrical Conductivity (EC) (dS/m)	pH	O. M %	Phosphorus (ppm)	Saturated Hydraulic Conductivity (Ks) (mm/h)	Potassium (ppm)	Saturation (%)
1.	0–6	1.8	7.5	–	6.7	35	135	38
2.	0–6	1.8	7.7	–	4.5	32	126	34
3.	0–6	1.7	7.5	–	5.3	34	131	38
4.	0–6	1.7	7.6	–	7.6	39	150	40

Table 3

Analysis of variance for fungal colony growth on Potato Dextrose Agar medium.

Source	DF	SS	MS	F	P
Error	2	0.50	0.25		
Fungal growth	8	74.30	9.28	3715.33	0.0000
Error	16	0.04	0.0025		
Total	26	74.84			

Table 4

Means of fungal colony growths on Potato Dextrose Agar medium.

Sr. No.	Fungi	Arithmetic Means
1	<i>Alternaria</i> spp.	5.70a
2	<i>Fusarium</i> spp	5.30b
3	<i>Curvularia</i> spp	3.30f
4	<i>Aspergillus</i> spp	4.10e
5	<i>Penicillium</i> spp	4.60c
6	<i>Haphlographium</i> spp	1.40 h
7	<i>Paecilomyces</i> spp	1.60 g
8	<i>Drechslera</i> spp	1.00i
9	<i>Phoma</i> spp	4.40d
LSD 0.1453		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

control (28 mm/ day) and lowest colony growth (1.00 mm/day) after adding Sulphur in PDA medium.

The results revealed that all fungi exhibited statistically higher growth on PDA medium. *Drechslera* fungi expressed lower growth (1 mm/day) as compared to *Haphlographium* (1.40 mm/day), *Paecilomyces* (1.60 mm/day), *Curvularia* (3.30 mm/day), *Aspergillus* (4.10 mm/day), *Penicillium* (4.60 mm/day), *Fusarium* spp. (5.30 mm/ day) and *Alternaria* spp. (5.70 mm/day) respectively. Metiram inhibited the growth of all fungi, i.e., *Phoma* (0.00 mm/day), *Aspergillus* (0.00 mm/day), *Alternaria* (1.00 mm/day), *Penicillium* (0.00 mm/day), *Fusarium* (0.00 mm/day) and *Lichenocomium* (0.00 mm/day) as compared to control. Similarly, application of Chlorothalonil + Procymidone caused a significant reduction in fungal growth, i.e., *Phoma* (0.00 mm/day), *Aspergillus* (1.00 mm/day), *Alternaria* (0.00 mm/day), *Penicillium* (0.00 mm/day), *Fusarium* (1.00 mm/day) and *Lichenocomium* (0.00 mm/day) as compared to control treatment.

Alternaria showed higher fungal growth of 1 mm/ day on PDA containing Sulphur, Mancozeb and Metiram, while no growth was observed on Chlorothalonil + Procymidone as compared to control (20 mm/ day). The Sulphur and Mancozeb inhibited the fungal growth, i.e., 1 mm/day of *Penicillium* on PDA and no colony was observed on PDA with Metiram and Chlorothalonil + Procymidone (0.00 mm/ day) as compared to control (20 mm/ day). Likewise, *Fusarium* colony growth was retarded by 0.00 mm/day on medium containing Sulphur and Metiram whereas Mancozeb and Chlorothalonil + Procymidone exhibited 1 mm/ day as compared to control (30 mm/ day). Three fungicides, i.e., Mancozeb, Metiram and Chlorothalonil + Procymidone retarded up to 0.00 mm/day fungal colony growth, whereas Sulphur inhibited the *Lichenocomium* growth by 2.00 mm/ day as compared to control 10.0 mm/ day (Table 5).

Table 5

The impact of different fungicides on fungal colony growth through poisoned food medium.

Sr. No.	Commercially available Fungicides	Fungal mycelial growth (mm/ day) on poisoned food medium					
		<i>Phoma</i> spp	<i>Aspergillus</i> spp	<i>Alternaria</i> spp	<i>Penicillium</i> spp	<i>Fusarium</i> spp	<i>Lichenocomium</i> spp
1	Sulphur	3.00b	1.00b	1.00b	1.00b	0.00c	2.00b
2	Mancozeb	0.00d	0.00c	1.00b	1.00b	1.00b	0.00c
3	Metiram	0.00d	0.00c	1.00b	0.00c	0.00c	0.00c
4	Chlorothalonil + Procymidone	1.00c	0.00c	0.00b	0.00c	1.00b	0.00c
5	Control	10.0a	28.0 a	20.0a	20.0a	30.0a	10.0a
LSD values		0.6904	0.8062	1.0614	0.3606	0.7008	0.4333

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

4. Discussion

The wilting, cankers, internal chlorosis and necrosis are the major nefarious disease symptoms observed after sever attack of multiple pathogens (Kumar 2013, Boland and Woodward 2021, Lloret and Batllori 2021). An earlier study (Kumar 2013) reported that wilt disease of shisham in Dehradun and Saharanpur district of western Uttar Pradesh was due to *Fusarium solani*.

Several researchers also isolated these fungi from shisham seeds. The fungi species included *Aspergillus*, *Penicillium*, *Rhizopus*, *Alternaria*, *Fusarium*, *Chaetomium*, *Drechslera* and *Curvularia*, *F. solani* and *Pallidoroseum* (Kumar 2013, Kumar 2014). Likewise, Appanah et al. (2000) isolated *Alternaria*, *Aspergillus* species and *Fusarium*, while *A. niger*, *A. flavus*, *A. tenuis*, *A. alternata*, *Chaetomium* spp., *D. australiensis*, *F. pallidoroseum*, *F. solani*, *Fusarium* spp., *Penicillium* spp., *Rhizopus* and *Geotrichum* spp., were observed by Khan et al. (2002). The study of (Mustafa et al., 2004) also observed *Rhizoctonia solani*, *F. solani*, *F. oxysporum*, *F. moniliformae*, *A. niger*, *A. alternate* and *Helminthosporium oryzae* from shisham tree after severe disease attack. The results of Rajput et al. (2010) are also in line with the current research who isolated *F. solani*, *F. moniliformae*, *F. equiseti*, *F. oxysporum*, *F. semitectum*, *R. solani*, *A. alternate*, *Curvularia lunata*, *A. niger* and *Penicillium* spp. from shisham.

Dieback disease is caused by number of biotic and abiotic factors and commonly known as syndrome due to complexity of pathogens attack (Parveen et al., 2019). The casual pathogen of dieback disease completes its reproduction in vascular bundles and blocks the translocation of water as well as other essential nutrients. The severe attack causes an extensive damage to shisham in South-Asian territories (Ijaz and Haq, 2021; Rehman et al., 2012a, b; Rizvi et al.,). Shisham tree is facing serious disease attack in Pakistan as well as various other countries of the world. Nevertheless, the severity of disease symptoms varies from plant to plant and country to country (Webb and Hossain 2005, Jones, 2021). Dieback caused extensive damages to the planted trees growing along water channels as well as on the edges of agricultural land (Jones, 2021, Kovač et al., 2021, New et al., 2021).

Several studies proved that dieback disease can only be prevented by the application of fungicides as seed treatment and soil modification with crop remnants. This complex disease was managed successfully by application of Bavistin and Captafol with less propagules per unit of soil. It is not feasible to restrict the soil-borne pathogens either with the help of chemicals or crop rotation from the infected soil. For this purpose, the number of factors, i.e., raising of healthy nursery, correct selection of plantation location having light textured soil, good drainage and sufficient humidity should be kept in mind to minimize this disease of shisham (Arif et al., 2021, Shah et al., 2021).

5. Conclusion

It is concluded from this study that several fungal species are responsible for shisham decline in the study area. The dieback and wilt are the major constraints in the successful production of shisham in different growing areas of Pakistan. The involvement of numerous fungi species indicated that this tree has a major extinction threat. There should be concrete efforts to protect this tree from these fungi species. This disease can only be managed by opting multiple approaches such as soil capacity enhancement via nutritional management, proper selection of nursery, proper selection of plantation sites, prevention of root injury, implementation of normal cultivation practices and avoiding the excessive application of fertilizers, insecticides, and fungicides.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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