Dieback disease of *Dalbergia sissoo* trees of some major areas of district Swabi

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Abstract
A survey of various areas of District Swabi including Saleem Khan, Nogram and Gulo Dheri was carried out in different seasons to find out the disease of *Dalbergia sissoo*. Different parts of the plant like roots, barks and leaves were collected and analyzed for disease causative agents and data was recorded. Maximum infection percentage of *Fusarium* sp., including *Fusarium solani* (55.55%), *Fusarium oxysporum* (55.55%) and *Rhizoctonia solani* (66.66%) was reported in roots of *Dalbergia* tree located in the area of Saleem Khan, Swabi. While the bark and leaves of the *Dalbergia* trees were found infected with *Macrophomina phaseolina*, showing maximum infection percentage (77.77%) in the area of Saleem Khan Swabi. Similarly the infection percentage of *Macrophomina phaseolina* was observed maximum in roots, barks and leaves i.e. 88.88%, 55.55% and 77.77% respectively in the region of Nogram Swabi. The site examined like Gulo Dheri shows that the infection percentage of *Macrophomina phaseolina* was highest in roots (88.88%) and leaves (100%) while the infection percentage of *Rhizoctonia solani* was highest in bark (77.77%).

Keywords: *Fusarium* sp; Infection; Pathogens; Sheesham; Survey

Introduction
*Dalbergia sissoo* is a very important and multiuse tree of South Asia [1]. It is very important medicinally [2, 3], and widely used by people traditionally to cure many diseases. The plant also shows antipyretic and analgesic properties [4]. The timber of *D. sissoo* is hard and is used in making furniture [5]. The ethanoic extract of *D. sissoo* has powerful anti-inflammatory effect [6]. The leaves, bark, and wood of *Dalbergia* tree are used as expectorant, anthelmintic, antipyretic and abortifacient [7]. People of India and Nepal used leaves extract to cure dysentery, sore throats, gonorrhea, heart problems and syphilis [8]. The leaves extract of *D. sissoo* has antioxidant activity [9]. *D. sissoo* has property of analgesic, anti-inflammatory and antipyretic [10]. *D. sissoo* tree also has the potential of nitrogen fixing that’s why it is considered as a very important tree [11, 12].

During the survey of a few areas of Punjab it is found that about 80% of *Dalbergia* trees along the canal bank are infected from the wilting and dieback disease and about 20-40% of the trees are infected along the highways and roadsides while the trees growing in the fields are less affected by the wilt and dieback diseases [13]. The maximum frequency of disease (i.e. 53%) was recorded in trees planted on irrigated lands while minimum frequency (i.e. 8.3%) was recorded on farmlands. The common pathogens were *F. solani*, *Botryodiplodia theobromae*, *Phytophthora cinnamomi* and *F. oxysporum* [14]. Dieback disease of *D.
sissoo is considered to be due to the pathogenic bacteria *i.e.* *Pseudomonas* [15]. The pathogenic bacteria, *Pseudomonas* and *Bacillus* play important role in causing Dieback disease of *Dalbergia* [16]. From last few years *Dalbergia* trees are suffering from wilting and dieback diseases [7, 8, 17], in Asia it is considered as the most bethink problem especially in Pakistan. *F. solani* was the causative agent of the dieback disease [19]. The dieback and wilt disease of *D. sissoo* is considered to be due to the pathogenic fungi, *B. theobromae*, *F. solani*, *P. cinnamomi*, *F. oxysporum* and *Ganoderma lucidum* [17, 20]. *F. solani* and *F. oxysporum* cause wilting of *Dalbergia* either alone or together [21]. Four species of *Aspergillus* i.e. *A. flavus*, *A. fumigatus*, *A. japonicus* and *A. teerius* cause failure of seeds to germinate [22]. Ceratocystis is the major pathogen of *D. sissoo* trees [23]. The distribution of different fungi vary on *Dalbergia* tree i.e. *F. oxysporum* is distributed frequently in stem and *R. solani* is distributed in leaves while *B. theobromae* is widely distributed in whole parts of the plant like roots, stem, twigs and bark. Thus *B. theobromae* is considered as the most frequent causative agent of dieback disease [24]. The present resistant species of *Dalbergia*, that show resistant to dieback are actually the progeny of susceptible variety that have undergone some changes in their genotype [25]. The oil extracted from *Putranjiva roxburghii* has antifungal property and stop the activity of *F. solani* and *F. oxysporum* [21]. Use of *Pseudomonas. koreensis* AS15 can raise disease resistant of *D. sissoo* [26]. Scientists worked on Dieback disease of *Dalbergia sissoo* and identified the different pathogens to cause the disease. While the fluctuations in data in the previous work was observed so that the aim of the present study is to identify the causative agents and actual reason of Dieback disease in *Dalbergia sissoo*.

**Methods and materials**

**Survey and collection of diseased samples**

A survey of different areas of Swabi district was carried out in order to collect the samples of *Dalbergia sissoo* trees showing symptoms of dieback disease (Figure 1). Samples were collected from road sides, along canals, water channels and also on agriculture lands. The diseased samples, including bark, leaves and roots, were kept separately in zip lock sterilized polythene bags, labeled and then brought to the laboratory for the isolation of associated pathogens [27]. The samples were placed in refrigerator at 4°C and isolation of associated pathogens was done within 24 hours.

**Isolation of associated pathogens from infected roots, leaves and bark**

Roots, leaves and bark were washed in the running tap water, cut into 1cm small pieces and treated by 1% Ca (OCl)\(_2\) solution. 1cm small parts of tap root, leaves and bark were then transferred on potato dextrose agar (PDA) having streptomycin (0.2 g/L and penicillin (100000 units/L) plates and incubated at 28 °C for five days.

**Identification of pathogens**

After incubation period the growth of fungi was observed on roots, barks and leaves pieces, isolated and identified by keys prescribed by [24, 28, 29]. Infection and colonization percentage of different pathogens were calculated by following formula.

Infection % = No of plants infected by pathogen / Total No. of plants × 100

Colonization % = No of root pieces colonized by pathogen / Total No. of root pieces of all Plants × 100
Figure 1. Trees of *Delbergia sissoo* showing symptoms in the area of Nogram located in District Swabi

**Results**

Among all the pathogens, the percentage infection of *M. phaseolina* was highest i.e. (88.88) in the roots of *D. sissoo* located in the area of Nogram and Gulo Dheri while in Saleem Khan, *R. solani* was found in highest percentage (66.66). *M. phaseolina* (44.44) in Saleem Khan while *R. solani* was (77.77) both in Nogram and Gulo Dheri. Maximum percentage infection of *F. solani* was recorded at the site of Nogram compared to other visited areas i.e. 77.77, 55.55 and 55.55 in Nogram, Saleem Khan and Gulo Dheri respectively. Similarly the sites of Saleem Khan, Nogram and Gulo Dheri the percentage infection of *F. oxysporum* was 55.55, 22.22 and 44.44 respectively. Percentage infection of *F. moniliforme* (11.11) and *Botrytis sp* (11.11) was only found in root isolated from the site of Saleem Khan and was not observed in Nogram and Gulo Dheri (Figure 2). While highest colonization percentage of *M. phaseolina*, *F. solani* and *R. solani* were 35.5, 26.66 and 28.85 respectively recorded in root in the area of Saleem Khan (Figure 3). The percentage infection of *M. phaseolina* (77.77) was highest in the bark of *D. sissoo* collected from Saleem Khan as compared to Nogram (55.55) and Gulo Dheri (66.66). While percentage infection of *F. solani* (55.55), *F. oxysporum* (55.55) and *R. solani* (77.77) was highest in the bark of Gulo Dheri compared to Nogram and Saleem khan (Figure 4). Similarly the highest colonization percentage of
M. phaseolina, was 46.66 in bark of the plant in the area of Nogram while F. solani (22.22) in Saleem khan and R. solani (35.55) in the area of Gulo Dheri were recorded. (Figure.5).
The percentage infection of M. phaseolina (100) was higher in leaves of D. sissoo collected from Gulo Dheri, compare to the tree located in the area of Saleem Khan and Nogram (77.77) and (77.77) respectively. While percentage infection of F. oxysporum (66.66) and F. moniliforme (44.44) was higher in leaves of Nogram than that of Gulo Dheri F. oxysporum (11.11) recorded while the site visited Saleem Khan showed percentage infection of F. oxysporum (33.33) only. The percentage infection of F. solani (44.44) was same in leaves of D. sissoo tree collected from Nogram and Gulo Dheri and (22.22) in the sites of Saleem Khan. Similarly the percent infection of R. solani was same and higher in the area of Saleem Khan and Nogram (66.66)) compared to the site of Gulo Dheri that was (55.55) (Figure 6). F. solani, M. phaseolina, R. solani and Botrytis spp percent colonization were recorded highest, 28.88, 62.22, 35.55 and 37.77 respectively in the area of Saleem khan (Figure 7).

Discussion
The pathogenic fungi like R. solani, Curvularia lunata, F. equiseti, F. moniliforme, F. oxysporum, F. solani, F. semitectum, Aspergillus niger, Alternaria alternate and penicillium are the major reason of Shisham decay in Pakistan [30].
Several pathogenic fungi such as Ganoderma lucidum, F. solani, R. solani, P. cinnamomi, C. lunata, A. niger, A. flavus, Colletotrichum gloeosporioides and A. alternate were isolated from the diseased parts of D. sissoo trees [31]. In present study the pathogens which were isolated from the roots, barks and leaves of Dalberga sissoo, collected from different areas of District Swabi i.e. Saleem Khan, Nogram and Gulo Dheri, were M. phaseolina, F. solani, F. oxysporum, F. moniliforme, R. solani and Botrytis sp. While the major pathogen that was present highly in percentage were M. phaseolina and F. solani which caused decline of Dalberga sissoo trees. Similar research was done by [17, 24, 32] that the main cause of dieback disease of Dalberga sissoo is F. solani. The pathogenic fungi, F. solani is mostly limited to roots of Shisham plants [33, 34]. The highest number of pathogens was recorded in the root samples than in twigs and bark samples [14]. F. solani was mostly found in roots and stem while Colletotrichum sp. was found only in stem [35]. In present study it was observed that the infection % of F. solani was highest in roots as compared to bark and leaves. Similarly F. solani inoculated in Shisham plants created maximum disease incidence, and produced prominent symptoms of the disease with internal browning of roots and stem. However, C. lunata and R. solani completely unsuccessful or affected very rare infection on test plants [36].
The incidence of disease is associated with biotype and zonal environmental factors. However, high genetic variability was found among the pathogenic isolates of F. solani, and it is the major cause of epidemic and determination of shisham dieback [37].
Figure 2. Infection % of fungal pathogens isolated from the roots of *Dalbergia Sissoo*

Figure 3. Colonization % of fungal pathogens isolated from the roots of *Dalbergia Sissoo*
Figure 4. Infection % of fungal pathogens isolated from the bark of *Dalbergia Sisso*.

Figure 5. Colonization % of fungal pathogens isolated from the bark of *Dalbergia Sisso*.
Figure 6. Infection % of fungal pathogens isolated from the leaves of *Dalbergia sisso* Tree

Figure 7. Colonization % of fungal pathogens isolated from leaves of *Dalbergia sisso*
Conclusion
It is concluded that the highest infection of *F. solani* was in roots as compared to bark and leaves. While incidence of *M. phaseolina* and *R. solani* were observed in all selected areas of district Swabi and the combination of these pathogens increased the severity of dieback disease of *Dalbergia sissoo*.

Authors’ contributions
Conceived and designed the experiments: G Parveen. Performed the experiments: S Zain. Analyzed the data: G Parveen & G Rasul. Contributed materials/ analysis/tools: G Parveen. Wrote the paper: G Parveen & Z Rahim.

References


