

Integrated Management of Die back Disease of *Dalbergia sissoo*

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ABSTRACT

Shisham, also known as sissoo (*Dalbergia sissoo*), holds significant value as a versatile tree with high economic importance; however, it has unfortunately fallen victim to several root pathogens. The paper aims to study and report findings carried out on dieback disease affecting Shisham, along with their corresponding integrated Pest Management (IPM) treatment methods. Various fungi, such as *Fusarium oxysporum*, are observed in the environment. Some of the fungi mentioned include *oxysporum*, and others. The diseases that sissoo causes, and *F. oxysporum* represent the primary pathogenic agents in Shisham, leading to both root rot and vascular wilt diseases, ultimately resulting in widespread mortality of this tree species. Nevertheless, cultivating sissoo as a reforested standalone plant without removing the stumps or the original plant's roots may lead to a severe issue of root rot. Proper field sanitation and effective field management are essential for controlling fungal diseases in Shisham trees. A suitable solution for controlling dieback of Shisham has not been found yet. It is necessary to implement the integrated pest management supported by the vermicompost to enhance the quality of soil and restrict the pathogenic fungi to save the shisham plant from the dieback disease.

Keywords: Shisham, Dieback disease, IPM, Pathogenic fungi, Vermicompost.

Introduction

Wilt and dieback are two of *Dalbergia sissoo*'s main illnesses. In a variety of quick successions, the dieback disease causes a significant loss of plants. The disease was mainly concerned with pathogenic rhizospheric fungus. It is rather tough to eradicate mycoflora from the sissoo growing lands, so the disease management assists in the protection of *D. sissoo* plants. The best strategies for the sick management suppose to be integrated disease management. The appropriate application of chemical and biological agents for illness downregulation is part of integrated disease management.

The pathogen-related fungus inhibits soil and infects plants through their roots. Therefore, a key component of disease control is managing the mycofloral diversity and richness of rhizospheric soil.

Management of dieback

Numerous corrective actions can still be performed to minimize harm and to avoid or lessen the die-back epidemic, even though its source is still unknown (Khan et al., 1965). Numerous chemical dieback management trials have been conducted by Idrees et al. (2006). To combat fungal dieback, a variety of compounds can be employed, including Topsin-M, Trimitox Forte, Scoral and Derosal, and Dithane M-45. Furthermore, appropriate results will be obtained with adequate agricultural technology, managerial expertise, and availability of these pesticides (Tiwari et al., 2020; Tripathi et al., 2020). They did not, however, report any success when the illness rate exceeded 25%. Therefore, the key to controlling dieback is early detection in the field and mitigation. It is unable to stop or reverse the disease's progression once dieback symptoms appear in a tree (Javaid et al., 2004). According to Ahmad et al. (1996), fungicides including Vitavax, Dithane M-45, Bavistin, and Benlate are frequently thought to have significant growth-suppressive effects on *F. solani*. Various studies were conducted by biologists to lessen the loss of shisham and regulate die-back. Bakshi (1976) and Bajwa et al. (2007) investigated the antagonistic effects of several fungal species, including *Trichoderma viride*, *T. koningii*, *T. pseudokoningii*, *T. areoviride*, *T. harzianum*, *Aspergillus fumigatus*, and *A. glaucus*, on dieback.

Selection of Bio-agents for Control:

Biological control of fungal diseases includes selection of one or more biological agents as an antagonist of the pathogens. Biological control is specialized and uses specific organism that interfere with the growth of pathogen. As per the literature known different species of *Tricoderma* was found to be as a promising biological control against soil borne plant parasitic fungus. *Tricoderma* is wide spread in nature and present as high population density in soil and plant debris. These are saprophytic fungus which can be quickly grown and easily cultured to produce large amount of conidia and spores which may be used as bio-control agents (Maczinger et al. 2002). Biological control

with the help of *Tricoderma* against pathogenic fungus has been found as effective method and cheapest source of disease controlling agents.

Isolation of *Tricoderma* from soil samples is quite difficult due to the high rate of growth of other fungus on PDA media. *Tricoderma* may be isolated from soil by using selective Agar medium (TSM) for quantitative isolation (Elad *et. al.* 1981). *Tricoderma* may be purchases and use directly and used as a bio-control. According to the Das *et. al.* (1997) for the mass multiplication of *Tricoderma* wheat bran is most suitable for the growth and sporulation. Harman *et. al.* (2004) reported that *Tricoderma* species is present as common free living fungi of soil and root ecosystem. These fungi show opportunistic and parasitic behavior on other fungi. They produce varieties of compounds which induces localized or systemic resistance. Plants get protected from different pathogenic fungus by responses similar to systemic resistance acquired by the plant. Root colonization of *Tricoderma* species enhances the root growth and development, produces resistance against many pathogenic fungus to the host plants (Hossain *et al.*, 2017).

The antagonistic properties of *Tricoderma* species was due to the production of some volatiles compounds which inhibits the growth of other fungi (Dennis and Webster 1971). According to Abdullah *et. al.*, (2013) the *Tricoderma viride* over grown rapidly over the colony of *Fusarium oxysporium* and suppress the growth of *Fusarium oxysporium*. *Tricoderma* species was found to be have inhibitory effect on hyphal growth of *F. oxysporium* in vitro condition (Wendu *et. al.* 2023). The production of volatile and non-volatile antibiotics produced by *T. harzianum* as a common effect suppresses the growth of *Fusarium culmorum* (Michrina *et. al.* 1995). *T. viride* and *T. harzianum* culture filtrates were found to have inhibitory effect to *F. moniliforme* and lesser inhibitory effect on *Aspergillus flavus* (Calistru *et. al.* 1997). According to Biswas (1999) *T. harzianum* have antagonistic effect of *F. oxysporum* due to excessive growth of *T. harzianum* over *F. oxysporum*.

Methods and Materials

Isolation of Pathogen and Bio-control agents

The phytopathogen of *Dalbergia sissoo* which is *F. oxysporium* were isolated and purified from the infected roots over PDA medium. The Bio-control agents i.e.

Trichoderma species were isolated as bio-control. In this case *T. harzianum* were isolated from soil over specialized TSM media and purified.

Screening of microbial antagonist against phytopathogen

The antagonistic behavior of *T. harzianum* were screened for the *F. oxysporium* and also for *Aspergillus niger* and *Rhizoctonia solanii*. The method was adopted as described by Skidmore and Dickinson (1976). The percentage inhibition of mycelial growth of pathogenic fungus was calculated by the method suggested by Vincent and Budge (1990).

Screening of Biocontrol agents for growth enhancement

Pot culture experiment was carried out in green house to check the efficacy of bio-control agents viz; *T. harzianum* against *F. oxysporium* and related fungus viz; *Aspergillus niger* and *Rhizoctonia solanii*. The Biometric observation such as height of plants, No. of leaves and No. of branches were recorded. *In vivo* experiment were performed to check the bio-control efficacy. To test the bio-control activity of *T. harzianum* in *D. sissoo* cutting under green house condition. The test with *D. sissoo* cutting was carried out in two phases by cross inoculation methods. Seedling inoculation includes aqueous inocula of pathogen and *T. harzianum* were prepared. In phase-I aqueous inocula of *T. harzianum* were first provided at the base cutting and further inoculated with pathogenic *F. oxysporium*. In phase-II the aqueous suspension of pathogenic *Fusarium* was inoculated at the cut base of seedling and then cross inoculated with antagonistic *T. harzianum* after 7 days.

Mechanism of Bio-control

Bio-controlling agents which produced as volatile organic compounds, non-volatile organic compound, HCN, Siderophore, Salicylic acid, Chitinase enzyme and assay of peroxidase (PO) controls the growth and proliferation of pathogenic fungi. These parameters are used as a test of bio-control mechanism of *T. harzianum* over *F. oxysporium* for the control of growth and development of *F. oxysporium*.

Soil amendment with organic nutrients:

Soil nutrients may be enriched by different organic nutrients which are half degraded product of biomass. These include green manure, humus, and vermicompost etc. These are rich in minerals such as Nitrogen, Potassium and Phosphorus. This is the primary nutrients need for growth and development of plants. The organic nutrients may be produced by the help of mineralizers such as bacteria and fungi under natural condition or may be produced by earthworm as a digestive product of green vegetation known as vermicompost. Vermicompost is supposed to be very good organic nutrients due to the presence of higher amounts of primary and secondary nutrients, humus and relative moisture. It supply required nutrients to the growing plants. As it does not contain chemical fertilizers thus the danger of salinity of soil is not under concern.

Vermicompost were prepared for the experiment to test the growth of *Dalbergia sissoo* plantlets under optimal nutritional environment to test the effect of *Fusarium* on pathogenesis over host *D. sissoo* plants. The vermicompost unit of University Department of Botany, T. M Bhagalpur University, Bhagalpur was used by following the standard methods. The cemented bed of 8foot × 1foot by ½ foot was filled with gravels of 5 cm average size. This was covered by sand and loamy soil mixture up to 6 cm. The exotic species of earthworm (*Eisenia foetida*) and indigenous species of earthworm (*Perionix excavates*) was used for inoculation and laid over soil layer. The fresh cow dung was laid over soil layer up to 6 cm height and the semi digested vegetable garbage, fallen leaves, chopped straw and other dead plant material were added to the tank. The whole system is finally covered with hay and water was sprinkle everyday to maintain the moisture content of 45 – 50 percent. The earthworm present in the bed along with the microorganism ultimately converts the biomass into nutrient rich vermicompost within 45 days.

In Vivo Experiments

To test the nutritional role of organic matter in the development of disease cause by *F. oxysporium* were performed with vermicompost soil. In the experiment four (4) different sets were prepared.

T₁ = Sterilized Soil T₂ = Sterilized soil + *Fusarium oxysporium* (pathogen)

T₃ = Sterilized soil + Vermicompost

T₄ = Sterilized soil + Vermicompost + *Fusarium oxysporium* (Pathogen)

The soil used in the experiment was prepared by mixing clay, sand into 2:1 ratio and sterilized at 121°C and 15 PSI. The earthen pots were also disinfected by soaking them into 5% Iodine solution overnight and washed with sterilized distil water before the transfer of sterilized soil. Vermicompost soil was also sterilized by autoclaving at 121°C for 3hrs. The earthen pots were prepared as per T₁, T₂, T₃ and T₄ the healthy plantlets were potted for the diseased assessments. The experiments were carried out in three (3) sets. The planted earthen pots were grown in poly-house University Department of Botany, T.M. Bhagalpur University, Bhagalpur. The experiments were initiated during the months of October 2011. The observations were recorded for Plants Height, No. of Leaves, No. of Branches. The observations were started to record after 45 days from plantation and last for about a year with regular observation at the regular intervals of three (3) months.

Soil amendment with Bio-agents

The soil amendments for the better growth and maintenance of *Dalbergia sissoo* seedling under in vivo condition may be done with bio-control agents *Tricoderma harzianum*. In this experiment six (6) different sets were considered.

T₁ = Control (Sterlized soil only), T₂ = Sterlized soil + *Fusarium oxysporium* (Pathogen)

T₃ = Sterlized soil+ *Fusarium oxysporium* (Pathogen)+*Tricoderma harizanium* (Biocontrol)

T₄ = Garden/Natural soil T₅ = Garden soil + *Fusarium oxysporium* (Pathogen)

T₆ = Garden soil+*Fusarium oxysporium* (Pathogen)+*Tricoderma harzianum* (Bio-control)

The soil used for experiment is a mixture of sand, clay and vermiculite in the ratio of 2:1:1. The soil mixture was sterilized at 15 PSI or 121°C temperature after cooling the different parts of soil were separated and prepared as T₁, T₂, T₃, T₄, T₅, and T₆ in separate earthen pots. The earthen pots were pre sterilized by soaking into 5% Iodine solution. The diseased free seedlings were planted in T₁ to T₆ earthen pots. The experiment was carried in three (3) sets. The planted pots were irrigated with sterile distil water and were grown under poly house of University Department of Botany, T. M Bhagalpur University, Bhagalpur. The observations were made after 45 days from the day of plantation. The observation were recorded for height of plants, No. of branches, and No. of leaves. The regular observations were made after three (3) months for one year. During the year of 2011 – 12 the experiments were initiated from the month of

October 2011. The environmental conditions were maintained in poly-house for the optimal growth of host plants.

Results and Discussion

The role of soil *Fusarium* and *Trichoderma* has been observed as a integrated management of the wilting diseased of *Dalbergia sissoo*. The experiments were carried out two different sets, each having triplicates of the similar experiments. The result showed was represented as mean value of the data obtained. In the 1st set of experiment sterilized soil was tested for disease appearance with the pathogenic fungi *F. oxysporum* and their remedy by amending the soil with antagonistic biopesticide fungi *Trichoderma harzianum*. In the 2nd set of experiment the similar experiments were carried out with the natural soil *F. oxysporum* and *T. harzianum*.

Percentage wilting of *Dalbergia sissoo* Treatment

		% Healthy plants	% wilted plants
T ₁	Sterilized Soil (Control)	100	00
T ₂	Sterilized Soil + <i>Fusarium oxysporum</i>	00	100
T ₃	Sterilized Soil + <i>F. oxysporum</i> + <i>Tricoderma harzianum</i>	58	42
T ₄	Natural Soil	89	11
T ₅	Natural Soil + <i>Fusarium oxysporum</i>	00	100
T ₆	Natural Soil + <i>F. oxysporum</i> + <i>Tricoderma harzianum</i>	54	46

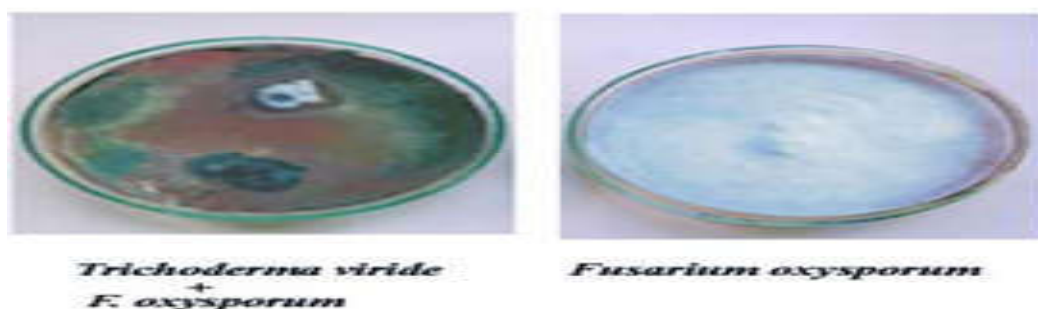


As a integrated pest management the biological pesticide such as *Tricoderma* were afferyly used in the present study to different species of *Tricoderma* viz;- *T. viride* and *T. harzianum* were tested.



Table : In vivo biological control of Fusarium oxysporum by *Tricoderma viride* and *T. harzinum* after 7 days of incubation

Sl. No	Antagonistic Fungus	Diameter of <i>F. oxysporum</i> (mm)	% inhibition of growth of <i>F. oxysporum</i>
01	<i>Tricoderma viride</i>	29.5	46.4
02	<i>Tricoderma harzianum</i>	25.2	58.0



T. viride was found to inhibit the growth of *F.oxysporium* to a minimum of 29.5mm which was 46.4% inhibition with respect to the pure culture of *F. oxysporium*. *T. harzianum* was found to inhibit the fungal colony of *F.oxysporium* to a minimum diameter of 25.2mm which is equal to 58% inhibition with respect to pure cultures of *F. oxysporium*.

Pathogenesis test were carried out with two most dominant fungus found in all the three sites under study

Table E:-Pathogenecity Test

Sl. No	Name of Fungi	Wilting
01	<i>A. niger</i>	-
02	<i>F. oxysporium</i>	+

The *A. niger* would not be able to develop wilt disease in the healthy seedling of *Dalbergia sissoo* while *F. oxysporium* develops to the sign of wilting disease in the healthy seedling of *Dalbergia sissoo*. This results confirms the association of *F. oxysporium* with the wilting disease of *Dalbergia sissoo* plants.

Table – F

The results of pot experiments for the Average Shoot eight (mm) Average No. of Branches and Average No. of Leaves develop in different treatment groups were taken as a mean value.

Table --- F **Treatment of *Dalbergia sissoo***

Sl. No	Treatment	Period (in months)								
		Average shoot height(mm)			Average No of branches			Average No. of leaves		
		06	09	12	06	09	12	06	09	12
01	T ₁ (control)	28.3	33.9	40.5	08	13	18	15	21	45
02	T ₂	25.2	27.3	28.0	04	05	05	10	12	12
03	T ₃	26.3	28.1	30.0	05	07	07	12	16	21
04	T ₄	27.8	30.0	32.4	06	06	09	15	19	30
05	T ₅	24.0	25.2	26.8	03	04	05	11	12	12
06	T ₆	25.1	27.1	32.3	08	08	10	11	16	20

T₁ = Sterilized Soil (control) T₂ = Sterilized Soil + *Fusarium oxysporium*

T₃ = Sterilized Soil + *Fusarium oxysporium* + *Tricoderma harzianum*

T₄ = Unsterilized Soil T₅ = Unsterilized Soil + *Fusarium oxysporium*

T₆ = Unsterilized Soil + *Fusarium oxysporium* + *Tricoderma harzianum*

Table – G **Treatment of *Dalbergia sissoo* with different soil condition**

Soil Condition	Av. Shoot height (mm)			Av. No.of branches			Av. no of leaves		
	06	09	12	06	09	12	06	09	12
T ₁ (Sterlized soil) (Clay:Sand:VC::2:1:1)	32.3	39.6	50.1	10	14	21	17	28	51
T ₂ (Natural sterilized soil)	28.1	33.4	40.6	09	13	19	15	22	43

T1 was taken as natural sterilized soil while T2 was amended with vermicompost in the ratio of Clay : Sand : Vermicompost as 2 : 1 : 1. The different growth parameter such as Average shoot height, Average No. of Branches and Average no of leaves were considered for the three different growth period viz;- 6, 9 and 12 months respectively.

As the Vermicompost regarded as organic nutrients for the growing plants it produces good growing condition for the different plants including *Dalbergia sissoo*. The minerals and physical condition such as pH, moisture, salinity created by vermicompost to the local rhizosperic environment becomes very beneficial for the growth of *Dalbergia sissoo* seedlings. Resulting in much better growth in the sence of average shoot height, average no. of branches and average no. of leaves with respect to natural garden soil.



Conclusion

Many species of fungi such as Fusarium solani, F. oxysporum, Ganoderma lucidum, Phellinus gilvus, Polyporus gilvus, Rhizoctonia solani, Polyporus spongiosum, are the reason of shisham decline. The principal reasons for tree wilting were linked with F.

Solani fungus, *G. Lucidum* was responsible for extreme root rot, and the main cause of dieback is not yet comprehended. It can also be minimized by combining approaches such as root injury prevention, soil capacity enhancement by nutrient control, the avoidance of any harmful use of fungicide and chemical fertilizers, and the implementation of normal cultivation practices. In order to check the sources of pathogens and avoid further transmission of the disease, the dead, wilted, and die-back plants should be uprooted. To replace the susceptible varieties, new shisham nurseries should be raised from seeds and cuttings of the resistant variety. The appropriate use of chemical fungicides against tree diseases entails an understanding of the epidemiology of the disease. Understanding epidemic dynamics, including the life cycle of the pathogen, provides a framework for forecasting disease outbreaks. The decline of shisham is not just the concern of Nepal and India, but also the entire subcontinent of India, such as Myanmar, Pakistan, Bhutan, Bangladesh, Sri Lanka, etc. A strong interaction case by networking is, therefore, necessary on the international level for monitoring and combating this problem. Therefore, further study on the long-term surveillance and evaluation of root pathogens for multiple causes associated with the decrease in the shisham population is recommended.

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