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Physiological studies on *Lasiodiplodia theobromae* and *Fusarium solani*, the cause of Shesham decline

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Abstract

Optimum temperature for the growth of the *Fusarium solani* and *Lasiodiplodia theobromae* fungi was 25 ºC. It was found that continuous light was more suitable than continuous darkness (24 hours) and 12 hours light plus 12 hours darkness for the growth of fungi. Two media were evaluated for best growth of the fungi i.e. *Fusarium solani* and *Lasiodiplodia theobromae, Fusarium solani* grew maximum on Poatao Dextrose Agar (PDA) medium but least grew on the water agar medium. Both the fungal species gave the best mycelial growth on PDA medium as compared to water agar medium.

Key words: *Fusarium solani*, growth medium, *Lasiodiplodia theobromae*, light, pathogenicity, temperature.

Introduction

Shisham (*Dalbergia sissoo* Roxb) is a deciduous tree of family *Papillionaceae* and have great economic value. The species is found in India, Nepal, Bhutan, Bangladesh, Pakistan and Afghanistan. It is also found in tropical to subtropical Africa and Asia, viz. Java, Nigeria, Mauritius, Sri Lanka, Kenya, Northern Zimbabwe, Palestine and South Africa (Tewari, 1994). *D. sissoo* is an important fuel wood, shade, shelter and fodder tree. It usually cultivated in forest plantations and along the canals, roadsides, railway lines, water channels and borders of the agricultural fields. The area under such plantation in Punjab is 154,886 ha, with an average annual production of 28,000 m^3 (Khan and 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 in Indo-Pakistan (Khan *et al*., 1956, Khan, 1960, 1961, Khan and Bokhari, 1970, Bagchee, 1952, Bakshi, 1954, Zakaullah, 1999). Mycologists have recorded 62 pathogenic species of fungi in sissoo. *Polyporus* and *Fusarium oxysporum* cause root rot and wilt respectively (Khan, 1989). *Fusarium solani* was isolated as a facultative parasite associated with wounds and on hosts weekends by unfavorable conditions. Baral (1995) traced the first report of sissoo die back in the plantations of Nepal. A new form of disease die back has been established in sissoo. This disease has been reached epidemic proportion in Bangladesh and other countries of South Asia.

Keeping in view the importance of shesham and its sudden death phenomenon which is the complex disease, present project has been designed to focus on its etiology and physiology of the associated pathogens with shesham decline with followings objectives:

1. Isolation and identification of associated pathogen with shesham decline.
2. Physiological studies of isolated pathogen.

It will provide the help to better understand the optimum conditions for the growth of associated pathogen.

Materials and Methods

Disease sample collection

A thorough survey of shesham growing regions of district Multan was carried out to reveal the symptoms and severity of dieback of shesham. The natural diseased plants showing the typical symptoms of disease were selected from the field and samples of stem portion of diseased shesham tree were collected for the isolation of associated pathogens.

Isolation of pathogens

Isolation of pathogen from these samples were carried out on potato dextrose agar (PDA) medium and on filter paper. The pathogens were examined under microscope and maintained the culture on PDA plates at room temperature (25 ± 2 °C). The associated pathogens of shesham decline were isolated from the infected stem pieces (Pathak, 1987). The infected stem was cut into small pieces 3-4 mm in size with the help of sterilized knife. The pieces were surface sterilized in 2% sodium hypochlorite solution for 3 minutes

followed by five washings with sterile distilled water. The surface sterilized pieces were then placed on three layers of well moistened filter paper in plastic Petri plates and PDA medium (Saleem and Nasir, 1991). All Petri plates were incubated at 25 ± 2 °C for seven days for isolation of associated fungi.

Pathogenicity

Ten healthy plants of sissoo were selected from nursery. A cut was made in the stem of 9 healthy plants with the help of a sterilized knife and inoculated with 1×2 cm block of the isolated culture of *Lasiodiplodia theobromae* and *Fusarium solani* isolated from naturally infected diseased plants in the field. Following this method a cut was made in the remaining one healthy plant to serve as a control and inoculated with 1×2 cm block of only PDA and wrapped with parafilm. Plants were monitored for the development of disease symptoms and pathogens were reisolated from stem of the test plants after seven days to confirm the pathogenicity according to Koch's Postulates (Saleem and Nasir, 1991).

Effect of different temperatures on mycelial growth of Pathogens.

Five milimeter culture discs were cut with sterilized cork borer from advancing margin of colonies of *L. theobromare* and *F. solani* and inoculated on PDA plates separately and incubated at 20, 25, 30, 35 and 40 °C.

This experiment was run in quadruplicate and mycelial growth was recorded for seven days. The experiment was conducted in completely randomized design (CRD) and the data were statistically analyzed (Steel and Torrie, 1986).

Effect of light and darkness on mean mycelial growth of Fungi.

To study the effect of light and darkness on mycelial growth of isolated fungi, 5 mm culture discs were cut with the sterilized cork borer from advancing margin of the colonies of *L. theobromae* and *F. solani* and inoculated on PDA plates separately for seven days. Carbon paper was used to wrap the Petri dishes for darkness. Fluorescent lamp was used for light exposure. All the Petri dishes were incubated at 25 ± 2 °C in quadruplicates under following conditions.

(a) Continuous light (24 hours).
(b) Alternating light and darkness (12 hours light + 12 hours darkness)
(c) Complete darkness (24 hours).

Effect of artificial growth media on pathogens

Mycelial growth of fungus was compared on two medium (2% PDA = Potato starch = 10 g, Dextrose =10 g, Agar = 10 g, Distilled water = 500 ml.) 2% Water Agar = Agar = 10 g, Distilled water = 500 ml.) and 2.5 ml Streptomycin was added to each medium to avoid bacterial contamination.

Five milimeter culture discs were cut with a sterilized cork borer from actively growing margin of colonies of *L. theobromae* and *F. solani* and inoculated on PDA plates and agar plates separately and incubated at 25 ± 2 °C for seven days for growth of associated fungi. The experiment was designed in complete randomized design in quadruplicate. The data were recorded and analyzed statistically (Steel and Torrie, 1980).

Results and Discussion

Effect of temperature on the mycelial growth of L. theobromae and F. solani

Impact of temperature on the growth of different fungi was different. The study revealed that 25 ± 2 °C was found to be most suitable temperature for mycelial growth of *L. theobromae* and *F. solani* fungi as the colony growth of *L. theobromae* and *F. solani* fungi at this temperature was 12. 4 and 25.58 mm in diameter respectively after seven days of incubation (Table 1).Above this temperature growth of both the fungal species was significantly low and was only 2. 5 and 3.81 mm at 40 °C. However at 30 °C the growth of both the fungal species did not decrease so much and it was 10.4 and 10.5 mm. At 35 °C and 20 °C the growth of fungus *L. theobromae* decreased less and it was 6.2 mm and 7.3 mm, respectively. Alam *et al.* (2001) also obtained the maximum growth of *L. theobromae* at 25±2 °C.

Effect of light and darkness on mycelial growth of L. theobromae and F. solani.

The effect of light and darkness on mycelial growth *of L. theobromae* and *F. solani* was significant but varied with the light duration (Table 2). Continuous light was found to be the most suitable for maximum growth of both the fungi. The colony diameter were 17.3 and 16.67 mm when both the fungal species were exposed to continuous light for seven days. The colony diameter in continuous darkness was significantly decreased as 6.7 mm in *L. theobromae* and 11.5 mm in *F. solani*. The colony diameter was 6.3 and 8.9 mm when exposure to alternating 12 hours light + 12 hours darkness. The fungus growth was

statistically different at continuous light and continuous darkness. Alam *et al.* (2001) obtained the maximum growth of *L. theobromae* under conditions of continuous light and less in continuous darkness.

Growth of L. theobromae and F. solani on water agar and PDA media.

To check the best growth of fungi *L. theobromae* and *F. solani* two different media Potato dextrose agar (PDA) and water agar were selected and incubated the both fungus for seven days. The colony diameter of both the fungi after seven days on PDA were 12.8 and 13.7 mm respectively (Table 3). There was significant difference in the growth of *F. solani* on the two media. Unique morphological and physiological features combined with the wide variety of disease caused on a large number of plants make these fungal species one of the most fascinating subjects for investigation.

Table 1: Effect of temperature on the growth of *Lasiodiplodia theobromae* and *Fusarium solani.*

Temperatures	*L.theobromae*	*F. solani*
	Mean mycelial growth (mm)	
20 ºC	7.37	6.99
25 ºC	12.42	25.58
30 ºC	10.42	10.54
35 ºC	6.19	3.23
40 ºC	2.54	3.81

Table 2: Effect of light conditions on the growth of *Lasiodiplodia theobromae* and *Fusarium solani.*

Light conditions	*L.theobromae*	*F. solani*
	Mean mycelial growth (mm)	
Continuous light	17.30	16.67
Alternate light+ darkness	6.37	8.96
Continuous darkness	6.78	11.52

Table 3: Effect of media on the growth of *Lasiodiplodia theobromae* and *Fusarium solani.*

Media	*L.theobromae*	*F. solani*
	Mean mycelial growth (mm)	
PDA	12.89	13.76
Water Agar	10.20	5.8

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