

## ***Fusarium semitectum* as a Dominant Seed-borne Pathogen in *Dalbergia sissoo* Roxb., Its Location in Seed and Its Phytopathological Effects**

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### **ABSTRACT**

*Dalbergia sissoo* Roxb. (*Shisham*) seeds naturally infected with *Fusarium semitectum* showed either dark brown discoloration with compressed hilar region (13.0-23.75%) or covered with white mycelial crust (0.50-6.25%) causing wilt disease. These seeds on incubation yielded pure growth of the pathogen. Seeds were characterized as asymptomatic and symptomatic (weakly and heavily infected) on the basis of severity of infection. Cleared whole mount preparation and their section of seed revealed (location of the pathogen in the seed) mycelium in various seed components, in asymptomatic it is restricted to layers of seed coat and it invades endosperm and embryonal axis in symptomatic seeds. The fungus gained entry in the seed either through hilar region or indirectly through the pores in seed coat. The pathogen is both externally and internally seed-borne. The internal inoculum affects seed germination, viability and caused high total (pre- and post emergence) losses from 15 to 75%. The pathogen is transmitted from seed to seedling causing heavy losses to the tree plantation.

**Key Words:** *Dalbergia sissoo*, *Fusarium semitectum*, pre- and post emergence losses, Phytopathological effect

### **INTRODUCTION**

*Fusarium semitectum* Berk. & Rav. is one of the dominant pathogen associated with seeds of *Dalbergia sissoo* Roxb. It is a common pathogen of crop plants particularly in tropical and sub-tropical countries and often a secondary invader of plant tissue causing serious storage rot of groundnut, banana, citrus, tomato and cucurbits (Booth, 1971). It is both internally and externally seed-borne causing wilting and damping-off disease. The pathogen is of great economic value affecting its plantation, it attacks the entire plant and affect vascular system causing pre- and post emergence mortality. It is often found to be associated with a disease complex and demonstrated as pathogenic in relation to sesame as detected by Mc Donald (1963). However, its pathogenicity was confirmed by artificial inoculation in healthy host tissue which resulted in disease. Ashour and El-Kadi (1959) stated that cultural studies demonstrated its contribution in the damping-off of tomato seedlings.

There is no information on the seed-borne nature of *F. semitectum* in *D. sissoo* seeds. Therefore, the present study was carried out to know the location of the pathogen, its role in disease transmission and their phytopathological effects.

### **MATERIALS AND METHODS**

Fifty one seed samples were collected from 9 locations of Jaipur in year 2000-2002, were subjected to dry seed examination and incubation tests by Standard Blotter

Method (SBM); Potato Dextrose Agar (PDA) plate test as recommended by ISTA (Anonymous, 1990). In dry seed examination besides normal, flat, brown, reniform, asymptomatic seeds various discolourations (dark brown, white, black, green) and deformities (shiny, dull) were observed. Seeds were incubated on moistened blotters both untreated and 3% chlorine pretreated for 5 min. in PDA test, pretreated seeds were spaced (10 seeds/plate) on petriplates containing PDA medium. Two samples were selected from University campus and seed market locations of sample nos. 1 and 45 having high incidence of *F. semitectum* respectively. These samples were used for location and transmission studies. The seeds were categorized as asymptomatic and symptomatic (weakly and heavily infected) seeds on the basis of severity of infection (Fig. 1A).

Location of the pathogen in different seed components was studied using component plating (10seeds/category/sample), hand cut and microtome sectioning (5seeds/category/sample) (Singh *et al.*, 1977). Transmission of seed-borne inoculum from seed to seedling/plant was studied using petriplate method and growing on water agar seedling symptom test (Anonymous, 1990). In petriplate method four replicates of 100 seeds (pretreated) were sown on moistened blotters (10seeds/plate) and in water agar test tube seedling symptom test (1 seed/test tube). Observations were made to record percent germination, incidence of pathogen, seedling symptom and seedling mortality.

## RESULTS AND DISCUSSION

All the 51 seed samples collected from 9 locations of Jaipur were either covered with white mycelial crust (0.50-6.25%) or were dark brown colored (13.0-23.75%) carrying infection of *Fusarium semitectum*. Such seeds on incubation yielded pure growth of the pathogen. The fungus was detected in 45, 42 and 18 samples with incidence in the range 12.0-72.0, 8.0-78.0% and 10.0-80.0% in untreated, pretreated seeds in SBM and PDA test respectively. Incidence was high in the samples from Seed market, Vidhan sabha bhawan, Bani park and University campus areas (Table 1). For the location of the pathogen in various parts of seed, component plating, cleared preparation and sectioning (handcut, microtome) were performed. In component plating, pathogen was detected in seed coat (25, 30%), cotyledons (40, 30%) and embryonal axis (20, 25%) of asymptomatic seeds. Whereas it varied from 50-65% on seed coat, 60-75% on cotyledons and 45-75% on embryonal axis of weakly and heavily symptomatic seeds of the samples with sample nos. 1 and 45 respectively (Table 2).

In cleared whole mount preparation of various parts of infected seeds hyphae was observed 30,40% in seed coat, 35,40% in cotyledons and 20,25% in embryonal axis of asymptomatic seeds of the 2 samples respectively. Inter- and intra- cellular mycelium was detected in symptomatic seeds (with weak and heavy infection) and it varied from 70-100% in seed coat, 50-100% in cotyledons and 40-90% in embryonal axis in 2 samples respectively (Table 2).

Handcut and microtome sections revealed infection with pathogen, 2 and 1 seed out of 5 seeds of the two samples

respectively of asymptomatic seeds. Few mycelial fragments and hyphal bits were observed in all the layers of seed coat and cotyledons (Figs. 1B, D). Pathogen was not recovered in the deeper tissues of the seed. Whereas sections of symptomatic seeds (with weak and heavy infection) revealed infection in all the components of seed. Weakly symptomatic seeds showed the presence of characteristic thin mycelium in epidermal and sub-epidermal layers of seed coat (Fig. 1E). The cells of seed coat were disintegrated with depleted food contents and become vacuolated with little and no cytoplasm. Heavy infection caused various degrees of deformation and disintegration in all the parts of seed. The cells of the seed coat became irregularly elongated, disfigured and vacuolated. Similar effects as detection of hyphal mat in the tissue and depletion of food content due to heavy infection of *F. oxysporum* in seeds of moth bean (Varma,1990), guar (Bhatia,1995), green gram (Sharma,1999) and sesame (Dubey, 2000). Paik and Do (1987) reported 7% infection of *F. moniliforme* in seed coat and 2% in the embryo of green gram seed. Mycelial fragments were also observed in the space between seed coat- cotyledons and between two cotyledons. Hilar region and cotyledonary cells were densely colonized (Fig. 1G). The cells of embryonal axis were distorted and occupied by inter- and intracellular mycelium. The pathogen gained the entry in the seed either through the hilar region or through the pores on the surface of seed coat (Figs. 1E-F).

**Table 1: Seed samples of *Dalbergia sissoo* infected with *Fusarium semitectum* in Jaipur**

Location	No. of Samples	Dry Seed Examination		In Standard Blotter Method		In PDA test
		Dark Brown	White Crusted	Untreated	Pretreated*	
Bani Park	3	3	2	3	3	-
Forest Training Centre	3	3	3	3	3	2
Jhalana Natural Garden	3	3	2	3	3	2
Police Academy	12	12	12	12	9	4
Sanganer	3	2	3	-	-	-
Seed Market	9	9	6	9	9	4
University Campus	12	12	12	12	12	4
Vidhan Sabha Bhawan	3	3	3	3	-	-
World Forestry Arboratum	3	3	1	1	3	2
Total	51	50	44	45	42	18

\* 3% Chlorine pretreatment for 5 min.

**Table 2: Incidence of *Fusarium semitectum* in various components of seed and percent disease transmission.**

Category	Ac. No. 1			Ac. No. 45		
	Asy.	Sym.		Asy.	Sym.	
		W Sym.	H Sym.		W Sym.	H Sym.
<b>Component Plating</b>						
Seed Coat	25	50	55	30	60	65
Cotyledon	40	75	100	30	60	75
Embryonal Axis	20	50	75	20	45	60
<b>Cleared Components</b>						
Seed Coat	30	70	80	40	80	100
Cotyledon	35	60	50	40	100	100
Embryonal Axis	20	55	40	20	90	80
<b>Disease Transmission (%)</b>						
Germination	85	50	10	80	65	10
Seedling Symptoms	40	15	5	40	45	5
Seedling Mortality	10	20	5	5	10	5
Disease Transmission	58.8	70.0	100.0	56.2	84.6	100.0

(**Abbrev.** Asy. = Asymptomatic; Sym. = Symptomatic; W Sym. = Weakly Symptomatic and H Sym. = Heavily Symptomatic)

During transmission studies, in petriplate method germination started after 48 hrs of sowing and became maximum as 85,80% in asymptomatic seeds, 50,65% in weakly symptomatic and 10,15% in heavily symptomatic seeds of sample 1 and 45 respectively (Fig.2). Due to heavy infection seeds were rotted and failed to germinate. These observations were supported by Varma, 1990 (moth bean); Bhatia, 1995 (guar) and Sharma, 1999 (green gram). On growing in test the germination was 80,100% in asymptomatic seeds, whereas it varied from 20-95% in symptomatic seeds (with weak and heavy infection) of both the samples on 8<sup>th</sup> day of incubation. The total loss and disease transmission varied from 20-25%, 15-75% and 43.8-80%, 66.7-100% in asymptomatic, weakly and heavily infected seeds of the two samples in both the test respectively (Table 2).

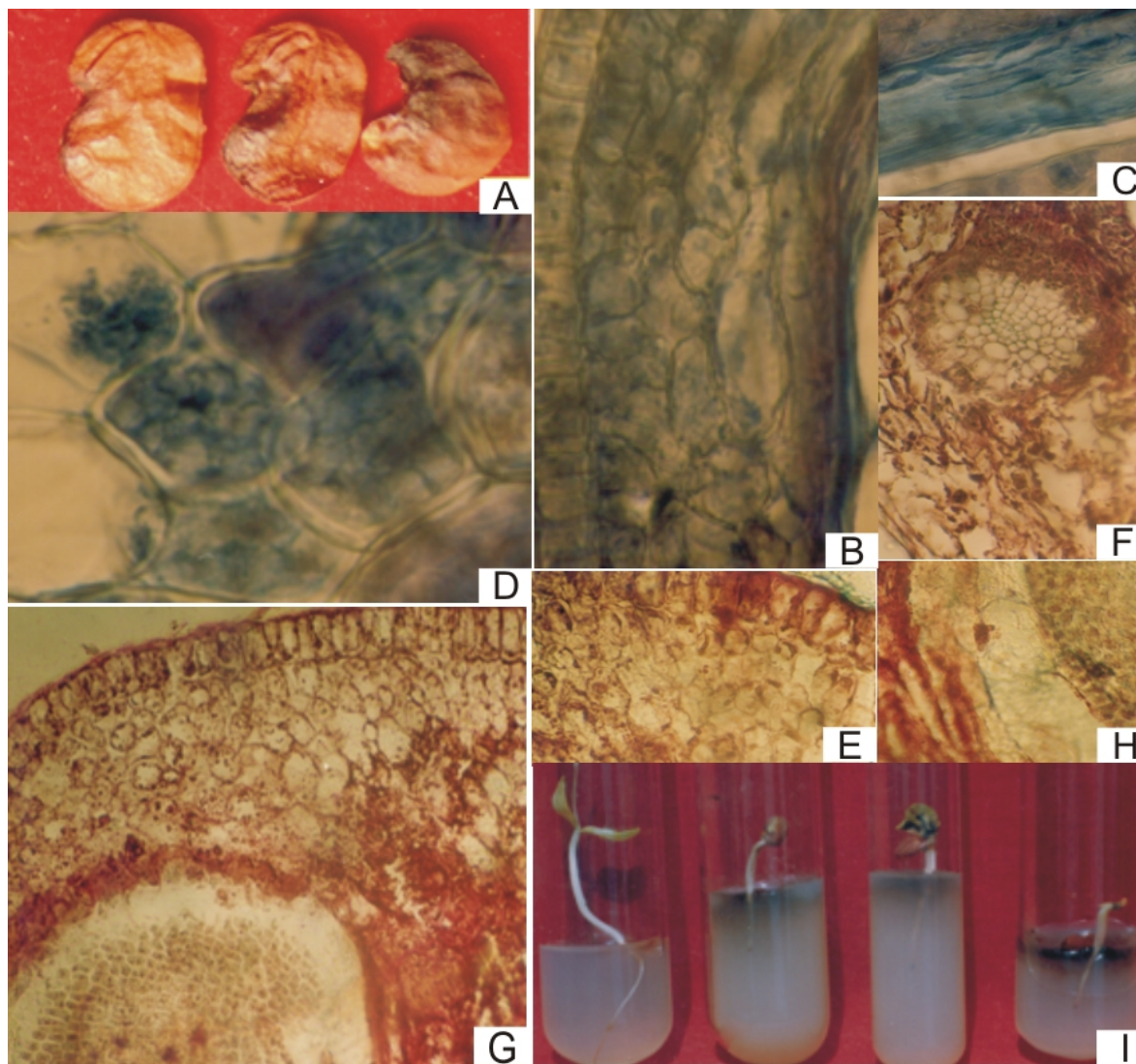
Initial signs of disease symptoms appeared on 4<sup>th</sup> day of incubation as radical browning; later the symptoms appeared on hypocotyls as browning and on cotyledonary leaves as formation of brown necrotic spots (Fig. 1I). Similar results as browning of leaves in the tip region and discoloration (yellowing) of first few basal leaves reported by Singh and Singh (1977). Similar symptoms on radicle and hypocotyls due to infection of *F. moniliforme* and *F. semitectum* have also been reported by Saxena and Sinha (1979). The severely infected seedlings were finally wilted. The pathogenicity was confirmed by artificial inoculation of

healthy plant parts resulted in the appearance of disease such studies were also supported by Ashour and El-Kadi (1959). Paik and Do (1987) confirmed the pathogenicity of *F. moniliforme*, *F. pallidoroseum* and *F. equiseti* in seedling via water agar test tube method.

The present study showed that infected seeds were asymptomatic or symptomatic (weakly and heavily infected). The pathogen was mostly found to be associated with seeds with white mycelial crust and dark brown discolorations (Fig. 1 A). The pathogen was both externally and internally seed-borne. The pathogen ramified the seed inter- and intracellularly and was correlated with severity of disease symptoms (Fig.1 I). Handcut sections of infected seeds revealed inter- and intra cellular mycelium in various layers of seed coat (Fig. 1 B). endosperm and the space between the seed coat and cotyledons (Fig. 1 C). Depletion in cell content and the hyphal fragments in and around the cells of cotyledons (Fig. 1 D). Microtome section of seeds infected with *F. semitectum* showing inter- and intracellular mycelium in cells of seed coat (Fig.1 E, G). and embryonal axis (Fig.1 H). In heavily infected seeds mycelial aggregation was detected in and around the cells of hilar region. The pathogen invades the seed either through hilar region or through the pores on the seed coat surface (Fig. 1 F). Seedborne fungal pathogens reduced seedling emergence and the quality of *Dalbergia nigra* seedlings in Brazil.

*Cladosporium cladosporioides*, *Colletotrichum crassipes*, *Fusarium semitectum*, *Phomopsis dalbergiae* and *Pestalotiopsis* sp. were the only fungi consistently isolated from seed samples obtained from a number of trees. Colonisation of both, pericarp and seed by these fungi starts

soon after pod formation and continues to increase to pod maturity. The findings of this study explain the heavy losses suffered by nurserymen due to low quality seedlings. This is the first report on economic loss due to these fungi on this host (Dhingra et al., 2003).



**Fig. 1. Histopathological and cytopathological localization of *Fusarium* in *Dalgerbia sissoo***

**A** During dry seed examination, seed showing discolorations caused by *F. semitectum* with scattered brown patches and compressed hilar region. X 18.

**B, C, D** Handcut sections of seeds of shisham seeds infected with *F. semitectum*. Inter- and intra cellular mycelium in various layers of seed coat, X 350 (**B**). Mycelial strands in endosperm and the space between the seed coat and cotyledons, X 700 (**C**). Depletion in cell content and the hyphal fragments in and around the cells of cotyledons, X 700 (**D**).

**E, F, G** Microtome sect of seeds infected with *F. semitectum*. T.S. part of seed showing mycelial bits in the seed coat X 350 (**E**). T.S. part of heavily infected seeds showing mycelial aggregation in and around the cells of hilar region, X 700 (**F**) Inter- and intracellular mycelium in cells of seed coat and embryonal axis X 700 (**G**), Mycelial strands in between seed coat and embryonal axis X 700 (**H**).

**I** Phytopathological studies in water agar seedling symptom test showing various degree of infection normal seedling and infected seedling.

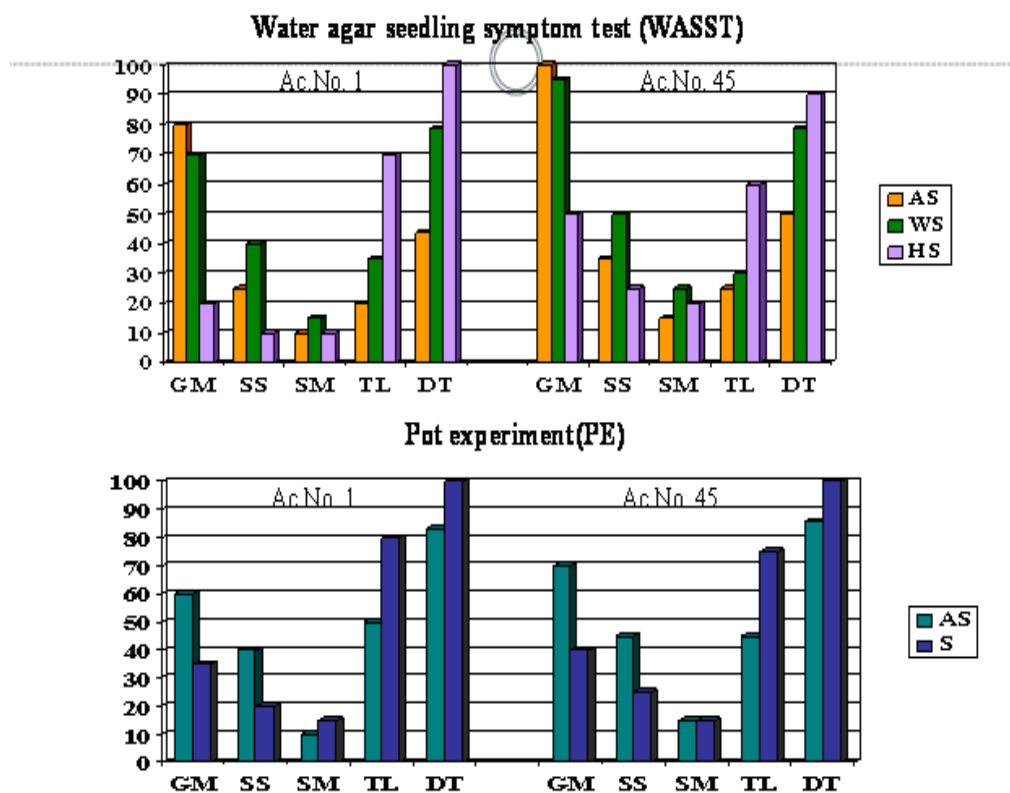


Fig. 2: Phytopathological effects of *F. semitectum* naturally infected seeds in WASST and PE

During transmission studies the pathogen moved from seed to seedling/plant, caused high pre- and post emergence losses, produced brown necrotic spots on cotyledonary leaves and browning of radical, hypocotyls (Fig. 1 I). Later the seedlings wilted and succumb to death. Similar observations were made by Sharma (1999) in green gram seeds. Kochler (1960) also reported seed as source of seedling infection in corn. Maximum infection frequency of 75.00% was exhibited by *F. solani* colonizing stem tissues followed by 47.39% from bark tissues, 29.83% from seeds and 15.62% from roots. The colonization percent of *F. solani* was highest as compared to other isolated fungi. Seed germination percentage was also reduced (50.00%) in soil infested with *F. solani* and seedling mortality was 93.33% followed by soil infested with *R. solani* (60.00%) with mortality rate (66.66%) and *C. lunata* (70.00%), seedling mortality rate of 42.85% as compared to *F. moniliforme* and *F. oxysporum*, respectively (Rajput et al., 2010).

## REFERENCES

**Anonymous (1990).** International rules of seed testing, International seed testing association. *Seed science and Technology*, **18**, 299-513.

**Bhatia A (1995).** Studies of important field and storage seed-borne fungi of Guar (*Cymopsis tetragonoloba* (L.) Taub.). Ph.D. Thesis University of Rajasthan, Jaipur.

**Booth C (1971).** The genus *Fusarium*. Common wealth Mycological institute, Kew, Surrey. The eastern Press Ltd., London, 237.

**Dhingra OD, Lustosa DC, Maia CB and Mesquita JB (2003).** Seedborne fungal pathogens of jacaranda (*Dalbergia nigra*) tree. *Seed Science and Technology* **3** 341-349

**Dubey A (2000).** Studies on seed-borne microorganisms of sesame (*Sesamum indicum* L.). Ph.D. Thesis University of Rajasthan, Jaipur.

**Kochler B (1960).** Corn stalk rot of Illinois. *Illinois Agriculture Experimental Station Bulletin* **658** 90.

**Kumar D (2000).** Studies on seed-borne microorganisms of pigeon pea (*Cajanus cajan* L.) Ph.D. Thesis University of Rajasthan, Jaipur.

**Paik SB and Do ES (1987).** *Fusarium* spp. Isolated from seed, root and cultivated soil of *Phaseolus dissimus* and their pathogenicity. *Korean Journal of plant pathology* **3** 8-12.

**Rajput NA, Pathan MA, Rajput AQ, Jiskani AA, Lodhi AM, Rajput SA and Khaskhali MI (2010).** Isolation Of Fungi Associated With Shisham Trees And Their Effect On Seed Germination And Seedling Mortality. *Pakistan Journal of Botany* **42** 369-374.

**Saxena RM and Sinha S (1979).** Field mycoflora in *Vigna radiate* (L.) Wilczek var. *radiate* and *Vigna mungo* in relation to pre-emergence and post-emergence mortalities. *Seed research* **7** 159-164.

**Sharma K (1999).** Studies on seed and seedling on green gram (mung bean) grown in Rajasthan. Ph.D. Thesis University of Rajasthan, Jaipur.

**Singh GP and Singh N (1968).** Blight of sugarcane seedling of Uttar Pradesh. *Indian Phytopathology* **21** 113-115.

**Singh D, Mathur SB and Neergaard P (1977).** Histopathology of sunflower seeds infected by *Alternaria tenuis*. *Seed science and technology* **5** 579-586.

**Varma R (1990).** Studies of seed-borne mycoflora and diseases of mothbean (*Vigna aconitifolia*) and cowpea (*V. unguiculata*) grown in Rajasthan. Ph.D. Thesis University of Rajasthan, Jaipur.