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Phytopathological and transmission studies of *Phyllactinia dalbergiae* in *Dalbergia sissoo* Roxb.

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Phyllactinia dalbergiae causes powdery mildew on *Dalbergia sissoo* Roxb. (shisham) in early stages of plants, pathogen takes entry in the host through the stomatal opening later, spread and establish itself in the host. The initial sign of symptom appears as the development of white powdery mass on the leaf surface later it covers the entire shoot finally plant succumbs to death. During survey powdery mildew was detected in the plantlets in the nursery, the infected plantlets were collected to study morphology, histopathology, pathogenicity and disease development under *invivo* and*invitro* condition. The infected part showed dense mycelial growth with erect conidiophore carrying single terminal conidium and brown-black coloured cleistothecia (fruiting body). The viability of the spore was detected to be 100% within 2-4 days in cavity slide containing nutrient medium through germ tube formation. During pathogenicity studies, healthy plants were inoculated with 15 days old culture of the pathogen to study the disease development. Cleared wholemount preparation and hand cut sections revealed the presence of intercellular mycelium in the epidermal and subepidermal (dorsal and ventral surface) cells of the leaf. Dense mycelial ramification caused lysis and disintegration of cells. The pathogen is transmitted through stomata causing heavy losses to the tree plantation.

Key words: *Dalbergia sissoo, Phyllactinia dalbergiae,* mildew disease

INTRODUCTION

Shisham (*Dalbergia sissoo* Roxb.) an important legume tree belonging to family Fabaceae, subfamily Papilionaceae is widely grown throughout the world and in India It is grown as the ornamental roadside tree and for medicinal purposes. Powdery mildew is caused by *Phyllactinia dalbergiae,* in early stages of plants leads to the formation of symptoms and then succumb to death. Powdery mildew in the country was reported for the first time in *Dalbergia sissoo* by *Phyllactinia dalbergiae* (Jackson, 1987) and later by Patil and Mahamulkar (1998). Wankhade and Peshney (1991) studied conidial survival and cross-infectivity of certain powdery mildew fungi, *viz.*, *Unicinula, Phyllactinia, Sphaerotheca* and *Erisyphe* from cultivated and wild plant. They also found spore germination in 12 hr. when grown in a suspension of 5% glucose with traces of citric acid in a cavity slide. Hiremath and Hiremath (1993) studied biochemical changes in leaves of *Dalbergia sissoo* by *Phyllactinia dalbergiae.*

During the field survey tree and seedlings in nurseries were found to be infected with*Phyllactinia dalbergiae.* Therefore, the present study was carried out on morphology, pathogenicity and disease development of *Phyllactinia dalbergiae in vivo* and *in vitro* conditions.

MATERIALS AND METHODS

The field survey was conducted for the collection of seed samples from 9 different locations of Jaipur. The execcive seedling loss was detected due to the incidence of powdery mildew disease caused by *Phyllactinia dalbergiae* .Samples of infected seedlings were collected and fixed in FAA for further studies. The detailed study was carried out on morphology, pathogenicity and disease development under *in vivo* and *in vitro* conditions.

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For morphological studies, the identification and isolation of pathogen was carried out by incubating the surface sterilized (3% sodium hypochlorite for 5 min.) leaves on moistened blotters at 26° ± 2 °C under 12/12 hr. alternating cycles of light and darkness for 7 days. Observations were made on the growth pattern, development, structure of mycelium, conidia and cleistothecia of the pathogen. *Phyllactinia dalbergiae* were transferred on Potato Dextrose Agar (PDA) medium and Richard's medium (broth) for 7 and 30 days respectively. Germination of conidia was studied by nutrient solution in cavity slide.

For determining the pathogenicity, healthy seedlings (5 seeds/ pot) 3-4 months old were artificially inoculated by spraying spore suspension (15 days old culture on PDA) of the pathogen. Observations were made on symptomatology, disease-development and survivability of plant on 2nd, 4th and 8th day of inoculation. Infected plants were incubated using blotter method to reisolate the fungus followed by cleared whole mount preparation and hand cut sections.

To study the disease development in naturally infected seedlings and plants histopathological studies were carried out to determine the establishment of the pathogen in the host tissue. The infected seedlings were subjected to cleared whole mount preparation, epidermal peeling, hand cut and microtome sectioning.

RESULTS AND DISCUSSION

During the field survey, *Phyllactinia dalbergiae* was found as a dominant pathogen on seedlings and plants of *Dalbergia sissoo* affecting the plantation. In India, powdery mildew caused by *Phyllactinia dalbergiae* was reported first time in *D. sissoo* by Jackson (1987). Therefore, infected seedlings were collected from nurseries and field. During morphological studies, infected plant parts were examined under stereo binocular microscope showed mycelial growth with densely emerging erect conidiophores, each with terminal single conidium. In later stages, cleistothecia (fruiting body) dark brown to black coloured spherical bodies with few appendages containing ascus and ascospores were observed on the leaf surface (Fig. 1 A). On incubation infected plant part gave pure growth of the pathogen, which on transfer on PDA and Richard's medium produces white mycelial mat after 8 and 15 days of incubation respectively. Formation of cleistothecia was observed in 18-20 days old culture in both the media (Fig. 1 C, D). Characteristic mycelium was hyaline, coenocytic, sparingly branched, hypophyllous with dense cytoplasm and prominent nucleus. On maturity, the hyphae were septate and uninucleate. Conidia

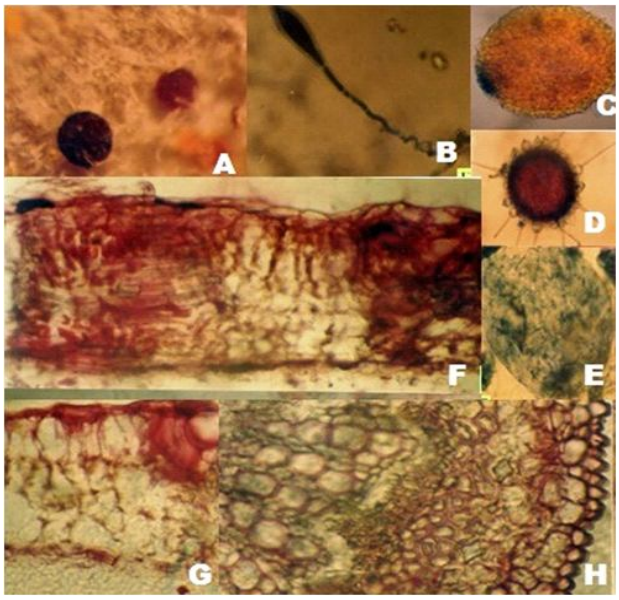

Fig. 1 : Morphological, cultural and histopathological studies of *P. dalbergiae* infecting *Dalbergia sissoo* Roxb. plants

Morphological and cultural studies (A-E): Cleistothecia (fruiting body) dark brown to black coloured spherical bodies with few appendages containing ascus and ascospores were observed on leaf surface (A); Characteristic Conidia develop singly on conidiophore. (B). Initially the developing cleistothecia appears as white pinhead like structure, later changes to reddish-orange (C); finally black (D) in colour at maturity and clesitothecia in culture (E).

Histopathological studies (F-H), Handcut and microtome transversed sections were made to study the extent of invasion of *P. dalbergiae* (F). Characteristic intercellular mycelium on both dorsal and ventral epidermal and sub-epidermal cells of leaf (G). Mycelium also invaded in the palisade cells of the mesophyll tissue (H).

develop singly on conidiophore. Conidiophores are long, hyaline, erect, unbranched and spirally coiled at the base producing single terminal conidium. Conidiophore consists of foot cell, middle intercalary and terminal generative conidiogenous cell. Conidia are one-celled, hyaline, thin walled and vacuolated. The shape varies from naviculate, obovate to pyriform with the rounded tip, measuring 47.8-114 µm X 14.7-33.1 µm (Fig. 1 B). The viability was 100% and germinated within 2-4 days in cavity slide containing nutrient medium. Germ tube generally developed from four parts viz., tip, near-tip, base, near-base of the conidium with one

or more germ tube produced from single conidium. Wankhade and Peshney (1991) studied conidial survival and cross-infectivity of certain powdery mildew fungi, *viz*., *Unicinula, Phyllactinia, Sphaerotheca* and *Erisyphe* from cultivated and wild plant. They also found that in a suspension of 5% glucose with traces of citric acid in a cavity slide under moist chamber, the spore germination in 12 hr. Similar observations were made by Banerjee *et al.* (1995) as they observed conidia of *Phyllactinia dalbergiae* causing powdery mildew of *D.sissoo* do not germinate in water but on a cellulose acetate film. Initially, the developing cleistothecia appear as white pinhead like structure, later changes to reddish-orange and finally black in colour at maturity. Mature cleistothecia measures 180-280 ìm in diameter, spherical or globose, covered by a thick hyphal wall consisting of 6-15 short, apical, rigid, simple, spear-like with bulbous base, pointed tip and mucilage-secreting appendages for anchoring the leaf surface. It encloses 10-30 ascus measuring 45-80 μm X 14-22 μm (Fig. 1 C, D).

During pathogenicity studies, 3-4 month seedlings were inoculated by spore suspension of the pathogen. After 2-4 days conidia start germinating by germ tube formation and in later stages (10-12 days) white powdery mass appears on the aerial parts of the infected plant showing symptoms. Plants were stunted with inhibited leaf primordial formation and finally defoliated. Cleared preparation and transverse hand cut sections of infected leaves revealed characteristic mycelium in epidermal and subepidermal layers (Fig. 1 F, G, H).

Disease development in naturally infected seedling was studied. The initial symptom of the disease appeared on leaves as deposition of white powdery mass on its ventral surface that gradually extended to dorsal surface, petiole, leaf base, stem or whole aerial part. Leaves became yellow to brown which initiated from margin finally leads to drying of leaves following defoliation. In advanced stages of infection cleistothecia development was observed on the leaf surface. Due to infection leaf primordia fail to emerge. Infected seedlings from the nursery were harvested at 10 days interval up to 90 days and incubated on moistened blotters. Similar symptoms on the host were also revealed by Jackson (1987) on *D. sissoo* and Smith (1999) by *Microsphaera pulchra* causing white mildew.

During histopathological studies, cleared wholemount preparation and epidermal peeling of infected plant parts *viz*., leaf and stem showed the presence of characteristic mycelium in epidermal cells that also transverse intercellularly in sub-epidermal layer and hyphae also transverse along the veins (Fig. 1 F). Handcut and microtome transverse sections were made to study the extent of invasion of *P. dalbergiae*. Characteristic intercellular mycelium was observed on both the surface (dorsal and ventral) epidermal and subepidermal cells of the leaf. Dense hyphae were seen near stomatal aperture (Fig. 1 G). Mycelium also invaded in the palisade cells of the mesophyll tissue. Infected stem revealed hyphal bits and fragments in the epidermal layer and in intercellular space of parenchymatous cortex (Fig. 1 H). Due to heavy infection the cells were lysed and disintegrated (Fig. 1 G). Mycelium was also detected near vascular elements. Lysis and disintegration of cells have been observed due to heavy infection. Dense hyphae were seen near the stomatal aperture.

In the present study, during the field survey, *P. dalbergiae* was found as a dominant pathogen on seedling and plants of *D. sissoo*. Therefore, infected plant material was collected to study symptomatology, isolation of pathogen, characterization of fungus in the host and in culture media, their histopathology and disease development.

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REFERENCES

Banerjee, K., Khatua, D.C. and Mukherjee, N. 1995. Assessment of some fungicides for the control of powdery mildew of *Dalbergia sissoo*. *Indian Journal of Forestry*, **18:** 121-124.

Hiremath, S.V. and Hiremath, P.C. 1993. Effect of blight and powdery mildew infection on *Dalbergia* leaves. *Karnataka Journal of agricultural Sciences*, **6:** 204-207.

Jackson, J.K. 1987. *Mannual of afforestation in Nepal*. Nepal-UK. Forest Research Project, Forest survey and research office, Department of forest, Kathmandu, Nepal.

Patil, M.S. and Mahamulkar, S.H. 1998. Studies on powdery mildews-III. *Journal of Mycology and Plant Pathology*, 29: 42-47.

Smith, V.L. 1999. First report of powdery mildew on *Cornus florida* in Connecticut caused by *Microsphoera pulchra*. *Plant disease*, **83:** 782 (En, 2 ref.)

Wankhade,S.V. and Peshney, N.L. 1991. Conidial survival and cross infectivity of certain powdery mildew fungi prevalent in Vidarbha. *Indian Journal of Mycology and Plant Pathology,* **22:** 195-196.