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FOLIAR BLIGHT DISEASE OF INDIAN SANDALWOOD (*SANTALUM ALBUM* L.) TREES CAUSED BY THE PATHOGEN *PESTALOTIOPSIS GUEPINII* (DESM.) STEYAERT

MUTHU KUMAR A.[1]*, V. SOUNDARARAJAN[2], REKHA J.[3], M. DIVYA BHARATHI[1]AND MAMATHA N.[1]

[1]Forest Protection Division, Institute of Wood Science and Technology,
[2]Information Technology Cell, Institute of Wood Science and Technology,
[3]Genetic and Tree Improvement Division, Institute of Wood Science and Technology, 18th Cross, Malleshwaram, Bengaluru, Karnataka, India

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Abstract – Symptoms of brown to dark brown irregular lesions along the leaf tip and margin was observed on young plants of Indian Sandalwood (*Santalum album* L.) at Institute of Wood Science and Technology, Bengaluru, Karnataka, India. Similar symptoms were observed in other parts of Sandalwood growing plantations of Karnataka. Sandalwood plants of 2 to 3 years old expressed similar kind of symptoms. The pathogen diminishes the lively leaf area, thereby leading to drying of plants in advance stages. The pathogen was isolated from infected leaves and cultured on Potato Dextrose Agar (PDA) for identification. Simultaneously symptoms were confirmed for pathogenicity test. The culture and microscopic characters revealed *Pestalotiopsis guepinii* (Desm.) Steyaert., as a causal organism of foliar blight disease in Indian Sandalwood. The identification of causal organism was confirmed by Agharkar Research Institute, Pune, India. As per literature, it was found, the present report on foliar pathogen *Pestalotiopsis guepinii* (Desm.) Steyaert., affecting Indian Sandalwood is a new record.

INTRODUCTION

Santalum album L. or East Indian sandalwood is a mistletoe parasite plant grows by tapping the xylem of their hosts. It is highly prized for their aromatic heartwood (Page and Tony *et al*., 2012). India has been the traditional leader for more than 5000 years in the production of sandalwood oil for perfumery and pharmaceuticals. Now, the annual produce of sandalwood in the country significantly reduced from 2287.8 tons between the year 1960-1965 to 366.74 tons in 1995-2000 (Arun Kumar *et al*., 2016) due to deadly spike disease caused by MLO (Mycoplasma Like Organism), illicit poaching, monopoly of sandalwood trade by the Indian Government and its consequences have resulted in severe exploitation, pushing *S. album* into the vulnerable class of the IUCN Red List (IUCN, 2012) (IUCN. 2012). However, other diseases of *S.album* also play a major factor in the decline in nursery and plantation produces (Nayar *et al*., 1980; Barry, 2002; Barbour *et al*., 2010).

S. album is prone to disease and a wide range of pathogens attack plants, especially in seedling and young plantation. *Fusarium, Rhizoctonia, Phytophthora,* and *Pythium* reported to cause root related diseases and *Ascochyta santali, Macrophorina phaseoli, Asterina congesta*, and *Sphaecelomiasantali* causes various foliar diseases in seedlings (Remadevi *et al.,* 2005; Muthu Kumar and Ashish Kumar Pandey 2016). Some of the mature trees are highly susceptible to spike disease,and the first report on spike was reported by McCarthy in the year 1899 (Mc Carthy, 1899) and recent incidence of spike disease was reported by Arun Kumar and Geetha Joshi in a one-year-old plantation in Hagalwadi village Gubbitaluk in 2012 (Arun Kumar and Geeta Joshi, 2012). Other diseases were *Fusarium oxysporium* causing wilt, cholorosis in seedling (Nayar *et al*., 1980) and canker in Anchipura area

sandalwood plantation, Channapattana Taluk of Karnataka (Nagaveni *et al.,* 2014), *Pseudoidium santalacearum* causing powdery mildew in Madhya Pradesh (Patel *et al*., 2015) and *Neofusicoccum parvumas* causing wilt and stem rot on *S.album* in a commercial plantation in Dongguan, Guangdong province (Wang *et al*. 2016).

During a recent survey related to assessment of health status of *S. album* in nursery and plantation, we had come across peculiar symptoms of brown to dark brown irregular lesions which later coalesce along the leaf tip and margin. This blight like symptoms were observed on *S. album* plants growing at the campus of Institute of wood science and technology, Bangalore. Similar symptoms were observed in other parts of Sandalwood growing plantations of Karnataka. Sandalwood plants of 2 to 3 years old expressed blight kind of symptoms. Foliar blight, most dreaded disease of agriculture crops and as well as plantation crops which considered to cause a significant reduction in yield (Saari, 1998; Tuset *et al*., 1999; Gruenwald *et al*., 2008). The onset of the disease is observed under a favorable climatic condition of rainfall associated with high relative humidity (95%), temperature (28-32 °C) and spread through wind (Rush and Lee 1983; Hennessy *et al*., 1990). Since there was no literature on foliar blight disease associated with *S.album*, the present work was to identify the causal organism and its pathogenicity.

MATERIALS AND METHODS

Isolation of pathogen

Infected leaves showing conspicuous symptoms of dark brown lesions near tip and leaf margin were collected. Sections of symptomatic leaves were surface sterilized with a 70% ethanol for 30 sec followed by 10% sodium hypochlorite for 60 sec and rinsed well with autoclaved sterile distilled water. The leaves were dried and placed on potato dextrose agar (PDA) medium with streptomycin. Inoculated plates were incubated at RT (Room Temperature - 24 ^{o}C) and observed periodically.

Further, the pure culture isolated was authenticated by getting identified atNational Fungal Culture Collection of India, Agharkar Research Institute, Pune, India.

Seedling growth condition

The seedling was raised from theseeds sowed on a sterile autoclaved sand bed. Before sowing the seeds were treated with 0.05% of gibberellic acid to increase the rate of germination. At 4 leaves stage seedling was subsequently transferred to root trainer and later to polybag with a potting mixture of sand: soil: compost (1:2:1 v/v/v). Four months old seedlings were used for pathogenicity test.

Pathogenicity test

The pathogenicity test was carried out by using conidial inoculum. Conidia for inoculum were washed from the surface of the plates and suspended in sterile distilled water. The inoculum was adjusted to 10^3 /ml by hemocytometer. Individual, uninfected leaves on 4 months old seedlings grown in the green house chamberwere used for the test. Conidia inoculum was sprayed on entire surface of the leaves and control leaves of seedling were sprayed with sterile water. The inoculated and control seedlings were immediately covered with plastic bags to maintain high humidity (e"60%). Development of typical disease symptoms was evaluated after 7-10 days of inoculation.

RESULTS

Observation of Symptoms

The leaf lesion first observed on the tip and progressed along the margin of the entire leaf which eventually coalesce the whole leaf area. The advancing edges of lesions were characteristically irregular, with a brown to a dark brown border between the necrotic area and the green tissue. Infection is confined only to leaf and later stages leaves abscise (Fig. 1).

Fig. 1. A-Infected branch of *S.album.* B-Lesions along margin of the leaf.

Culture observation

The infected leaf partwas inoculated on PDA and

incubated at 25 °C for five days. The microscopic observation of inoculated culture repeatedly produced similar conidial characters, which was consistently isolated. Spores of isolated culture were observed after 20 days of incubation on PDA. Based on the morphology of conidia [7] and result obtained from Agharkar Research Institute, Pune the isolate was identified as *Pestalotiopsis guepinii* (Desm.) Steyaert. *P. guepinii* produced an orange to pink colored colonies with whitish aerial mycelium, cottony textured, raised elevation andfiliform margin. Conidia were pigmented, 3 transverse septa, two apical appendages and a basal appendageon PDA medium (Fig: 2)

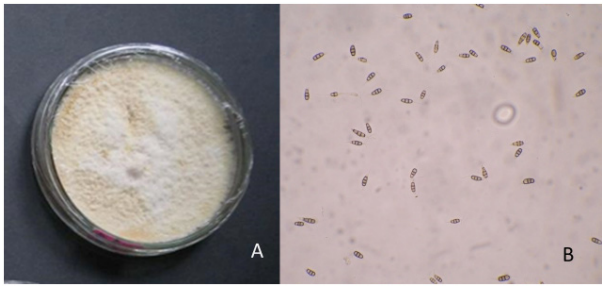

Fig. 2. A P.guepiniiculture colony. B Conidia of P.guepinii

Pathogenicity Test

The pathogenicity test exposed *P.guepinii* causes leaf blight in *S.album*. The symptoms observed after 7-10 days on seedling were similar to those initially observed on the infected plants in field (Fig. 3). No symptoms developed on the leaves sprayed with sterile water. *P. gueipini* was consistently isolated from the surface sterilized leaves of inoculated plants signpostsits pathogenicity.

Fig. 3. **A** Infected plant showing blight symptoms after 7 days. **B** Healthy plant - control

DISCUSSION AND CONCLUSION

S.album is an economically important species with ecological, socio cultural and religious significance. They play a vital role in the cultural heritage of people of Southern India, exclusively in the state of Karnataka. IWST being a center of excellence for sandalwood in India, execute research on *S. album* extensively. *S. album* is distributed within the campus in the form of nurseries, naturally grown trees and planted saplings. Recently, *S. album* was found to be associated with various disease causing causal organisms, e.g., *Fusarium*, *Alternaria* and *Pythium* responsible for foliar and root disease (Muthu Kumar and Ashish Kumar Pandey, 2016). Nevertheless, the extent of cultivation of *S. album* is being drastically increasing in southern regions of India, also in Northern regions where *S. album* was never grown as plantation crop. The change in micro climatic factors and introduction of *S. album* as agroforestry crop with other tree species in newer arenas could have paved way for emergence of novel pathogens, e.g., Canker disease of Sandalwood in the region of Karnataka, Southern India (Nagaveni *et al*., 2014) and Powdery mildew disease in Madhya Pradesh, Northern India (Patel *et al.* 2015).

Foliar blight disease is common in agriculture and any other tree crops. The saplings and plants of Sandalwood had never encountered with pathogen of foliar blight, also profound literature survey maintains the same. Young plants and saplings of *S. album* at IWST was recently observed with symptoms of foliar blight, and subsequently the pathogen was isolated and authenticated at National Fungal Culture Collection of India, Agharkar Research Institute, Pune, India as *Pestalotiopsis guepinii* (Desm.) Steyaert. The pathogen, *Pestalotiopsis guepinii* (Desm.) Steyaert., is favored by high relative humidity with sub-optimal temperature and rainfall as predicted (Hennessy *et al.* 1990). Stressed and susceptible plants are more prone to foliar infections. Henceforth, influence of prevailing micro-climatic factors plays a major role in incidence of disease.

The causal organism *P.guepinii* is an anamorphic

member of the family Amphisphaeriaceae (Jeewon Rajesh *et al*., 2003). The species is distributed in tropical and subtropical ecosystems (Maharachchikumbura *et al*. 2016; Tejesvi *et al*., 2007) and occur on a wide range of substrata (Jeewon Rajesh *et al*., 2003). *P. guepinii* is considered to be a weak parasite (Rivera *et al*., 1991) not to be confined to particular hosts (Jeewon Rajesh *et al*., 2003; Carl and Bartlett, 1922) and named based on host and disease symptoms (El-Badawy, F. Noha *et al*., 2012). In this contest the present identification of *P.guepinii* causing a foliar blight in *S.album* is a new record in Indian sub-continent.

Conflict of Interest

The authors declare there is no conflict of interest.

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