The Pharma Innovation Journal 2021; 10(3): 1010-1013

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2021; 10(3): 1010-1013

www.thepharmajournal.com
Received: 20-09-2020
Accepted: 26-12-2020

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First report of *Myrothecium roridum* causing leaf spot on bael (*Aegle marmelos* Correa.) from India

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Abstract
Beal (*Aegle marmelos* L. Correa) is native across the Indian subcontinent and is found wild throughout the Indian Peninsula. Cultivation of bael is a lucrative venture because of its high pharmaceutical importance. The deciduous tree with trifoliate aromatic leaves offering of bael leaves is a compulsory ritual of the worship of lord Shiva. The importance of bael lies in its curative properties, make the tree one of the most useful medicinal plant of India. Bael plants suffer from a number of fungal foliar bacterial diseases namely root rot, collar rot, wilt, Alternaria leaf spot, bacterial shot hole, fruit canker, sooty mould and gummosis. In bael nursery/plant, disease appeared during post rainy season, disease symptoms recorded first during rainy season (July- August). Initial symptom were recorded on leaves as small, circular in shape which are brown in colour but later on these spots enlarges and cover the more area up to 40 mm in diameter and later become dark brown with grayish centre. Chlorosis around the lesions may be seen and concentric rings are produced on the middle of the spot on the leaves. Characteristic symptoms of this disease are formation of shot hole due to shedding of necrotic tissues of the leaves. In severe condition number and size of lesions increases, black sporodochia with white and marginal mycelia tuffs bearing black in colour spores masses were observed in the older lesions. This type of symptoms incited by *Myrothecium roridum* was identified as a causal organism. The pathogenicity test of the fungus was established. Fungus produces creamy white colonies on PDA which are 1.5 cm diameter after 5 days at 25+2 ^{0}C. Concentric pattern of sporodochia of *Myrothecium roridum* which are black in colour formed on PDA after 32 days of inoculation. Conidia were aseptate, hyaline, rod shaped and rounded to both ends.

Keywords: *Aegle marmelos*, *Myrothecium roridum*, leaf spot

Introduction
Bael (*Aegle marmelos* L. Correa) is one of the most utilitarian medicinal plants of India; it grows under adverse agro-climatic conditions. It is known by different names in different parts like Bengal quince, golden apple, stone apple, Bael and Sirphalmaredu (Andhra Pradesh), Bael (Bengal), Bil (Gujarat, Himachal Pradesh), bael (Hindi), bilpatra, kumbala, malura (Karnataka), Vilwam (Kerala), Bilwa (Sanskrit), Kuvalum (Tamil Nadu). The deciduous tree with trifoliate aromatic leaves offering of bael leaves is a compulsory ritual of the worship of lord shiva. It has capacity to adapt successfully to a wide range of habitat from arid, semiarid to mesophytic conditions and a wide temperature tolerance (from -7 ^{0}C to 48 ^{0}C). The ripen bael fruits are popular among people because of the delicious fruit pulp, which is ideal for making jam, syrup and pudding. Bael possesses many medicinal values and therefore used as an ingredient in ayurvedic herbal medical preparations. The fruits, bark, leaves, seeds and roots of bael contain bioactive compounds such as coumarin, xanthotoxol, imperatorin, aegelineandmarmeline. These compounds can provide anticancerous, antidiabetic, antimicrobial, antifertility, immunogenic and insecticidal activities. Bael is also essential as a species for reforestation, especially in the unfertile marginal lands. Bael seeds possess a unique fatty acid (12 hydroxyoctadec-cis 9-enoic acid or ricinoleic acid) a convertible item into biodiesel (Pathirana *et al.* 2020) [4]. The importance of bael lies in its curative properties, make the tree one of the most useful medicinal plant of India. Bael plants suffer from a number of fungal foliar and bacterial diseases namely root rot, collar rot, wilt, Alternaria leaf spot, bacterial shot hole, fruit canker, sooty mould and gummosis. (Anonymous, 2019) [1].

Materials and Methods
The lab work was done in the Department of Plant Pathology and field experiment was conducted at Main Experiment Station, Horticulture situated at main campus of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.).

This centre is situated between 26.47^{0}N latitude to 82.12^{0}E longitude and at an altitude of 113 m above mean sea level on Faizabad-Raibarelly road about 42 Km away from Faizabad city. The location falls under Indo-Gangatic plains of Eastern Utter Pradesh of North-India.

Isolation of pathogen

The diseased leaves were collected from the nursery seedling /adult plant of bael showing distinct characteristics of symptoms, selected for the isolation of the pathogen. The leaves were washed with fresh distilled sterilized water in order to remove the dust particles and surface contaminants. The washed leaves samples were cut into small bits with some healthy portions with the help of flamed razor blade and forceps. The bits were surface sterilized by dipping them in 0.1 per cent mercuric chloride solution for 10-15 seconds under aseptic conditions followed by 3 to 4 washing with sterilized water to remove the traces of mercuric chloride. These bits were de-moisturized by placing them between folds of sterilized filter paper. One such bits were placed separately into sterilized culture tubes containing Potato Dextrose Agar (PDA) under aseptic conditions. These tubes were properly marked with glass marker and incubated at 25 + 1^{0}C.

Pure culture of pathogen

Pathogens were purified by using single spore isolation technique. Ten ml of clear, filtered 2% water agar was poured into sterile Petri-plates and allowed to solidify. Dilute spore suspension was prepared in sterilized distilled water from 30 days old culture. One ml of such suspension was spread uniformly on agar plate. These plates were incubated at 25 ± 1^{0}C for 20 hours and examined under microscope to locate single conidium and marked with permanent marker on the surface of the plates. Single conidium was transferred to Petri-plates having PDA with the help of cork borer under aseptic conditions and incubated at 25 ± 1^{0}C.

Pathogenicity test of Myrothecium roridum

Seeds of bael were surface sterilized with 0.1% mercuric chloride and sown in 15 cm diameter earthen pots containing sterilized soil @ 5 seeds/pot. They were allowed to grow for a month. In each pot 2 plants were maintained. Prior to inoculation, the plants were predisposed to 95% humidity for 24 hours. Thereafter, they were inoculated by conidial suspension having 1x10^6 spores/ml with the help of automizer. After inoculation, the plants were kept in the glass house. Control plants were also maintained by spraying sterile distilled water alone. After symptoms appeared the organism was re-isolated from artificially infected leaves and the culture obtained was compared with the original culture.

Results and Discussion

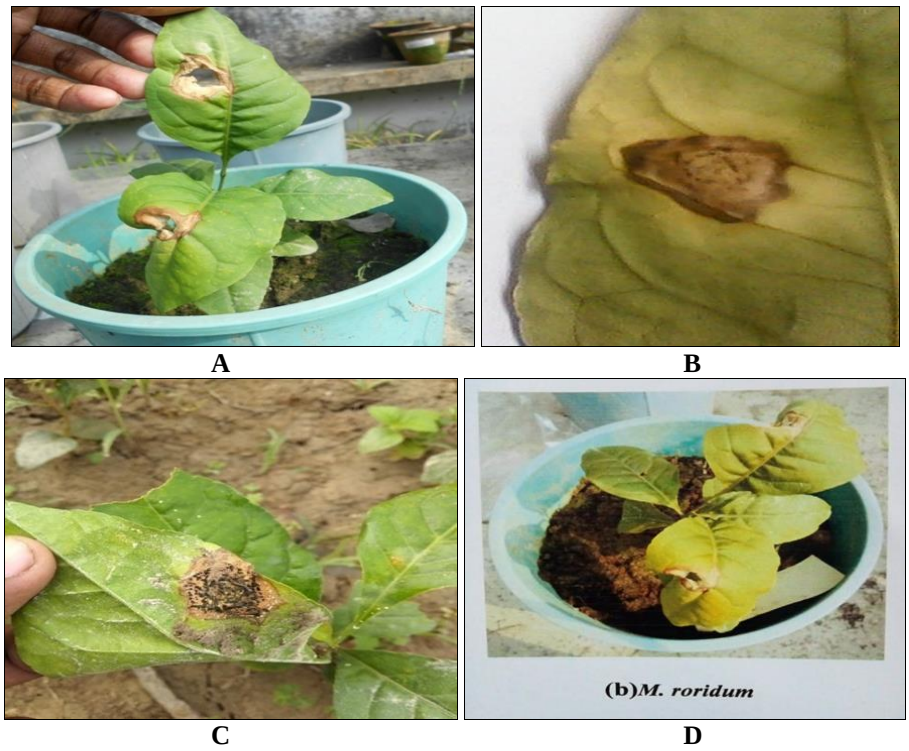
Isolation, Identification and Pathogenicity test

After 4 days of incubation on PDA medium, it was found that the fungal mycelium was emerging out from infected leaf portions. This was further sub cultured for the generation of pure culture and was send to Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi for identification. The pathogen was identified as *Myrothecium roridum* with Id No. 9556.14.

Pathogenicity was tested, using *M. roridum* culture isolated from bael leaves and inoculated on healthy seedlings. The inoculated plants produced symptoms within 7-10 days period of artificial inoculation. Symptoms produced on inoculated plants are similar to those observed in the field. Cultural characters of the test organism which were re-isolated and grow on PDA were similar to the previous culture. After pathogenicity test of *Myrothecium roridum* with disease of bael was associated and confirmed in artificial inoculation trials.

Symptoms

This species is responsible for causing leaf spot disease in bael. The initial symptoms were found on leaves as small, circular in shape which are yellowish brown in colour but later on these spots enlarges and cover the more area up to 40 mm in diameter and later become dark brown with grayish centre. Chlorosis around the lesions may be seen and concentric rings appears in the middle of the spot are characteristic symptoms. Disease symptoms recorded first during rainy season (July- August), 15-20 days after symptom production concentric rings are produced. Characteristic symptoms of this disease are formation of shot hole due to shedding of necrotic tissues of the leaves (Fig.-1A,B,C,D). In severe condition number and size of lesions increases, black sporodochia with white and marginal mycelia tuffs bearing black in colour spores masses were observed in the older lesions (Fig.-1B). These symptoms were consistence with those of *Myrothecium roridum* causing leaf spot on *Coffea canephora* (Silva *et al.*, 2014) [5].



A B C D

Fig 1(A,B,C,D): A- *Myrothecium roridum* leaf spot on bael, B- Sporodochia produce in lower surface of the Myrothecium infected leaves, C- Myrothecium spot sporodochia on mid rib of the leaves, D- Myrothecium present on midrib covered whole surface of the leaves produces necrosis symptom and shot hole

Microscopic features

Fungus produces creamy white colonies on PDA which are 1.5 cm diameter after 5 days at 25+2 ^{0}C (Fig.-2). Concentric pattern of sporodochia of *Myrothecium roridum* which are black in colour, 32 days after inoculation sporodochia was developed and fungus produces different growth pattern which are seen in fig.-3(A, B, C). Conidia were aseptate, hyaline, rod shaped and rounded to both ends (Fig.-4 A&B). Mangandi *et al.*, 2007[3]; Zhang, *et al.*, 2011[6]; Li BJ, *et al.*, 2014[2] and Silva *et al.*, 2014[5] were observed different types of microscopic observation i.e. conidia were hyaline and cylindrical with rounded ends at both the ends, greenish to black in mass and 5 to 6 µm and 1 to 2 µm wide in different crops caused by *Myrothecium roridum.*

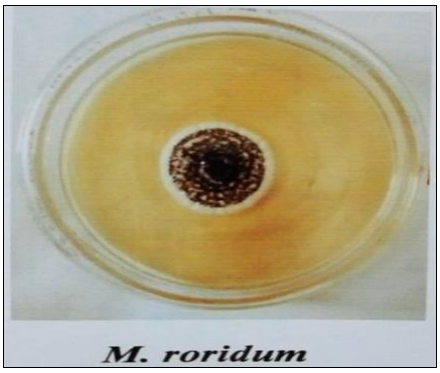
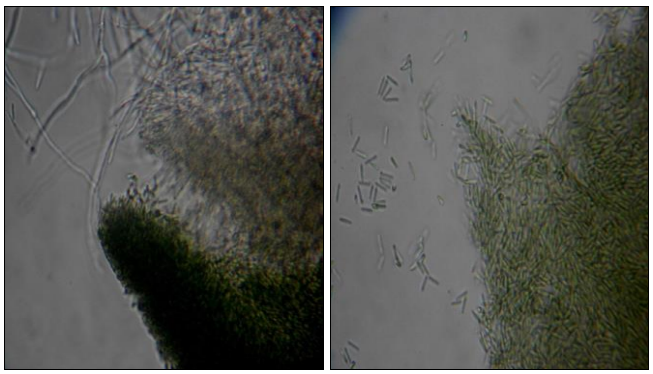


Fig 2: Growth pattern of *Myrothecium roridum* after inoculation with black sporodochia

A B C

Fig 3: (A, B, C): Growth pattern of *Myrothecium roridum* and production of black sporodochia after 32 days of inoculation



A B

Fig 4: A & B: *Myrothecium roridum* mycelium, sporodochia and conidia

Conclusion

The present studies, there is no reports of earlier from India in disease of bael caused by *Myrothecium roridum*. Thisspecies is responsible for causing leaf spot disease in bael. The initial symptoms were found on leaves as small, circular in shape which are brown in colour but later on these spots enlarges and cover the more area up to 40 mm in diameter and later become dark brown with grayish centre. Chlorosis around the lesions may be seen and concentric rings appears in the middle of the spot are characteristic symptoms. Disease symptoms recorded first during rainy season (July- August), 15-20 days after symptom appear, concentric rings are produced and formation of shot hole due to shedding of necrotic tissues of the leaves. In severe condition number and size of lesions increases, black sporodochia with white and marginal mycelia tuffs bearing black in colour spores masses were observed in the older lesions. The fungus *Myrothecium roridum* causing leaf spot on bael have been reported first time from India. The review of literature showed that it is a new record on bael from India.

Acknowledgements

Authors are thankful to Director, Central Institute of Arid Horticulture, Bikaner and Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI New Delhi for identification of the pathogen with Id No. 9556.14.

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