

Original Research Article

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## First Report on Cross-Infection of Coffee Leaf Spot Pathogen *Myrothecium roridum* on Black Pepper

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### ABSTRACT

*Myrothecium roridum*, a fungal plant pathogen is known to infect the leaves and stem of coffee seedlings in the nursery and leaves and berries of the plants in the field. The pathogen has a very wide host base infecting many agricultural and horticulture crops, ornamentals, gymnosperms and weeds. As black pepper is a major intercrop grown in the coffee plantations, the present study was under taken to test the ability of *M. roridum* to infect black pepper (*Piper nigrum* L.). Two isolates of *M. roridum*, isolated both from coffee nursery and field was inoculated on the leaves of pepper vines. The results indicated that the coffee isolates of *M. roridum* could infect the leaves of the pepper vines. Two days after of inoculation, the pathogen expressed the symptoms on the inoculated leaves of pepper vines similar to that of *Myrothecium* leaf spot observed on coffee. The sporodochia could be observed on the inoculated pepper leaves on both lower and upper surface 8 days after inoculation and were arranged concentrically on the affected area. The artificial inoculation confirmed the ability of the pathogen *M. roridum* existing on coffee can infect the pepper vines and cause crop loss. As per our knowledge this is the first report of *M. roridum* found pathogenic on pepper vines.

#### Keywords

Black pepper,  
Coffee, Cross-  
infection, Leaf spot,  
*Myrothecium*

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### Introduction

Coffee is a perennial plantation crop, cultivated in the tropics and sub-tropics of the world. The produce of coffee is internationally traded second to petroleum products and contributes about Rs.4,600 crores of foreign exchange to the national exchequer annually, apart from providing

employment for more than 6 lakh people involved in the coffee industry (Anon., 2018). The roasted beans from fruits of the coffee plant are used mainly as a non-alcoholic beverage by several hundred million consumers throughout the world. The genus *Coffea* belongs to the economically important botanical family Rubiaceae. Arabica (*Coffea arabica* L.) and robusta (*Coffea canephora*

Pierre ex Froehner) are the two major species of *Coffea* that are commercially cultivated in India (Wrigley, 1988; Anon., 2014; Ranjini *et al.*, 2018).

The first record on infection of *Myrothecium roridum* on coffee plant was reported from Colombia in 1951 and Costa Rica in 1961 (Schier and Zentmyer, 1968). *M. roridum* is a cosmopolitan plant pathogen with wide host range causing leaf spot and necrosis on many agricultural crops such as cotton, tomato, cacao, coffee, potato, soybean, cucurbits as well as various ornamental plants (Preston, 1935; French, 1989; Yum and Park, 1990; Kim *et al.*, 2003; Kyung *et al.*, 2014). In India, the *Myrothecium* fungal infection on coffee was considered as minor disease in the past but in recent years the leaf spot and stem necrosis disease caused by *Myrothecium* on coffee is posing a major problem mainly during continuous rainy season (Daivasikamani *et al.*, 2016). The disease is widely spreading in the coffee nurseries and field of Karnataka State and is observed on both the cultivated species of *Coffea*. The fungus infects foliage and stem of coffee seedlings in the nursery and the leaves of coffee plants in the field. Leaves and stem of coffee seedling infected with *M. roridum* initially show water soaked circular necrotic spots which gradually spread (Fig. 1 and 2). Black fruiting bodies are also noticed on the infected area (Fig. 3).

In India, coffee is mainly grown under shaded conditions with many intercrops like pepper, areca, cardamom, banana etc. Black pepper (*Piper nigrum* L.) is a major intercrop cultivated in most of the coffee growing areas. Cultivation of pepper in coffee plantation fetches an additional income to the farmer. As *M. roridum* is reported to have a wide host range, the present study was undertaken to find out the susceptibility of pepper vines by the pathogen *M. roridum* existing on coffee.

## Materials and Methods

### Collection of samples

The infected leaves showing typical leaf spot symptoms of *Myrothecium roridum* were collected from coffee seedlings and on coffee plants (*Coffea arabica* cv. Sln.13 ) both in the nursery and field of Central Coffee Research Institute (CCRI), Balehonnur situated at an elevation of 823-914 m above MSL and longitude 75°28<sup>1</sup>E and latitude 13°22<sup>1</sup>N in Chikkamagaluru district of Karnataka State, India. The infected samples were thoroughly washed with running tap water and then immediately examined under a compound microscope for preliminary identification of the pathogen.

### Isolation of the fungus

Isolation of the fungus was made by tissue isolation technique (Aneja, 2012). The coffee leaves exhibiting moderate to severe disease symptoms in the field and nursery were collected and cut into small bits with the help of a sterilized blade separately. Bits of diseased tissues were washed with sterilized distilled water and then disinfected with 1% sodium hypochlorite solution for two minutes followed by 2 to 3 thorough washing with sterilized water. The selected bits of diseased tissues both from nursery and field were transferred directly on the surface of potato dextrose agar containing Petri plates under aseptic conditions. Inoculated Petri plates were then incubated at 25 °C. The resulting fungal cultures were purified after eight days of incubation by single spore isolation. The isolated fungus produced white buff colony on PDA medium with white flat mycelium producing concentrically arranged sporodochia (Fig. 4).

Morphological characters of the fungus were studied by Nikon SMZ-800 stereo-binocular and Nikon Eclipse E-600 research

microscope. On the basis of morphological characters, the causal fungus was identified as *Myrothecium roridum* by comparing with the fungal culture collections maintained in the Division of Plant Pathology, CCRI.

### **Pathogenicity test**

To test the pathogenicity of *Myrothecium roridum* isolated from infected coffee leaves on black pepper, the pepper vines which were grown in the CCRI farm was used for the study. The pathogenicity test was conducted by following mycelial disc inoculation method (Aneja, 2012). The surface of the pepper leaves to be inoculated was sterilized with 1% sodium hypochlorite with a cotton swab. Five mm culture disc from 10 days old pure culture of *M. roridum* was cut and placed on the lower surface of the leaves and similarly the coffee leaves were also inoculated with both the isolates of the pathogen as a standard check (Fig. 5 & 6). The leaves were covered with polypropylene bags for 48 hours to maintain humidity. Control plants were also maintained by placing plain agar disc on the pepper leaves. Observations at an interval of every 24 hours after inoculation was recorded up to 10 days by recording maximum and minimum temperature and for disease symptoms expression by the pathogen.

### **Results and Discussion**

The average maximum and minimum temperature recorded during the experiment period was 27 °C and 12 °C respectively. Both the isolates of *M. roridum* obtained from coffee nursery and field were able to infect all the inoculated leaves of pepper vine and the symptoms expressed by the pathogen on pepper leaves are similar to that expressed on coffee. The un-inoculated leaves remained healthy. Two days after inoculation (DAI), the inoculated leaves of pepper (*Piper nigrum* L.) started exhibiting the symptoms similar to

that of coffee with water soaked lesion around the inoculated site (Fig. 7). Greyish to dark brown lesions could be observed on 4 DAI (Fig. 8). The sporodochia could be observed on the inoculated pepper leaves on both lower and upper surface on 8 DAI and were arranged concentrically on the affected area (Fig. 9 and 11). Inoculated and infected leaves detached from the plant and defoliated on 10 DAI (Fig. 10)

The pathogen *M. roridum* was re-isolated from the inoculated and infected leaves of black pepper on PDA plates and the fungal colonies grown after incubation period was morphologically identical to the inoculated isolates of coffee, thus proving the Koch's postulates.

The studies of Mangandi *et al.*, (2007) and Kim *et al.*, (2003) revealed that regional change in the weather results in local growing conditions which is more favorable to *M. roridum* infection and also facilitates a broader host range for the fungus. On coffee, infection of *M. roridum* could be observed by Silva and Pinto in 2016 on *Coffea canephora* seedlings. Silvaldo *et al.*, (2007) observed stem canker and leaf necrosis on coffee seedlings in Rio de Janeiro state by *M. roridum*. From India, Nagaraj and George (1958) reported the *Myrothecium* disease observed on coffee seedlings as "Target leaf spot" disease. Further, he reported that the pathogen could infect the coffee plants and the berries under field conditions. Nirmala Kannan and Muthappa (1982) reported the *Myrothecium* disease as "Tip blight of coffee". *M. roridum* was recently reported as an endophyte of the gymnosperm, *Pinus albicaulis* at high elevation in Oregon (Worapong *et. al.*, 2009). McLean and Sleeth (1961) from their studies reported that relatively high temperatures and frequent rain events are prerequisite for disease development.



**Fig.1** Myrothecium leaf spot on coffee leaves



**Fig.2** Stem necrosis on coffee seedling



**Fig.3** Microscopic view of Sporodochia on coffee leaves (20X)



**Fig.4** Axenic culture of *M. roridum*



**Fig.5** Inoculation on the pepper leaves



**Fig.6** Inoculation on coffee leaves



**Fig.7** Water soaked lesions



**Fig.8** Greyish dark brown lesions



**Fig.9** Sporodochia on the inoculated area



**Fig.10** Detached pepper Leaf



**Fig.11** Microscopic view of sprodokia on pepper leaf

Jordan *et al.*, (2018) confirmed the pathogenicity of *M. roridum* on pepper (*Capsicum annuum*) in United States and reported that 25 °C temperature and 75% relative humidity were favorable for the development of disease. Chase and Poole (1984) found that 21 °C to 27 °C was optimum for disease development in *Dieffenbachia maculate* and temperatures of 30 °C or higher inhibited lesion formation by *M. roridum*. Although most inoculation studies have used temperatures in the range of 25 °C, Fitton and Holliday (1970) reported that optimum temperature for conidial germination by *M. roridum* is 28 °C and relative humidity is also another important requirement for infection and disease development. Singh *et al.*, (2003) observed the maximum disease intensity of *M. roridum* in the first fortnight of September (45.6%) when the average atmospheric temperature,

relative humidity and rain were 27 °C, 84.7% and 11.4 mm respectively. Tomar (2008) reported that the favourable climatic conditions for the development of *Myrothecium* blight in cotton includes a mean air temperature between 24.5 °C and 28.8 °C, relative humidity between 45 to 69% and cumulative rainfall in the range of 251 to 522 mm. There is report that based on the pathogenicity of *M. roridum* on the weeds, it could also be used as a bio-herbicide.

The present study revealed that the fungus *M. roridum* isolated from the leaves of coffee seedlings and field plants could infect the leaves of pepper vines and vice-versa indicating the virulence and potential of the pathogen. As black pepper (*Piper nigrum* L.) is a major intercrop grown in most of the coffee plantations of India, it is important to monitor the incidence of *Myrothecium* disease

not only on coffee but also on pepper particularly during continuous rain to keep the pathogen at bay and to take up appropriate control measures so as to realize maximum crop.

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