

Original Research Article

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First Report on Host Range of *Myrothecium roridum* Infecting Arabica Coffee (*Coffea arabica* L.) in India

Madhu S. Giri*, A. P. Ranjini, Santoshreddy Machenahalli,
M. Sudha and S. Daivasikamani

Division of Plant Pathology, Central Coffee Research Institute,
Coffee Research Station - 577 117, Chikkamagaluru District, Karnataka, India

*Corresponding author

ABSTRACT

Coffee is one of the most popular non-alcoholic beverages consumed all over the world. The coffee industry offers lively hood for millions of people directly and indirectly among the coffee growing countries. The coffee crop is known to be affected by many diseases at different seasons. Earlier, the pathogen *Myrothecium* was known to cause stem necrosis and leaf spot disease on coffee seedlings in the nursery. In the recent years, there was a gradual increase in the incidence of leaf spot and fruit rot of Arabica coffee caused by *Myrothecium* in the field condition during rainy season. During the visit to coffee plantations in Chikkamagaluru and Hassan districts, the plants and weeds growing in and around the coffee plantation were observed for the presence of leaf spot with typical symptoms caused by *Myrothecium*. A total of eleven different plant species viz., *Colocasia* sp., *Alternanthera bettzickiana*, *Remusatia vivipara*, *Phlebodium aureum*, *Alternanthera brasiliana*, *Polypodium triseriale*, *Urtica* sp., *Artocarpus heterophyllus*, *Tarenna asiatica*, *Impatiens dasysperma* and *Hibiscus rosa-sinensis* with typical symptoms of *Myrothecium* were identified. Based on the morphological character the fungal cultures were identified as *Myrothecium roridum*. The development of typical symptoms of *Myrothecium* on the Arabica coffee leaves cross-inoculated with pure fungal culture indicated the cross infecting ability of the fungus isolated from different host plants. As per our knowledge this is the first report indicating the cross infecting ability of the *Myrothecium roridum* cultures on Arabica coffee leaves isolated from different host plants, majority of them were weed plants commonly grown in and around coffee plantation.

Keywords

Coffee, Cross inoculation, Leaf spot, *Myrothecium roridum*, Weed

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Introduction

Coffee is one of the most important economic plantation crop of tropical and sub-tropical climate of the world. Coffee in India is mainly cultivated in the hilly tracts of Western and Eastern Ghats. This perennial plantation crop

cultivated under agro-forestry ecosystem provides livelihood to millions of people involved in the coffee production chain and it is one of the major sources of foreign exchange earnings for most of the coffee producing countries.

The two commercially cultivated species of *Coffea* viz., *Coffea Arabica* L. (Arabica coffee) and *C. canephora* Pierre ex A. Froehner (Robusta coffee) are affected by many fungal diseases. Some of the fungal diseases are restricted to the early stage of nursery and usually causes seedling loss which inturn hamper the establishment of healthy plantation.

Among the nursery diseases, the leaf spot and stem necrosis caused by *Myrothecium roridum* Tode ex Fr. which occurs during rainy season under favourable weather condition causes huge mortality of seedlings. Earlier, this disease was considered as minor and restricted to nursery (Daivasikamani *et al.*, 2016). But, in the recent years it was noticed that the occurrence of leaf spot and stem necrosis was very severe in the nursery which resulted in huge mortality of coffee seedlings. Similarly, it was also noticed that there is a drastic increase in the incidence of leaf spot and berry rot caused by *Myrothecium roridum* in field conditions during rainy season in the recent past indicating the changes in the dynamics of disease development and spread.

Myrothecium roridum is a common soil inhabiting fungus with a relatively wide host range that includes such agronomic crops as cotton, tomato, cocoa, coffee, potato, soybean and cucurbits as well as various ornamental plants (Ponnappa, 1970; Chase, 1983; Bruton, 1996; Kim *et. al.*, 2003; Kyung *et al.*, 2014) and causes leaf spot and stem necrosis. In India, Nagaraj and George (1958) reported the *Myrothecium* disease on coffee seedlings as “Target Leaf Spot”. Further, he reported that the same pathogen could infect the leaves and berries of coffee plants in field condition. Later, Nirmala Kannan and Muthappa (1982) reported the *Myrothecium* disease as “Tip blight of coffee”.

In the recent past, increased incidence of *Myrothecium* leaf spot and berry rot was observed on field grown Arabica plants in many plantations of Chikkamagaluru, Hassan and Kodagu districts of Karnataka during rainy season. *Myrothecium roridum* infecting other agricultural and horticultural crop is known to have a wide host range. In view of this, a study was initiated to investigate the collateral or alternate hosts and efforts has been made to identify the weed plants with typical symptoms of *Myrothecium* infection during the visit to coffee plantation under rainy season which acts as additional source of inoculums for disease spread. The result of the study will enable to develop suitable weed management practices and to contain the disease during rainy season.

Materials and Methods

Collection of samples

The infected leaves showing typical leaf spot symptoms of *Myrothecium roridum* were collected from the plantation of various coffee zones of Chikkamagaluru and Hassan districts (Table 1). The infected leaf samples were brought to the Plant Pathology Laboratory, Central Coffee Research Institute (CCRI) and were examined under stereo-microscope (Nikon SMZ - 800) for preliminary identification of the pathogen.

Isolation of pathogen

From the infected leaves, the pathogen was isolated by transferring sporodochia directly from the infected host tissue to the Petri-dishes (90 mm diameter) containing Potato Dextrose Agar (PDA) and incubated at 25±1°C. The colonies of the fungus was further purified by single spore culture technique and maintained on PDA medium at 25±1°C.

Proving pathogenicity on coffee / Cross inoculation on coffee

Twenty days old active cultures of the pathogen with sporodochia cultured on PDA medium were used for inoculation. Culture discs (9 mm diameter) of *Myrothecium* isolated from different host plants were prepared by using cork borer. Such culture discs were used for inoculation. Leaves selected for inoculation were surface disinfected with 70% ethanol. Leaves of Arabica coffee plants in the field were inoculated at multiple sites with culture discs of *Myrothecium* on lower surface of the leaves. Further, inoculated discs were covered with a thin layer of absorbent cotton to avoid desiccation of culture discs.

The inoculated leaves were then kept moist for 10 days by covering the branches with polypropylene bags to create high humidity. Similarly, un-inoculated control was

maintained by placing PDA discs on the lower side of the leaves. Observations on the development of disease symptoms and reaction of the host to pathogen were recorded every day.

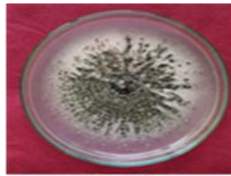
Results and Discussion

The stereo-microscopic observation of leaves of different host plants viz., *Colocasia* sp., *Alternanthera bettzickiana*, *Remusatia vivipara*, *Phlebodium aureum*, *Alternanthera brasiliana*, *Polypodium triseriale*, *Urtica* sp., *Artocarpus heterophyllus*, *Tarenna asiatica*, *Impatiens dasysperma* and *Hibiscus rosa-sinensis* collected from coffee plantation of different locations suspected to be infected with *Myrothecium* showed the presence of black coloured sporodochia (Figure M. r. 1 to M. r. 11). The details of host plant and locations from where they collected are presented in table 1.

Table.1 *Myrothecium roridum* infected leaf samples collected from different host plants and their locations

Isolate	Host plant	Family	Location	District
M. r. 1	<i>Colocasia</i> sp.	Araceae	Hanthuru	Chikkamagaluru
M. r. 2	<i>Alternanthera bettzickiana</i>	Amaranthaceae	CCRI farm	Chikkamagaluru
M. r. 3	<i>Remusatia vivipara</i>	Araceae	CCRI farm	Chikkamagaluru
M. r. 4	<i>Phlebodium aureum</i>	Polypodiaceae	CCRI farm	Chikkamagaluru
M. r. 5	<i>Alternanthera brasiliana</i>	Amaranthaceae	TEC, Mudigere	Chikkamagaluru
M. r. 6	<i>Polypodium triseriale</i>	Polypodiaceae	TEC, Mudigere	Chikkamagaluru
M. r. 7	<i>Urtica</i> sp.	Urticaceae	TEC, Mudigere	Chikkamagaluru
M. r. 8	<i>Artocarpus heterophyllus</i>	Moraceae	TEC, Sakaleshpura	Hassan
M. r. 9	<i>Tarenna asiatica</i>	Rubiaceae	Taluvane	Chikkamagaluru
M. r. 10	<i>Impatiens dasysperma</i>	Balsaminaceae	Hiregadde	Chikkamagaluru
M. r. 11	<i>Hibiscus rosa-sinensis</i>	Malvaceae	Ossuru	Hassan

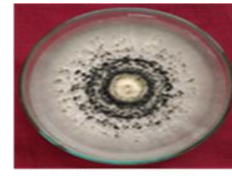
Fig.1 Pure cultures of *Myrothecium roridum* and their respective host plants



M r. 1



M r. 2



M r. 3



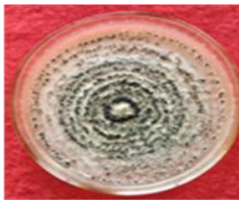
Colocasia sp.



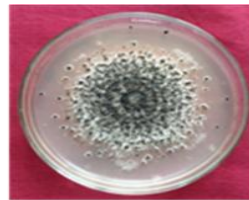
Alternanthera
betzickiana



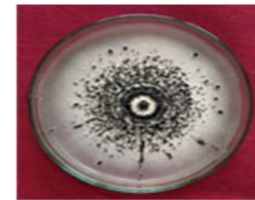
Remusatia
vivipara



M r. 4



M r. 5



M r. 6



Phlebodium
aureum



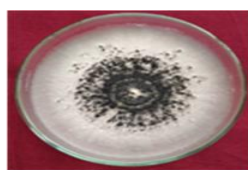
Alternanthera
brasiliana



Polypodium
triseriale



M r. 7



M r. 8



M r. 9



Urtica sp.



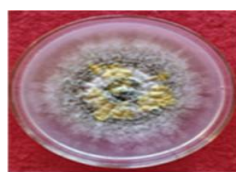
Artocarpus heterophyllus



Tarenna asiatica



M r. 10



M r. 11



Impatiens dasysperma



Hibiscus rosa-sinensis

Isolation of pathogen

Fungal cultures were obtained from lesions and cultivated on PDA medium and incubated at 25±1°C in the dark. In the culture, the fungal colonies reached 90 mm diameter on PDA 20 to 22 days after incubation. Initially colonies of the isolates were white, floccose, wrinkled, somewhat raised at the centre. Sporulation occurred throughout the colony in concentric greenish black zones. These zones consisted of cluster of conidiophores forming sporodochia. Conidia were rod shaped. The characteristics of the fungus were consistent

with those reported for *M. roridum* by earlier investigators (Seebold *et al.*, 2005; Mangandi *et al.*, 2007; Han *et al.*, 2014; Ben *et al.*, 2015; Ranjini and Rajanaika, 2018).

Cross inoculation of cultures on Arabica coffee

In cross inoculation study, all healthy Arabica coffee leaves showed typical symptoms 7 to 10 days after inoculation, while un-inoculated control leaves remained symptomless. The typical symptoms were dark brown, circular or sub-circular lesions on leaf surface. Lesions

expanded gradually. Later, the infected leaves turned yellow and abscised. At later stage, black sporodochia with white mycelial tufts were produced. The same fungus was re-isolated from inoculated plants, but not from the un-inoculated controls.

Myrothecium roridum has an extensive host range, covering 294 species host plant (http://nt.ars-agrin.gov/fungal_databases) and 23 genera of foliage plants which were considered as potential hosts of *M. roridum* (Chase, 1987). Quezado-Duval *et al.*, (2010) also found out some unfamiliar hosts of *Myrothecium* fungi in Brazil. In their study they have isolated *M. roridum* and *M. verrucaria* from three vegetable plants (Sweet pepper, tomato and cucumber), four ornamental species (*Spathiphyllum wallisi*, *Solidago Canadensis*, *Anthurium andraeanum* and *Diffenbachia amoena*) and a weed belonging to solanaceae family (*Nicandra physaloides*) and confirmed pathogenicity.

Similarly, Ranjini *et al.*, (2019), artificially inoculated the pathogen *M. roridum* to the leaves of black pepper vines that is existing on coffee and confirmed its ability to infect black pepper vines.

The present study revealed that *Myrothecium roridum* isolated from different host plants growing in and around coffee plantation can infect leaves of Arabica coffee plants and produce necrotic leaf spots. Even though there are numerous reports of *M. roridum* as a pathogen of coffee seedlings, to our knowledge, this is the first report in India and world indicating the cross infecting ability of *M. roridum* causing leaf spots on Arabica coffee which were isolated from different host plants growing in and around coffee plantation.

This study also provides a hint for the increased incidence of necrotic leaf spot and

berry rot caused by *Myrothecium* in the field condition during monsoon season in the recent years. As the pathogen has got fairly wide host range, timely action is needed to restrict its spread on coffee through maintenance of sanitation in the plantation by keeping the plantation free from weeds.

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