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Leaf spot and stem necrosis disease of Coffee seedlings caused by *Myrothecium roridum* Tode ex Fr. in India

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The leaf spot and stem necrosis disease was observed in severe form on coffee seedlings during 2016 in nursery at Central Coffee Research Institute, Balehonnur, Chikkamagaluru District, Karnataka State, India. Samples were collected from the affected seedlings and the fungus was isolated, purified, identified and characterized as *Myrothecium roridum* Tode ex Fr. The disease caused by the fungus *M. roridum* on leaves of coffee seedlings in India was wrongly reported as "Target leaf spot "and "Tip blight of coffee" by earlier workers and they considered it then as a minor disease. The present study revealed that the fungus *M. roridum* could infect both the leaves and young stem of coffee seedlings at nursery stage. Further, identification, confirmation and characterization of the causative organism from this study enable in better understanding of the pathogen and its interaction with the host (coffee plant) which in future help to develop strong control strategies against the disease.

Key words: Coffee, leaf spot, stem necrosis, *Myrothecium roridum*

INTRODUCTION

Coffee is a perennial plantation crop, mainly cultivated in the hilly tracts of Western and Eastern Ghats of India. 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. 2017). 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.* 2017).

Coffee is mainly propagated through seeds, especially the arabica coffee. The coffee seedlings are raised and maintained in the poly bag (basket) nursery up to 6-8 months before they are planted in the field where the plant would survive for a period of 45-60 years. Hence, it is very important to raise healthy, vigorous and disease free seedlings in a perennial crop like coffee (Anon, 2014).

Hither to the coffee seedlings in the nursery were affected by collar rot and brown eye spot diseases caused by fungi. But in recent years the leaf spot and stem necrosis disease caused by *Myrothecium* sp. is posing a major problem mainly during continuous rainy season. (Daivasikamani *et al.* 2016). The disease is widely spreading in the coffee nurseries of Karnataka State and is observed on both the cultivated species of *Coffea*. The fungus infects foliage and stem of coffee seedlings. In view of the above, the present study was carried out to understand the etiology of the fungus to know the host pathogen interaction.

The pathogen

The fungus *Myrothecium* is an economically important genus of the Family: Incertae sedis, Order: Hypocreales, Class: Sordariomycetes. It is reported that *Myrothecium* species are common

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soil inhabitants in temperate and tropical regions of the world. (Domsch *et al*.1980). Presently, there are several species of *Myrothecium*, most of them are reported as saprophytes. Only *M.* roridum Tode ex Fr. is considered as an expressive plant pathogen (Tulloch, 1972; Ahrazem *et al.* 2000).

Myrothecium roridum is a cosmopolitan plant pathogen with a wide host range causing leaf spot and necrosis on many economically important crop plants (Preston, 1935; French, 1989; Yum and Park, 1990; Kim *et al.* 2003). The stem necrosis and leaf spot disease caused by the fungus *M. roridum* is considered as one of the major diseases of coffee seedlings in Brazil, Colombia, Costa Rica and Guatemala since 1960s. There are few reports available on *M. roridum* incidence on coffee seedlings from India. Nagaraj and George (1958) reported the *Myrothecium* disease observed on coffee seedlings as “Target leaf spot” disease. While, Nirmala Kannan and Muthappa (1982) reported the *Myrothecium* disease as “Tip blight of coffee”.

Symptoms of the disease

The pathogen infects both stem and leaf of coffee seedlings. The symptoms are

On leaves

Infected leaves initially show water soaked circular necrotic spots which gradually spread. These spots then become brown with concentric rings. In severe condition 2 to 3 such spots coalesce to form irregular necrotic patches and may even cover the entire lamina of the leaf. Black fruiting bodies are noticed on the under surface of the affected leaf all along the concentric rings of the necrotic spot. Occasionally the centre of the lesion cracks irregularly, ruptures and some of the affected tissues drop down during the rainy season (Fig.1, 5).

On stem

The infected seedling shows water-soaked brown to grey discoloration on the tender stem indicating necrosis above the soil (Fig.2). Sometimes 2 to 3 such lesions are noticed on the same stem. The infected region later shows cushion shaped black fruiting bodies surrounded by white mycelia. Affected seedlings gradually start wilting and then die.

MATERIALS AND METHODS

Collection of specimen

During 2016 season, leaves and stem of coffee seedlings showing characteristic symptoms of *Myrothecium* infection were collected from the nursery at Central Coffee Research Institute (CCRI), Balehonnur situated at an elevation of 823-914 m above MSL and longitude 75°28 E and latitude 13°22 N in Chikkamagaluru district of Karnataka State, India.

Isolation of the fungus

Samples collected from the nursery were examined in the laboratory of plant pathology division under stereomicroscope and the infected leaf or stem of coffee seedlings were selected and cut into 1 cm^2 pieces and processed further. Samples were washed with tap water and surface sterilized with 1% sodium hypochlorite solution for two minutes followed by 2-3 thorough washing with sterilized water. The processed samples were aseptically transferred to 90 mm Petri plates containing 20 ml of solidified Potato Dextrose Agar medium (PDA). These inoculated Petri plates were incubated at 25°C for 2-3 days and the fungus was isolated. The isolated fungus was purified by transferring actively growing mycelium from the colony margin (Jhonston and Booth, 1983; Aneja, 2012).

Identification of the fungus

Morphological characters of the fungus were studied with Nikon SMZ-800 stereo-binocular and Nikon Eclipse E-600 research microscope. Key identification features of the fungus such as colony colour and shape; mycelial morphology, sporodochia production; conidia formation; conidia colour, size and shape were studied as described by CMI and Mycobank, Korea (Fitton and Holiday, 1970; Mycobank #142164). The fungal culture isolated separately from leaves and stem of coffee seedlings, actively growing mycelium was transferred to PDA slants and incubated at 25°C for 7 days. The PDA slants with the developing fungal culture were sent to Indian Type Culture Collection (ITCC), Identification/Culture Supply Services, Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, India for further confirmation and identification up to species level.

Pathogenicity test (Koch's postulates)

Pathogenicity of the fungus was confirmed by application of Koch's postulates. Seeds of *Coffea arabica* cultivar Chandragiri were sown in the seed bed. After 45 days of sowing, the topee stage seedlings were transferred to poly bags filled with one kg of nursery mixture (jungle soil, farm yard manure and sand in the ratio of 6:3:1). The seedlings were maintained in the nursery and when they attained 2-4 leaves, the pathogencity test was conducted by following mycelial disc inoculation method (Aneja, 2012). The surface of the 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 *Myrothecium* sp., was cut and placed on the lower surface of the leaves and the stem of the seedling with and without injury. The seedlings were covered with polypropylene bags for 48 hours to maintain humidity. Control plants were also maintained by placing plain agar disc leaves and stem. Maximum and minimum temperature during the experiment period was 27 °C and 12°C respectively. Symptoms were observed for every 48 hours after inoculation up to 10 days.

RESULTS AND DISCUSSION

Identification of the fungus

The isolated fungus produced white buff colony on PDA medium with white flat mycelium producing concentrically arranged sporodochia (Fig.3). Cultures covered the 90 mm Petri plate after 20 days of incubation period at the 25° C. The lower surface of the Petri plates with PDA medium on which the fungus is growing indicated the colour change of rosy buff or yellow. Sporodochia is a pin head size, polymorphic, with variable size, dark green or greenish black in colour which is surrounded by white mycelium and bears a mass of slimy green to black conidia (Fig.4). The sporodochia are often grown in concentric rings on the Petri plate giving carom coin appearance. The conidia are hyaline, rod shaped or narrowly ellipsoidal, unicellular tip rounded and base slightly truncate. Confirmation and identification up to species level of the fungus was carried out. The fungus isolated from arabica coffee seedlings was identified as *Myrothecium roridum* Tode ex Fr. with the identification reference [I.D. No.10.636.17 (CCRI-3)] by the IARI, New Delhi. The sporodochia formed by *M. roridum* on *Pepromia quandrangularis* from Korea were black and surrounded by white tufts of mycelium as was reported by Kyung *et. al.* (2014). Kim *et al*. (2003) found that the *M. roridum* infecting water melon produced white colonies with flat mycelium producing black sporodochia. A survey conducted in different ecological zones of Punjab on bitter gourd indicated that highest incidence of *Myrothecium* leaf spot disease in mixed cropping zone compared to single cropping zone (Sumera *et al.* 2017).

In India, occurrence of this disease was very rarely reported till the year 2005 and it was considered as a minor disease of coffee. However, since five years, the incidence of this disease is increasing and is causing concern for rising of coffee seedlings in the nursery for field planting. A preliminary assessment on the incidence of this disease in the coffee nurseries varied from as low as 10% to a maximum of 83.25% resulting in mortality of seedlings in most of the coffee regions throughout Karnataka (Anon, 2008).

Pathogenicity test

Symptoms of disease like the one appeared on the coffee seedlings were noticed on all the inoculated plants whereas the un-inoculated seedlings remained healthy. Both injured and non injured seedlings could take up the infection after two days of inoculation. Details are presented in the Table1.

On the leaves

Water soaked lesion around the inoculated site could be observed after two days of inoculation (DAI). On the injured leaves necrotic area spread rapidly than the uninjured leaves and reached up to 5 cm on 6 DAI. The sporodochia could be observed on the inoculated leaves on 8 DAI and were arranged concentrically on the affected area. Inoculated and infected leaves detached from the plant on 10 DAI.

On the stem

The inoculated seedling injured on the stem took the infection on 2 DAI whereas uninjured seedling took the infection on 4 DAI. The sporodochia could be observed on 8 DAI in both injured and uninjured

seedling. Finally, on 10 DAI the seedlings were dead due the breakage of the stem. The pathogen could infest the seedling without injury and

Fig.1: Irregular *Myrothecium* leaf spots with concentric rings on upper surface of coffee leaves (16X)

Fig. 2: Necrosis of the stem on the collar region of coffee seedling (16X)

symptoms on both the injured and uninjured seedlings did not make much difference in the appearance of the symptoms. The pathogen was re-isolated from inoculated seedlings produced the similar colonies of the pathogen which was isolated from the naturally infected seedlings earlier. From Brazil, Silva and Pinto (2016) reported that the *M. roridum* could infect *Coffea canephora* seedling. Silvaldo *et al.* (2007) observed stem canker and leaf necrosis in Rio de Janerio state.

Fig. 3: Pure culture of the *M. roridum* on PDA medium (16X)

Fig. 4 : Sporodochium of *M.roridum* with phialides and conidia (400X)

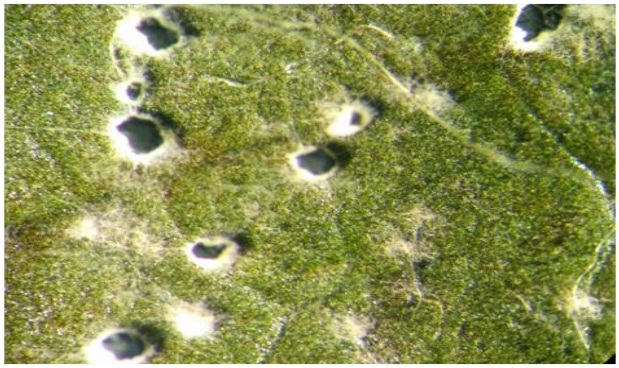

Fig.5: Sporodochia on lower surface of coffee leaf (10X)

Table 1: Symptoms expressed by coffee seedlings after inoculation of *Myrothecium roridum*

Details of inoculation	Days After Inoculation	Symptoms observed
Lower surface of leaves without injury	2	Water soaked lesion
	4	Greyish brown lesion of 1cm developed
	6	Greyish brown lesion of 3 cm with concentric rings
	8	Sporodochia on upper and lower surface of the leaf
	10	Premature defoliation
Lower surface of leaves with injury	2	Water soaked lesion
	4	Greyish brown spot of 2 cm width
	6	Greyish brown spot increased up to 5 cm with concentric rings
	8	Sporodochia on the lower and upper surface of the leaf and ruptured in the middle of lesion
	10	Premature defoliation
Inoculation on the stem without injury	2	Water soaked lesion
	4	Girdling of the stem
	6	Greyish brown necrotic spot
	8	Sporodochia on the necrotic lesion
	10	Death of the seedling
Inoculation on the stem with injury	2	Girdling on the stem
	4	Greyish brown necrotic lesion
	6	Necrosis on the stem
	8	Sporodochia on the necrotic lesion
	10	Death of the seedling

Cabral *et al*. (2009) proved the pathogenicity of *M. roridum* on many of the cucurbits such as gherkin, cucumber, squash, pumpkin, water melon and melon etc. Quezado Duval *et al.,* (2010) had reported that the pathogen *M. roridum* caused foliar spots in many cultivated plants. Kyung *et al.* 2014 reported that the disease could be observed on leaf and stem of the *Peporomia quadrangularis* on inoculation after 7 days of incubation. Nasreen and Ghaffar (2009) reported that *Myrothecium* could cause the seed rot, damping off, root rot of vegetable and plantation crop across the world.

Though the leaf spot disease on coffee seedlings was reported from Colombia as early as in 1951 and Costa Rica in 1961, the control measures suggested was spraying of cuprous oxide on to the seedlings. But it failed as the copper-based fungicide caused constriction on the stem which could break due to strong winds (Schier and Zentmyer 1968). However, as the same disease is observed on seedlings in the coffee nurseries across India which is severe and needs control measures. The present study indicated that the pathogen *M. roridum* could infect the leaves and tender stem of coffee seedlings and when the seedling attain 3months of age the stem becomes hard and the pathogen cannot infect stem of the seedling only the leaves of the seedling will be affected especially during the rainy season.

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