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Cultural and morphological variability among *Myrothecium roridum* isolates causing *myrothecium* leaf spot disease collected from soybean growing area of Chhattisgarh

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Abstract

Myrothecium leaf spot of soybean caused by *Myrothecium roridum*. *Myrothecium* leaf spot of soybean is occurring in almost all the major soybean growing areas of India causing about 30 per cent yield loss. Initial symptoms of the disease appear as small round or oval, brown spots with dark brown margin on leaves in the infected plant. In cultural and morphological variability studies of 20 isolates of *Myrothecium roridum*. The maximum radial growth (38.9mm) was observed in isolate Mr9 at 5 DAI and minimum radial growth (25.9mm) was observed in isolate Mr14. Maximum mean mycelial growth (88.5mm) was observed in isolate Mr9 and minimum mean mycelial growth (43.9mm) was observed in isolate Mr14. Sporulation color of *M. roridum* were recorded PDA. Seven isolates were found black in color, two isolates brownish black color, three isolate light green color, five isolates dark green color and three isolates produced white color sporulation. Mycelial growth pattern of all the isolates were observed on PDA out of which eleven isolates categorized in circular margin with smooth surface and nine isolates were categorized in irregular with rough surface. Thirteen isolates showed concentric ring and remaining seven isolates were without concentric ring. The highest conidia size (6.24×1.45µm, length × width) was recorded in isolate Mr5 and lowest conidia size (2.48× 0.61µm, length × width) was recorded in isolate Mr2. Conidia of nine isolates were cylindrical with round end shape, eight isolates were rod shaped with round end and three isolates were narrowly ellipsoid with round end shape.

Keywords: *Myrothecium* leaf spot, soybean, *Myrothecium roridum*, survey, surveillance

Introduction

Soybean (*Glycine max.* L. Merrill) belonging to family Leguminaceae is designated as miracle bean established its potential as an industrially vital and viable oilseed crop in many areas of India. Leaf spot of soybean caused by *Myrothecium roridum* Tode ex. Fries is an important disease, which occurred in epidemic proportion entailing into colossal losses to soybean crop in Madhya Pradesh (Shrivastava and Khan, 1994, Singh and Shrivastava, 1994) [6, 7]. *Myrothecium* leaf spot of soybean is occurring in almost all the major soybean growing areas of India causing about 30 per cent yield loss (Shrivastava and Khan 1994) [6]. The disease severity of *myrothecium* leaf spot soybean was in the range of 35 to 45% and disease incidence of *myrothecium* leaf spot soybean was in the range of 30 to 55% (Singh and Shrivastava, 1994) [7]. *Myrothecium roridum* is ordinary soil fungi, and survive in this environment as saprophytes in decaying plant tissues (Ellis, 1971). Initial symptoms of the disease appear as small round or oval, brown spots with dark brown margin on leaves in the infected plant. Marissonia *et al.* (2008) [4] reported the variability of 53 isolates of *Myrothecium roridum* obtained from melon (*Cucumis melo*) fields in the State of Rio Grande do. Rajender *et al.* (2013) collected twenty-five isolates of *Alternaria helianthi* in pure form from Directorate of Oilseeds Research, Rajendranagar, Hyderabad for cultural, morphological and pathogenic characterization. Singh *et al.* (2014) [5] reported variability in *Alternaria solani* isolates on the basis of cultural and morphological characters. Kumar *et al.* (2015) [3] evaluated the cultural and morphological variation of 12 isolates of *Colletotrichum capsici* collected from different locations. These isolates were grown on different media and were characterized for colony morphology.

Methods and Material

Experimental site

All the laboratory experiment were carried out at the Department of Plant Pathology, IGAU, Raipur.

General procedure followed

In general, in each Petri dish about 15-20 ml of potato dextrose agar medium was poured, supplemented with streptomycin in order to check the unwanted bacterial contamination. Wherever growth studies were conducted five mm disc of pure culture of *Myrothecium roridum* Tode ex. Fries. By the help of cork borer, was used for inoculation of medium in Petri dish. The inoculated plates were incubated in the 25 °C for three days. Observation for the growth and sporulation were recorded at 10 to 15 days after inoculation.

Media used

Following medium was used during laboratory studies on *Myrothecium roridum* Toed ex Fries.

Table 1: Show the composition and Quantities

Media	Composition	Quantities
Potato dextrose agar	Potato (peeled and sliced)	200 g
	Dextrose	20 g
	Agar-Agar	20 g
	Distilled water	1000 ml

Collection of diseased sample

The naturally infected leaf of soybean crop with the myrothecium leaf spot symptoms were collected from different soybean growing area in Chhattisgarh. Collected samples were brought to the laboratory for critical examination of the symptoms for the identification studied under compound microscope & isolation of the pathogen.

Isolation, purification & identification of test fungus

The infected leaves of soybean were cut into small pieces, surface sterilized with 0.1% mercuric chloride (HgCl₂) solution followed by three washing with sterile distilled water and placing in moist chamber than after 1 to 2 days fungal mycelium growth were seen than finally small bits of fungus kept on the previously poured and solidified potato dextrose agar medium in Petri plates for isolation of the pathogen. The plates were incubated at 25 °C in an incubator. The plates were observed after mycelial growth from the inoculated mycelium bits. Mycelial were then sub-cultured, purified by hyphal tip method and maintained culture on PDA slant & Petri plate kept on incubator at 25 °C. All the growth characters were recorded and compared with the standard reports publish for confirmation.

Isolates

The total twenty isolates were isolated from the samples collected from different soybean growing areas of Chhattisgarh and designated are as given in Table 2.

Cultural and morphological study of *Myrothecium roridum*

The twenty isolates of *Myrothecium roridum* was grown in PDA medium for the cultural and morphological study. The colony growth starts in 3-4 days at 25 °C under darkness on PDA. The cultural and morphological character (radial growth and colony character) were recorded 5 days, 10 days, and 15 days after inoculation. Slides were prepared from 18 days old culture and number of conidia and the shape and size of conidia were recorded.

Result

Cultural and morphological characteristics of *Myrothecium roridum*

Measurement of the mycelial growth was taken for 20 different isolates of *Myrothecium roridum* from purified culture when inoculated on PDA plates.

Radial growth

Result presented in Table 3, Fig. 1 and Plate 1 showed the variation in mycelial growth of different isolates of *M. roridum*. The maximum radial growth (38.9 mm) was observed in isolate Mr9 at 5 DAI followed by Mr5 (36.3 mm), Mr8 (35.8 mm), Mr19 (35.5 mm), Mr2 (35.5 mm), Mr11 (35.3 mm), Mr7 (33.7 mm), Mr20 (32.6 mm), Mr16 (32.5), Mr1 (32.2 mm), Mr15 (32.0 mm), Mr13 (30.6 mm), and Mr12 (30.5 mm). The minimum radial growth (25.9 mm) was observed in isolate Mr14 at 5 DAI. The maximum radial growth (70 mm) was observed in isolate Mr2 at 10 DAI followed by Mr5 (68.3 mm), Mr19 (67.5 mm), Mr1 (65.5 mm), Mr9 (65.5 mm), Mr15 (63.6mm), Mr4 (62.3mm), Mr13 (62.2mm), Mr20 (60.5mm), and Mr16 (60.3mm). The minimum radial growth (40.5mm) was observed in isolate Mr14 at 10 DAI. The maximum radial growth (88.5mm) was observed in isolate Mr9 at 15 DAI followed by Mr12 (85.6mm), Mr3 (83.2mm), Mr19 (78.2mm), Mr15 (76.2mm), Mr5 (76.1mm), Mr12 (75.6mm), Mr13 (75.5mm) and Mr1 (75.2mm). The minimum radial growth (62.6mm) was observed in isolate Mr10 at 15DAI. But the maximum mean mycelial growth (88.5mm) was observed in isolate Mr9 followed by Mr2 (63.8mm), Mr19 (60.4mm), Mr5 (60.1mm), Mr1 (57.3mm), Mr16 (56.1mm), Mr20 (55.8mm) and Mr4 (55.2mm). The minimum mean mycelial growth (43.9mm) was observed in isolate Mr14 followed by Mr6 (45.4mm), Mr10 (46.4) and Mr18 (46.6mm). During the studies of cultural variability among isolates of *Myrothecium roridum*, most of the isolates of *Myrothecium roridum* were showed variation in radial growth (Figure 1). Talhinhos *et al.* (2005) [8] reported the variation in radial growth among isolates of *Colletotrichum capsici*.

Sporulation color

Isolates of *M. roridum* showed great variability in sporulation color on PDA medium. Seven isolates (Mr1, Mr9, Mr10, Mr11, Mr12, Mr18 and Mr20) exhibited Black color, two isolates (Mr2 and Mr17) were brownish black color, three isolates (Mr4, Mr7 and Mr16) light green color, five isolates (Mr5, Mr6, Mr14, Mr15 and Mr19) dark green color and three isolates (Mr3, Mr8 and Mr13) were white in color (Table 4 and Plate 2).

Colony character

Colony character presented in Table 5 and Plate 1 indicated that all the isolates differ in their colony character. Margin pattern of all isolates were categorized in two pattern i.e. circular with smooth surface and irregular with rough surface. Mycelial growth pattern of isolates Mr1, Mr2, Mr3, Mr4, Mr5, Mr6, Mr7, Mr14 Mr15 Mr19 and Mr20 grew with circular margin with smooth surface and Mr8, Mr9, Mr10, Mr11, Mr12, Mr13, Mr16, Mr17 and Mr18 isolates were growing with irregular and rough surface (Table 4 and Plate 2).

Concentric ring

Out of 20 isolates, thirteen isolates (Mr1, Mr4, Mr5, Mr6, Mr7, Mr9, Mr10, Mr11, Mr12, Mr14, Mr15, Mr19 and Mr20) showed concentric ring and remaining seven isolates (Mr2, Mr3, Mr8, Mr13, Mr16, Mr17 and Mr18) were by without concentric ring (Table 4 and Plate 2).

Cleia *et al.*, (2009) [1] reported variability on five isolates of *M. roridum* on the basis of presence and absence of concentric ring. Kumar (2015) [3] also found the significantly different in morphological characteristics of *Colletotrichum capsici* viz. colony diameters, colony morphology, pigmentation, radial growth, colony color, mycelial growth pattern and sporulation. Singh *et al.* (2014) [5] reported variability in *Alternaria solani* isolates on the basis of cultural and morphological characters.

Table 2: Designation of *M. roridum* isolates and their place of collection

S. No.	Isolates	Place
1	Mr1	Rajnandgaon
2	Mr2	Ghumka
3	Mr3	Saja
4	Mr4	Semra
5	Mr5	Khairagarh
6	Mr6	Lohara
7	Mr7	Bhorampur
8	Mr8	Chhuhikhadan
9	Mr9	Gandai
10	Mr10	Atariya
11	Mr11	Kachnar
12	Mr12	Kawarda
13	Mr13	Darampura
14	Mr14	Mungeli
15	Mr15	Chatarkhar
16	Mr16	Bemetra
17	Mr17	Pendridihi
18	Mr18	Odhiyakla
19	Mr19	Biranpur
20	Mr20	Raipur

Table 3: Radial growth of *Myrothecium roridum* on PDA medium

S. No.	Isolates	Radial growth (mm)		
		5 DAI	10 DAI	15 DAI
1	Mr1	32.2	65.5	75.2
2	Mr2	35.5	70.5	85.6
3	Mr3	28.3	50.3	83.2
4	Mr4	30.2	62.3	73.3
5	Mr5	36.3	68.3	76.1
6	Mr6	27.5	43.4	65.5
7	Mr7	33.7	54.5	71.2
8	Mr8	35.8	49.5	68.3
9	Mr9	38.9	65.5	88.5
10	Mr10	30.2	46.5	62.6
11	Mr11	35.3	50.2	72.2
12	Mr12	30.5	58.2	75.5
13	Mr13	30.6	62.2	75.6
14	Mr14	25.9	40.5	65.5
15	Mr15	32.	63.6	76.2
16	Mr16	32.5	60.3	70.1
17	Mr17	29.3	55.2	70
18	Mr18	26.6	47.7	65.5
19	Mr19	35.5	67.5	78.2
20	Mr20	32.6	60.5	74.5

Table 4: Cultural variability of different isolates of *Myrothecium roridum* on potato dextrose agar

Isolates	Colony character			
	Sporulation color	Circular/irregular	Smooth/rough	Concentric zone
Mr1	Black	Circular	Smooth	concentric zone
Mr2	Brown black	Circular	Smooth	Without concentric zone
Mr3	White	Circular	Smooth	Without concentric zone
Mr4	Light green	Circular	Smooth	concentric zone
Mr5	Dark green	Circular	Smooth	Concentric c zone
Mr6	Dark green	Circular	Smooth	Concentric zone
Mr7	Light green	Irregular	Rough	Concentric zone
Mr8	White	Irregular	Rough	Without concentric zone
Mr9	Black	Irregular	Rough	Concentric zone
Mr10	Black	Irregular	Rough	Concentric zone
Mr11	Black	Irregular	Rough	Concentric zone
Mr12	Black	Irregular	Rough	Concentric zone
Mr13	White	Irregular	Rough	Without concentric zone
Mr14	Dark green	Circular	Smooth	Concentric ring zone
Mr15	Dark green	Circular	Smooth	Concentric ring zone
Mr16	Light green	Irregular	Rough	Without concentric zone
Mr17	Brown black	Irregular	Rough	Without concentric zone
Mr18	Black	Irregular	Rough	Without concentric zone
Mr19	Dark green	Circular	Smooth	Concentric zone
Mr20	Black	Circular	Smooth	Concentric zone

Table 5: Morphological variability of different isolates of *Myrothecium roridum*

Isolates	Conidia size (µm) (Length × Width)	Conidia shape
Mr1	4.94-5.49(5.21)×1.06-1.59(1.32)	Cylindrical with round end
Mr2	2.32-2.64(2.48)×0.54-0.69(0.61)	Rod shaped with round end
Mr3	4.66-5.34(5.00)×1.19-1.40(1.27)	Cylindrical with round end
Mr4	4.78-5.58(5.18)×0.91-1.23(1.07)	Cylindrical with round end
Mr5	5.83-6.66(6.24)×1.23-1.68(1.45)	Cylindrical with round end
Mr6	2.53-3.91(3.22)×0.69-0.86(0.77)	Narrowly ellipsoid with round end
Mr7	5.26-6.08(5.67)×1.30-1.80(1.55)	Cylindrical with round end
Mr8	4.47-6.11(5.29)×1.00-1.28(1.14)	Cylindrical with round end
Mr9	2.09-3.05(2.57)×0.51-0.82(0.66)	Rod shaped with round end
Mr10	2.43-3.70(3.18)×0.53-1.08(0.80)	Narrowly ellipsoid with round end
Mr11	5.40-6.23(5.81)×1.26-2.30(1.78)	Cylindrical with round end
Mr12	2.51-3.29(2.90)×0.74-0.84(0.79)	Rod shaped with round end
Mr13	2.62-3.04(2.83)×0.70-0.82(0.76)	Rod shaped with round end
Mr14	2.47-3.05(2.75)×0.68-0.88(0.78)	Rod shaped with round end
Mr15	2.47-2.82(2.64)×0.52-0.77(0.64)	Rod shaped with round end
Mr16	2.47-3.08(2.77)×0.64-0.75(0.69)	Rod shaped with round end
Mr17	2.45-3.27(2.86)×0.64-0.75(0.69)	Rod shaped with round end
Mr18	5.53-5.95(5.74)×1.02-1.32(1.17)	Cylindrical with round end
Mr19	5.02-6.00(5.51)×1.35-1.65(1.50)	Cylindrical with round end
Mr20	3.02-3.81(3.41)×0.63-1.01(0.85)	Narrowly ellipsoid with round end

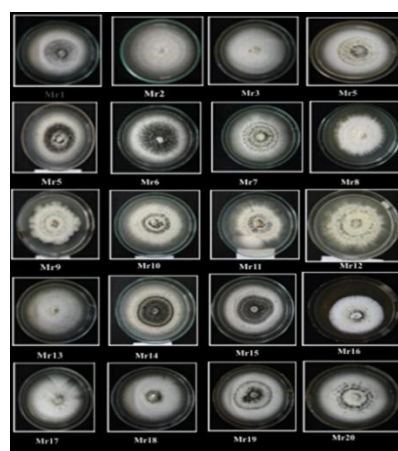


Plate 1: Cultural variability of different isolates of *M. roridum* on PDA medium

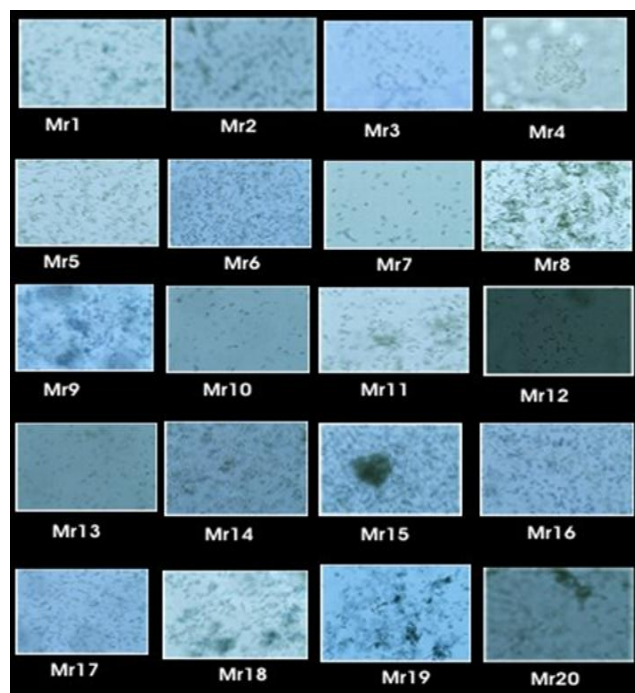


Plate 2: Isolates of *Myrothecium roridum* showing the conidial variation

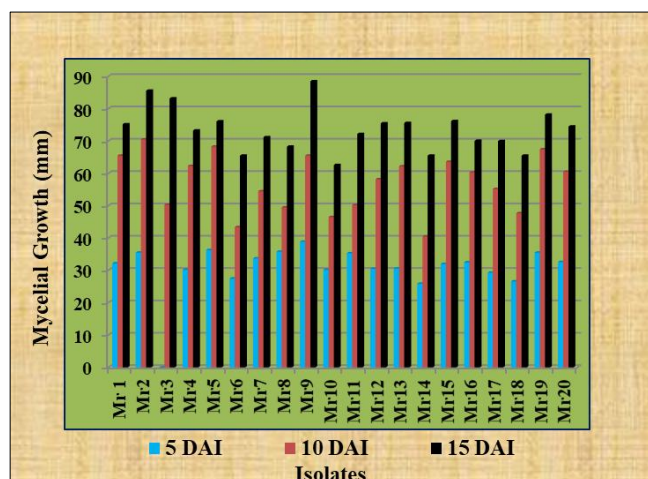


Fig 1: Mycelial growth of *Myrothecium roridum* on PDA medium

Morphological variability

The results presented in Table 5 and Plate 3 indicated that the conidial size, conidial shape, and average number of conidia per microscopic field were differ among isolates. The highest conidia size ($6.24 \times 1.45 \mu\text{m}$, length \times width) was recorded in isolate Mr5 followed by Mr11 ($5.81 \times 1.78 \mu\text{m}$), Mr18 ($5.74 \times 1.17 \mu\text{m}$), Mr7 ($5.67 \times 1.55 \mu\text{m}$). The lowest conidia size ($2.48 \times 0.61 \mu\text{m}$) was recorded in isolate Mr2 followed by Mr15 ($2.64 \times 0.64 \mu\text{m}$), Mr16 ($2.69 \times 0.65 \mu\text{m}$), Mr14 ($2.75 \times 0.78 \mu\text{m}$).

Out of 20 isolates, conidia of nine isolates (Mr1, Mr3, Mr4, Mr5, Mr7, Mr8, Mr11, Mr18 and Mr19) conidia were cylindrical with round end shape, eight isolates (Mr2, Mr9, Mr12, Mr13, Mr14, Mr15, Mr16 and Mr17) were rod shaped with round end and three isolates (Mr6, Mr10 and Mr20) were narrowly ellipsoid with round end shape.

Present finding are in accordance with Cleia *et al.*, (2009) ^[1] they observed the cylindrical with round end shape of conidia of *Myrothecium roridum*. They also reported the size of conidia ranges from $6.4\text{-}7 \times 1.7\text{-}2.3 \mu\text{m}$. Hong *et al.* (2013) ^[2] reported narrowly ellipsoid with rounded ends conidia of

Myrothecium roridum isolates conidia. The average size of 100 conidia was $6.25 \pm 0.04 \times 1.63 \pm 0.02 \mu\text{m}$.

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