The Bioscan 13(3): 795-798, 2018
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

EFFECT OF CULTURE MEDIA AND TEMPERATURE ON MYCELIAL GROWTH AND SPORULATION OF *Myrothecium roridum*, THE LEAF SPOT AND STEM NECROSIS PATHOGEN OF COFFEE SEEDLINGS

A. P. RANJINI* AND RAJA NAIKA[2]
[1]Division of Plant Pathology,
Central Coffee Research Institute, Coffee Research Station - 577 117, Chikkamagaluru , Karnataka, INDIA
[2]Department of Applied Botany, Kuvempu University,
Jnana Sahyadri, Shankaraghatta - 577 451, Shivamogga, Karnataka, INDIA
e-mail: ccpranju@gmail.com

KEYWORDS
Myrothecium roridum
Coffee seedlings
Leaf spot
Stem necrosis
Culture media
Temperature

Received on :
09.07.2018

Accepted on :
21.08.2018

***Corresponding author**

ABSTRACT
The fungus *Myrothecium roridum* is a potential pathogen which causes leaf spot and stem necrosis on coffee seedlings in the nursery. *In vitro* studies were carried out to find out the best culture medium for the better growth and sporulation of the fungus. Nine different media were used for the studies. They are *viz.,* Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Malt Extract Agar (MEA), Yeast Extract Agar (YEA), Czapekdox Agar (CZA), Hansen's Agar (HA), Potato Carrot Agar (PCA), Glucose Peptone Agar (GPA) and a semi artificial medium-Coffee Leaf Extract Agar (CLEA). Growth and sporulation of the pathogen was also studied at different temperature levels *viz.,* 10, 15, 20, 25, 30 and 35ºC. The study indicated that Coffee Leaf Extract Agar is the most suitable medium and recorded maximum radial growth of 88.16 mm followed by Potato Dextrose Agar (74.83 mm) 15 days after incubation (DAI), as the better growth media for culturing the fungus *M. roridum*. Highest sporulation was observed in PCA and YEA. The present study also clearly indicated that 25ºC is the optimum temperature and recorded maximum radial growth of 89.37 mm 15 DAI and sporulation of the fungus was at the maximum level.

INTRODUCTION

Fungi are sensitive to nutrition and environmental factors for their growth and sporulation. Composition of growth media and temperature greatly influence on mycelia growth and development of fungi. The fungus *Myrorthecium roridum* causes leaf spot and stem necrosis disease on coffee seedlings and could be seen in severe form since five years in India. The disease is observed in severe form during continuous rains without a dry spell (Daivasikamani *et al.,* 2016; Anon., 2014).

The symptoms on the infected leaves of coffee seedlings 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.

The infected stem of coffee seedling shows water-soaked brown to grey discoloration on the tender stem indicating necrosis above the soil. 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 die (Ranjini and Rajanaika, 2018). *M. roridum* not only affects coffee but it has wide host range from sea weeds, plantation crops, vegetable crops, gymnosperms and also ornamentals (Kim *et al.*, 2003; Yum and Park, 1990; Cabral *et al.,* 2009; Tewari *et al.*,1977 and Kyung *et al.*, 2014). The present study was undertaken to find out the best suitable growth medium and optimum temperature required for better growth and sporulation of the fungal pathogen *M. roridum*.

MATERIALS AND METHODS

Study area and sample collection

Samples of leaves and stem of coffee seedlings infected by the fungus *M. roridum* were collected from the nursery at Central Coffee Research Institute (CCRI), Balehonnur situated at an elevation of 823-914 m MSL and longitude 75º28'E and latitude 13º22'N in Chikkamagaluru district of Karnataka State, India during the monsoon season of 2016.

Isolation of pathogen

Infected portion of the leaf or stem as the case may be of coffee seedlings were selected and cut into 1 cm^2 pieces. Then soaked in 1% sodium hypochlorite solution for surface sterilization followed by 2 to 3 thorough washing with double distilled water. Dried with filter paper and were aseptically transferred to 90 mm Petri plates containing 20 ml of solidified

PDA. These inoculated Petri plates were incubated at 25ºC for 2-3 days and the fungus was isolated. The isolated fungus was purified by transferring actively growing mycelium from the colony margin (Dingra and Sinclai, 1995).

Effect of culture media on growth of *M. roridum*

Nine different culture media were used to assess the growth of mycelia and conidial production of the fungus *M. roridum*. The different medium used for the experiment are; Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Malt Extract Agar (MEA), Yeast Extract Agar (YEA), Czapekdox Agar (CZA), Hansen's Agar (HA), Potato Carrot Agar (PCA), Glucose Peptone Agar (GPA) and also a semi artificial medium-Coffee Leaf Extract Agar (CLEA). Individual culture medium was prepared (250 ml), autoclaved at 120ºC and at 15 psi for 20 min. The composition and preparation of the media was done following the procedures as described by Ronald (2010) and Aneja (2012). Twenty ml of each culture medium was poured into Petri plates and kept for solidification. Then 0.8 mm fungal disc was cut using sterilized cork borer from seven days old culture and transferred to the center of the Petri plate. The inoculated Petri plates were incubated at 25ºC for 15 days. Three replicates were maintained for each treatment. Radial growth of *M. roridum* was measured in millimeter by using ruler on 7 and 15 days after inoculation (DAI) or until the fungal mycelia covered the full Petri plate as described by Pradnyarani and Kulkarni (2015).

Effect of culture media on sporulation of *M. roridum*

To determine the influence of media on sporulation, the fungus was grown in different media as described above (culture media) for a period of 15 days. From each culture medium, spores of the fungus was harvested by taking 5 mm disc from centre, middle and periphery to represent the whole culture plate and placed in a test tube containing 10 ml of sterilized water separately and the culture was mixed thoroughly before microscopic spore count. A drop of spore suspension was placed on the glass slide and a total of ten microscopic fields were counted under 400x magnification and average conidia per microscopic field (MF) was calculated and were graded as Excellent (51–80 spores/MF), Good (21– 50 spores/MF), Medium (11–20 spores/MF) and Poor (5–10 spores/MF) using the methodology of Pradnyarani and Kulkarni (2015).

Effect of temperature on mycelia growth of *M. roridum*

Twenty ml of PDA medium was prepared and then it was poured into the Petri plates and kept for solidification. Then 0.8 mm fungal disc was taken from seven days old culture and transferred to the center of the Petri plates and sealed with parafilm. With four replicates, the inoculated Petri plates were incubated at six different temperature levels of 10, 15, 20, 25, 30 and 35ºC in an incubator. Radial growth of *M. roridum* was measured in millimeter by using ruler on 7 and 15 days after inoculation or until the mycelia growth covered the full plate in any one of the treatment (Ramteke and Kamble, 2011).

Effect of temperature on sporulation of *M. roridum*

To determine the effect of temperature on the sporulation, the pathogen was cultured at different temperatures as described above for a period of 15 days. The conidial count was recorded and average counts from four replicates were graded as described earlier.

RESULTS AND DISCUSSION

Effect of culture media on growth and sporulation of *M. roridum*

The observations recorded on the radial growth of the fungus on 7 and 15 DAI are presented in table 1 and fig. 1.

The mycelial growth of the fungus varied significantly in different culture media. The radial growth of the fungus was maximum on CLEA (88.16 mm) and was 74.83 mm on PDA medium after 15 DAI. On both these media the colony morphology of the fungus was similar with white myelial growth and the conidia were arranged concentrically. There was good sporulation of the fungus in both the media. In PSA, MEA, HA and GPA media, not much difference was observed in radial growth of the fungus. However, there is difference in the colony morphology and sporulation characters of the fungus in those media studied. On HA medium, radial growth was thin and sporulation could not be observed even 15 DAI whereas in MEA the mycelial growth was compact, thick cottony mass but without sporulation. In GPA and PSA media moderate mycelial growth was recorded with minimum sporulation. However, on YEA and PCA media there was abundant sporulation but the mycelia growth was thick cottony mass

Table 1: Effect of different culture media on mycelial growth and sporulation of *M. roridum*

Sl.No.	Microbial culture media	Mycelia growth (mm)			Sporulation
		7 DAI	15 DAI	Mean	
1	Potato Dextrose Agar (PDA)	36.33(37.07)*	74.83(59.89)	55.58	+ + +
2	Potato Sucrose Agar (PSA)	27.16(31.39)	68.5(55.94)	47.83	+ +
3	Malt Extract Agar (MEA)	34.16(35.76	68.16(55.66)	51.16	+
4	Yeast Extract Agar (YEA)	26.66(31.09)	53.33(46.91)	39.99	+ + + +
5	Czapedox Agar (CZA)	0.00(0.00)	0.00(0.00)	0.00	-
6	Hansen's Agar (HA)	32.66(34.86)	64.33(53.33)	48.49	+
7	Potato Carrot Agar (PCA)	25.66(30.44)	50.5(45.29)	38.08	+ + + +
8	Glucose Peptone Agar (GPA)	34.5(35.97)	69.00(56.17)	51.75	+ +
9	Coffee Leaf Extract Agar (CLEA)	43(40.97)	88.16(69.91)	65.58	+ + +
	S. Em. ±	0.53	0.90	-	-
	CD @ 1%	2.17	3.66	-	-

*Figures in parentheses are arcsine values; + + + + : Excellent (51–80 spores/MF), + + + : Good (21–50 spores/MF), + + : Medium (11–20 spores/MF), + : Poor (5-10 spores/MF), - : No sporulation

Table 2: Effect of temperature on mycelial growth and sporulation of *M. roridum*

Temperature (°C)	Mycelial growth (mm)			Sporulation
	7 DAI	15 DAI	Mean	
10	8.42 (16.80)*	8.62(17.07)	8.52	-
15	10.00(18.40)	20.80(27.18)	15.40	+
20	17.75(24.8)	38.00(38.05)	27.80	+ +
25	43.62(41.3)	89.37(70.98)	66.40	+ + + +
30	31.00(33.8)	60.62(51.13)	45.80	+ + +
35	8.12(16.5)	8.75(17.20)	8.43	-
S. Em. ±	0.52	0.40	-	-
CD @ 1%	2.10	1.64	-	-

*Figures in parentheses are arcsine values; + + + + : Excellent (51–80 spores/MF), + + + : Good: (21–50 spores/MF), + + : Medium (11–20 spores/MF), + : Poor (5-10 spores/MF), - : No sporulation

Figure 1: Effect of culture media on growth and sporulation of *M. roridum*

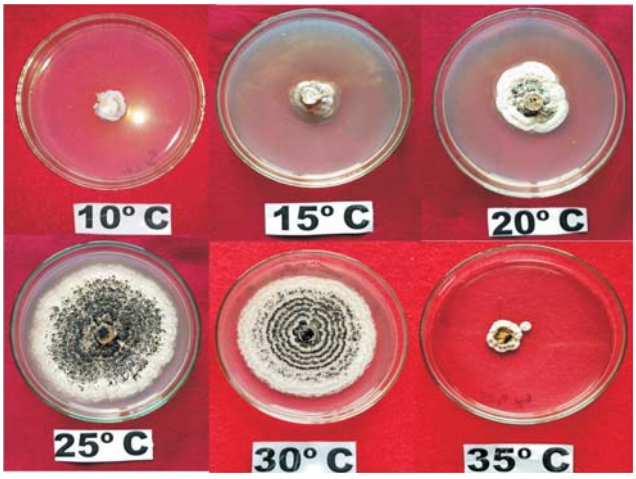

Figure 2: Effect of temperature on growth and sporulation of *M. roridum*

with slow growth. In CZA medium there was no initiation of mycelia growth and hence no sporulation of the fungus.

Talukdar and Dantre (2013) reported that the best media for the growth *M. roridum* was PSA and maxiumun condia production was reported in MEA. Okonowo *et al.* (2010) also found in their studies that PSA medium was the best medium for the growth and MEA medium for sporulation of the fungus *M. roridum*. However, the present study indicated that most suitable medium for culturing the pathogen *M. roridum* causing leaf spot and stem necrosis on coffee seedlings was Coffee Leaf Extract Agar (CLEA) followed by Potato Dextrose Agar (PDA). Both these media were good at stimulating the mycelial growth and sporulation of the fungus.

Effect of temperature on mycelial growth and sporulation of *M. roridum*

Observations recorded at different temperature levels on the growth and sporulation of *M. roridum* on 7 and 15 DAI are presented in the table 2 and fig. 2. The data was statistically analysed and were found significant (CD @ 1%).

The temperature for maximum growth of *M. roridum* ranged from 25ºC to 30ºC where colonies of the fungus covered maximum surface area of the Petri plates after 15 days of incubation. A significant relationship between the temperature ranges tested and growth of the fungus was detected, demonstrating that temperature fluctuations induce changes in the mycelial growth of *M. roridum*. No sporulation of the fungus was observed in colonies growing at 10ºC and 35ºC but mycelial growth was initiated and arrested after three days of inoculation, whereas in the intermediate temperature ranges, differences could be observed. Colonies incubated at 25ºC and 30ºC were found having active growth and sporulation of the fungus whereas in those growing at 15ºC and 20ºC, sporulation and mycelial growth was scanty. The colony colour of the fungus remained same without any colour chage in all the temperature range tested. However, the mycelial growth and sporulation of the fungus differed with each temperature range.

In 1970 Chauhan and Suryanaryana reported that 25ºC was the best temperature for the growth and sporulation of *M. roridum*. The present study also confirmed their report and revealed that 25ºC is the optimum temperature level for best growth and sporulation of the fungus *M. roridum*. Worapong *et al.* (2009) found that conidial germination was highest at 28ºC and lowest at 12ºC. Whereas, Talukdar and Dantre (2013) stated that sporuation of *M. roridum* was high at 30°C.

ACKNOWLEDGEMENT

The authors wish to thank the Director of Research, Coffee Board for providing the required facilities to carry out the experiments and to the Divisional Head and Scientists of Plant

Pathology Division, Central Coffee Research Institute (CCRI), Chikkamagaluru District, Karnataka State, India for their co-operation during the study.

REFERENCES

Aneja, K. R. 2012. Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International (P) limited, New Delhi. PP. 607.

Anonymous. 2014. Coffee Guide – A Manual of Coffee Cultivation. Published by Coffee Board of India. PP. 262.

Cabral, C. S., Henz, G. P., Moreira, A. J. A. and Reis, A. 2009. New cucurbitaceous hosts of *Myrothecium roridum* in Amazonas State, Brazil. *Tropical Plant Pathology*, **34(16)**: 402-405.

Chauhan, M. S. and Suryanarayana, D. 1970. Effect of temperature, pH and light on the growth and sporulation of *Myrothecium roridum,* the causal organism of leaf spot disease of cotton in Hariyana State. *Indian Phytopath*., **23**: 660-663.

Daivasikamani, S., Ranjini, A. P. and Raghuramulu, Y. 2016. Diseases of Coffee and their management. In: Plant Pathogens and their management. Ed: P. C. Trivedi, Avaishkar Publishers, Distributors, Jaipur, Rajasthan, India. PP. 19-50.

Dingra, O. D. and Sinclai, B. J. 1995. Basic Plant Pathology Methods. Lewis Publishers, London. PP. 215.

Kim, D. K., Bae, D. W., Lee, S. C., Han, K. S. and Kim, H. K. 2003. Detection of Myrothecium leaf spot, a new disease of watermelon. *Plant Pathol. J.*, **19**: 200–202.

Kyung, S. H., Seung, K. C., Hyeong, H. K., Sung, C. l., Jong, H. P., Myoung, R. C. and Mi-Jeong, P. 2014. First Report of *Myrothecium roridum* causing leaf and stem rot disease on *Pepromia quandrangularis* in Korea, *Mycobiology*, **42(2):** 203-205.

Okonowo, W. O., Gbenle, G. O., Osuntoki, A. A. and Adekunle, A. A. 2010. Media studies on *Myrothecium roridum* Tode: A potential bio control agent for water hyacinth. *J. Yeast and Fungal Research,* **1(4):** 55-61.

Pradnyarani, N. and Kulkarni, M. S. 2015. Effect of different pH, temperature and solid media on growth of purple blotch of onion caused by *Alternaria porri ellis*. *The Bioscan,* **10(4):** 1839-1843.

Ranjini, A. P. and Rajanaika. 2018. Leaf spot and stem necrosis disease of coffee seedlings caused by *Myrothecium roridum* Tode ex Fr. in India. *J. Mycopathol., Res.,* **56(1):** 5-10.

Ramteke, P. K and Kamble, S. S. 2011. Physiological studies in *Fusarium solani* causing rhizome rot of ginger (*Zinger officinale* Rosc.).*The Bioscan*. **6(2):** 195- 197.

Ronald, M. A. 2010. Handbook of Microbiological Media. CRC Press. Taylor and Francis Group. PP. 1957.

Talukdar, D. and Dantre, R. K. 2013. Physiological studies on *Myrothecium roridum* causing leaf spot of soybean. *Indian Phytopathology*, **66 (2):** 224-225.

Tewari, J. P. and Skoropad, W. P. 1977. *Myrothecium roridum*, a potential pathogen of rapeseed and mustard in Alberta. *Canadian Plant Disease Survey,* **57:** 37-41.

Worapong , J., Sun, J. and Newcombe, G. 2009. First report on *Myrothecium roridum* from a Gymnosperm. *North American Fungi*, **4 (6):** 1-6.

Yum, K. J. and Park, E. W. 1990. Soybean leafspot disease caused by *Myrothecium roridum* Tode ex Fries. *Korean J. Plant Pathol*., **6:** 313–317.