Physiological Studies of *Myrothecium Roridum*: An Inciting Agent of Myrothecium Leaf Blight in Cotton

T. Umamaheswari, S.K. Beura, A. Sahoo\(^1\), S. Pattanayak\(^1\)  
10.18805/IJARe.A-5575

**ABSTRACT**

**Background:** Cotton is one of the most important cash crops in India. It is also called as “White gold” because of its agricultural, industrial importance. Myrothecium leaf blight is an emerging disease in cotton which is growing consistently throughout the country and has been reported to cause significant losses in major cotton growing tracts of Odisha. Considering the importance of the disease, the present investigation has been taken up to study the physiology of the fungus.

**Methods:** An experiment was conducted in vitro during 2016-18 to investigate the effect of temperature and pH on the growth of *M. roridum*. Seven different nutrient media viz. Potato Dextrose Agar (PDA) medium, Potato Sucrose Agar (PSA) medium, Host Leaf Extract Agar (HEA) medium, Potato Carrot Agar (PCA) medium, Czapek'sdox Agar (ZA) medium, Richard's Agar (RA) medium and Oat meal agar medium (OMA) were tested under *in-vitro* conditions to ascertain a suitable medium for the growth of *M. roridum*.

**Result:** The investigation in laboratory conditions has revealed that out of seven nutrient media tested, maximum radial growth was recorded in Potato Sucrose Agar (PSA) with an average radial growth of diameter 76.06 mm and the least growth was recorded on Richard's Agar with a diameter of 61.09 mm and the minimum average growth of 17.36 mm was recorded at 36°C. Studies on pH revealed that that the fungus grows well in neutral and slight alkaline medium (6.5 - 8.0).

**Key words:** Blight, Cotton, *Myrothecium*.

**INTRODUCTION**

Cotton is one of the most important cash crops in India, which is primarily valued for its extra ordinary strength, fine and durable fibre. The Indian textile industry consumes a diverse range of fibres and yarn. The production potential of the crop has not been fully exploited due to biotic and abiotic factors. The crop suffers from various diseases *i.e.* bacterial blight, grey mildew, alternaria leaf spot, myrothecium leaf spot, collar rot and wilt etc., of which foliar diseases take a heavy toll (Hosagaudar *et al.*, 2008). Among all the foliar diseases the incidence of Myrothecium leaf blight is growing consistently throughout the country and has been reported to cause significant losses in major cotton growing tracts of Odisha. The disease has been reported to cause loss in seed cotton up to 15-20 per cent (Taneja *et al.*, 1989, Tomar *et al.*, 2010).

The initial symptom of Myrothecium leaf blight incited by *Myrothecium roridum* appear as small round or oval, brown spots with dark brown margin surrounded by zones of translucent areas forming concentric rings on leaves of the infected cotton plants. After a few days, dark green sporodochia surrounded by a rim of white hair like mycelia are formed particularly in the region where rings are formed. The mycelium of the fungus was hyaline branched and non-septate. The conidia are formed in the cluster from the phialids and are single celled, ovoid to elliptical with tapering ends. The mycelium of the fungus was branched and non-septate. Conidia hyaline or olive green to slightly dark, one-celled, ovoid to elongate or rod shaped with rounded ends measuring 2.0 to 2.5 × 5.5 to 6.5 µm. (Talukdar and Jagdish Prasad, 2013). Saccardo (1886) described the morphology of fungus *Myrotheciumroridum* Tode ex.Fries infecting soyabeen as” sporodochia discoid or flat, black, white fringed, conidia cylindrical with blunt ends, 8-10 x 2 µm, biguttulate, pale olivaceous".

Tomar, (2005) have studied the morphology of several isolates and observed that, the isolates show minor variation in the measurements of conidia. The nutrient composition and the type of carbon source used play a major role in the growth of the pathogen. Hence, studies were conducted to ascertain a suitable medium for the growth of *M. roridum* which plays an important role in physiological study of the fungus. The experiment was carried out in the laboratory, Department of Plant Pathology, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar and AICRP on cotton, Bhawanipatna during 2016-17 and 2017-18.
**MATERIALS AND METHODS**

**Isolation of the Pathogen**

The experiment was carried out in the laboratory, Department of Plant Pathology, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar and AICRP on cotton, Bhawanipatna during 2016-17 and 2017-18. Myrothecium infected cotton leaf samples were collected from AICRP on Cotton, Bhawanipatna and Central Research Farm, Orissa University of Agriculture and Technology, Bhubaneswar. The diseased samples exhibiting typical symptoms of Myrothecium leaf spot were collected and examined under the microscope which revealed the presence of characteristic mycelia and conidia of the fungus. The diseased samples were isolated and purified by single spore and hyphal tip methods. The culture thus obtained was sub-cultured on PDA (Potato dextrose Agar) slants and allowed to grow at 28±1°C for ten days.

**Cultural characteristics of causal pathogen in different media**

Seven different nutrient media were tested under *in-vitro* conditions to ascertain a suitable medium for the growth of *M. roridum*. The media used were Potato Dextrose Agar (PDA) medium, Potato Sucrose Agar (PSA) medium, Host Leaf Extract Agar (HEA) medium, Potato Carrot Agar (PCA) medium, Czapek’s dox Agar (ZA) medium, Richard’s Agar (RA) medium and Oat meal Agar medium (OMA). Each culture medium is prepared by suspending the requisite amount of components in 1000 ml of distilled water and subsequently boiled to dissolve the medium completely. Then the pH of the medium was adjusted to 7.0 by using 0.1 N sodium hydroxide or 0.1N hydrochloric acid before sterilization. The media were sterilized in an autoclave at 15 psi for 15 min at 121.6°C.

The pathogen was cut into 5 mm discs from the periphery of actively growing colony with sterilized cork borer and transferred to the centre of each plate containing the culture medium. All the inoculated Petriplates were incubated at 28±2°C in BOD incubator.

**Effect of temperature on growth of the fungus**

The experiment was conducted to find out the most suitable temperature for the growth of Myrothecium. The sterilized poured Petri plate with potato dextrose media were inoculated with 5 mm disc of the test pathogen of seven days old culture. The Petriplates were incubated at 24°C, 26°C, 28°C, 30°C, 32°C, 34°C and 36°C. Three replications were maintained for each treatment and observation for radial growth was recorded at 7th day and 15th day after inoculation.

**Measurement of the mycelia growth of the fungus**

The diameter of the fungal colonies was measured fifteen days after inoculation by taking mean values of three replications. Each replication involves measurement of diameter twice *i.e.* vertically and horizontally and its average was recorded.

**Effect of pH on growth of the fungus**

This experiment was conducted with an objective to determine the optimum pH for the growth and sporulation of the test fungus. Seven levels of pH viz., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 replicated thrice were employed using potato dextrose broth medium. The initial pH was adjusted with 1N HCl or 1N NaOH solutions. The pH requirements were measured with photo volt model 110 electric pH meter before autoclaving the medium. The flasks were sterilized and inoculated with five millimeter disc of the culture of Myrothecium. The inoculated flasks were incubated on bench top for fifteen (15) days at room temperature (approximately 27 ± 3°C). The culture media were decanted and each flask medium was separately filtered using known weighed Whatman filter paper No.1 to recover the mycelia after washing several times with distilled water. The mycelia were oven dried at 80°C until constant dry weight was obtained. The dried mycelia were weighed using electrical mettler balance. The average of the triplicates was recorded. The experiment was done under *in-vitro* condition and the data was analyzed by using Complete randomized design (CRD).

**RESULTS AND DISCUSSION**

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

The effect of media on the growth of *Myrothecium roridum* was tested by using seven different culture media under *in-vitro* conditions to ascertain a suitable medium for the growth of the pathogen. Observations on radial growth of the mycelium were recorded after fifteen days of inoculation and the result obtained was presented in Table 1. The data reveals that all the media supported the growth of the fungus to various degrees (Plate 1). The maximum growth rate was recorded on potato sucrose agar (PSA) media with an average radial growth of diameter 76.06mm after an incubated period of fifteen days followed by host extract agar (HEA) 69.77mm. The minimum growth was recorded on Richards agar with an average radial growth of diameter 23.66 mm. The result showed that, the radial growth of *M. roridum* in different media was significantly different from each other.

**Table 1: Effect of different nutrient media on radial growth of *Myrothecium roridum***

| T. No | Media            | Average radial growth (mm) |
|-------|------------------|-----------------------------|
| T1    | Host Extract Agar| 69.76*                      |
| T2    | Potato Dextrose Agar | 64.40            |
| T3    | Czapek's Agar    | 62.63                      |
| T4    | Richard's Agar   | 23.66                      |
| T5    | Potato Carrot Agar| 53.86                      |
| T6    | Oat meal Agar    | 51.96                      |
| T7    | Potato Sucrose Agar| 76.06                      |
|      | SE(m)±            | 2.287                      |
|      | CD (0.05)        | 7.005                      |

*Mean of three replication.
Physiological Studies of Myrothecium Roridum: An Inciting Agent of Myrothecium Leaf Blight in Cotton

The average maximum radial growth of 61.09 mm was recorded at 28°C followed by 55.61 mm at 26°C. The minimum average growth of 17.36 mm was recorded at 36°C.

Effect of pH on the growth of the fungus

The effect of pH of medium on the growth of Myrothecium roridum was investigated under laboratory condition to ascertain a suitable pH for the growth of fungus. The fungus M. roridum was grown on potato dextrose broth for 15 days (Plate 2). Growth media with a pH ranging from 5.0 to 8.0 was prepared as described under “Material and methods.

It can be inferred from the Table 3 that the mycelial growth of M. roridum varied with the changing level of pH. Results of this study revealed that the fungus Myrothecium roridum grew more profusely in neutral medium and also in weak alkaline medium of 6.5-8.0. The highest mean dry mycelial weight of 494.63 mg was obtained at pH 6.5. This was followed by 472.19 mg at pH 7.0 and 432.27 mg at pH 7.5. The lowest mean dry weight of 264.71 mg was obtained at a pH 5.0.

In this study the mycelial growth of Myrothecium roridum was recorded to be maximum on Potato sucrose agar (PSA) media, followed by Host extract agar (HEA), Potato dextrose agar (PDA), Czapek’s agar (ZA), Potato carrot agar (PCA), Oat meal agar (OMA) and Richards agar (RA). The growth of the fungus was more on natural media when compared to artificial media. Arya (1956) has reported that the best surface growth with copious sporulation was obtained on Potato dextrose agar followed by Oat meal agar, Czapek’s agar and Richard’s agar. Growth of Myrothecium roridum on different media was studied extensively by Munjal (1960). He observed that Oat Meal, Potato dextrose agar, Richard’s medium, Brown’s medium and Czapek’s medium were suitable for the growth of fungus. The growth of the fungus was more on natural media when compared to artificial media. Very little sporulation occurred on Czapek’s medium. This study corroborated with the findings of Hettiarachchi et al., (1983); Murakami et al., (1998); Kim et al., (2003); Lee et al., 2008 and Duval, 2010.Taneja et al., (1993) observed that growth and sporulation of M. roridum isolates varied significantly at different temperature. The average maximum radial growth of 61.09 mm was recorded at 28°C followed by 55.61 mm at 26°C. The minimum average growth of 17.36 mm was recorded at 36°C.

Effect of temperature on the growth of fungus

An experiment was conducted in-vitro to investigate the effect of temperature on the growth of M. roridum. The radial growth of M. roridum was recorded at 7 and 14 days after incubation and the data obtained is presented in Table 2.

It is evident from the table that the growth of M. roridum varied significantly at different temperature. The average maximum radial growth of 61.09 mm was recorded at 28°C followed by 55.61 mm at 26°C. The minimum average growth of 17.36 mm was recorded at 36°C.

| Treatments | pH range | Average growth (mm) |
|------------|----------|---------------------|
| T1         | 5.0      | 264.71*             |
| T2         | 5.5      | 280.57              |
| T3         | 6.0      | 355.52              |
| T4         | 6.5      | 494.63              |
| T5         | 7.0      | 472.19              |
| T6         | 7.5      | 432.27              |
| T7         | 8.0      | 384.36              |

SE(m)± 17.472  CD (0.05) 53.111

T1, Host Extract Agar; T2, Potato Dextrose Agar; T3, Czapek’s Agar; T4, Host’s agar; T5, Potato Carrot Agar; T6, Oat meal agar; T7, Potato sucrose Agar

Plate 1: Radial growth of Myrothecium roridum in different media.

Plate 2: Effect of pH on the growth of M. roridum

Table 2: Effect of different temperature on radial growth of M. roridum.

| Treatments | Temperature | Radial growth (mm) | Average growth (mm) |
|------------|-------------|--------------------|---------------------|
| T1         | 24°C        | 30.76*             | 50.68               |
| T2         | 26°C        | 34.26              | 55.61               |
| T3         | 28°C        | 40.86              | 61.09               |
| T4         | 30°C        | 23.70              | 32.70               |
| T5         | 32°C        | 19.43              | 24.36               |
| T6         | 34°C        | 16.70              | 20.86               |
| T7         | 36°C        | 13.53              | 17.36               |

SE(m)± 1.205 1.571 CD (0.05) 3.689 4.812

*Mean of three replication.

Table 3: Effect of pH on radial growth of M. roridum.

| Treatments | pH range | Average growth (mm) |
|------------|----------|---------------------|
| T1         | 5.0      | 264.71*             |
| T2         | 5.5      | 280.57              |
| T3         | 6.0      | 355.52              |
| T4         | 6.5      | 494.63              |
| T5         | 7.0      | 472.19              |
| T6         | 7.5      | 432.27              |
| T7         | 8.0      | 384.36              |

SE(m)± 17.472  CD (0.05) 53.111

*Mean of three replication.
from cotton were best on Richard’s solution and potato broth medium. Chlorine inhibited the growth of microorganisms and therefore, it was used in the formulation of some disinfectants and germicides (Davis et al., 1994; Gottardi and Nagl, 2005). The mycelia growth of M. roridum was least favour ed on Czapek-Dox agar medium. This medium has been reported as a poor medium for the growth of fungi (Nwodo, 2007). This may be due to an inhibition in the growth of the fungus as a result of the chlorine present in the form of potassium chloride in the Czapek-Dox agar medium.

This study was also in support with the findings of Okunowo et al. (2010) who used seven culture media i.e. potato dextrose agar (PDA), malt extract agar (MEA), potato sucrose agar (PSA), sabouraud agar (SA), potato carrot agar (PCA) and Czapek-Dox agar (ZA) and water hyacinth agar (WHA) as growth medium. They observed that maximum growth of the fungus was recorded in potato sucrose agar (PSA) media, while minimum growth was in Czapek-Dox agar (ZA) media.

The Temperature is one of the important factors that govern the distribution, growth reproduction and survival of the fungus. An effort was made to know the optimum temperature for the growth of the fungus. It was observed that Maximum fungal growth was observed at 28°C and the least growth was recorded at 36°C.

The observations were in agreement with Taneja et al. (1993) who observed that growth and sporulation of M. roridum isolates from cotton was found to be optimum at temperature 28°C. Singh, (2001) reported that on Mung bean leaf maximum number of spores was recorded at 28°C. Tomar et al. (2006) studied the effect of temperature on the growth and sporulation of ten different isolates of M. roridum and observed that 24 to 32°C was recorded as the optimum temperature for growth of all isolates.

The effect of pH on the growth of M. roridum was investigated under laboratory conditions to find a suitable pH for the growth of fungus. The mycelial growth of M. roridum differed with the change of pH levels. The highest mean of dry mycelial weight was recorded at pH 6.0 and the lowest was obtained at pH 5.0 broth medium.

Munjal (1960) reported that pH range of 4.5-6.0 is suitable for the growth of the fungus. Chauhan and Suryanarayana (1970) made extensive studies on the factors affecting the growth and sporulation of M. roridum in the laboratory and observed that the best growth and sporulation was observed at pH 6.0. Taneja et al., (1993) observed that pH range of 6.0 is suitable for optimum growth and sporulation of M. roridum. Similarly Okunowo et al. (2010) reported that pH range of 5.5 to 8.6 was suitable for growth and sporulation of the fungus.

CONCLUSION
In the present investigation the mycelia growth was most favoured on Potato Sucrose Agar (PSA) media with an average radial growth of diameter 76.06mm and the least growth was observed on Richard’s agar i.e. 23.66mm. The mycelium growth was observed to be maximum at 28°C and the minimum average growth was recorded at 36°C. However, it was found that longer incubation period is required for sporulation and sporodochia formation. It was observed that the fungus grows well in neutral and weak alkaline medium (6.5 - 8.0). The highest mean of dry mycelial weight 494.63 mg was obtained at pH 6.5 broth medium and the least was recorded at pH 5.0 with a dried mycelial weight of 264.71mg.

Based on these findings it may be concluded that the fungi grows well on natural media than artificial media and the fungus grows well in neutral and slight alkaline medium (6.5-8.0). These observations can further be used in mycological research for development of management measures.

REFERENCES
Arya, H.C. (1956). On a new leaf spot disease of guar (Cyamopsis tetragonoloba) caused by Myrothecium roridum, Indian phytopathology. 9: 174-181.
Chauhan, M.S. and Suryanarayana, D. (1970). Effect of temperature, pH and light on growth and sporulation of Myrothecium roridum the causal organism of cotton in Haryana state, Indian Phytopathology. 22 : 6.
Davis, C.P., Shiraltif, M.E., Trieff, N.M., Hoskins, S.L. and Warren, M.M. (1994). Quantification, qualification and microbial killing efficiencies of antimicrobial chlorine-based substances produced by iontophoresis, Antimicrobial agents and Chemotherapy. 38(12): 2768-2774.
Duvel, A.M.Q., Henz, G.P., Lima, P.M.L., Medeiros, A.R., Miranda, B.E.C., Pfenning, L.H. and Reis, A. (2010). Brazilian Journal of Microbiology. 41(1): 246.
Gottardi, W. and Nagl, M. (2005). Chlorine covers on living bacteria: the initial step in antimicrobial action of active chlorine compounds. Journal of Antimicrobial agents and Chemotherapy. 55(4): 475-482.
Hettiarachchi, S., Gunasekera, S.A. and Balasooriya, I.(1983).Leaf spot disease of Water Hyacinth in Sri Lanka, Journal of Aquatic Plant Management. 21: 62-65.
Hosagoudar, G.N., Chattannavar, S.N. and Kulkarni, S. (2008). Survey for foliar diseases of Bt Cotton Karnataka, Journal of Agriculture Sciences. 21: 139-140.
Kim, D.K, Bae, D.W., Lee, S.C., Han, K.S., Kim, H.K. (2003). Detection of Myrothecium Leaf Spot, A New Disease of Watermelon, Journal ofPlant Pathology. 19(4): 200-202.
Lee, H.B., Kim, J., Hong, K. and Kim, C. (2008). Evaluation of a Fungal Strain, Myrothecium roridum F0252, as a Bioherbicide Agent. Journal of Plant Pathology. 24(4): 453-460.
Munjal, R.L., (1960). A commonly occurring leaf spot disease caused by Myrothecium roridumTode ex Fr. Indian Phytopathology. 13: 150-15.
Physiological Studies of *Myrothecium Roridum*: An Inciting Agent of Myrothecium Leaf Blight in Cotton

Wastes in media formulation, African Journal of Biotechnology. 6(3): 243-246.

Okunowo, W.O., Gbenle, G.O., Osuntoki, A.A., Adekunle, A.A. and Ojokuku, S.A. (2010). Production of cellulolytic enzymes by a phytopathogenic *Myrothecium roridum* and some avirulent fungal isolates from water hyacinth, African Journal of Biotechnology. 9(7): 1074-1078.

Saccardo, P.A. (1886) *Sylloge Fungorum*. 4: 750.

Singh, S.N. (2001). Effect of temperature and relative humidity on sporulation of three different isolates of *Myrothecium roridum* on mungbean. Annals of Plant Protection Sciences. 9(1): 148-150.

Taneja, N.K. and Raj, S. (1993). Growth and sporulation of *Myrothecium roridum* as affected by different media, temperature, pH and light under in vitro conditions, Journal of Cotton Research and Development. 7(1): 158-163.

Talukdar, D. and Prasad, J. (2013). Isolation and Pathogenicity of *Myrothecium roridum* causing Myrothecium leaf spot of Soyabean, Bioinfolet. 10(3A): 915 -918.

Taneja, N.K. and Sheo, Raj, (1989). Studies on the Myrothecium leaf spot disease of cotton, *Ph.D. Thesis* Dr. Y.S. Parmar University, Journal of Horticulture and Forestry, Solan, (H.P.) Pp. 108.

Tomar, D.D. and Shashtry, P.P. (2006). Efficacy of fungicides against recovery of *Myrothecium roridum* in cotton seed, International Journal of Agriculture Sciences. 2(2): 408-410.

Tomar, D.S., Shastry, P.P. and Chauhan, A.K.S., (2010). Yield loss relationship with incidence and intensity of Myrothecium leaf blight of cotton, Indian Journal of Agriculture Sciences. 80: 845-847.

Tomar, D.S. (2005). Studies on the Myrothecium blight of cotton (Gossypium Spp.) caused by *Myrothecium roridum* Tode, *PhD. Thesis* Dr. B.R. Ambedkar University, Agra (U.P.)