J. Cotton Res. Dev. **30** (1) 90-96 (January, 2016)

Morphological and histopathological studies of *Myrothecium roridum* on cotton

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ABSTRACT: Myrothecium leaf blight (Myrothecium *roridum* Tode Ex Fries) is a major production constraint of cotton an important fiber crop grown in East Nimar of Madhya Pradesh. Infection related aspects and histopathology of Myrothecium roridum on cotton plant were investigated. The fungus was identified as *Myrothecium roridum* with characteristic morphology. The symptoms have been documented. Myrothecium blight affected cotton plants showed systemic and non systemic symptoms. Sporodochia has been observed to be restricted up to the epidermis only. An aggregate of fungal mycelium forming stromatic masses in the intercellular spaces was seen. A hymenium layer is formed on the surface. Typical fructifications (sporodochia) are formed that are discoid / irregular shaped without setae on which the conidia are formed.

Key words : Isolation, Identification, Pathogenecity, Histopathology, *M. roridum*

Myrothecium leaf blight of cotton (*Gossypium* spp) caused by *Myrothecium roridum* Tode Ex Fries is an important foliar disease and causes severe loss in the seed cotton yield in East Nimar of Madhya Pradesh (Anonymous, 2004). The disease till the last decade was of minor importance but is now becoming a major constraint in the economic cultivation of cotton in Central India (Patil and Ghoderao, 2002). The mode and extent of spread and the site of colonization of the parasite into host tissues gives a basic understanding of the host parasite relationship. As studies on isolation and identification of pathogen, prove the Pathogenecity, symptomatology, mode of infection by Myrothecium roridum and histopathology of infected leaves of cotton plants are lacking this investigation was made.

MATERIALS AND METHODS

Morphological studies: The leaf samples from each location showing typical spots were collected in polythene samples bags. The samples were collected from Khandwa, Singot, Chhindwara, Badnawar, Dhar, Ratlam, Khargone, Badwani and Burhanpur. Attempts were made to isolate the associated organism for subsequent pathogenecity tests. Isolations were attempted on potato-dextrose agar medium from the young as well as mature lesions. The tissue with the spot was surface sterilized with 0.1 per cent mercuric chloride solution, washed in sterile water twice by serial transfers. The surface sterilized/MLB infected tissue bits were plated on potato dextrose agar medium. A pinch of streptomycin sulfate was added to avoid bacterial contamination. Simultaneously sporodochia from the host tissue were directly transferred to potato-dextrose agar slants. The isolated fungus in each case was further purified by single spore technique and maintained on PDA at 27 $\pm 1^0$ C. The morphological and cultural characters of the nine isolates designated as MR_a , MR_b. MR_c , MR_d , MR_e , MR_f. MR_g , MR_h and MR_i were studied and documented for the identification of the isolated fungus. A representative sample of the isolated fungus was sent to the Department of Plant Pathology,

JNKVV, Jabalpur for confirming the identity.

Pathogenecity tests : The pathogenecity of *Myrothecium roridum* isolates *viz.,* MR_a , MR_b. MR_c , MR_d , MR_e , MR_f. MR_g , MR_h and MR_i was attempted on young and healthy *Gossypium hirsutum* cv JK 4 plants grown under greenhouse conditions. The inoculum of the pathogen was prepared in sterile distilled water at a concentration of 1 x 10^6 conidia/ml from 7-10 day old colonies growing on Potato Dextrose Agar medium. Sterile distilled water was added to the agar grown culture and the surface of the colonies was scrapped to harvest the conidia. The conidial suspension was centrifuged at 3000 rpm for 2-3 minutes. The mass of conidia was re-suspended in sterile distilled water and conidia concentration was determined with a haemocytometer and adjusted to desired concentration. Healthy plants of *G. hirsutum* variety JK-4 were grown in 15 cm earthen pots filled with a mixture of sandy soil and farmyard manure (3:1 v/v). Thirty day old healthy plants were sprayed in triplicate with conidial suspensions with a fine atomizer. The plants sprayed with sterile distilled water served as control. Both inoculated and control plants were covered with a polythene bag for 24 hours and then transferred in the greenhouse for development of symptoms. The pathogen was re-isolated and the identity of *M. roridum* was checked.

Documentation of symptoms: The plants showing leaf spots and the lesions invariably exhibiting the association of *Myrothecium roridum* were critically examined for various phenotypic manifestations of the disease. The symptoms developing on healthy plants inoculated with a pathogenic isolate of *M. roridum* were also documented. To assess the variability in the symptoms, crop growing under six different agro climatic situations of Madhya Pradesh as detailed below was surveyed. Under these situations, symptoms on plants infected with *M. roridum* were critically examined and documented. The six different situations where the symptoms of *Myrothecium* leaf blight on cotton were examined and documented are S1= Deep soil + Heavy rainfall ; S2= Deep soil + Low rainfall ; S3= Medium soil + Heavy rainfall ; S4= Medium soil + Low rainfall ; S5= Shallow soil + Heavy rainfall ; S6= Shallow soil + Low rainfall.

Histopathological Studies: The host-pathogen relationship was studied in infected leaves. Fresh green leaves on healthy plants were inoculated with viable spores of *Myrothecium roridum* and covered with polythene bags to maintain humidity for 24 hours. With the initiation of symptoms, infected leaves were collected and fixed. Mature lesions were also fixed for subsequent examination. Cross sections from the fixed material were examined.

Killing, fixing and sectioning: Infected leaves were collected and fixed in a fixative. The fixative used was Formalin Acetic Acid Alcohol (FAA) solution (Johanson's,1940). The material was preserved in the FAA for further microscopic studies. The fixed material to be sectioned was appropriately trimmed with a razor blade. A leaf piece with carrot pith was positioned in Radical Hand and Table Microtome model RMT 5 (Radical Instrument India Ltd., Mumbai). To get the desired plane of the section, the universal joint of the clamp was manipulated while the thickness gauge was set to 0.01 mm (10 µ). The sections were placed on slides and stained with lacto-phenol cotton blue for 2 hours. A clean microscope cover glass no 1 (English cover glass, size 22 x 40mm) was placed on the section before being examined under high power objective lens with a total magnification of 10x X 45x.

RESULTS AND DISCUSSION

Morphological studies: The isolated fungus from each location (nine locations designated as MR_a , MR_b. MR_c , MR_d , MR_e , MR_f. MR_g , MR_h and MR_i) was subjected to detail morphological studies. The observations on the microscopic studies have been presented through Table 1. The mycelium of the fungus consists of slender, profusely branched hyaline hyphae. The hyphae are intertwined and contorted with blunt ends. The vegetative hyphae tend to curve inwards. The cells contain granular protoplasm with occasional presence of oil globules. There is no distinction between vegetative and reproductive hyphae. The conidiophores are erect, branched septate, hyaline with tapering axis. Each branch of the conidiophore terminates in a whorl of phialids. The phialids are clavate in whorls of two to eight at the apex of the main axis or its branches. The phialids measure 5-8 x 1.5 -2.0 µ. The phialids form a closely packed hymenium like layer in the synnema.

The conidia are produced from the phialids and remain clustered together forming a dark black compact mass on the head of the synnema. The conidia are embedded in a gelatinous matrix. The conidia are cylindrical or very slightly tapering with rounded ends, unicellular, hyaline at first gradually turning into pale green. The conidia measures 6-8 µ x 1.5 – 2.0 µ in diameter. The growth on the potato-dextrose agar medium was white and cottony. Synnematic rings (sporodochia) are formed which imparts a typical zonate appearance to the fungal growth. The consecutive rings are initially with a greenish tinge finally turning dark in colour. The sporodochia were picked from the medium and examined microscopically (40x10x). The sporodochia are burried in the medium. They are discoid in shape often coalescing to form large masses. They are green at first, becoming black typically white rimmed, without setae arising from the mycelial mass.

The above description was compared with those given by Saccardo (1886), Munjal (1960) and Arya (1966) and it is found that the fungus under study is identical with *Myrothecium roridum* Tode Ex Fr. with very minor differences like the size of conidia which is slightly smaller. The organism is therefore identified as *Myrothecium roridum* Tode Ex Fr.

The comparative study of the fungus morphology of the nine isolates reveal that they do not show any significant variation among themselves. The general characters of the mycelium, conidiophores, conidia and colony characters on PDA were almost same. However, there were some minor variations in the measurements of the conidia among different isolates (Table 2).

Myrothecium roridum has been known to show variability in some crops (Arya, 1959). There are scanty reports of the presence of variability in cotton (Taneja *et.al.*, 1990, Munjal,1960). In the present study, attempts were also made to look for variation in nine isolates collected from different locations during the survey on the basis of cultural characters on potato dextrose agar medium and measurements of the conidia. The study reveal that they do not show any significant variation among themselves. The general characters of the mycelium, conidiophores, conidia and colony characters on PDA were almost same. However there were some minor variations in the measurements of the conidia among different isolates. Similar finding were also reported by Brooks (1945) , Arya (1959) and Taneja *et.al.* (1990) have indicated the existence of pathotypes in *M. roridum*. Taneja *et.al.*,(1990) have reported that five pathotypes are prevalent on cotton in India. On the other hand ,Chase (1983) while working with seven isolates of *M. roridum* could not provide any evidence of variability .

Pathogenecity tests: The isolates of *M. roridum* from all the nine locations viz MR_a , MR_b.

Table 1. Characterization of *M. roridum* isolated from different locations of cotton growing tract of M.P. on P.D.A.

Colony characters	MR_a	MR_b	MR_c	MR_d	MR_e	MR_f	MR_g	MR_h	MR_i
Growth in 3 days (mm)	27.00	26.66	27.33	28.66	26.66	25.33	26.33	28.33	28.00
Formation of synnema	5 days	7 days	5 days	6 days	5 days	5 days	6 days	5 days	5 days
Concentric rings	Present	Present	Present	Present	Present	Present	Present	Present	Present
Intensity of sporulation	Copious	Copious	Copious	Copious	Copious	Copious	Copious	Copious	Copious
Mycelial growth	Fluffy	Fluffy	Fluffy	Fluffy	Fluffy	Fluffy	Fluffy	Fluffy	Fluffy
Mycelium	Hyaline & Unicellular	Hyaline & Unicellular	Hyaline & Unicellular	Hyaline & Unicellular	Hyaline & Unicellular	Hyaline & Unicellular	Hyaline & Unicellular	Hyaline & Unicellular	Hyaline & Unicellular
Conidiophore	Non distinct	Non distinct	Non distinct	Non distinct	Non distinct	Non distinct	Non distinct	Non distinct	Non distinct
Spore mass	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark
Sporodochia	Discoid	Irregular	Discoid	Discoid	Irregular	Discoid	Discoid	Discoid	Irregular
Conidiafeatures	Biguttulats Cylindrical with blunt ends	Biguttulats Cylindrical with blunt ends	Biguttulats Cylindrical with blunt ends	Biguttulats Cylindrical with blunt ends	Biguttulats Cylindrical with blunt ends	Biguttulats Cylindrical with blunt ends	Biguttulats Cylindrical with blunt ends	Biguttulats Cylindrical with blunt ends	Biguttulats Cylindrical with blunt ends

MR_c , MR_d , MR_e , MR_f. MR_g , MR_h and MR_i were highly pathogenic on the *G. hirsutum* cultivar JK 4 under greenhouse conditions. The symptoms of the disease began to appear on the third/ fourth day in the form of small spots, which later formed typical zonate leaf spots. The sporodochia were visible from the seventh day. The control plants were free from the disease. The re-isolation from artificially infected tissue on PDA consistently yielded *M. roridum* in case of all the nine isolates thus fulfilling Koch's postulates. Chouhan and Chouhan (1984) have also reported that the genus *Myrothecium* has been observed on plants of more than twenty important agricultural crops causing different types of symptoms.

Documentation of Symptoms: The *Myrothecium roridum* infected plants were critically examined to document the manifestations of the disease. The symptoms have been presented through. The symptoms of the disease were observed on all aerial parts of the plant and more conspicuously on leaves (Plates 8, 9 & 10), bracts and green bolls. In the beginning the disease appears in the form of minute dark spots usually 1-2 mm in diameter. The spots gradually increase in size. The spots are characterized by the presence of broad violet to brown margins surrounded by zones of translucent areas giving an appearance of concentric rings.

Table 2. Measurements of the conidia of various isolates

Name of the isolate	Measurements of the conidia [µ]	Mean size [µ]
MR_a	4.33-7.30 X 1.24-2.06	6.26 X 2.08
MR_b	5.17-6.78 X 0.99-2.17	5.94 X 1.93
MR_c	4.73-7.54 X 1.37-2.20	6.72 X 1.81
MR_d	4.10-5.71 X 0.87-1.99	5.74 X 1.39
MR_e	6.25-7.64 X 1.37-1.99	6.90 X 1.57
MR_f	5.18-7.11 X 1.40-2.0	6.12 X 1.66
MR_g	5.03-7.60 X 1.13-2.08	6.05 X 1.50
MR_h	5.76-7.60 X 0.87-2.16	6.34 X 1.89
MR_i	5.03-7.65 X 1.30-2.10	6.25 X 1.76

After a few days fructifications of the pathogen are formed as dark green coloured sporodochia with white mycelial margins arranged in concentric rings Adjacent spots sometimes coalesce to give blight like appearance covering a large area of the leaf lamina. In some cases, the area covered by the necrotic lesions dried and withered giving rise to shot hole symptoms. In severe situations it was observed that leaf dries and detaches from the plant. On the bracts, typical violet coloured spots develop which gradually enlarges. On the green bolls, light brown coloured spots are formed which later turn into dark colour. The symptoms were recorded in all the three species of cotton vz. *Gossypium arboreum, G. herbaceum* and *G. hirsutum* that are cultivated in Madhya Pradesh.

To assess, the variability if any in the symptom across six different agro-climatic situations of Madhya Pradesh where cotton is grown in a sizeable area, the crop was examined and the symptoms were documented and are presented in Table 3. The observations clearly reveal that the same types of lesions are produced in all the situations. Under all the situations, similar lesions were observed both on the bracts as well as green bolls. Shot hole formation was recorded in all the situations except S1 (Deep soil + Heavy rainfall). Sporodochia formation on the translucent area giving rise to zonate leaf spots was recorded in all the six situations. The symptoms documented during the present studies are in consonance to those recorded by Munjal (1960), Shrivatava and Singh (1973) and Taneja (1989).

Histopathological studies : The Histopathological observations on the association of *Myrothecium roridum* has been depicted through illustrations. The transverse sections of infected cotton leaves reveal the formation of sporodochia with conidiophores and conidia on the surface of the leaf. Sporodochia has been observed to be restricted up to the epidermis only. They are

Table 3. Comparative study of symptoms of *Myrothecium* leaf blight in various agro climatic situations of Madhya Pradesh

Symptoms	S-1	S-2	S-3	S-4	S-5	S-6
Presence of zonate spots	+	+	+	+	+	+
Shot hole formation	-	+	+	+	+	+
Typical spots on bracts	+	+	+	+	+	+
Typical spots on green bolls	+	+	+	+	+	+
Formation of sporodochia	+	+	+	+	+	+

discoid / irregular shaped without setae on which the conidia are formed. Aggregates of fungal mycelium forming stromatic masses in the intercellular spaces has been observed. A hymenium layer on the surface from which the sporodochia are formed. Similar histopathological relationship between *M.roridum* and the host plant has been reported for cotton, guar and hollylock (Munjal, 1960; Arya, 1956; Barpete , 1986). The pathogen after entering the host tissue has been reported to colonize by inter and intra cellular mycelia. Disintegration and necrosis of invaded cells occurred after the pathogen had established itself (Laxminarayana, 1974). Barpete (1986) observed that the sporodochia of *M. roridum* in hollyhock are formed on the surface only. Munjal (1960) has reported the formation of stromatic masses inside the host tissue below the epidermis, a few strands of mycelia emerge either by rupturing the epidermis or through the stromata and give rise to sporodochia on the surface on which the conidia are formed.

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Received for Publication : May 20, 2014
Accepted for Publication : January 16, 2015