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ISOLATION, IDENTIFICATION AND PATHOGENICITY ASSESSMENT OF *Myrothecium roridum* CAUSING LEAF SPOT DISEASE IN SOYBEAN

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ABSTRACT

Soybean (*Glycine max* L. Merrill) is an important oilseed and protein-rich cropcultivated widely in India and other parts of the world. Its production is affected by several diseases, among which Myrothecium leaf spot caused by *Myrothecium roridum* Tode ex Fries can cause significant damage under favourable conditions. The present study was undertaken to isolate, identify and confirm the pathogenicity of *M. roridum* associated with leaf spot of soybean and to study its survival on decayed leaf tissues. Diseased soybean leaves showing characteristic leaf spot symptoms were collected from the research farm of the Department of Plant Pathology, College of Agriculture, IGKV, Raipur, Chhattisgarh. The pathogen was isolated on potato dextrose agar (PDA), purified by the hyphal tip method and maintained at 25 ± 2 °C. The isolate produced white, cottony colonies with dark green to black sporodochia. The phialides were hyaline, cylindrical and branched, while the conidia were hyaline, single-celled, non-septate, cylindrical to rod-shaped with rounded ends, measuring 2.19 × 7.16 µm. Based on these cultural and morphological characteristics, the pathogen was identified as *M. roridum*. Pathogenicity was tested on healthy 20-day-old soybean plants using a conidial suspension. Characteristic symptoms appeared within 3–7 days after inoculation, beginning as small, circular to oval brown lesions with dark margins that gradually enlarged and coalesced. Black sporodochia developed on infected tissues during the later stages of infection. The pathogen was successfully re-isolated from artificially infected tissues, confirming its pathogenicity and fulfilling Koch's postulates. In the detached leaf assay, abundant black sporodochia developed on decayed leaf tissues within 36 hours and persisted on dried leaf debris, indicating the ability of *M. roridum* to survive on decayed soybean leaves through sporodochia. The study confirms *M. roridum* as the causal agent of Myrothecium leaf spot of soybean and provides information on its cultural, morphological, pathogenic and survival characteristics.

***Key words*:** Soybean, *Myrothecium roridum*, Leaf spot,

Introduction

Soybean *(Glycine max* L. Merrill) a member of the family Leguminaceae, is widely recognized as a "miracle bean" because of its immense industrial, nutritional, and economic importance. It is one of the most important oilseed crops cultivated in several regions of India and across the world. India ranks fourth globally in soybean cultivation, with an area of 11.25 million hectares, an annual production of 9.30 million metric tons, and an average productivity of 8.30 quintals per hectare (Kumari *et al.*, 2025). Soybean is considered complete protein due to its well-balanced composition of essential amino acids (Singer *et al.*, 2019). Because of its high protein and oil content, soybean is extensively used to produce vegetable oil and alsoserves as a valuable source of nutrition for humans, livestock, poultry, and fish (Shashank *et al.*, 2015). Soybean is an ancient leguminous crop believed to have originated in China, where its cultivation and utilization as a food source dates back more than 6,000 years (Krishnamurthy, 1975). Over time, soybean cultivation expanded to many parts of the world, and today it holds a prominent place among the major oilseed crops

due to its wide adaptability and high productivity (Agarwal *et al.*, 2013).

Despite its economic importance, soybean production is severely affected by several diseases caused by fungal, bacterial, and viral pathogens. Among these, leaf spot caused by *Myrothecium roridum*Tode ex. Fries is considered an important disease of soybean. The disease has been reported in epidemic form in several soybean-growing regions of India, particularly in Madhya Pradesh, leading to considerable yield losses (Shrivastava and Khan, 1994; Singh and Shrivastava, 1994). Myrothecium leaf spot has been recorded in almost all major soybean-growing areas of the country and is capable of causing yield losses of up to 30 per cent (Shrivastava and Khan, 1994). The severity of the disease generally ranges between 35 and 45 per cent, while the incidence may vary from 30 to 55 per cent (Singh and Shrivastava, 1994) *Myrothecium roridum* is a soil-inhabiting fungus that commonly survives as a saprophyte on decomposing plant materials (Domsch*et al*., 2007). Although it primarily exists as a saprophyte, it is also capable of causing diseases in plants, particularly affecting the aerial parts of several host species (Ahrazem *et al.*,2000; Domsch *et al.*, 2007). This fungus is considered a facultative parasite and has been reported to infect a wide range of plants, including vegetables, fruits, and ornamental crops (Murakami and Shirata, 2005). Considering the economic importance of soybeanand the increasing occurrence of this fungal disease, the present study was undertaken to identify the pathogen and to study its cultural and morphological variability. The initial symptoms of the disease appear as small, circular to oval brown lesions with dark brown margins on infected leaves, which may enlarge and coalesce under favorable environmental conditions, ultimately reducing the photosynthetic area of the plant and affecting yield. Singh *et al.*, 2025 reported that the disease begins with small, round or oval brown spots having dark brown margins on infected leaves.

Materials and Methods

Collection of diseased Sample

Soybean leaves naturally infected with symptoms of Myrotheciumleaf spot were collected from the research farm of theDepartment of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. The collected samples were brought to the laboratory for detailed examination of symptoms, identification, and isolation of the pathogen.

Isolation, Purification and Identification of the Fungus

The diseased leaves of soybean were cut into small pieces and surface sterilized using 0.1% Sodium hypochlorite (NaOCl) solution. The sterilized pieces were then washed three times with sterile distilled water and placed in a moist chamber. After 1–2 days, fungal mycelial growth was observed. Small portions of the growing fungal mycelium were then transferred onto previously poured and solidified potato dextrose agar (PDA) medium in Petri plates for isolation of the pathogen. The inoculated plates were incubated at 25°C. After sufficient mycelial growth appeared, the culture was sub-cultured and purified using the hyphal tip method. The key characteristics, including colony colour and shape, as well as sporodochia, Mycelia and conidia were studied. The purified culture was maintained on PDA slants, Petri plates and kept in an incubator at 25°C. The cultural and growth characteristics of the fungus were recorded and compared with standard published descriptions for confirmation of the pathogen. Based on the morphological characteristics and molecular analysis, the pathogen was identified as *Myrothecium roridum* Tode ex. Friesasfirst report of *M. roridum* causing leaf spot on common bean (*Phaseolus vulgaris* L.) in China (Zhao *et al.*, 2009).

Testing of Pathogenicity

Twenty-day-old healthy soybean plants grown in pots were selected for the pathogenicity test. A conidial suspension of the fungus was prepared from a 20-day-old culture grown on potato dextrose agar medium. The suspension was uniformly sprayed on the foliage of soybean plants using a hand atomizer to ensure proper inoculation, while control plants were sprayed with sterile distilled water only. After inoculation, both the inoculated and uninoculated (control) plants were covered with

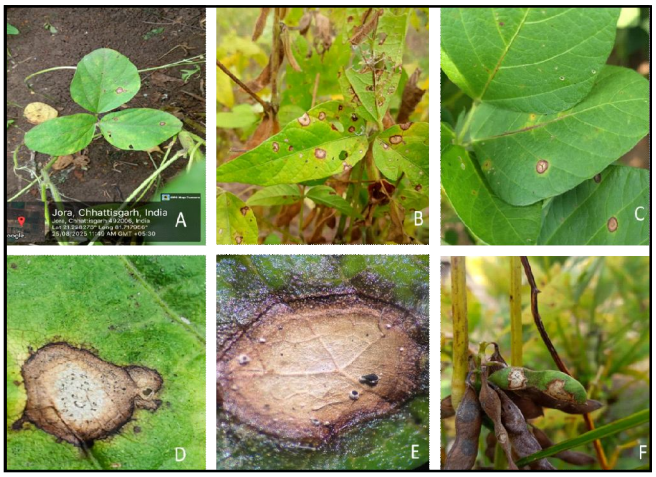


Fig. 1: Field symptoms of Myrotheciumleaf spot: (A-C) Small, circular to oval, brown necrotic lesions with dark brown margins developed on the leaves. (D) Close up of a single leaf displaying discrete brown lesions. (E) Black sporodochia formed on brown necrotic spots. (F) Necrotic lesions developed on the pods.

perforated polythene bags to maintain high humidity. The polythene bags were removed after five days, and the plants were subsequently observed at regular intervals for the development of disease symptoms. When characteristic symptoms appeared, the pathogen was re-isolated from the infected leaf tissues, and the culture obtained was compared with the original culture to confirm the identity of the pathogen and to fulfill Koch's postulates.

Survival of *Myrothecium roridum* in decayed leaves

Healthy soybean leaves were surface sterilized with 1% sodium hypochlorite for 2 minutes, rinsed thoroughly with sterile distilled water, and air-dried under aseptic conditions. The leaves were placed in sterilized Petri plates lined with two layers of moist sterile blotter paper. A 5 mm mycelial disc of *Myrothecium roridum*, obtained from the actively growing margin of a 10-day-old culture, was inoculated onto the upper surface of each leaf after slight wounding. The Petri plates were maintained under moist conditions by periodically adding sterile distilled water to the blotter paper and incubated at laboratory temperature. The inoculated leaves were allowed to dry and undergo natural decay, and were examined at regular intervals for the formation and persistence of sporodochia. The presence of sporodochia on the decayed leaf debris was recorded as evidence of survival of *M. roridum*. Three leaves were maintained as replications, while leaves inoculated with sterile PDA discs served as the control.

Results and Discussions

Symptomatology

The initial symptoms appear as small, round to oval brown spots with dark brown margins on the leaves of infected plants.In some cases, as the lesions develop further, shot holes are formed on the leaves. Later on number of spots were formed get enlarged and merged to form irregular shape spot and severely infected leaves become dry. The center of the leaf spots appeared light gray, and black sporodochia were present on some older leaf spots.Symptoms were also observed on pods, where black sporodochia were sometimes formed. The initial symptoms of *Myrothecium* leaf spot in soybean plants appear as small round or oval, brown spots with a dark brown margin on the leaves (Dewangan *et al.*, 2019). These symptoms were also consistent with those reported for leaf spot of *Coffee canephora* caused by *Myrothecium roridum* (Silva *et al.*, 2014). Small, round to oval brown lesions with dark brown margins were observed on infected cotton leaves, often surrounded by translucent zones forming concentric rings (Umamaheswari *et al.*, 2022). The symptoms of *Myrothecium* leaf spot on soybean appear as distinct leaf spots with dark edges and relatively lighter centers on the infected leaves (Hauden shield *et al.*, 2018). The infected leaves developed round to oblong dark brown lesions with clear concentric rings, measuring about 8–10 mm in diameter (Jordan *et al.*, 2018). Leaves of watermelon showed numerous round to oblong brown lesions with distinct concentric rings (Seebold *et al.*, 2005). Leaf spots appeared circular to subcircular, dark brown in colour with distinct concentric zones, measuring about 10–20 mm in diameter (Zhang *et al.*, 2011).

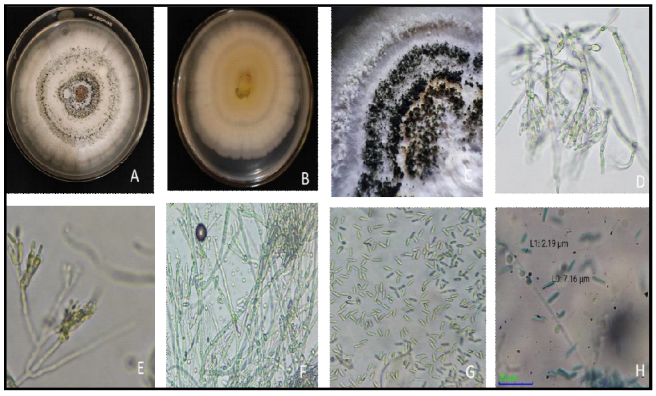


Fig. 2: (A-B) Front and back view of culture plate of the pathogen(C)View of Sporodochium in plate (D-E) Phialides and attachment of conidia to conidiogenous cell (F) Mycelium (G-H) Rod-shaped, hyaline conidia.

Isolation, purification and Identification of pathogen

After four days of incubation on PDA medium, fungal mycelium was observed emerging from the infected leaf tissues. The pathogen was purified using the single hyphal tip technique. The purified culture was subsequently multiplied and maintained on PDA at 25 ± 2 °C in a BOD incubator. On PDA, the isolate produced white, cottony colonies followed by dark green to black sporodochia.

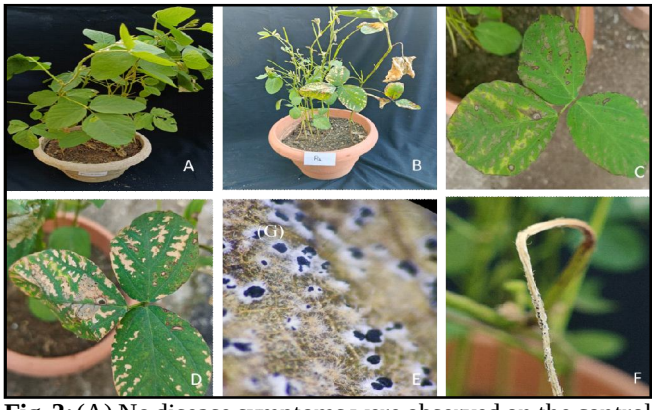


Fig. 3: (A) No disease symptoms were observed on the control plant. (B-D) Leaves of the infected plant developed small, brown, oval lesions with dark margins. (E)Sporodochia appeared on infected leaves (F) Sporodochia structures formed on infected twigs.

Phialides were hyaline, cylindrical and branched. The colony covered about 65–70 mm of the PDA plate within 15 days at 25 ± 1 °C. Conidia were hyaline, single-celled, non-septate, cylindrical to rod-shaped with rounded ends, measuring 2.19 × 7.16 µm (width × length). These morphological characteristics confirmed the pathogen as *M. roridum*. Conidia observed on *Salvia* spp. were hyaline, cylindrical with rounded ends, and measured approximately 7.4 × 2.0 ìm (Mangandi *et al.*, 2007). *Myrothecium roridum* was isolated from coffee berries. Conidiophores were branched two to three times with hyaline, cylindrical phialides. Conidia were single-celled, cylindrical to ellipsoid, hyaline to pale olive green, and turned black in mass (Giri *et al.*, 2019). Colonies were floccose, white to buff, producing sporulation in concentric zones. Olivaceous-black to black sporodochia developed on the colony surface with viscid masses of conidia. Conidia were narrowly ellipsoid with rounded ends, measuring 6.25 ± 0.04 × 1.63 ± 0.02 ìm (Hong *et al.*, 2013). Conidia were hyaline to olive green or slightly dark, single-celled, ovoid to elongate or rod-shaped with rounded ends, measuring 2.0–2.5 × 5.5–6.5 ìm, as reported by D. Talukdar and Jagdish Prasad (2013). Rod-shaped, hyaline conidia with rounded ends were observed, measuring on average 6.8 × 2.5 µm (Y.J. Zhao *et al.*, 2010).

Testing of Pathogenicity

The pathogenicity test was conducted using a culture of *M. roridum* isolated from infected soybean leaves. The pathogen was inoculated onto healthy seedlings. The inoculated plants developed disease symptoms within 3-7 days after artificial inoculation. In the attached leaf pathogenicity test, symptoms started appearing after 3 days of inoculation. Numerous small, round to oval, grayish necrotic spots were observed on the inoculated leaves. The lesions gradually became darker after 5 days of inoculation. By the 7th day, the lesions enlarged and merged together, resulting in drying of the infected leaves. Black sporodochia were also observed on the infected leaves and twigs during the later stage of infection. The symptoms observed on the inoculated plants were similar to those recorded under natural field conditions. The pathogen was re-isolated from the infected tissues of inoculated plants and cultured on PDA medium. The cultural characteristics of the re-isolated fungus were found to be like those of the original culture, thereby confirming the association of *Myrothecium roridum* with the disease through artificial inoculation. The bael plants were inoculated with a conidial suspension (1×10^6 spores ml^{-1}) using an atomizer and maintained in a glasshouse. Control plants were sprayed with sterile distilled water. After symptom development, the pathogen was re-isolated from the infected leaves and compared with the original culture to confirm pathogenicity (Kumar and Singh, 2021). The pathogenicity of *Myrothecium roridum* on salvia plants was confirmed by spraying a conidial suspension (1×10^5 conidia ml^{-1}), followed by covering the plants with plastic bags and incubating them in a growth chamber at 28 °C for 7 days. Mangandi *et al.*, (2007).

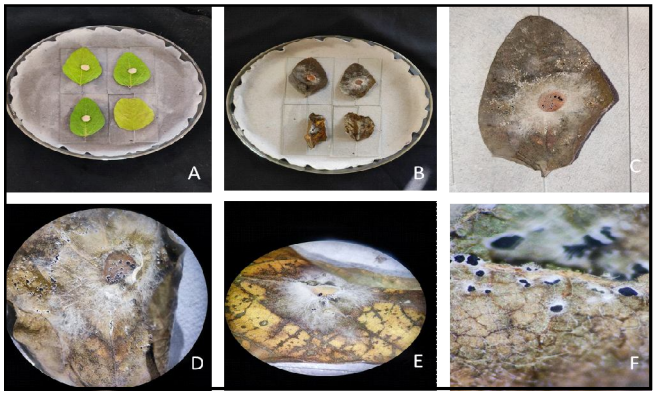


Fig. 4: (A) Leaves of soybean before inoculation(B-F) Leaf surface showed fungal growth, sporodochia formation.

Survival of *Myrothecium roridum* in decayed leaves

In the detached leaf assay, abundant black sporodochia developed on the decayed leaf tissue within 36 hours of inoculation. With disease progression, the leaves gradually dried, while the sporodochia remained intact on the dried leaf debrissuggesting that *M. roridum* can survive on decayed leaves as sporodochia. (Kuti *et al.*, (1989) demonstrated the pathogenicity of *M. roridum* on detached muskmelon leaves.

Conclusion

The present study confirmed that *Myrothecium roridum* is the causal agent of leaf spot disease of soybean. The pathogen was successfully isolated from infected soybean leaves and identified based on its cultural and morphological characteristics, including colony features, sporodochia, mycelium, and conidia. Pathogenicity tests on healthy soybean leaves produced typical leaf spot symptoms, thereby fulfilling Koch's postulates. The findings establish the association of *Myrothecium roridum* with soybean leaf spot

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