

Identification and pathogenicity of *Corynespora cassiicola* causing leaf spot disease of cowpea (*Vigna unguiculata*) in Assam

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During a field survey conducted on fungal foliar diseases of vegetable crops in Assam, India, cowpea, *Vigna unguiculata* plants were found to exhibit symptoms of leaf spot disease. The affected plants displayed greyish-brown lesions on the leaves and black necrotic patches on the stems. Fungi isolated from the symptomatic tissues were identified as *Corynespora cassiicola* by morphological observation and molecular characterization through ITS-rDNA sequence analysis. Pathogenicity tests further confirmed that this fungus is the causative agent of the disease. The study represents first report of *C. cassiicola* causing leaf spot disease on cowpea in Assam. These findings underscore the potential threat posed by this pathogen to cowpea cultivation in the region, highlighting the need for further research and effective management strategies.

Keywords : *Corynespora cassiicola*, ITS rDNA analysis, Leaf spot, Pathogenicity, *Vigna unguiculata*

INTRODUCTION

Cowpea, *Vigna unguiculata* L. (Fam: Fabaceae) often referred to as a “poor man’s meat” is one of the most widely cultivated legumes, especially in tropical and subtropical regions, due to its nutritional benefits and adaptability to harsh growing conditions. It contains high protein ranging between 22% and 33%, making it a critical source of essential amino acids such as lysine and tryptophan (Alemu *et al.* 2019; Dania and Gbadamos, 2019). The crop is a staple food, contributing significantly to food security by providing an affordable alternative to animal-based proteins. In addition to its nutritional value, cowpea also plays a vital role in sustainable agricultural systems due to its ability to fix atmospheric nitrogen through a symbiotic relationship with *Rhizobium* bacteria, improving soil fertility and reducing the need for synthetic fertilizers (Abdel-Monaim and Atwa, 2019). Despite these benefits, cowpea production is often limited by biotic stresses, particularly diseases caused by fungal

pathogens that can lead to substantial yield losses by affecting various parts of the plant, including roots, shoots, leaves, and pods.

Among the major pathogens reported on cowpea are *Alternaria alternata*, *Colletotrichum capsici*, *Corynespora cassiicola*, *Fusarium cicero*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Curvularia lunata*, which collectively threaten both the quantity and quality of the produce (Roy and Basu, 2021). Leaf spot diseases, in particular, are a significant concern as they reduce photosynthetic efficiency, weaken the plant, and can lead to premature defoliation, ultimately affecting seed development and yield.

In recent years, *Corynespora cassiicola*, a widespread pathogen known to infect a variety of crops, has emerged as a serious threat to cowpea cultivation. It has been reported as the causative agent of leaf spot disease in several economically important crops such as cucumber, soybean, tomato, sweet pepper, and eggplant (Zhang, 2018; Gao *et al.* 2011). The fungus is capable of surviving on a wide range of host plants, which complicates its management in agricultural systems. In India, *Corynespora cassiicola* has

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been reported as a pathogen on cowpea in West Bengal (Roy *et al.* 2015), however, there are no prior records of its occurrence from Assam.

The present study aims to characterize the fungal pathogen causing leaf spot disease on cowpea in Assam. The symptomatic plants were observed in fields located near Gerua (N: 6° 21' 14.66", E: 92° 14' 47.50"), Morigaon district of Assam. The pathogen was characterized based on its morphological observation and molecular characterization. Further, pathogenicity tests were conducted to confirm the fungus as the causative agent.

MATERIALS AND METHODS

Sample Collection and Pathogen Isolation

Diseased leaves were collected from cowpea plants showing symptoms of greyish-brown spots from fields near Gerua (N: 6° 21' 14.66", E: 92° 14' 47.50"), Morigaon, Assam. To isolate the pathogens, surface sterilization was performed by dipping the symptomatic leaves in 70% ethanol for one minute, followed by immersion in 0.2% sodium hypochlorite for 2 minutes. The leaves were then rinsed thrice with sterile distilled water and cut into small sections (5 × 5 mm²). The cut sections were placed on Potato Dextrose Agar (PDA) plates and incubated at 28 ± 2°C for seven days. Emerging fungal hyphae were sub-cultured and subsequently pure cultures were obtained for further study.

Identification and phylogenetic analysis

The cultural and morphological characteristics of the pathogen were observed on PDA after a 7-day incubation period at 28±2° C. The size and structure of the conidiophores and conidia were examined using a compound microscope, with measurements conducted on 20 samples each, observed with an optical microscope (Primo Star®, ZEISS, Göttingen, Germany) at 400× magnification. For molecular identification, total genomic DNA was extracted, and the internal transcribed spacer (ITS) region was amplified and sequenced using the ITS1/ITS4 primer pair, as described by White *et al.* (1990). The resulting ITS sequences were compared with existing

sequences in the GenBank database using BLAST analysis to determine the closest similarity percentage. Phylogenetic relationships were analysed using the Maximum Likelihood method and the Kimura-2 parameter model in MEGA 11 software (Tamura *et al.* 2021). Bootstrap analysis was conducted with 1000 replicates to validate the results in the phylogenetic tree.

Pathogenicity assay

Pathogenicity tests were conducted on healthy cowpea plants by inoculating 20 µL of conidial suspension (1 × 10⁶ conidia/mL) of the fungus at needle puncture wound sites made with a sterile scalpel (Kou *et al.* 2024). The inoculated plants were covered with transparent perforated polyethylene bags and moistened with distilled water to maintain a humid environment. Simultaneously, healthy uninoculated cowpea plants were used as control groups. Both inoculated and control plants were kept under greenhouse conditions to ensure adequate relative humidity (85%). After 7 days, the inoculated plants displayed characteristic leaf spot symptoms, whereas the control group remained symptom-free. The fungus was re-isolated from the inoculated plants, and its identity was confirmed through morphological and molecular analyses as described earlier.

RESULTS AND DISCUSSION

During the field survey conducted on foliar fungal diseases of economically important vegetables crops of Assam, cowpea plants was observed to display symptoms of leaf spot disease which was characterized by greyish-brown lesions on the leaves and black necrotic patches on the stems. The associated fungus was isolated from the symptomatic tissues and a distinct morphological feature of the pathogen was observed on PDA medium after 7-day incubation period at 28±2° C. The causal organism was designated as 'MCY1' and colonial trait of the isolate on PDA medium, exhibited circular with raised edges and rough texture, displaying colours that varied from greyish to whitish-grey, with a brownish-black reverse. The conidiophores were solitary, erect, and pale brown, measuring 48-189 × 2-4 µm, and

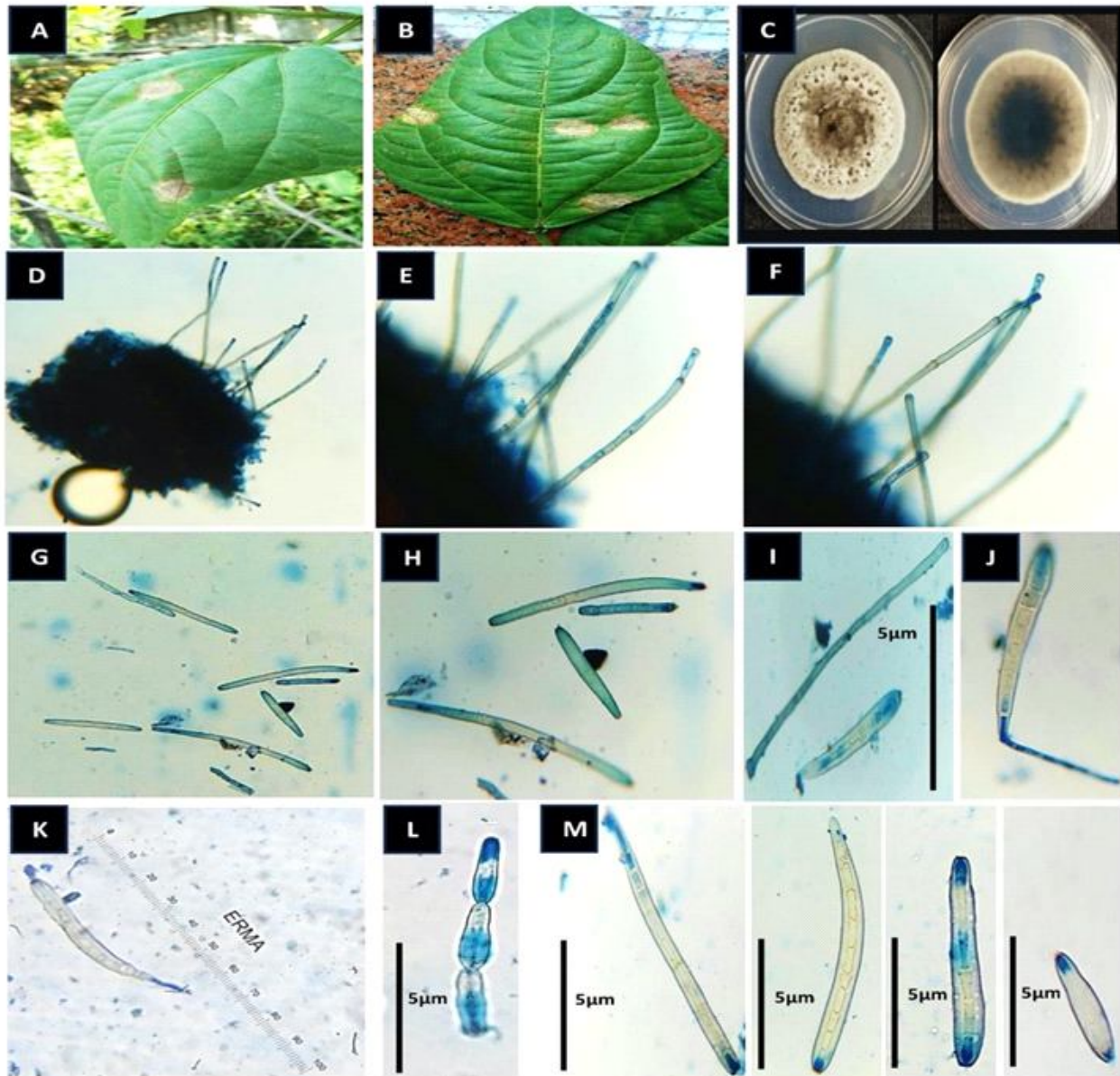


Fig. 1 : Symptoms of leaf spot disease on *V. unguiculata*, and the morphology of the pathogen, *Corynespora cassicola* isolate MCY 1: (a-b) Symptom of leaf spot observed in the field; (c) Front and reversed view of the isolate on PDA medium; (D-F) conidiophores; (G-M) Conidia. Scale bars = 5mm

were found to be 3-5 septate and unbranched, either straight or slightly curved. The conidia exhibited a cylindrical to obclavate shape, were subhyaline to pale olivaceous brown with smooth walls, measuring $30-93 \times 5-10 \mu\text{m}$, and had 0-20 pseudo septa, forming either singly or in chains (Fig. 1). These morphological characteristics aligned with previous descriptions of *Corynespora cassicola*.

Furthermore, molecular characterization was considered necessary for precise identification

of the fungus. For this purpose, the internal transcribed spacer region (ITS) was amplified using the ITS1 and ITS4 primers. The obtained sequences were deposited in GenBank with accession numbers PP789711. BLASTn search revealed that the ITS gene sequence of the fungal isolate MCY1 exhibited 99–100% similarity with *Corynespora cassicola* (GenBank accession numbers MT775478 and MW255069). Phylogenetic analyses was carried out using Maximum Likelihood method in MEGA 11 with the Kimura 2-parameter model and the

generated tree indicated that isolate MCY1 clustered closely with *C. cassiicola* (Fig. 2). Thus the molecular analysis supported the initial morphological identification. To satisfy Koch's postulates, pathogenicity tests were performed on healthy cowpea plants by inoculating 20 µL of a conidial suspension (1×10^7 conidia/mL) of the isolate MCY1. Meanwhile, young, healthy uninoculated cowpea plants were maintained as control groups. Within seven days, the inoculated plants exhibited symptoms of leaf spot disease. The lesions began as small, greyish-brown spots with distinct brown halos, similar to those observed in the field. As the disease progressed, the lesions coalesced, forming large, irregularly shaped spots, while the control plants remained asymptomatic (Fig. 3). The successful re-isolation of the pathogen from the infected leaves fulfilled Koch's postulates and conclusively established *C. cassiicola* as the causal agent of the disease. To best of our knowledge, this study represents the first documentation of *C. cassiicola* causing leaf spot disease on cowpea in Assam, underscoring the potential threat posed by this pathogen to cowpea cultivation in the region.

Previous studies have identified *C. cassiicola* as the causative agent of leaf spot diseases from various crops, including pepper, kiwifruit, and cotton in Korea, China, and India, respectively (Kwon *et al.* 2001; Yuan *et al.* 2014; Salunkhe *et al.* 2019). This pathogen is known for notorious for its wide host range, which complicates its management in agricultural systems. The ability of *C. cassiicola* to infect multiple crops not only makes it a formidable threat to diverse agricultural practices but also facilitates its persistence in various environments, contributing to its potential for significant economic losses. The economic impact of *C. cassiicola* is particularly concerning given the reliance of small-scale farmers in Assam on cowpea as a crucial source of protein and income. As this pathogen emerges in the region, it poses a serious threat to cowpea cultivation, which is vital for food security and livelihoods. In light of this situation, the development of integrated disease management strategies becomes imperative. Such strategies may include the identification and use of resistant cultivars that can withstand infection, the implementation of

proper field sanitation practices to reduce pathogen loads in the environment, and the exploration of biological control agents that could offer eco-friendly alternatives to chemical fungicides. By focusing on these approaches, farmers can mitigate the impact of *C. cassiicola* on their crops and ensure the sustainability of cowpea production in Assam. Moreover, ongoing research is essential to monitor the spread of this pathogen, understand its biology and ecology, and develop effective management practices that align with sustainable agricultural principles.

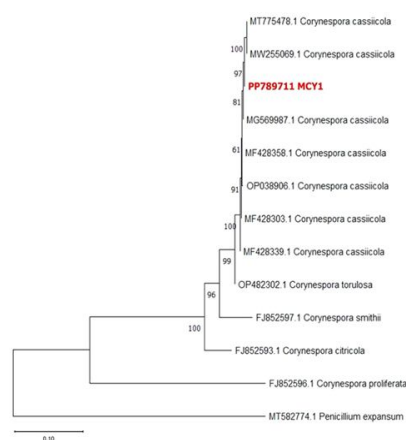


Fig. 2: Phylogenetic tree of *Corynespora* spp., including the isolate MCY1, based on ITS rDNA sequences using the maximum likelihood method and Kimura 2-parameter model with MEGA11 software. Bootstrap values, expressed as a percentage of 1000 replicates, are indicated at branch points. The strain MCY1 is highlighted in red

CONCLUSION

The identification of *C. cassiicola* as the causal agent of leaf spot disease on cowpea in Assam, India for the first time marks a significant finding of this study. Utilizing morphological, molecular, and pathogenicity analyses, the research clearly established the pathogen's involvement in the disease symptoms observed in the field. As cowpea serves as a vital protein-rich crop in the region, the emergence of *C. cassiicola* poses a considerable threat to its cultivation. This finding highlights the urgent need for further research focused on effective disease management strategies. Future efforts should prioritize monitoring the pathogen's spread, investigating resistant cultivars, and developing eco-friendly control measures to safeguard cowpea production.

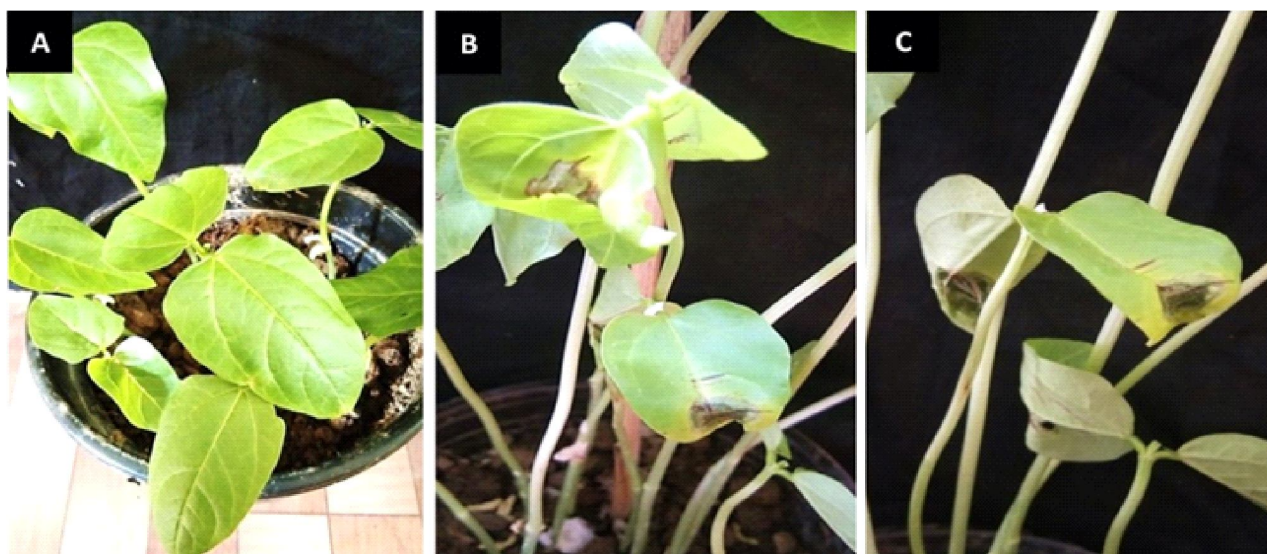


Fig . 3 : Pathogenicity of *Corynespora cassicola* (isolate MCY1): (A) Control plant; (B-C) plant inoculated with the pathogen (isolate MCY1) showing the diseased symptoms.

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DECLARARTION

Conflict of Interest. Authors declare no conflict of interest.

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