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# First report of leaf spot caused by Colletotrichum siamense on Ocimum tenuiflorum in India.

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**Research Article**

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# Abstract

A leaf spot disease of Indian basil has been observed in Kalyani, West Bengal during the month of September 2022–2023. The disease symptoms appeared as light to dark brown circular spots with irregular margins which rarely coalesced and covered the entire leaf surface. The pathogen was identified through morphological and molecular analyses as an ascomycetes fungus *Colletotrichum siamense*. The leaf spot causes serious damage to leaf yield with severity estimated up to 30–50%. This is the first record of *C. siamense* causing leaf spot on Indian basil in the country.


# Full Text

Indian basil (*Ocmium tenuiflorum*. L) is an aromatic perennial shrub in the *Labiaceae* family, widely grown in the tropical regions of Southeast Asia (Cohen 2014). It has curative properties against cough, cold, bronchitis, respiratory syndrome (Chandra et al. 2016; Mukherjee and Mondal 2023). Even such valuable medicinal crop is not immune to diseases.

*Colletotrichum* leaf spot of basil has been reported first in India from Kerala (Nilamudeen et al. 2013), but the causal agent has been identified as *Colletotrichum gloeosporeoides.* Other instances of leaf spot in basil caused by *Colletotrichum* spp. report the causal agents to be *Colletotrichum ocimi* of the *Colletotrichum destructivum* species complex (Cacciola et al. 2020). There have been no reports of leaf spot in basil caused by *C. siamense*.

The disease was observed during September 2022-2023 in the Medicinal Plants Garden (22°59'21"N latitude and 88°27'18"E longitude) of All India Coordinated Research Project on Medicinal and Aromatic Plants and Betel vine, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India which comes under the Gangetic New Alluvial plain zone and subtropical climatic condition. The severity was about 30-50 % during the peak incidence period of September and October, 2022-2023.

Symptoms initially appeared as necrotic water soaked spots on the surface of the leaves which later turned dark brown to black. The spots ware mostly circular with irregular margins and numerous spots developed on the leaves which rarely coalesced with each other (Fig. 1). These spots (n = 60) were 2.00 ± 0.83 mm (Range: 0.94 - 5.37 mm) in length and 1.22 ± 0.82 mm (Range: 0.91 - 5.59 mm) in breadth. The disease mainly occurred in the monsoon season with hot and humid climate prevailing.

Pathogenicity test was conducted in shade-net house. Healthy and mature leaves of 10 potted basil plants were sprayed with a conidial suspension ($10^5$ conidia/ml) from 14-15 days old culture of *C. siamense*. 3 healthy potted plants were sprayed with sterilized distilled waterto serve as control. The inoculated leaves were covered properly using moist polythene bags and maintained at 27±1°C. After 5-6 days, symptoms expressed on inoculated leaves were studied followed by re-isolation of the pathogen to fulfil Koch’s postulate. The experiment was replicated thrice to confirm symptomatology. The plants

showed infection on the leaves 5-7 days after inoculation and the re-isolated pathogen was same as the *Colletotrichum* spp. observed (Fig. 2).

Pathogen was isolated in September, 2022-2023 on PDA medium incubated at 27 ± 1$^{o}$C. The fungus produced cottony, fluffy raised, white to grey mycelium with smooth margin on PDA media incubated at 27 ± 1$^{o}$C and full plate growth within seven days after inoculation. The pathogen produced hyaline, unicellular, smooth walled, straight, cylindrical, bullet shaped conidia on PDA media. Size of the conidia ranged from 7.6 to 13.9 µm (Mean 11.9 ± 1.0 µm) in length and from 2.9 to 4.9 µm (Mean: 3.7 ± 0.4 µm) in breadth. Setae were not or rarely formed in PDA media.

For comparative study, the fungal pathogen was also grown on Modified Mathur's Agar Media (Tu 1985) and incubated at 27 ± 1$^{o}$C. The result revealed that the fungus produced flat to fluffy, white to dark grey mycelium with shallow centre and full plate growth was observed after 7 days of inoculation. Conidia were hyaline, unicellular, smooth walled and cylindrical measuring 12.22 to 20.54 µm (Mean 16.88 ± 1.66 µm) in length and 3.06 to 4.84 µm (Mean 3.88 ± 0.37 µm) in breadth. There was formation of orange coloured conidiomata (acervulus) which possessed abundant conidia and setae. Setae in MMM measured 27.94 to 64.59 µm (Mean 46.69 ± 9.11 µm) in length and 1.73 to 4.93 µm (Mean 3.48 ± 0.655 µm) in thickness (Fig. 3).

Comparing the fungal growth on two medium (PDA and MMM), it was observed that on PDA the pathogen produced more mycelium mass and showed moderate sporulation whereas on MMM mycelium growth was poor but numerous conidia and setae were observed. The shape of conidia was similar on both the medium however; on MMM the size of conidia was somewhat larger than that on PDA. The fungus was submitted in the National Agriculturally Important Microbial Culture Collection (NAIMCC) of ICAR- National Bureau of Agriculturally Important Microorganisms (NBAIM) in Kushmaur, Uttar Pradesh, India, and a culture collection number of NAIMCC-F-04497 was obtained.

The pathogen species was identified by sequencing the internal transcribed spacer (ITS) (White et al. 1990) and β-tubulin (TUB2) (Glass and Donaldson 1995) regions. A sequence of 557 bp from the ITS region and 447 bp from the TUB2 region was obtained. The sequences were concatenated and compared with related sequence through blast and phylogenetic analysis. The results were confirmed to be *Colletotrichum siamense*. The ITS rDNA (OP886681) and TUB2(OQ446139)sequence were deposited in GenBank. The ITS and TUB2 regions of the studied fungal isolate were analysed with nucleotide BLAST search in NCBI GenBank database to determine its phylogenetic relationships with other closely related fungal species (Fig. 4). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model with a discrete Gamma distribution to model evolutionary rate differences among sites (Tamura 1992). Evolutionary analyses were conducted in MEGA11 (Tamura et al. 2021).

The *Colletotrichum gloeosporeoides* species complex involves multiple species that includes *Colletotrichum fruticola* and *Colletotrichum siamense* among others. Many of the species are unable to

be reliably distinguished using ITS, thus another gene i.e, TUB2 has been used for identification.

After confirming the pathogenicity of the test organism, we report *Colletotrichum siamense* as the causal agent of leaf spot on tulsi at Kalyani, West Bengal, India by studying morphological characters coupled with molecular characterization and phylogenetic analysis of ITS and TUB2 regions.

Anthracnose of Luffa sponge gourd is caused by *C. siamense*. Morphologically, the colony colour of *C. siamense* was white and gradually turned grey white, containing dense aerial mycelium. Conidia were hyaline, unicellular, aseptate, long elliptic to cylindrical, and measured 12.24–28.57 × 4.08–8.29 µm (Li et al. 2022).

*Colletotrichum* isolates of chilli anthracnose in India were studied and the morphology of *C. siamense* was compared. It was observed that *C. siamense* had cottony, pale yellowish to pinkish mycelium with fusiform to cylindrical conidia of dimensions 10.2 ± 1.49 × 3.6 ± 0.5 µm. (Sharma and Shenoy 2014)

*Colletotrichum siamense* has also been observed on *Viburnum odoratissimum* as leaf blotch. Colonies on PDA were white to pale greenish-grey with dense and fast growing aerial mycelia and the reverse was grey-green at the centre. Conidia were single celled, hyaline, cylindrical, round at both ends, with dimensions 16.2 ± 1.1 × 5.8 ± 0.3 µm. Setae were observed in acervuli having pale to dark browncolour, straight or curved, with acute tips, and dimensions 104.4 ± 17.4 × 3.1 ± 0.5 µm. (Li et al. 2023)

After comparing the morphological and phylogenetic characteristics of the pathogen with other reported isolates of *C.siamense* it was found that the pathogen was highly similar. Hence, the causal agent of leaf spot disease of Indian basil was identified as *Colletotrichum siamense.*

# Declarations


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### Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Soumik Mukherjee, Siddharth Singh and Susmita Oraon. The first draft of the manuscript was written by Soumik Mukherjee, Ankur Mukhyopadhyay and Goutam Mondal and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Conceptualization: Goutam Mondal. Methodology: Soumik Mukherjee, Siddharth Singh, Susmita Oraon. Formal analysis and investigation: Soumik Mukherjee, Siddharth Singh, Ankur Mukhyopadhyay. Writing-original draft preparation: Soumik Mukherjee, Goutam Mondal. Writing-review and editing: Ankur Mukhyopadhyay. Supervision: Goutam Mondal.

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**Competing interests:** The authors declare no competing interests.

**Ethical Approval:** Ethical approval was not required for this study, as it only involved plant samples.

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# Figures



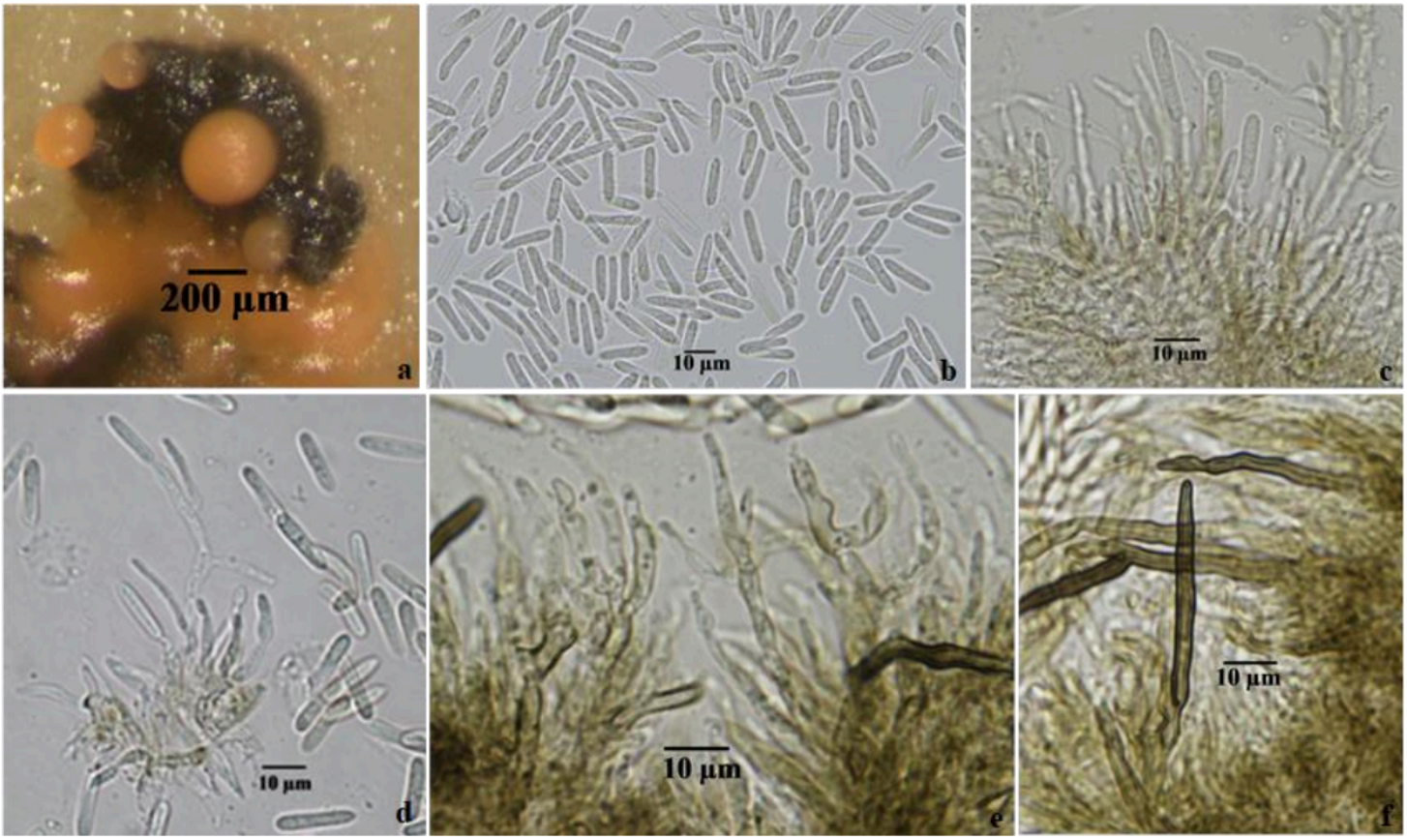

**Figure 1**

Symptoms of basil leaf spot caused by Colletotrichum siamense (a) field condition and (b,c) stereo microscope (scale bar = 1000 µm)



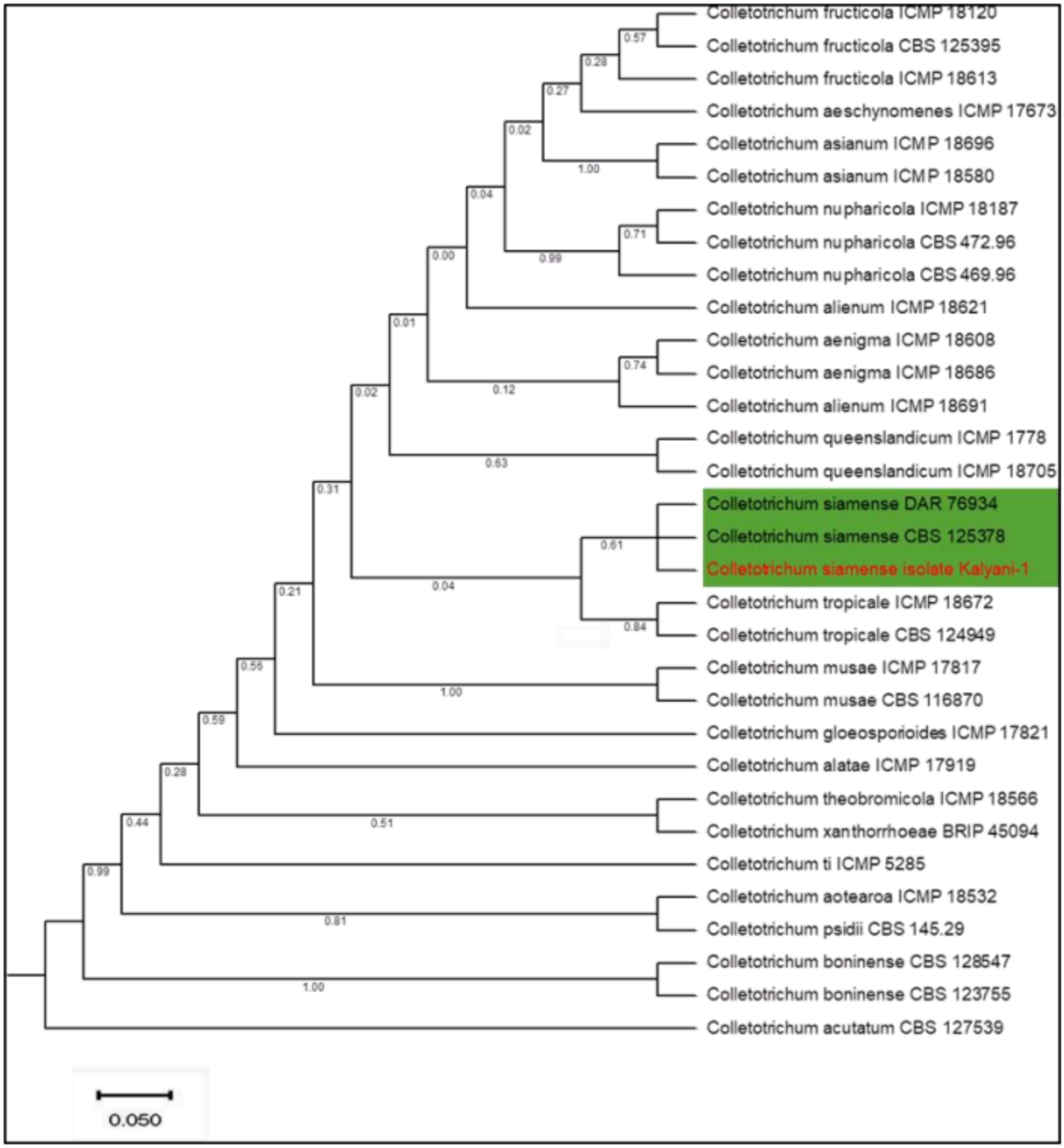

**Figure 2**

Pathogenicity test (a) pathogen inoculated plant in sterile condition (b) symptom on diseased leaf (c) comparison of healthy and diseased leaf




## Figure 3

*Colletotrichum siamense* on MMM media (a) Conidiomata (scale bar = 200 µm) (b) Conidia (scale bar = 10 µm)(c-d) Conidiophores with conidia (scale bar = 10 µm) (e) Conidiophores branching (scale bar = 10 µm) (f) Setae (scale bar = 10 µm)




## Figure 4

Phylogenetic tree constructed using Maximum Likelihood method and Tamura 3-parameter model with a discrete Gamma distribution to model evolutionary rate differences among sites. The sequence with *Colletotrichum siamense* isolate kalyani-1 is marked in red, while the *C. siamense* clade is marked in green. *Colletotrichum acutatum* is used as outgroup. This analysis involved 32 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a

total of 908 positions in the final dataset. The bootstrap values are provided on the branches (Scale bar = 0.05)