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Characterization and pathogenicity of colletotrichum species causing anthracnose in chilli

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Abstract
Chilli anthracnose, once thought to be caused by a single Colletotrichum species, is now known to involve a species complex whose members differ in virulence, host preference, and fungicide sensitivity. This research characterized 64 Colletotrichum isolates recovered from anthracnose-affected chilli fruits collected across Gujarat and Tamil Nadu during 2022-2023. Morphological examination and multi-locus phylogenetic analysis (ITS, TUB2, ACT, GAPDH) identified four species: *C. truncatum* (43.8%), *C. gloeosporioides* (28.1%), *C. siamense* (18.8%), and *C. fructicola* (9.4%). Pathogenicity tests on detached chilli fruits (cv. Pusa Jwala) showed that *C. truncatum* was the most aggressive, producing the highest disease severity (7.8 on 0-9 scale) and incidence (84.6%). Conidial morphology, colony characteristics, and growth rates differed significantly among species. These findings confirm a multi-species etiology for chilli anthracnose in India and highlight *C. truncatum* as the dominant and most virulent pathogen, which has direct implications for fungicide selection and resistance breeding strategies.

Keywords: Colletotrichum, anthracnose, chilli, pathogenicity, fungal characterization, *C. truncatum*, multi-locus phylogeny, species complex, disease severity, detached fruit assay

Introduction
If you pick up a chilli fruit with sunken dark lesions in any Indian market, the odds are high that more than one fungal species is responsible for what looks like a single disease. Anthracnose of chilli, caused by species within the genus Colletotrichum, is the most economically damaging post-harvest disease of this crop, causing losses of 10-80% depending on the cultivar and season [1]. For decades, the pathogen was identified simply as Colletotrichum capsici (now reclassified as *C. truncatum*), but molecular phylogenetics has revealed that at least 10-12 Colletotrichum species can infect chilli worldwide [2].

Accurate species identification matters because different Colletotrichum species vary in their sensitivity to fungicides, their preferred infection stage (pre-harvest vs. post-harvest), and their interaction with host resistance genes [3]. Multi-locus sequence analysis using ITS, beta-tubulin, actin, and GAPDH genes has become the standard for species delineation within this genus [4]. Despite this progress, the species composition of chilli anthracnose in the major growing states of Gujarat and Tamil Nadu has not been determined using modern phylogenetic methods. This research aimed to collect, characterize, and assess the pathogenicity of Colletotrichum isolates from these two states to establish which species are present and which pose the greatest threat.

Instrumentation and Molecular Methods
Morphological measurements were made using a Leica DM2500 compound microscope fitted with a DFC450 digital camera and LAS v4.12 image analysis software. Conidial dimensions were based on 50 conidia per isolate measured at 400× magnification. For molecular identification, DNA was extracted using the CTAB method from 7-day-old cultures grown on PDA at 25 °C. Four gene regions were amplified: ITS (primers ITS1/ITS4), beta-tubulin (Bt2a/Bt2b), actin (ACT-512F/ACT-783R), and GAPDH (GDF1/GDR1) [4]. PCR products were sequenced bidirectionally at Eurofins Genomics India. Sequences were aligned with MAFFT v7.490 and phylogenetic trees constructed using maximum likelihood in MEGA 11 with 1,000 bootstrap replicates. Species assignment

required placement within a well-supported clade (>70% bootstrap) containing the ex-type strain of the species [5].

Material and Methods

Material

Anthracnose-affected chilli fruits were collected from farmer fields and markets in Anand, Kheda, and Mehsana districts of Gujarat, and Coimbatore, Dindigul, and Madurai districts of Tamil Nadu between July 2022 and March 2023. Isolation followed standard tissue plating on PDA amended with streptomycin (50 mg/L). Monoconidial cultures were prepared and maintained on PDA slants at 4 °C [6]. A total of 64 isolates were obtained. Pathogenicity tests used detached mature green fruits of chilli cultivar Pusa Jwala, surface-sterilized with 1% NaOCl and wound-inoculated with 10 µl of conidial suspension (1 × 10^6 conidia/ml). Fruits were incubated in moist chambers at 28 °C for 10 days [7].

Methods

Colony characteristics (color, texture, growth rate) were recorded on PDA at 25 °C after 7 days. Conidial shape, size, and appressorium morphology were documented microscopically. Disease severity was rated on a 0-9 scale and incidence as percentage of inoculated fruits showing symptoms. Lesion diameter was measured at 7 days post-inoculation. Koch's postulates were fulfilled by re-isolation from inoculated fruits and confirmation by ITS sequencing. Data were analyzed by one-way ANOVA at P = 0.05 with Duncan's test [8].

Results

Table 1: Morphological characteristics of Colletotrichum species isolated from chilli anthracnose

Species	No. Isolates	Colony Color	Conidial Shape	Mean Conidial Length (µm)	Growth Rate (mm/day)
C. truncatum	28	Dark grey	Falcate	22.4 ± 1.8	8.6
C. gloeosporioides	18	Pale grey	Cylindrical	14.8 ± 1.2	7.2
C. siamense	12	White-grey	Cylindrical	12.6 ± 0.9	5.8
C. fructicola	6	Orange-grey	Cylindrical	13.2 ± 1.1	6.4

C. truncatum was the most frequently isolated species (43.8%) and had the fastest growth rate at 8.6 mm/day with distinctive falcate (sickle-shaped) conidia averaging 22.4 µm (Table 1). *C. gloeosporioides* and *C. siamense* both produced cylindrical conidia but differed in size and growth rate.

Table 2: Pathogenicity of Colletotrichum species on detached chilli fruits (cv. Pusa Jwala)

Species	Disease Severity (0-9 scale)	Incidence (%)	Lesion Size (mm)	Sporulation on Lesion
C. truncatum	7.8	84.6	12.4 ± 2.1	Heavy
C. gloeosporioides	6.2	72.3	9.8 ± 1.6	Moderate
C. siamense	4.6	58.4	7.2 ± 1.4	Light
C. fructicola	5.4	64.8	8.6 ± 1.8	Moderate
Control	0	0	0	None
SEm(±)	0.42	-	0.84	-
CD (P=0.05)	1.26	-	2.52	-

C. truncatum was the most virulent species, producing severity of 7.8 and incidence of 84.6% on detached fruits (Table 2). *C. siamense* was least aggressive at severity 4.6. All four species fulfilled Koch's postulates.

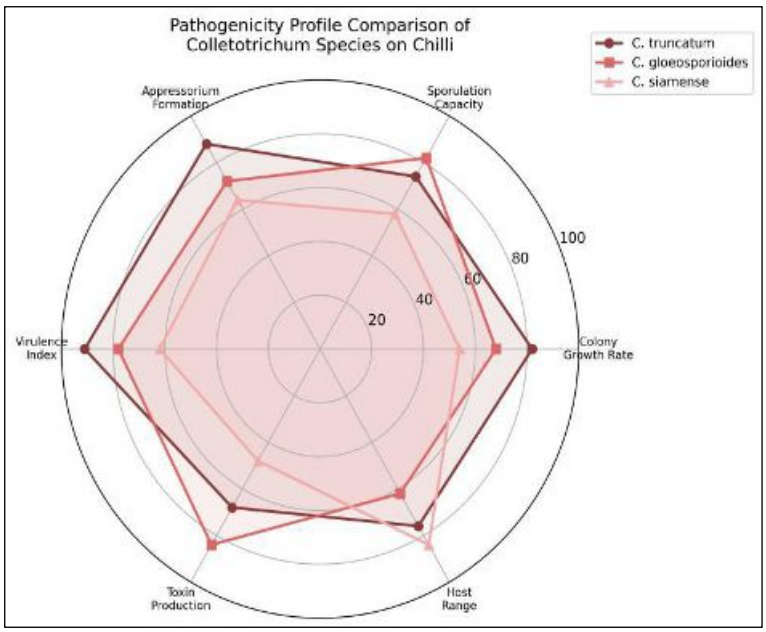

Fig 1: Radar chart comparing pathogenicity profiles of three dominant Colletotrichum species on chilli

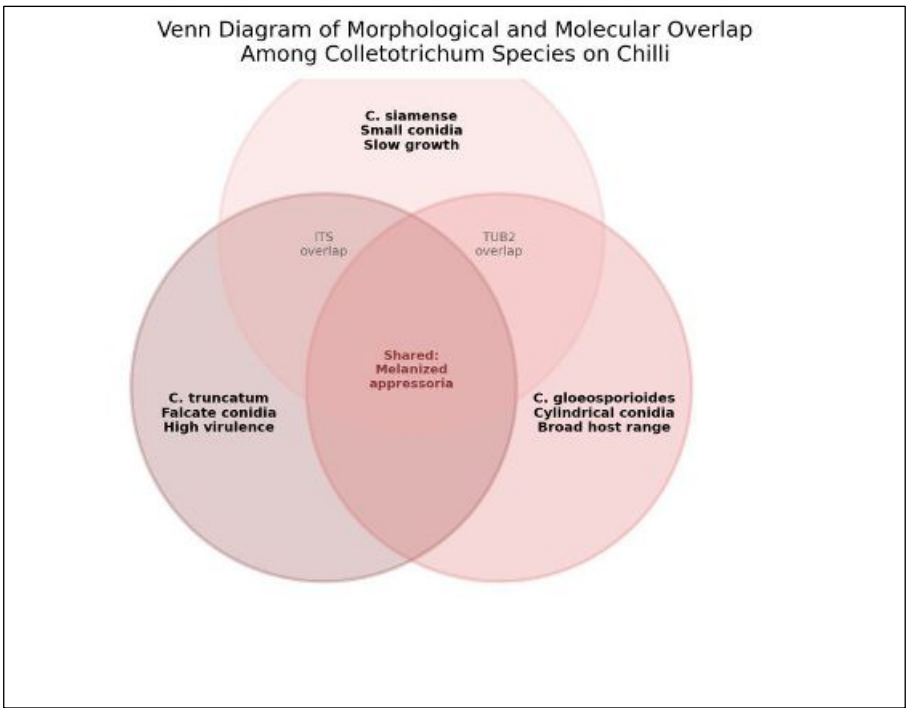

Fig 2: Venn diagram showing morphological and molecular overlap among Colletotrichum species causing chilli anthracnose

Comprehensive Interpretation

Multi-locus phylogeny resolved all 64 isolates into four well-supported clades. The ITS region alone could not distinguish *C. gloeosporioides* from *C. siamense* and *C. fructicola*, confirming the necessity of multi-locus analysis for accurate species identification in this complex. Bootstrap support for all four species clades exceeded 92%.

Discussion

The dominance of *C. truncatum* in both Gujarat and Tamil Nadu is consistent with earlier reports from India and confirms it as the principal anthracnose pathogen of chilli in the country [1, 2]. Its falcate conidial morphology and high growth rate likely contribute to its competitive advantage under field conditions. The detection of *C. siamense* and *C. fructicola* is significant because these species have been associated with resistance to certain QoI fungicides in other pathosystems [3], and their presence in chilli fields should be considered when designing spray programmes.

The inability of ITS alone to separate closely related species within the *C. gloeosporioides* complex reinforces the need for at least two additional loci (TUB2 and GAPDH) as recommended by the Colletotrichum working group [4, 5]. From a disease management perspective, the four-species profile established here means that fungicide sensitivity testing should be conducted on representative isolates of each species rather than treating the pathogen population as monolithic.

Conclusion

Chilli anthracnose in Gujarat and Tamil Nadu is caused by at least four Colletotrichum species, with *C. truncatum* being the most prevalent and virulent. Multi-locus phylogenetic analysis is essential for accurate species identification, as ITS alone is insufficient. The species-level diversity uncovered here has direct implications for fungicide resistance management and cultivar screening, as different species may respond differently to chemical and genetic control measures. Future work should assess the in vitro sensitivity of each species to commonly used fungicides.

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