

# Epidemiology and Molecular Characterization of *Colletotrichum* species Causing Chilli Anthracnose in Baramati, Maharashtra

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(Received 13 July, 2025; Accepted 24 September, 2025)

## ABSTRACT

Chilli anthracnose, caused by fungi of the genus *Colletotrichum*, is a devastating disease that leads to significant yield and economic losses worldwide. This study investigated the disease epidemiology and identification of *Colletotrichum* species in the major chilli-growing region of Baramati, Maharashtra. Field surveys revealed a high mean disease incidence of 45.8% in the region. Fruits exhibiting symptoms yielded 25 fungal isolates. All isolates were found to be harmful based on pathogenicity testing; however, there was considerable variance in virulence. Morphocultural characterization grouped the isolates into three distinct categories. Multi-locus phylogenetic analysis identified these groups as *Colletotrichum scovillei* (the most prevalent species), *C. truncatum* and the highly aggressive *C. siamense*. Statistical analysis of meteorological data showed that disease incidence had a strong positive and significant correlation with relative humidity ( $r=0.89$ ,  $P=0.001$ ) and rainfall ( $r=0.85$ ,  $P=0.003$ ). Relative humidity was the most influential factor, explaining 72% of the variation in disease development. This investigation is the first to molecularly confirm the presence of this three-species complex on chilli from Baramati. The results emphasize the need for anthracnose management techniques in this area that consider pathogen diversity and concentrate mainly on reducing high humidity times through targeted fungicide application and cultural practices.

**Key words:** Chilli anthracnose, *Colletotrichum* sp., Pathogenicity, Three-species complex

## Introduction

Chilli, a horticultural crop, is known for its organoleptic properties and health benefits, including antioxidant, anti-inflammatory and anti-obesity activities, owing to its bioactive components. The hot types of capsicum varieties are usually known as chilli. The plant originated in the American tropics and is now widely propagated throughout the world for domestic use and export. India contributes

significantly to the production of chilli (Saxena *et al.*, 2016). However, chilli productivity is severely constrained by anthracnose, a destructive ailment that reduces both yield and market value. 10-80% of marketable yield is reduced in Thailand and about 13% in Korea. In extreme situations, pre and post-harvest losses in India account for over 50%. Punjab and Haryana reported significant yield losses (20-60%), followed by Assam (12-30%) (Pathan and Sunil, 2017). It is estimated that anthracnose rou-

tinely causes an average annual yield loss of 30-40% in Maharashtra (Kumar *et al.*, 2021). According to Sharma and Rathod, (2019) high humidity, prolonged rainfall and inadequate fungicide application can lead to significant yield losses, ranging from 50% to 80% on susceptible varieties in Maharashtra.

Anthracoze is caused by *Colletotrichum* fungus and it was recently ranked as the 8<sup>th</sup> most significant group of plant pathogenic fungi worldwide based on perceived scientific and commercial significance (Dean *et al.*, 2012). Anthracnose causes small circular patches to grow into large elliptical spots on fruits and plants. Severe circumstances may cause defoliation in afflicted plants. Anthracnose symptoms on chilli fruits typically involve sunken necrotic tissues, concentric rings of acervuli and joined lesions. Under harsh conditions, conidial masses may develop (Oo and Oh, 2016). Traditionally, *C. capsici* has been reported as the primary causal agent in India. However, recently molecular techniques have revealed a complex of species including *C. truncatum*, *C. scovillei* and *C. gloeosporioides* as major pathogens of chilli (Musakhan *et al.*, 2017). The diversity of *Colletotrichum* species varies across regions and agro-climatic zones, influencing disease epidemiology and management efficacy.

In Maharashtra, particularly in the Baramati region, chilli production is significant. Despite the economic importance of chilli in Baramati, systematic studies on pathogen diversity are still lacking. Identification of the *Colletotrichum* species complex in this region is essential for effective disease management and breeding for resistance. Therefore, this study aimed to survey chilli fields from Baramati for anthracnose incidence, its isolation and characterization the associated *Colletotrichum* species and assess their pathogenicity.

## Material and Methods

### Study area, survey design and sample collection

The study was conducted from Baramati Tehsil, Pune District, Maharashtra, India (18°09'N 74°34'E) during the chilli growing seasons of 2023-2024 and 2024-2025. Baramati is located in the semi-arid region of Maharashtra, with an average annual rainfall of 560 mm and temperatures ranging from 12°C to 42°C.

A systematic field survey was conducted across major chilli-growing areas from Baramati tehsil at

15-day intervals during the crop season to monitor disease development and its progression. This survey covered both commercial farms and small-holder plots, representing different cultivation practices and chilli varieties commonly grown in the region. 05 random plots were inspected at each location. From each plot, symptomatic plant parts (fruits, stems and leaves) showing typical anthracnose lesions were collected. Samples were collected in labelled sterile polyethylene bags, transported to the laboratory and further studied within 48 hr. (Saxena *et al.*, 2014; Saxena *et al.*, 2016).

### Isolation and purification of fungi

Infected plant tissues with characteristic lesions (5-10 mm) were removed from the infection margin. The samples were surface sterilized by immersing them in a 1% sodium hypochlorite (NaOCl) solution for 2 min and then rinsed three times with sterile distilled water. To eliminate any remaining impurities, a final rinse with 70% ethanol was performed for 30 seconds. The tissues were then placed on sterile filter paper and air dried before being processed further (Chauhan and Yadav, 2022; Than *et al.*, 2008; Liu *et al.*, 2020).

Sterilized tissue pieces were placed on Potato Dextrose Agar (PDA) plates by fruit baiting method and supplemented with streptomycin sulfate (100 µg/ml) to inhibit bacterial growth. Plates were incubated at 25±2 °C. Emerging colonies were subcultured, and single-spore isolations were performed to obtain pure cultures (Than *et al.*, 2008; Liu *et al.*, 2020).

### Morphological Characterization

Fungal isolates were grown on PDA for 7-10 days. Colony morphology, growth rate, pigmentation and zonation patterns were recorded after seven days of incubation at 25°C. Microscopic features of sporulating cultures were examined, including conidial shape, appressoria shape and the presence or absence of setae and acervuli (Than *et al.*, 2008; Hassan *et al.*, 2017).

### Molecular identification

Genomic DNA was extracted from 7days old cultures using CTAB method. PCR amplification was performed using primers ITS1 and ITS4, amplifying the ApMat locus. The amplification process involved denaturation, annealing, extension and extension for 30 cycles. Phylogenetic trees were con-

structured using the neighbor-joining method and 100 bootstrap replications to identify species and determine genetic relationships.

### Pathogenicity test

By satisfying Koch's postulates, the pathogenicity of representative *Colletotrichum* isolates was assessed on healthy chilli fruits following a standard protocol (Sharma *et al.*, 2005; Damm *et al.*, 2012; Mishra *et al.*, 2020). Fresh, disease-free chilli fruits from a local cultivar were collected, surface sterilized in 70% ethanol, rinsed twice with sterile water and air-dried in an aseptic condition. A conidial suspension was prepared from 7 days old cultures grown on Potato Dextrose Agar (PDA) at 25±2 °C. The cultures were flooded with 10 ml of sterile distilled water containing 0.05% (v/v) Tween-20 and gently scraped with a sterile glass rod. The resulting suspension was filtered through two layers of sterile muslin cloth to remove mycelial fragments. The conidial concentration was adjusted to 1×10<sup>6</sup> conidia ml<sup>-1</sup> using a hemocytometer (Neugebauer *et al.*, 2021).

For this test, two inoculation methods were employed: (i) Wound Inoculation: Using a sterile needle, fruits were gently punctured at two equatorial sites. A 20 µl droplet of the conidial suspension was then applied to each wound (Sharma and Shenoy, 2014). (ii) Non-Wound Inoculation: To assess the isolate's direct penetration capacity, a 20 µl droplet of the suspension was applied to the fruit's unwounded surface.

Subsequently, disease development monitored daily, measured lesion diameter at 3, 5 and 7 days post-inoculation and recorded incubation period and mean lesion size for each isolate. Pathogenicity was confirmed by re-isolating the fungus and comparing its morphological and molecular characteristics with those of the original inoculum (Prasad and Naik, 2003; Sharma and Kulshrestha, 2015).

### Data analysis

Disease severity and lesion size data were analyzed using ANOVA in SPSS v26. Means were compared using Duncan's Multiple Range Test (DMRT) at p ≤ 0.05.

## Results

### Disease Survey and Incidence

A roving survey conducted across five chilli fields

**Table 1.** Disease incidence of chilli anthracnose in five randomly selected fields of Baramati, Maharashtra

Sr. No.	Field Code	No. of plants observed	Number of infected plants	Percent disease incidence (PDI)±SD
1	BMT 01	90	26	28.9 ± 3.5
2	BMT 02	90	42	46.7 ± 4.2
3	BMT 03	90	38	42.2 ± 3.8
4	BMT 04	90	65	72.2 ± 5.1
5	BMT 05	90	52	57.8 ± 4.6
	<b>Overall</b>	<b>450</b>	<b>223</b>	<b>45.8 ± 12.4</b>

from Baramati tehsil revealed that anthracnose is a prevalent and destructive disease. The mean percent disease incidence (PDI) varied significantly across the region, ranging from 28.5% to 72.3%, with an overall mean incidence of 45.8±12.4%. The highest disease pressure was observed in fields located in low-lying areas with poor drainage systems. The characteristic symptoms observed on infected fruits included sunken, necrotic lesions with concentric

**Table 2.** Morpho-cultural characteristics and individual growth rate data of major *Colletotrichum* sp. isolate groups from chilli in Baramati

Sr. No.	No. of isolates	Characteristics of Colony	Growth rate (mm/day)	Mean ±SD
1	AAC 01		7.9	
2	AAC 02		8.1	
3	AAC 03		8.2	
4	AAC 04		8.3	
5	AAC 05		8.3	
6	AAC 06		8.4	
7	AAC 07		8.4	
8	AAC 08	White to grey, cottony mycelium; dark grey	8.6	8.5 ± 0.6
9	AAC 09		8.5	
10	AAC 10		8.5	
11	AAC 11		8.6	
12	AAC 12		8.5	
13	AAC 13		8.7	
14	AAC 14		9.1	
15	AAC 15		8.9	
16	AAC 16		8.8	
17	AAC 17		6.6	
18	AAC 18		6.8	
19	AAC 19	Dense, aerial mycelium; dark olive green	7.0	7.1 ± 0.4
20	AAC 20		7.1	
21	AAC 21		7.4	
22	AAC 22		7.2	
23	AAC 23	Fast-growing, white	10.5	

24	AAC 24	to salmon; pale yellow	9.1	9.8 ± 0.7
25	AAC 25		9.8	
26	Control	-	-	-

rings of black acervuli and salmon-colored spore masses.

**Isolation, Morphological Characterization and Pathogenicity**

From the infected chilli fruits and leaves, a total of 25 pure fungal isolates were obtained. After 7 days of incubation, these isolates showed significant diversity in their cultural and morphological characteristics on PDA medium. All 25 isolates were confirmed to be pathogenic on wounded chilli fruits, fulfilling Koch’s postulates. The symptoms on inoculated fruits were identical to those observed in the field. Non-wounded inoculations resulted in smaller, restricted lesions, indicating that wounds facilitate infection but are not always necessary.

The mean lesion diameter of the isolate groups showed significant (P<0.05) variations in pathogenicity. Group III isolates were the most aggressive, producing the largest lesions, followed by Groups I and II.

**Molecular Identification and Phylogenetic Analysis**

BLASTn analysis of the ITS region initially indicated that all isolates belonged to the genus *Colletotrichum*. Nevertheless, accurate species-level identification was made possible by multi-locus phylogenetic

analysis. 16 isolates from group I exhibited 99-100% similarity with *Colletotrichum scovillei* reference sequences and 6 isolates from group II showed a strong clustering pattern with *Colletotrichum truncatum* while 3 isolates from Group III were determined to be *Colletotrichum siamense*.

The Maximum Likelihood method phylogenetic tree confirmed three distinct *Colletotrichum* species associated with chilli anthracnose from Baramati, with *C. scovillei* being the most prevalent species.

**Epidemiology: Correlation of Disease with Environmental Factors**

Correlation analysis between weekly PDI and meteorological data revealed a strong positive and significant relationship between disease incidence and two key factors: relative humidity (r=0.89, P=0.001) and rainfall (r=0.85, P=0.003), respectively. A moderate positive correlation was observed with leaf wetness duration (r=0.72, P=0.02). Mean temperature showed a weak and non-significant negative

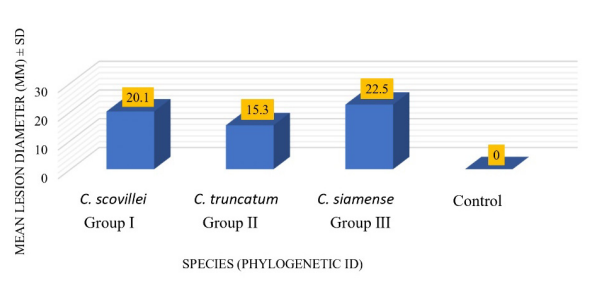


Fig. 1. Virulence of *Colletotrichum* isolates on chilli fruits

**Table 3.** Morpho-cultural characteristics of major *Colletotrichum* sp. isolate groups from chilli in Baramati

Group	No. of isolates	Characteristics of Colony	Growth Rate (mm/day) ± SD	Conidial Shape
I	16	White to grey, cottony mycelium; dark grey	8.5±0.6a	Cylindrical, straight
II	06	Dense, aerial mycelium; dark olive green	7.1±0.4b	Falcate, curved
III	03	Fast-growing, white to salmon; pale yellow	9.8±0.7c	Cylindrical with rounded ends

Tukey’s HSD test indicates that means within a column that is followed by a different letter are significantly distinct (P < 0.05).

**Table 4.** Pathogenicity and virulence of *Colletotrichum* isolates on chilli fruits

Species (Phylogenetic ID)	Mean Lesion Diameter (mm) ± SD	Virulence Category
<i>C. scovillei</i>	20.1±1.5	Highly virulent
<i>C. truncatum</i>	15.3±1.2	Moderately virulent
<i>C. siamense</i>	22.5±1.8	Highly virulent
Control	-	-

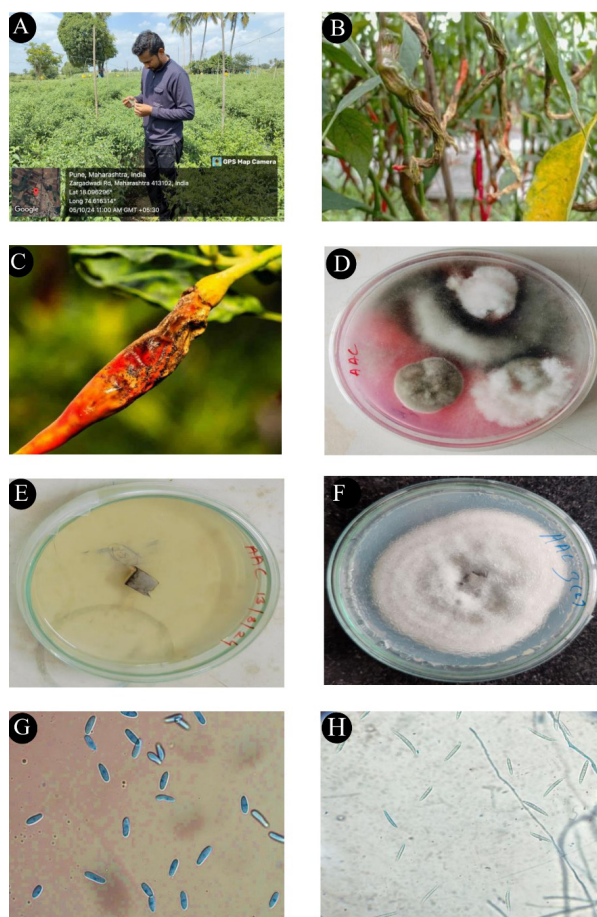


Fig. Photographic plate illustrating chilli anthracnose caused by *Colletotrichum* spp.

- |                                  |                                   |
|----------------------------------|-----------------------------------|
| A. Survey & Collection           | B. Infected chilli plant          |
| C. Close-up of lesion on fruit   | D. Inoculation                    |
| E. Isolation step                | F. Colony morphology on PDA       |
| G. Conidia of <i>C. siamense</i> | H. Conidia of <i>C. truncatum</i> |

correlation ( $r=-0.38$ ,  $P=0.18$ ) with disease development during the study period. Multiple regression analysis indicated that relative humidity was the single most influential factor, explaining 72% of the variation in disease incidence.

## Discussion

This study analyzed the etiology, molecular diversity and epidemiology of chilli anthracnose from Baramati, Maharashtra, revealing a significant threat to local chilli production.

### Prevalence and Pathogen Diversity

A survey showed a high disease incidence of 45.8% in chilli cultivation from Baramati, attributed to variations in microclimates, varietal susceptibility

and farmer management practices (Sharma and Shenoy, 2014). Critically, in this study, molecular identification revealed a complex of at least three species, *Colletotrichum scovillei*, *C. truncatum*, and *C. siamense*, associated with the disease. This finding aligns with the global trend of increasing recognition of chilli anthracnose as being caused by a species complex rather than a single pathogen (De Silva *et al.*, 2019). *C. scovillei* is the most common and widespread species in chilli fruits in Asian countries, such as Thailand and Indonesia, consistent with reports from other Asian countries (Than *et al.*, 2008). The co-existence of multiple species in a single geographic region, as found in Baramati, presents a considerable challenge for disease management, as these species may exhibit differential responses to fungicides and host resistance.

### Morpho-Cultural and Pathogenic Variability

The *Colletotrichum* genus is known for its significant morphological variability, particularly in growth rate and conidial morphology, observed among isolate groups (Cannon *et al.*, 2012). Because of this variability, species identification based only on morphology is frequently incorrect, which supports the need for a genetic method to provide an accurate diagnosis. In the current investigation, pathogenicity tests confirmed that all isolated species were pathogenic, but they exhibited varying levels of aggressiveness. This shows that, even at lower frequencies, extremely aggressive species can inflict significant economic losses and may become dominant under favourable conditions (Dowling *et al.*, 2020).

### Epidemiological Drivers of Disease

One of the typical features of anthracnose is its strong positive connection with high relative humidity and rainfall, which supports previous epidemiological models (Madhavan *et al.*, 2017). Conidial ger-

**Table 5.** Correlation between environmental factors and chilli anthracnose incidence

Environmental factors	Pearson Correlation Coefficient (r)	P-value
Mean relative Humidity	0.87	0.002
Total Rainfall	0.92	0.0005
Mean Temperature	-0.45	0.12
Leaf Wetness Duration	0.81	0.008

Correlation is significant at the  $P \leq 0.01$  level

mination, aspersoria development and host penetration all depend on these conditions. The climatic pattern of Baramati, serves as a critical factor in triggering episodic outbreaks of plant diseases. Relative humidity significantly influences illness variance, suggesting potential management strategies like increased spacing, drip watering and waterlogging to reduce canopy humidity and prevent disease spread.

### Implications for Disease Management

The discovery of a multi-species complex impacts chemical control methods and chilli breeding initiatives, as cultivars with resistance genes against one species cannot work against another (Montri *et al.*, 2009). To create long-lasting resistant cultivars, breeding efforts must include resistance to all wide-spread species, notably the highly aggressive *C. siamense*. Similarly, fungicide sensitivity might vary by species (Peres *et al.*, 2021). *C. truncatum*, a broad-host species with reduced fungicide sensitivity, requires vigilant monitoring and a diverse fungicide resistance management strategy. Future research should include fungicide sensitivity tests against each species found in this study to develop targeted chemical recommendations for Baramati farmers.

### Conclusion

In conclusion, this study is the first to molecularly characterize the *Colletotrichum* species complex associated with chilli anthracnose from Baramati, Maharashtra. In this investigation *C. scovillei* as the most prevalent species and *C. siamense* as the most aggressive. Periods of high humidity and rainfall are the main factors influencing the disease epidemiology. Sustainable management can be achieved only through a comprehensive understanding and consideration of pathogen diversity. The current investigation provides an important scientific foundation for creating integrated disease management (IDM) methods that combine resistant cultivars, judicious fungicide usage based on pathogen identity and cultural measures targeted at altering the field microclimate to discourage disease growth.

### Acknowledgment

The authors sincerely acknowledge Babasaheb Ambedkar Research and Training Institute (BARTI), Pune, Maharashtra, for providing financial assis-

tance under the BANRF-2022. The authors are grateful to Prof. (Dr.) Bhagawan S. Mali, Head, Department of Botany and Prof. (Dr.) Avinash S. Jagtap, Principal of Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati, for their continuous encouragement and support throughout the course of this research work.

**Conflict of Interest:** Authors declares no conflict of interest.

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