



Cultural and Physiological Variability in *Colletotrichum musae*-Causal Agent of Banana Post-Harvest Fruit Rot under Hill and Plain Agro-Ecosystems of Meghalaya

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Abstract: The study investigated the fungal pathogens contributing to the banana fruit rot complex, identifying *Colletotrichum musae* as significant causative agent. Ten *C. musae* isolates obtained from different market localities were subjected to cultural and physiological variability analyses. The isolates exhibited characteristics such as hyaline, aseptate, cylindrical conidia with dimensions ranging from 7.03-10.77 μm (length) and 3.02-3.77 μm (width), while acervuli sizes varied from 53.6 to 78.99 μm in diameter. Among tested media, Oat meal agar proved most suitable for *C. musae* growth, followed by potato dextrose agar, with Richard's agar showing sparse mycelial density. Martin's rose bengal agar demonstrated the lowest average radial growth and, thus, was deemed unsuitable. Colony colours were predominantly salmon white, greyish white, or completely white across the five media. Temperature studies revealed optimal radial growth for *C. musae* at temperatures between 15 and 27°C, with 27°C recording maximum growth. No growth occurred at 4 and 40°C. The pH of 6, 7, and 8 supported optimal growth, while extremes below 6 or above 8 were unfavourable. The slightly acidic pH of 6 was identified as the most suitable for *C. musae* growth when using PDA as a basal medium.

Keywords: *Musa* spp., *Colletotrichum musae*, Variability, Temperature, pH

Currently, India contributes 0.3% to the total global banana exports, holding the 21st position among exporters. India is responsible for 19.37% of the global banana production, cultivating 33.06 million tons across an area of 0.924 million hectares (Anonymous 2020). Bananas are cultivated in the elevated terrain of Meghalaya, located in the north-eastern region of India, particularly in mid-hill areas, primarily in the Ri-Bhoi district and to a lesser extent in the East Khasi Hills district. In Meghalaya, bananas are cultivated over 7,264 hectares, with specific regions like Ri-Bhoi covering 949 hectares and East Khasi Hills spanning 795 hectares. The overall state production amounts to 94,603 metric tons, with Ri-Bhoi contributing 17,614 metric tons and East Khasi Hills contributing 9,074 metric tons (Anonymous 2019). Significant losses occur during the transportation of bananas to markets, primarily due to the ripening process, leading to post-harvest and storage diseases such as anthracnose, crown rot, and cigar end rot, among others. These diseases pose significant challenges to the marketability and export value of bananas. Anthracnose and crown rot, in particular, emerge as substantial post-harvest concerns for bananas intended for export. The overall quality of the produce is influenced by a combination of environmental factors and both pre- and post-harvest conditions. Banana fruit rot disease results from a

combination of fungal genera, comprising *Colletotrichum musae*, *Musicillium theobromae*, *Lasiodiplodia theobromae*, *Nigrospora sphaerica*, *Cladosporium* sp., *Acremonium* sp., *Ceratocystis paradoxa*, *Penicillium* sp., *Aspergillus* sp., and numerous *Fusarium* spp. (Snehalatharani et al 2021)

The post-harvest fruit rot of bananas is prevalent in Meghalaya and no investigations were conducted on the morphological, cultural and physiological variability of different isolates of *C. musae* collected from diverse agro-ecosystems in the state. The current research was undertaken to evaluate the morphological, cultural, and physiological variations among *C. musae* isolates gathered from both hilly and lowland banana varieties in Meghalaya.

MATERIAL AND METHODS

The ten isolates were acquired from ten diverse market locations in the two districts of Meghalaya, specifically, Ri-Bhoi (Khanapara, Bymihot, Nongpoh, Bhoirymbong, and Umsning) and East Khasi Hills (Bara bazaar, Jalupara, Laitumukrah, Polo bazar, and Sohra) during the year 2019-20. Each of these ten isolates was sourced from distinct banana varieties namely 'Saeel', 'Sahnsniang', 'Kait Syiem', 'Kait Khar', 'Kait Mon', 'Borton', 'Kait Kaji', 'Jahaji', 'Malbhog' and 'Chini Champa' (Table 1) and were designated with isolate codes Cm-1 to Cm-10 respectively (Table 2). The

banana cultivars 'Saeel,' 'Sahsniang,' 'Kait Syiem,' 'Kait Khar,' 'Kait Mon,' 'Borton,' and 'Kait Kaji' are categorized as hill varieties, whereas 'Jahaji,' 'Malbhog,' and 'Chini Champa' are predominantly found in the lowland regions of Meghalaya, particularly in areas contiguous with Assam. *Colletotrichum musae* isolates were purified on PDA slants and subsequently stored in a refrigerator at 4°C. Spores of *C. musae* isolates were placed on a clean glass slide. These spores were thoroughly mixed with lactophenol to achieve a uniform spread and then covered with a cover slip. Fifty conidia and acervuli from each of the ten isolates were measured under a high-power objective using an ocular and stage microscope.

The growth characteristics of *Colletotrichum musae* were examined on five solid media, namely, PDA, Czapek's Dox agar (CDA), Oat meal agar (OMA), Richards's agar (RA), and Martin's rose bengal agar (MRBA). The composition of these various media was sourced from "Dictionary of the Fungi" by Ainsworth and Bisby (1961) and "Plant Pathological Methods, Fungi and Bacteria" by Tuite (1969). All the media were sterilized at 121°C for 15 minutes. Then, 20 ml of each

medium was poured into individual Petri plates. These plates were inoculated aseptically with a 5 mm disc taken from the outer edge of an actively growing culture. Subsequently, the plates were incubated in the dark at a temperature of 27±2°C for seven days. Every treatment was duplicated three times. Observations were made regarding colony size seven days after inoculation. Details such as colony colour, appearance, shape, zonation, and acervuli production were documented at the same seven-day mark. The data regarding radial growth were then subjected to statistical analysis using SPSS 16.0 software.

The pathogen's growth was assessed at various temperatures, including 4, 15, 27, and 40°C. 20 ml of sterilized PDA medium was dispensed into Petri plates with a diameter of 90 mm. These plates were then aseptically inoculated with a 5 mm disc from a seven-day-old culture of the pathogen. The Petri plates were placed in incubators set at different temperatures, and each treatment was repeated three times. After a seven-day incubation period, observations were made to record the colony diameter. PDA served as the base medium, with its pH adjusted using 0.1N NaOH (alkali) or 0.1N HCl (acid). The pH levels tested ranged from 4.0 to 9.0. The culture was introduced into Petri plates with a diameter of 90 mm, each containing 20 ml of the base medium. These plates were then kept in an incubator at a temperature of 27±2°C for period of 7 days. Three replicates were maintained for each pH level of the medium. Following the 7-day incubation period, observations regarding the colony diameter were documented.

RESULTS AND DISCUSSION

The characteristic symptoms caused by *C. musae* on banana fruits involve sunken lesions that are adorned with acervuli displaying a salmon colour. As the fruits ripen, these sunken areas progress into brown spots with acervuli taking on an orange hue. The average size of the conidia and

Table 1. GPS coordinates of the surveyed locations

Place of collection	Coordinates
Sohra	25.2702° N, 91.7323° E
Laitumkhrah	25.5707° N, 91.8977° E
Jhalupara	25.5739° N, 91.8701° E
Barabazar	25.5724° N, 91.8745° E
Polo Bazar	25.5812° N, 91.8882° E
Bhoirymbong	25.7095° N, 92.0219° E
Umsning	25.7478° N, 91.8889° E
Nongpoh	25.8699° N, 91.8337° E
Byrnihut	26.0515° N, 91.8696° E
Khanapara	26.1213° N, 91.8208° E

Table 2. Morphological variability among isolates of *C. musae*

Isolate	Place of collection	Variety	Length of conidia (µm)	Breadth of conidia (µm)	Diameter of acervuli (µm)
Cm-1	Sohra	Saeel	7.76	3.59	66.23
Cm-2	Laitumkhrah	Sahsniang	10.77	3.65	67.90
Cm-3	Jhalupara	Kait Syiem	8.83	3.02	66.30
Cm-4	Barabazar	Kait khar	8.07	3.24	68.56
Cm-5	Polo Bazar	Kait mon	9.69	3.36	70.60
Cm-6	Bhoirymbong	Borton	7.25	3.16	53.60
Cm-7	Umsning	Kait Kaji	9.29	3.77	78.99
Cm-8	Nongpoh	Jahaji	7.03	3.61	61.36
Cm-9	Byrnihut	Malbhog	8.74	3.49	60.23
Cm-10	Khanapara	Chini Champa	9.36	3.21	62.35

acervuli of the ten isolates were computed, and photomicrographs were captured to illustrate the typical morphology of the fungus (Fig. 1a-t). The conidia shapes in all *C. musae* isolates were transparent and cylindrical. The size of these conidia ranged from 7.03×3.61 to 10.77×3.65 μm (Table 2). Every *C. musae* isolate generated transparent cylindrical conidia. The acervuli diameter (excluding setae) varied between 53.6 and 78.99 μm (Table 2). Saurabh et al (2023) documented that the conidia were oblong to cylindrical in shape and measured 6 to 18 μm in length and 3 to 6 μm in width. Lim et al (2002) documented aseptate, hyaline, predominantly ellipsoid conidia of *C. musae*. Jagana et al (2017) stated that the conidia size ranged from 11.43 to 16.27 x 3.86-5.47 μm. Saurabh et al (2023) indicated that the acervuli (including setae) measured between 111 to 450 μm in length and 42 to 180 μm in width, with setae dimensions of 82-125 μm in length and 3-5 μm in width. Consequently, the actual acervuli size can be calculated as ranging from 29 to 325 μm, which aligns with the findings of our study. The morphological variations observed in the current study validate the findings of these earlier research studies.

Radial growth, colony colour, appearance, shape, zonation, acervuli production, and consistency of the culture were recorded in the five different media viz., Potato dextrose agar (PDA), Martin's rose bengal agar (MRBA), Oat meal agar (OMA), Richard's agar (RA), Czapek's Dox agar (CDA) following a seven day incubation period. Nearly all the colonies from the isolates displayed a white or salmon-white coloration on OMA, Martin's rose bengal MRBA, CDA and Richard's agar RA. In PDA few isolates exhibited a slightly greyish-white mycelium, while the majority of isolates appeared white even on PDA medium. The average radial diameter was highest in OMA medium, 8.9 cm for the Cm-1 isolate, followed closely by isolates Cm-3 and Cm-5 in PDA medium, with a growth of 8.83 cm (Table 3). Thangamani et al (2011) observed the greatest average colony diameter on PDA medium, followed by OMA medium. Across PDA, OMA, RA, CDA, and MRBA media, the colonies exhibited colours ranging from white to pink for different isolates. The present observations align with the results reported by Thangamani et al (2011).

The growth and development of any pathogen are primarily influenced by temperature and pH of medium. The examination of the growth of the *C. musae* pathogen at varying temperatures indicated a preference for a temperature range between 15 and 27°C across all isolates (Table 4). The majority of isolates exhibited the highest average radial growth at a temperature of 27°C. The fungus displayed no mycelial growth at temperatures of 4°C and 40°C. Unnithan et al (2017) observed peak growth of *C. musae* was at 30°C (9 cm).

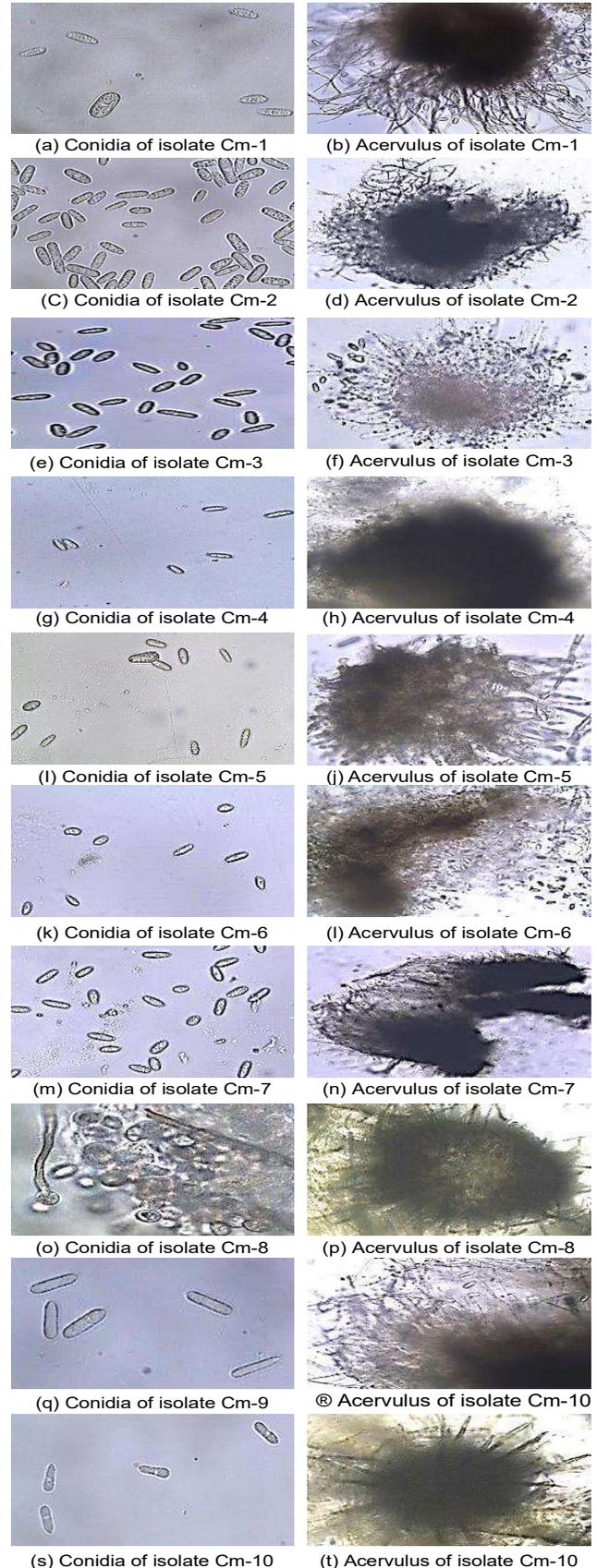


Fig. 1. Conidia and acervuli of *C. musae* isolates

Table 3. Radial Growth (cm) of *C. musae* in different media

Isolate	PDA	RBA	OMA	RA	CDA
Cm-1	8.70 ^{ab}	6.00 ^c	8.90 ^a	8.23 ^{bc}	7.8 ^b
Cm-2	8.80 ^a	7.23 ^b	7.77 ^{de}	8.50 ^{ab}	7.93 ^b
Cm-3	8.83 ^a	7.40 ^b	7.97 ^d	8.50 ^{ab}	7.23 ^c
Cm-4	8.07 ^c	8.26 ^a	7.67 ^e	8.80 ^a	8.17 ^{ab}
Cm-5	8.83 ^a	4.47 ^e	7.70 ^e	7.87 ^{cd}	7.00 ^{cd}
Cm-6	7.10 ^f	5.03 ^d	8.60 ^b	8.43 ^{ab}	6.83 ^d
Cm-7	7.40 ^e	5.70 ^{±c}	8.33 ^c	8.80 ^a	8.53 ^a
Cm-8	7.77 ^d	7.13 ^b	7.03 ^f	7.70 ^{de}	7.00 ^{cd}
Cm-9	7.00 ^f	4.23 ^e	8.80 ^{ab}	8.47 ^{ab}	7.23 ^c
Cm-10	8.50 ^b	4.20 ^e	7.73 ^{de}	7.40 ^e	6.67 ^d

Table 4. Radial growth (cm) of *C. musae* at different temperatures (*NG= No growth)

Isolate	4°C	15°C	27°C	40°C
Cm-1	NG	4.67 ^d	8.70 ^{ab}	NG
Cm-2	NG	2.67 ^f	8.80 ^a	NG
Cm-3	NG	4.20 ^e	8.83 ^a	NG
Cm-4	NG	2.90 ^f	8.07 ^c	NG
Cm-5	NG	4.20 ^e	8.83 ^a	NG
Cm-6	NG	5.50 ^b	7.10 ^f	NG
Cm-7	NG	6.60 ^a	7.40 ^e	NG
Cm-8	NG	4.10 ^e	7.77 ^d	NG
Cm-9	NG	5.10 ^c	7.00 ^f	NG
Cm-10	NG	4.07 ^e	8.50 ^b	NG

Table 5. Radial growth (cm) of *C. musae* at different pH levels

Isolate	pH4	pH5	pH6	pH7	pH8	pH9
Cm-1	4.80 ^c	7.53 ^{bc}	8.93 ^a	8.70 ^{ab}	8.70 ^{ab}	5.80 ^{bc}
Cm-2	4.87 ^{bc}	8.63 ^a	8.70 ^{bc}	8.80 ^a	6.50 ^d	6.00 ^{ab}
Cm-3	2.70 ^f	7.70 ^b	7.97 ^d	8.83 ^a	8.70 ^{ab}	4.90 ^d
Cm-4	4.83 ^{±bc}	5.13 ^f	7.73 ^e	8.07 ^c	4.17 ^{bc}	5.73 ^c
Cm-5	5.20 ^b	5.60 ^e	8.67 ^{bc}	8.83 ^a	8.77 ^a	4.70 ^d
Cm-6	3.23 ^e	3.80 ^g	5.63 ^g	7.10 ^f	4.17 ^f	3.60 ^f
Cm-7	4.83 ^{bc}	5.00 ^f	6.30 ^f	7.40 ^e	4.27 ^f	4.13 ^e
Cm-8	2.87 ^f	3.63 ^g	5.40 ^h	7.77 ^d	8.37 ^c	6.10 ^a
Cm-9	3.60 ^d	6.87 ^d	8.60 ^c	7.00 ^f	4.83 ^e	2.20 ^g
Cm-10	6.10 ^a	7.33 ^c	8.83 ^{ab}	8.50 ^b	4.67 ^e	1.90 ^h

The isolate Cm-1 exhibited the highest growth at pH 6 (8.93 cm) followed by isolates Cm-3 and Cm-5 at pH 7 (8.83 cm). Consequently, pH levels 6 and 7 were identified as the optimum range for the favourable growth of *C. musae*. Isolate Cm-10 displayed the minimal radial growth (1.9 cm) at pH 9, while Cm-3 exhibited the least growth (2.7 cm) at pH 4. pH levels below 6 and above 8 were unsuitable for the optimal growth of the fungus (Table 4). Jagana et al (2017)

documented the highest dry mycelial weight of *C. musae* at pH 6.0 (398 mg), which was comparable to pH 7.0 (383 mg), followed by 8.0 (377.33 mg). Similarly, Yang et al (2000) observed that the peak growth and spore germination of *C. musae* occurred at pH 6 and 7, respectively.

CONCLUSION

Oat meal agar and potato dextrose agar were the optimal

substrates for the proliferation of *Colletotrichum musae*. Additionally, the research identifies the optimal thermal range for the growth and development of the fungus to be between 15 and 27°C, while temperatures of 4 and 40°C inhibit its growth. The fungus exhibits optimal survival at a neutral pH range of 6-7, with growth cessation occurring at pH levels outside this range. Future research could explore the genetic and biochemical responses of the fungus to varying temperatures and pH levels which could provide insights into its adaptability and resilience at various climatic conditions. Additionally, developing more effective control strategies for managing *Colletotrichum musae* in agricultural settings could benefit from these findings, particularly by manipulating environmental conditions for storage and transportation of the fruits from fields to the markets to inhibit fungal growth.

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