

Identification and Host Range Tests of *Colletotrichum* sp. Associated with the Manalagi Mango Cultivar

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ABSTRACT

Anthrachnose is a significant disease in mango production and other tropical fruits, often causing major yield reductions. The disease is caused by the fungal pathogen *Colletotrichum* sp. could infect many fruits commodities included mango. This study aims to identify morphological *Colletotrichum* spp. associated in Manalagi Mango and its host-range test among the fruits. Two isolates (MM1 and MM2) were obtained from symptomatic mango fruits and characterized based on macroscopic and microscopic morphological features. Both isolates exhibited rapid mycelial growth and cylindrical conidia, consistent with characteristics of the *Colletotrichum* species. Host-range tests were performed on citrus, chayote, tomato, and cayenne pepper. MM1 showed limited host range, while MM2 infected all tested hosts with higher aggressiveness, especially on tomato and cayenne pepper. The results confirmed the wide host adaptability of *Colletotrichum* spp. and their potential for host-range among fruit commodities. Accurate identification and host-range assessment are critical for effective anthracnose disease management, and future studies should incorporate molecular tools for precise species delineation.

Keywords: Anthracnose; *Colletotrichum*; Host Range; Manalagi Mango; Morphological Identification

INTRODUCTION

Mango is one of the most popular fruit commodity that has expanded more than 100 countries, especially Indonesia (Rattanakreetakul et al., 2023). Mango productivity reached out 3.2 million tons/year, Indonesia holds the fourth position globally in mango production (Badan Pusat Statistika, 2026; Benatar et al., 2021). But one of the biggest obstacle that caused mango yield loss is *Colletotrichum* spp. *Colletotrichum* spp. is globally recognize for infecting mango diseases with potential yield losses of reaching up to 100% (Nurlaelita et al., 2024). In addition to fruit, the pathogen can also infect leaves, flowers, and

inflorescences. A 100% disease incidence rate has been noted during the blossoming stage, particularly in situations with high humidity levels (Bincader et al., 2021; Tovar-Pedraza et al., 2020). Any stage of mango fruit can be infected (Benatar et al., 2021; Giblin et al., 2018; Mo et al., 2018; Wu et al., 2020). On premature fruit, usually easy to dropped from the trees, while the mummification was typically found in young fruits. On mature fruit, symptoms manifest as small black circular spots that expand into irregular, necrotic, sunken lesions with dark to black coloration (Rattanakreetakul et al., 2023).

Colletotrichum spp can infect a broad hosts. The potency of cross-inoculation can occur on fruits and vegetables being important to investigate. They were available in the field and market make the potential for cross-inoculation (Suparman et al., 2018). A previous cross-infection study demonstrated that *C. siamense* can cause anthracnose in banana, apple, citrus, and avocado; *C. asianum* in avocado, papaya, apple, and citrus; *C. gloeosporioides* in citrus and apple; and *C. sloanei* in apple, guava, citrus, and papaya. Several species, including *C. siamense*, *C. asianum*, *C. gloeosporioides* and *C. sloanei*, are known to be associated with anthracnose in tropical fruit crops. The potency of the cross-infection test showed considerable variability in pathogenicity of the *Colletotrichum* isolates (Zhafarina et al., 2021).

The significance of correctly identifying fungi is reflected in their distinct physical and functional characteristics. One popular technique for recognizing an object based on its characteristics is image-based identification. Morphological and textural traits are the most frequently used elements in identification based on a microbiological image especially for *Colletotrichum* fungal pathogens (Pramesti et al., 2022). The effectiveness of disease control and prevention is largely determined by precise pathogen identification and host-range information. The objective of this study is to identify the morphological characterization of *Colletotrichum* spp. on mango cultivar manalagi and investigate the host-range potency toward others host.

MATERIALS AND METHODS

a. Study location and sample collection

The study was conducted from July to December 2024 at the Laboratory of Plant Protection, Department of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional “Veteran” Yogyakarta, Special Region of Yogyakarta, Indonesia. The anthracnose symptoms on mango cultivar manalagi (*Mangifera indica*) was obtained from local market in Klaten, Central Java, Indonesia.

b. Isolation of *Colletotrichum* spp.

Mango cultivar manalagi (*Mangifera indica*) exhibiting anthracnose symptoms were surface-cleaned with 70% alcohol with gently rubbing the fruit surface. The area between the healthy and infected tissues was taken out 1cm×1cm and cultured on Potato Dextrose Agar (PDA) medium on a Petridish. The cultures were maintained at room temperature for 4-7 days. Then the culture was purified with single-spore isolation technique. The culture on PDA was cutting 1x1cm followed by the addition of 500 µL sterile water into a 1.5 mL microtube to make suspension of its fungi. Then it was mixed by the vortex. The suspension obtained was spread onto fresh PDA medium and incubated at room temperature for 12–24 hours. A sterile needle was used to remove the germinated conidium viewed under a microscope observation before it was transferred to a fresh PDA media and incubated at room temperature. The fungal cultures were then subcultured onto fresh PDA medium in another Petri dish for further testing.

c. Macroscopic and microscopic features analysis

The morphological characters were analyzed by macroscopic and microscopic features. The parameters of macroscopic were following Zhafarina et al. (2021). They were aerial view, reverse view, colony texture, concentring ring, conidiomata, blackspot and growth rate. For the microscopic parameters were colonial shape and size. The conidial size was measured by choosing 50 random conidia after 14 days of incubation.

d. Host-range assay

The mandarin citrus sample was subcultured on PDA medium in a Petri dish and incubated at room temperature for 7 days. The healthy post harvest commodities were prepared which untreated and un-waxed to do cross-inoculation assay. The surface of the post harvest commodities was cleaned with gently rubbing the fruit surface using 70% alcohol. Disinfected post harvest commodities were kept in a plastic container lined with tissue paper and sprayed with sterile water to maintain the relative humidity 95%. The post harvest commodities were inoculated using a wound methods by puncturing the middle part of the post harvest commodities with a sterile needle. Then the mycelial disk of fungal samples was placed onto the wound. The inoculated post harvest commodities were maintained in the box at 30-35°C under a 12-hour light and dark cycle.

The post-harvest commodities used in hos-range test were citrus (*Citrus reticula*), mango (*Mangifera indica*), chayotte (*Sechium edule*), tomato (*Solanum lycopersicum*) and cayenne pepper (*Capsicum annum*) with five replicates per commodities. The diseases severity was evaluated based on progression of lesions on symptomatics area. The formula was following Montri et al. (2009)

$$\text{Disease severity (\%)} = \frac{\text{Lesion length}}{\text{Fruit length}} \times 100$$

The data were analyzed used analysis of variance ($p < 0.05$) with Least Significant Difference (LSD) Test for multiple range test using STAR.

RESULTS AND DISCUSSION

a. Anthracnose symptoms and *Colletotrichum* sp characterization on manalagi mango

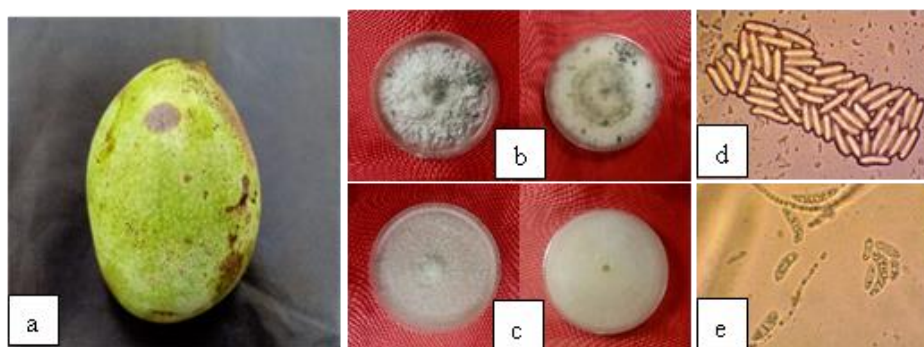


Figure 1. Anthracnose symptoms on Manalagi Mango (a); mycelium characters of MM1 isolate (b); and MM2 isolate (c); conidium characters of MM1 (d); and MM2 (e)

Table 1. Mycelium morphological characterization from Mango isolates

No	Isolates	Mycelium Morphological Characterization						Growth Rate (cm/day)
		Aerial Views	Reverse Views	Colony Texture	Concentric Ring	Conidiomata	Black Spot	
1	MM-1	white greyish	white greyish flat	cottony	-	-	v	1.190476
2	MM-2	White	white flat	velvety	-	-	-	1.0625

Table 2. Conidia characters from Mango isolates

No	Isolates	Conidial Shape	Conidial Shape (μm)			
			Length	Range	Width	Range
1	MM-1	Cylindrical	14.96	10.48-18.01	4.02	3.21-4.99
2	MM-2	Cylindrical	12.364	8.53-18.22	4.21	3.36-4.86

The anthracnose symptoms on unripened fruits were collected from Manalagi Mango located in Klaten, Central Java. The visual symptoms appeared on unripened fruit as small, with a dark brown to black coloration, circular spots, along with irregularly shaped, necrotic, sunken lesions varying from brown to black in color shown on Fig 1(a). In cases of severe infection, extensive rotting of the fruit was evident, and under conditions of high humidity, spore masses ranging from bright orange to pale in color became visible. Earlier infection on fruits was easily dropped from the tree (Rattanakreetakul et al., 2023).

The mycelia appearance of *Colletotrichum* sp. were shown at Fig 1(b,c) and Table 1. MM1 isolate displayed a circular growth with a dense, cottony texture. Initially, the mycelium appears white to grayish-white, becoming darker or slightly greenish-gray at the center as the colony matures. The surface may show concentric ring patterns was present. Its colony margin was regular and well-defined. While MM2 isolate displayed uniform growth pattern and the mycelium appeared dense with a velvety texture. The mycelium color was predominantly white to pale yellowish. Its colony margin was regular and entire, with no evidence of irregular or lobed edges. Both of the two isolates were consistent with morphological traits commonly reported for *Colletotrichum* sp. Mycelia growth rate of MM1 was 1.190476cm/day, while MM2 was 1.0625cm/day. This mycelium growth rate were belongs to fast group rate. Fuentes- (Fuentes-Aragón et al., 2020) reported that the mycelial morphology of *Colletotrichum* isolates associated with mango was highly diverse, showing distinct differences in colony colors like white grey, dark grey and many more; texture and growth rate, zonation across species indicating significant phenotypic variability even within the *Colletotrichum gloesporioides* species complex.

Conidia appearance of MM1 and MM2 showed at Fig 1 (d,e) and Table 2. MM1 isolate had cylindrical to slightly fusiform in shape, with both ends rounded or sometimes slightly pointed. Conidial dimensions ranged from 10.48–18.01 µm in length and 3.21–4.99 µm in width. While MM2 isolate formed like asci, ascospore appearance. Asci was cylindrical. Conidial dimensions ranged from from 8.53 to 18.22 µm in length and 3.36 to 4.86µm in width. Similar studies was carried out by Wan et al. (2021) showing *Colletotrichum chongqingense* microscopic morphological that cylindrical asci containing eight spores were observed, with sizes varying among samples. The ascospores were hyaline, aseptate, and smooth-walled, arranged biserially, and cylindrical to narrowly fabiform in shape with rounded to slightly acute ends, appearing straight or slightly curved. Earlier, similar studies were carried out by Joshi et al. (2023) who isolated *C. gloesporioides* formed whitish colony and conidia were smooth, hyaline and sub cylindrical with round end. MM1 isolates was belongs to *C. gloesporioides*. According to mycelium and microscopic morphological characters showed that the two isolates were belongs to *Colletotrichum* species. Additionally, *Colletotrichum* species was mentioned that the wide variety of conidia sizes in *Colletotrichum* species allowed for morphological findings to overlap. *Colletotrichum* species from various hosts would show distinct colonies and conidial morphology; in addition to the host, the environmental factors affecting isolate growth such as growing media, temperature, light, may also have an impact (Hayden et al., 1994; Shivas et al., 2016). The diversity of *Colletotrichum* subspecies had wide variability, moreover identification using colony and conidia morphology were difficult to distinguish until species level. Thus, molecular analysis is necessary for accurate identification of *Colletotrichum* at the species level (Zhafarina et al., 2021).

b. Host-Range Assay of *Colletotrichum* sp. Associated Manalagi Mango

Table 3. presents the percentage of fruit length infected toward five commodities i.e. citrus, mango, chayote, tomato and cayenne pepper following inoculation with two isolates (MM1 and MM2). Based on Table 3, the results indicate significant differences in host susceptibility and infection progression between the two isolates. Even within the same species, isolates can differ in their genetic and physiological characteristics, resulting in different pathogenicity levels. Moreover, the interaction between individual isolates and host plants may influence infection success and disease development. Thus, isolates of the same *Colletotrichum* species may exhibit different levels of aggressiveness toward different host species or cultivars (De Silva et al., 2021; De Souza et al., 2013) . For MM1 inoculation, no infection was observed on cayenne pepper throughout day 10 after inoculation. It was suggesting non-host status of cayenne pepper. Tomato exhibited the highest infection level, reaching 53.93% at day 10 after inoculation. It was supposed to be susceptible toward MM1 isolate. While moderate infection levels were observed on mango with the percentage of fruit infected 39.82% and citrus 24.50%, whereas chayote showed relatively low infection percentages. In contrast, MM2 inoculation resulted in infection on all tested commodities. MM2 isolate demonstrated a broader host range. Cayenne pepper and tomato were the most susceptible with infection reaching 96.51% and 94.85% respectively on day 10 after inoculation. Citrus and mango showed intermediate levels of infection with the percentage of fruit infected 62.95% and 48.93%. While chayote displayed the lowest infection rate. Both of MM1 and MM2 were able to infect their original hosts as well as other hosts.

There would be the cross-infection potential among the commodities. The two isolates were capable of producing anthracnose symptoms not only in their original host, but also on another commodities. Previous report Lakshmi et al. (2011), Suparman et al. (2018), Zhafarina et al. (2021) reported the potency of *Colletotrichum* species could infect others non-original hosts with varying severity symptoms. In this study, MM2 was more aggressive isolate with a wider host than MM1. Isolates of *Colletotrichum* species showed greater aggressiveness on other tested fruits than on their original host (Lakshmi et al., 2011). In contrast, earlier studies indicated that isolates of *Colletotrichum* species tend to be more aggressive on their original host than on other tested hosts (Hayden et al 1994). *Colletotrichum* spp. have varying capacities to infect different types of commodities, because different host types have distinct compositions (Lakshmi et al., 2011). Tomato and cayenne pepper were identified as the most susceptible hosts in this study, while chayote consistently showed the least infection. Host susceptibility to *Colletotrichum* infection was related to the amount of antifungal inhibitors present in the host tissues (Zhafarina et al., 2021).

Table 3. Percentage of Fruit Length Infected per Day on Different Hosts Inoculated with *Colletotrichum* sp.

MM1 inoculation	Fruit Length Infected (%) / day									
	1	2	3	4	5	6	7	8	9	10
Citrus	0 ^a	0 ^a	9.79 ^a	12.82 ^{ab}	13.71 ^a	14.01 ^{bc}	15.49 ^{bc}	17.04 ^{bc}	17.04 ^{bc}	24.50 ^{bc}
Mango	0 ^a	0 ^a	7.90 ^a	11.27 ^{bc}	13.98 ^a	17.86 ^{ab}	22.71 ^{ab}	24.95 ^{ab}	35.06 ^a	39.82 ^{ab}
Chayote	0 ^a	0 ^a	0 ^b	9.21 ^c	9.43 ^b	11.25 ^c	12 ^c	12.75 ^c	12.93 ^c	13.29 ^{cd}
Tomato	0 ^a	0 ^a	0 ^b	16.03 ^a	17.28 ^a	20.57 ^a	26.54 ^a	27.86 ^a	27.86 ^{ab}	53.93 ^a
Cayenne Pepper	0 ^a	0 ^a	0 ^b	0 ^d	0 ^c	0 ^d	0 ^d	0 ^d	0 ^d	0 ^d
MM2 inoculation	1	2	3	4	5	6	7	8	9	10
Citrus	0 ^a	0 ^a	14.61 ^c	17.6 ^c	19.97 ^c	22.81 ^c	26.77 ^c	27.23 ^c	27.23 ^b	62.95 ^b
Mango	0 ^a	0 ^a	0 ^d	5.09 ^d	9.68 ^d	15.23 ^{cd}	23.69 ^c	24.10 ^c	37.26 ^b	48.93 ^c
Chayote	0 ^a	0 ^a	0 ^d	1.13 ^d	7.59 ^d	9.92 ^d	10.42 ^d	10.62 ^d	10.90 ^c	11.83 ^d
Tomato	0 ^a	0 ^a	23.04 ^b	31.25 ^b	39.95 ^b	58.92 ^b	77.25 ^b	77.62 ^b	90.81 ^a	94.85 ^a
Cayenne Pepper	0 ^a	0 ^a	39.42 ^a	44.62 ^a	58.87 ^a	80.07 ^a	88.69 ^a	89.58 ^a	90.36 ^a	96.51 ^a

*Means with the same letter in each column are not significantly different from each other based on LSD

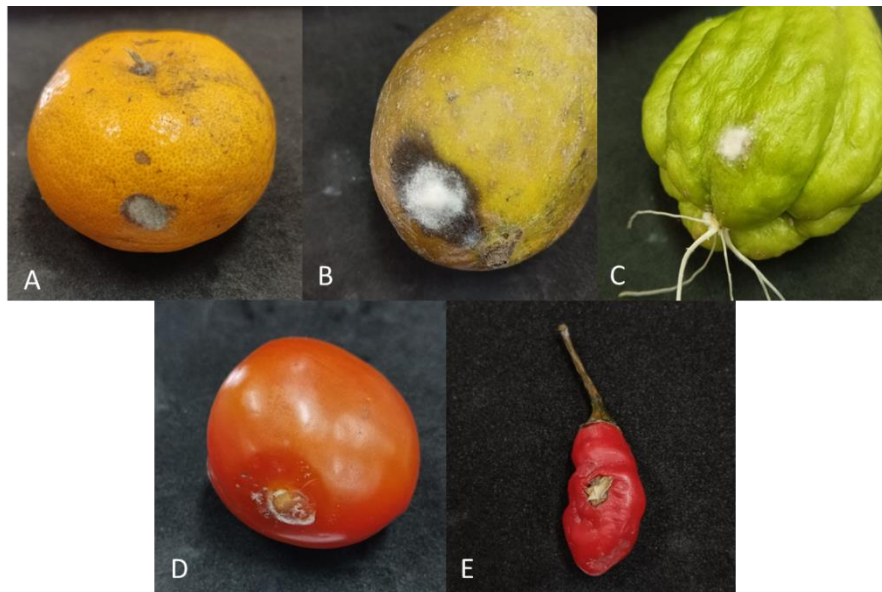


Figure 2. Symptoms of MM1 inoculation on varied hosts/non-host (a) anthracnose symptoms on citrus; (b) mango; (c) chayote, (d) tomato; (e) asymptomatic on cayenne pepper.

Figure 2 and Figure 3 were anthracnose symptoms caused *Colletotrichum* species originally obtained from mango that inoculated onto different hosts commodities. MM1 isolate were able to infect all the commodities showing anthracnose symptoms except the cayenne pepper. While MM2 isolates were able to infect all the commodities. On citrus (Fig 2.A. and Fig 3.A.) typical anthracnose symptoms were observed, characterized by sunken, circular lesions with grayish centers and darkened margins. But the percentage of citrus length infected because of MM2 inoculation was more severe than MM1. Typical anthracnose symptoms on citrus fruits and leaves include sunken patches with a brilliant brown to black surface. A mass of spores was present on dry symptoms (Shidiq et al., 2024).

On Mango (Fig 2.B. and Fig 3.B.) exhibited pronounced necrotic lesions accompanied by dense white mycelial growth indicating active colonization and sporulation by the pathogens. The symptoms was brown to black spots and irregular. The symptoms shown was similar with first symptoms founded on manalagi mango. On the surface of the ripe fruit as well as the fruit starting the climacteric phase, there were signs of dark-brown sunken spots to a huge chlorotic lesion that combined to form a larger necrotic region (Benatar et al., 2021). On chayote (Fig 2.C. and Fig.3.C) developed only a small, superficial white spot at the point of inoculation, suggesting a limited or early-stage infection. The fruit symptoms caused by all species were similar, showing sunken lesions, light to brown coloration, and spore mass formation (Vieira et al., 2024). On tomato (Fig.2.D and Fig.3.D) showed a localized sunken lesion with brown discoloration at the inoculation site, consistent with early anthracnose development. According to Shahriar et al. (2023) on tomato, anthracnose infection usually starts as small, dark, water-soaked spots that sink into the tissue and eventually merge into broader soft lesions. In contrast, cayenne pepper (Fig 2.E) did not develop any visible symptoms throughout the observation period, indicating potential resistance or non-host status to the MM1 *Colletotrichum* isolate. While based on Fig.3E showing the severe anthracnose symptoms accompanied by dense white mycelial growth on the surface. The anthracnose-causing isolates induce highly consistent symptomatology on chili pepper fruit. Initial infection is characterized by inward wrinkling of the affected tissue, which progresses to the formation of brown necrotic lesions with irregular circular margins. Subsequently, minute black acervuli develop centrally within the lesions. In the absence of intervention, the infected tissue undergoes desiccation and collapse (Budi et al., 2023). Overall, our results demonstrated that both MM1 and the MM1 *Colletotrichum* isolates are more aggressive on post-harvest commodities with thin skins and on their original host compared to those with thicker skins. This finding is consistent with Ulilalbab et al. (2025), highlighting that host structural characteristics and origin play a crucial role in determining pathogen aggressiveness and infection success.

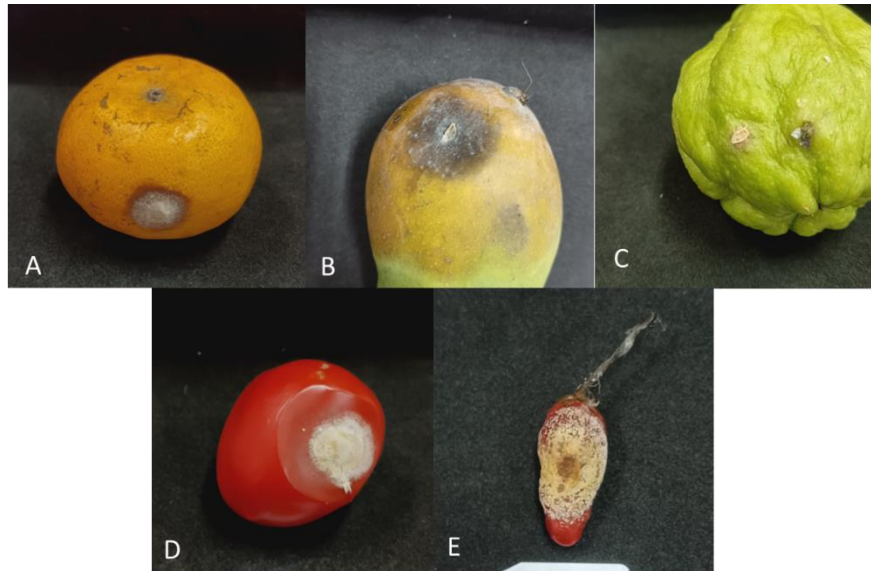


Figure 3. Symptoms of MM2 inoculation on varied hosts/non-host (a) anthracnose symptoms on citrus; (b) mango; (c) chayote, (d) tomato; (e) cayenne pepper.

CONCLUSIONS

This study successfully identified two *Colletotrichum* isolates (MM1 and MM2) associated with anthracnose symptoms in Manalagi mango based on macroscopic and microscopic morphological characteristics. Both isolates exhibited typical traits of *Colletotrichum* species, including cylindrical conidia and rapid mycelial growth. Host-range assays revealed that the isolates, particularly MM2, possessed a broad host range, infecting not only mango but also citrus, chayote, tomato, and cayenne pepper. MM2 demonstrated higher aggressiveness and infection severity across multiple hosts compared to MM1. The differential host responses suggest variability in pathogenicity among isolates and indicate the potential risk of cross-infection among tropical fruits. These findings underscore the importance of accurate morphological identification and host-range testing in managing anthracnose disease and developing effective quarantine or crop rotation strategies. For more definitive species-level identification, molecular characterization is recommended in future studies.

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