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Diversity of morphology, pathogenicity, and host range of *Colletotrichum* spp. associated with chili anthracnose in East Priangan, Indonesia

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Abstract. *Hodiyah I, Suryaman M, Hartini E, Juhaeni AH, Laksana BY, Aisyah, Benatar GV. 2024. Diversity of morphology, pathogenicity, and host range of* Colletotrichum *spp. associated with chili anthracnose in East Priangan, Indonesia. Biodiversitas 25: 533-541.* Chilies, in Indonesia, encounter several obstacles that impact their production and quality. Chili production in Indonesia has seen oscillations, partly due to the detrimental effects of anthracnose disease produced by the *Colletotrichum* fungus species. The disease has a detrimental effect on the quality of fruit, foliage, and plants, resulting in unpredictable yields and significant losses, especially during the rainy season. Although chilies has important economic value in Indonesia, more comprehensive studies are needed on chili anthracnose in the East Priangan area, West Java Province, Indonesia. The purpose of this research was to specifically investigate the incidence of chili anthracnose and to isolate the pathogen responsible for the disease. *Colletotrichum* isolates were subjected to morphological characterization and pathogenicity tests to assess their virulence. In addition, the research evaluated the possible spectrum of hosts that these isolates might infect in order to determine their effect on other commodities. In East Priangan, chili anthracnose disease was found everywhere with severe disease incidence. Highest disease incidence was recorded in Garut District (100%), and the lowest was in Ciamis District (50.5%). The isolation results showed that a total of 18 diverse isolates were isolated from three chilli species at different altitudes. Macroscopic and microscopic characteristics showed variability, with growth rates ranged from 0.24 to 3.7 mm per day and diverse conidia shapes and sizes. The pathogenicity test confirmed the virulence of 17 isolates, causing symptoms with concave circular wound diameters ranged from 6.88 to 61.08 mm. Host range results demonstrated the pathogenicity of specific isolates to tomatoes, strawberries, and grapes, with strawberries exhibited higher severity levels (28.05 to 56.38 mm lesions). Gaining comprehensive knowledge about the variety and widespread occurrence of chili anthracnose in this particular area is essential for formulating effective measures to minimize its adverse effects on chili cultivation and guarantee the long-term viability of the chili sector in Indonesia. To our knowledge, this is the first study of chili anthracnose in the East Priangan region.

Keywords: Anthracnose, chilies, *Colletotrichum*, incidence, macroscopic, microscopic, severity, virulence

INTRODUCTION

Chili is an essential commodity in many countries, especially in Asia, which is the center of origin for chilies. This commodity has high economic value because it is one of the primary consumption materials for society and is a raw material for industry and health (Sativa et al. 2017). Capsaicin is the main biologically active compound found in chili, responsible for the pungent and spicy taste. It has been shown to provide several health advantages, such as anti-inflammatory and antioxidant qualities (Lu et al. 2020).

Chilies have high demand in the Indonesian consumer market and are the main ingredient in various traditional dishes, with a per capita requirement of 3.7 kg. In 2022, people used 636.56 thousand tons of big chilies at home, which is 6.78% more (40.42 thousand tons) than in 2021. Additionally, 71.33% of households used big chilies. For cayenne pepper, household consumption in 2022 was 569.65 thousand tons, a 7.86% increase (41.51 thousand tons) from 2021. The participation rate for households using cayenne pepper was 75.77%. Chilies are also an important export commodity for Indonesia (Irjayanti et al. 2023). According to data from BPS-Statistics Indonesia, chili exports from Indonesia in 2020 reached 25.81 million, up 69.86% or US$10.36 million from 2019.

Chili production in Indonesia continues to grapple with various factors contributing to yield losses. The interplay of biotic and abiotic variables significantly influences the fluctuations in chili production in Indonesia. There are biotic elements, such as plant disease, that exert an influence on the volatile production of chili. Anthracnose is the primary disease affecting chili plants, leading to inconsistent yield. According to Anggrahini et al. (2020), during the rainy season, the chili crop can experience complete yield losses due to attacks by anthracnose pathogen infection.

Anthracnose, a prevalent disease affecting chili plants, is mainly caused by a range of *Colletotrichum* fungal species, including *C. acutatum*, *C. capsici*, *C. coccodes*, *C. dematium*, *C. scovillei*, *C. siamense*, and *C. gloeosporioides*. Anthracnose, a fungal infection, impacts nearly every above-ground component of the chili plant, with its main consequence being fruit decay in both green

and red stages (Saxena et al. 2016). Characteristic signs of anthracnose on chili fruit include the development of dark spots and depressed necrotic tissue displaying concentric rings of acervuli. Initially, the spots on the fruit surface are small, slightly sunken, and dark yellow, progressing to darken and assume a circular or angular sunken appearance with concentric rings of acervuli. These areas often exhibit moisture and give rise to pink to orange conidial masses. Severely affected fruits undergo a color transformation, turning either straw-colored or pale white (Than et al. 2008; Mongkolporn and Taylor 2018; Kiran et al. 2020). This particular ailment induces harm to the fruit, foliage, and chili plants, thereby resulting in a decrease in both the amount and quality of the harvested stems (De Silva et al. 2017).

The reporting of the prevalence rate of chili anthracnose disease in Indonesia is still lacking. To date, there has been a notable absence of research on the disease infection in the East Priangan region. Further extensive research is required to investigate the diversity of *Colletotrichum*, the causal agent of the disease in Indonesia. The objective of this research was to conduct a comprehensive investigation of anthracnose disease in chilli in the East Priangan and to isolate the pathogen and characterize its morphological characteristics. The study further seeks to assess the virulence of isolates, evaluate their impact on various hosts, and provide crucial insights for formulating effective measures to sustain chili cultivation in Indonesia.

MATERIALS AND METHODS

Chili anthracnose exploration and prevalence

Anthracnose disease was explored by field survey to identify and select those parts of chilies that showed symptoms of the disease. Exploration was carried out in June-October 2023 and occurred at productive chili planting centers in the East Priangan region consisting Garut District, Tasikmalaya District, Tasikmalaya City, Ciamis District, Banjar City, and Pangandaran District of West Java Province, Indonesia. Two sub-districts were selected in each city/district, with two planting points identified in each sub-district, resulting in a total of 24 sampling points per location. From each sampling point, thirty chilies representing various species were randomly selected. Subsequently, chilies were grouped based on the species, ensuring consistency within each city/district. A total of 120 chilies were randomly selected to determine the incidence and severity of chili anthracnose disease for each city/district. Fruit, leaf, and twig with anthracnose symptoms disease were selected, preserved, and then sent to the laboratory for further analysis. The disease incidence and disease intensity were determined using the following formula according to Lakshmi and Prasad (2011) with slight modifications: Incidence (%) = (Total infected fruit / Total fruit sample) x 100. Disease intensity was first determined through an assessment to fruit that shows anthracnose symptoms and quantified using the following formula:

$$IP = \frac{\sum (n \times v)}{N \times Z} \times 100$$

Where: IP: Disease Severity; n: number of samples at each attack level; v: scales at each attack level; N: number of samples observed; Z: highest score. Disease severity was scored on a 0-to-9 scale, where 0 was no infection, 1 was 1-2% of the fruit area showing necrotic lesion or a more significant water-soaked lesion surrounding the infection site, 3 was >2-5% of the fruit area shows necrotic lesion, acervuli may be present, or water-soaked lesion up to 5% of the fruit surface, 5 was >5-15% of the fruit area shows necrotic lesion, acervuli present, or water-soaked lesion up to 25% of the fruit surface, 7 was >15-25% of the fruit area shows necrotic lesion with acervuli, and 9 was >25% of the fruit area shows necrosis, lesion often encircling the fruit, and abundant acervuli.

Isolation and morphological characterization

Isolation of *Colletotrichum* spp. initially was done by cutting infected plant parts with a sterile scalpel measuring (± 1 cm) in the Laminar Air Flow. Sample pieces were disinfected by dipping them in 1% sodium hypochlorite for 5 seconds, then washing with sterile water and drying them on sterilized tissue paper. The direct planting technique (Malloch 1997) was used to isolate *Colletotrichum* spp., which sample piece was placed using sterile tweezers into a Petri dish containing 9 mL PDA medium, and incubated at room temperature (± 28°C) for three days. Fungi grew with the characteristics of *Colletotrichum* were purified by single conidia isolation (dilution factor 10-3) according to the method of Choi et al. (1999). 1 mL of the suspension was taken with a micropipette spread on PDA media and incubated at room temperature (± 28°C) for three days.

Macroscopic and microscopic characteristics of pure isolates (10 days after incubation) were identified following Weir et al. (2012), through observations with a light microscope Olympus CX 23 (Olympus Corporation, Tokyo, Japan). The colony diameter of each isolate with three replications was calculated every day using a digital caliper on the reverse side for seven days after incubation to measure the average daily growth. Observation of shape and size of conidia was carried out by observing the conidia suspension in a microscope. Then, 100 conidia were randomly selected to measure their length and width. Appressorium formation was observed by growing colonies of *Colletotrichum* on a water agar medium using the slide culture technique (Siddiquee 2017), then incubated at 28°C for three days and observed using a microscope. Microscopic feature measurements were made with a micrometer-calibrated microscope lens, microscope camera Optilab (PT. Miconos, Yogyakarta, Indonesia), and Image Raster 3 software.

Pathogenicity test

Pathogenicity tests were conducted on 18 isolates based on the method of Anggrahini et al. (2020) with slight modifications. Isolates were inoculated into healthy *Capsicum annum* fruits to confirm fungal pathogenicity. The surface of healthy fruits was sterilized using 70% alcohol and then rinsed with sterilized distilled water 3

times. The fruits were wounded by inserting a sterile needle 1 mm deep (6 punctures) in a circle with a diameter of 5 mm. Pathogen inoculation was done by placing mycelial discs (5 mm diameter) of 5-day-old *Colletotrichum* culture on the wounded fruit and then incubated in a closed plastic container. Humidity was regulated by wetting sterile cotton with sterile water in the container. The symptomatic diameter, measured ten days after inoculation, was determined diagonally across eight points on the wound edge using a digital caliper. A disease severity scale of *Colletotrichum* spp. infected all chili fruits was used to score the severity levels according to Montri et al. (2009). A pathogenicity test was developed utilizing a Completely Randomized Design (CRD) with five replicates, and the experiment was conducted thrice for validation.

Host range test

The representative *Colletotrichum* isolates based on typical morphology features from each city/district were chosen for testing host range with other fruit commodities such as tomatoes, strawberries, and grapes, according to Zhafarina et al. (2021), with slight modifications. These hosts were strategically selected based on their economic importance and susceptibility to *Colletotrichum*, aligning with the objectives of our research to assess the pathogen's impact on relevant agricultural crops in West Java. Healthy and physiologically ripe hosts were prepared and then sterilized by rubbing 70% alcohol on the surface of the fruit. The sterilized host was placed in a plastic container with a sterile tissue base moistened with sterile water (95% relative humidity). Hosts were inoculated with *Colletotrichum* using wounding method (De Silva 2017). The method was carried out by perforating the host with a sterile needle in the middle of the fruit and then inoculating with a 5 mm disc of *Colletotrichum* mycelium (5 days of culture). Inoculated samples were incubated in sterile containers at room temperature and 12 hours in dark/light conditions. The experiment was done three times, with three fruits of each host inspected using the protocol for each isolate. Infection was measured based on the formation of symptoms in the host and assessed according to Montri et al. (2009).

RESULTS AND DISCUSSION

Disease symptoms in the field

Symptoms of chili anthracnose, as shown by the red arrow in Figure 1, were observed in the chili growing area in East Priangan, West Java Province, Indonesia. Symptoms of blackish-brown necrosis were often found on the fruit, leaves, and twigs of cayenne peppers (Figure 1.B-F) and large chilies (Figure 1.A). A small number of symptoms on twigs were also observed. On chili fruit, masses of orange conidia were observed, while on leave, concentric zones of black microsclerotia were observed (Figure 1.A-C, E, F).

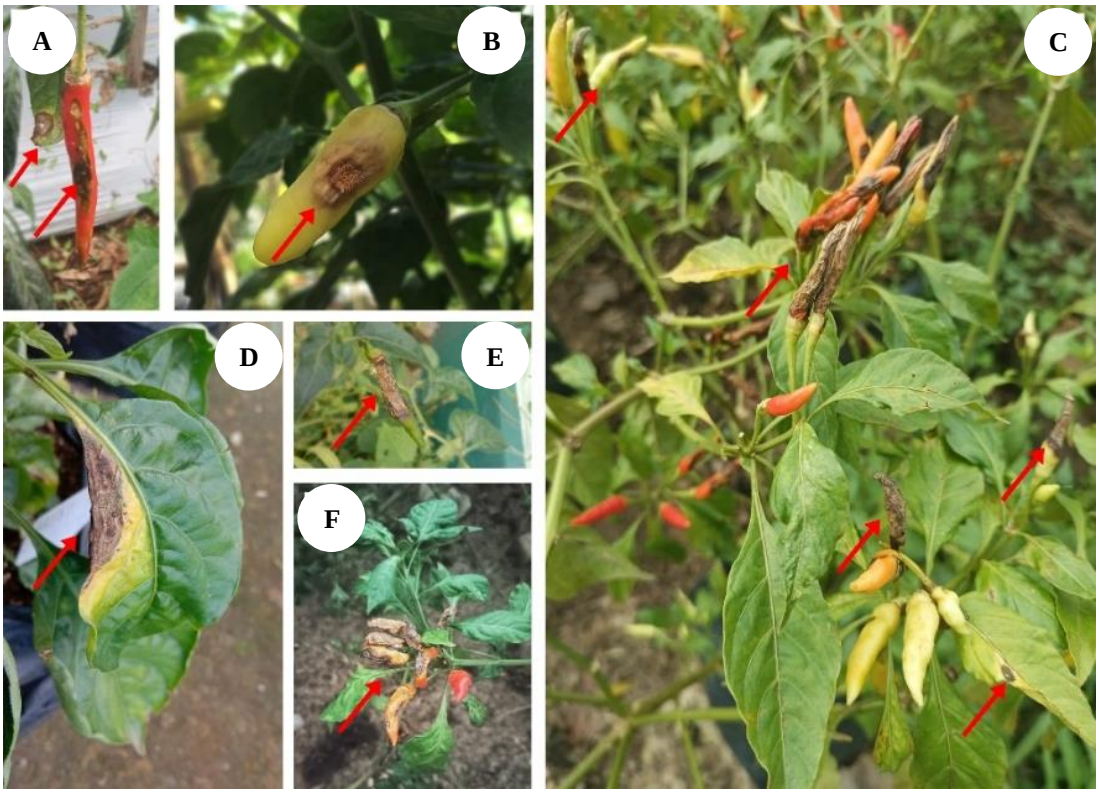

Figure 1. Symptoms of anthracnose on chilies in East Priangan of West Java Province, Indonesia. Symptoms appeared on fruit (A, B, C, E, F) and leaves (A and D). Red arrows indicate symptoms

Symptoms of anthracnose in chili fruit include round and concave lesions with concentric rings produced from black acervuli and orange conidial masses (Than et al. 2008). The disease causes significant damage to ripe fruits in the field and during storage under favorable circumstances, compounding the crop's yield loss and total output (Saxena et al. 2016). On the leaves, symptoms show as little brown or black water-soaked patches bordered by a light brown or yellow hallow edge. These spots are initially small, then enlarged, eventually merged and to form a huge lesion. These necrotic spots cause defoliation of the crop's leaves. The initial signs on the stem according to Manda et al. (2020) were brown patches, which led to necrosis of twigs and, in extreme cases, plant dieback. On the necrotic surface of the twigs, acervuli are also discovered.

Disease incidence and severity

Results of incidence and severity of chili anthracnose disease in East Priangan showed variations between regions (Figure 2). Based on the incidence data, almost all regions had a high incidence rate of more than 50%, with Garut District reaching an incidence rate of 100%. The lowest (50.5%) incidence was recorded in Ciamis District. Apart from that, severity of chili anthracnose disease showed that the damage was also significant, especially in Tasikmalaya District, with a severity level reaching 43.9%. Even though the survey was conducted during the dry season, data on the incidence and severity of chili anthracnose disease indicated that most chili plants in East Priangan were infected. Anthracnose disease was discovered in all chili-producing areas in Bali, with an average incidence of 63% and an average disease severity of 68%. Variation in disease incidence and severity is commonly caused by differences in chili cultivar, cultural practices, climatic conditions, locales, and *Colletotrichum* species (Khalimi et al. 2020).

Colletotrichum, a causal pathogen of anthracnose, may exist as minute black microsclerotia in the soil and as acervuli on plant debris throughout the season; therefore, it can infect plants even during the dry season. These microsclerotia are small, black, spherical structures created by the fungus as a survival mechanism, as described by Baysal-Gurel and Subedi (2014). Microsclerotia are resting structures that may remain in the soil for years and are stimulated to germinate by root exudates (Santhanam 2014). Acervuli, on the other hand, are little, saucer-shaped structures that generate spores. The germination rate of acervuli inside diseased plant debris is also an essential element in the transmission and dispersion of plant diseases (Siébou et al. 2019).

Colletotrichum isolate

The results of isolation revealed that total 18 isolates obtained from three chili species, including *Capsicum frutescens*, *C. annum*, and *C. chinense*, were grown across various elevations, ranging from lowlands to highlands (Table 1).

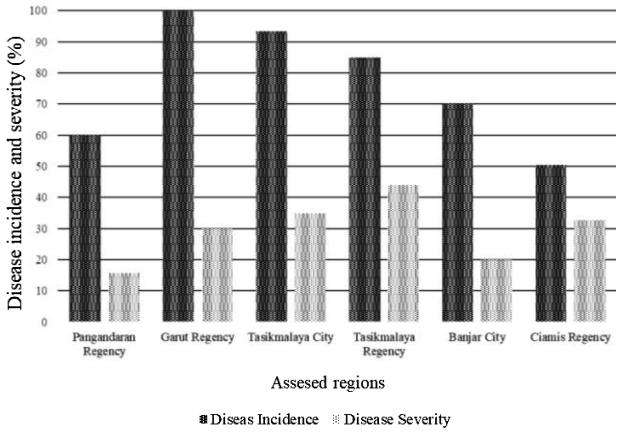

Figure 2. Disease incidence and severity of chili anthracnose in East Priangan of West Java Province, Indonesia

Table 1. *Colletotrichum* isolates obtained from chili with anthracnose symptoms in East Priangan of West Java Province, Indonesia

Isolates name	Host	Isolation sources	Locations	Latitude	Longitude	Altitude (m asl)
CCG2	*Capsicum frutescens*	Fruit	Sindanggalih, Karangtengah, Garut District	-7.1762367	108.0425615	1157
BCG1	*Capsicum annum*	Fruit	Sindanggalih, Karangtengah, Garut District	-7.1762367	108.0425615	1157
BCGTR	*Capsicum frutescens*	Fruit	Sindanggalih, Karangtengah, Garut District	-7.1762367	108.0425615	1157
CMRCS	*Capsicum frutescens*	Fruit	Cisayong, Cisayong, Tasikmalaya District	-7.2559484	108.1249764	663
CMRDCS	*Capsicum frutescens*	Fruit	Cisayong, Cisayong, Tasikmalaya District	-7.2559485	108.1249764	663
RJPCBMD	*Capsicum annum*	Leaf	Dawaagung, Rajapolah, Tasikmalaya District	-7.2492804	108.1249764	448
RJPCBMT	*Capsicum annum*	Twig	Dawaagung, Rajapolah, Tasikmalaya District	-7.2492805	108.1249764	448
ACT2	*Capsicum frutescens*	Fruit	Mugarsari, Tamansiari, Tasikmalaya City	-7.3907719	108.2511952	363
MGCMKB	*Capsicum annum*	Fruit	Mugarsari, Tamansiari, Tasikmalaya City	-7.3907721	108.2511952	363
THDP1	*Capsicum chinense*	Fruit	Mugarsari, Tamansiari, Tasikmalaya City	-7.3907722	108.2511952	363
LBKCMRB	*Capsicum frutescens*	Fruit	Kelapasawit, Lakbok, Ciamis District	-7.4128407	108.6616515	14
LBKCMRB2	*Capsicum frutescens*	Fruit	Kelapasawit, Lakbok, Ciamis District	-7.4128407	108.6616515	14
LBKCMRB4	*Capsicum frutescens*	Fruit	Kelapasawit, Lakbok, Ciamis District	-7.4128407	108.6616515	14
WBJR1	*Capsicum frutescens*	Fruit	Waringinsari, Langgensari, Banjar City	-7.359872	108.637076	17
WBJR2	*Capsicum frutescens*	Fruit	Waringinsari, Langgensari, Banjar City	-7.359872	108.637076	17
WBJR4	*Capsicum frutescens*	Fruit	Waringinsari, Langgensari, Banjar City	-7.359872	108.637076	17
LKPCBMB	*Capsicum annum*	Fruit	Langkaplancar, Langkaplancar, Pangandaran District	-7.5568711	108.4003198	402
LKPCBMD	*Capsicum annum*	Leaf	Langkaplancar, Langkaplancar, Pangandaran District	-7.536259	108.4627936	447

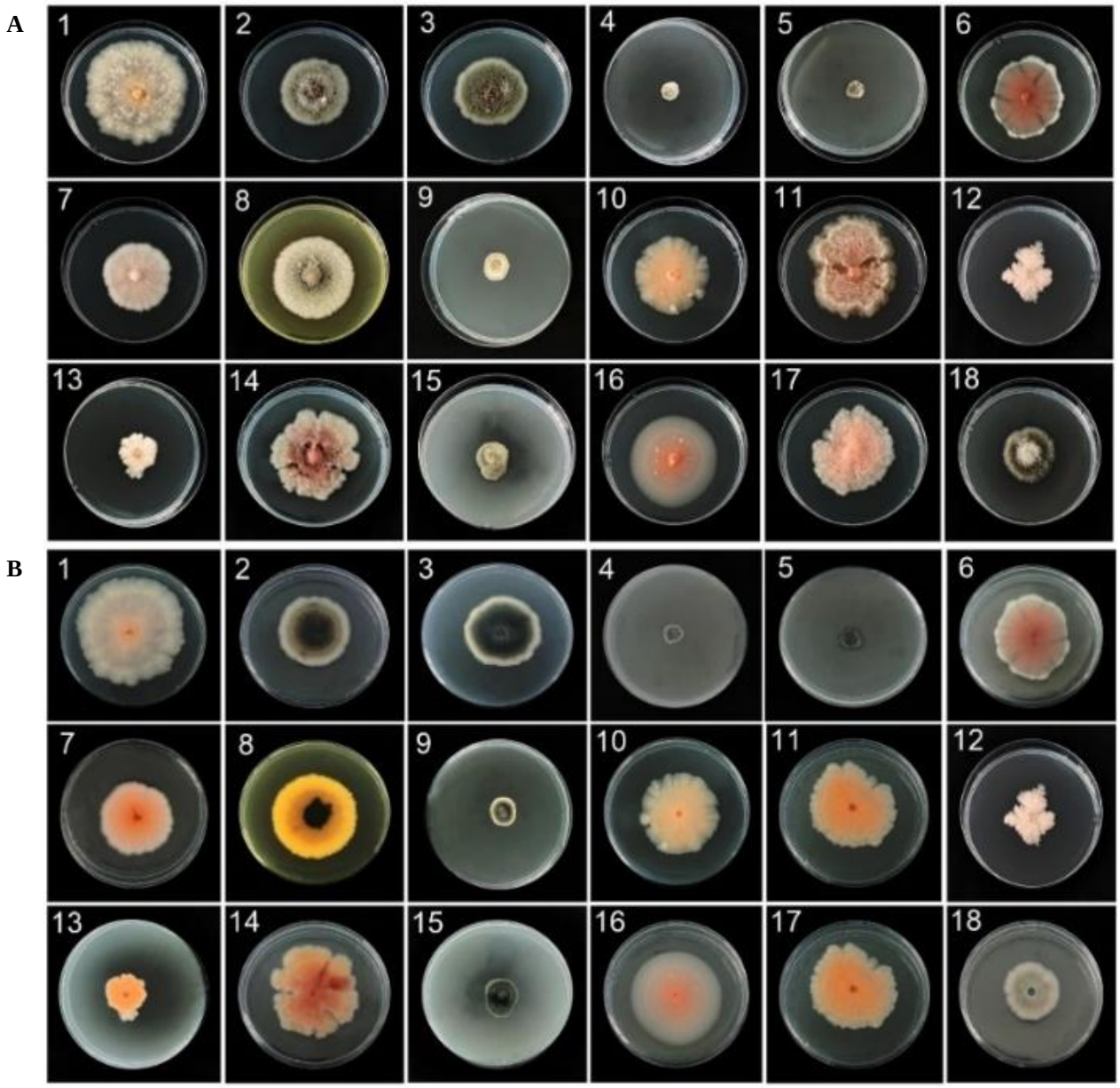

Figure 3. Appearance of 18 colonies of *Colletotrichum* spp. on PDA. Description: A. front colony; B. reverse colony; CCG2, BCG1, BCGTR, CMRCS, CMRDCS, RJPCBMD, RJPCBMT, ACT2, MGCMKB, THDP1, LBKCMRB, LBKCMRB2, LBKCMRB4, WBJR1, WBJR2, WBJR4, LKPCBMB, and LKPCBMD

Of these isolates, 13 were obtained from fruit, 4 from leaves, and 1 from twigs. Specifically, 6 isolates were collected from the lowlands (14-17 m above sea level), 9 isolates from the midlands (363-663 m above sea level), and 3 isolates from the highlands (1157 m above sea level). Anggrahini et al. (2022) reported that *Colletotrichum*, the causal agent of chili anthracnose in *C. annum* and *C. frutescens*, is isolated from symptomatic chili fruit and leaves at altitudes ranging from 5 to 343 m above sea level. Furthermore, according Almeida et al. (2017) and Sánchez-Sandoval et al. (2021), anthracnose disease is detected in *Capsicum chinense* in Brazil and México.

Morphological characteristic

The macroscopic characteristics of each isolate exhibit different diversity, as detailed in Table 2 and Figure 3. The front colonies of the isolates typically present a sparse and cottony texture with varying colors such as pale pink, grayish white, pale orange, salmon, or gray. Conidiomata were observed on the front colonies of isolates CCG2, BCG1, CMRDCS, RJPCBMD, and MGCMKB, displayed a pale yellow or pale orange colors. The reverse colonies exhibited gray-white, salmon, pale yellow, or reddish-white colors. The growth rate of each colony averaged between 0.24 and 3.7 mm per day. Isolates CCG2, BCGTR, RJPCBMD, RJPCBMT, ACT2, THDP1, LBKMRB, WBJR1, WBJR4, and LKPCBMB demonstrated a fast

growth category (2.1-3.7 mm per day). Moderate growth was observed in isolates BCG1, LBKCMRB2, LBKCMRB4, LBKCMRT, and WBJR2 (1.2-1.8 mm per day), while slow growth was observed in isolates CMRCS, CMRDCS, MGCMKB, and LKPCBMD (0.24-0.9 mm per day).

Colletotrichum species isolated from chili exhibited a variety of morphological traits. The characteristics of *Colletotrichum* colony may vary depending on the species and the growing conditions. For example, blue-light irradiation in the presence of photoacid can confer changes to the colony morphology of the plant pathogen *Colletotrichum gloeosporioides* (Simkovitch et al. 2017). In another study, the cultural and morphological characteristics of *Colletotrichum kahawae* isolates vary with respect to altitude (Yoganie et al. 2022). The growth rate of *Colletotrichum* varies depending on several factors such as temperature, culture media, and the presence of other compounds. Most *Colletotrichum* species grow at temperatures ranging from 10°C to 35°C, with only *C. musae* showing vigorous mycelial growth at 5°C (Salotti et al. 2022). The radial growth rate of each *Colletotrichum* species at different temperatures was measured, and it was found that milder temperatures are more suitable for this fungal genus (Mello et al. 2004).

Variability was also observed in the microscopic characteristics of each isolate, as listed in Tables 3-4 and Figure 4. CCG2, CMRDCS, and RJPCBMD isolates showed falcate conidia with slightly pointed ends. Conidia were cylindrical with blunt ends in isolates BCG1, BCGTR, ACT2, THDP1, LBKCMRB, LBKCMRB2, LBKCMRB4, WBJR2, WBJR4, LKPCBMB, and LKPCBMD. CMRCS, RJPCBMT, MGCMKB, and WBJR1 had fusiform conidia with blunt ends. Each isolate had a mean conidia length range of 6.43-26.37 µm and a conidia width of 1.53-3.35 µm. Acervulus and setae were observed only in isolates that had falcate conidia. Appressoria was also observed in almost all isolates except BCGTR isolates. Appressoria had an average length of 7.17-12.17 µm and a width of 5.12-8.06 µm, displaying an ovoid or slightly irregular shape and a blackish-brown color. Anggrahini et al. (2020) found that *Colletotrichum* spp. infecting chili plants have fusiform to cylindrical conidia with two pointy or somewhat blunt ends or crescent forms with length and width sizes ranging from 9.02-19.38 µm x 2.37-8.57 µm.

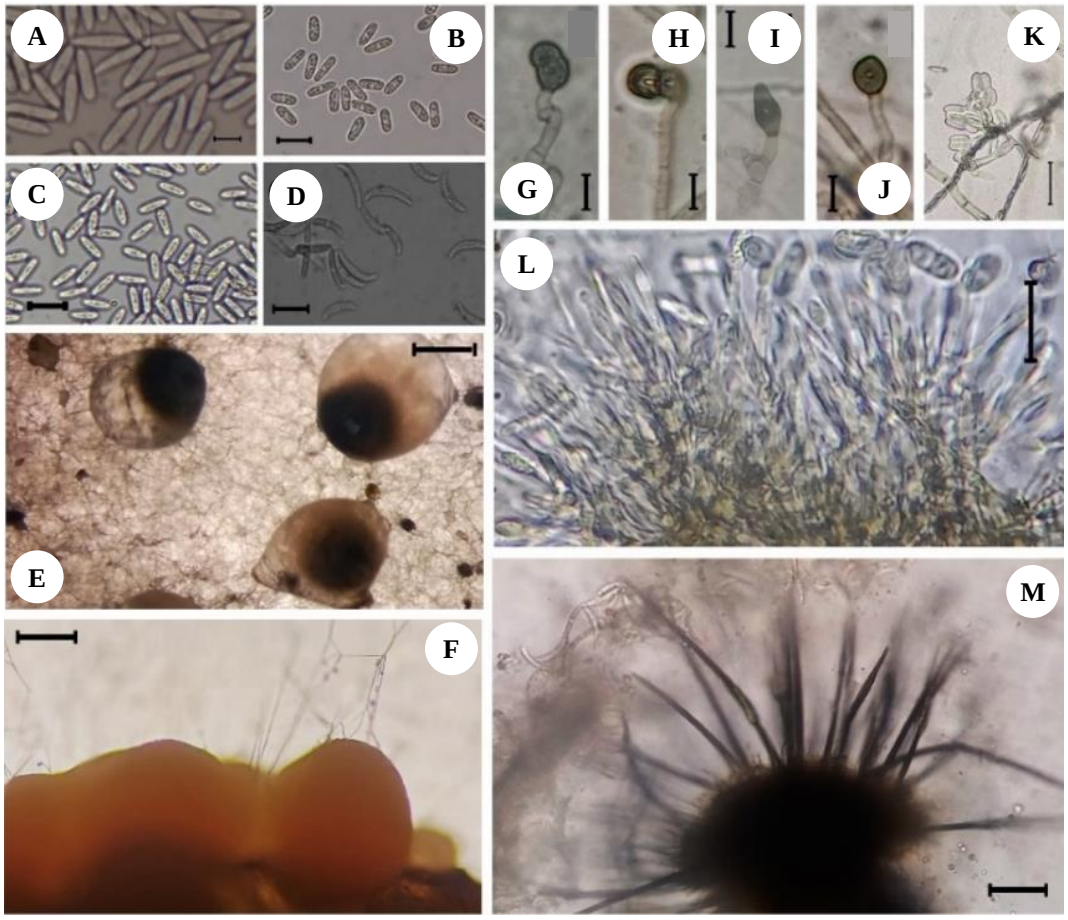

Figure 4. Microscopic characteristics of *Colletotrichum* spp. Description: A. long cylindrical conidia; B. cylindrical conidia; C. fusiform conidia; d. falcate conidia; E-F. conidiomata; G-J. appressoria; K-L. conidiogenous cells; M. acervulus. The black scale bar indicates a size of 10 µm

Table 2. Macroscopic characteristics of *Colletotrichum* spp.

Isolates name	Colony texture	Front colony	Reverse colony	Conidiomata	Growth rate (mm/day)	Growth speed
CCG2	Sparse	Pale pink	Reddish white	Present	3.2	Fast
BCG1	Cottony	White grey to olivaceous-grey	Greyish white	Present	1.7	Moderate
BCGTR	Cottony	White grey to olivaceous-grey	Greyish white	Absent	2.3	Fast
CMRCS	Cottony	White grey to olivaceous-grey	Greyish white	Absent	0.29	Slow
CMRDCS	Cottony	White grey to olivaceous-grey	Greyish white	Present	0.24	Slow
RJPCBMD	Sparse	Pale pink	Reddish white	Present	3.3	Fast
RJPCBMT	Sparse	Pale pink	Reddish white	Absent	2.29	Fast
ACT2	Cottony	White grey	Greyish white	Absent	2.8	Fast
MGCMKB	Cottony	White grey to olivaceous-grey	Blackish grey	Present	0.5	Slow
THDP1	Sparse	Pale orange to salmon	Salmon	Absent	2.1	Fast
LBK CMRB	Cottony	Pale orange to salmon	Salmon	Absent	3.7	Fast
LBK CMRB2	Cottony	Pale orange to salmon	Salmon	Absent	1.6	Moderate
LBKCMRB4	Cottony	Pale orange to salmon	Salmon	Absent	1.6	Moderate
LBKCMRT	Sparse	Pale orange to salmon	Salmon	Absent	1.8	Moderate
WBJR1	Sparse	Pale pink	Reddish white	Absent	3.2	Fast
WBJR2	Cottony	White grey to olivaceous-grey	Greyish white	Absent	1.2	Moderate
WBJR4	Sparse	Pale pink	Reddish white	Absent	2.7	Fast
LKPCBMB	Cottony	Pale orange to salmon	Pale yellow	Absent	2.6	Fast
LKPCBMD	Cottony	White grey to olivaceous-grey	Greyish white	Absent	0.9	Slow

Table 3. Microscopic characteristics of *Colletotrichum* spp.

Isolates	Conidia							
	Shape and color	Length (µm)				Width (µm)			
		Min	Max	Mean	St. Dev.	Min	Max	Mean	St. Dev.
CCG2	Falcate with two ends slightly acute, hyaline	11.33	17.15	14.13	1.54	1.53	3.08	2.02	0.31
BCG1	Cylindrical with two ends obtuse, hyaline	6.83	11.98	9.28	1.23	2.12	4.7	3.05	0.54
BCGTR	Cylindrical with two ends obtuse, hyaline	6.51	12.6	10.14	1.51	2.22	4.88	3.31	0.66
CMRCS	Fusiform with two ends slightly acute, hyaline	6.23	11.21	8.80	1.13	2.06	3.83	2.71	0.51
CMRDCS	Falcate with two ends sligthly acute, hyaline	9.65	18.38	13.68	2.14	1.57	3.23	2.31	0.42
RJPCBMD	Falcate with two ends slightly acute, hyaline	7.99	23.73	14.09	3.76	1.35	3.83	2.47	0.5
RJPCBMT	Fusiform with two ends slightly acute, hyaline	4.45	8.91	6.43	1.26	1.63	3.38	2.17	0.43
ACT2	Cylindrical with two ends obtuse, hyaline	7.63	14.76	9.8	1.64	1.18	4.13	2.62	0.69
MGCMKB	Fusiform with two ends slightly acute, hyaline	6.18	9.86	7.72	1.04	1.68	3.17	2.27	0.3
THDP1	Cylindrical with two ends obtuse, hyaline	4.59	9.78	7.34	1.12	1.31	3.1	2.24	0.4
LBKCMRB	Cylindrical with two ends obtuse, hyaline	5.18	11.19	8.12	1.55	1.41	2.88	2.19	0.37
LBKCMRB2	Cylindrical with two ends obtuse, hyaline	13.29	26.37	19.63	3.14	2.27	5.05	3.49	0.68
LBKCMRB4	Cylindrical with two ends obtuse, hyaline	4.96	11.08	7.76	1.36	1.15	1.99	1.53	0.19
WBJR1	Fusiform with two ends slightly acute, hyaline	6.47	15.35	10.47	2.01	1.87	2.98	2.53	0.34
WBJR2	Cylindrical with two ends obtuse, hyaline	8.95	14.65	11.58	1.69	1.76	2.94	2.41	0.29
WBJR4	Cylindrical with two ends obtuse, hyaline	7.44	13.6	9.43	1.63	1.88	2.94	2.4	0.3
LKPCBMB	Cylindrical with two ends obtuse, hyaline	10.19	17.6	14.2	2.03	2.42	4.16	3.35	0.52
LKPCBMD	Cylindrical with two ends obtuse, hyaline	13.58	19.69	16.38	1.83	1.87	4.33	3.06	0.63

Pathogenicity test

The results of pathogenicity exhibited that of the 18 isolates, 17 isolates were found pathogenic, with various virulence categories, ranging from the virulent to the high virulent category (Table 5). Typical anthracnose symptoms appeared 2 days after inoculation, while BCGTR isolate did not induce any symptoms. The symptoms of concave circular wounds had varying diameters, ranging from 6.88 to 61.08 mm. All isolates were subsequently reisolated and exhibited the same morphological characteristics as before, confirming the fulfillment of Koch's postulates. *Colletotrichum* is a fungal genus containing hemibiotrophic phytopathogens highly variable in host and tissue specificities. When *Colletotrichum* species infect their hosts, they establish biotrophic and necrotrophic lifestyles. To colonize host tissues, pathogens of *Colletotrichum* species generally form appressoria, which penetrate the host's epidermis using penetration pegs. Identifying virulence factors and characterizing their evolution is critical for controlling *Colletotrichum* disease and understanding the fundamental mechanisms of host-pathogen interaction (Liang et al. 2018; Benatar et al. 2021; Liang et al. 2021; Guo et al. 2023).

Table 4. Characteristic of appressoria of *Colletotrichum* spp.

Isolates	Appresoria								
	Length (µm)				Width (µm)				Shape and color
	Min	Max	Mean	St. Dev.	Min	Max	Mean	St. Dev.	
CCG2	3.8	9.61	7.17	2.46	3.56	6.73	5.32	1.17	Ovoid to slightly irregular, brown to dark
BCG1	5.39	9.37	7.83	1.47	4.65	6.44	5.81	0.74	Ovoid to slightly irregular, brown to dark
BCGTR	-	-	-	-	-	-	-	-	-
CMRCS	7.77	12.65	9.8	1.84	3.95	8.27	6.02	1.66	Ovoid to slightly irregular, brown to dark
CMRDCS	6.45	9.71	8.28	1.17	3.13	6.41	5.16	1.45	Ovoid to slightly irregular, brown to dark
RJPCBMD	10.39	16.56	12.17	2.66	4.74	11.57	8.06	2.77	Ovoid to slightly irregular, brown to dark
RJPCBMT	7.54	13.69	10.97	2.27	4.11	7.35	5.89	1.19	Ovoid to slightly irregular, brown to dark
ACT2	6.18	10.05	7.51	1.66	5.31	7.12	5.96	0.7	Ovoid to slightly irregular, brown to dark
MGCMKB	7.38	11.46	9.47	1.68	4.28	6.2	5.42	0.83	Ovoid to slightly irregular, brown to dark
THDP1	7.32	11.73	9.71	2.06	3.97	7.04	5.21	1.18	Ovoid to slightly irregular, brown to dark
LBKCMRB	7.01	10.04	8.74	1.48	4.29	6.15	517	0.84	Ovoid to slightly irregular, brown to dark
LBKCMRB2	6.58	10.39	8.62	1.66	4.58	6.09	5.36	0.54	Ovoid to slightly irregular, brown to dark
LBKCMRB4	6.1	10.35	8.87	2.01	5.54	8.44	6.37	1.18	Ovoid to slightly irregular, brown to dark
WBJR1	6.93	10.66	8.36	1.43	5.72	6.49	5.92	0.32	Ovoid to slightly irregular, brown to dark
WBJR2	6.59	11.81	9.64	2.14	3.95	6.29	5.12	0.94	Ovoid to slightly irregular, brown to dark
WBJR4	9.55	12.89	11.3	1.3	5.57	8.01	6.7	0.89	Ovoid to slightly irregular, brown to dark
LKPCBMB	9.35	13.91	11.69	1.89	5.53	6.84	6.1	0.59	Ovoid to slightly irregular, brown to dark
LKPCBMD	9.09	11.47	10.5	0.9	4.83	8.35	6.28	1.63	Ovoid to slightly irregular, brown to dark

Note: -: Not found

Table 5. Pathogenicity of *Colletotrichum* spp.

Isolates	Score			Disease Severity (%)	Lesion diameter (mm)	LD. ST. Dev.
	U1	U2	U3			
Control	0	0	0	0	0	0
ACT2	5	5	3	48.14	7.66	0.97
BCG1	7	7	5	70.37	21.06	6.38
BCGTR	0	0	0	0	0	0
CCG2	9	9	9	100	61.08	18.23
CMRCS	5	5	5	55.55	17.23	3.18
CMRDCS	5	3	3	40.74	8.45	3.86
LBKCMRB	5	3	5	48.14	14.18	8.99
LBKCMRB2	3	3	3	33.33	6.88	0.85
LBKCMRB4	5	5	5	55.55	8.5	2.08
LKPCBMB	5	7	7	70.37	22.8	10.51
LKPCBMD	5	5	3	48.14	12.21	5.65
MGCMKB	5	7	5	62.96	14.31	6.83
RJPCBMD	5	5	5	55.55	9.18	1.94
RJPCBMT	3	5	3	40.74	7.73	1.68
THDP1	7	7	7	77.77	27.46	5.5
WBJR1	5	5	5	55.55	14.46	1.2
WBJR2	5	3	5	48.14	8.56	2.6
WBJR4	5	5	3	48.14	9.9	2.49

Note: *LD.ST.Dev.: Standard Deviation of Lesion Diameter

Table 6. Host range of *Colletotrichum* spp.

	Isolates						
	Control	THDP1	WBJR4	RJPCBMT	LKPCBMD	BCG1	LBKCMRB
Plants	**Isolate pathogenicity**						
Tomato	-	+	+	+	+	+	+
Strawberry	-	+	+	+	+	+	+
Grape	-	+	+	-	+	+	+
	Lesion diameter (mm)						
Tomato	0	26.78	11	7.08	15.12	24.53	19.2
Strawberry	0	47.78	28.05	43.07	44.17	56.38	37.72
Grape	0	6.1	5.42	0	6.32	6.78	1.12

Note: +: Presence; -: Absence

Host range

The results of cross-infection using representative isolates from each region (Table 6) revealed that THDP1, WBJR4, LKPCBMD, BCG1, and LBKCMRB isolates were pathogenic to tomatoes, strawberries, and grapes. In contrast, the RJPCBMT isolate did not exhibit pathogenicity on grapes. The lesions observed had diameters ranging from 7.08 to 26.78 mm on tomatoes, from 28.05 to 56.38 mm on strawberries, and from 1.12 to 6.78 mm on grapes. Based on the data, it was evident that *Colletotrichum* isolates from chili had a higher level of severity infection in strawberries than the other two fruits.

Conducting host range testing is essential in studying plant diseases as it enables the prediction of host range, characterization of plant pathogens, understanding of pathogen epidemiology, developing of disease management methods, and clarifying of host range concept (Morris and Moury 2019). The specific reasons for this discrepancy require careful analysis, and potential factors contributing to higher disease severity in strawberries may include variations in host susceptibility, pathogen adaptation to different hosts, or differences in environmental conditions influencing the interaction between the pathogen and host. Occasionally, while doing pathogenicity testing with *Colletotrichum* species, the fruit infected with the fungus does not exhibit any symptoms. An instance of this can be seen in research conducted in Korea, which showed that healthy fruits deliberately infected with *Colletotrichum*

scovillei did not exhibit any symptoms, possibly due to low virulence (Oo et al. 2017).

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