Resistance of cayenne pepper varieties (Capsicum frutescens) to anthracnose disease (Colletotrichum gloeosporioides) isolates from swampy areas

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Resistance of cayenne pepper varieties (*Capsicum frutescens*) to anthracnose disease (*Colletotrichum gloeosporioides*) isolates from swampy areas

Ketahanan varietas cabai rawit (*Capsicum frutescens*) terhadap penyakit antraknosa (*Colletotrichum Gloeosporioides*) isolat asal lahan rawa

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ABSTRACT
Anthracnose, a primary disease in chili plants caused by the pathogen fungus *Colletotrichum* sp., has proven significant losses by affecting harvests and inducing fruit decay. Control measures using synthetic pesticides have been implemented but yielded unsatisfactory results. The utilization of resistant varieties stands as a pertinent approach in early control efforts to minimize harvest losses. Additionally, the use of resistant varieties is a crucial component of integrated disease control implementation. This study aims to investigate the resistance levels of ten chili varieties commonly grown in swampy areas against specific isolates of *Colletotrichum* sp. present in swampy locations. The research, conducted in a randomized complete design in a greenhouse, tested ten varieties of cayenne pepper typically cultivated in swampy areas. The results revealed that the Hiyung variety is Susceptible, while Bara, Dewata 43 F1, Tiung Tanjung, Genie, Sekar, and CR-9 varieties exhibit moderate resistance. Conversely, Tiung Ulin, Alip, and Sret varieties are classified as resistant. The incubation period for tested cayenne pepper varieties varied, ranging from 3.5 to 5.3 days. A longer incubation period indicates greater resistance to anthracnose in chili varieties. The selection of resistant varieties is a pivotal step in anthracnose management, not only to minimize harvest losses but also to support an effective integrated control approach.

ABSTRAK
Antraknosa, penyakit utama pada tanaman cabai yang disebabkan jamur patogen *Colletotrichum* sp., terbukti menimbulkan kerugian signifikan karena dapat menginfeksi hasil panen hingga menyebabkan kerusakan buah yang parah. Meskipun telah dilakukan upaya pengendalian menggunakan pestisida sintetis, hasilnya belum memuaskan. Penggunaan varietas tahan menjadi pendekatan yang sangat relevan dalam strategi pengendalian dini untuk meminimalkan kerugian hasil panen. Selain itu, integrasi varietas tahan menjadi unsur krusial dalam implementasi pengendalian penyakit secara terpadu. Penelitian ini bertujuan untuk menyelidiki tingkat ketahanan sepuluh varietas cabai yang umumnya ditanam di lahan rawa terhadap isolat *Colletotrichum* sp. yang spesifik di lokasi lahan rawa. Penelitian ini dilakukan di rumah kaca dengan rancangan acak lengkap untuk menguji sepuluh varietas cabai rawit yang biasanya ditanam di lahan rawa. Hasil penelitian mengungkapkan bahwa varietas Hiyung tergolong rentan, sementara varietas Bara, Dewata 43 F1, Tiung Tanjung, Genie, Sekar, dan CR-9 bersifat moderat; sedangkan varietas Tiung Ulin, Alip, dan Sret tergolong tahan. Masa inkubasi bervariasi antara 3.5-5.3 hari untuk beberapa varietas cabai rawit yang diuji. Semakin panjang masa inkubasi, semakin tinggi tingkat ketahanan varietas cabai terhadap penyakit antraknosa. Pemilihan varietas tahan menjadi langkah kunci dalam pengelolaan antraknosa, tidak hanya untuk meminimalkan kerugian hasil panen, tetapi juga untuk mendukung pendekatan pengendalian terpadu secara efektif.

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INTRODUCTION

Chili is one of the horticultural crops extensively cultivated in Indonesia. There are three common types of chili grown in Indonesia, i.e., chili pepper (*Capsicum annum* var L.), curly chili (*Capsicum annum* var L.), and cayenne pepper (*Capsicum frustescens* L.). In 2018, among these chili types, cayenne pepper proved to have the highest production in Indonesia, reaching 1.34 million tons (BPS Indonesia, 2019). However, like other crops, pest attacks have become an inseparable part of the chili cultivation process, leading to a decline in both quality and quantity, causing significant losses for farmers. Anthracnose is a major disease affecting chili plants and significantly impacting chili production worldwide, including Indonesia (Than et al., 2008; Widodo & Hidayat, 2018). Anthracnose disturbances have posed a significant obstacle to economic growth in every planting season (Widodo, 2007).

Anthracnose attacks have been shown to cause yield losses in chili crops ranging from 10-80% during the rainy season and 2-35% during the dry season (Widodo & Hidayat 2018). Aside from causing severe harvest losses, anthracnose epidemics can also diminish product quality (Salotti et al., 2023). Initially, infected fruits develop sunken lesions, followed by the appearance of dark gray to black spots with brown edges. In the middle of the symptoms, acervuli resembling small blackish spots emerge. Severe infections result in fruit wrinkling, drying, and rotting (Mariana, et al., 2021a). High losses occur due to the presence of the pathogen in both post-harvest and pre-harvest periods, leading to the loss of 10-80% of marketable chili fruit (Than et al., 2008). During pre-harvest in the field, the primary pathogenic fungus is dispersed by the wind, and windy rain significantly aids the increased development of anthracnose. The disease continues to evolve post-harvest as it spreads from infected to healthy fruits through contact during storage. The disease worsens when there are injuries to healthy fruits.

The *Colletotrichum* sp. fungus is not only the cause of chili fruit rot (de Silva et al., 2019, Sutomo et al., 2022) but has also been proven to be the culprit behind diseases affecting various horticultural plants and fruits worldwide. In China, 11 different species of *Colletotrichum* have been identified as the cause of anthracnose in chili plants (Diao et al., 2017). In Indonesia, 7 out of the 24 reported *Colletotrichum* species in Asia have been identified (de Silva et al., 2019). According to the research by Anggrahini et al. (2020), four different *Colletotrichum* species were successfully molecularly identified in Yogyakarta, *viz. C. scovillei*, *C. truncatum*, *C. siamense*, and *C. makassarii*.

Currently, chili plants are extensively cultivated in both lebak swamp and tidal swamp areas. However, chili cultivation in lebak swamp areas offers a specific advantage—chili plants can be grown during the dry season, while simultaneously other agroecosystems experience drought. Therefore, in lebak swamp areas, chili cultivation can occur outside the usual planting season, leading to higher selling prices. In lebak swamp regions, utilizing the surjan system, a locally adaptive cultivation technique for swampy areas, chilies are typically planted on raised beds (Hayati & Hardarani, 2019). Raised beds, also known as tukungan or embankments, elevate the soil to prevent inundation during high tide or standing water in lebak swamp areas (Nursyamsi & Noor, 2015). Chilies are also commonly grown in type B tidal swamp areas, where tidal flooding occurs only during significant high tides (Hartoni & Shafriani, 2023).

South Kalimantan is one of the provinces where chili plants are most commonly found in lebak swamp and tidal swamp areas. However, anthracnose consistently proves to be a serious issue faced by farmers every planting season. Anthracnose disease in chilies has been identified in local cayenne pepper varieties, such as Hiyung, cultivated in several locations in South Kalimantan (Budi & Mariana, 2016). In the year 2020, the incidence of anthracnose in lebak swamp areas reached 100%, with an average disease severity level of 43.7%. The average disease incidence rate was also higher in chili cultivation in the tidal swamp areas of the Marabahan Subdistrict, reaching 57.54%.

The research findings of Mariana et al. (2021b) demonstrate that *Colletotrichum* isolates from the swampy area in Hiyung Village exhibit resistance to the fungicide containing propineb, commonly used by farmers when symptoms of plant infection arise. Farmers also frequently employ chlorothalonil-based fungicides at double the recommended dosage;

however, it has been proven ineffective in mitigating anthracnose issues in chilies (Hajijah et al., 2022). In addition, the potential escalation of anthracnose is heightened not only through seed transmission but also via contact between fruits during storage. Rainwater splashes, residues of diseased plants in the soil, and surface water flow contribute to the disease spread (Oo & Oh, 2016). Epidemiologically, anthracnose follows a polycyclic life cycle where asexual spores (conidia), transported by water splashes, play a crucial role in the initiation and spread of the epidemic (Salotti et al., 2023). Plant materials infected with *C. acutatum*, even if symptomless, can serve as a source of infection and strongly correlate with disease occurrences both pre-and post-harvest (Debode et al., 2015).

Farmers have employed various control measures to tackle the *Colletotrichum* sp. issue due to the significant potential for losses. In an effort to minimize these losses, farmers often opt for the control of this disease using synthetic pesticides. However, the use of synthetic pesticides should ideally be restricted further due to the potential residue they leave on agricultural products and in the environment, posing risks to both humans and other non-target organisms (Purnama & Mutamima, 2023; Malhat et al., 2023; Syafrani et al., 2022). Therefore, alternative methods are essential for anthracnose control, particularly in field settings. One such approach involves the use of resistant plant varieties, commonly known as resistant varieties.

Control through resistant plants can be easily and compatibly integrated with other control techniques. In addition to being environmentally friendly, the cultivation of resistant varieties also reduces control costs, minimizes pesticide residues on products, and diminishes risks for farmers during field applications (Shahzaman et al., 2015; Rahoo et al., 2017). The resistance genes possessed by resistant varieties enable plants to withstand pathogen attacks even in conditions conducive to rapid disease development. The resistance of PBC932 to *C. acutatum* is governed by two dominant genes at the green fruit stage and two recessive genes at the ripe fruit stage, demonstrating the inheritance of monogenic dominant resistance against *C. truncatum* (Ridzuan et al., 2018). The HpmsE032 marker can be considered valuable in selecting resistant genotypes originating from the PBC80 line (Rout et al., 2023).

The resistance of chili varieties to anthracnose has been extensively studied, yet the evaluation of certain varieties cultivated by farmers in swampy areas remains crucial, especially concerning their resistance to *Colletotrichum* sp. in the presence of commonly used fungicides, and the prevalence of anthracnose in swampy fields, as observed in the main cayenne pepper cultivation center of Banjar, particularly the Hiyung variety (Mariana et al., 2021a). Hence, this study aims to evaluate the resistance levels of ten chili varieties commonly grown in swampy areas against *Colletotrichum* sp. isolates originating from swampy fields. The ten evaluated varieties include Bara, Hiyung, Dewata 43 F1, Tiung Tanjung, Tiung Ulin, Genie, Sekar, Alip, Sret, and CR-9, which are the most cultivated cayenne pepper varieties by farmers in swampy areas based on the initial survey results of this research. The testing employs *Colletotrichum* sp. isolates sourced from Hiyung chili in the swampy fields of Hiyung Village. Hiyung chili is the most extensively cultivated variety by farmers in South Kalimantan.

MATERIALS AND METHODS

The evaluation of the resistance of these ten test varieties was conducted in the greenhouse and the Phytopathology Laboratory of the Faculty of Agriculture, Lambung Mangkurat University, Indonesia (3°26'26.3"S 114°50'43.6"E). The test plants consist of ten cayenne pepper varieties commonly cultivated in the chili cultivation centers of tidal swamp fields in Antaraya Village, Barito Kuala Regency, South Kalimantan, and in lebak swamp fields in Hiyung Village, Tapin Regency, South Kalimantan. The ten varieties include Hiyung, Tiung Ulin, Alip, Sret, Bara, Dewata 43 F1, Tiung Tanjung, Genie, Sekar, and CR-9. The test plants were grown in polybags using soil from swampy fields supplemented with organic fertilizer at a soil-to-organic-fertilizer ratio of 2:1. The research was performed in a greenhouse with a completely randomized design, consisting of ten treatments repeated three times, and each experimental unit containing two chili plants.

Isolation and preparation of Colletotrichum sp. *inoculum from Hiyung chili*

Samples displaying symptoms were collected from the Hiyung chili cultivation site in the swampy fields of Hiyung Village, Central Tapin Regency, South Kalimantan Province. Chili peppers exhibiting early signs of pathogen attack or those not fully covering the fruit surface with symptoms were used as the inoculum source. The *Colletotrichum* isolation procedure followed the method described by Hu et al. (2022), wherein chili peppers with anthracnose symptoms were cut into 5 mm × 5 mm pieces on the fruit area between the diseased and healthy parts. Subsequently, they were dipped in 70% alcohol for 30 seconds to eliminate contamination on the outer surface and rinsed three times in sterile water. The dried chili fruit pieces were cultured on potato dextrose agar (PDA) supplemented with streptomycin sulfate (100 mg/L) at a concentration of 2 mL/L.

Observation of macroscopic characteristics involved visual examination of the colony, encompassing cultural morphology such as colony color and edge shape, surface colony characteristics, acervulus color, and colony growth rate. Microscopic observations were conducted under a Leica DM300 microscope (Leica Microsystems Pte Ltd, USA). The observed microscopic morphological characteristics of the pathogen included the shape and character of conidia, as well as the presence of setae. The results of both macroscopic and microscopic observations were then validated through relevant literature on the fungal colonies of the *Colletotrichum* sp. genus (de Silva, 2019).

Testing chili resistance levels

Small polybags filled with a homogeneous mixture of soil and organic fertilizer (2:1) were planted with chili seeds soaked in water for 24 hours (Debbarma et al., 2018). The soil was perforated to a depth of 1-2 cm, and two chili seeds were placed inside, followed by covering with soil. The chili seedlings were nurtured until they had four leaves and were three weeks old. Seedlings with four leaves were then transferred to larger polybags measuring 35 × 35 cm. Before the transfer, the planting medium was perforated to a depth of ±5 cm. Transplantation involved selecting healthy and uniformly grown chili seedlings, ensuring the soil adhering to the root system was transferred along with the seedling, and closing the hole with the planting medium. The newly planted seedlings were immediately watered to keep the soil moist and provided with shade. Additionally, backup plants were prepared to replace any primary plants that died or were affected by pests.

Inoculation of *Colletotrichum* sp. begins by creating an inoculum suspension. This is performed by adding 10 mL of sterile water to a Petri dish containing the *Colletotrichum* sp. culture. The contents of the dish are then spread evenly using a triangular spreader. Subsequently, the 10 mL suspension is transferred to an Erlenmeyer flask containing 90 mL of sterile water and homogenized using an orbital shaker (Orbital shaker TS 330 A, TIT, Taiwan) at a speed of 150 rpm for 15 minutes. The suspension is prepared at a concentration of 10^6 spores/mL, determined using a hemacytometer (Mapienfeld, Germany). Healthy chili peppers to be inoculated are first wounded using a sterile needle. Inoculation is performed by spraying 10 mL of the fungal suspension per plant over the entire surface of the chili plants. Afterward, the topsoil is covered with a damp cloth, and the plants are covered with transparent plastic for 2 days. After 2 days, the cover is removed, and the plants are placed in a shaded area (Dzung et al., 2017; Srisapoom et al., 2021).

The parameters observed in this study include the incubation period duration, calculated from inoculation to the appearance of the first symptoms (Leclerc et al., 2014), disease incidence (Syukur et al., 2007), and infection rate (van der Plank, 1963). Disease incidence observation commenced one day after inoculation and continued until the susceptible check plant (Hiyung variety) displayed anthracnose symptoms with intensity exceeding 40%, classifying it as susceptible. Disease incidence (DI) was measured using the formula based on Syukur et al. (2007):

$$DI = \frac{n}{N} \times 100\% \quad (1)$$

where:

DI = Disease incidence, %

n = Number of infected fruits, fruits

N = Total number of observed fruits, fruits

Establishing criteria/degrees of chili plant resistance to anthracnose attacks is based on disease incidence (Andika, 2020). The determination of chili plant resistance criteria to anthracnose is grounded in the disease incidence outlined in Table 1 (Palupi et al., 2015).

Table 1. Criteria for chili plant resistance to anthracnose

Disease incidence (%)	Criteria
$0 \leq X \leq 10$	Highly resistant
$10 \leq X \leq 20$	Resistant
$20 \leq X \leq 40$	Moderat
$40 \leq X \leq 70$	Susceptible
>70	Highly Susceptible

The disease infection rate is a measure of the pathogen's rapid development over time. The polycyclic formula is used to determine the disease infection rate (Van der Plank, 1963) with the calculation formula:

$$R = \frac{2,3}{t2-t1} \log 10 \frac{X2(1-X1)}{X1(1-X2)} \quad (2)$$

where:

R = Infection rate, %/day

x1 = percentage of disease incidence at the first observation, %

x2 = percentage of disease incidence at the second observation, %

t1 = time of the first observation, day

t2 = time of the second observation, day

RESULTS AND DISCUSSIONS

Anthracnose symptoms

The isolates identified as the causative agent of anthracnose exhibit nearly identical symptoms. Initially, the infected part of the chili pepper fruit undergoes inward wrinkling (Figure 1A), subsequently transforming into brown spots (Figure 1B) with irregularly formed circles. In the middle, small black spots emerge (Figure 1C), and if left untreated, the infected part of the fruit dries up (Figure 1D). These findings align with the research of Kiran et al. (2020), stating that anthracnose-affected chili pepper fruits exhibit black spots, and concave necrotic tissue with concentric rings of acervuli. Oo & Oh's (2020) study reports typical symptoms of anthracnose in chili pepper fruits, including concave necrotic tissue with concentric acervuli rings and merging lesions. This begins with the development of indentations and progresses to the appearance of dark gray to black spots with brownish edges. Additionally, according to Soesanto (2019), symptoms of the disease in chili pepper fruits are characterized by small, round, slightly submerged yellowish spots that later turn brown. Furthermore, Almeida et al. (2017) state that severe disease infestations can cause the fruits to shrink and completely dry up, even though the initial symptoms consist of small brown lesions with clearly defined circular edges.

Symptoms of the affected fruits found in Hiyung Village begin with the formation of indentations, followed by the appearance of dark gray to black spots. In the middle of the symptoms, there are small spots with a dark black color. The spots are surrounded by brown edges (Figure 1D). Further infection results in the fruits wrinkling, drying, and rotting. The development of anthracnose symptoms in each test variety does not show significant differences, both in the early and advanced symptoms (Figure 2). However, the number of affected fruits varies among different varieties. In the Hiyung variety, more chili pepper fruits are affected (42.8%), while the Sret variety has only 17.11% affected fruits (see Table 1).

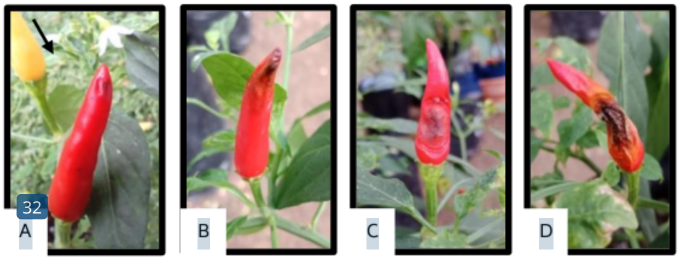

Figure 1. The sequence of anthracnose disease symptoms after inoculation with *Colletotrichum* sp

Figure 2. Anthracnose symptoms resulting from inoculation on hot chili peppers for each test variety: A. Hiyung, B. Dewata 43 F1, C. Bara. D. Tiung Tanjung. E. Genie. F. CR-9 G. Alip. H. Stret. I. Tiung Ulin. J. Sekar

Microscopic observations reveal conidia growing from conidiophores present on acervuli (Figure 3C). The conidia are cylindrical, and straight, with both ends blunt (Figure 3D and 3E). The diagnosis of the plant disease causative agent begins with Koch's postulates procedure. Factors observed include the symptoms and macroscopic and microscopic characteristics of pure isolates derived from those symptoms (Riley et al., 2002). Based on the symptom description by Liu et al. (2016), the symptoms of the Hiyung variety are indicative of anthracnose disease. Anthracnose symptoms on hot chili peppers manifest as dark brown to black spots, concave lesions with numerous black acervuli on the surface, and a plethora of dirty white conidia masses in humid conditions.

The colonies of pure isolates obtained from symptomatic Hiyung chili pepper fruit with anthracnose are greenish-gray and exhibit a cotton-like mycelium. The back of the petri dish shows a diurnal zonation resembling concentric rings with the presence of aerial mycelium (Figure 3Aii). Initially, the colony is white and gradually turns light gray, then dark greenish-gray. This aligns with the findings of Widodo & Hidayat (2018), where among three types of *Colletotrichum* isolates from chili peppers, morphotype 2 exhibits a cotton-like mycelium, grayish color with an olive gray to dark gray base (Figure 3B). Molecular characterization identified morphotype 2 as *C. gloeosporioides*. Similar observations were reported by Than et al. (2008) for *C. gloeosporioides* colonies from chili peppers, ranging from whitish-gray to dark gray. Some isolates displayed diurnal zonation with aerial mycelium in pale to blackish-gray, while others produced even and palpable aerial mycelium. This is consistent with Sheu & Wang's (2005) research, which found that *C. gloeosporioides* isolates from chili peppers exhibited colors ranging from white to gray and dark olive green. The colony's rapid growth, reaching 90 mm in 9 to 10 days at an average rate of 9.95 mm per day, is notable. *C. gloeosporioides* colonies showed significantly faster growth compared to other *Colletorichum* species, as reported by Than et al. (2008), with *C. gloeosporioides* isolates growing at 11.0 mm per day to 11.2 mm per day, significantly outpacing the other groups—*C. capsici* isolates at 7.1 mm per day and *C. acutatum* isolates at 5.8 mm per day.

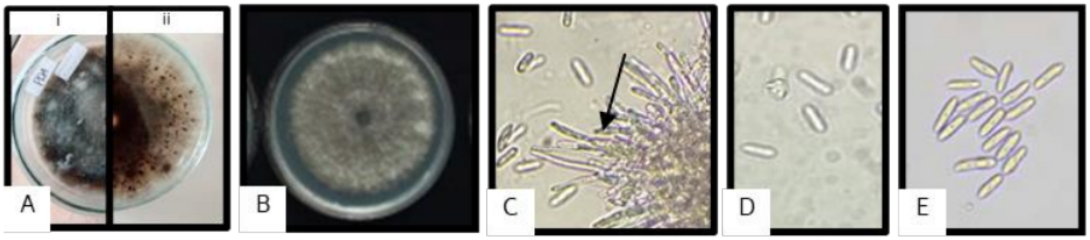

Figure 3. Isolate from symptomatic Hiyung chili pepper fruit, A: *Colletotrichum* sp. isolate on (i) front and (ii) back, B: *C. gloeosporioides* isolate (Widodo & Hidayat, 2018), C: acervuli with conidiophores D. conidia of *Colletotrichum* sp., E: conidia of *C. gloeosporioides* (Widodo & Hidayat, 2018)

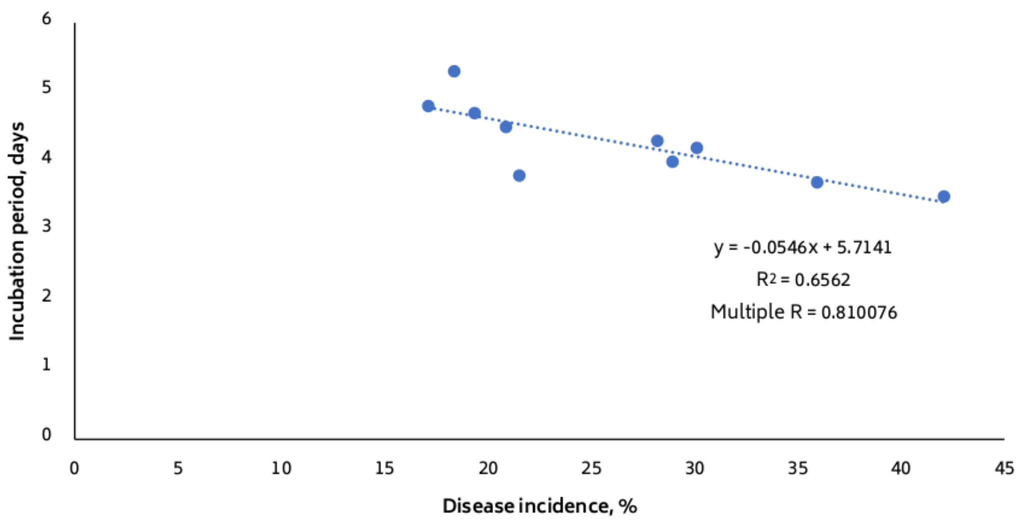

Figure 4. Regression relationship between disease incidence and incubation period

The results of microscopic morphological observations indicate that the conidia are cylindrical with slightly blunt and transparent ends, and no setae were found, suggesting that the fungus belongs to *C. gloeosporioides*. According to de Silva (2019), the conidial shape is a characteristic used to determine *Colletotricum* species. *C. gloeosporioides* has cylindrical conidia with blunt ends, distinguishing it from *C. acutatum* (which has pointed conidia) and *C. truncatum* (which has falcate conidia). Besides conidial shape, the presence of setae is rare in *C. gloeosporioides*, and its conidia are straight cylinders with blunt ends on both sides. The isolate of *C. gloeosporioides* has cylindrical, straight conidia with blunt ends, appearing white to gray, and exhibits scattered black acervuli spots throughout (Mariana et al., 2021a). In contrast, according to Widodo & Hidayat (2018), the conidia of *C. gloeosporioides* are cylindrical with rounded ends on both sides.

Correlation of incubation period with resistance levels of varieties

Incubation period observations were conducted daily after inoculating the plants with *Colletotrichum* sp. until the appearance of the first symptoms. Based on the obtained data, each studied type of hot pepper has a different incubation period. The data analysis results for the incubation period indicate that Tiung Tanjung, Sekar, Dewata 43 F1, Bara, and CR-9 did not differ from Hiyung. Conversely, Genie, Alip, Sret, and Tiung Ulin varieties were significantly different from Hiyung (Table 2). Hiyung had an incubation period of 3.5 days, followed by Tiung Tanjung at 3.7 days, and Sekar at 3.8 days. Dewata 43 F1 had an incubation period of 4.0 days, Bara at 4.2 days, CR-9 at 4.3 days, Genie, and Alip each at 4.5 days, Sret at 4.8 days, and Tiung Ulin had the longest incubation period at 5.3 days.

The regression test results between the incubation period and disease incidence show a very strong correlation with a value of R = 0.810076, consistent with the findings of Putra et al. (2022), stating that if the correlation coefficient (Multiple R) is between 0.60 – 0.899, there is a strong correlation between variables x and y, i.e., between the incubation period and disease incidence. The correlation resulted in an equation and coefficient of determination (R^2) indicating the percentage of the incubation period's influence on disease incidence. The linear equation is $y = -0.0546x + 5.7141$ with $R^2 = 0.6562$ (Figure 4). This analysis indicates that the incubation period influences 65% of disease incidence, while the remaining percentage is influenced by other factors. The negative impact value in the regression equation suggests that the longer the time it takes for the pathogen to enter and produce symptoms in plants, the lower the disease incidence.

The incubation period is influenced by the pathogen's ability to attack the plant until symptoms are produced, determined by the plant variety's inherent resistance to pathogen attacks (Mongkolporn & Taylor, 2018). In susceptible hosts, the biotrophic phase with extensively branched primary hyphae lasts only 72 hours after initial infection before transitioning to the necrotrophic phase. In the necrotrophic phase, secondary narrow filamentous hyphae grow rapidly from the tips of primary hyphae, attacking surrounding cells quickly, and resulting in anthracnose symptoms (de Silva, 2017b). The incubation period of anthracnose caused by *Colletotrichum* sp. varies between different varieties, and their resistance levels also differ. Mishra et al. (2017) conducted a study to test the resistance of two chili cultivars, Teja Jhal (TJ) and Bhut Jolokia (BJ), to anthracnose. The highly susceptible chili genotype TJ infected by *C. truncatum* exhibited symptoms as early as 3 days post-inoculation, rapidly developing from 26.2 ± 0.6 mm to 29.41 ± 0.2 mm. In the resistant variety BJ, the incubation period was 5 days post-inoculation, and symptom development was slow, ranging from 1.18 ± 0.13 mm to 1.21 ± 0.21 mm. The resistance and susceptibility of a variety are determined by the incidence rate of the disease it causes. A shorter incubation period indicates a higher disease incidence rate and increased vulnerability to pathogen attacks. In more susceptible varieties, the incubation period is shorter, and conversely, in more resistant varieties, it is longer, as indicated in this study by the strong correlation value of 0.810076 between the incubation period and disease incidence. The incubation period is longer with higher resistance and shorter with lower resistance (Mora et al., 2015). The chili genotype CB-EL in the study by Andarwening & Matra (2020) demonstrated the highest resistance among other genotypes, with a leaf curl yellow virus disease incidence rate of 8.89% and the longest incubation period, ranging from 21 to 50 days.

Disease incidence and plant resistance level

All evaluated varieties of cayenne pepper exhibited a daily increase in anthracnose disease incidence. Additionally, the percentage of disease incidence varied across all evaluated disease types. Hiyung variety recorded the highest disease incidence rate, accounting for 42.08%, while the lowest disease incidence occurred in the Sret variety, with only 17.11% (Figure 5).

Each chili pepper species under study exhibits different resistance levels to anthracnose disease. The disease incidence percentages for Tiung Ulin, Alip, and Sret, at 18.37%, 19.36%, and 17.11% respectively, fall under the resistant category. On the other hand, with disease incidence percentages of 30.11% for Bara, and sequentially 28.94%, 35.93%, 20.87%, 21.52%, and 28.20% for Dewata 43 F1, Tiung Tanjung, Genie, Sekar, and CR-9, they are classified into the moderate resistance group. Meanwhile, the Hiyung variety falls into the Susceptible category with a disease incidence percentage of 42.08% (Table 2). Field observations on farmers' land indicate that Tiung Tanjung and Hiyung are the most commonly found chili pepper varieties in swampy cultivation areas. Hence, it is not surprising that these varieties exhibit a higher disease development rate compared to others, as the pathogens have adapted to both varieties in swampy fields.

Table 2. Average disease incidence, resistance category, incubation period, and average infection rate (n = 40)

Variety	Disease Incidence (%)	Resistance Level	Incubation period	Average Infection Rate
Bara	30.11	Moderat	4.2[abc]	0.132
Hiyung	42.08	Susceptible	3.5[a]	0.181
Dewata 43 F1	28.94	Moderat	4.0[abc]	0.125
Tiung Tanjung	35.93	Moderat	3.7[ab]	0.165
Tiung Ulin	18.37	Resistant	5.3[d]	0.109
Genie	20.87	Moderat	4.5[bc]	0.113
Sekar	21.52	Moderat	3.8[ab]	0.139
Alip	19.36	Resistance	4.7[cd]	0.117
Sret	17.11	Resistance	4.8[cd]	0.115
CR-9	28.20	Moderat	4.3[abc]	0.13

Note: Numbers followed by the same letter indicate no significant difference based on DMRT at α = 5% level.

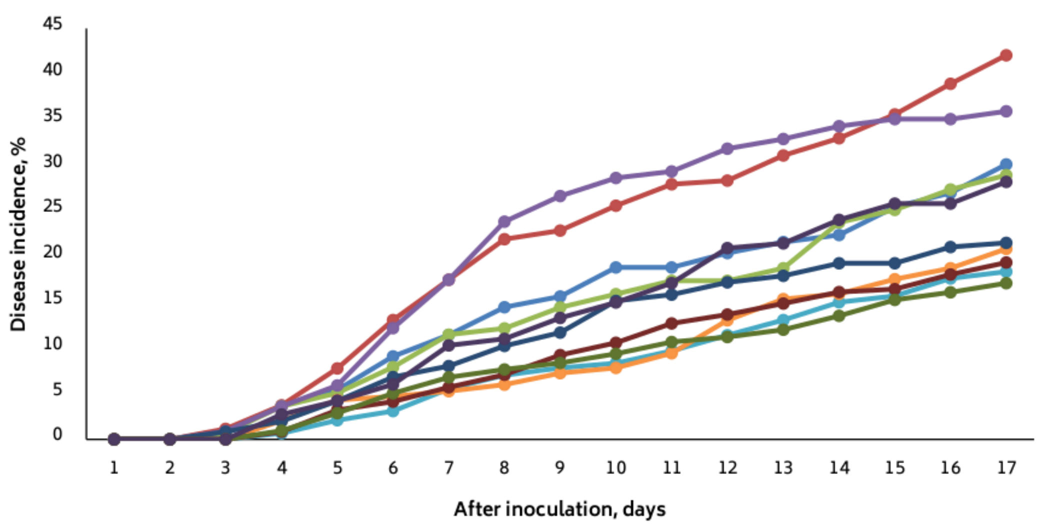

Figure 5. The progression of anthracnose disease incidence in the tested varieties: Bara (sky blue), Genie (orange), Hiyung (red), Sekar (dark blue), Dewata (lime), Alip (brown), Tiung (purple), Sret (green), Tiung Ulin (blue-green), CR9 (black)

In this study, it has been demonstrated that each variety exhibits distinct categories of resistance. This is presumed to occur due to the plant's defense system, regulated by the genes inherent in each variety, both before and after successful pathogen entry. Six genes contributing to the resistance of the large chili variety *Capsicum annuum* cv. Nokkwang against *Colletotrichum siamense* include cytochrome P450, PepCYP, thionin-like (PepThi), defensin (J1-1), pepper thaumatin-like (PepTLP), MADS-box (PepMADS), and pepper esterase (PepEST) genes (Cui et al., 2023). Another study by Perdani et al. (2021) on six chili varieties revealed differing levels of resistance to *C. acutatum* and *C. gloeosporioides*. Resistance to *Colletotrichum* species is governed by specific gene families and biochemical interactions through specific enzymes and secondary metabolites produced during the host-pathogen interaction (de Silva, 2017a and de Silva, 2017b). Genes involved in the resistance of chili varieties to anthracnose include those producing antimicrobial peptides such as defensin, lipid transfer proteins, and protease inhibitors. Quantification of secondary metabolites during the interaction between resistant *C. annuum* and *C. siamense* reveals high concentrations of caffeic acid and chlorogenic acid, with differential expression dependent on fruit development stages and time after inoculation (incubation period) (Cui et al., 2023).

According to Prasath and Ponnuswami (2008), chili genotypes resistant to anthracnose exhibit higher levels of phenol content and active enzymes compared to non-resistant genotypes (such as ortho-dihydroxy phenol, peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase). The enzyme phenylalanine ammonia-lyase (PAL), crucial for the formation of phytoalexins and phenolics, is associated with an increase in phenolic compound content in plants. The resistance of chili fruits to *C. gloeosporioides* is attributed to the hypersensitive reaction (HR) (Kim et al., 2004). Differences in the resistance categories of chili plants are also suspected to result from mechanical resistance factors or regulation of morphological structures by expressed genes, such as cuticle layer thickness. Enhanced resistance through silica application can lead to thickening of cell walls and cuticles. Additionally, an increase in phenol levels or the outcome of various combined mechanisms can also be influencing factors (Jayawardanaa et al., 2016).

Differential disease infection rates in various plant resistance levels

In this study, each tested variety of cayenne pepper exhibited varying rates of anthracnose disease infection. The infection rates were as follows: Bara chili variety at 0.132 units per day, Hiyung variety at 0.181 units per day, Dewata 43 F1 variety at 0.125 units per day, Tiung Tanjung variety at 0.165 units per day, Tiung Ulin variety at 0.109 units per day, Genie variety at 0.113 units per day, Sekar variety at 0.139 units per day, Alip variety at 0.117 units per day, Sret variety at 0.115 units per day, and CR-9 variety at 0.130 units per day (Table 2). The highest infection rate occurred in the Hiyung variety with a value of 0.181 units per day, while the slowest infection rate was recorded in the Tiung Ulin variety with a value of 0.109 units per day. This indicates that in the Hiyung variety, there is an average increase of 0.181 fruits affected by the disease every day, whereas in the Tiung Ulin variety, the increase is 0.109 fruits per day. The progression of the anthracnose disease infection rate can be observed in Figure 6.

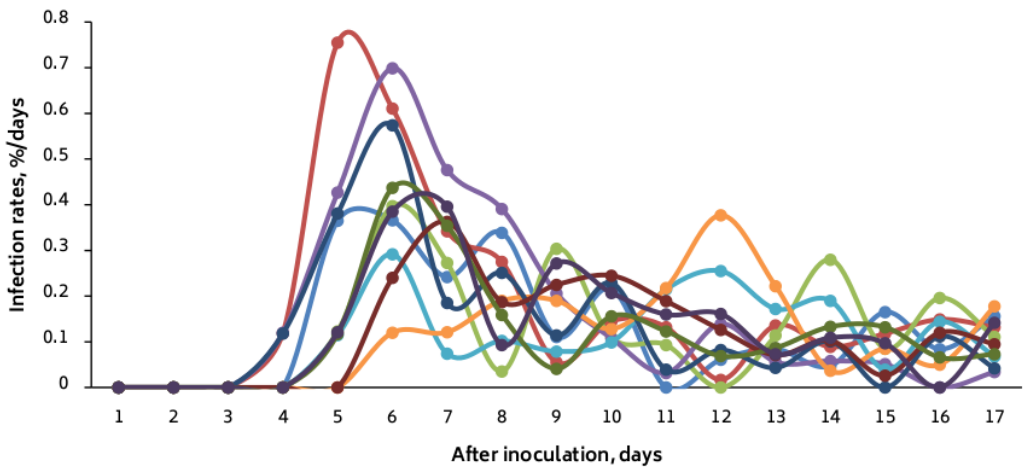

Figure 6. Development of anthracnose disease infection rates in several tested varieties of cayenne pepper: Bara (sky blue), Genie (orange), Hiyung (red), Sekar (dark blue), Dewata (lime), Alip (brown), Tiung (purple), Sret (green), Tiung Ulin (blue-green), CR9 (black)

Infection rate is influenced by resistant genes present in the host used by the pathogen to counteract virulence genes. These genes control one or more pathogenicity traits, including the infection rate. Based on the research data, the infection rates vary among tested varieties of cayenne pepper. The differences in disease infection rates in each tested variety align with variations in disease incidence and plant resistance levels (Table 2). According to Nutter (2007), this simple calculation provides an estimate of how the infection rate delays the time required to reach a specific disease intensity level (such as the onset of the disease).

In the Hiyung variety, the disease infection rate is higher compared to the other tested hot pepper varieties. This implies that a higher disease infection rate leads to a faster development of the pathogen population per unit of time, resulting in increased susceptibility of the variety. This observation is supported by the regression analysis results (Figure 7), showing that a faster infection rate corresponds to a higher disease incidence, with a high coefficient of determination ($R^2 = 0.815$). This indicates that 81.5% of the disease incidence is influenced by the pathogen's infection rate. Nutter (2007) asserts that one of the factors affecting R (disease infection rate) is the resistance of the host plant. The disease infection rate is a measure of the rate of development of the pathogen population per unit of time or the rate of growth of the pathogen population.

Based on the research data, each evaluated variety of cayenne pepper exhibits different levels of anthracnose infection. Resistant varieties can reduce the occurrence of the disease. The regression equation describing the influence of disease incidence on the infection rate is $y = 25.729x + 6.5063$. This indicates that the higher the infection rate, the higher the disease incidence, making the variety more susceptible. According to Meena et al. (2011), in Indian mustard plants (*Brassica juncea*), the Varuna cultivar is more Susceptible compared to the Rohini cultivar, as the infection rate on leaves and pods of the Varuna cultivar is higher than that of the Rohini cultivar.

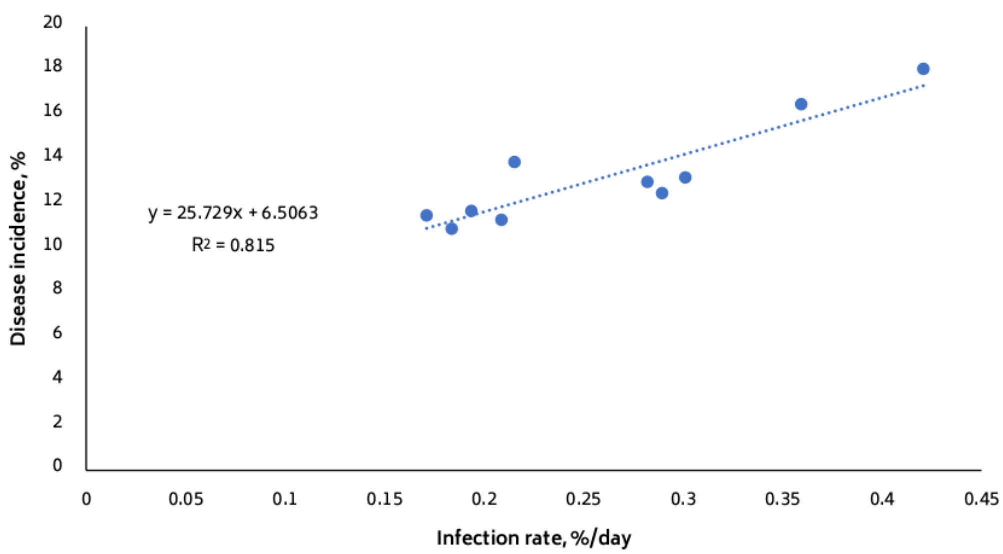

Figure 7. Regression Relationship between Disease Incidence and Infection Rate

Preliminary observations on each test variety indicate a higher infection rate, which then decreases until the end of the observation period (Figure 6). This aligns with the findings of Dhiman et al. (2022), who reported that bean plants (Phaseolus vulgaris) exhibit higher resistance in older plants compared to younger ones against anthracnose caused by *C. lindemuthianum*. This phenomenon is referred to as Adult Plant Resistance (APR). The research by Mongkolporn &

Taylor (2018) supports this, demonstrating that the age of chili plants influences their resistance. Mature chili fruits exhibit greater resistance to anthracnose. Young, green fruits (30–45 days after flowering) show more resilience than mature red and harvest-ready fruits (45–55 days after flowering). The anthracnose infection rate in various varieties of cayenne pepper in this study ranges from 0.109 units/day in Tiung Ulin with a disease incidence of 18.17%, lower than the infection rate in Hiyung, which is 0.18 units/day with a disease incidence of 42.08%. These findings are lower than the results of a study conducted by Prihatiningsih (2020), where the anthracnose infection rate in local Baturaden chili varieties in Kemutug Lor Village showed the highest disease intensity at 76% with an infection rate of 0.345 units per day.

CONCLUSION

The resistance categories to anthracnose disease among the ten cultivated cayenne pepper varieties in the swampy fields are classified as Susceptible (Hiyung variety), Moderate (Bara, Dewata 43 F1, Tiung Tanjung, Genie, Sekar, and CR-9 varieties), and Resistant (Tiung Ulin, Alip, and Sret varieties). The incubation period varies among cayenne pepper varieties, with an average ranging from 3.5 to 5.3 days. Varieties with a longer incubation period tend to exhibit greater resistance to anthracnose disease. The infection rate of anthracnose disease differs across the tested cayenne pepper varieties. Higher infection rates correlate with increased disease incidence, indicating greater susceptibility in those varieties.

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Resistance of cayenne pepper varieties (Capsicum frutescens) to anthracnose disease (Colletotrichum gloeosporioides) isolates from swampy areas

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