Anthracnose Disease on Vegetables Crops in Serang Village, District of Karangreja, Purbalingga Regency

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Abstract. Various vegetables are widely cultivated in Serang Village, District of Karangreja, Purbalingga Regency. This village is located at 1350 above sea level. Vegetable productions in Serang are often constrained by the presence of diseases caused by pathogenic fungi *Colletotrichum* sp. called anthracnose. However, there was no data regarding anthracnose attack in vegetable crops in Serang. This study aimed to find out the types of vegetables that were attacked by anthracnose disease as well as the intensity of the attack in vegetable crops in Serang Village, Karangreja District, Purbalingga Regency. Vegetables with anthracnose were purposively selected from 10 different locations. The anthracnose disease was identified based on their symptoms and analyzed descriptively. The results showed that five different vegetable crops suffer from anthracnose. The intensity of anthracnose ranges from 32% up to 52%. Those intensity percentages indicate that a large part of vegetable crops in Serang Village was attacked by anthracnose attack in Serang Village. The data is essential for the management of vegetable crops in Serang Village.

Key words: anthracnose, vegetable plants, percentage of damage

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INTRODUCTION

Serang Village, District of Karangreja, Purbalingga Regency, is located at 1,350 meters above sea level (masl) and has an area of 3,052.44 ha. It has monthly rainfall ranges from 235-274 mm with air temperature ranging between 18°C and 24°C. The climate condition of Serang Village is very suitable for horticulture crops (Central Bureau of Statistics, 2018).

Approximately 43.26% (1,245.07 ha) of dry areas of Serang Village are utilized by the farmer to grow vegetables. Therefore, vegetable crops in Serang Village provide a significant contribution to vegetable production in the District of Karangreja. However, vegetable production (ton) of the District of Karangreja has fluctuated from 2016 to 2017, depending on the commodity. The production of several commodities had decreased in 2017, while others had increased (Central Bureau of Statistics, 2016; 2018).

Vegetable production decline in Serang Village could be due to the fungal infection. According to Soesanto et al. (2019), fungal infection is among the limiting factor in crop production. In the case of vegetable crops, a fungal infection might cause anthracnose disease. Previous reports from Rojas et al. (2010) and Rampersad (2011) proved that anthracnose is a common disease in vegetable crops. Other studies also reported that anthracnose had caused huge losses on vegetable production (Syukur et al., 2011; De Silva et al., 2017). Production loss in vegetable crops caused by anthracnose ranges between 20% and 90% Balitbangtan (2016). Even the latest study proved that production loss due to anthracnose disease might reach 100% in fruit (Diao et al., 2017)

Anthracnose disease in vegetable crops is caused by *Colletotrichum* infection (Cannon et al., 2012; Dean et al., 2012; Udayanga et al., 2013). *Colletotrichum* infection starts by conidia attachment to the surface of the host plant. The first hyphae penetration does not do significant damage to the host, but the damage occurred when the next hypha is growing and forms intercellular webs (Syukur et al., 2011). The characteristics of the fruit affected by this pathogen include shrinking, runny, and having a concave black circle. The expanded circle is slimy with orange spores mass (Herwidyarti et al., 2013). Other symptoms found in fruit are drying and rotting (Diao et al., 2017).

Many studies have done to observe anthracnose in various vegetable crops (Herwidyarti et al., 2013; Palupi et al., 2015; Sudirga, 2016) from several locations. However, no scientific publication was available about anthracnose disease on vegetable crops in Serang Villages, District of Karangreja, Purbalingga Regency. Therefore, it was crucial to study anthracnose infection in vegetable crops in those areas.

This study aimed to find out the types of vegetables that were attacked by anthracnose disease as well as the intensity of the attack in the vegetable crops in Serang Village, Karangreja District, Purbalingga Regency. Information obtained from this study is essential as a scientific reference for vegetable cultivation management in that area.

METHODS

Research Location and Time

The survey was conducted at vegetable crops owned by communities in Serang Village, District of Karangreja, Purbalingga (Figure 1). Samples examinations were conducted at Mycology and Phytopathology Laboratory of Biology Faculty, Jenderal Soedirman University, Purwokerto. The samples were collected in two periods (27 April to 4 May and 10 June to 15 June 2019). Laboratory examination had conducted from 12 June to 10 August 2019.



Figure 1. Sampling locations in Serang Village

Sampling Technique

Samples collection technique used was purposive random sampling based on the abundance of vegetable plants at ten different places (Figure 1). The percentage of disease was calculated based on the formula from Palupi et al. (2015) as follows, $Kp = (n/N) \ge 100\%$

Remarks: Kp: Percentage of disease n: The number of infected plants N: The number of observed plants

Disease identification was performed based on signs and symptoms observed. Afterward, the obtained signs and symptoms were referred to several references, including Haggag (2010) and Avasthi et al. (2011).

Research Parameter

Two research parameters had measured during the study. These parameters were divided into primary and secondary parameters. The main parameter consisted of a type of plant and the percentage of the disease caused by fungi. The supporting parameter consisted of air temperature, air humidity, and soil acidity (pH).

Fungal Isolation and Identification

The samples of an infected plant from each location were brought to Mycology and Phytopathology Laboratory for fungal isolation and identification. The isolated fungi were rejuvenated to obtain a colony. The colony was then subjected to macroscopic and microscopic identification. Macroscopic identification was conducted to observe the shape, color, surface, edge, and distribution of the colony. Microscopic observation included the type of hyphae (insulated or non-insulated), shape and color of the spore, shape, color, and size of the conidia by referring to Avasthi et al. (2011). Each isolated pathogen was inoculated in the same vegetable plant (Koch Postulate test) to find out whether the characteristics of the same as of the previously isolated pathogenic fungi were present or not. Then, the process was continued with the second macroscopic and microscopic identification

Temperature and humidity measurements were carried out in the morning (07.00 WIB) and afternoon (17.00 WIB) using a Thermo hygrometer every day for one month. Meanwhile, the measurement of soil pH was carried out at each plot in all sampling locations using a soil tester.

Data Analysis

Data about the percentage of disease was presented in the table. All the symptoms and colony characteristics were documented and presented in pictures. All the data were analyzed descriptively through reference comparison.

RESULTS AND DISCUSSION

Vegetable crops in Serang Village and anthracnose infection

During the field observation, there were ten species of vegetable plants grown by the farmer in Serang Village, District of Karangreja, Purbalingga Regency (Table 1). It can be seen in Table 1 that a high variable of vegetables was grown in Serang Village. A total of 11 vegetable crops was grown in the District of Karangreja. It is mean that Serang Village supports for approximately of 90.91 % of total vegetables grown in Karangreja (Central Bureau of Statistics, 2017). An area of 671.28 ha of Serang Village was utilized for vegetable production. The area is 31.52% of the total area of vegetable crops farming in the District of Karangreja, with a total of 2.130 ha (Central Bureau of Statistics, 2018). That means that Serang Village provides high support to total vegetable production in the District of Karangreja, Purbalingga Regency. However, several species were susceptible to anthracnose disease, which might disrupt vegetable production in the District of Karangreja.

Table 1. The vegetables cultivated in Serang Village,District of Karangreja, Purbalingga Regency

Location	Vegetables		
II, IX	Tomato (Lycopersicum esculentum Mill.)		
III, X	Red Chili (Capsicum annuum L.)		
V, VII	Spring Onion (Allium fistulosum L.)		
IV, VI	Beans (Phaseolus vulgaris L.)		
VIII, X	Chili (Capsicum frutescens L.)		
VI, VIII	Chinese cabbage (Brassica pekinensia L.)		
I, X	Mustard greens (<i>Brassica rapa</i> var. <i>parachinensis</i> L.)		
V, VII	Celery (Apium graveolens L.)		
II, IV	Lettuce (Lactuca sativa L.)		
VIII, III	Chayote (Sechium edule (Jacq.)		

Signs and Symptoms of the Anthracnose in Each Vegetable Species

Careful observation found that fungi infected five out of ten vegetable species. The sign and symptoms of fungi infection in each vegetable crop and infected organ is summarized in Table 2. The observed symptom of fungal infection, as observed in all vegetables, shows a strong indication that fungi infect all vegetables from a single genus, namely *Colletotrichum*.

Table 2. List of infected vegetables, organs, sign, and symptom

Vegetable	Organ	Sign and Symptom
Tomato (Lycopersi-	Fruit	Dark-brown spot
cum esculentum Mill.)		
Red chili (Capsicum	Fruit	Blackish-brown
annuum L.)		patch
Spring onion (Allium	Leaf	Brown concave
fistulosum L.)		spots with yellow-
		ish halo
Beans (Phaseolus	Fruit	Runny, blackish-
vulgaris L.)		brown circular,
		and sunken spots
Cayenne chili (Capsi-	Fruit	Dry, brown or
cum frutescens L.)		black circular or
		curve patches

In the case of tomato fruit, the infection is possibly caused by *Colletotrichum coccodes* species. *C. coccodes* is thought to be the species that infect tomato fruit in Serang Village based on the similarity of the anthracnose symptoms in tomato fruit infected by *Colletotrichum coccodes* reported by Liu et al. (2011). According to Liu et al. (2011), tomato fruit infected by *Colletotrichum coccodes* will have many rounded small spots with a sunken area. The spots grow and become more prominent with dark-brown color. In the center of a big spot, there is a concentric ring. A bulk of spore forms the ring. Similar symptoms had also explained by Ben-Daniel et al. (2012) in potato and tomato.

The argumentation of *C. coccodes* infection in tomato fruit in Serang Village was supported by the data from macroscopic and microscopic observation of the isolated fungi. The fungi have white-yellowish color and cottony surface colonies. The colony has a concentric distribution pattern, insulated hyphae, noninsulated conidia, and crescent-shaped conidia with a dimension of 8-10 μ m x 2-3 μ m (Figure 1). The observed characters are similar to the characters of *C. coccodes* as previously explained by Sharma and Kulshrestha (2015) that *C. coccodes* has grey to pink mycelium and no sexual phase and the fungal colony has a light yellow color.



Figure 1. *Colletotrichum* sp. colony isolated from tomato on PDA medium; (A) top surface of pure culture; (B) bottom surface of pure culture; (C) Conidia (40x10)

The symptom observed in red chili fruit (Table 2) proved that the chili is suffering from a disease called fruit rot or anthracnose, which is caused by *Colleto-trichum* sp. The finding of this study is similar to anthracnose symptoms reported by Ratulangi et al. (2012) and Ramdan et al. (2019). Both previous studies stated that *Colletothricum* infection in red chili might generate brownish-black spots with a diameter reaches 1.2 cm on the surface of the fruit. According to Srideepthi et al. (2017) and Sharma and Shenoy (2014), anthracnose is one of the diseases in chili plants caused by *Colletotrichum* sp. This disease can generate necrotic tissue in sunken lesions on the surface of infected fruit that can spread out rapidly under high humidity.

The decision that *Colletotrichum* infection has occurred in red chili crops in Serang Village was strengthening by the characteristic of the isolated fungi. Macroscopic observation showed that the isolated fungi had a colony with a yellowish-white top surface and yellow to the brown coloration of the bottom surface when they were grown on the PDA medium. The top surface of the colony was cottony with jagged colony edges, and concentric colony distribution patterns. The data from microscopic observation also supported additional support. During the observation, isolated fungi from chili had singlecelled, hyaline, cylindrical, and non-insulated conidia.

Furthermore, the conidia had a rounded tip and narrowed base with a conidia dimension of 7-8 μ m in length and 3-5 μ m in width. Conidia lie on conidiophore, which has 2-3 bulkheads (Figure 2). The characteristics of the fungus isolated from chili samples are similar to the characteristic of *Colletothricum* isolated from *Capsicum frutescens*, as reported by Anggaraeni et al. (2019).



Figure 2. *Colletotrichum* sp. colony isolated from red chili on PDA medium; (A) a top surface of pure culture; (B) a bottom surface of pure culture; (C) Conidia (40x10)

The symptoms of anthracnose disease on spring onion also indicated that Colletotrichum had infected the vegetable. The determination was based on the characteristic that is similar to the symptoms of anthracnose as reported by Kurniasih et al. (2014) on spring onion (Allium fistulosum). In their study, Kurniasih et al. (2014) observed that Colletothricum sp. infection in spring onion form irregular and concave white spots. Afterward, the spot became brown or black-brown coloration and formed a rupture at the infection point, causing the leaf to become dry and fall (Kurniasih et al. 2014). Moreover, Gautam (2014) stated that Colletotrichum sp. causes irregular sunken white spots with brown or black color. In severe cases, Colletotrichum sp. might cause necrosis on infected leaves.

Macroscopic observation of fungi infecting spring onion strengthens our decision that Colletotrichum has infected that spring onion in Serang Village. The fungi had a white colony with a serrated edge, and the colony spread out radially. Microscopic observations proved that the fungi have cylindrical, rounded, and not-insulated hyaline conidia. The size of the conidia was 7-13 μ m x 2-3 μ m. According to those colony characteristics, the fungi infecting spring onion in Serang Village was detected as *Colletotrichum* sp. (Figure 3).



Figure 3. *Colletotrichum* sp. colony isolated from spring onion on PDA medium; (A) top surface of pure culture; (B) bottom surface of pure culture; (C) conidia (40x10)

The finding of this study is similar to the characteristic of *Colletotrichum* sp. reported by Damm et al. (2009), who stated that Colletothricum isolated from spring onion has hyaline (colorless) conidia with the size ranges between 17-18 x 3-4 μ m. Similar conidia characteristic of *Colletotrichum* sp. isolated from *Allium fistullosum* was also reported by Kurniasih et al. (2014). The characters include colorless conidia (hyaline), blunt edges, ovoid shape, and non-insulated with a size of 15 μ m x 5 μ m.

The disease symptoms found in common beans from Serang Village were similar to the characteristic of anthracnose as previously reported by Ramdan et al. (2019) that were the presence of blackish-brown small patches on the surface of the fruit. The symptoms indicated that the common beans were infected by *Colletotrichum* sp (Ramdan et al. 2019). Therefore, we determined that common beans in Serang Village were suffered an attack from *Colletotrichum*.

The macroscopic characteristics of fungi infecting common beans were a white color in the top surface of the colony and yellowish-white color on the bottom surface of the colony. The colony was cottony with jagged edges, and concentric distribution (Figure 4). The characteristics of fungi samples are similar to the characteristic of *Colletotrichum* sp isolated from beans from India (Masoodi et al. 2013) and Indonesia Puspitasari et al. (2014). Both earlier studies described that *Colletotrichum* sp. infecting common beans have white-greyish up to blackish-grey colors. According to Thangamami et al. (2011), distribution patterns of *Colletotrichum* sp. are concentric with a smooth surface.

Microscopic observation on the fungi isolated from infected common beans showed that the fungi had insulated hyphae. Other characteristics of the fungi were branched and insulated conidiophores, hyaline, and crescent conidia with a length of 9.5 μ m and a width of 3 μ m. Characteristics of the fungi, as observed during the study, are similar to the characteristics of *Colletothricum* sp as describe by Mota et al. (2016), that the conidia of *Colletotrichum* sp. are either cylindrical, crescent form with both pointed ends, or slightly fusiform curved. The size of the conidia was 9.05-28.94 μ m in length and 2.93-9.3 μ m in width.



Figure 4. The colony of *Colletotrichum* sp. isolated from common beans on PDA medium; (A) Top surface of pure culture; (B) Bottom surface of pure culture; (C) Conidia (40x10)

The disease symptoms found in cayenne pepper indicated that the pepper had been attacked by anthracnose disease. We were convinced that the disease is anthracnose. It is based on the fact that the observed characteristic is similar to the characteristic of anthracnose in cayenne pepper as previously reported by Anggraeni et al. (2019). Further examination of the isolated fungi proved that the pepper was infected by *Colletothricum* (Anggraeni et al. 2019). Based on the similarity of the symptom, as reported by Anggraeni et al. (2019), we were convinced that Colletotrichum also infected the cayenne pepper in Serang Village.

The fungi had been isolated and were cultured on PDA media. Macroscopically, growing fungi had a white color on the top surface and a yellowish-white color on the bottom surface. The colony had a cottony surface with jagged edges and a concentric spreading pattern (Figure 5). Those colony characteristics are consistent with the characters of *Colletothricum* sp, as reported by Suwardani et al. (2014), who stated that the *Colletotrichum* sp has a smooth colony surface with the subtle and concentric distribution. Masoodi et al. (2013) stated that the colony color *Colletotrichum* sp are white to blackish gray in the upper surface and yellowish-white to brownish color in the bottom surface.

Microscopic observation showed that the colony has both hyaline and insulated hyphae. It has also insulated conidiophore. Other characteristics of the fungi are hyaline, non-insulated, and crescent moon conidia. The conidia have a dimension of 9-11 μ m in length and 3-4 μ m in width. The observed characteristic of the isolated fungi is similar to the microscopic characteristics of *Colletotrichum* sp. as described by Sudirga (2016). Therefore, it can be stated that the fungi isolated from chili belong to *Colletotrichum* sp. Moreover, Suwardani et al. (2014) stated that *Colletrotrichum* sp. has insulated and hyaline or colored hyphae.



Figure 5. *Colletotrichum* sp. colony isolated from chili on PDA medium; (A) top surface of pure culture; (B) a bottom surface of pure culture; (C) conidia (40x10)

The Intensity of Anthracnose Infection

Each sampling location and vegetable species suffers a different intensity of anthracnose infection. The infection intensity ranged between 32% and 52% (Table 3). Those values indicated that high intensity of anthracnose infection occurred in vegetable crops in Serang Village, District of Karangreja, Purbalingga Regency. That high value means that anthracnose becomes a severe problem of Serang Village and might threaten the vegetable production in that village.

Among five vegetable species grown in Serang Village, the lowest and the highest anthracnose intensity were found on common beans (32%) and red chili pepper (52%), respectively. According to Leonard (2001), infection intensity below 50% is not harmful to the host plant, while infection above 50% is over the limit and considered dangerous for the host plant. In that case, anthracnose infection needs to be controlled (Leonard, 2001). The highest percentage of anthracnose disease was found in vegetable crops of cayenne pepper and red chili pepper, reaching a percentage of 50% and 52%, respectively. That findings indicate that anthracnose infection on the cayenne and red chili pepper exceeds the limit (50%). Therefore, anthracnose infection on the cavenne and red chili pepper in Serang Village must be controlled. Otherwise, it will harm the plants and reduce the production of both vegetable species.

Environmental factors such as temperature, humidity, and soil pH significantly affect fungi development and spore dispersal. It was observed that adjacent planting among different vegetable species facilitates the spore to spread out from one location to another location. Therefore, it was reasonable that the fungi attached many vegetable species grown in Serang Village. Efforts to control anthracnose disease in Serang Village were only by using the fungicides, crop rotation, and mulch. Local farmers had applied mulch in their vegetable crops, but various weeds species were left to grow around the vegetable farm. The presence of weeds harms the vegetables due to nutrient and water use competition. It might interfere with the physiological processes of vegetable plants and make them easily attacked by anthracnose. According to Matruti et al. (2013), nutrients input influences the growth rate and the ability of plants to defend against disease. Nutrients such as phosphorus affect the development of the disease. Phosphorus can increase resilience by increasing nutrient balance in plants and allowing them to avoid pathogen infections.

Table 3. The intensity of anthracnose infection on each type of vegetable in Serang Village, District of Karangreja, Purbalingga Regency

Vagatablas	Loca-	Frequency	Intensity
vegetables	tion	(times)	(%)
Tomato (Lycoper-	T	22	44
sicum esculentum	I	24	48
Mill.)			
Red chili (Capsi-	Ι	21	42
<i>cum annuum</i> L.)	II	26	52
Spring onion (Alli-	Ι	19	38
um fistulosum L.)	II	23	46
Bean (Phaseolus	Ι	20	40
vulgaris L.)	II	16	32
Chili (Capsicum	Ι	17	34
frutescens L.)	II	25	50

This study provides the first scientific information about quantitative data on anthracnose attacks on vegetables in Serang Village, District of Karangreja, Purbalingga Regency. The information is crucial as a scientific basis for designing vegetable culture management in those areas.

CONCLUSION

Based on the results, it can be concluded that five species of vegetable crops in the Serang Village District of Karangreja, Purbalingga Regency, were infected by Colletothricum and suffered from anthracnose. High intensity of anthracnose disease in vegetable crops in Serang Village needs to be controlled, especially for cayenne and red chili pepper.

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