

## RESPONSE OF SIX CHILI VARIETIES TO ANTHRACNOSE DISEASE CAUSED BY *Colletotrichum acutatum* AND *C. gloeosporioides*

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### ABSTRACT

**Response of six chili varieties to anthracnose disease caused by *Colletotrichum acutatum* and *C. gloeosporioides*.** Chili is one of the horticultural commodities with high economic value. Chili production is constrained by anthracnose diseases. Losses due to anthracnose can reduce the fruits quality and yields lose. This study aims to determine the resistance of several chili varieties to anthracnose. Genetic material was used six varieties of chili. Ripe chilies were inoculated with two types of *Colletotrichum* isolates, i.e. *C. acutatum* and *C. gloeosporioides*. The experiment was arranged in a factorial randomized block design with three replications. The first factor were chili varieties: Laris, SSP, Habanero, Cibinong, Ekasari, and Kopay. The second factor was two types of fungal isolates. Observations were made on the incidence and severity of disease due to anthracnose. The results showed that both fungal isolates were effective in causing anthracnose disease in chilies. Habanero was very susceptible to anthracnose. Laris and Ekasari were moderately resistant to anthracnose diseases. These findings are important to develop new *Capsicum* cultivars that are more adaptive to anthracnose disease.

**Key words:** anthracnose, *Capsicum*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*

### INTRODUCTION

Chili is a horticultural commodity that has high economic value. The demand and price of chilies can increase due to certain moments or because of the weather. The main cause of the high price of chilies is the extreme weather factor (Rusmadi, 2017). Chili production is always low during the rainy season because most of the lowlands are planted with rice, and in upland, farmers do not planting chili because of the high failure rate (Kementrian Pertanian, 2020). This makes chilies becomes one of the inflation-forming commodities (Nugrahapsari & Arsanti, 2018; Rahmanta & Maryunianta, 2020; Rusmadi, 2017). Every difference in the price of red chilies increases at 1%, its will increase inflation by 0.18% (Rahmanta & Maryunianta, 2020) with low volatilization at curly chilies (Nugrahapsari & Arsanti, 2018). National chili production is 2.53 million tons with productivity of 16.4 tons/ha (BPS, 2019).

*Colletotrichum* infection on chilies is still the main cause of yield losses. The infection of pathogens can reduce yield up to 50% (Than *et al.*, 2008a). Anthracnose is one of the main diseases in chilies (Muamaroh *et al.*, 2018; Ratulangi *et al.*, 2012; Syukur

*et al.*, 2009; Tefa *et al.*, 2015; Suryadi *et al.*, 2018). This disease is generated from the fungi of the genus *Colletotrichum*. The spores can mobilize freely and widely spread through water splashing either from watering and rain (Rashid *et al.*, 2015; Than *et al.*, 2008a).

The *Colletotrichum* spp. is a hemibiotrophic pathogen, which means that it requires plant tissue to complete its life cycle. Spores will attach to the host surface and germinate to form a germ tube which develops into the appressorium, then penetrate the cuticle and epidermal cell wall (Boddy, 2016). After penetration occurs, primary vesicles and hyphae infection form, then secondary hyphae spread and kills the host cell (Sharma & Kulshrestha, 2015). *Colletotrichum* produces particular infection characteristic symptoms: germ tubes, appressoria, intracellular hyphae, and secondary necrotrophic hyphae (Oo & Oh, 2016). *C. acutatum* has growth with secondary conidia formed after appressoria germination and quiescent infections (Than *et al.*, 2008a).

Simple field control by maintaining a sanitary environment could not completely eradicate the disease. Performing good agricultural practices by keeping the planting environment clean from weeds, maintaining an

aeration system so that there is no stagnant water, reducing ineffective plant branches, and using healthy seeds of superior varieties has been reported to reduce the risk of yield loss to anthracnose disease. Integrated management practices combining cultural, mechanical, chemical, and biological control will be the best method to be performed (Oo & Oh, 2016; Rashid *et al.*, 2015).

Resistant chili varieties are one of the most important components of good agricultural practices. Until now, there has not been reported a chili variety that fully resistant to anthracnose. *Capsicum* breeding to produce resistant varieties due to of multiple *Colletotrichum* sp. has also not been successful (Rashid *et al.*, 2015; Than *et al.*, 2008a). Anthracnose resistance is reported to exist in several local or wild varieties, but this needs more investigation. The use of resistant cultivars is the cheapest, easiest, safest, and most effective means of controlling crop diseases (Oo & Oh, 2016). Currently, anthracnose control still uses chemicals, but some have applied organic materials. The incidence and severity of anthracnose of chili disease can significantly be reduced by organic matter combination (Rashid *et al.*, 2015). Combining the use of straw mulch, the application of intercropping, the use of biological fertilizers, the NPK application by 40% can control anthracnose (Hersanti *et al.*, 2016). This research aimed to study the incidence and severity of anthracnose in *Capsicum*.

## MATERIALS AND METHODS

**Research Site.** The experiment was carried out at the Research Center for Biotechnology LIPI from July to September 2020.

**Experimental Design.** The treatment was arranged factorial in a randomized block design with three replications. The first factor was the six varieties of chilies. The second one was two types of *Colletotrichum* fungi. The variables observed were the incidence and severity of the disease.

**Chili Varieties.** The genetic material was used six varieties of chilies: Laris, SSP, Habanero, Cibirong, Ekasari and Kopay. These varieties were a collection of Research Center for Biotechnology LIPI. The germplasm comes from the results of previous research collaboration, direct purchases, and farmers. An amount of 10 plants of each variety were planted in polybags and maintained in a greenhouse. The maintenance was carried out by the recommended dosage fertilization and

optimally pests and diseases controlling. The fruit used for the experiment was the second harvest.

**Fungal Isolates of *Colletotrichum* spp..** Two *Colletotrichum* species were used in this study, there were *C. acutatum* and *C. gloeosporioides*. Both isolates were obtained from the Laboratory of Plant Pathology, Department of Plant Pests and Diseases, Faculty of Agriculture, Gadjah Mada University. However, the number and origin of the isolates were not disclosed.

**Assessment on the Response of Chili Varieties to the Infection of *Colletotrichum* spp..** The Assessment was performed on the healthy chilies of each variety. An amount of 7 chilies with a uniform level of fruit maturity was used as the sample for each treatment. The fruit was sterilized using 70% alcohol and 1% sodium hypochloride then washed by dipping in sterile water 3 times at 1 minute. The fruits were arranged in a sterile plastic tray with sized 21 × 16 × 2.5 cm. The bottom of the tray was covered by two-layer sterile wet tissue to keep moisture during incubation. The pre-sterilized net wire was put inside the tray to keep the fruit from touching the wet tissue. After that, the tray was covered by a plastic wrap. Amount of 2 µL of the pathogenic conidia suspension containing 5 × 10<sup>5</sup> conidium/mL was injected into the chilies using a 10 µL pre-sterilized microtip. This step was to initiate the fruit tissue wounding and facilitated conidium penetration. This was suggested by Alves *et al.* (2015) that since non-wounded treatment caused no lesions of *Colletotrichum*, after drying the epidermis of each fruit was marked on the surface at four equidistant points, where four lesions of approximately 3 mm in depth. The chilies were incubated at room temperature for 7 days in dark conditions. Observations were conducted at the end of the experiment by scoring anthracnose and counting the number of symptomatic fruits. Disease incidence was analyzed using formula:

$$DI = \frac{n}{N} \times 100\%$$

DI = disease incidence (%);  
n = number of fruits symptomatic with anthracnose spots;  
N = total fruits observed .

Disease Severity (DS) was calculated based on the scoring method (Table 1) and analyzed using formula:

$$IP = \frac{\sum_{i=1}^k v_i \times n_i}{N \times V} \times 100\%$$

- $n_i$  = number of fruits attacked in each attack category;  
 $v_i$  = the numeric value of the attack category;  
 $N$  = number of fruits observed;  
 $V$  = the highest attack category numeric value.

**Cluster Analysis.** The cluster analysis using Past version 2.15 software (Wahyuni *et al.*, 2013). Clustering was performed by paired group method and Euclidean similarity, followed by bootstrap analysis with 100 replications.

**Data Analysis.** The data of disease incidence and disease severity were analyzed by Anova and continued with least significant difference (LSD) test at 5%. All the analysis was performed using the Statistix version 8.0 (Paradisa *et al.*, 2020).

## RESULTS AND DISCUSSION

**The Response of Chili Varieties to *Colletotrichum* spp..** The analysis of variance result showed that types of varieties and types of pathogens as well as their interaction significantly influenced the disease severity caused by infection of *Colletotrichum* spp. (Table 2). The disease severity caused by the two pathogens in the six varieties tested was 67.36% (Table 3). Habanero

Table 1. Anthracnose disease scoring (Montri *et al.*, 2009)

Score	Resistance level	Symptoms details
0	Highly resistant	No infection
1	Moderately Resistant	1–2% of the fruit area shows necrotic lesion or a larger water-soaked lesion surrounding the infection site
3	Resistant	>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	Moderately susceptible	>5–15% of the fruit area shows necrotic lesion, acervuli present, or water-soaked lesion up to 25% of the fruit surface
7	Susceptible	>15–25% of the fruit area shows necrotic lesion with acervuli
9	Highly susceptible	>25% of the fruit area shows necrosis, lesion often encircling the fruit; abundant acervuli

Table 2. The result of analysis of variants of the anthracnose disease severity

Source	Degree of freedom	Mean square
Group	2	12.85
Variety (V)	5	1235.78*
Pathogen (P)	1	177.82*
V x P	5	111.75*
Error	22	29.49
Coefisien of Varian	8.09	

Numbers to follow with sign \* was significant different in F test at 0.05 level.

Table 3. Anthracnose disease severity (%) on six varieties of chili

Variety	Disease severity (%)	
	<i>C. acutatum</i>	<i>C. gloeosporioides</i>
Laris	52.67 f	55.33 ef
SSP	74.67 c	59.67 ef
Habanero	100.00 a	89.67 b
Cibinong	63.33 de	70.00 cd
Ekasari	57.33 ef	57.33 ef
Kopay	69.67 cd	59.00 ef

Numbers to follow with same letter(s) was not significant different in LSD test at 0.05 level.

is the most susceptible to anthracnose disease severity in all types of pathogens with the highest percentage of disease severity, 100.00% (*C. acutatum*) and 89.67% (*C. gloeosporioides*). Meanwhile, Laris showed a significantly lower severity level compared to other varieties, 52.6% (*C. acutatum*) and 55.33% (*C. gloeosporioides*). In this study, *C. acutatum* and *C. gloeosporioides* generated the same virulence in Ekasari, which was shown by disease severity that was almost the same. The *C. acutatum* was more virulent than *C. gloeosporioides* in SSP, Habanero and Kopay. Meanwhile, Laris and Cibinong produced a higher disease severity in *C. gloeosporioides* (Tabel 3). Differences in *Colletotrichum* virulence in each variety may correlate with pathogen to variety interactions. In the olive plants, *C. acutatum* infection showed more virulent than *C. gloeosporioides* (Talhinhas *et al.*, 2015). However, *C. gloeosporioides* infection has been reported to be more virulent than *C. acutatum* in peaches (Zaitlin *et al.*, 2000). The difference in disease severity might also be due to differences in conidia germination and appressoria formation (Talhinhas *et al.*, 2015). The appressoria formation of *C. acutatum* produced predominantly fusiform conidia (Than *et al.*, 2008b) with ovoid appressorial shape (Than *et al.*, 2008b; Živkovic *et al.*, 2010; Abera *et al.*, 2016), while *C. gloeosporioides* produced cylindrical conidia (Than *et al.*, 2008b) with irregularly (Than *et al.*, 2008b) or cylindrical (Abera *et al.*, 2016) appressorial shape.

The disease incidence of two pathogens in all varieties was 100%. All the varieties that were inoculated showed anthracnose symptoms. This proved that the two types of inoculants had the same ability to the anthracnose incidence. Ibrahim *et al.* (2017) reported that virulence of *C. acutatum* on chili depend on the isolates and chili varieties. *C. acutatum* and

*C. gloeosporioides* had capability to infect all of the parts of the chili plant. In the case of chili fruit, both of *Colletotrichum* had the potential to cause symptom in all of developmental stages of chili, starting from young and mature green fruits (Rashid *et al.*, 2015; Than *et al.*, 2008a). *C. acutatum*, *C. capsici*, and *C. gloeosporioides* produced symptoms of anthracnose and lesions that were not significantly different in size from another (Than *et al.*, 2008b). The information about level of existence and aggressiveness pathogen isolates is important when choosing suitable isolates to screen germplasm and lines for resistance in plant breeding programs (Montri *et al.*, 2009).

**Anthracnose Disease Scale in Chili.** The disease scale used in this study refers to Montri *et al.* (2009) with a score of 0 to 9, which is determined based on the percentage of spot size to the overall size of the fruit, the appearance of symptoms in infected tissue (necrotic or wet spots), and the presence of acervulus. The results of this experiment, there were no chilies with a disease scale of 0 and 3 (Table 4). A scale of 5 was found in Laris, Cibinong, and Ekasari which were infected with *C. acutatum*. In addition, it was also found in Laris, SSP, Ekasari and Kopay which were infected with *C. gloeosporioides*. Chili fruit with a scale of 5 had symptoms of necrotic spots of 5–10% of the fruit size or wet spots of 25% of the fruit surface and usually found acervulus in the spots that appear. The SSP and Kopay infected with *C. acutatum* and Cibinong infected with *C. gloeosporioides* had a disease scale of 7 because found necrotic spots of 15–25% with the appearance of acervulus. Habanero inoculated with the two types of *Colletotrichum* spp. was categorized as disease scale 9 because it was more than 25% symptomatic of necrosis with many acervulus and the

Table 4. The classification of anthracnose resistance in six varieties of chili

Pathogen	Varieties	Score	Categories
<i>C. acutatum</i>	Laris	5	Moderately susceptible
	SSP	7	Susceptible
	Habareno	9	Highly susceptible
	Cibinong	5	Moderately susceptible
	Ekasari	5	Moderately susceptible
	Kopay	7	Susceptible
<i>C. gloeosporioides</i>	Laris	5	Moderately susceptible
	SSP	5	Moderately susceptible
	Habareno	9	Highly susceptible
	Cibinong	7	Susceptible
	Ekasari	5	Moderately susceptible
	Kopay	5	Moderately susceptible

spots usually surround the chillies so that it is. Table 4 showed that each variety could respond differently to the pathogens that were inoculated. Cibinong is more susceptible to *C. gloeosporioides* than *C. acutatum*. In contrast, Kopay was more susceptible to *C. acutatum*. Laris and Ekasari showed more resistant to anthracnose than other varieties. Genotypes with partial resistance will result in lower infection rates to reduce the number of inoculums in the field (Than *et al.*, 2008a). The inheritance of chili resistance trait to *C. acutatum* is not influenced by the maternal effect, but is controlled by genes in the cell core (Rosidah *et al.*, 2014). Kirana *et al.* (2014) reported that there was the different responses from the six red chili genotypes of crossing parent after inoculation using *C. acutatum* pure culture.

**Cluster Analysis Results.** Variations in resistance in the tested chili varieties to anthracnose disease were described in Figure 1. The varieties were divided into 3

groups of resistance to anthracnose disease. The first group was Laris and Ekasari with a moderate resistant level to both *Colletotrichum* spp.. This result was in line with Rosmayati (2008) report, showing Laris was resistant to anthracnose based on field test results. The second group consists of SPP, Kopay, and Cibinong. However, even though they were in one group but Cibinong showed more susceptible than SPP and Kopay. Group 3 only had 1 member, i.e. Habanero variety. The Habanero variety was very susceptible to both *Colletotrichum* species. Differences in resistance between chili varieties can occur due to genetic (Kirana *et al.*, 2014), maternal effects (Rosidah *et al.*, 2014), and environmental factors such as number of inoculums in the field (Than *et al.*, 2008a).

## CONCLUSION

The results of this study concluded that *C. acutatum* and *C. gloeosporioides* both caused

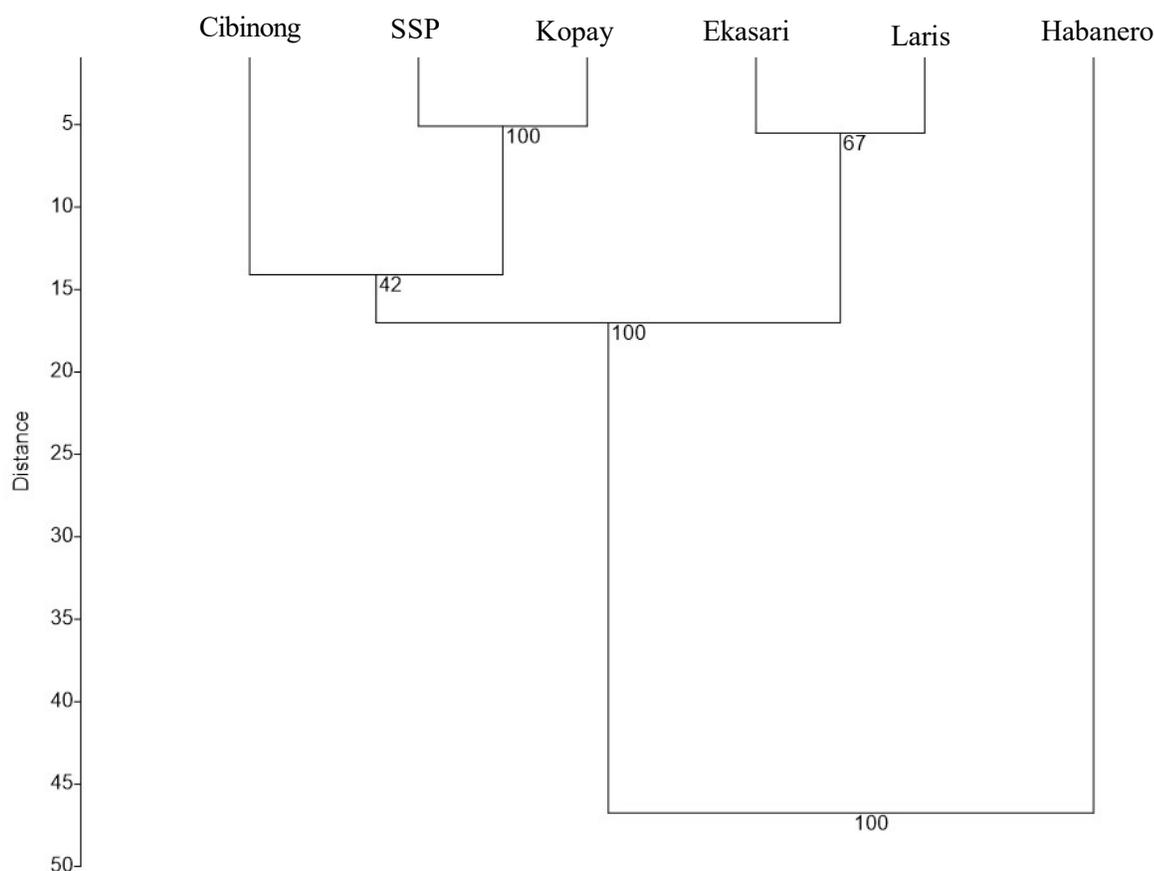


Figure 1. Dendrogram clustering between chilli varieties to anthracnose disease. The dendrogram was developed using PAST version 2.15 software (Wahyuni *et al.*, 2013) by paired group method and Euclidean similarity with 100 replications of bootstrap analysis.

anthracnose disease in *Capsicum* with moderate to high severity. Laris and Ekasari were varieties with moderately susceptible to anthracnose. Meanwhile, Habanero was highly susceptible to anthracnose.

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