Jurnal Perlindungan Tanaman Indonesia, Vol. 28, No. 1, 2024: 46–57
DOI: 10.22146/jpti.87342
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Potential Antagonists *Trichoderma viride* as Biofungicide, Plant Spacing, and Agricultural Lime Application to Suppress Anthracnose on Chili

Arifin Tasrif[1)]*, Yuliar[2)], Dwiwanti Sulistyowati[1)], Endang Krisnawati[1)], Bayu Adirianto[1)], & Dwi Sugiharti[3)]

[1)]*Bogor Agricultural Development Polytechnic*
Jln. Aria Surialaga No. 1, Cibalagung, Bogor, West Java, 16119 Indonesia

[2)]*Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN)*
Jln. Raya Jakarta KM 46, Cibinong, Bogor, West Java 16911 Indonesia

[3)]*Center for Diagnostic Standard of Agricultural Quarantine*
Jln. Pemuda No. 64, Kav. 16−17, Rawamangun, Jakarta 13220 Indonesia

**Corresponding author. E-mail: arifintasrif59@gmail.com*

Received July 26, 2023; revised October 25, 2023; accepted April 2, 2024

ABSTRACT

Anthracnose caused by *Colletotrichum capsici* and *C. gloeosporioides* on chili is a disease that can reduce up to 80% of yield. Fungicide application has not been able to eliminate infection because *Colletotrichum* can spread due to splashing of water, especially during the rainy season. The use of antagonistic fungi against *Colletotrichum* spp. has been widely published but is still limited to the laboratory and greenhouse settings, while field conditions are unpredictable. This study aims to identify the potency of *Trichoderma viride* that can be used as a biofungicide to control anthracnose in chili and to determine aspects of agronomic that can reduce the risk of anthracnose in chili. Samples of stems, leaves, and fruits were collected from infected plants located at the experimental farm of Bogor Agricultural Development Polytechnic. The experiment was conducted using Randomized Complete Design and Randomized Complete Block Design. The percentage of disease intensity of the fungus *C. capsici* and the intensity of anthracnose in chili both under screen house and open fields. Effects of different fertilizer on plant height, fruit weight, number of fruits, and the percentage of intensity of *C. capsici* were also observed and analyzed. Results showed that four fungi isolates were able to be identified, including *Penicillium* sp., *Aspergillus flavus*, *T. viride*, and *C. capsici*. *In vitro* analysis showed *T. viride* ability to suppress *C. capsici* growth to up to 71%. The fungus *T. viride* with a density of 7×10^6 CFU/mL suppressed the development of anthracnose by 59 to 87% under screen house conditions. However, under field conditions, the fungus *T. viride* was not able to suppress the development of anthracnose. Agronomic aspects such as plant height, number of fruits and production, and productivity of chili did significantly affect anthracnose.

Keywords: *Colletotrichum*; efficacy; field; incidence; intensity

INTRODUCTION

Chili (*Capsicum annuum* L.) is an important commercially grown vegetable cultivated in Indonesia. According to Sulandari (2004) various species of chili have been domesticated, but only *C. annuum* and *C. frutescens* have economic potential. Besides that, it is also known as cayenne pepper (*Capsicum frutescens* L.). *Capsicum frutescens* is a type of vegetable that has small fruit with a spicy taste. The increasing demand of cayenne peppers every year due to the various types of Indonesian cuisines that use chili as an important ingredient, everyday household consumption, market demand, and foreign exports.

Anthracnose caused by *Colletotrichum* spp. in chilies will occur when the fruit is ripe, causing a decrease in the number and quality of chilies. Anthracnose is also called "pathek" disease (Herwidyarti *et al.*, 2013). Yield losses due to anthracnose can reach 80% if conditions are favorable for the development of the pathogen (Than *et al.*, 2008). *Colletotrichum* conidia can be distributed by wind

causing fast transmission across chili fields or neighboring fields. *Colletotrichum* pathogen can occur in the vegetative phase of chili until just before harvest (Saxena *et al.*, 2016).

Previous studies have reported that antagonistic fungi can be used as biological agents against anthracnose in several fruits and vegetables. Siregar *et al.* (2007) reported that the fungus *Trichoderma harzianum* can control the fungus that causes anthracnose in chili. *Trichoderma harzianum* and *Gliocladium roseum* can be used as biological agents against *C. acutatum* and *C. gloeosporioides* that cause anthracnose in fruits (Živković *et al.*, 2010). Furthermore, Dharmaputra *et al.* (2015) reported that three filamentous fungi isolates (*Plectosphaerella cucumerina* and *Aspergillus flavus*) and yeast isolates (*Issatchenkia orientalis*) have the ability to inhibit the growth of *C. capsici* more than 70%, based on *in vitro* laboratory scale.

In line with above point of views, it is necessary to conduct further study by looking for various biocontrol agent that could be potential to become antagonistic to pathogens causes of anthracnose and the effect of some agronomic aspects that can reduce the level of intensity of *Colletotrichum capsici*. This study aims to analyze the potential antagonism of *Trichoderma viride* as a bio fungicide against anthracnose on chili and explored plant characteristics of chili plants that can reduce the intensity of anthracnose on chili in the screen house and field settings.

MATERIALS AND METHODS

Antagonist *Trichoderma* dan *Colletotrichum* Isolation

Samples of anthracnose infected and healthy chilies were obtained from the chili planting area located at the Bogor Agricultural Development Polytechnic Experimental Garden. *Colletotrichum* sp. were isolated from infected chili leaves and fruits. Samples for the exploration of antagonistic fungi were taken from healthy plant roots, stem, leaves, and fruits. Isolation of antagonistic fungi was carried out using direct seed plating technique. Fresh plant leaves and fruit were washed under tap water for 10 minutes, dried with sterile tissue. Then, samples were sterilized with 5% NaOCl for 1 minute, twice using 70% alcohol for 1 minute, then rinsed twice with sterile distilled water for 1 minute. Isolation of healthy and infected plant tissues were carried out using pour plate method on Potato Dextrose Agar (PDA) medium containing chloramphenicol (100 mg/L). Samples of plant parts and symptoms of chili infected with anthracnose were wiped using a tissue paper treated with 70% ethanol, then the fruit was rinsed with sterile distilled water and air dried. Skin tissue and flesh between the diseased and healthy parts (5 mm × 5 mm) were sampled. A total of 5 pieces of skin tissue and fruit flesh were placed on PDA containing chloramphenicol (100 mg/L) in Petri dishes (5 pieces per Petri dish), then incubated at room temperature (28 ± 2 °C) for 7 days.

Purification and Mass Production of Antagonist and Fungus Isolates *Colletotrichum* spp.

These cultures were separated into different Petri dishes containing PDA medium based on their growth characteristics, then incubated at room temperature (28 ± 2 °C) for 7 days. Colony growths were examined for growth characteristic and color and later subcultured from single conidia according to Ilyas *et al.* (2006), Iqbal *et al.* (2017), and Widodo and Hidayat (2018). These subcultures then were incubated at room temperature (28 ± 2 °C) for 14 days.

Identification of Antagonistic and Fungal Pathogen

Petri dishes containing different pure cultures were identified by visual examinations (macroscopic) and observed under light-microscope (microscopic). The pathogens were identified based on their cultural, color, and morphological characters. Fungal culture grown on PDA plates were taken on a glass slide and observed with a microscope for the presence of *Colletotrichum* spp., *Trichoderma* sp. as well as other fungus.

Identification of *Trichoderma* antagonist isolates and other fungi that have potential as biological agents and were not pathogenic in chili were identified morphologically accordingly to Ilyas *et al.* (2006), Iqbal *et al.* (2017), Rodrigues *et al.* (2007), Varga and Samson (2008), while identification of *Colletotrichum* spp. were carried out based on de Silva *et al.* (2019), Kumar *et al.* (2015), and Rangkuti *et al.* (2017).

Four species fungal identified were confirmed namely *Trichoderma viride*, *Penicillium* sp., *Aspergilus flavus*, while anthracnose pathogens were identified as *C. gloeosporioides* and *C. capsici*. However, further

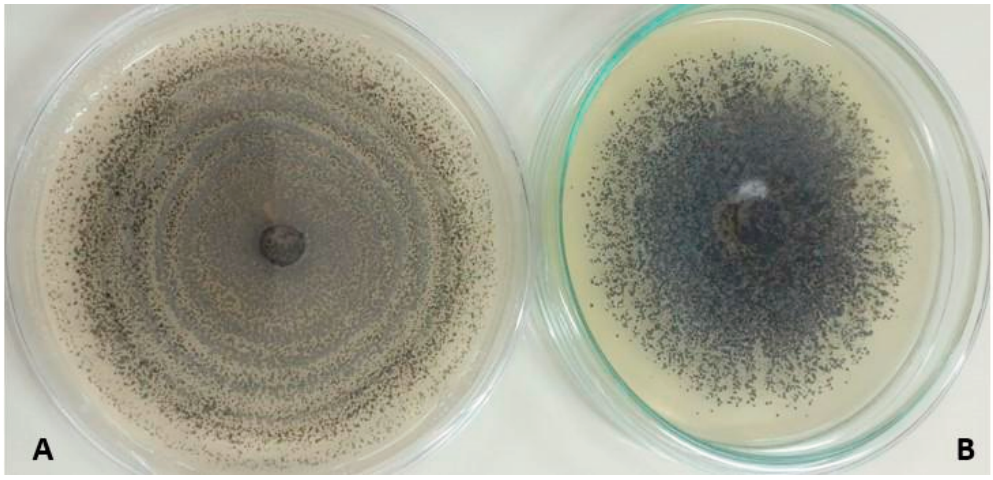

Figure 1. Pure sub-culture of *Colletotrichum capsici* (A) at seven days after inoculation (DAI) and *Trichoderma viride* (B) at 3 DAI

studies on anthracnose pathogen was only focused on *C. capsici*, while potential biocontrol agent was focused on *T. viride* as shown on Figure 1. These two fungi colonies had different growth characteristics.

In Vitro Antagonist *T. viride* on *C. capsici*

The antagonistic test in this study was carried out using multiple culture methods with an *in vitro* ratio of 1:1 in one confrontation dish or a modified co-culture method (Johnson, 1957 as cited in Widyastuti, 2007). *Trichoderma* antagonist colonies were inoculated in confrontation dishes prior to entering *C. capsici* with an incubation period of 14 days. Then the antagonist isolate was grown in a confrontation dish on the opposite side at a distance of five cm from the pathogenic fungi colony. The isolates were measured every two days until the 14th day since the two isolates were put together. Inhibition zones are lengths of region in the confrontation cup that are not overgrown by the two mutually exclusive isolated antagonists. Inhibition is calculated using a formula by Rohana (1998). The experiment was arranged using Completely Randomized Design (CRD) with five replicates.

Trichoderma viride Antagonist against *C. capsici* under Screen House Settings

Results of the *in vitro* antagonistic tests between isolates against *C. capsici* showed a high antagonistic level, then it was propagated and used in chili cultivation in screen house settings. One one-month old chili seedling was grown in plastic pots with 10 kg of soil. The treatment was prepared using Complete Randomized Design (CRD) with five replicates.

Inoculation with *T. viride* isolates was applied on 50 day old plants with three fruits on each plant with a suspension solution of *T. viride* isolates at a concentration of 7×10^6 CFU/mL. *Trichoderma* inoculation was done by coating fruits using a brush. The *T. viride* was then diluted into a half-concentrated solution with a concentration of 3.5×10^3 CFU/mL, and a quarter solution with a conidia density of 1.75×10^2 CFU/mL (Juraimi *et al.*, 2005). One week after inoculation with antagonist *T. viride*, the chilies were inoculated with *C. capsici* solution with a concentration of 5×10^6 CFU/mL by coating fruits using a brush.

Cultivation Efforts to Reduce the Risk of Anthracnose under Field Conditions

The experiment was carried out between February to August 2022 at the Agricultural Experimental Garden of Bogor Agricultural Development Polytechnic, West Bogor District, Bogor City and Gardens at Palasari Village, Cijeruk District, Bogor Regency. The experiment was arranged as a Nested Design with two experimental factors and three replications. The first factor is liming practices, namely T0 (0 t/ha), and T1 (3 t/ha). The second factor was planting spacing, namely Ps1 (plant spacing [40×60 cm]), Ps2 (plant spacing [50×60 cm]), and Ps3 (plant spacing [50×70 cm]), resulting in 18 treatment combinations with five plants samples for each combinations in 1.2 m×3 m planting beds with organic fertilizer/manure of 30 t/ha. Plant seedlings

that had four to five leaves were planted during the afternoon.

Plant Maintenance and Harvesting

Maintenance activities include replanting, inorganic fertilization, irrigation, pest and disease control. Stitching was done a week after transplanting (WAT) by replacing dead chili with old plants. Inorganic fertilizer in the form of Nitrogen (N), Phosphorus (P), and Kalium (K) (16-16-16) was applied every week in the vegetative phase by using a concentration of 10 g/L and applied as much as 250 mL per plant. Fertilization using NPK (10-55-10) during the generative phase with concentration 2 g/L by spraying plants once a week. Pest control was carried out at any time when symptoms of attack were seen. Chili harvesting was done when the plants were around 70–120 days after planting (DAP). Harvesting was done by picking the fruit at the physiological ripe stage (60–90%).

Plant height measurement was done every two weeks. Measurement of the diameter of the largest outermost plant canopy was carried out every two weeks during the generative phase. Measurement of plant height from the base of stem to the tip of the plant was done from the age of two weeks after planting (WAP) every two weeks. Plant production was determined by the number of fruits from the first harvest to the last harvest for each plant and each plot.

Statistical Analysis

Observations were made one week after the next application and it was repeated up to four times with an interval of seven days. Disease Incidence (DI) and Intensity (I) were used to analyze experiment under screen house and field conditions according to Nurbailis *et al.* (2017), with formula:

$$DI = \frac{A}{B} \times 100\%$$

DI is Disease Incidence; A is number of fruits with anthracnose symptoms; and B is number of fruits observed. The intensity of anthracnose was determined based on the formula from Zadoks and Schein (1979) as follows:

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

I= Intensity of disease, n = number of infected fruits, v = numerical value for each category, N = the total observed fruits, and Z= the highest score value.

Scores based on anthracnose intervals on chilies (Herwidyarti *et al.*, 2013) are as follows: 0: no symptoms of anthracnose inoculated with *C. capsici*; 1: symptoms of anthracnose less 20% on the chili fruits; 2: symptoms of anthracnose 20—40%; 3: symptoms of anthracnose 41–60%; 4: symptoms of anthracnose 61–80%; and 5: symptoms of anthracnose 80–100%.

RESULTS AND DISCUSSION

Potential Antagonis *T. viride* on *C. capsici*

In vitro study identified three species of antagonistic fungi against anthracnose in chili, namely *Penicillium* sp., *Aspergillus flavus*, and *T. viride* (Table 1). *Trichoderma viride* significantly suppressed the growth of *Penicillium* sp. and *C. capsici* of 62.80 up to 71.23% at 14 DAI (Table 1 and Figure 2). Furthermore, the inhibition ability of *T. viride* by these three isolates tested tends to be stable due to the speed of development of *T. viride* compared to the three isolates tested.

Table 1. Average *Trichoderma viride* inhibition of isolates tested at 14 days after inoculation (DAI)

No.	Isolates	Average of Suppression*)
1	*Penicillium* sp.	62.801[a]
2	*Aspergillus flavus*	70.122[b]
3	*Colletotrichum capsici*	71.231[b]
4	*Trichoderma viride* (Control)	79.603[c]

*) Averages followed by the same letter are not significantly different at the 0.1% based on LSD test level

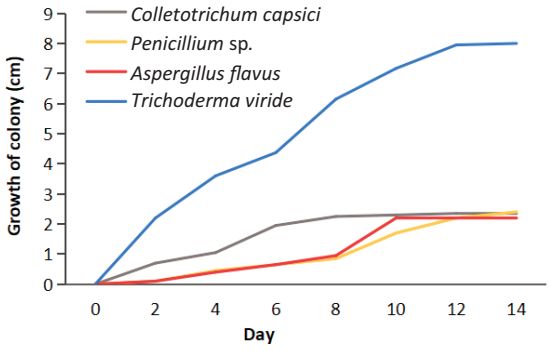

Figure 2. *Colletotrichum capsici* colony growth at 14 days after inoculation (DAI)

Antagonistic Study of *T. viride* against *C. capsici* on Chili under Screen House Settings

Antagonist test of *T. viride* against *C. capsici* on chili plants under screen house settings showed that the inoculation of *T. viride* (T2) with a concentration of 7×10^6 CFU/mL was only able to suppress disease intensity of by *C. capsici* by 60% and similar to control treatment (T1)(Table 2). However, the inoculation of the fungus *T. viride* at half strength and quarter concentration solutions was not significantly different from the disease intensity of *C. capsici*. In this follow-up study, the focus was only on the *T. viride* isolate against *C. capsici* because it showed a higher level of suppression compared to the other two isolates of *Penicillium* sp. and *A. flavus*.

Table 2. Disease incidence of anthracnose on chili at 28 days after inoculation (DAI)

Treatment	Concentration	Disease of Incidence*)
T1	Without treatment	40 a
T2 inoculum of *T. viride*	7×10^6 CFU/mL	60 ab
T3 inoculum of *T. viride*	3.5×10^3 CFU/mL	80 bc
T4 inoculum of *T. viride*	1.75×10^2 CFU/mL	87 c

*) Averages followed by the same letter are not significantly different at the 0.1% based on LSD test level

Trichoderma fungus inoculation with a solution density of 7×10^6 CFU/mL was only able to produce disease intensity of 13.33% and was not significantly different from treatment T1 (negative control). Thus the ability of the fungus *T. viride* suppressed the development of disease intensity in chili plants by 86.67% (Table 3). Treatment with quarter strength and half of full concentrations showed an increasing trend of disease progression at 7 days after inoculation (DAI) and continued to increase exponentially up to 28 DAI, with a rate of disease progression by 22%–41%. In contrast, treatment of full strength and control began to increase from 7 to 21 DAI (Figure 3 and 4).

Agronomic Effort to Suppress Anthracnose on Chili

Plant height and canopy diameter. Plant spacing did not significantly affect chili plant height indicating that all plant spacing used in this experiment had used recommended plant spacing. Meanwhile, lime application showed significant effects with plant height increasing respective to lime concentrations. Lime application as a soil amendment will change pH close to neutral and optimize metabolic response (Table 4). There was no interaction between plant spacing treatment and the application of agricultural lime on plant height of chili plants. Lime application

Table 3. Disease intensity of anthracnose on chili at 28 days after inoculation (DAI)

Treatment	Concentration	Average of Disease intensity (%)*)
T1 (control) without treatment	Without treatment	3.503 a
T2 (full strength) inoculum of *T. viride*	7×10^6 CFU/mL	13.332 ab
T3 (half strength) inoculum of *T. viride*	3.5×10^3 CFU/mL	22.221 bc
T4 (quarter strength) inoculum of *T. viride*	1.75×10^2 CFU/mL	41.332 c

*) Averages followed by the same letter are not significantly different at the 0.1% based on LSD test level

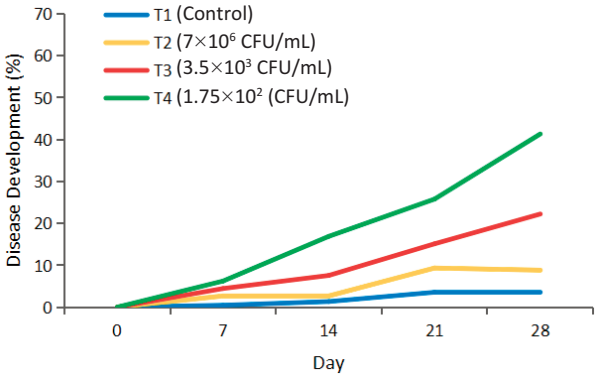

Figure 3. The average development of anthracnose up to 28 days after inoculation (DAI)

Table 4. Plant spacing and liming effects on chili plant height at 16 weeks after planting (WAP)

Treatments	Liming (t/ha)	
Plant Spacing (cm)	T0 (Control)	T1(3 t/ha)
40×60	66.121	72.221
50×60	63.130	69.521
50×70	64.902	63.501
Average	64.71[a]	68.414[b]

Note: Different letters in the same row are significantly different at 5%.

Figure 4. Anthracnose symptom on 7 to 28 days after inoculation (DAI) under screen house condition (various concentration of *Trichoderma viride*)

and plant spacing showed no significant difference in diameter of chili plant canopy. Even though there was no significant difference, there was still a tendency that liming showed a higher response to crown diameter than without lime (Table 5).

Table 5. Plant spacing and liming effects on the crown diameter of chili plants at 20 weeks after planting (WAP)

Treatment	Liming (t/ha)	
Plant Spacing (cm)	T0 (Control)	T1(3 t/ha)
40×60	68.203	72.721
50×60	69.212	74.122
50×70	67.401	71.601
Average	68.272[a]	72.815[a]

Note: Different letters in the same row are significantly different at 5%.

Number of fruits, fruit production, and productivity. The results showed that there was no significant difference between plant spacing treatments. This showed that the use of smaller or larger plant spacing was not able to increase the number of fruits. However, lime treatment was able to increase the number of fruits (Table 6). Lime application significantly increased the number of fruits by increasing effectiveness of nutrient absorption due to neutral soil pH levels and better plant metabolism (Table 7). Plant spacing treatments showed no significant difference in chili productivity (Table 8). This indicated that the provision of agricultural lime

Table 6. Plant spacing and liming treatment on number of chili

Treatment	Liming (t/ha)	
Plant Spacing (cm)	T0 (Control)	T1 (3 t/ha)
40×60	27.101	48.812
50×60	31.210	48.702
50×70	31.521	50.012
Average	29.944[a]	49.175[b]

Note: Different letters in the same row are significantly different at 5%.

Table 7. Plant spacing and liming treatment on chili production

Treatment	Liming (t/ha)	
Plant Spacing (cm)	T0 (%)	T1 (%)
40×60	542	976
50×60	624	974
50×70	630	1000
Average	598.666[a]	983.333[b]

Note: Different letters in the same row are significantly different at 5%.

Table 8. Plant spacing and liming treatment on the productivity

Treatment	Liming (t/ha)	
Plant Spacing (cm)	T0 (%)	T1 (%)
40×60	2179845	3920267
50×60	2506400	3915045
50×70	2622083	4166667
Average	2,436.109[a]	4.000,660[b]

Note: Different letters in the same row are significantly different at 5%.

was needed to increase the production and productivity of chili as much as 1.5 times higher than without liming.

Disease incidence of anthracnose on chili. Plant spacing did not significantly affect anthracnose intensity on fruit. On the other hand, lime application showed higher rates of anthracnose (Table 9). This showed that lime has no effect on reducing anthracnose on chilies.

Table 9. Plant spacing and liming treatment on anthracnose intensity

Treatment	Liming (t/ha)	
Plant Spacing (cm)	T0 (%)	T1 (%)
40×60	10.910	35.401
50×60	8.902	22.411
50×70	12.910	39.402
Average	10.907a	32.405b

Note: Different letters in the same row are significantly different at 5%.

Discusssion

Potential of *T. viride* as Biofungicide to Control *C. capsici* of Chili

In vitro test results of isolates of *T. viride* showed that *Trichoderma* was able to inhibit *C. capsici*, *Aspergillus flavus*, and *Penicillium* sp. by 71% after 14 DAI. According to Muliani *et al.* (2019) the use of *Trichoderma* spp. on day 5 was able to inhibit the development of anthracnose on cayenne pepper (*Capsicum frustescens* L.) up to 65%. Furthermore, Khairul *et al.* (2017) reported that the colony of the fungus *Trichoderma* sp. was capable of inhibiting the growth of *Colletotrichum capsici* colonies with an average inhibition percentage of 2.82% on the third day after inoculation; 70.28% on the fourth day after inoculation and 100% on the fifth day after inoculation.

Colletotrichum spp. growth inhibition by *Trichoderma* is due to its ability to produce antibiotic compound such as harzianic acid, alamethicin, tricholin, peptaibols, 6-penthyl-α-pyrone, massoia lactone, viridian, gliovirin, glisoprenins, hiptelidic acid, trichodermin, dermadin and others (Sundari *et al.*, 2014). Furthermore Supriati *et al.* (2010) stated that *Trichoderma* sp. act as microparasites for other fungi by growing around the pathogenic mycelium. Inhibition of *Trichoderma* sp. against *C. capsici* is thought to be due to the composition of the outer wall of *Colletorichum* hyphae which causes this pathogen to be easily degraded by the chitinase enzyme (Afrizal *et al.*, 2013). *Colletotrichum* hyphae walls have a texture made of chitin (β1,4 N acetylglucosamine) (Purnomo, 2010). The chitinase enzyme produced by *Trichoderma* sp. causes the hyphal walls of the pathogen *C. capsici* to be dissolved causing inhibited growth and later mortality (Lubis *et al.*, 2018). Furthermore, recent study shows that four species of *Colletotrichum* spp. on chili, namely *C. scovelli*, *C. truncatum*, *C. siamense*, and *C. makassarii* have been identified (Anggrahini *et al.*, 2020).

The ability of the fungus *T. viride* suppressing the development of anthracnose in screen house conditions was more successful (41% disease intensity) when compared to open field conditions (100% disease intensity) presumably influenced by several factors such as average temperature, humidity and rainfall during the study. Besides that, the type of media, the density of inoculum and the method of application are thought to have an effect on the ability to inhibit *Colletrichum* spp. Nindya (2018) reported that the application of *Trichoderma* sp. with the method of flushing the soil is effective in suppressing the development of anthracnose disease in chilies with an effectiveness category of 91%. Furthermore, Nurhidayati *et al.* (2015) suggested that *T. harzianum* with coconut water media and stored for two months was able to have a significant effect on the number of spores, the viability of *T. harzianum* spores and the intensity of anthracnose disease (*Colletotrichum* sp.) in large chilies in the field. Likewise Supriati and Djaya (2016) stated that the average effectiveness value of the biological agents *T. harzianum* and Actinomycetes of >69% indicated a good quality. Thus, the addition of corn flour in PDA media increases the population of Actinomycetes and *T. harzianum* that increases the number of populations of biological agents applied to plants to inhibit disease development and affect the success of controlling anthracnose disease in chilies.

Inhibition levels of *T. viride* against *C. capsici* in controlled environmental conditions at the highest tested concentration at 28 DAI was able to inhibit

the intensity of anthracnose by 87%. These results were in line with Khairul *et al.* (2017) suggesting that the 21 DAI observations are of the lowest intensity with a concentration of 80 mL/L of 18.36% and gave a significant effect compared to other treatments. The antagonistic ability of the fungus *T. viride* can be used as an alternative to reduce the intensity of anthracnose. Antagonistic activity of the fungus *T. viride* to inhibit the growth of *C. capsisi* presumably due to the composition of the outer wall of the hyphae (Afrizal *et al.*, 2013).

Several factors influence the successful application of *Trichoderma* sp. including application time. The application time must be right, for example when the weather is clear. Planting chili plants during the rainy season can also support disease development to develop more rapidly (Sriyanti *et al.*, 2015). The pathogen *C. capsici* on chili plants can reduce production by up to 60% to even failure depending on environmental conditions (Arofahsari, 2015). According to Sitompul (1995), *Trichoderma* sp. application has different effects in the field. However, the application of *T. viride* in this study resulted in milder infections compared to without the application.

On the other hand, results showed that *T. viride* isolates were ineffective in the field to suppress anthracnose (disease intensity ranging from 87% to 100%) and was caused by several factors, such as time and method of induction of *T. viride* on plants, weather conditions (rain and wind) as well as fertilizer application and plant spacing. This is in line with the results of research by Ibrahim *et al.* (2019) that suggests low effectiveness if efficacy are <50% and good effectiveness if efficacy is >50%. The high content of fiber and carbohydrates can be a potential source of nutrients and carbon for the growth of the fungus *Trichoderma* sp., such as hydrogen (H), carbon (C), oxygen (O), phosphorus, nitrogen, sulfur and calcium. Elements CHO are three important elements that are widely available in organic matter that function as energy sources, cell-forming materials and electron acceptors to produce energy for fungi (Ibrahim *et al.*, 2019). Results from this study demonstrates *T. viride* potential as a potential biological agent against anthracnose in chili production, but to increase its effectiveness further studies on isolate methods using appropriate growth media and stickers together with optimize utilization should be done.

Relations between Chili Agronomic Parameter and Anthracnose

Plant spacing did not significantly affect plant height as they were all recommended plant spacing for chili cultivation. The use of narrow plant spacing can increase yields, as long as limiting factors can be avoided to eliminate or reduce competition between plants (Mayadewi, 2007). While wide plant spacing results in optimum LAI to take longer to reach but the yield per area is low (Nawangsih *et al.*, 2003).

Plant spacing 30×100 cm resulted in higher yields per chili plants but lower yields per hectare compared 20×50, 30×50, and 20×100 cm (Aminifard *et al.*, 2010 as cited in Laili *et al.*, 2020). Using a rather wide plant spacing of chili aims to reduce humidity and increase air circulation that later result in larger fruits (Agricultural Extension Center for Food Crops and Fisheries, 2021; Suparwoto & Waluyo, 2022). Giving lime showed a real response, namely chili plants were higher than without liming. The effect of applying lime as a soil conditioner when the degree of soil acidity (pH) is acidic or alkaline conditions will result in close to neutral soil pH and better nutrient uptake by roots and plant metabolic responses. This was shown by higher plant growth when lime was applied than without application. Although the application of lime and plant spacing showed no significant effects on the diameter of the chili plant canopy, there was still a numerically higher crown diameter after lime application due to axillary branches in the crown to continue to grow and later produce more fruit.

Number of Fruits, Fruit Production, and Productivity

No significant differences were demonstrated from the different treatment combinations. This indicates that smaller or larger spacing was not able to increase the number of fruits, but the lime treatments did due to the increase of nutrient absorption and plant metabolism because of neutral soil pH. Similar effects of lime application and planting space were found on the chili plant production per production area. The plant spacing (100×80 cm) of pepper did not affect the wet weight of fruit per plot but affected fruit fresh weight per plant (Aminifard *et al.*, 2010).

This implies that the application of agricultural lime can increase the production and productivity of chili to approximately 1.5 times higher compared to without lime application.

Based on the observed agronomic parameters, different planting spaces did not significantly affect the anthracnose intensity of infested fruits. However, lime application actually showed higher rates of anthracnose infection by up to ~3-folds. This demonstrates that lime application has no effects in reducing anthracnose infestation on chili.

CONCLUSION

The antagonist fungus *T. viride* has potential as a biofungicide against anthracnose in chili production based on its ability to inhibit the growth *C. capsici* by 71% *in vitro*. The use of the fungus *T. viride* (full strength) can suppress the development of anthracnose by 59% to 87% under screen house conditions. However, this fungus was not able to suppress the anthracnose under field conditions. The application of agricultural lime increased plant height, number of fruits and production, and productivity of chilies; however, did not reduce the disease intensity.

ACKNOWLEDGEMENTS

This study was funded by the Strategic Project of Bogor Agricultural Development Polytechnic in Fiscal Year 2022. Of that, we would like to thank Dr. Detia Tri Yunandar, Former Director of Bogor Agricultural Development Polytechnic and the Head of Research and Community Empowerment for their approval. We also would like to thank Dr. Sriyanto for his valuable assistance and permission to use the Laboratory of Plant Disease of the Office of the Agricultural Quarantine Standard Test Center. We also would like to thank Mr. Taufik Bahtiar for his assistance on the data analysis.

LITERATURE CITED

Afrizal, Marlina, & Susanti, F. (2013). Kemampuan Antagonis *Trichoderma* sp. terhadap Beberapa Jamur Patogen *In Vitro* [The Ability of Antagonist *Trichoderma* sp. against Some Pathogenic Fungus *In Vitro*]. *Jurnal Floratek*, 8(1), 45–51. Retrieved from https://jurnal.usk.ac.id/floratek/article/view/860

Aminifard, M.H., Aroiee, H., Karimpour, S., & Nemati, H. (2010). Growth Yield and Characteristic of Paprika Pepper (*Capsicum annuum* L.) in Response to Plant Density. *Asian Journal of Plant Sciences*, 9(5), 276–280. https://doi.org/10.3923/ajps.2010.276.280

Agricultural Extension Center for Food Crops and Fisheries (2021). *Pengendalian Pathek pada Cabai*. UPTD Balai Penyuluhan Pertanian Tanaman Pangan dan Perikanan Wilayah III, Seyegan Sleman. https://bp4seyegan.slemankab.go.id/pengendalian-pathek-pada-cabai.slm

Akbar, A.R. (2020). *Pengaruh Pupuk Kalium terhadap Infeksi* Colletotrichum capsici *pada Tanaman Cabai Rawit (Effects of Potassium on the Infection of* Colletotrichum capsici *on Cayenne Pepper)* [Bachelor thesis]. Indralaya, Indonesia: Jurusan Hama Penyakit Tumbuhan, Program Studi Proteksi Tanaman, Fakultas Pertanian, Universitas Sriwijaya.

Anggrahini, D.S, Wibowo, A., & Subandiyah, S. (2020). Morphological and Molecular Identification of *Colletotrichum* spp. Associated with Chili Anthracnose Disease in Yogyakarta Region. *Jurnal Perlindungan Tanaman Indonesia*, *24*(2), 161–174. https://doi.org/10.22146/jpti.58955

Arofahsari, D.N. (2015). *Viabilitas dan Efektivitas Biofungisida Berbahan Aktif* Trichoderma harzianum *untuk Mengendalikan Penyakit Rhizoctonia pada Tanaman Kedelai* [Bachelor thesis]. Jember, Indonesia: Program Studi Agroteknologi Fakultas Pertanian Universitas Jember.

Choi Y.W., Hyde, K.D., & Ho, W.H. (1999). Single Spore Isolation of Fungi. *Fungal Diversity*, *3*, 29–38. Retrieved from https://www.fungaldiversity.org/fdp/sfdp/FD_3_29-38.pdf

de Silva, D.D., Groenewald, J.Z., Crous, P.W., Ades, P.K., Nasruddin, A., Mongkolporn, O., & Taylor, P.W.J. (2019). Identification, Prevalence and Pathogenicity of *Colletotrichum* species Causing Anthracnose of *Capsicum annuum* in Asia. *IMA Fungus*, *10*(1), 8. https://doi.org/10.1186/s43008-019-0001-y

Dharmaputra, O.S. Sudirman, L.I., & Fitriani, M. (2015). Mikobiota pada Buah Cabai untuk Pengen-

dalian Hayati *Colletotrichum capsici* [Mycobiota on Chilli Fruits for Biological Control of *Colletotrichum capsici*]. *Jurnal Fitopatologi Indonesia*, *11*(5), 150–158. https://doi.org/10.14692/jfi.11.5.150

Herwidyarti, K.H., Ratih, S., & Sembodo, D.R.J. (2013). Keparahan Penyakit Antraknosa pada Cabai (*Capsicum annuum* L.) dan Berbagai Jenis Gulma. *Jurnal Agrotek Tropika*, *1*(1), 102–106. https://doi.org/10.23960/jat.v1i1.1925

Ibrahim, A., Abdel-Razzak, H.A., Wahb-Allah, M., Alenazi, M., Alsadon, A., & Dewir, Y.H. (2019). Improvement in Growth, Yield, and Fruit Quality of Three Red Sweet Pepper Cultivars by Foliar Application of Humic and Salicylic Acids. *HortTechnology*, *29*(2), 170–178. https://doi.org/10.21273/HORTTECH04263-18

Iqbal, S., Ashfaq, M. Malik, A.H., Inam-ul-haq, Khan, K.S. & Mathews, P. (2017). Isolation, Preservation and Revival of *Trichoderma viride* in Culture Media. *Journal of Entomology and Zoology Studies*, *5*(3), 1640–1646. Retrieved from https://www.entomoljournal.com/archives/?year=2017&vol=5&issue=3&ArticleId=1998

Ilyas, M., Rahmansyah, M., & Kanti, A. (2006). *Seri Panduan: Teknik Isolasi Fungi*. Jakarta, Indonesia: LIPI Press.

Juraimi, A.S, Tasrif, A., Kadir, J., Napis, S., & Sastroutomo, S.S. (2005). Phytotoxicity and Field Efficacy of *Exserohilum longirostra* Jc/Min the Control of Barnyardgrass Ecotypes (*Echinochloa crus-galli* var. *crus-galli* (L.) Beauv). *BIOTROPIA*, (24), 20–29. https://doi.org/10.11598/btb.2005.0.24.172

Khairul, I, Montong, V.B., & Ratulangi, MM. (2017). Uji Antagonisme *Trichoderma* sp. terhadap *Colletotrichum capsici* Penyebab Penyakit Antraknosa pada Cabai Keriting Secara *In Vitro*. *Cocos*, *9*(6), 1–8. Retrieved from https://ejournal.unsrat.ac.id/index.php/cocos/article/view/20109

Kumar, S., Singh, V., & Garg, R. (2015). Cultural and Morphological Variability in *Colletotrichum capsici* Causing Anthracnose Disease. *International Journal of Current Microbiology and Applied Sciences*, *4*(2), 243–250. Retrieved from https://www.ijcmas.com/vol-4-2/Saket%20Kumar,%20et%20al.pdf

Laili, F.N., Kurniastuti, T., & Puspitorini, P. (2020). Respon Pertumbuhan dan Hasil Tanaman Cabai Merah Keriting (*Capsicum annuum* var. *longun* L.) terhadap Pemberian Dosis Pupuk NPK dan Bokashi. *VIABEL: Jurnal Ilmiah Ilmu-Ilmu Pertanian*, *14*(1), 37–43. https://doi.org/10.35457/viabel.v14i1.999

Lubis, J.I., Yusriadi, & A. Rizali. (2018). Uji Daya Hambat *Trichoderma* spp. Isolat Kabupaten Kapuas Kalimantan Tengah terhadap *Colletotrichum* spp. pada Cabai. *Agrotek View: Jurnal Tugas Akhir Mahasiswa*, *1*(3). Retrieved from https://ppjp.ulm.ac.id/journals/index.php/agv/article/view/706

Mayadewi, N.N.A. (2007). Pengaruh Jenis Pupuk Kandang dan Jarak Tanam terhadap Pertumbuhan Gulma dan Hasil Jagung Manis [Effect of Farm Manure Materials and Plant Spacing on Weed Growth and Sweet Corn Yield]. *AGRITOP*, *26*(4), 153–159. Retrieved from https://ojs.unud.ac.id/index.php/agritrop/article/view/3069

Muliani, S., Sukmawi, & Nildayanti. (2019). Efektifitas Cendawan Endofit dan *Trichoderma* spp. terhadap Penyakit Busuk Pangkal Batang Lada (*Phytophthora capsici*) di Pembibitan. *Jurnal Agroplantae*, *8*(1), 27–31. Retrieved from https://ppnp.e-journal.id/agro/article/view/13

Nawangsih, A.A., Imdad, H.P., & Wahyudi, A. (2003). *Cabai Hot Beauty*. Jakarta, Indonesia: Penerbit Swadaya.

Nindya, N.S. (2018). Uji Efektifitas Metode Aplikasi Jamur Antagonis *Trichoderma* sp. terhadap Penyakit Antraknosa (*Colletotrichum capsici*) pada Tanaman Cabai (*Capsicum annum*) [Bachelor thesis]. Gorontalo, Indonesia: Fakultas Pertanian, Universitas Negeri Gorontalo.

Nurbailis, Martinius, & Naipinta, R. (2017). Kesintasan Beberapa Jamur Antagonis pada Buah Cabai dan Potensinya dalam Menekan Penyakit Antraknosa yang Disebabkan oleh *Colletotrichum*

gloeosporioides [Persistence of Several Antagonistic Fungus on Chilli and its Potential to Suppress Anthracnose Disease Caused by *Colletotrichum gloeosporioides*]. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, *17*(2), 162–169. https://doi.org/10.23960/j.hptt.217162-169

Nurhidayati, S., Majid, A., & Mihardjo, P.A. (2015). Pemanfaatan Biofungisida Cair Berbahan Aktif *Trichoderma* sp. untuk Mengendalikan Penyakit Antraknosa (*Colletotrichum* sp.) pada Cabai di Lapang. *Berkala Ilmiah PERTANIAN*. Retrieved from https://repository.unej.ac.id/bitstream/handle/123456789/71506/SITI%20NURHIDAYATI.pdf?sequence=1

Purnomo, H. (2010). *Pengantar Pengendalian Hayati*. Yogyakarta, Indonesia: CV Andi.

Rangkuti, E.E., Wiyono, S., & Widodo. (2017). Identifikasi *Colletotrichum* spp. Asal Pepaya [Identification of *Colletotrichum* spp. Originated from Papaya Plant]. *Jurnal Fitopatologi Indonesia*, *13*(5), 175–183. Retrieved from https://journal.ipb.ac.id/index.php/jfiti/article/view/19640

Rodrigues, P., Soares, C., Kozakiewics, Z., Paterson, R.R.M., Lima, N., & Venâncio, A. (2007). Identification and Characterization of *Aspergillus flavus* and Aflatoxins. In A. Méndez-Vilas (Ed.), *Communicating Current Research and Educational Topics and Trends in Applied Microbiology* (pp. 527–534). Lisbon, Portugal: Badajoz, Formatex.

Rohana, I. (1998). Efektifitas Penggunaan *Trichoderma harzianum* dan Fungisida Mankozeb untuk Pengendalian *Rhizoctonia solani* Penyebab Penyakit Lodoh pada *Acacia mangium* [Bachelor thesis]. Bogor, Indonesia: Fakultas Kehutanan, Institut Pertanian Bogor.

Saxena, A., Raghuwanshi, R., Gupta, V.K., & Singh, H.B. (2016). Chilli Anthracnose: The Epidemiology and Management. *Frontiers in Microbiology*, *7*, 1527. https://doi.org/10.3389/fmicb.2016.01527

Siregar, A.N., Ilyas, S., Fardiaz, D., Murniati, E., & Wiyono, S. (2007). Penggunaan Agens Biokontrol *Bacillus polymyxa* dan *Trichoderma harzianum* untuk Peningkatan Mutu Benih Cabai dan Pengendalian Penyakit Antraknosa. *Jurnal Penyuluhan Pertanian*, *2*(2), 105–114. https://doi.org/10.51852/jpp.v2i2.228

Sitompul, S.K. (1995). *Evaluasi Keefektifan Penghambatan Beberapa Agens Biokontrol terhadap Pertumbuhan* Marasmius palmivorus *Sharples* [Bachelor thesis]. Bogor, Indonesia: Institut Pertanian Bogor.

Sriyanti, N.L.G, Suprapta. D.N., & Suada, I.K. (2015). Uji Keefektifan Rizobakteri dalam Menghambat Pertumbuhan Jamur *Colletotrichum* spp. Penyebab Antraknosa pada Cabai Merah (*Capsicum annuum* L.) [Effectiveness of Rhizobacteria to Inhibit the Growth of *Colletotrichum* spp. the Cause of Antracnose on Red Chilli (*Capsicum annuum* L.]. *Jurnal Agroekoteknologi Tropika (Journal of Tropical Agroecotechnology)*, *4*(1), 53–64. Retrieved from https://ojs.unud.ac.id/index.php/jat/article/view/12501

Sulandari, S. (2004). *Kajian Biologi, Serologi, dan Analisis Sidik Jari DNA Virus Penyebab Penyakit Daun Keriting pada Cabai* [Doctoral dissertation]. Bogor, Indonesia: Institut Pertanian Bogor.

Sundari, A., Khotimah, S., & Linda, R. (2014). Daya Antagonis Jamur *Trichoderma* sp. terhadap Jamur *Diplodia* sp. Penyebab Busuk Batang Jeruk Siam (*Citrus nobilis*). *Protobiont Jurnal Elektronik Biologi*, *3*(2), 106–110. Retrieved from https://jurnal.untan.ac.id/index.php/jprb/article/view/5517

Suparwoto., & Waluyo (2022). Penerapan Teknologi Proliga pada Cabai Merah di Lahan Kering Kabupaten Ogan Ilir Sumatera Selatan [Application of Proliga Technology on Red Chilies in Dry Land, Ogan Ilir District, South Sumatra]. *Jurnal Ilmu Pertanian Agronitas*, *4*(1), 178–186. Retrieved from https://www.ejournal.unitas-palembang.ac.id/index.php/ags/article/view/383

Supriati, L., & Djaya, A.A. (2016). Pengendalian Penyakit Antraknosa pada Tanaman Cabai Merah Menggunakan Agen Hayati *Trichoderma harzianum* dan Actinomycetes [The Control Anthracnose Disease on Red Pepper with Involve Agents *Trichoderma harzianum* and Actinomycetes]. *Jurnal AgriPeat*, *16*(1), 20–26.

Supriati, L., Mulyani, R.B., & Lambang, Y. (2010). Kemampuan Antagonisme Beberapa Isolat *Trichoderma* sp. Indigenous terhadap *Sclerotium*

rolfsii secara *in Vitro*. *Jurnal Agroscientic*, *17*(3), 119–122.

Than, P.P., Prihastuti, H., Phoulivong, S., Taylor, P.W.J., & Hyde, K.D. (2008). Chilli Anthracnose Disease Caused by *Colletotrichum* Species. *Journal of Zheijiang University SCIENCE B*, *9*(10), 764–778. https://doi.org/10.1631/jzus.B0860007

Varga, J., & Samson, R.A. (Ed.) (2008). *Aspergillus in the Genomic Era*. Ed. I. Wageningen, NL: Wageningen Academic Publishers.

Widodo, & Hidayat, S.H. (2018). Identification of *Colletotrichum* Species Associated with Chili Anthracnose in Indonesia by Morphological Characteristics and Species-Specific Primers. *Asian Journal of Plant Pathology*, *12*(1), 7–15. https://doi.org/10.3923/ajppaj.2018.7.15

Widyastuti, S.M. (2007). *Peran* Trichoderma *spp. dalam Revitalisasi Kehutanan di Indonesia*. Yogyakarta, Indonesia: Gadjah Mada University Press.

Zadoks, J.C., & Schein, R.D. (1979). *Epidemiology and Plant Disease Management*. New York (Etc.): Oxford University Press.

Živković, S., Stojanović, S., Ivanović, Ž., Gavrilović, V., Popović, T., & Balaž, J. (2010). Screening of Antagonistic Activity of Microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. *Archives of Biological Sciences*, *62*(3), 611–623. https://doi.org/10.2298/ABS1003611Z