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DOI : https://doi.org/10.22146/jpti.87342

Reference : Mak-661

To appear in : *Jurnal Perlindungan Tanaman Indonesia*

Received date : 26 July 2023

Revised date : 25 October 2023

Accepted date : 2 April 2024

This is an early version of Accepted Manuscript, which has been through the Jurnal Perlindungan Tanaman Indonesia peer-review process and is available shortly after acceptance as our service to the community. The edited manuscript will be published after technical editing, formatting, and proofreading. Please note that minor changes to the text and/or graphics might be introduced during technical editing, which could affect the content. Terms & Conditions and the Ethical Guidelines of the Journal still apply.

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Research Article

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

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ABSTRACT

Anthracnose caused by *Colletotrichum capsici* and *C. gloesporium* on chili is a disease that can reduce chili yields up to 80%. Control with fungicide has not been able to provide maximum results, because *Colletotrichum* can spread due to splashing of water, especially in the rainy season. The use of antagonistic fungi against *Colletotrichum* spp. has been widely published but is still limited to the laboratory and greenhouse scale, 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 infected plants of stems, leaves, and fruits were collected from 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. While testing the effect of cultivation aspects using different types of fertilizer on plant height, fruit weight, number of fruits, and the percentage of intensity of *C. capsici* were analyzed. The results of this study shown that four isolates of fungi have been identified such as *Penicillium* sp., *Aspergillus flavus*, *T. viride*, and *C. capsici*. In vitro analysis shown the ability of *T. viride* to suppress the growth of the *C. capsici* up to 71%. The fungus *T. viride* with a density of 7×10^6 CFU/mL can suppress 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 were not significantly effect on anthracnose.

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

INTRODUCTION

Chili (*Capsicum annuum* L.) is one of the important vegetables that are cultivated commercially in Indonesia. According to Sulandari (2004) various species of chili that have been domesticated, but only *C. annuum* and *C. frutescens* which have economic potential.

Besides that, it is also known as cayenne pepper (*Capsicum frutescens* L.). (*C. frutescens*) is a type of vegetable that has small fruit with a spicy taste. Seeing the need for cayenne pepper every year increases due to the increasing variety and variety of types of dishes in Indonesia that use chili as an ingredient, ranging from household needs, market demand, even to the need for 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* conidium can be scattered by the wind so that the transmission is very fast and can even be spread evenly on chili 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. *T. 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 bio-control 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 antagonistic of *Trichoderma viride* that can be used as a bio fungicide control of anthracnose on chili and to analyze aspects of chili cultivation that can reduce the intensity of anthracnose on chili in the screen house and field conditions.

MATERIAL DAN METHODS

Antagonist *Trichoderma* dan *Colletotrichum* Isolation

Samples of chili infected with anthracnose and healthy were obtained from the chili planting area located at the Bogor Agricultural Development Polytechnic Experimental Garden. Samples for isolating the Colletotrichum fungus were leaves and fruit of chili

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plants that were attacked by pathogens. Samples for the exploration of antagonistic fungi were taken from healthy plant roots, stem, leaves, and fruits. Samples was put in a plastic bag and labeled. Isolation of antagonistic fungi was carried out using direct seed plating technique. Fresh plant leaves and fruit was washed under tap water for 10 minutes, dried with sterile tissue. Then, the sample was sterilized with 5% NaOCl for 1 minute, twice 70% alcohol for 1 minute, then rinsed twice with sterile distilled water for 1 minute. Isolation of healthy plant (leaf, stem and fruits) and those with showing symptoms on stems, leaves, and fruits 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 on a tissue paper treated with 70% ethanol, then the fruit was rinsed with sterile distilled water and air dried. Then, cut the skin tissue and flesh between the diseased and healthy parts (5 mm × 5 mm). 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 in to different Petri dish containing PDA medium based on their growth characteristics, then incubated at room temperature (28 ± 2°C) for 7 days. These colony growths were examined based on growth characteristic and color were then sub-culture from single conidia as suggested by Ilyas *et al*. (2006), Iqbal *et al*. (2017), and Widodo and Hidayat (2018). These sub-cultures 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. A full of fungal culture grown on PDA plates were taken on a glass slide and observed with 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 as suggested by Ilyas *et al*. (2006), Iqbal *et al*. (2017), Rodrigues *et al*. (2007), Varga and

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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).

The results of identification confirmed that four species fungal were identified namely *Trichoderma viride*, *Penicillium* sp. *Aspergilus flavu*s, while anthracnose pathogen were *C. gloeosporeides* and *C. capsici*. However, for further study 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 were different growth characteristics.

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

The antagonistic test in this study was carried out using multiple culture method 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 14^{th} day since the two isolates were put together. The zone of inhibition is the length of the region in the confrontation cup that is not overgrown by the two mutually exclusive isolate antagonist. Collected data is made by measuring the length of the empty zone. Percent inhibition is calculated by formula used by Rohana (1998). The experiment was arranged using Completely Randomized Design (CRD), the fungus *Trichoderma* isolates were treated and repeated five times.

***Trichoderma viride* Antagonist against *Colletotrichum capsici* under Screen House Conditions**

The results of the in-vitro study of antagonistic isolates against the fungus *C. capsici* showed a high antagonistic level, then it was propagated before being used as an antagonistic isolate in chili cultivation. The use of the antagonistic study was carried out in a screen house condition. Chili plants were grown in plastic pots measuring 10 kg of soil. Each pot was planted with one chili seedling of one month after sowing. The treatment was prepared using Complete Randomized Design (CRD), repeated five times.

Inoculation with *T. viride* isolates was carried out when the plants were around 50 days old by selecting three fruits for each plant with a suspension solution of *T. viride* isolates at a concentration of 7×10^6 CFU/mL (full strength). *Trichoderma* inoculation is done by coating the fruit using a brush. The *T. viride* was then diluted into a half-concentrated solution with a

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concentration of 3.5×10^3 CFU/mL (half strength), and a quarter solution with a conidia density of 1.75×10^2 CFU/mL (quarter strength) as suggested by Juraimi *et al*. (2005). One week after inoculation with antagonist *T. viride*, then the chilies were inoculated with *C. capsici* solution with a concentration of 5×10^6 CFU/mL (full strength) by means fruit coating using a brush.

Cultivation Efforts to Reduce the Risk of Anthracnose under Field Conditions

The experiment was carried out in 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 in a Nested Design with two experimental factors and three replications. The first factor is in the form of liming, namely T0 (without liming), and T1 (liming 3 t/ha). The second factor was the plant spacing treatment, namely Ps1 (plant spacing [40×60 cm]), Ps2 (plant spacing [50×60 cm]), and Ps3 (plant spacing [50×70 cm]), so there were 18 experimental units. Five plants were taken as observation samples in each experimental unit. Chili planting: the beds were made 1.2 m × 3 m in size and then given agricultural lime according to the treatment, as much as T0 (without liming) or T1 (calcification of 3 t/ha), and the provision of organic fertilizer/manure of 30 t/ha. The distance between planting holes according to the treatment, namely T1 (40 cm×60 cm), T2 (50 cm× 60 cm) or T3 (50 cm× 70 cm). Chili seeds were ready to be planted when they have 4 to 5 leaves by transferring them to planting hole. Planting chili seeds should be done in the afternoon. Seeds that were planted and selected for uniform growth, not attacked by pests and diseases, and fresh green leaf color.

Plant Maintenance and Harvesting

Maintenance activities include replanting, inorganic fertilization, irrigation, pest and disease control. Stitching was done at 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 given 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) in the generative phase with concentration 2 g/L, the fertilizer solution was sprayed per plant once a week. Pest control was carried out at any time when symptoms of attack were seen. While, Chili harvesting was done when the plants around 70–120 days after planting (DAP). Harvesting was done by picking the fruit at the physiological ripe stage (60–90%).

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Plant height measurement was done from every two week. Measurement of the diameter of the largest outermost plant canopy was carried out every two weeks during generative phase. While, 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) at every two weeks. Plant production such as number of fruits and calculation of the number of fruits obtained from the first harvest to the last harvest for each plant and each plot.

Statistical Analysis

Observations were made one week after 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:

$$DI = \frac{A}{B} \times 100\% \qquad (1)$$

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

$$I = \frac{\sum (n \times v)}{N \times Z} \times 100\% \qquad (2)$$

Where: 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*

The results of the in vitro study found three species of antagonistic fungi on anthracnose-in chili. The three antagonistic fungi such as *Penicillium* sp., *Aspergillus flavus,* and *T. viride* (Table 1).

The results of the ANOVA test (Table 1 and Figure 2) show that *T. viride* significantly different and able to suppress the growth of the fungus *Penicillium* sp. and *C. capsici* of 62.80 up to 71.23% at 14 DAI. Furthermore, the inhibition ability of *T. viride* to the three

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isolates tested tend to be stable (Figure 2). This was due to the speed of development of *T. viride* compared to the three isolates tested.

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

The results of activity of the antagonist test of *T. viride* against *C. capsici* on chili plants under screen house conditions (Table 2) showed that the inoculation of *T. viride* (T2) with a concentration of full-strength solution with a density of $7{\times}10^6$ CFU/mL was only able to show the disease intensity of by *C. capsici* by 60% or showing the same disease intensity as the control treatment (T1). However, the inoculation of the fungus *T. viride* at the concentration of half strength and quarter strength solution 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 comparing to the other two isolates of *Penicillium* sp. and *A. flavus.*

Table 3 shows the intensity of anthracnose in chili plants at 28 DAI. *Trichoderma* fungus inoculation with a solution density of $7{\times}10^6$ 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%.

Treatment with quarter strength and half strength concentrations showed an increasing trend of disease progression at 7 days after inoculation (DAI) and continued to grow exponentially up to 28 DAI, with a rate 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

Chili plant height showed no significant difference in the plant spacing treatment. This shows that all plant spacing used in this experiment had used recommended plant spacing, so that it did not show any environmental stress which was indicated by an etiolation response (response to lack of light intensity). While giving lime showed a real response, namely chili which were higher than without liming. The effect of applying lime as a soil amendment when the degree of soil acidity (pH) is acidic or alkaline conditions will result in an increase or decrease in pH close to neutral, so that the plant's metabolic response becomes better. The component of plant growth (plant height) were higher in the liming treatment than without

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liming (Table 4). There was no interaction between plant spacing treatment and the application of agricultural lime to the plant height of chili plants.

Lime application 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).

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 number of fruits. However, lime treatment was able to increase number of fruits (Table 6). Giving lime increased number of fruit and significantly different from without liming. Giving lime can increase effectiveness of nutrient absorption, because soil pH conditions become more neutral, so plant metabolism becomes better, marked by a greater number of fruits compared to without liming.

The results of observing number of fruits, the treatment in spacing did not show a significant difference in the production of chili plants. However, the application of lime showed a significantly different in production response which was higher than without liming (Table 7). This showed that the provision of lime was very necessary at the location of this study because it was able to neutralize soil pH, so that the effectiveness of nutrient uptake and plant metabolism increases resulting in increased chili production.

Plant spacing treatments showed no significant difference in chili productivity. Giving agricultural lime can increase the productivity of chili (Table 8). The increase of production due to the administration of lime, the productivity response was also the same. This indicated that the provision of agricultural lime was needed to increase the production and productivity of chili as much of 1.5 times higher than without liming.

Disease Incidence of Anthracnose on Chili

The treatment of plant spacing did not show a significant difference in the intensity of fruit attacked by anthracnose. On the other hand, giving lime showed a higher rate of anthracnose (Table 9). This showed that giving lime has no effect on reducing anthracnose on chilies.

Discussion

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

In-vitro test results of isolates of *T. viride* showed the inhibition of the fungus

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Trichoderma against *C. capsici, Aspergillus flavus*., and *Penicillium* sp. able to suppress 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 in cayenne pepper (*Capsicum frustescens* L.) up to 65%. Furthermore, Khairul *et al.* (2017) reported that the colony of the fungus *Trichoderma* sp. 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.

The growth inhibition of *Colletotirchum* spp. due *Trichoderma* produced antibiotic compound such as harzianic acid, alamethicins, tricholin, peptaibols, 6-penthyl-α-pyrone, massoilactone, 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. this causes the hyphal walls of the pathogen *C. capsici* dissolved causing the growth of the pathogen to be inhibited and then die (Lubis *et al*., 2018). Further more, 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 a storage time of 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

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Actinomycetes >69% indicated a very good category. Thus it can be stated that the addition of corn flour in PDA media increases the population of Actinomycetes and *T. harzianum*, the number of populations of biological agents applied to plants so that they can inhibit disease development and affect the success of controlling anthracnose disease in chilies.

The inhibition level of *T. viride* against *C. capsici* in more controlled environmental conditions (temperature and humidity) with a range of *T. viride* (full strength) with a duration of 28 DAI was able to inhibit the intensity of anthracnose by 87%. The results are in line with Khairul *et al.* (2017) suggesting that the 21 DAI observations are of intensity the lowest 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 the disease in anthracnose caused by *C. capsici.* 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 C. *capsici* which causes this pathogen to be easily degraded by the chitinase enzyme. With the presence of this enzyme, *Trichoderma* sp. can suppress anthracnose on chili plants (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 sunny, not raining so that the ability of *Trichoderma* sp. to work can be maximized. Planting chili plants during the rainy season can also support disease development to develop more rapidly (Sriyanti *et al*., 2015). the pathogen *C. capsici* in chili plants can reduce production by up to 60%. As well as in optimal environmental conditions it can cause farmers to fail to harvest (Arofahsari, 2015). In observations in the field, the development of anthracnose has different attacks. According to Sitompul (1995) where the application of *Trichoderma* sp. has a different effect on the field, this is due to environmental conditions, humidity, and also the nutrient content in each soil is different so that the results obtained are not the same for each place. However, the application of *T. viride* in this study which was applied more often obtained milder results than without the application, so that basically *T. viride* was able to have a positive effect if the application was right, the concentration was right, and the age of *T. viride* was still productive or not too long and the microparasites in it are still effective.

On the other hand, the results of field trials which describe the ineffectiveness of *T. viride* isolates. suppressing the development of anthracnose (disease intensity ranging from 87% to 100%) 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

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spacing. This is in line with the results of research by Ibrahim *et al*. (2019); suggests that based on the value of effectiveness it is categorized that chili plant leaves in the field are in a bad category because their efficacy value is 50% and chili efficacy is in the good category if it has a value of 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. A mold really need essential nutrients include hydrogen, carbon (C), oxygen (O), phosphorus (H), nitrogen, sulfur and calcium in growth. 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).

The results of experiments that have been carried out both antagonistic study of the fungus *T. viride* against *C. capsici* that causes anthracnose in chili plants in the controlled environment for utilizing *T. viride* as a potential biological agent by further studying identification for storing isolates using appropriate growth media and stickers, and efficient utilization.

Aspects of Chili Agronomic on Anthracnose

In the different plant spacing experiments, there was no significant difference in plant height. In accordance with the results of the study that variations in plant spacing did not affect plant height in chili plants. The plant spacing used in this experiment has used recommended plant spacing for chili cultivation. The use of narrow plant spacing can increase yields, as long as limiting factors can be avoided so that competition between plants does not occur (Mayadewi, 2007). While wide plant spacing causes the optimum LAI to be slow to reach, but the yield of unit area is low (Nawangsih *et al*., 2003).

Plant spacing of 30 × 100 cm causes yields per plant of chili peppers to be higher, but yields per hectare are lower than plant spacing of 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, which is about 65–70 cm (70 cm is better) and planting in a zig-zag manner, this aims to reduce humidity and air circulation is quite smooth because the distance between plants is wider, another advantage is that the fruit will grow bigger (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 an increase or decrease in pH close to neutral, resulting in better nutrient uptake by roots and plant metabolic responses. Shown by the plant growth component (plant height) is

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higher in the liming treatment than without liming. Although the application of lime and plant spacing showed no significant difference in the diameter of the chili plant canopy, there was still a tendency that liming showed a higher response to crown diameter than without lime. The tendency of the crown diameter to be higher, in which each part of the axillary branch in the crown is a place for chili fruit to grow, so that the wider the diameter of the crown, the more fruit will be produced.

Number of Fruits, Fruit Production, and Productivity

The results showed that there was no significant difference between the treatments spacing. This shows that the use of smaller or larger spacing is not able to increase the number of fruits. However, the treatment of giving lime can increase the number of fruits. Giving lime increased the number of fruit significantly different from without liming. Giving lime can increase the effectiveness of nutrient absorption, because the soil pH conditions become more neutral, so that plant metabolism becomes better, marked by a greater number of fruits compared to without liming.

As with the results of observing the number of fruits, treatment of differences in plant spacing did not show a significant difference in the production of chili plants. However, the application of lime showed a significantly different production was higher than without liming. This shows that the application of lime as a soil conditioner is very necessary at the location of the research area because it is able to neutralize soil pH, so that the effectiveness of nutrient uptake and plant metabolism increases resulting in increased chili production. The plant spacing (100×80 cm) of pepper did not affect the wet weight of fruit per plot but affect wet weight of fruit per plant (Aminifard *et al.*, 2010). Differences in plant spacing showed no significant difference in chili productivity. Giving agricultural lime can increase the productivity of chili. This indicates that the provision of agricultural lime is needed to increase the production and productivity of chili will be able to increase production 1.5 times higher than without liming.

Based on the aspect of agronomic study showed that treatment of differences in plant spacing did not show a significant difference in the intensity of fruit attacked by anthracnose. however, giving lime actually showed a higher rate of anthracnose attack, the percentage was up to three times. This shows that giving lime has no effect on reducing anthracnose attacks on chili.

CONCLUSIONS

The antagonist fungus *T. viride* has potential as a bio fungicide in chili. This is evidenced

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by the ability to inhibit the growth of the fungus *C. capsici* of 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, it could not able to reduce the disease intensity.

ACKNOWLEDGEMENTS

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

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APPENDIX

LIST OF TABLES

Table 1. Average *Trichoderma viride* inhibition of isolates tested 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}

*)The average followed by the same letter are not significantly different at the 0.1% LSD test level

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

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

*)The average followed by the same letter are not significantly different at the 0.1% LSD test level

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}/mL	41.332^{c}

*)The average followed by the same letter are not significantly different at the 0.1% LSD test level

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Table 4. Treatment of plant spacing and liming 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	64.71a	68.414b

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

Table 5. Treatment of plant spacing and liming 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	68.272a	72.815a

Note: Similar letters in the same row are not significantly different at 5% level

Table 6. Treatment of spacing and liming of the 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% level

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Table 7. Treatment of plant spacing and liming of chili production

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

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

Table 8. Treatment of spacing and liming on the productivity of chili plants

Treatment	Liming (t/ha)	
Plant Spacing (cm)	T0 (%)	T1 (%)
40×60	2,179,845	3,920,267
50×60	2,506,400	3,915,045
50×70	2,622,083	4,166,667
Average	2,436.109a	4.000,660 b

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

Table 9. Treatment of plant spacing and liming on fruit intensity attacked by anthracnose

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	32.405 b

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

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LIST OF FIGURES

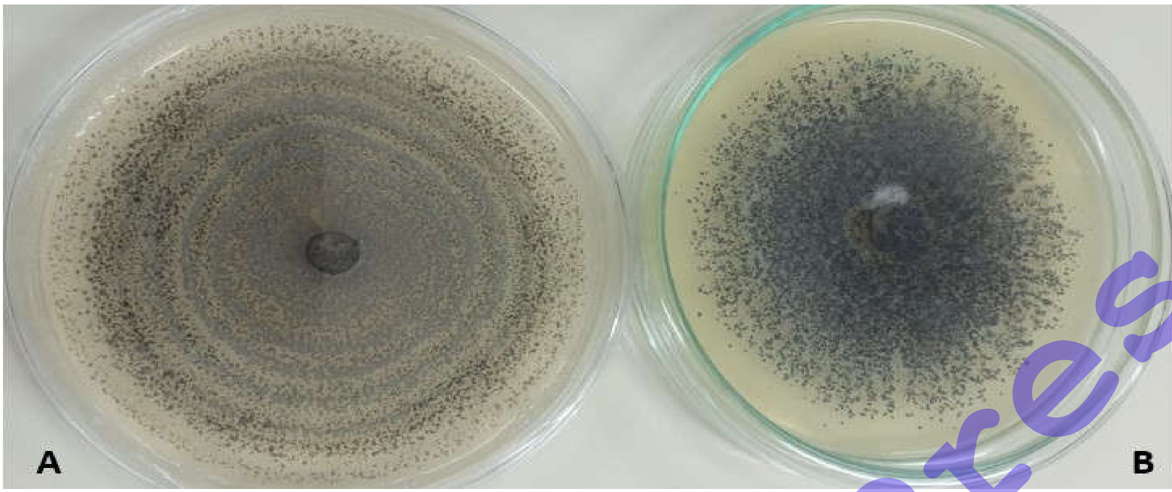

Figure 1. Pure sub-culture of *Colletotrichum capsici* (A) at seven days after inoculation (DAI) and *Trichoderma viride* (B) at three DAI

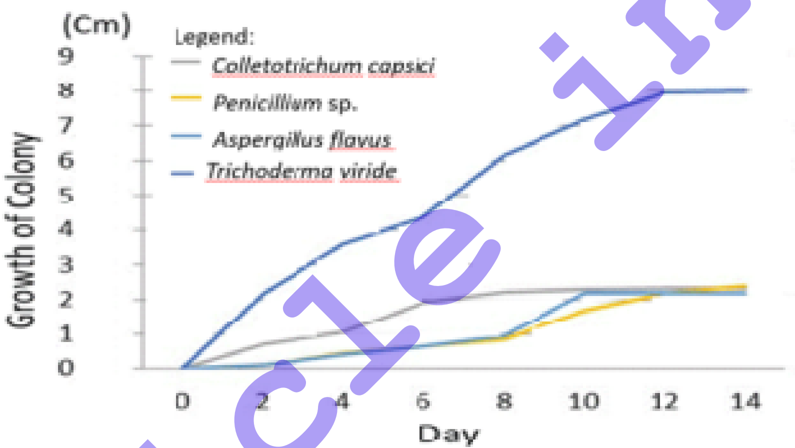

Figure 2. Graph of antagonist and *Colletotrichum capsici* at 14 days after inoculation (DAI)

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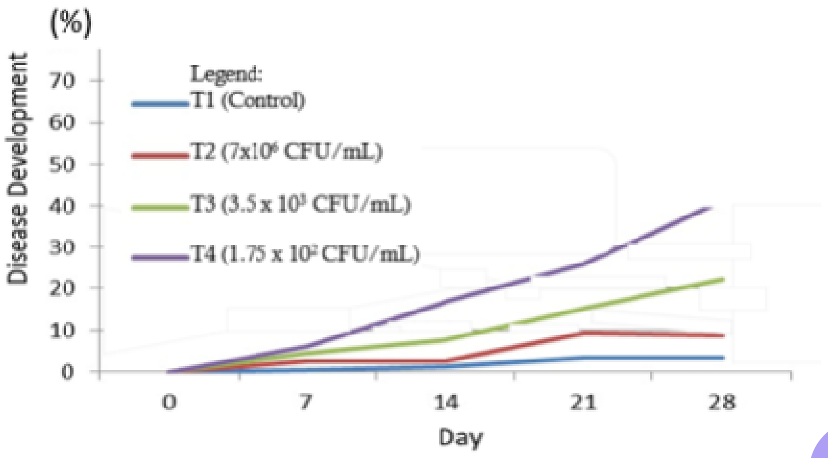

Figure 3. The average development of anthracnose up to 28 days after inoculation (DAI)

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

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