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EFEKTIVITAS JAMUR ANTAGONIS *Gliocladium sp.* DAN *Trichoderma harzianum* UNTUK MENGENDALIKAN *Colletotrichum spp.* PADA TANAMAN CABAI *(Capsicum annuum* L.*)*

EFFECTIVENESS OF ANTAGONISTIC FUNGI *Gliocladium sp*. AND *Trichoderma harzianum* TO CONTROL *Colletotrichum* spp. on chili (*Capsicum annuum* L.)

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ABSTRAK
Produktivitas cabai baik dari segi kualitas maupun kuantitas terkendala oleh serangan penyakit antraknosa yang disebabkan oleh *Colletotrichum* spp. Pestisida umumnya digunakan untuk mengendalikan penyakit tersebut. Penelitian dilakukan untuk mendapatkan jamur antagonis dan dosis efektif yang mampu menghambat pertumbuhan *Colletotrichum* spp. Uji antagonis dilakukan secara in vivo menggunakan metode langsung dengan tiga ulangan. Uji penghambatan disusun dalam Rancangan Acak Kelompok Lengkap (RAKL) dengan 7 perlakuan: Kontrol, *Gliocladium sp* (150, 250, dan 350 mL / tanaman) dan *T. harzianum* (150, 250, dan 350 mL / tanaman) dengan empat ulangan dan tiga sampel dari 20 tanaman per perlakuan. Hasil penelitian menunjukkan bahwa persentase penghambatan *T. harzianum* terhadap *Colletotrichum* spp. adalah 55% sedangkan *Gliocladium sp.* adalah 70,33% dalam uji antagonis. Rerata intensitas penyakit pada 11 hingga 32 DAI pada kontrol masing-masing adalah 5,61 ± 0,71% hingga 20,30 ± 1,35%. Ini secara signifikan lebih tinggi daripada *Gliocladium sp*. 350 mL / Tanaman (1,94 ± 0,45% hingga 11,15 ± 0,87%), *T. harzianum* 350 mL / Tanaman (1,10 ± 0,58% hingga 8,68 ± 0,91%), dan *T. harzianum* 250 mL / Tanaman (2,28 ± 0,43% hingga 8,75 ± 0,79%). Tidak ada perbedaan yang signifikan pada rata-rata berat segar hasil buah cabai merah per tanaman. *T. harzianum* dan *Gliocladium sp.* efektif dalam mengendalikan serangan penyakit antraknosa pada cabai. Dosis yang paling efisien untuk mengendalikan penyakit antraknosa adalah pemberian *T. Harzianum* atau *Gliocladium sp* dengan dosis 250 mL / tanaman.

Kata Kunci: Cabe, Anthracnose, *Gliocladium, Trichoderma, Colletotrichum*

ABSTRACT
The productivity of chili in terms of both quality and quantity is suppressed by the attacks of anthracnose disease caused by *Colletotrichum* spp. Pesticides is commonly used to control the disease. Researched was conducted to determine the antagonistic fungi and the effective dose in inhibiting the growth of the *Colletotrichum* spp. Antagonistic tests were performed in vivo using the direct method with three replicates. Inhibition test was arranged in a Complete Randomized Block Design (RCBD) with 7 treatments: Control, *Gliocladium* sp (150, 250, and 350 mL/plant) and *T. harzianum* (150, 250, and 350 mL/plant) with four replicates and three samples out of 20 plants per treatment. The results showed that the percentage of inhibition of *T. harzianum* against *Colletotrichum*

sp. is 55% while *Gliocladium* sp. is 70.33% in antagonistic test. The mean of disease intensity at 11 up to 32 DAI on control is 5.61 ± 0.71% up to 20.30 ± 1.35 % respectively. It is significantly higher than *Gliocladium* sp. 350 mL/Plant (1.94 ± 0.45 % up to 11.15 ± 0.87 %), *T. harzianum* 350 mL/Plant (1.10 ± 0.58 % up to 8.68 ± 0.91 %), and *T. harzianum* 250 mL/Plant (2.28 ± 0.43 % up to 8.75 ± 0.79 %). No significant different is observed on mean of fresh weight of red chili fruit yield per plant. *T. harzianum* and *Gliocladium sp.* fungi are effective in controlling the attack of anthracnose disease in chili. The most efficient dose to control anthracnose disease is the application of *T. Harzianum* or *Gliocladium* sp at the dose of 250 mL/plant.

Key Words: Chilli, Anthracnose, *Gliocladium, Trichoderma, Colletotrichum.*

INTRODUCTION

The productivity of chili is suppressed by the attacks of anthracnose disease caused by *Colletotrichum*. Production and quality of red chili decreased into 45-60%. In mature plants it can be causing dieback, then followed by further infection in fruit. (Hidayat *et. al.*, 2004). Invention of disease resistant cultivars has been conducted in the last decades (Palupi *et. al.,* 2015), however the disease is still the main obstacle for increasing chili production and pesticides application is commonly done with implication of the reduction of soil quality. *Gliocladium* sp. and *Trichoderma harzianum* is an antagonistic fungi that can play a role in suppressing population or plant pathogenic activity. *Gliocladium* sp. can produce gliovirin and viridian which are functional antibiotics. While *Trichoderma harzianum* can produce chitinase and B-1.3-glucanase enzymes, with the antagonistic process (Gonzales, *et. al.,* 2012). Istokirini (2008) reported that endophytic fungi isolated from the roots of chili plants, are *Trichoderma* sp. and *Gliocladium* sp. They are able to inhibit *Colletotrichum* sp. directly. While Wilia *et al.,* (2012) states that the suppression of anthracnose disease by the fungi *Acremonium* sp., *F. oxysforum* CT1, and *F. solani* CJ1 is more effective than by *Trichoderma* sp. and *Gliocladium* sp., but there was no antibiotic mechanism between the endophytic fungi and *C. acutatum* in multiple culture tests. Therefore, further research is needed to find out the inhibitory ability of *Trichoderma harzianum* and *Gliocladium* sp in controlling anthracnose disease caused by *Colletotrichum* spp.

METHODOLOGY OF RESERCH

Research was carried out in vitro for antagonistic tests and in vivo for testing the effectiveness of pathogen inhibition. Antagonistic test was conducted by direct inhibition test based on the 2014 National Standardization Agency on PDA media. Seven days old *T. harzianum* and *Gliocladium* sp. were taken using a 0.5 cm diameter cork borer from the edge of the colony. Each of *T. harzianum* or *Gliocladium* sp. then inoculated on a PDA with a distance of 2 cm from the edge of 9 cm diameter petri dish and *Colletotrichum* spp from the edge on the opposite side with three replicates. Observation was conducted every 24 hours for 7 days by measuring the radius of each fungi colony. The magnitude of the effect of inhibiting biological agents on pathogens is calculated using formula.

$Z = \frac{(r1-r2)}{r1}$ x 100%

Z: antagonistic agent inhibition (%)
r1: radius of pathogenic colony without antagonistic agents (control)
r2: radius of pathogenic colony with antagonistic agent
In vivo experiment was carried out in a Complete Randomized Block Design (RCBD) with 7 treatments: Control, *Gliocladium* sp (150, 250, and 350 mL/plant) and *T. harzianum* (150, 250, and 350 mL/plant) with four replicates. Three chili plants (nonresistant variety of TM 999 F1) out of 20, were used as sample of each treatment. Chili plants were planted in field with 30 x 40 cm spacing, and sprayed with pure culture of *Colletotrichum* spp. suspension by added 250 mL of sterile distilled water, filtered then added 750 mL of sterile distilled water when they began to bear fruit at the age of 70 days. Inoculation of biological agents was carried out by spraying suspension of *Gliocladium* sp and *T. harzianum* on chili plants a day before the plants were inoculated with *Colletotrichum* spp. The intensity of the disease attack were observed every 7 days in the morning, from 11 up to 32 day after inoculation (DAI). The intensity of the pathogen attack is calculated using the following formula:

$$I = \frac{a}{a+b} \times 100\%$$

I = intensity of attacks
a = Number of plant parts (chili fruit) attacked
b = Number of healthy plant parts (chili fruit)
The analysis was carried out using analysis of variance (ANOVA) and Duncan's Multiple Range Test at 95% confidence level (α = 0.05). Data transformation was performed according to the nature of the data.

RESULTS AND DISCUSSIONS

Antagonistic Test

Fungi antagonistic test results of *Colletotrichum* spp. with *T. harzianum* or *Glioladium* sp. can be seen in Figure 1.

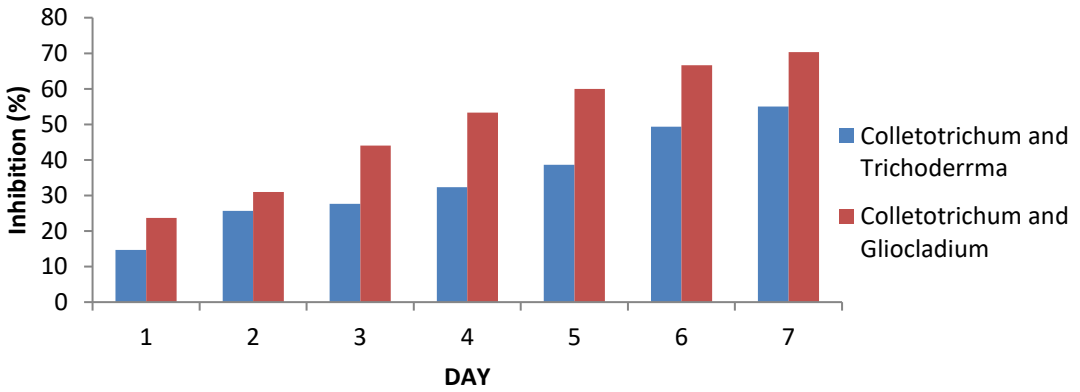

Figure 1. Antagonistic performance of *Gliocladium* sp and *T. harzianum* against *Colletotrichum* spp.

T. harzianum Gliocladium spp. are able to suppress up to 55.00% and 70.33% respectively (Figure 1.). The antagonism mechanism of *T. harzianum* is

as an antibiosis while the mechanism of *Gliocladium* spp. is by competition. *T. harzianum* produces enzymes and antibiosis compounds that can inhibit or even kill pathogens. These antibiotic compounds are gliotoxin, glyoviridin and trichodermin which are very strong pathogens-growth inhibitor. It is also reported that *T. harzianum* is capable of producing volatile and non-volatile antibiotic compounds (Meena *et. al.*, 2017). These compounds affect and inhibit many functional systems and make pathogens vulnerable. Toxins released by *T. harzianum* is not limited on growth inhibition but even eliminate *Colletotrichum* spp.on the PSA media and also on the chili (Ismail & Tenrirawe, 2012). The same result also found on *Botryodiplodia sp*. By Octaviani *et. al.* (2015). *T. harzianum* and *Gliocladium sp.* antagonism with the direct method able to inhibit the growth of *Botryodiplodia sp*. with the average 52.53% and 46.46% respectively on PDA for 7 days of observation. The mechanism that occurs between *Gliocladium* sp. against *Colletotrichum* spp. is competition. The competition mechanism occurs at the beginning of its growth due to rapid growth of *Gliocladium* sp. compared to *Colletotrichum* spp.

The Effectiveness of Pathogen Inhibition

Intensity of anthracnose disease in the red chili increase from 11 DAI up to 32 DAI with the highest attack on control treatment. Significantly different to control treatment is observed from 11 DAI up to 32 DAI for *Gliocladium* sp. at dose of 350 mL/plant. Significant effect of *T. harzianum* increase from 11 DAI up to 32 DAI, at a dose of 350 mL/plant and 250 mL/plant (Table 1.). Lowest disease intensity is found on the application of *T. harzianum* 350 mL/Plant at 11 DAI and it is not significantly to *T. harzianum* 250 mL/Plant, *Gliocladium* sp. 350 mL/Plant, and *Gliocladium* sp. 250 mL/Plant at 25 and 32 DAI.

Table 1. Means (± SE) intensity of disease (%) of anthracnose

Treatment	Day after inoculation (DAI)			
	11	18	25	32
Control	5.61 ± 0.71 [a]	13.10 ± 0.73 [a]	19.41 ± 1.44 [a]	20.30 ± 1.35 [a]
Gliocladium sp. 150 mL/Plant	6.51 ± 0.65 [a]	12.35 ± 1.20 [a]	18.03 ± 1.40 [a]	18.03 ± 1.40[ab]
Gliocladium sp. 250 mL/Plant	4.61 ± 0.66 [a]	10.82 ± 1.14 [a]	13.10 ± 1.32[b]	13.68 ± 1.30[bc]
Gliocladium sp. 350 mL/Plant	1.94 ± 0.45 [b]	7.63 ± 0.73 [b]	10.78 ± 1.00[b]	11.15 ± 0.87[c]
T. harzianum 150 mL/Plant	4.90 ± 0.62 [a]	12.24 ± 1.10 [a]	17.78 ± 1.24[b]	18.14 ± 1.39[a]
T. harzianum 250 mL/Plant	2.28 ± 0.43 [b]	5.65 ± 0.74 [b]	8.55 ± 0.85 [b]	8.75 ± 0.79[c]
T. harzianum 350 mL/Plant	1.10 ± 0.58 [c]	5.44 ± 1.01[b]	8.68 ± 0.91[b]	8.68 ± 0.91[c]

Note: Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ (DMRT).

The intensity of the attack of anthracnose disease in 32 DAI red chili with control treatment was categorized as moderate because it was above 11% and below 25%, the treatment of *Gliocladium* sp. a dose of 150 mL/plant, 250 mL/plant and 350 mL/plant is categorized as moderate because it is above 11% and below 25% whereas in *T. harzianum* treatment a dose of 150 mL/plant is categorized moderate because it is above 11% and below 25%, *T. Harzianum* doses 250 mL/plant and 350 mL/plant are categorized as light because the intensity of the attack is less than 11%. The intensity of attacks on the control, is

higher than that of *Gliocladium* sp. 350 mL /plant, *T.harzianum* 250 mL /plant, *T. harzianum* 350 mL /plant. The intensity of anthracnose disease shows that the treatment of *T. harzianum* and *Gliocladium sp*. can suppress the growth of pathogenic *Colletotrichum* spp. The suppression is due to the antagonism of *T. harzianum* and *Gliocladium* sp. Research conducted by Supriyati & Djaya (2016) with the application of *T. harzianum* and *Actinomycetes* 250 $mL.L^{-1}$ has the same effectiveness to suppress the attack of anthracnose in red chili with an effectiveness value> 69% (very good).

Mixing of *T. harzianum* in bioactivator application of legundi leaf extracts of fluids and tablets is able to suppress the incident of Fusarium wilt disease and yield on Onion (Sudantha *et. al.*, 2018). Baker & Cook (1974) states that the effectiveness of antagonists generally occurs in three types, there are (1) antibiosis and lysis, (2) competition or competition and (3) parasitism and predation. Antibiosis is the process of inhibition of an organism by secondary metabolites produced by other organisms, antibiotic activity generally inhibits growth and possibly kills other organisms, whereas lysis usually causes damage, decomposition or decomposition of biological substances. Fungi have evolved different strategies to increase their competitiveness for nutrient acquisition toward other microorganisms and to protect themselves from predation by animals. Similar to plants, the main defense strategy of fungi is chemical defense, i.e., the production of toxins impairing the growth, development, or viability of the antagonists by the fungus (Künzler, 2018).

According to the intensity of the disease, it can be assumed that reduction of yield may be occured. However, there was no significant difference in the mean of fresh weight of chilies fruit yield in the control, *T. harzianum* and *Gliocladium* sp treatment (Table 2.). This is because the intensity of the disease is light up to moderate so it has no significant effect on the fresh weight of chilies fruit yield. Resistance test on 14 resistant lines of chili to the anthracnose disease is also acquired the same result. Disease intensity is ranged from 1.11% up to 20.52% for high resistant and moderate resistant lines respectively. It also has quite low effect on chili fruit production. Fruit chili weight decrease from 165.24 g into 91.58 g (Palupi, *et. al.,* 2015). It is obviously seen that the application of *T. harzianum* and *Gliocladium* sp. have the same effect to use of resistant cultivar. It can be assumed that the application of those biological agents on resistant cultivar is able to provide multiple protection to anthracnose disease instead of pesticides applications.

Table 2. Means (± SE) of fresh weight of red chili fruit yield per plant (g)

Treatment	**Average (Gram)± SE**
Kontrol	100.50 ± 5.57[a]
Gliocladium sp. 150 mL/Plant	107.42 ± 6.21[a]
Gliocladium sp. 250 mL/ Plant	96.50 ± 7.54[a]
Gliocladium sp. 350 mL/ Plant	117.08 ± 8.04[a]
*T. harzianum*150 mL/ Plant	98.33 ± 5.89[a]
*T. harzianum*250 mL/ Plant	122.67 ± 7.13[a]
*T. harzianum*350 mL/ Plant	98.75 ± 5.56[a]

Note: Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ (DMRT).

CONCLUSION

Trichoderma harzianum and *Gliocladium* fungi are effective in controlling the attack of anthracnosa disease in red chili. The most efficient dose to control anthracnose disease is the application of *T. Harzianum* or *Gliocladium* sp at the dose of 250 mL/plant.

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