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The effectiveness of fungi *Gliocladiumfimbriatum* and *Trichoderma viride* to control fusarium wilt disease of tomatoes (*Lycopersicum esculentum*)

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ABSTRACT

Tomato (*Lycopersicum esculentum*) is a horticultural commodity with high economic value. However, serious disease management is needed to get good production with better fruit quality. Wilt disease caused by Fusarium is constraining tomato production. This study was aimed to measure the effectiveness the use of *Gliocladium fimbriatum* and *Trichoderma viride* to control fusarium wilt disease. This study was conducted in Food Crop and Holticultural Plant Protection Institute of West Papua and Laboratory of Plant Diseases of Agricultural Faculty, Papua University, Manokwari. This study was designed using factorial complete randomized design consisting of two treatment factors and repeated three times. The experiment findings indicated that servo variety was better compared to other tested varieties as indicated by better plant height, low fusarium wilt disease intensity, and higher fruits quantity and weight. *Trichoderma* and *Gliocladium* were capable of controlling the development of fusarium wilt disease in tomatoes plant, but the effectiveness of *Trichoderma* (53.68%) was better controlling the pathogen compared to *Gliocladium* (48.89%). Interaction between the treatments of Servo variety and fungi *Trichoderma* resulted in better effect both to lower disease intensity and higher fruits quantity and weight.

Key words: *Fusarium oxysporum, Gliocladium fimbriatum,* Tomatoes, *Trichoderma viride.*

INTRODUCTION

Tomato (*Lycopersicum esculentum*) is a very beneficial agricultural commodity due to its vitamins and minerals content. As mineral source, tomato fruits can be beneficial to bone and dental formation (lime and phospor substance), whereas the iron (Fe) contained in tomatoes can play a role in the formation of red blood cells hemoglobin. In addition, tomatoes contain potassium substance which is very useful to reduce high blood pressure (Cahyono, 2005). Therefore, the demand on tomato commodity keeps increased with the growth of population and people awareness on health.

Tomato production in Indonesia is low, 6.3 tons/ha as compared to other countries such as Taiwan, Saudi Arabia, and India at 21 ton/ha, 13.4 ton/ha, and 9.5 ton/ha, respecti vely. Low tomato production in Indonesia due to inappro priate variety, suboptimal cultivation, or uneffective pest and disease control (Kartapradja and Djuariah, 1992). Wilt disease caused by *Fusarium oxysporum* is the most important disease of tomato in Manokwari infecting all planted varieties.

Chemical pest controls have been used, but this strategy has negative effects to the environmental and leaft harmful residue. In addition, pesticides is expensive and not available in remote areas such as in West Papua province with limited accessibility.

The environmental friendly pest and disease controll is needed to minimize the negative impacts of chemical utilization. Compared to synthetic pesticides, the use of biological control agents is harmless particularly when it is applied during harvesting and post-harvesting period (Soesanto, 2008).

Biological control agents such as *Trichoderma viride* and *Gliocladium fimbriatum* are safe alternative to control soil-borne and seed-borne pathogens. These antagonist agents have the ability in controlling pathogens either by producing inhibitory compounds or compete for limited nutrients (Semangun, 1993). Several study findings indicated that the antagonist agents could suppress plant disease development from various pathogen attacks such as *Verticillium* sp. in strawberries (Suryawan *et al.*, 2017), *Elsinoe batatas* in sweet potatoes (Martanto *et al.*, 2015), *Botrytis cinerea* in beans (Papavizas, 1985), *Botrytis cinerea* in tomatoes (Neill *et al.*,1996), *Phytium ultimum* and *Rhizoctonia solani* in radish (Cliquet and Scheffer, 1996).

The utilization of these two antagonist agents is also capable of providing plant nutrients required to support vegetative organ growth or reproductive growth through decomposition process of organic substance provided in planting medium (Hartal, 2010).

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Considering the above problems, this research was conducted with the aim to study the effectiveness of *Gliocladium fimbriatum* and *Trichoderma viride* to control wilt disease of tomato caused by *Fusarium oxysporum*.

MATERIALS AND METHODS

The present research was conducted during February – June 2016 at Laboratory of Pest and Disease University of Papua (latitude 00°70‘76“S, longitude 134°06‘79“E, altitude 126) and of Food Crop and Holti cultural Plant Protection Institute of West Papua Indonesia (latitude 00°55‘25“S, longitude 134°02‘38“E, altitude 12). This study used factorial complete randomized design consisting of antagonist factors and varieties, repeated three times. Antagonist factors consisted of A0 (without antagonist fungus), A1 (with *Gliocladium fimbriatum*) and A2 (*Trichoderma viride*). Whereas the variety factors consisted of V1 (Permata variety), V2 (Lentana variety), V3 (Betabila variety), and V4 (Servo variety).

Antagonists and their application: Isolates of *Gliocladium fimbriatum* and *Trichoderma viride* were reproduced in husk bran medium at ratio 3:1. The antagonist substrates were applicated into the soil at the dosage of 50 g/polybag at 1 week after planting. The density of *Trichoderma viride* spores was 2.45 x 10^7 spore/ml water, whereas the *Gliocladium fimbriatum* 4.78 x 10^7 spore/ml water.

Pathogens and their inoculation: The isolates of *Fusarium oxysporum* was multiplied in corn sand medium at the ratio of 3:1. The pathogen inoculation at the density of 1.51 x 10^7 spore/ml water was conducted on the 7th day after the application of the antagonist agents.

Observation variables

Disease intensity: Disease intensity was calculated according to the percentage of occurring disease symptoms. Disease intensity was observed 3 weeks after planting, repeated 8 times at the observation interval of 1 week. Disease intensity was calculated based on the following formula :

$$DI = \frac{\sum_{i=0}^{n} (n \times v)}{Z \times N} \times 100\%$$

DI = Disease intensity, n_i = Number of leaves in each infection category, v_i = Scale value of each infection category, N = Number of observed leaves, Z = Highest scale value. Category of attacks agreed upon was based on Soesanto *et al*. (2010) as follow:

0	=	No symptomatic leafs
1	=	Yellowing leaves symptom 0-20 %
2	=	Yellowing leaves symptom 21- 40 %
3	=	Yellowing leaves symptom 41-60 %
4	=	Yellowing leaves symptom 61-80 %
5	=	Yellowing leaves symptom >80 %

Antagonist effectiveness: Antagonist effectiveness can be calculated by the formula of Abbott (Ditjen, 2004)

$$EI = \left[\frac{Ca - Ta}{Ca}\right] x\ 100\ \%$$

El = effectiveness of Antagonist Agent (%), Ca =Intensity of disease in control/without treatment, Ta = Intensity of disease with treatment/after application.

Plant height: Plant height was measured from land surface to the highest leaf or bud, at the age of 3-9 WAP (Weeks After Planting).

Number of flower per plant: Observation on flower number was conducted starting from the very first flower to last flower appeared. Observation was conducted every two weeks.

Number of fruits per plant (fruits/plant): Healthy fruits were calculated during harvesting.

Fresh fruit weight per plant (gr/plant): Fresh and good fruits were harvested and weighted during harvesting time.

Data analysis: The collected data were analyzed by variance analysis, and when there was a significant difference, continued by LSD test at the confidence interval of 95% (Gomez and Gomez, 2010). The antagonist effectivity test was presetend by tabulation method.

RESULTS AND DISCUSSION

Plant height: The best height was shown by the Servo variety (67.65 cm) and the lowest height was shown by the Permata variety (54.69cm). The application of antagonist had a significant effect on plant height on the observation at 3-8 WAP and there was significant difference between treat ment. The height of plant treated with treatment *Trichoderma* resulted in the highest height (75.60 cm) and the lowest was at the treatment without antagonist (37.91 cm). Tomato plants innoculated with *Fusarium* without the addition of antagonist microorganisms had very slow growth compared to those inoculated with antagonist (Table 1). The combination of A1V4 resulted in the best plant height (81.43 cm) and the lowest in combination of A0V1 (18.58 cm (Table 2).

The difference in plant height occurred due to the genetic propertices owned by the individual variety. Sopialena (2015) suggested that a plant variety reaches the maximum height growth according to its genetic properties. Sunarjono (1977) added that some of the tomato plant varieties can reach height up to more than 1 m and some grow determinate, namely growth shorter than 40 cm.

The application of antagonist effects the plant height. *Trichoderma* treatment caused the plant height higher compared to the treatment of *Gliocladium* and without antagonist. *Trichoderma*, in addition to its ability in supressing the pathogen growth, it is also a microorganism that dissolve phosphate which can be used by the plant, so the growth of the plant can be favored. This is in accordance with Setiadi (1989) who stated that the existence of

Table 1: The effect variety and antagonist on plant height

Treatment	Plant height (cm)						
(variety)	**3 WAP**	**4 WAP**	**5 WAP**	**6 WAP**	**7 WAP**	**8 WAP**	**9 WAP**
Permata	14.63c	22.31b	31.40b	41.39c	47.09b	50.47b	54.69b
Lentana	18.66b	30.01a	37.16a	4579b	53.26a	56.75a	62.75a
Betavila	18.20b	26.49a	35.75a	47.41ab	52.18ab	59.37a	66.85a
Servo	21.09a	29.88a	36.74a	50.84a	56.19a	60.97a	67.65a
LSD 95% :	2.30	3.95	3.98	4.36	5.18	5.96	7.03
A0	14.17c	16.49c	18.51c	24.85b	26.79b	31.72b	37.91b
A1	18.26b	30.18b	41.30b	55.54a	64.28a	68.66a	75.43a
A2	22.00a	34.85a	45.97a	58.70a	65.47a	70.29a	75.60a
LSD 95% :	1.99	3.42	3.45	3.78	3.49	5.16	6.09

Note : Numbers followed by same letter in same column are not significantly different according to
LSD 95% test. A0=Without Antagonist, A1= *Gliocladium*, A2 = *Trichoderma*.
WAP = Weeks After Planting

Table 2: Combination of variety and antagonist application on plant height, disease intensity, number of flowers, number of fruits and fruits weight.

Treatment	Plant height		Disease intensity (%)		Number of flower	Number of fruit	Fruit weight(g)
	8 WAP	**9 WAP**	**8 WAP**	**9 WAP**			
A0V1	15.58c	18.58d	46.06a	75.63	0.00	0.00d	0.00
A0V2	31.08b	35.58c	43.61ab	59.73	0.00	0.00d	0.00
A0V3	40.85b	48.49b	30.70ab	59.40	0.00	0.00d	0.00
A0V4	39.38b	48.74b	25.90bc	56.79	0.10	0.09d	0.25
A1V1	63.58a	71.50a	18.30cd	33.79	1.75	0.25cd	2.83
A1V2	68.25a	73.12a	15.26cd	29.50	0.50	0.66b	3.33
A1V3	70.49a	76.35a	20.36bc	40.05	0.91	0.00d	0.00
A1V4	72.33a	81.43a	19.75cd	25.22	1.75	1.25a	9.08
A2V1	72.27a	73.99a	28.96ab	31.30	1.16	0.58bc	9.00
A2V2	70.92a	79.27a	29.75ab	34.00	1.08	0.66b	7.00
A2V3	66.78a	75.69a	12.80d	29.68	1.50	0.17d	1.58
A2V4	71.19a	72.78a	20.21cd	21.50	2.00	1.33a	1.41

Note : A0=Without antagonist, A1= *Gliocladium*, A2 = *Trichoderma,* V1= Permata variety,
V2= Lentana variety, V3= Betavila variety and V4= Servo variety.
WAP = Weeks After Planting

Trichoderma in the soil has a synergistic effect with bacteria that dissolve phosphate.

At 9 WAP, the combination with the best plant height, namely *Gliocladium* and Servo variety, whereas the combination without antagonist with Permata variety indicated the lowest plant height. According to Iskandar and Pinem (2009), the presence of *Gliocladium*, in addition to be able to supress the development of disease, it can also provide nutrients for plant, so that the plant can grow normally.

Disease intensity (%): Disease symptoms began to occur at 3 WAP. The highest intensity was observed in Permata variety (46.90%) and the lowest intensity was observed in Servo variety (34.52%). Disease intensity without treatment was higher (62.89%) compared to those with *Gliocladium* (32.14%) and *Trichoderma* treatment (29.13%). Effectiveness of *Trichoderma* was higher (53.68%) compared to *Gliocladium* (48.89%) (Table 3). At 8 WAP, disease intensity was the lowest in combination of A2V3 (12.80%) and the highest in combination of A0V1 (46.06%). At 9 WAP indicated that the most of the tested plants showed green yellow and dry (Table 2).

Disease intensities in the tested tomato varieties were all below 50%. Disease intensity for Servo variety in 9 WAP showed the lowest disease intensity (34.52%) and the highest in Permata variety (46.90%). This is due to the application of *Gliocladium* and *Trichoderma* can supress pathogen *Fusarium* in tomato plants. According to Purwantisari and Hastuti (2009) and Semangun (1991) *Trichoderma* sp. and *Gliocladium* sp. were very important antagonist for biological control. Belete *et al*., (2015) and Yulia *et al*., (2017) mentioned that the fungus *Trichoderma* spp. had many mechanisms in the pathogen control process. Trichoderma spp. suppress pathogens by various mechanisms such as competition, mikoparasit, producing antibiotics, and resistant induction. In addition Ekowati *et al*. (2009) and Harni *et al*., (2017) that secondary metabolite of Trichoderma

Table 3: The effect of variety and antagonist on disease intensity

Treatment	Disease intensity (%)						
(variety)	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP
Permata	8.50a	11.84a	15.42a	18.52a	20.93	31.11a	46.90a
Lentana	4.87ab	9.06ab	7.29b	10.40b	19.58	29.54a	41.07ab
Betavila	5.08b	9.92a	8.38b	14.46ab	17.54	21.28b	43.04ab
Servo	3.77b	6.30b	11.46ab	12.26b	15.34	21.95b	34.52b
LSD 95% :	*2.58*	*3.11*	*4.76*	*4.57*	*6.21*	*5.79*	*10.87*
A0	10.73a	22.92a	23.72a	27.62a	32.00a	36.57a	62.89a
A1	3.37b	3.00b	4.07b	7.59b	9.85b	18.42b	32.14b
A2	2.52b	1.92b	4.12b	6.52b	13.19c	22.93b	29.13b
LSD 95% :	2.24	2.69	4.12	3.95	5.37	5.01	9.41
			Antagonist effectiveness (%)				
A1	68.59	68.91	82.84	72.51	69.21	49.63	48.89
A2	76.51	91.62	82.63	76.39	58.78	37.29	53.68

Note : Numbers followed by same letter in same column are not significantly different according to LSD 95% test. A0=Without Antagonist, A1= *Gliocladium*, A2 = *Trichoderma*
WAP = Weeks After Planting

Table 4: The effect of variety and antagonist on the number of flowers, fruits, and weight of fruits per plant

Treatment (variety)	Number of flower	Number of fruits	Fruits weight (g)
Permata	0.97ab	0.27bc	3.94ab
Lentana	0.52b	0.44b	3.44bc
Betavila	1.16ab	0.05c	0.52c
Servo	0.88ab	0.88a	6.92a
LSD 95% :	0.46	0.23	3.11
A0	0.03b	0.02b	0.06c
A1	1.50a	0.54a	3.81b
A2	1.16a	0.68a	7.25a
LSD 95% :	0.39	0.20	2.69

Note : Numbers followed by same letter in same column are not significantly different according to LSD 95% test. A0=Without Antagonist, A1= *Gliocladium*, A2 = *Trichoderma*

ininhibit the development of pathogens is through protein denaturation, both structural and functional on pathogen cells.

Trichoderma treatment in the last observation indicated low disease intensity (29.13%), followed by *Gliocladium* (32.14%) and without antagonist (62.89%). Taufik (2010) suggested that *Trichoderma* sp. has the ability to compete for space and food and capable of supressing the pathogen development in the soil and plant tissues, and induce immunity and inactivation of pathogenic enzymes. Papavizas (1985) suggested that *Trichoderma* sp. produces Trichodermin and *Gliocladioum* sp. produces gliotoxin and viridin which were toxic to pathogens. Ditjen (2004) added that *Trichoderma* sp. could supress the pathogen growth by twining the pathogen hypha, releasing enzyme β-(1-3) gluconase and chitinase that could invade the host cell wall. The effectiveness of *Trichoderma* in controlling Fusarium wilt disease was higher (53.68%) compared to *Gliocladium* (48.89%).

Number of flowers, number of fruits and fruits weight. The highest flower number was observed in Betavila variety (1.16) and the lowest one in Lentana variety (0.52). Number of fruits per plant was highest in Servo variety (0.88) and lowest in Betavila variety (0.05). The best fruits weight was observed for Servo variety (6.92 g), and the hightest one in Betavila variety (0.52 g). Antagonist treatment had the effect on number of flowers, number of fruits, and fruits weight. Number of flowers in *Trichoderma* treatment showed best result (1.16), whereas the lowest results was observed in the treatment without antagonist (0.03). Number of fruits in *Trichoderma* treatment showed the best results (0.68) and the lowest in treatment without antagonist (0.02). Fruits weight in treatment of *Trichoderma* showed best results (7.25 g), and the lowest in treatment without antagonist (0.06 g) (Table 4).

Interaction occurred only in number of fruit. The highest number of fruits was observed in combination of A2V4 (1.33). The highest number of flowers was observed in combination of A2V4 (2.00). The best fruit weight was observed in combination of A2V4 (11.41 g) (Table 2).

Pathogen treatment without antagonist caused the pathogen easily attacked the tomato plants and affected the number of fruits and fruits weight, resulting in the lower production. Application of *Gliocladium* and *Trichoderma* affected the number of flower, fruit and fruit weight.

Antagonist can decompose organic matters derived from rice husk, and in the decomposition process, either *Trichoderma* sp. or *Gliocladium* sp. change the element in the soulble form so it can be absorbed by the plant. Broto *et al*. (1994) suggested that rice husk used as treatment medium could provide many inorganic and organic components (cellulose, lignin, chitin, carbohydrate, N and lipid). The available carbohydrates and celluloses are utilized by the *Trichoderma sp*. and *Gliocladium* sp. as energy source and carbon sorce in the decomposition process. According to Suryawan *et al*. (2017) potassium element in rice husk that can increase plant resistance to disease and antagonist able to inhibit disease progression.

CONCLUSION

Study findings indicated that Servo variety is better than other tested varieties as indicated by the higher plant height, lower disease intesity, and higher number of fruits and fruits weight. *Trichoderma* and *Gliocladium* are capable of supressing the development of Fusarium wilt disease in tomato plants, but the effectiveness of *Trichoderma* (53.68%) is better in controlling pathogen compared to *Gliocladium* (48.89%). Interaction between treatment of Servo variety and fungi *Trichoderma* resulted in better effect in lower disease intensity, and higher number of fruits and fruits weight.

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