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Antagonist Test Of *Trichoderma harzianum* to *Fusarium oxysporum* as Causes Of Withering Disease In Plants Tomato (*Lycopersicum esculentum*) In Vitro

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Abstract

Fusarium oxysporum is a very harmful fungus because it can attack tomato plants from germination to maturity. Control of fusarium wilt disease, generally only limited to sanitation, biological control by using a biological control agent (APH) Trichoderma harzianum is an alternative to reduce the use of chemical pesticides in disease control. The experiment used a completely randomized design (CRD), with in vitro testing of Trichoderma harzianum against Fusarium oxysporum from stem, leaf, flower and fruit organs of tomato plants. Trichoderma harzianum in in vitro conditions was able to suppress the growth of Fusarium oxysporum which infects fruit by 100%, tomato flower organs 92,2%, tomato leaf organs 59,0% and from tomato plant stem organs 50,2%.

Keywords: T.harzianum, F.oxysporum, in vitro test

INTRODUCTION

Tomato (*Lycopersicum esculentum* Smith) is a vegetable which is widely cultivated in Indonesia commercially. Tomatoes are classed as highland vegetables, as good quality is only achieved at an altitude of 800 meters above sea level. In the highlands, the formation of good fruit and the withered attack of *fusarium* is reduced. However, tomatoes can be cultivated in lowlands.

The rate of tomato production per hectare in Sorong Regency of West Papua Province in 2019 amounted to 5.8 tons / ha. The result is lower when compared to the national production potential which reaches 10-16 tons / ha. One of the factors which affect the production and quality of tomatoes is the attack of pests and diseases. The disease which most affects tomato crops in tomato plantations is *fusarium* withered disease caused by *fusarium oxysporum* fungus and is one of the main diseases in tomato crops (Sorong District Agriculture and Food Crops Office, 2020).

Fusarium oxysporum is a very detrimental fungus because it can attack tomato plants from germination to adulthood. Although known as soil contagious pathogens, these fungal infections are not only in root but can also infect other organs such as stems, leaves, flowers, and fruit, for example through wounds. Transmission of diseases other than with spores found in the soil can also be with spores carried by wind and water. (Mulyaman *et al*. 2007). Jamur *Fusarium oxysporum* forms a polypeptide called litcomarasmin, a toxin that interferes with the permeability of plant plasma membranes. In addition, it also forms a simpler compound, *furic* acid, and produces pectic enzymes,

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especially *pectinmetilesterase* (PME) and *depolymerase* (DP). PME removes methyl in the pectin chain into pectic acid. Depolymerase breaks down the pectic acid chain into *polygalaconides* of varying molecular weights. These enzymes break down the pectin material present in the cell wall of xylem. Fragments of pectic acid enter the xylem vessels which then form a colloidal mass containing non-pectin material that can clog the vessels. The vascular file will become brown because the phenols that are released into the vascular file. These phenols by the enzyme *phenoloxide* produced by the host plant will be polymerized into brown melanin. This colored material is mainly absorbed by the *berlignin* xylem vessels that cause the distinctive brown color in *Fusarium* withered diseases (Gaumann and Jaag 1947 *in* Semangun, 2007).

Control of fusarium withered diseases in the field is generally performed by farmers in Sorong Regency only limited to sanitation because chemical control using synthetic fungicides costs a lot when compared to the economic value of tomatoes. On the other hand, the use of synthetic chemical compounds in control efforts carried out over a relatively long period of time, impacting environmental ecosystems, can kill natural enemies and cause pathogens to become resistant.

Biological control by utilizing biological controlling agents (APH) is an alternative to reducing the use of synthetic chemical pesticides. The use of APH is growing because it is able to limit the growth and development of OPT for a relatively long time and has the advantage in maintaining the balance of agricultural environmental ecosystems. *Trichoderma harzianum* is one of the potential fungi to be developed as APH in an effort to find alternatives to the use of synthetic chemical pesticides in plant disease control.

Trichoderma harzianum is a type of non-mycorrhizal fungus that can be found in almost all kinds of soil and in various habitats. *Trichoderma* grows very well and is abundant in the soil around healthy rooting and is beneficial by attacking pathogens that exist around plant rooting (Widyastuti et al. 2001). This mushroom also acts as a biodecommiser because it is able to utilize organic matter in nature, especially cellulose as a source of carbon and energy for its living needs (Winarsih and Syafrudin, 2001). *Trichoderma harzianum* fungus is known to have a high antagonistic ability in inhibiting the development of contagious soil pathogenic fungi. The antagonistic mechanism that occurs cannot be explained with certainty, but it is estimated that there are three phenomena that work synergistically, namely competition of growing space and nutrition, antibiosis mechanisms and hyphae system interactions (Nugroho et al, 2003).

RESEARCH METHODOLOGY

This experiment was organized in the Complete Random Design (RAL), which consisted of 5 treatments and was repeated 5 times with the treatment tested in the following:

IMT = Pure isolate *Trichoderma harzianum* (control)

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TFb = *Trichoderma harzianum* to *Fusarium oxysporum* from the stem organs of the tomato plant
TFd = *Trichoderma harzianum* to *Fusarium oxysporum* from the leaf organs of the tomato plant
TFbg = *Trichoderma harzianum* to *Fusarium oxysporum* from the flower organs of tomato plants
TFbu = *Trichoderma harzianum* to *Fusarium oxysporum* from the fruit organs of the tomato plant

1. *Isolated Trichoderma harzianum*

The isolated Trichoderma harzianum was obtained by taking soil samples ± 100 gr from five randomly determined points around the rooting of a healthy tomato plant at a depth of 0-20 cm. Examples of soil were then homogenized and made a dilution solution until the dilution series was 10-3. The results of each dilution of $^{10-1}$, 10-2 and 10-3 in a pipette of 1 ml were then poured into PDA media by *pour plate* method and incantated at room temperature 28 ° C for six days. Pure isolated *Trichoderma harzianum* was obtained by isolating a cut measuring 5x5 mm from the mycelium of *trichoderma harzianum* fungus on the PDA media of identification (hyphae fragmentation method) and then *incussed* at room temperature. Rejuvenation of the isolate was carried out when the isolate has met the petri dish (7 days).

2. *Fusarium oxysporum* Isolate

Tomato plants in the field which showed symptoms of *fusarium oxysporum* fungus were taken and put into a plastic canteen and taken to a laboratory for identification. To find out the cause of the disease caused by fungi was done by scraping the part of the plant that shows symptoms of disease with a needle and observed under a microscope. When the disease-causing fungus was *Fusarium oxysporum*, it was then isolated into PDA media.

Identification of plant organs which were not found fungal spores was finished by cutting the part of the plant showing the symptoms of disease measuring 1 cm then washed with water and rinsed with 70% alcohol. The piece was isolated into a petri dish based on moist filter paper and placed into incubation for five days and if there were hyphae in the plant pieces, it could be ascertained which the cause of the disease was mold. Fungal hyphae grown on PDA media and placed into incubation for five days was later identified to confirm the disease-causing fungus was *fusarium oxysporum* fungus. The next identified mushroom was propagated and rejuvenated by the hyphae fragmentation method (5x5 mm) of *fusarium oxysporum* fungus and grown in PDA media.

3. Antagonist Test

Antagonist test was carried out by a double test method named pieces of mycelium isolate *fungus Fusarium oxysporum* with the size including 5x5 mm origin of certain organs of tomato plants and pieces of mycelium isolate *Trichoderma harzianum*

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size 5x5 mm placed on PDA media with the distance between the two isolates 30 mm. Each treatment was repeated 5 times. As both positive and negative controls, pieces of pure mycelium isolate *Trichoderma harzianum* were grown on any PDA medium. Extensive observations of mycelium *Trichoderma harzianum* and *Fusarium oxysporum* were made from day 1 to day 7 after testing. The percentage of *T. harzianum* antagonistic ability to control *Fusarium oxysporum* from each tomato plant organ was calculated using the formula put forward by Pamekas et al (1997), as follows:

$$\% \text{ antagonist} = \frac{\text{area of } mycelium\ T.\ harzianum \text{ on the day of observation } (\text{mm}^2)}{\text{area of anatogist test room } (\text{mm}^2)} \text{ x } 100\%$$

Data Analysis

Data research results were analyzed by using variant analysis with the F (ANOVA) test. Interpretation of the data was provided in the way of further testing using the BNJ test at the level of 0.05% to match the influence between treatments.

RESULTS AND DISCUSSION

Results

Extensive area Mycelium *Trichoderma harzianum* in antagonistic test to *fusarium oxysporum*

The average area of mycelium of *trichoderma harzianum* fungus (mm^2) in petridis dishes in antagonistic tests against *Fusarium oxysporum* fungus until day 7 observation, the largest area of mycelium was found in *the trichoderma frarenum* antagonist test to *Fusarium oxysporum* isolated from the fruit organs of tomato plants (TFbu) (Table 1.).

Table 1. Different Test of Average area of miselium *Trichoderma harzianum* (mm^2) in antagonist test to *Fusarium oxysporum*. Observation days of 1-7.

Treatment	Observation results						
	1 HSI	2 HSI	3 HSI	4 HSI	5 HSI	6 HSI	7 HSI
TFb	1,140	1,240	1,340^{a}	1,520^{ab}	2,440^{bc}	4,020^{ab}	5,020^{b}
TFd	1,500	1,620	1,820^{a}	2,120^{a}	2,980^{ab}	4,420^{ab}	5,900^{b}
TFbg	2,060	2,160	2,760^{a}	5,280^{a}	6,980^{a}	8,340^{a}	9,220^{a}
TFbu	2,240	2,340	3,540^{a}	6,700^{a}	8,320^{a}	9,980^{a}	10,000^{a}
BNJ	-	-	3,106	5,095	4,165	4,538	2,952

Note: The average value of the same letter followed is no different from the BNJ test in the confident level of 0.05.

Based on the results (Table 1.), the area of mycelium in the *trichoderma harzianum* fungal antagonist test to *Fusarium oxysporum* from the observation time of 3-7 after the test showed the area of mycelium *T. harzianum* to *F. oxysporum* from the organs of tomatoes was no different from the organs of tomato flowers, on observation days 5 -7 area mycelium *T. harzianum* to *F. oxysporum* from the organs of tomatoes is different from the leaf organs and stems of tomato plants.

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The average area of *mycelium Trichoderma harzianum* under *in vitro* antagonist tests from days 1 to 7 on each antagonist test treatment varies. The average area of mycelium *T. harzianum* to *F. Oxysporum*, which was reduced until the observation of day 7, was found in the antagonist test on the tomato stem organ (5,020 mm2). The largest average area of mycelium *T. Harzianum* is against *F. oxysporum* in the fruit organ of tomatoes (10,000 mm2). The area of mycelium *T. harzianum* in the antagonist test room *in vitro*, showed that there is an inhibitory ability of the fungus against fungal pathogens of plant diseases. The inhibition is caused by the exposure to nutrients contained in the antagonistic test medium for its survival in the form of carbohydrates, proteins, essential amino acids, minerals and micro-elements such as phosphorus (P), magnesium (Mg) and Potassium (K), vitamin C (ascorbic acid), some B vitamins (thiamine, niacin, vitamin B6) and some antibiotic compounds produced by *T. harzianum* for inhibition of the development of F. fungus *oxysporum*. Soesanto (2008), stated that *Trichoderma harzianum* produces several antibiotics, including *peptaibolic* antibiotics that work synergistically with the enzyme *ß* (1,3) *glucanase*, a compound 3-(2-hydroxypropyl)-4-(2-hexadienil)-2(5H) furanon that helps the inhibition process against *Fusarium oxysporum* and alkyl pyroon compounds (6-n-pentil-2H-piran-2-on or 6PP) are fungicidal and able to change the spread of mushroom biomasa with a wide range. Free amino acids such as aspartic acid, glutamic acid, alanine, leusin and valine as well as two other positive ninhydrin compounds produced by *Trichoderma harzianum in vitro* can also decrease pathogenic fungal pathogenic pathogens.

Antagonis *Trichoderma harzianum* against *Fusarium oxysporum* is thought to have involved the mechanism of mycoparasitism. *Trichoderma harzianum* begins to form hyphae branches that grow towards *Fusarium oxysporum* at the growth stage of chemotrophs. The recognition stage is special, for instance, antagonistic mushrooms only attack certain pathogens. According to Soesanto (2008), the growth of *trichoderma harzianum* mycelium towards pathogenic mycelium is stimulated by the presence of α proteins that bind to chitins that bind to the chitin constituents of the pathogenic cell wall. Furthermore, at the attachment stage, hifa mushroom *Trichoderma harzianum* can grow along the host hyphae or smelt around the host hyphae. The penetration rate formed from *trichoderma harzianum* hyphae will perforate the cell wall or break down the cell wall of pathogens that produce enzymes in the entangling cell wall.

The Percentage of Antagonists *in vitro Trichoderma harzianum* to *Fusarium oxysporum*

In *vitro* antagonism *of Trichoderma harzianum* to *Fusarium oxysporum* isolated from various tissues of tomato plants, resulting in a percentage difference of antagonism. Observations and calculations of the average percentage of antagonism *of Trichoderma harzianum* in *in vitro* antagonistic tests to *Fusarium oxysporum* are presented in Figure 1.

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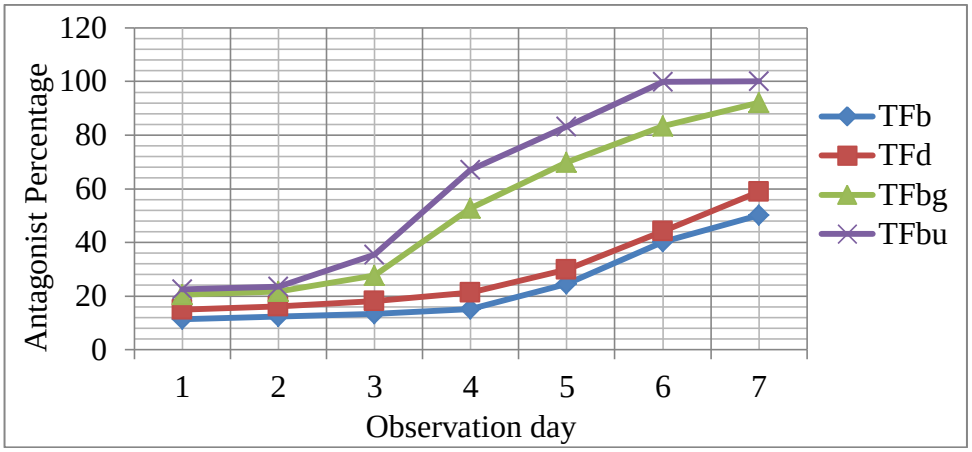

Figure 1. Percentage of antagonis *Trichoderma harzianum* against *Fusarium oxysporum* from various organs of tomato plants

The Percentage of *frazianum Trichoderma* antagonists against *Fusarium oxysporum* isolated from various organs of tomato plants in in-vitro testing time up to 7 days of testing, the highest percentage to *Fusarium oxysporum* fungus isolated from fruit organs (100%) and percentage of antagonists from other tomato plant organs are 92.2% of the flower organs, 59% of the leaf organs and 50.2% of the stem organs.

Day 6 of the *trichoderma harzianum* antagonist test to *Fusarium oxysporum*, the highest percentage of antagonists was found in *Fusarium oxysporum* isolated from tomato plant tissue (99.8%), and the percentage of *frazianum Trichoderma* antagonists against *Fusarium oxysporum* from tomato plant flowers by (83, 2015). 4%), from the tomato plant leaf network (44.2%) and from the stem tissue of the tomato plant (40.2%).

Discussion

Exploration of *fusarium oxysporum* mushrooms from tomato plants is conducted at the time of flowering and fruiting plants. The results of exploration, found several organs from tomato plants that show symptoms of fusarium wither disease, namely stem organs, leaves, bunga and fruit. This exploration is based on the Mulyaman's statement et al (2007), *fusarium oxysporum* mushrooms can attack tomato plants ranging from germination to adulthood. Although known as a soil contagious pathogen, the mushroom can also infect other parts of the plant. It is because the transmission of diseases in addition to spores contained in the soil can also be with spores carried by wind and water then infect plants through wounds.

The results of the observations and calculations of the average area of *trichoderma harzianum* mycelium in *in vitro* antagonist test ranging from day 1 to day 7 can be for each antagonist test treatment varies. The average area of mycelium *Trichoderma harzianum* is lowest until day 7 of the antagonistic test against *Fusarium oxysporum* from the stem organ (5020 mm^2). Uji antagonists to *Fusarium oxysporum* from leaf organs by 5900 mm^2 and *Fusarium oxysporum* from flowers by 9220 mm^2.

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The average area of mycelium *Trichoderma harzianum* was highest in antagonistic tests to *Fusarium oxysporum* found in the mother organ (for example, 10000 mm^2).

The area of miselium *Trichoderma harzianum* in antagonistic tests to *Fusarium oxysporum* has not filled the test room (petri dish) on the last day of observation, this term is thought to be due to competition of growing space and nutrients. The competition occurs when there are two or more microorganisms that directly require the same nutrients. The competition seen in the antagonistic test room between *Trichoderma harzianum* and *Fusarium oxysporum* is due to the need of these mushrooms to the nutrients contained in the antagonistic test medium for its survival. Imas and Setiadi (1987), stated that the competition of microorganisms in a space or place is due to the need for nutrients in the form of carbohydrates, proteins, essential amino acids, minerals and micro-elements such as phosphorus (P), magnesium (Mg) and Potassium (K), vitamin C (ascorbic acid), some B vitamins (thiamine, niacin, vitamin B6). Carbohydrates and sugars have a role as sources of carbon to produce energy and also for the biosynthesis of carbon compounds. Carbohydrates are remodeled into certain organic acids and carbon dioxide. This reshuffle involves extracellular enzymes that are bound to the cell wall and only a few soil organisms can perform the remodel, one of which is *Trichoderma harzianum*. Soesanto (2008), sugars and carbohydrates are utilized by *Trichoderma harzianum* as a source of carbon that has a role as a precursor of secondary metabolites to inhibit germination of pathogenic mushroom spores.

The area of mishapum *Trichoderma harzianum* tends to be greater than the area of mycelium *Fusarium oxysporum* in the results of antagonistic tests, this is thought to be due to the ability of *Trichoderma harzianum* to produce certain organic acids that cannot be utilized *fusarium oxysporum* and the ability of the *Trichoderma harzianum* to produce secondary metabolites in the form of antibiotics that inhibit the germination of fusarium *oxysporum* mushroom spores. The results of this test are in accordance with Soesanto's opinion (2008), that *Trichoderma harzianum* produces several antibiotics, including peptaibol antibiotics that work synergistically with the enzyme *ß* (1,3) *glukanase*, a compound 3-(2-hydroxypropyl)-4-(2-hexadienil)-2(5H) furanon that helps the process of inhibition against *Fusarium. oxysporum* and pyron alkyl compounds (6-n-pentil-2H-piran-2-on or 6PP) are fungicidal and are able to change the spread of mushroom biomesa in a wide range. Free amino acids such as aspartic acid, glutamic acid, alanine, leusin and valine as well as two other positive ninhydrin compounds produced by *Trichoderma harzianum in vitro* can also decrease pathogenic fungal pathogenic pathogens.

Trichoderma harzianum antagonist test results to *Fusarium oxysporum*, resulting in the lowest antagonistic percentage up to day 7, which is 50.2% in tomato plants. It is claimed to be due to the high ability of competition and the growth of mycelium from the pure isolate *Fusarium oxysporum* derived from the fruit. Based on the results of extensive observations of daily mycelium until day 7, the growth of *fusarium*

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oxysporum isolates that are isolated from tomatoes produces an area of mycelium that is best when compared to the area of myelium isolated from other tomato plant organs.

The higher competition ability of *Fusarium oxysporum* isolated from tomatoes is thought to be supported by the biotic environmental factors from which these mushroom isolates originated. According to Freeman *et al.* (2009), mycelium mushrooms grow very quickly according to the number of organic molecules they absorb from their growing medium. Organic molecules are used as a source of nutrients including for the formation of hyphae cell walls. The optimal absorbing of organic molecules by *Fusarium oxysporum* that infects fruit organs will also optimize the formation of the mushroom hyphae cell wall, for example the formation of a cell wall that is as compact (tight) and thick.

The solid and thick cell wall of hyphae is thought to be the defense of *fusarium oxysporum* mushrooms when faced with attacks from *Trichoderma harzianum. Trichoderma harzianum* takes longer to kill its host in the process of mycoparasitism as well as in the process of antibiosis.

CONCLUSION

Trichoderma harzianum under *in vitro* conditions is able to suppress the growth of *Fusarium oxysporum* which infects tomato plants with different antagonistic percentages, where the inhibition of *Fusarium oxysporum* isolated from fruit tissue by 100%. The inhibition ability of other plant organs in a row is from the tomato flower organ by 92.2%, the tomato leaf organ by 59.0% and from the stem organ of the tomato plant by 50.2%.

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