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Jl. STKIP - Kel. Naram, Singkawang
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+62562 4200584 journal@stkipsingkawang.ac.id

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TABLE OF CONTENTS

ARTICLES

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The Effect of Mushrooms *Trichoderma* sp. and Its Secondary Metabolites on Suppression of *Fusarium* sp. and Growth of Tomato Plants (*Solanum lycopersicum* mill.)

Agus Suyanto[1,*)], Agnes Tutik Purwani Irianti[2], Hamdani[3], Ismail Astar[4], Dwi Nurteto[5]
Universitas Panca Bhakti Pontianak, Pontianak, Indonesia
agussuyanto@upb.ac.id[1], agnestutik@upb.ac.id[2], hamdani@upb.ac.id[3], ismailastar@upb.ac.id[4], dwinurtertoo@gmail.com[5]
[*)]Corresponding author

Keywords:
Trichoderma sp., *Fusarium* sp., Tomato, Secondary Metabolites

ABSTRACT
This study aims to determine the interaction effect of the type and dose of Trichoderma sp. and its secondary metabolites on suppression of Fusarium sp. wilt disease and its effect on the growth of tomato plants (Solanum lycopersicum mill.). This study used a Randomized Block Design (RBD) which consisted of two factors. The first factor is the type of application (J) which consists of three levels of treatment, namely: J1 = Trichoderma sp., J2 = Secondary metabolites. J3 = Trichoderma sp. + Secondary metabolites, while the second factor is the application dose (D) which consists of three treatment levels, namely: D1 = 10 ml/plant, D2 = 20 ml/plant, D3 = 30 ml/plant. The treatment was repeated 3 times to obtain 81 tomato plants and added 9 control treatment plants. The parameters observed were the percentage of wilt disease, plant height, stem diameter, number of leaflets, and number of branches. The results showed that in laboratory testing, the mushrooms Trichoderma sp. and secondary metabolites can inhibit the growth of the mushrooms Fusarium sp. In research in the field, treatment with Trichoderma sp., secondary metabolites, and Trichoderma sp. + secondary metabolites, with various spore densities and applied 7 days before planting can reduce the wilting percentage to 0%. The interaction treatment of type and application dose had a very significant effect on the number of leaflets, and had a significant effect on stem diameter but had no significant effect on plant height and number of branches. The highest results on the variable plant height and number of leaflets were found in the J3D3 treatment with a height of 82.67 cm and a number of leaflets of 85.11 strands. The highest results on the stem diameter variable were found in the J1D3 treatment with a diameter of 6.59 mm, while the variable number of branches was found in the J3D1 treatment with 1.35 branches.

International Journal of Multi Discipline Science (IJ-MDS)
Vol. 4 No. 2 (2021)
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INTRODUCTION

Cultivation of tomato plants has major obstacles, especially the presence of disease. The disease that causes a lot of harm and attacks tomato plants is wilt disease caused by the fungal pathogen *Fusarium* sp. *Fusarium* wilt disease is one of the limiting factors for tomato production because it causes damage and death to tomato plants, so it can be a threat to tomato farmers. Tomato production can be reduced by up to 30% and in the rainy season, it can reach 60% due to *Fusarium* wilt disease (Purwati, 2009). *Fusarium* wilt disease on tomato plants has been reported to cause great losses in East Java with an attack rate of 23% (Bustaman, 1997).

Efforts to control *Fusarium* wilt disease that have been carried out include the use of synthetic/inorganic chemical fungicides. Inorganic chemical pesticides have proven to be very effective in controlling plant pests and pathogens, but the use of inorganic chemical pesticides that are not controlled and carried out continuously results in environmental pollution, resistance, and the destruction of several beneficial organisms (Adriansyah, Hamawi, & Ikhwan, 2015). Biopesticides are an alternative to reduce the use of synthetic chemical pesticides. Natural pesticides and biological agents are still considered safe for the environment and able to cope with plant pathogen attacks (Adriansyah, Hamawi, & Ikhwan, 2015). Disease control using biopesticides still has problems because there is still a lack of knowledge about appropriate and effective application doses in controlling plant pathogens.

Trichoderma sp. is a type of fungus that can be found on forest soil and agricultural land. As a Biological Control Agent, *Trichoderma* sp. is quite effective against several types of pathogens including *Fusarium, Sclerotium, Phytium, Phytophtora, Armilaria, Colletroticum, Rigidoporus,* and *Rhizoctonia* (BPTP Central Java, 2012). Previous research has also shown *Trichoderma* sp. can effectively inhibit and prevent Fusarium wilt disease in tomato plants (Esrita, Ichwan, & Irianto, 2011; Ambar, 2013; Hardianti, Rahayu, & Asri, 2014; Antara, Rosmini, & Panggeso, 2015; Sopialena, 2015; Ghufron, Nurcahyanti, & Wahyuni, 2017).

Trichoderma sp. potential as an antagonist for plant pathogens, because it produces antibiotics, quickly masters space and nutrients as well as parasites. *Trichoderma* sp. has the potential to produce secondary metabolites that are antibiotics, namely Viridin and Trichomidin (Adriansyah, Hamawi, & Ikhwan, 2015). Viridine and Trichomidin can inhibit growth or even kill other fungi. Secondary metabolites of *Trichoderma* sp. as a source of important compounds for the development of antimicrobial compounds in carrying out sustainable agriculture.

This research is an initial study in the development of biopesticides with active ingredients from secondary metabolites *Trichoderma* sp. and has the aim of knowing the effect of the application interaction type and dose of *Trichoderma* sp. and its secondary metabolites on suppression of *Fusarium* wilt disease and its effect on the growth of tomato plants.

METHOD

Research design

This research was conducted from May to July 2017, at the Balai Proteksi Tanaman Perkebunan (BPTP) Pontianak and the Green House of the Faculty of Agriculture, Panca Bhakti University, Pontianak. This study used a Randomized Block Design (RBD) pattern consisting of two factors. The first factor was the type of application (J) which consists of three levels of treatment, namely:
J1 = *Trichoderma* sp.
J2 = Secondary metabolite
J3 = *Trichoderma* sp. + Secondary metabolites
The second factor was the application dose (D) which consists of three levels of treatment, namely:
D1 = 10 ml/plant

D2 = 20 ml/plant
D3 = 30 ml/plant
From these two factors, nine treatment combinations were obtained as follows: J1D1, J1D2, J1D3, J2D1, J2D2, J2D3, J3D1, J3D2, J3D3. The nine treatment combinations were each repeated three times and each replication consisted of 3 plants. Then the number of plants 3 x 3 x 3 x 3 = 81 plants.
As a comparison, one more treatment was made, namely Control without *Trichoderma* sp. and secondary metabolites, but infected with the fungus *Fusarium* sp. consisting of 9 plants. So the total plants are: 81 + 9 = 90 plants. The conidia density of the pathogenic fungus *Fusarium* sp. is equal to 1 x 10^6 /ml. Conidia density of *Trichoderma* sp. that is equal to 1 x 10^6 /ml. The parameters observed were the percentage of wilt disease, plant height, stem diameter, number of leaflets, and number of branches.

1. Percentage of Wilting Disease

Observation of the percentage of wilt disease caused by *Fusarium* sp. at the research site, conducted every 7 days. Observations started from week 1 after planting until week 4 when the plants flowered, and the percentage of wilt attacks from each experiment was determined by calculating the number of wilted plants divided by the number of plants used. The formula used is as follows (Antara, Rosmini, & Panggeso, 2015):

$$PS = \frac{n}{N} \text{ x } 100\%$$

Description:
PS = Attack percentage (%)
n = Amount infected plants
N = Amount plants used

2. Plant Height (cm)

Measurement of plant height was carried out by measuring tomato plants from the base of the stem to the highest growing point using a ruler. Measurements were made after the plant began to flower or in the generative phase.

3. Stem Diameter (mm)

Measurement of stem diameter was carried out when the plant was 40 DAP or when the plant began to flower (generative phase) using a caliper.

4. Number of Leaflets (pieces)

Counting the number of leaflets was carried out in the generative phase or when the plant was 40 DAP and the leaves counted were leaflets that had been completely formed.

5. Number of Branches (branches)

Counting the number of branches was done when the plant was 40 DAP or when the plant began to flower (generative phase), the primary branch was counted.

The data obtained were tested with the *F* test and further tested using the 5% Honest Significant Difference (HSD) test.

Preparation of Inoculum

1. Making PDA (Potato Dextrose Agar)

The manufacture of PDA media was carried out by mixing 19.5 grams of the manufacturer's PDA powder in 500 ml of distilled water. Mixing was done by cooking a solution of distilled water that has been mixed with the manufacturer's PDA on the stove while stirring continuously until the PDA powder dissolves and the solution boils. After mixing, the PDA was sterilized using an autoclave together with the Petri dish to be used, the sterilization time was ± 30 minutes. After being sterilized, the PDA was poured into the Petri dish and left for 10 minutes until the PDA hardened.

2. Making SPE (Sugar Potato Extract)
To make a 500 ml SPE, it took 100 grams of potatoes, 10 grams of dextrose, and 510 ml of sterile water (aquades). To make an SPE, first, the potatoes were washed, then peeled and cut into cubes. After that, the potatoes were boiled using 500 ml of sterile water (aquades) for ± 30 minutes until the potatoes were soft. Once soft, then filtered and only the extract was taken. Then boiled again the potato extract earlier and added Dekstros. Because of the process of boiling potatoes for ± 30 minutes, a lot of water evaporates. Furthermore, the potato extract was added with distilled water up to 500 ml. After that, the sugar potato extract was put into a sterile Erlenmeyer, then covered with cotton and aluminum foil. Furthermore, the Sugar Potato Extract (SPE) was sterilized using an Autoclave.

3. Preparation of *Fusarium* sp.
Fusarium sp. fungal isolate was isolated from tomato plants showing *Fusarium* wilt symptoms. Specimens obtained from the field, the stems were cleaned with 70% alcohol, thinly sliced, and then isolated on PDA media and incubated at room temperature. After growing, identification was carried out under a microscope. When you had obtained *Fusarium* sp., purification was carried out by taking the tip of the fungus using an ose needle, then isolating it on new sterile PDA media. It was carried out in an aseptic isolation box and then incubated. Purification was carried out 2-3 times until pure isolates were obtained.

Furthermore, the Koch Postulate test was carried out by infecting the pathogen *Fusarium* sp. that obtained from tomato plants that were attacked by *Fusarium* wilt toward healthy tomato plants and the tomato plants should cause the same symptoms as the initial plants that were attacked by *Fusarium* sp. then the infected tomato plant was isolated and there must be the fungus *Fusarium* sp. that grows.

4. Provision of *Trichoderma* sp. and secondary metabolites
Fungus *Trichoderma* sp. used was *Trichoderma harzianum* isolate of Bengkayang which was propagated by the BPTP Pontianak. In the first treatment, using fungus *Trichoderma* sp., the method of providing it was by growing *Trichoderma* sp. in rice media for 7 days, after which it was dissolved in sterile water (aquades). The second treatment, using secondary metabolites. How to provide it was by growing the fungus *Trichoderma* sp. in SPE media and shaking at 180 rpm for 7 days for *Trichoderma* sp. secrete secondary metabolites. After 7 days, the SPE fluid was centrifuged to separate the secondary metabolites from the conidia and other carriers. In the third treatment, *Trichoderma* sp. + secondary metabolites, the method of preparation was the same as the second treatment, but the difference was that the SPE fluid does not need to be centrifuged.

Planting Preparation
1. Seeding seeds
The seeds selected were Servo varieties. The media was used for the nursery is a mixture of alluvial soil and chicken manure in a ratio (1:1). Tomato seeds were soaked for 15 minutes in water to remove dormancy. Then sown in the nursery. Maintenance was carried out during the nursery by watering 1-2 times a day.

2. Grow media setup
The growing medium was used for tomato plants is alluvial soil that is clean of grass and plant roots. The alluvial soil media was air-dried for about a week and then sifted. Then put in a polybag with a size of 30 x 40 cm as much as 8 kg.

3. Dolomite and basic fertilizer
Liming is done because alluvial soil has a low soil pH. Liming was carried out 21 days before planting using dolomite lime 7.7 g/polybag. Lime was given by spreading it evenly, then the planting media was mixed with basic fertilizer, namely chicken manure (80 g/polybag) and NPK (1.2 g/polybag).

4. Giving *Fusarium* sp.
Inoculation of pathogenic fungi was carried out by watering *Fusarium* sp. in the planting hole with a density of 1×10^6 conidia/ml and given as much as 20 ml per planting hole and applied 14 days before planting. The number of conidia was calculated using a Haemocytometer under a microscope.

5. Giving of secondary metabolites and *Trichoderma* sp.
Biofungicide inoculation was carried out by pouring bio fungicide in the planting hole according to the treatment and applied 7 days before planting. 1 ml of *Trichoderma* sp. contains 1 x 10^6 conidia.

6. Transfer of seeds or planting
Transfer of seedlings was done after the seedlings are 21 days old in the nursery. Planting was done in the afternoon to avoid the heat of the sun which can cause the seedlings to wither.

7. Marker
Plant marker was carried out with the intention that the plants do not collapse when blown by the wind or other disturbances. The marker was carried out after the plants were 4-5 days old after being planted in polybags, using bamboo which was split into several parts, which were ± 100 cm long.

8. Plant maintenance
Maintenance carried out was weed control and watering. Weed control was done so that weeds did not interfere with the growth of tomato plants. Watering tomato plants was done so that the growing medium remains moist and the plants did not lack water.

RESULTS AND DISCUSSION

Koch's Postulates Test
In this study, Koch's Postulate test was used to ensure that the fungal pathogen *Fusarium* sp. obtained is actually a pathogen that causes wilt disease in tomato plants. Koch's Postulate technique includes four stages, namely association, isolation, inoculation, and re-isolation. Koch's Postulate test can be seen in Fig. 1.

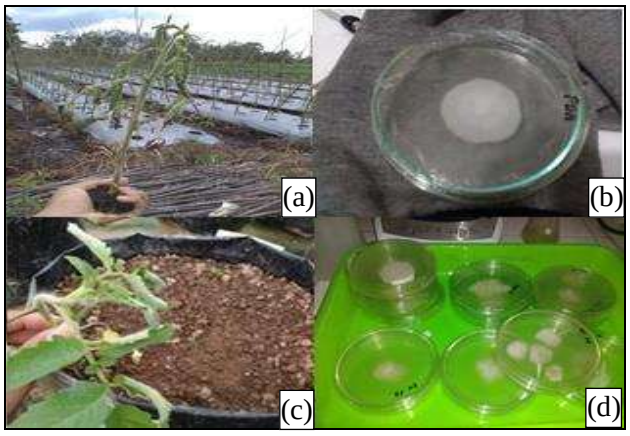

Fig. 1 *Koch's Postulate Test: (a) Association, (b) Isolation, (c) Inoculation, (d) Re-isolation*

From the results of Koch's Postulate test carried out, it proved that the fungus *Fusarium* sp. The results obtained are actually pathogenic fungi that cause wilt disease in tomato plants.

***Trichoderma* sp. Antagonist Test toward *Fusarium* sp.**
In the antagonist test stage, the fungus *Trichoderma* sp. and the fungus *Fusarium* sp. grown on PDA media in the same Petri dish. It is hoped that the fungus *Trichoderma* sp. can inhibit the fungus *Fusarium* sp. The results of the antagonist test showed that the *Trichoderma* sp. can inhibit the growth of the fungus *Fusarium* sp. The results of the antagonist test can be seen in Fig. 2.

International Journal of Multi Discipline Science (IJ-MDS)
Vol. 4 No. 2 (2021)
e-ISSN: 2615-1707. Page: 62-76

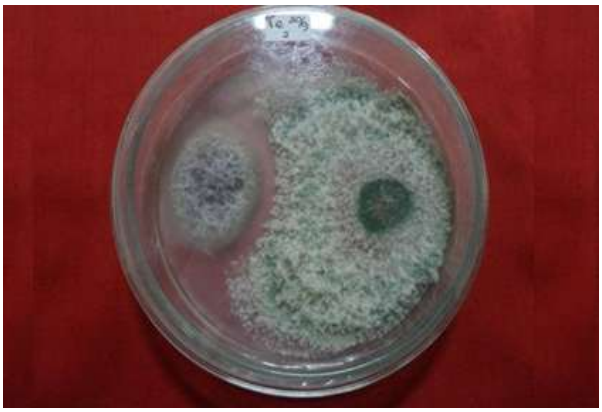

Fig. 2 *Antagonist Test of Trichoderma sp. with the Pathogen Fusarium sp.*

The antagonistic process occurred due to the competition that occurs between two types of fungi that are grown side by side. This competition results in the same needs from each fungus, namely the need for a place to grow and the nutrient media used to grow plants (Ara et al., 2012; Dwiastuti, Fajri, & Yunimar, 2015). At first, the hyphae of the fungus *Trichoderma* sp. met and wrapped around the hyphae of the fungus *Fusarium* sp., then the fungus *Trichoderma* sp. secrete a clear liquid in the form of secondary metabolites that are toxic to the fungus *Fusarium* sp., so that the fungus *Fusarium* sp. stunted growth and even die. Hyphae intervention by *Trichoderma* sp. results in changes in chemical elements and particles in the cell wall so that it can affect the permeability of the pathogen cell wall (Nohemi, Jose, & Alfredo, 2012). Antagonistic hyphae that successfully intervene and penetrate will absorb food essence so that the hyphae of pathogenic fungi can shrink and die (Purwantisari & Rini, 2009).

Trichoderma is one type of microbe that can inhibit the growth of pathogens by producing biologically active compounds in vitro. The active compounds include alkaloids, paxillin, lolitrems, and steroid tetranone (Sudhanta & Abdul, 2011). *Trichoderma* sp. controls the pathogen *Fusarium* sp. through space and nutrient competition, mycoparasitism, and antibiosis as a result of the release of antibiotics or chemical compounds, producing metabolites inhibiting pathogenic spores, and direct contact (Benitez et al., 2004; Dwiastuti, Fajri, & Yunimar, 2015; Istikorini, 2002).

Inhibition Test of Secondary Metabolites of *Trichoderma* sp.

This test was carried out through media poisoning (PDA). It was hoped that the pathogenic fungus *Fusarium* sp. cannot grow on the poisoned medium. In testing the inhibitory power of the secondary metabolites of *Trichoderma* sp., it was found that the secondary metabolites could inhibit the growth of the fungus *Fusarium* sp. The results can be seen in Fig. 3.

In PDA media that had been given secondary metabolites from *Trichoderma* sp. fungus, *Fusarium* sp. did not grow, whereas in media that did not use secondary metabolites, the fungus *Fusarium* sp. grows. This proved that the secondary metabolites produced by the fungus *Trichoderma* sp, are effective in controlling the pathogen *Fusarium* sp.

Trichoderma sp. has the potential to produce secondary metabolites that are antibiotics, namely Viridine and Trichomidin. Viridine and trichomidin can inhibit growth or even kill other fungi. Viridine and trichomidin can produce chitinase enzymes, where these enzymes can degrade chitin (Adriansyah, Hamawi, & Ikhwan, 2015), where secondary metabolites in the form of toxins for pathogenic fungi, can directly suppress the growth of pathogenic fungi *Fusarium* sp. In addition, Lone, Mohd., and Subzar (2012) stated that *Trichoderma* sp. produce antibiotic compounds trichodermin, trichidermol, harzianolide, and enzymes such as glucanase and chitinase that can destroy the cell walls of pathogenic mold hyphae by degrading polysaccharides and chitin in the cell walls.

International Journal of Multi Discipline Science (IJ-MDS)
Vol. 4 No. 2 (2021)
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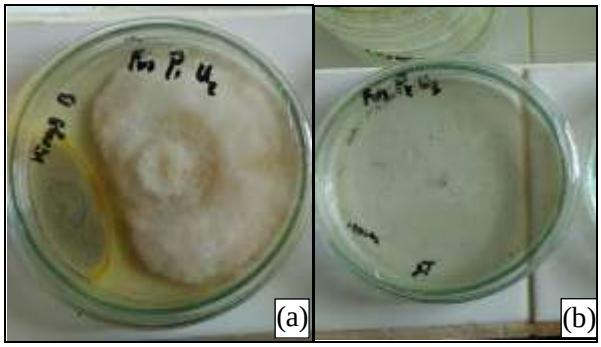

Fig. 3 *Inhibitory Test of Secondary Metabolites of Trichoderma sp. with the Pathogen Fusarium sp.: (a) Without Secondary Metabolites, (b) Using Secondary Metabolites*

Field Observation

1. Attack Percentage (%)

Observation of the attack percentage variable was carried out once every 7 days. The first observation (7th day), from all treatments, types, and doses were observed, there was no wilt disease caused by the pathogenic fungus *Fusarium* sp., while in control plants there were two plants that showed symptoms of *Fusarium* wilt.

The control plants first showed wilting symptoms on the lower leaves then spread to the top, then black spots appeared on the stems, then the plants withered and eventually died. Tomato plants that died after splitting the stems showed black spots in the bundle area, which were then taken to the laboratory for isolation. After being isolated and grown on PDA media, *Fusarium* sp. The growth can be seen in Fig. 4. This proved that the tomato plants died due to the pathogenic fungus *Fusarium* sp.

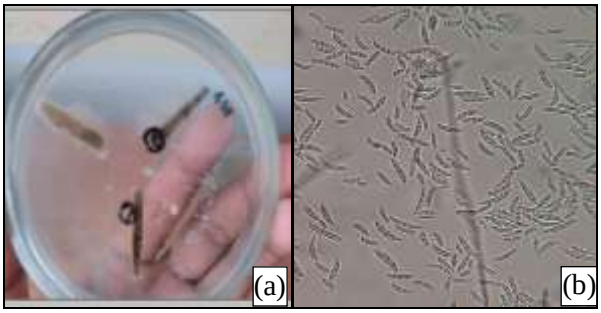

Fig. 4 *(a) Fungi Fusarium sp. Isolated from Control Plant, (b) Conidia Fusarium sp.*

In the second, third, and fourth observations, all treatment plants did not show any symptoms of *Fusarium* wilt, but in the third week, some leaves showed symptoms of chlorosis. Symptoms of chlorosis on the leaves were seen in treatment J1D1 with the highest chlorosis level of 20% and the lowest level of chlorosis was found in treatment J1D2 which was 11%, J2D1 was 8.12% and J2D2 was 4.12%, while the other treatments did not show symptoms of chlorosis.

Observation of the percentage of attacks on treatment with *Trichoderma* sp., secondary metabolites, and *Trichoderma* sp. + secondary metabolites, with various spore densities and applied 7 days before planting can reduce the wilting percentage to 0%. This proved that the fungus *Trichoderma* sp., secondary metabolites, and *Trichoderma* sp. + secondary metabolites were very effective in controlling the fungal pathogen *Fusarium* sp. The results of this study were in line with the research results of Hardianti, Rahayu, and Asri (2014) which stated that giving *T. harzianum* at 7 days before planting could reduce the wilting percentage to 0%.

Giving *Trichoderma* sp., secondary metabolites, and *Trichoderma* sp. + secondary metabolites in the growing media given 7 days before planting are thought to inhibit or even kill the fungus *Fusarium* sp. which is in the ground. Therefore, laboratory testing was carried out for each treatment, to prove that the fungus *Fusarium* sp. is no longer in the ground.

First, soil samples were taken from polybags for each treatment, then brought to the laboratory for identification. The identification results showed that there were several colonies of *Trichoderma* sp. that growth seen was in the treatment of J1 (*Trichoderma* sp.) and J3 (*Trichoderma* sp. + secondary metabolites). Meanwhile, in treatment J2 (secondary metabolites) no *Trichoderma* sp. grows. The results of the soil sample test can be seen in Fig. 5.

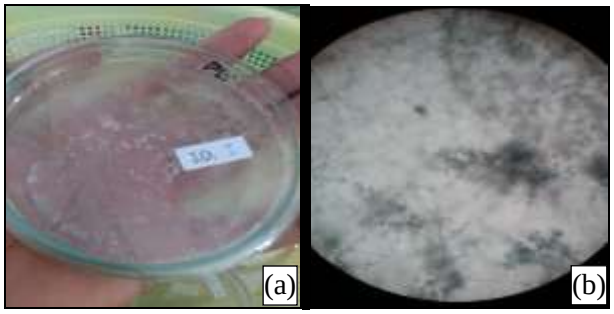

Fig. 5 *(a) Trichoderma sp. Isolated from the Treated Soil Sample, (b) Trichoderma sp.*

From all soil samples taken, no *Fusarium* sp. growing on the ground. So it could be said that the fungus *Fusarium* sp. was no longer present in the soil because it had been controlled by the fungus *Trichoderma* sp. and secondary metabolites.

Differences in inhibiting properties between the three types of applications used also affect the process of inhibiting the fungal pathogen *Fusarium* sp. In treatment J1 (*Trichoderma* sp.), the process was inhibited through nutritional competition, mycoparasitism, and antibiosis as a result of the release of antibiotics or chemical compounds (Istikorini, 2002). *Trichoderma* sp. must grow and develop first to inhibit and control the fungal pathogen *Fusarium* sp.

In treatment J2 (secondary metabolite), the process was inhibited by directly poisoning the pathogen *Fusarium* sp. with chemical compounds such as enzymes and toxins. Secondary metabolites are antibiotics, namely Viridin and Trichomidin (Adriansyah, Hamawi, & Ikhwan, 2015). While in treatment J3 (*Trichoderma* sp. + secondary metabolites), the inhibiting process is a combination of the two. Secondary metabolites can directly inhibit the fungal pathogen *Fusarium* sp. and the fungus *Trichoderma* sp. grow and develop so that it can serve as a guard toward pathogen attack in the future.

Field observations on the J1D1 treatment showed that there were still symptoms of chlorosis on the leaves. Symptoms of chlorosis are probably due to the attack of the pathogen *Fusarium* sp. which has not been fully controlled by the fungus *Trichoderma* sp., because the fungus *Trichoderma* sp. given was still too little, namely 10 ml or half of the recommended dose. *Trichoderma* sp. can also be influenced by environmental factors that can affect its growth, so it takes time for the fungus *Trichoderma* sp. to breed and also need time to control the fungal pathogen *Fusarium* sp.

Plant Height (cm)

Measurement of plant height was carried out at the end of the study or when the plant began to flower (generative phase) using a ruler. Plant height was measured from the base of the stem (root neck) to the highest growing point. The data from the analysis of diversity can be seen in Table 1.

International Journal of Multi Discipline Science (IJ-MDS)
Vol. 4 No. 2 (2021)
e-ISSN: 2615-1707. Page: 62-76

Table 1
Effect of Application Type and Dosage on Tomato Plant Height

Source of Diversity	DB	JK	KT	F count	F table	
					5%	1%
Group	2	568.40	284.20	10.62**	3.63	6.23
Treatment	8	1249.59	156.20	5.84**	2.59	3.89
J Treatment	2	294.19	147.09	5.50*	3.63	6.23
D Treatment	2	642.13	321.07	12.00**	3.63	6.23
J x D Treatment	4	313.27	78.32	2.93^{tn}	3.01	4.77
Error	16	428.17	26.76			
Total	**26**	**2246.16**		**KK = 7.11%**		

Description: (tn) = No effect; (*) = Have a real impact; (**) = Very real impact

Based on the results of the analysis of diversity on tomato plant height, it showed that the interaction treatment of type and application dose had no significant effect on tomato plant height. The type of treatment showed a significant effect on plant height. While the dose treatment showed a very significant effect on plant height. To find out each difference in the level of treatment for the type of application, an Honest Significant Difference (HSD) test was carried out at a 5% confidence level that can be seen in Table 2.

Table 2
HSD Test of Effect of Application Type Treatment on Tomato Plant Height (cm)

Treatment	Average	Different
J1	69.63	a
J2	71.30	ab
J3	77.31	b
HSD 5% = 6.29		

Note: Numbers followed by the same letter in a column are not significantly different at the 5% HSD test level

Based on the results of the HSD in Table 2, it showed that the highest mean of tomato plant height was found in the treatment type application J3 (*Trichoderma* sp. + secondary metabolites) which was 77.31 cm and significantly different from treatment J1 (*Trichoderma* sp.) which was 69.63 cm, but not significantly different from treatment J2 (secondary metabolite) ie 71,30 cm. To find out each difference in the level of application dose treatment, an HSD test was carried out at a 5% confidence level that can be seen in Table 3.

Table 3
HSD Test of Effect of Application Dosage Treatment on Tomato Plant Height (cm)

Treatment	Average	Different
D1	66.04	a
D2	74.72	b
D3	77.48	bc
HSD 5% = 6.29		

Note: Numbers followed by the same letter in a column are not significantly different at the 5% HSD test level

Based on the results of the HSD in Table 3, it showed that the highest mean of tomato plant height was found in the application dose treatment of D3 (30 ml) which was 77.48 cm and significantly different from D1 (10 ml) which was 66.04 cm, but it was not significantly different from treatment D2 (20 ml) which was 74.72 cm. In species treatment, the highest mean of tomato plant height was found in treatment J3 (*Trichoderma* sp. + secondary metabolites), but it was not significantly different from treatment J2 (secondary metabolites). When viewed from the type, treatment J3 had two types of control, the first is *Trichoderma* sp. control the pathogen *Fusarium* sp. through nutritional competition, mycoparasitism, and antibiosis as a result of the release of antibiotics or chemical

compounds (Istikorini, 2002). The second control method was secondary metabolites, where the secondary metabolites were antibiotics, namely Viridin and Trichomidin. Viridine and Trichomidin can produce chitinase enzymes, where these enzymes can degrade chitin which makes up cell walls. In treatment J2 (secondary metabolites) only had one type of control, namely secondary metabolites in the form of enzymes and toxins, so that they can directly control the pathogenic fungus *Fusarium* sp., so that the growth of tomato plant height is not disturbed.

In treatment J3 was significantly different from treatment J1 (*Trichoderma* sp.), where treatment J1 only had one type of control, namely using *Trichoderma* sp. Trichoderma sp. controls the pathogen *Fusarium* sp. through nutritional competition, mycoparasitism, and antibiosis as a result of the release of antibiotics or chemical compounds (Istikorini, 2002). But the fungus *Trichoderma* sp. must grow first to can control the fungal pathogen *Fusarium* sp. so that the growth of tomato plants is not disturbed.

In the dose treatment, the highest mean of tomato plant height was found in the D3 treatment, which was 30 ml, where the dose was related to the number of conidia given, which was 3 x 10^7. In the D1 treatment (10 ml) it was significantly different from the D2 and D3 treatments, where the D1 treatment amounted to conidia is 1 x 10^7, the dose is too low or too little to control the fungal pathogen *Fusarium* sp. which is in the ground. So that the control has not been maximized and the growth of tomato plant height is still disturbed. Besides being able to be used as a biocontrol against the attack of the pathogen *Fusarium* sp., *Trichoderma* sp. can also act as a biological fertilizer that can increase plant growth, increase the absorption of active minerals, and other nutrients from the soil (Antara, Rosmini, & Panggeso, 2015; Cornejo et al., 2009; Ghufron, Nurcahyanti, & Wahyuni, 2017). *Trichoderma* sp. is also able to decompose organic compounds, degrade organic compounds and increase the availability of nutrients in the soil (Lehar, 2012; Esrita, Ichwan, & Irianto, 2011). Research conducted by Murdiono (2015) found that Trichoderma sp. which was inoculated 1 week before planting stimulated the reshuffle of coarse materials such as plant residues from land cultivation to provide N nutrients that can encourage the formation of fruit and seeds in soybean plants. Adequate plant nutrients will support plant growth. This is because Trichoderma sp. is one of the fungi that can decompose soil organic matter such as N, P, K, and other nutrients that are compounded with Al, Fe, and Mn so that they can be used for plant growth (Hardianti, Rahayu, & Asri, 2014). degrade organic compounds and increase the availability of nutrients in the soil (Lehar, 2012; Esrita, Ichwan, & Irianto, 2011). Research conducted by Murdiono (2015) found that *Trichoderma* sp. which was inoculated 1 week before planting stimulated the reshuffle of coarse materials such as plant residues from land cultivation to provide N nutrients that can encourage the formation of fruit and seeds in soybean plants. Adequate plant nutrients will support plant growth. This is because *Trichoderma* sp. is one of the fungi that can decompose soil organic matter such as N, P, K, and other nutrients that are compounded with Al, Fe, and Mn so that they can be used for plant growth (Hardianti, Rahayu, & Asri, 2014).

Stem Diameter (mm)

Measurement of stem diameter was carried out at the end of the study or when the plant began to flower (generative phase) using a caliper. Analysis of the diversity of stem diameter measurements can be seen in Table 4.

Based on the results of the analysis of diversity on the average stem diameter of tomato plants, it was shown that the interaction between the type and dose of application had a significant effect on the stem diameter of tomato plants. For the type and dose treatment, each indicated that the treatment had no significant effect on the stem diameter of tomato plants. To find out the difference in the level of treatment of this interaction, the HSD test was carried out at a 5% confidence level that can be seen in Table 5.

International Journal of Multi Discipline Science (IJ-MDS)
Vol. 4 No. 2 (2021)
e-ISSN: 2615-1707. Page: 62-76

Table 4
Effects of Treatment Types and Application Doses on Tomato Plant Stem Diameter

Source of Diversity	DB	JK	KT	F Count	F table	
					5%	1%
Group	2	1.50	0.75	3.26^{tn}	3.63	6.23
Treatment	8	5.33	0.67	2.89*	2.59	3.89
J Treatment	2	0.11	0.06	0.24^{tn}	3.63	6.23
D Treatment	2	1.20	0.60	2.61^{tn}	3.63	6.23
J x D Treatment	4	4.02	1.00	4.35*	3.01	4.77
Error	16	3.69	0.23			
Total	**26**	**10.53**		**KK = 7.79%**		

Description: (tn) = No effect; (*) = Have a real impact

Table 5
HSD Test of Effect of Interaction of Type and Application Dosage on Tomato Plant Stem Diameter (mm)

Type	Dose		
	D1	**D2**	**D3**
J1	5.12 a (a)	6.53 a (b)	6.59 a (b)
J2	6.14 ab (a)	6.08 a (a)	6.48 a (a)
J3	6.52 b (a)	5.81 a (a)	6.25 a (a)
HSD 5% = 1.40			

Notes: Letters in brackets are read horizontally, lowercase letters without brackets are read vertically, numbers followed by the same letter are not significantly different according to the HSD Test at the 5% level

From the results of the HSD test in Table 5, it showed that the highest average stem diameter was found in the J1D3 treatment of 6.59 mm and significantly different from the J1D1 treatment, but not significantly different from the J1D2, J2D3, and J3D3 treatments. According to Semangun (2007), the fungus *Fusarium oxysforum* infects through the roots, especially through wounds. *Fusarium oxysforum* fungus can also infect roots that do not have wounds, especially at the tips of the roots. The fungus develops briefly in the parenchyma tissue, then settles and develops in the bundle of vessels.

It was known that *Trichoderma* sp. can also function as decomposing organisms. It was likely that it can act as a supplier of macro and micronutrients for plants, which in turn can affect the growth of tomato plant stem diameters to be greater. According to Hersanti et al. (2009), the fungus *Trichoderma* sp., apart from being able to be used as a biocontrol against the attack of the *Fusarium oxysporum* pathogen, can also act as a biological fertilizer known as "Plant Growth Promoting Fungi".

Number of Leaflets (pieces)
Measurement of the number of leaves was carried out at the end of the study or when the plant began to flower (generative phase) by counting all the leaflets. Analysis of the diversity of the measurement data for the number of leaves can be seen in Table 6.

The results of the analysis of diversity in Table 6 showed that the interaction of type and dose of application has a very significant effect on the number of leaflets. The type and dose of each treatment also had a very significant effect on the number of leaflets. To find out the difference in the level of interaction treatment, an HSD test was carried out at a 5% confidence level and the results can be seen in Table 7.

International Journal of Multi Discipline Science (IJ-MDS)
Vol. 4 No. 2 (2021)
e-ISSN: 2615-1707. Page: 62-76

Table 6
Effect of Interaction of Type and Application Dosage on Number of Leaflets of Tomato Plants

Source of Diversity	DB	JK	KT	F Count	F table	
					5%	1%
Group	2	1076.65	538.32	15.97**	3.63	6.23
Treatment	8	1754.78	219.35	6.51**	2.59	3.89
J Treatment	2	481.09	240.55	7.14**	3.63	6.23
D Treatment	2	523.29	261.65	7.76**	3.63	6.23
J x D Treatment	4	750.39	187.60	5.57**	3.01	4.77
Error	16	539,20	33.70			
Total	**26**	**3370.63**		**KK = 7.43%**		

Description: (**) = Very significant effect

Table 7
HSD Test of Effect of Interaction of Type and Application Dosage on the Number of Tomato Leaves (strands)

Type	Dose		
	D1	**D2**	**D3**
J1	56.89 a (a)	80.89 a (b)	80.67 a (b)
J2	74.22 b (a)	81.33 a (b)	79.33 a (a)
J3	84.56 c (a)	79.78 a (a)	85.11 a (a)
HSD 5% = 7.06			

Notes: Letters in brackets are read horizontally, lowercase letters without brackets are read vertically, numbers followed by the same letter are not significantly different according to the HSD Test at the 5% level

From the results of the HSD test in Table 7, it showed that the lowest number of leaves was in the J1D1 treatment with a total of 56.89 strands and significantly different from J2D1, J3D1, J1D2, and J1D3. While the highest number of leaves was found in the J3D3 treatment with a total of 85.11 strands and was not significantly different from the other treatments.

The J3D3 treatment (*Trichoderma* sp. + secondary metabolites with a dose of 30 ml) resulted in the highest mean number of leaves, which was 85.11 leaves. While the lowest number of leaves was produced by treatment J1D1 (*Trichoderma* sp. with a dose of 10 ml) with the lowest mean number of leaves being 56.89 leaves.

Nutrients are one of the factors that affect leaf development and it is also known that the growth of a plant is indirectly related to its nutrient requirements. *Trichoderma* sp. besides being able to be used as a biocontrol for plant growth, can also be used as an organism that decomposes organic matter (Lakitan, 1993). According to Antara, Rosmini, and Panggeso (2015), increasing the dose of *Trichoderma* sp. caused a decrease in the percentage of leaves affected by *Fusarium* wilt disease. The decrease in the percentage of leaves attacked by *Fusarium oxysporum* will affect the results of the photosynthesis process in the leaves, increasing the number of leaves.

Number of Branches (branches)
Measurement of the number of branches was carried out at the end of the study or when the plant began to flower (generative phase) by counting the number of branches formed. Analysis of the diversity of the measurement of the number of branches can be seen in Table 8.

International Journal of Multi Discipline Science (IJ-MDS)
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e-ISSN: 2615-1707. Page: 62-76

Table 8
Effects of Giving Types and Dosages of Applications on the Number of Branches of Tomato Plants

Source of Diversity	DB	JK	KT	F Count	F table	
					5%	1%
Group	2	0.05	0.03	1.22^{tn}	3.63	6.23
Treatment	8	0.32	0.04	1.94^{tn}	2.59	3.89
J Treatment	2	0.07	0.04	1.75^{tn}	3.63	6.23
D Treatment	2	0.04	0.02	0.86^{tn}	3.63	6.23
J x D Treatment	4	0.21	0.05	2.57^{tn}	3.01	4.77
Error	16	0.33	0.02			
Total	**26**	**0.71**		**KK = 12.04%**		

Description: (tn) = No effect

Based on the results of the analysis of diversity on the number of branches of tomato plants, the type and dose treatment showed that the treatment had no significant effect. In the interaction treatment, the type and dose of application had no significant effect on the number of branches of tomato plants. Furthermore, to see the average number of branches produced by each treatment interaction can be seen in Fig. 6.

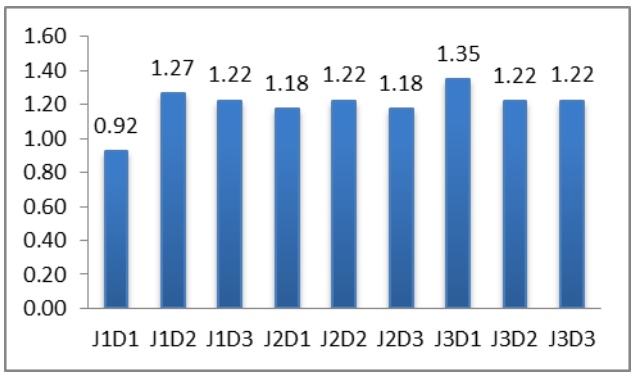

Fig. 6 *Effect of Application Type and Dosage on Number of Tomato Branches*

Based on Fig. 6, it showed that the J3D1 treatment (*Trichoderma* sp. + secondary metabolites with a dose of 10 ml) produced the highest average number of branches, namely 1.35 branches. While the lowest number of branches was produced by treatment J1D1 (*Trichoderma* sp. with a dose of 10 ml) with the lowest average number of branches, namely 0.92 branches, but treatment J1D1 was not significantly different from treatment J3D1 and to all treatments.

The type and dose of application and their interactions did not significantly affect the number of branches. This is because observations were only made to the stage of tomato plant growth (vegetative) and not to production (generative), while tomato plants will continue to grow and produce branches along with the generative phase. Tomato plants have many branches, tomato plant branches will continue to grow even though the plants have fruited so that the overall tomato plant is in the form of a shrub (Rismunandar, 2001).

CONCLUSIONS

Based on the results of research that had been done that the fungus *Trichoderma* sp. and its secondary metabolites can inhibit growth suppress the percentage of fungal attack *Fusarium* sp. up to 0%. In addition, the highest results on the variables measuring plant height and number of leaves were found in the J3D3 treatment with a height of 82.67 cm and a number of leaves of 85.11 strands. The highest results on the stem diameter variable were found in the J1D3 treatment with a diameter of 6.59 mm, while the variable number of branches was found in the J3D1 treatment with 1.35 branches.

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