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Antagonistic activity of *Trichoderma* spp. Against *Fusarium oxysporum* dan *Fusarium solani* Causes Yellow Disease in Pepper (*Piper Nigrum* Linn)

Resti Provilia[1], Rahmad Lingga[1*], Tri Wahyuni[2]

[1][Department of Biology, Universitas Bangka Belitung, Indonesia]; hanifhamzahtri@gmail.com
[2][Plant Protection Agency, Province of Bangka Belitung, Indonesia]; puterianeirra@gmail.com

Abstract.[Bangka Belitung is a pepper producing area known as the Muntok White Pepper. One of the main challenge in the cultivation of pepper is attackingof pathogens. The decrease in pepper is caused by yellow disease due to the attack of the fungus *Fusarium solani* and *Fusarium oxysporum*. The control of yellow disease is carried out using biological agent such as*Trichoderma* spp. This study aimed to obtain local isolates of *Trichoderma* spp. from the pepper garden in CengkongAbang Village as a biological agent and to measure the inhibition of *Trichoderma* spp. on the growth of *Fusarium oxysporum*and*Fusarium solani* in vitro. The research methods used were the isolation, identification and antagonism of *Trichoderma* spp. against *Fusarium oxysporum*and *Fusarium solani*. The result of isolation soil samples from the healthy pepper rhizosphere and yellow diseased pepper obtained 74 isolates of *Trichoderma* spp. The identification of 74 isolates obtained 7 types of *Trichoderma* spp. encoded THA, THB, THC, THD, THE, THF, THG. Fungal antagonist test of *Trichoderma* spp. against *Fusarium oxysporum* produced the highest percentage of inhibition in THB2Mb and THB2La isolates of 7.13% and 7.1%, while the highest percentage of inhibition from *Trichoderma* sp. against *Fusarium solani* was obtained in THD1Ib and TSD2Mb isolates at 7.33% and 7.23%.]

Keyword:[*Fusarium oxysporum, Fusarium solani*, Pepper, *Trichoderma* spp, Yellow disease]

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1 Introduction

Pepper is an important spice in Indonesian society and is known as the king of spices. The main pepper producing areas in Indonesia are Lampung, Bangka, West Kalimantan, East Kalimantan, Bengkulu, Southeast Sulawesi, and South Sulawesi [1]. Indonesia is a member of the International Pepper Community (IPC) world society. The productivity of Indonesian pepper is still below 1000 kg/ha, while other countries are already more than 2000 kg/ha [2]. The world

*Corresponding author at: Department of Biology, Universitas Bangka Belitung, Indonesia
E-mail address: [hanifhamzahtri@gmail.com]

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demand for pepper is increasing due to the high consumption of pepper in the traditional European market. Pepper is widely used as a spice in cooking and has developed in various industrial needs such as the food and cosmetic industry [3]. Bangka Belitung Islands Province is one of the pepper producing areas, known as Bangka white pepper (Muntok White Pepper). Around 82% of the people of CengkongAbang Village work as farmers who rely heavily on the pepper plantation sector. CengkongAbang village is also classified as a line of pepper production sector known as Muntok white pepper. Therefore, CengkongAbang Village was chosen as a sampling place according to predetermined criteria. Pepper varieties that are widely cultivated in CengkongAbang Village are petaling 1 and MerapinDaun Kecil varieties [4].

In 2016, the decrease of production of Indonesian white pepper from Bangka Belitung reached 29-38% [5]. According to the Agricultural Statistics Agency (2018) pepper production decreased in 2017-2018 from 34,278.1 tons to 32,811.1 tons [6]. The condition caused by the decrease of area of the acreage and the disturbance of pests and diseases of pepper. Pepper diseases that are considered very detrimental are stem base blight caused by *Phytoptoracapsici*, and yellow disease by *Fusarium solani* and *Fusarium oxysporum*. Yellow disease is reported to be found in the Bangka and West Kalimantan areas. According to Munif&Sulistiawati (2014) the percentage of yellow disease in CengkongAbang Village reached 28%, from 33.33% of the average incidence of yellow disease in Bangka regency [7].

The knowledge of Pepper Farmers in Bangka Belitung were low inyellow disease control in pepper. However, some farmers use a chemical pesticides to control yellow disease in pepper. The continuous use of pesticides can have a negative impact on the environment and human health. Chemical pesticides can leave harmful residues on the resulting pepper products, especially for export purposes that are very concerned about health and environmental aspects [8]. Thus, it is necessary to apply a new approach to disease control in environmentally sound crops such as the use of biological agents. *Trichoderma* fungus is an antagonistic fungus that has the potential to be a biological agent. The condition for an organism to be said as a biological agent is has the ability of antagonism in inhibiting the growth of other organisms [9]. Based on the results of research in Bangka Belitung, bamboo rhizosphere *Trichoderma* isolates limited the growth of pathogens that cause yellow disease in pepper [10]. One of the results of the study revealed that with the application of the fungus *Trichoderma* sp. it turns out that it can control yellow disease in pepper in petalingbangka village [8].Based on this, this study was carried out to determine the ability of *Trichoderma* spp. in inhibiting the growth of *Fusarium* spp. fungus, as well as obtaining *Trichoderma* spp. isolates which have the potential to be biological agents controlling *Fusarium* spp. fungi.

2. Research Methodology

This research was conducted from August 2019 to March 2020. The research was conducted at the UPTD Plant Protection Center of CengkongAbang Village, West Mendo District, Bangka Regency. The criteria for pepper garden used as research studies were pepper plants that were at least 1.5 years old and the land area ± 0.5-1 ha.

Sampling

Sampling was carried out at 2 plantation locations of CengkongAbang Village Farmers. Soil sampling was carried out through the diagonal random sampling method from Kurniasari[11]. This method was carried out by making 5 sampling plots in a pepper field measuring 10x10 m as shown in (Figure 1). Soil samples were taken as many as 2 samples from the rhizosphere of healthy pepper plants and the rhizosphere of sick pepper at a depth of 5- 10 cm which was carried out purposely. The next stage of each sampling plot was measured abiotic factors. The measured parameters were soil temperature, soil pH and soil moisture.

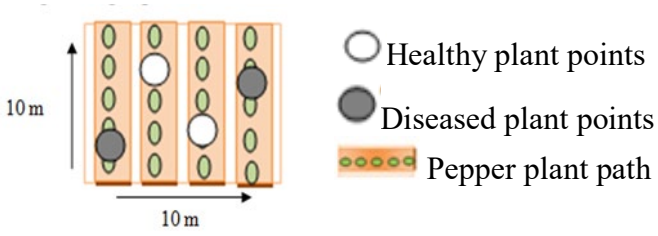


Figure 1. Plot Sampling

Isolation of Trichoderma.

Isolation of *Trichoderma* spp. was carried out using the method of radiant dilution. The soil sample was weighed by 1 gram, then diluted in a test tube containing 9 ml of sterile aquades to 10-3. The dilution sample was then homogenized, then taken as much as 1 ml, then poured into a petri dish. The treatment was carried out as many as 3 times and the results of inoculation were incubated for 7 days at room temperature.

Purification of Trichoderma.

The purification technique is carried out by sorting out the growing parts of the colony one by one. Colonies that are Trichoderma are cut to a size of 1x1 cm, then inoculated on sterile PDA media. The treatment was carried out as much as 3 repeats and isolates were incubated for 7 days at room temperature.

Identification Trichoderma spp.

Identification of Trichoderma spp. is carried out through observations macroscopically and microscopically. The observed macroscopic parameters are the shape and color of the

colony. The microscopic parameters observed are hyphae, conidiophore, conidia and fialid forms. Microscopic observations are carried out by making direct preparations and slide cultures that are observed using a microscope. Identification was carried out based on identification books by Watanabe (2002) and Kubicek et al (1998) [12; 13].

***Propagation of isolates of Trichoderma* spp.**

The propagation technique was carried out by taking 1 ose conidia, then inoculated on a new sterile PDA medium. The treatment was carried out as much as 3 repeats, and incubated for 7 days at room temperature

Trichoderma spp. Antagonist Test against Fusarium solani and Fusarium oxysporum

The antagonist test of the fungus *Fusarium* against *Trichoderma* spp. was carried out using the double culture method. Each isolate of*Fusarium* and *Trichoderma* spp. was placed face to face with a distance between isolates of 3 cm as (Figure 2). Isolates were incubated for 7 days at room temperature. Observations were made for 7 days, by measuring the radius of the *Fusarium* colony that grew towards *Trichoderma* spp. and the radius of the *Fusarium* colony towards the edge of the cup as a control.

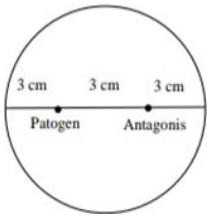


Figure 2. Double culture on petri dish

The antagonist test was carried out using a 2-factorial Complete Randomized Design (RAL) which was carried out as many as 3 replays. Here is an example of RAL with the double culture method, Pathogens: *Fusarium oxysporumand Fusarium solani* are coded (Fa, Fb). Biological agents: *Trichoderma* spp. isolates of the rhizosphere of healthy pepper are coded (TH), *Trichoderma* spp. isolates of the rhizosphere of sick pepper are coded (TS). Antagonistic test treatment was carried out on each isolate of *Trichoderma* spp. and *Fusarium* of different types.

Data Analysis

Data analysis was carried out by calculating the percentage of inhibitory activity of antagonistic fungi against pathogenic fungi using the following formula:

$$PA = \frac{d1 - d2}{d1} \times 100\%$$

Source: Cikita *et al* 2016

Note:

PA = Antagonist Percentage (%)

d1 = Radius of *Fusarium* colony towards the edge of the cup (cm)

d2 = Radius of *Fusarium* colony towards Colony *Trichoderma* spp. (cm)

Trichoderma spp. isolates of antagonistic test results from the rhizosphere of healthy and diseased pepper were then analyzed using a differential test (F test) using the SPSS 16.0 program.

3. Result and Discussion

Trichoderma spp. *isolates*

The results of the exploration of *Trichoderma* fungi at 2 garden sites from the rhizosphere of healthy pepper plants and yellow disease pepper plants were 74 isolates (Table 1). The number of isolates obtained from garden site 1 were 35 isolates of *Trichoderma*, including 28 isolates from the rhizosphere of healthy pepper plants, while 7 isolates were the the isolates from rhizosphere of the yellow-diseased pepper plant. From the location of the garden 2, 39 isolates of *Trichoderma* were obtained, including 28 isolates from the rhizosphere of healthy pepper plants, while 11 were the isolates of rhizospheres of yellow diseased pepper plants.

Table 1 *Trichoderma*isolated from Cengkong Abang Village Farmer's Garden

Locations	**Plots**	***Trichoderma* spp isolates.**		**Numbers**
		healthy plants	Yellow diseased plants	
Garden 1	1	5	2	35 Isolates
	2	6	2	
	3	5	0	
	4	6	2	
	5	6	1	
Garden 2	1	6	1	39 Isolates
	2	6	1	
	3	6	2	
	4	5	3	
	5	5	4	
Numbers		**56**	**18**	**74 isolates**

Based on the data on (Table 1) it can be concluded that *Trichoderma* was mostly found in the rhizosphere of healthy pepper plants compared to the rhizosphere of yellow disease pepper plants. This was because healthy plants have a good plant resistance response in suppressing the attack of disease-causing pathogens. Defense reactions can come from plant cells that come into contact with pathogens by sending signals to plants to respond (induced resistance) or systemic resistance through hormonal activity throughout the plant.In diseased plants the root system has suffered a damage from infection by parasitic nematodes and pathogenic fungi of yellow disease. Pathogenic fungi will form colonization of the root rhizosphere area, so that the physiological resistance of the plant is low. Factors that cause plants to be susceptible to disease are low soil fertility, low soil moisture or low soil moisture content [14]. These conditions causes the nematode population to grow, so that the level of

damage due to nematode infection is even greater which triggers the entry of pathogenic bacteria and fungi that cause yellow disease through the roots of plants [15].

Isolates of *Trichoderma* sp. from the rhizosphere healthy pepper was thought to be endophytic. This was supported by the research of Kusumawardani *et al* (2015) that *Trichoderma* exists as an endophytic fungus in pepper plants[16]. *Trichoderma* endophytes are parasitic against other pathogenic fungi, which can grow covering the entire surface of the medium including pathogens. According to Munif & Kristina (2012) the population of endophytic microbes in healthy plants is higher than that of diseased plants[15]. Endophytic microbes, both fungi and bacteria, serve to suppress the growth of disease-causing pathogens [15]. Less effective cultivation methods such as over-fertilization, improper use of pesticides, timing, concentration, type of pesticides, and the way they are applied, can affect the density of endophytic microbial populations in the roots. Therefore, in diseased plants that are often given pesticides in excess can cause some microbes to die, both disease-causing microbes, saprophytic microbes, both endophytic and antagonistic (Harni *et al* 2007 referred to in Munif & Kristina (2012)[15].

Abiotic Data

The presence and growth of the fungus *Trichoderma* in the field can be influenced by biotic and abiotic factors. The results of measuring abiotic data from 2 locations of pepper plantations of farmers in Cengkong Abang Village presented in Table 2.

Table 2 Abiotic Data Measurement Results

Location	Abiotic	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Garden 1	Soil temperature (°C)	26	26,5	26	27	26,5
	Soil moisture (%)	32,4	33,4	32,6	35	35
	Soil pH	6,9	6,9	7	6,7	7
Garden 2	Soil temperature (°C)	27	25	29	28	28
	Soil moisture (%)	57	45	36	40	40
	Soil pH	7	7	6,9	6,6	6,7

Based on the data on (Table 2) the results of measuring the average abiotic factor could support the growth of *Trichoderma*. *Trichoderma* can grow at a temperature of 7-41 °C, in media cultures it can grow at a temperature of 25-30 °C, does not grow at a temperature of 35 °C. *Trichoderma* grows optimally on moist soils, on dry soils the population of *Trichoderma* will decrease. *Trichoderma* spp. will form chlamydospores to survive in dry, nutrient-poor environments and develop again if environmental circumstances are already favorable [11].At the site of the garden 1 in plots 1 and 3 the soil moisture obtained is lower compared to other plots. According to Kurniasari et al (2019) stated that *Fusarium* is able to live at optimum

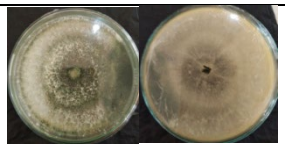
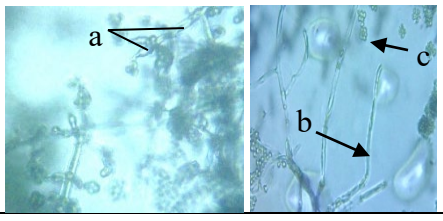
temperatures of 31 °C to 37 °C, suitable on acidic soils between pH 4.5-6, and can survive on dry soils compared to moist soils[11]. Another factor that causes the low presence of *Trichoderma* in the soil is influenced by the type of soil and the level of soil fertility. Mixed sandy soils with lepung or light soils can favor the growth of nematodes. This soil type has large soil pores, air and water in the soil are sufficient, so nematodes can move freely and can move from one plant to another [15].

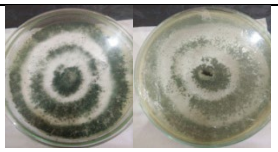
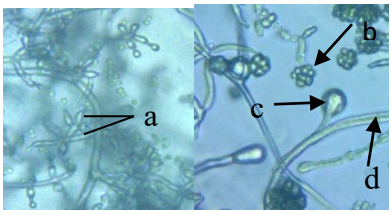
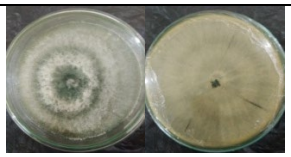
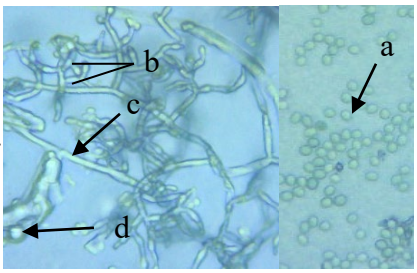
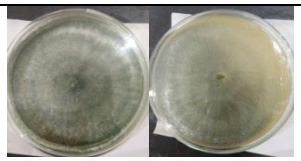
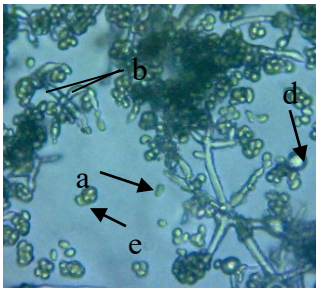
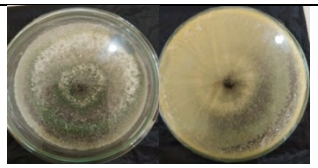
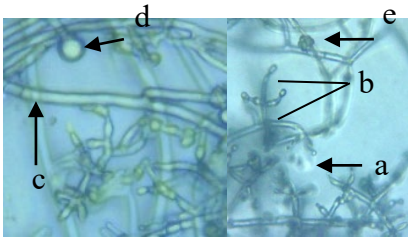
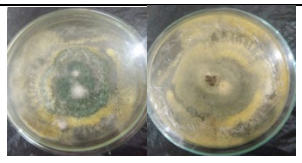
The results of observations in the study showed that the location of garden 2 had a poor carepractise. The factor was characterized by the tightness of growing weeds. This phenomenon can adversely affect the growth rate of pepper, so it is susceptible to disease. This was supported by the results of isolation, that *Trichoderma* isolates from the rhizosphere of yellow diseased pepper plants are more prevalent at site 2. According to Munif & Kristiana (2012) the population of *Meloidogyne* spp. in diseased plants is higher compared to healthy plants[15].This is because parasitic nematodes can multiply better in the roots of diseased plants, because plants that have less food substances will encourage nematodes to develop compared to plants that provide optimal food.

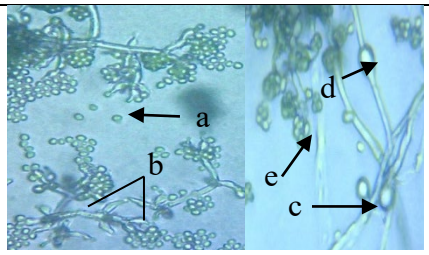
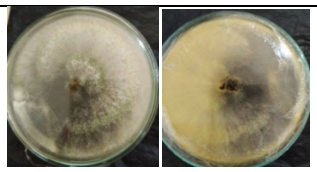
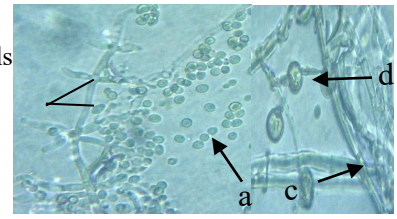
Trichoderma spp. Identity

The results of the identification of *Trichoderma* spp. Based on macroscopic and microscopic characters obtained 7 types of *Trichoderma* from 74 isolates. The identification technique was carried out by observing the macroscopic features of the 74 *Trichoderma* isolates obtained. Isolates that macroscopically have the same features are grouped in the same 1 type. Some types of isolates that have the same features macroscopically are observed microscopically in morphology. The isolate requirement to be identified is to produce the growth of *Trichoderma* colonies with a regular colony shape and color in petri dishes up to 7 days of age. Identification of *Trichoderma* from soil samples at 2 pepper garden sites resulted in the same type of *Trichoderma*, but the number of *Trichoderma* isolates produced in each sample was different. The following are the identification results of the *Trichoderma* isolates, listed in (Table 3):

Table 3.*Trichoderma* spp. Isolates Identification Results

Code	Macroscopic and Microscopic Features	Figure	Species Similarities
THA1 TSA1 THA TSA2	The shape of the colony is round, the color of the colony is green- dark green. The back view is slightly yellowish brown. Fialids are short, thick and dense, hyphae hyaline, berseptat, hyaline conidia, oval, branched conidiophores, chlamydospores are pale brown, intercalar sometimes subterminal location, oblong shape, oval. The distinctive feature has elongated hyphae, tapering towards the apex, the berseptat is called setae. Information: (a) phialid and conidiophore; (b) *setae* and septate; (c) conidium.	 	*Trichoderma hamatum*

THB1 THB2 TSB1 TSB2	The form of colonies is concentric, the color of the colony is yellow- dark green. The back view is dull green. Fialids are short, thick, hyaline hyphae, insulated, conidia are round, oval, light green color, branched conidiophores, hyaline-colored chlamyospores, intercropellaal location sometimes terminal, spherical shape. Information : (a) phialid and conidiophore; (b) conidium; (c) chlamidospore; (d) hyphae.	 	*Trichoderma harzianum*
THC1 THC2 TSC1 TSC2	The shape of the colony is round, concentric, the color of the colony is green- dark green. The rear view is gray, slightly yellowish. Fialids are short, tapered, curved at the ends, hyaline hyphae, berseptat, oval conidia, dark green color, branched conidiophores, hyaline-colored chlamydospores, intercalar location, round shape, oval. Information: (a) conidia;(b) phialid and conidiophore;(c) septate hiphae; (d) chlamidospore.	 	*Trichoderma atroviridae*
THD1 THD2 TSD1 TSD2	The shape of the colony is round, densely fibrous sheaves, the color of the colony is toska-green green. The back view is green. Fialids are short, hyaline hyphae, berseptat, oval conidia, green color, branched conidiophores, pale brown chlamydospores, intercalar location, rounded shape. Information: (a) conidia; (b) phialid and conidiophore;(d) chlamidospore; (e) conidium.	 	*Trichoderma viridae*
THE1 THE2	The shape of the colony is round, concentric, the color of the colony is pale yellow- green. The back view is slightly yellowish brown. Fialids are long, slender, tapered, at the ends, hyphae hyaline, berseptat, oval conidia, hyaline color, branched conidiophores, pale brown chlamydospores, terminal location sometimes subterminal, oval shape. Information: (a) conidia; (b) phialid and conidiophore;(c) septate hyphae; (d) chlamidospore; (e) conidium.	 	*Trichoderma koningii*
THF1 THF2 TSF1	The shape of the colony is rounded, there are also concentric, the color of the colony is yellow – dark green. The back view is slightly yellowish in color with green patches. Fialids are short, thick, hyaline hyphae, berseptat, oval conidia, green		*Trichoderma aureoviridae*

TSF2	color, branched conidiophores, pale brown chlamydospores, intercallar location, oval shape. A characteristic feature of the spore period is found in each fialid. Information : (a) conidia; (b) phialid and conidiophore;(c) septate hyphae; (d) chlamidospore; (e) conidium.		
THG1	The shape of the colony is round, concentric, the color of the colony is light green. The rear view is slightly yellowish-white. Fialids are slightly long, tapered at the ends, hypaline hyphae, berseptat, oval conidia, green color, branched conidiophores, pale brown chlamydospores, intercalar location, rounded shape. Chlamydospores have smooth walls and there is a circle on the inside. Information: (a) conidia; (b) phialid and conidiophore;(c) septate hyphae ; (d) chlamidospore.	 	*Trichoderma pseudokoningii*

Information: TH (*Trichoderma* from the rhizosphere of a healthy pepper plant); TS (*Trichoderma* of the rhizosphere of the diseased pepper plant); types of *Trichoderma* spp. (A: *Trichoderma* sp.1); (B: *Trichoderma* sp.2); (C: *Trichoderma* sp.3); (D: *Trichoderma* sp.4); (E: *Trichoderma* sp.5); (F: *Trichoderma* sp.6); (G: *Trichoderma* sp.7); (1= Garden 1); (2= Garden 2).

The following are the macroscopic and microscopic characteristics of the 7 types of *Trichoderma* spp. based on the literature, including:

a. *Trichoderma* sp.1 (THA1, THA2, TSA1, TSA2), has similar features to the species *Trichoderma hamatum*. The results of observations supported by Watanabe (2002) stated that the colonies in the PDA were green[12]. A distinctive feature of this species has short, thick, densely arranged fialids, distinctive features of the species setae-like hyphae of curved shape, tapering towards the apex, gilded. Conidia are hyaline, ellipsoidal or ovate. Pale brown, subglobose or ellipsoidal chlamydospores.
b. *Trichoderma* sp. 2 (THB1, THB2, TSB1, TSB2), have a characteristic resemblance to the species *Trichoderma harzianum*. The results of observations supported by Watanabe (2002) and Kubicek *et al* (1998) stated that the colonies were yellow- dark green. A distinctive feature of this species has fialids numbering 3 verticillates; short, thick. Conidia, globose, subglobose/ ovoid. Branched conidiophores resemble pyramids. Chlamydospores are located intercalar, sometimes the terminals are generally round, hyaline in color, and smooth-walled.
c. *Trichoderma* sp. 3 (THC1, THC2, TSC1, TSC2), have a characteristic resemblance to the species *Trichoderma atroviridae*. The results of these observations supported by Kubicek *et al* (1998) stated that the Colony grew rapidly (5-8 cm)[13]. Konidia quickly turn dark green. A distinctive feature of this species has fialids of 2-4 verticillate or solitary, often curved at the ends. Subglobose conidia form, dark green color. The inverted colony is colorless, or yellowish or dull, the smell resembles the aromatics of a coconut.
d. *Trichoderma* sp. 4 (THD1, THD2, TSD1, TSD2), bear similarities to the species *Trichoderma viridae*. The results of observations supported by Kubicek *et al* (1998) stated that the peculiarities of this species have conidiations in the form of dense fiber beams or more effuse, colonies are toska green,

fialids are usually solitary or number 3- verticillate, sometimes there are paired[13]. Conidiophores are paired, Conidia coagulate, ellipsoidal shape, green in color.

e. *Trichoderma* sp. 5 (THE1, THE2), bears a characteristic resemblance to the species *Trichoderma koningii*. The results of this observation supported by Watanabe (2002) stated that the colony was pale yellow- green[12]. Conidiiophores are hyaline-colored, branched, the peculiarity of this species has the form of long fialids, slender, tapering towards the apex. Hyaline conidia color, ovate shape, ellipsoidal. Chlamydospores are pale brown, subglobose form.

f. *Trichoderma* sp. 6 (THF1, THF2, TSF1, TSF2), have a characteristic resemblance to the species *Trichoderma aureoviridae*. The results of observations supported by Watanabe (2002) and Kubicek *et al* (1998) stated that the colony was yellowish-green, expanding; the inverted colony is striking brown due to the production of needle-shaped crystals in the agar culture[12;13]. The colony grows slowly (1-2.5 cm), pale green conidia, obovoid form, ovate, branched conidiophores. The peculiarity of this species has short and thick fialids numbering 2-3 verticillates and carrying a spore mass on each of them. Chlamydospores are pale brown, subglobose form, vowed.

g. *Trichoderma* sp. 7 (THG1), has a characteristic resemblance to the species *Trichoderma pseudokoningii*. Results supported by Watanabe (2002) and Kubicek *et al* (1998) state that colonies grow fast (6-7 cm), inverted colonies are usually colorless [12;13]. The peculiarity of this species has colonies of light green color, fialids numbering 2-5 verticillate, solitary, more irregular. Conidia phialosporous, pale green color, ellipsoidal, ovate. Chlamydospores are brown, subglobose form.

Antagonistic activity of Trichoderma isolates

Based on the observation of the antagonist test and the calculation of the percentage of inhibitory power, it was obtained that isolates with an inhibitory power of < 50% have low antagonisticactivity[17]. According to Amaria *et al* (2013) the percentage of the highest inhibitory power was categorized as having a value of >70%[17]. The *Trichoderma* spp. isolates tested for antagonistic ability against *Fusarium* were 74 isolates that derived from the rhizosphere of healthy pepper plants and rhizospheres of yellow disease pepper. Here are some isolates of *Trichoderma* spp. from the rhizosphere of healthy pepper plants and rhizospheres of diseased pepper that were potentially in antagonistic tests and had a certain inhibitory diameter.

Table 4Percentage of inhibitory power of *Trichoderma* spp. against *Fusarium* on day 7 on PDA media

		Percentage of inhibitory power (%) ± SD		
No	Isolates	*Fusarium oxysporum*	Isolat	*Fusarium solani*
1	THD_2Ib	6,53 ± 0,57	TSF_2Lb	6,73 ± 0,25
2	TSD_2Lc	6,87 ± 0,47	THC_2Ka	6,73 ± 0,76
3	TSB_2Ja	6,87 ± 0,45	THE_1Ka	6,87 ± 0,67
4	THB_2Ic	6,67 ± 0,87	THF_2Kb	6,77 ± 0,23
5	TSB_2Lc	6,93 ± 0,21*	TSD_2Lc	7,03 ± 0,06
6	THB_2Kb	6,97 ± 0,31	TSA_1Jb	6,9 ± 0,85
7	THB_2Jb	**7,03 ± 0,25**	THE_2Mc	**7,17 ± 0,06**
8	THB_2La	**7,1 ± 0,2**	THD_2Ib	**7,17 ± 0,38**

9	THB_2Kc	**7,03 ± 1,01**	THD_2Lc	7,07 ± 0,15
10	THB_2Ka	6,67 ± 1,4	TSD_2Mb	7,23 ± 0,21*
11	THB_2Mb	**7,13 ± 0,35***	THB_1Jc	6,7 ± 0,17
12	TSB_2Ka	6,87 ± 0,64	THB_1Ic	6,7 ± 0,36
13	TSB_2Kc	6,33 ± 0,76	THD_1Ib	**7,33 ± 0,06***
14	THD_2Lc	6,43 ± 0,23	TSB_1Ia	6,5 ± 0,3
15	THB_2La	6,57 ± 0,6	THD_1Mb	6,5 ± 0,36
16	THB_2Ja	6,5 ± 0,7	TSA_1Ja	6,47 ± 0,12
17	THF_2Kb	6,3 ± 0,44	THB_1Lb	6,57 ± 0,15
18	THC_2Lb	6,23 ± 0,25	TSB_1La	6,67 ± 0,25
19	THC_1Ia	6,3 ± 1,14	THB_1Kb	6,43 ± 0,12
20	THC_1Jb	6,27 ± 0,47	THB_1Kc	6,43 ± 0,67

Information: TH (*Trichoderma* from the rhizosphere of a healthy pepper plant); TS (*Trichoderma* of the rhizosphere of the diseased pepper plant); jenis*Trichoderma* spp. (A: *Trichoderma* sp.1); (B: *Trichoderma* sp.2); (C: *Trichoderma* sp.3); (D: *Trichoderma* sp.4); (E: *Trichoderma* sp.5); (F: *Trichoderma* sp.6); (G: *Trichoderma* sp.7); (1= Garden location 1); (2= Garden location 2); (I,J,K,L,M= Plot 1,2,3,4,5); Repetition (a,b,c= 1,2,3); **bold** (Highest inhibitory diameter for healthy rhizosphere *Trichoderma*); underline (Highest inhibitory diameter for *Trichoderma* rhizosphere pain); sign (*) the highest inhibitory diameter of the entire isolate.

The results of the *Trichoderma* spp. isolate antagonist test from a healthy rhizosphere that had the highest inhibitory power against *Fusarium oxysporum* were found in THB2Mb isolates of 7.13%, THB2Jb, THB2Kc of 7.03% and THB2La of 7.1%. The highest inhibitory power for *Trichoderma* spp. isolates against *Fusarium solani* was found in the THD1Ib isolate of 7.33%, and THD2Ib, THE2Mc, had a percentage resistance of 7.17%. *Trichoderma* spp. isolates from the rhizosphere of diseased pepper had a percentage of moderate inhibitory power in the antagonistic test against *Fusarium oxysporum* found in TSB2Lc isolates with a percentage of resistance of 6.93%, while TSB2Ja, TSD2Lc, TSB2Ka had an inhibitory power of 6.87 %. The *Trichoderma* spp. antagonist test against *Fusarium solani* resulted in the highest percentage of inhibitory power in TSD2Lc isolates of 7.03%, while TSD2Mb inhibitory power was 7.23%.

The following is the appearance of the *Trichoderma* spp. fungus antagonist test of the healthy and sick rhizosphere against *Fusarium oxysporum* and *Fusarium solani* day 7 as in Figure 3-4.

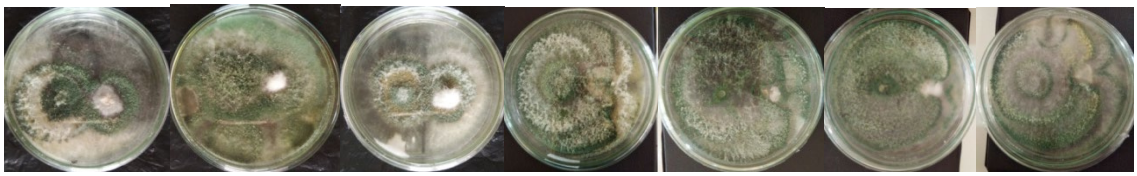


Figure 3. Results of the 7th day antagonist test of *Trichoderma* spp. isolates of the rhizosphere are healthy against *Fusarium* (A) THB2Mb (B) THB2Jb (C) THB2Kc (D) THB2La, (D) THD1Ib (E) THD2Ib (F) THE2Mc

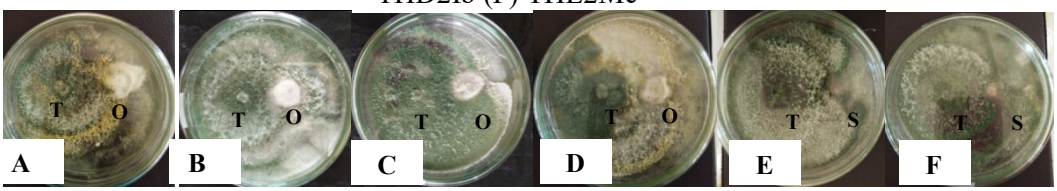


Figure 4. Results of the 7th day antagonist test of *Trichoderma* spp. isolates of rhizosphere pain against *Fusarium* (A) TSB2Lc (B) TSB2Ja (C) TSD2Lc (D) TSB2Ka (E) TSD2Lc (F) TSD2Mb

Based on the LSD test of isolates THB2Mb, THB2Jb, THB2Kc, THB2La of the healthy rhizosphere obtained markedly different results in inhibiting the growth of *Fusarium oxysporum*, while the isolates of THD1Ib, THD2Ib, THE2Mc had markedly different results in inhibiting the growth of *Fusarium solani*. The LSD test results from the TSB2Lc, TSB2Ja, TSD2Lc, TSB2Ka isolates from the disease rhizosphere obtained markedly different results in inhibiting the growth of

Fusarium oxysporum, while the TSD2Mb and TSD2Lc isolates had a marked effect in inhibiting the growth of *F.solani*.

Based on the results of antagonistic tests, the isolates of TSD2Mb and TSB2Lc had the potential to inhibit the growth of *F. oxysporum* and *F. solani* fungi. This was thought to be affected by the symptoms of yellow disease at the root were relatively mild. This condition was supported by environmental factors during the study due to the lack of rain. According to (Prasojo 2017) plants that lack water and nutrients can cause stunted plant growth which encourages the onset of disease[18]. In nutrient-poor environmental conditions, drought, *Trichoderma* spp. will form chlamydospores to survive and develop again if environmental conditions are already favorable [19].The results of the antagonist test could also be influenced by the growing medium used. The media used in the study was PDA (*potato dextrose agar*) media. PDA media is a common media used to grow microorganisms, both fungi and bacteria [20].According to Suryanti *et al* (2017) the use of media for fungal growth can have a high influence in producing antibiotics and show optimum inhibitory power[1]. PDA media contains potato extract which is a source of carbohydrates, dextrose sugar as a good nutritional addition to cultures, while agar is a good growing place for cultures because it contains enough water [20].

Trichoderma (THB, TSB) hada similarities macroscopically and microscopically to *T. harzianum* species. *T. harzianum* has a high antifungal activity compared to other types of *Trichoderma*[9]. These mushrooms can produce lytic enzymes and antifungal antibiotics. Antifungi and lytic enzymes actively play a role in degrading pathogenic cells that cause the lysis of pathogenic cells and secrete trichotoxins that can kill pathogenic fungi [21]. Reported by Achmad (2010) degradation of the hyphae cell wall of the fungus *F. oxysporum* by the chitinlytic enzyme of *T. harzianum* is used as a source of carbon in the culture medium, then the antagonist fungi will secrete chitinase and 1,3-B-glucanase into the medium[22]. Based on the results of antagonistic tests in the study, the growth of the fungus *T. harzianum* was faster than *Fusarium*.This phenomenon suggests that there is competition in the antagonist test. Antagonistic fungi are more competitive in utilizing growth space and nutrients in the same medium. This was in accordance with the statement reported by Yuniati (2005) that the species of *Trichoderma* spp. that are often used as biological agents are T. *harzianum, T. viridae*, and *T. koningii* which are widespread in various cultivated plants[23].

Trichoderma (THD, TSD) had a similarities macroscopically and microscopically to *T. viridae.* Based on antagonistic tests in the study, it showed that the growth of *T. viridae* fungus was faster than *Fusarium*. This was because *T. viride* fungus was a type of fungus that was cellulolytic because it could produce cellulase so that its growth was fast. *T.viride* also had a high production of chitinase as an antagonism. *Trichoderma* (THE) had a similarities macroscopically and microscopically to the species *T. koningii*. The fungi *Trichoderma koningii, Trichoderma atroviridae* and *T. harzianum* had

an antagonistic mechanism as mycoparasites, that was, hooked hyphae that were able to entangledand penetrated the hyphae of the host fungus so that the host hyphae undergo vacoulation, lysed and eventually disintegrated[19]. The fungus *T. koningii* also produces secondary metabolites, namely Alkyl pyrones and antibiotics in the form of isonitriles, harzianolide, peptaibols. Alkyl pyrones are antifungal folatil compounds that can inhibit the germination and growth of pathogenic fungal mycelia. Isonitriles and peptaibols are antibiotic compounds that fungi produce to inhibit the growth of pathogenic fungi. According to Berlian *et al* (2013) Harzianolide produced by *T. koningii* and *T. harzianum* can suppress spore germination and chlamydospores of the fungus *F. Oxysporum*[19].

The F test aimed to determine whether *Trichoderma* isolates in healthy and diseased rhizospheres had an influence or not in inhibiting the growth of the fungus *F. oxysporum* and *Fusarium solani*.

Table 5. TheF Test Analysis

Location	Rhizosphere healthy pepper plant		Rhizosphere yellow diseased pepper plant	
	Fusarium oxysporum	*Fusarium solani*	*Fusarium oxysporum*	*Fusarium solani*
1	0,000a	0,040a	0,026a	0,021a
2	0,014a	0,002a	0,096b	0,060b

Information: notation (a) differs markedly; (b) no real difference

Based on the results of the F test analysis, it was obtained that the *Trichoderma* spp. isolates of the rhizosphere of a healthy pepper plant had more influence in inhibiting the growth of the fungi *F. oxysporum* and *F. solani*, compared to the *Trichoderma* spp. isolates of the rhizosphere of the diseased pepper plant. *Trichoderma* spp. isolates of the healthy rhizosphere had potential isolates in antagonistic assays. This was because plants had a resistance mechanism to suppress pathogen attacks in the form of hormonal activity [14]. The roots of pepper plants have interactions with endophytic fungi. It is proved almost certainly that there is not a single type of plant that is not associated with the endophytic microbes[7]. Endophytic fungi play a direct role by removing antibiotic metabolites, or secreting enzymes capable of destroying pathogenic cells, while indirectly by increasing the resistance of host [14].

Based on the results of the antagonist test, there were several *Trichoderma* spp. isolates from the rhizosphere that had less effect in inhibiting the growth of the fungi *F. oxysporum* and *F. solani*. This was suspected to be because environmental factors in the rhizosphere area of diseased pepper plants could supported the development of nematodes, resulting in the incidence of yellow disease through the penetration of plant roots by nematodes. According to Suryanti *et al* (2017) yellow disease is caused by a complex of nematodes and fungi *Fusarium oxysporum* and *Fusarium solani*[1]. The nematode *R. similis* is the main factor that causes damage to the roots so that the roots are wounded. Damage from root cells, triggers

Meloidogyne spp. to enter the root tissue resulting in the roots swelling. The penetration of *M. incognita* into the roots of the plant produces wounds that occur mechanically, as well as chemically occurring wounds. *M. incognita* is able to produce cellulase and pectinase enzymes that can hydrolyze the cell walls of plants, thereby facilitating the entry of parasitic fungi, such as *Fusarium oxysporum* and *F. solani*. The interaction between *M. incognita* and *F. oxysporum* in pepper plants showed a higher degree of wilting [1]. *Fusarium solani* infection in root tissue is able to produce phytotoxin compounds that are translocated to the leaves, resulting in symptoms in the form of chlorosis. The toxins produced by *F. solani* are isomarticins and dihydrofusarubin which resulted in impaired chloroplast formation [1].

4. Conclusions, Suggestions and Recommendations

The study obtained 7 species of *Trichoderma* spp. encoded THA, THB, THC, THD, THE, THF, THG. The *Trichoderma* spp. isolate antagonist test which produced the highest inhibitory power against *Fusarium oxysporum* was found in THB2Mb and THB2La isolates, while the highest inhibitory power against *Fusarium solani* was produced by THD1Ib, TSD2Mb. Further research needs to be carried out field tests (in vivo) to determine the antagonism ability of *Trichoderma* spp. isolates in vitro in inhibiting *Fusarium* fungus that causes yellow disease in pepper plants.

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