Exploration and Effectiveness of *Trichoderma* sp. from Jember and Trenggalek, East Java, Indonesia Cacao Plantation as A Biological Control of *Phytophthora palmivora*

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Abstract. Fruit rot disease is very damaging to cacao pods, which is caused by *Phytoptora palmivora*. The attack rate of *P. palmivora* varies. In Java, losses due to this disease reduce yields by 90 %. *P. palmivora* is a soil–borne pathogen. It is currently included in the Kingdom Chromista. Control with fungicides is not successful at this time, the alternatives is biologis control with *Trichoderma* sp. This research used a Completely Randomized Design (CRD) which was arranged in factorial with two factors. The first factor was *Trichoderma* sp. the second factor was *P. palmivora*. All treatment combinations were repeated three times. *Trichoderma* sp. antagonist test to *P. palmivora* was analyzed using Analysis of Variance (ANOVA) and then further tested using a 5 % BNJ. *Trichoderma* sp. origin from Jember and Trenggalek districts, East Java, Indonesia were able to act as antagonists against *P. palmivora* with the highest inhibitory of 78 %. In comparison, the lowest inhibitory was 70 % of isolates from Jember district, East java, Indoe. Characteristics of *Trichoderma* sp. The origin of Trenggalek Regency and Jember Regency, East Java, Indonesia in inhibiting the growth of *P. palmivora* has the same species, namely *Trichoderma harzianum*.

Key words: Biologis control, environmentally friendly, fruit rot, increase cacao tree productivity, *Theobroma cacao* L.

1 Introduction

Cacao (*Theobroma cacao* L.) is one of the primary export commodities of Indonesia where over the past 5 yr, Indonesia has provided about 1 951 270 ha area of cacao plants. Unfortunately, its productivity level in the same period results in only around 655 kg ha^{-1}. In comparison, the productivity of cacao in Malaysia reaches about 1 800 kg ha^{-1}, around 800 kg ha^{-1} in Ivory coast, while in Ghana it reaches about 360 kg ha^{-1}.

Indonesia is the third–largest cacao producing country in the world after Ivory Coast and Ghana. Total cacao land of 1 400 000 ha in 2008 could contribute to the state of IDR 1 800 × 10^6 [1]. Currently, cacao development areas in Indonesia include South

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Sulawesi, West Sulawesi, Southeast Sulawesi, Central Sulawesi, West Papua, East Java, Lampung, West Sumatra, North Sumatra, and Aceh. Nationally, domestic cacao production reaches 700 000 t yr^{-1}, which is contributed the most by Sulawesi at around 70 % [9]. However, increasing productivity and quality of cacao have experienced many obstacles, including pests and disease attacks on cacao plants.

One of the critical diseases in cacao plants is cacao pod rot caused by *Phytophthora palmivora* [(E. J. Butler) E. J. Butler]. The disease can reduce cacao production by 32.6 % to 99 %. The distribution of this disease is widespread; its pathogenic diversity is also a threat to the decline in cacao production [14] Rates of *P. palmivora* attacks vary, more than 10 % occur in Peninsular Malaysia and 80 % to 90 % in Cameroon. In Java, losses due to this disease reduce yields 33 % to 50 % [4]. The threat of rapid fruit rot disease develops in the cacao planting area, so first precautions need to be taken. In areas that have been infected with the disease can be controlled with biological agents that are environmentally friendly. The use of biological agents is very useful and positive in controlling fruit rot disease caused by *P. palmivora* [10]. According to [2], good biological agents are agents originating from areas where the disease in question is found. Therefore, in this study *Trichoderma* sp. indigenous East Java, Indonesia which is used as a biofungicide against pathogens, especially against *P. Palmivora* by *in vitro* testing.

Up to now, research used *Trichoderma* from the collection but not from the same location as the pathogen, so there is a possibility that there is a mismatch of environmental conditions that will affect the antagonistic ability of *Trichoderma* sp. itself in controlling *P. palmivora*. [12] Used *Trichoderma* sp. isolates from the collection; then it could suppress pathogens. [4] In vitro test of the three types of fungus *Trichoderma* were found on cacao plantations that *Trichoderma* sp., *Trichoderma harzianum* Rifai, and *Trichoderma viride* Pers., showed that *T. viride* was the most capable of inhibiting the growth of the fungus *P. palmivora* with the highest percentage of 71.95 %. Four antagonists [*T. asperellum* (SF04), *T. virens* (255C1), *T. harzianum* (THP) and *T. longibrachiatum* (4088)] were tested, All Trichoderma significantly ($p \leq 0.05$) reduced the incidence and severity of disease. The 4088 (*T. longibrachiatum*) isolate was the best controller agent of *P. palmivora* in postharvest [7]. The purpose of this study was to determine the effectiveness of *Trichoderma* sp. isolates from Jember and Trenggalek districts, East Java, Indonesia against causes of cacao pod rot (*P. palmivora*).

2 Material and methods

This study began in December 2018 until March 2019. This study used a Completely Randomized Design (CRD). CRD was arranged factorial with two factors. The first factor was the type of *Trichoderma* sp., the second factor was the type of *P. palmivora*, all treatment combinations were repeated three times. The treatments given were:

T: *Trichoderma* sp. (TR1: *Trichoderma* sp. origin of Suruh cacao plantation, Trenggalek, East Java, Indonesia; TR2: *Trichoderma* sp. origin of Karangan cacao plantation Trenggalek, East Java, Indonesia; JB1: *Trichoderma* sp. origin of PUSLITKAKAO cacao plantation, Jember, East Java, Indonesia; JB2: *Trichoderma* sp. origin of Banjarsari cacao plantation, Jember, East Java, Indonesia).

P: *P. palmivora* (P1: *P. palmivora* from Suruh cacao plantation, Trenggalek, East Java, Indonesia; P2: *P. palmivora* from Karangan cacao plantation, Trenggalek, East Java, Indonesia; P3: *P. palmivora* from Puslitkoka (*Pusat Penelitian Kopi dan Kakao Indonesia* – Indonesian Coffee and Cacao Research Institute) cacao plantation, Jember, East Java, Indonesia; P4: *P. palmivora* from Banjarsari cacao plantation, Jember, East Java, Indonesia).

2.1 The exploration of the fungus *Trichoderma* sp.

Fungus *Trichoderma* sp. explored from cacao plantations in the Trenggalek and Jember districts, East Java, Indonesia. The point of taking soil was determined by the criteria for healthy cacao growing conditions (without symptoms of disease affecting the cacao plant). After the cacao plant was determined, it then digs up the soil as deep as 15 cm [3]. The isolation method of *Trichoderma* sp. used the dilution plate method.

2.2 Exploration and Isolation of *P. palmivora* pathogen

P. palmivora was isolated from diseased cacao with blackish brown spots. The area of the pathogen exploration same as *Trichoderma* sp. exploration. The surface of the cacao fruit was sterilized with tissue with 70 % alcohol. Approximately 0.5 cm of flesh was taken from the tissue, then planted into the Water Agar media and incubated for 4 d at room temperature. After 4 d, the mycelia was purified on V8 agar medium (200 mL of V8 juice, 800 mL of distilled water, 1 g of $CaCO_3$ and 20 g of Agar)

2.3 Identify fungi

The fungus identification was carried out after the fungus grew the colony, the fungus *Trichoderma* sp. and *P. palmivora* were identified using a microscope so that the type of *Trichoderma* and *P. palmivora* was known by means of a colony in the observation of the microscope observed based on a journal or book so that the type of *Trichoderma* sp. as well as the identification of pathogens *P. palmivora*.

2.4 Koch postulate test

Healthy cacao pods were sterilized with sterile water, 0.5 % Clorox, and then washed with sterile water. The surface of the cacao fruit is then injured using a needle and then inoculated with *P. palmivora*. Covered using a cotton cloth and taped. The disease incidence was determined based on the symptoms that appear. The results of the postula koch test were then isolated using V8 media and compared with origin *P. palmivora* isolate from exploration. If they showed the same characteristics, the postula koch test can prove the symptoms *P. palmivora* attack.

2.5 *Trichoderma* sp. against pathogenic *P. Palmivora*

Trichoderma sp. against *P. palmivora* cacao pod rot using a dual method on PDA media. Pieces of *P. Palmivora* isolates aged sevendai was placed with a distance of 2 cm from the edge of the petri dish then on the opposite side, was placed isolates of *Trichoderma* sp. with a range of 2 cm from the edge of the petri dish. The observation of the growth pathogen of *P. Palmivora* was carried out from one day to 7 d after the test.

The growth radius of *P. palmivora* was observed by measuring the growth of colonies of each fungus. Inhibition presentation using the Formula (1):

$$Z = (R1 - R2) / R1 \times 100 \% \qquad (1)$$

where Z: Percentage of inhibition, R1: Radius of *P. palmivora* without *Trichoderma* sp. (Control), R2: *P. palmivora* radius with *Trichoderma* sp.

The data were obtained then analyzed using the Analysis of Variance (ANOVA), if there were significantly different effects, further tests would be carried out using a 5 % Tukeys.

3 Result and discussions

3.1 Exploration *Trichoderma* sp. results from plantation in Trenggalek Regency and Jember Regency, East Java, Indonesia

Based on the exploration results of *Trichoderma* sp. from the cacao plantation area in Suruh District and Karangan District, Trenggalek Regency, East Java, Indonesia. Likewise, from the cacao plantations in PUSLITKAKAO and Banjaarsari District, Jember Regency, East Java, Indonesia each site was *Trichoderma* sp.

Microscopic appearance of *Trichoderma* sp. isolates, namely TR1, TR2 (Trenggalek), and JB1, JB2 (Jember) were green hyphae, short phyalid, greenish conidia, oval–shaped and there was also some conidia formed in clusters of light green on the surface of the conidiophore. Phyalid had a length of $\pm$ 11.1 μ and conidiophore branches of $\pm$ 13.4 μ. There were many upright, branched conidiophores, vertically arranged branches, short thick phyalids, colony on potato Dextrose Agar media was dark green and round. The diameter of the colony reaches more than 9 cm within 5 d. The characters from several isolates origin namely TR1, TR2, JB1, and JB2 showed the characteristics of *T. harzianum* [15, 6]

T. harzianum is generally found in hot climates, the optimum temperature for growth of *T. harzianum* is 15 °C to 30 °C, but the optimum growth is at 30 °C and for maximum temperatures of 30 °C to 36 °C. Normal growth of *T. harzianum* is at pH 3.7 to 4.7 [13]

The benefits of *T. harzianum* was most often used in biological control because it has several comparative advantages compared to other organisms. A wide range of environments, mycoparasitic and able to compete in gaining space and producing antibiotics and enzymes that harm to the pathogens [13].

3.2 *P. palmivora* exploration

P. palmivora was isolated from diseased cacao with blackish–brown spots. Fruit exploration was carried out in cacao plantation areas where in the same area with *Trichoderma* sp. All isolates that were obtained characterized as *P. palmivora*. The characteristic of *P. palmivora* isolates a a rounded edge colony. There were four forms of ovoid sporangia, limoniform, obturbinate, and obpyriform. Sporangium length 40 μm to 62 μm and width 28 μm to 43 μm, have papillae, short pedicels, and simple simpodiabranching models. This criterion was as described by Stamps [13].

3.2.1 Koch's postulates for identification test

Tests were carried out on healthy cacao pods, then given *P. palmivora* pathogens (exploration results) and observed the symptoms of the pathogen as presented in Figure 1.

Fig. 1. Postulate test of fungus identification on healthy cacao

Based on Figure 1, *P. palmivora* disease attack on healthy cacao fruits showed the same symptoms as cacao pods affected by the disease. Koch postulates was used to prove the isolates obtained the causative agents of the observed disease symptoms.

Koch's postulatewas also used as a method to identify fungi that cause signs of cacao pod rot (*P. palmivora*).

3.3 Antagonistic test of *Trichoderma* sp. against *P. palmivora*

An in vitro antagonist test was a way to evaluate the ability of antagonists (biological control agents) in a narrower scope and controlled environmental conditions (in vitro). The aim was to determine the potential or effectiveness of biological control agents in inhibiting the growth and development of pathogens [3]. The results of the antagonist test were presented in Table 1.

Based on the Tukey's test, the type of *Trichoderma* sp. TR1P1 showed the highest antagonicity (78 %) on 7 d observations against *P palmivora* on PDA medium, It was significantly different from *Trichoderma* sp. JB2P3 (70 %) on 7 d observations. The origin of *T. harzianum* isolate did not significantly influence the inhibition of *P. palmivora* from two different regions. The result of research was not in line with the results of the research of [2], that good controlling agents to control the disease were those from the same area with pathogen isolates. Antagonistic of all *T. harzianum* was no significant difference. It was likely due to the origin of the isolates being the same from the Java region, which has almost the same ambient temperature and humidity.

The difference in antagonism of *Trichoderma* sp. against *P. palmivora*, indicates the diversity of *Trichoderma* sp. who have been tested for their abilities as antagonists. This occurs a significant difference in the sporulation ability of each treatment type *Trichoderma* sp. following the results of Darmono's research [5], six isolates of *Trichoderma* sp. from the tested nature shows a difference in the ability to suppress *P. palmivora*.

According to [12] in general the mechanism of *Trichoderma* spp. in suppressing pathogens, namely as a micoparasitic and aggressive competitor. Initially, hypha *Trichoderma* spp. grow elongated, then convolve and penetrate the host fungal hyphae so that the host hyphae are vacuated, lysis, and finally destroyed. According to Harman [8] *Trichoderma* spp. penetrate the host cell wall with the help of cell wall degrading enzymes, namely chitinase, glucanase, and protease, then use the contents of the host hyphae as a food source. *Trichoderma* spp. also produce antibiotics such as gliotoxins and viridian when they penetrate cell wall.

Table 1. Antagonist test *Trichoderma* sp. to *P. palmivora*

Treatment	Disease intensity % in day after inoculation (d)						
	1	2	3	4	5	6	7
TR1P1	55 a	62 a	65 abc	66 ab	69 abc	72 ab	78 b
TR1P2	44 a	53 a	68 c	69 b	72 c	76 b	76 ab
TR1P3	59 b	62 a	65 abc	65 ab	70 bc	77 b	77 ab
TR1P4	58 b	58 a	66 abc	66 ab	68 abc	70ab	75 ab
TR2P1	48 a	57 a	65 abc	67 ab	69 abc	72 ab	75 ab
TR2P2	52 a	59 a	67 bc	67 ab	70 bc	74 ab	76 ab
TR2P3	47 a	56 a	65 abc	65 ab	68 abc	73 ab	75 ab
TR2P4	46 a	55 a	65 abc	66 ab	66 abc	72 ab	72 ab
JB1P1	52 a	62 a	66 abc	68 ab	70 bc	74 ab	77 ab
JB1P2	48 a	56 a	63 abc	66 ab	66 abc	75 ab	75 ab
JB1P3	45 a	55 a	62 abc	65 ab	65 abc	72 ab	74 ab
JB1P4	42 a	58 a	60 abc	63 ab	64 abc	74 ab	74 ab
JB2P1	44 a	55 a	58 a	62 a	64 abc	73 ab	75 ab

Table 1. Continued

Treatment	Disease intensity % in day after inoculation (d)						
	1	2	3	4	5	6	7
JB2P2	55 a	56 a	58 a	62 a	62 ab	70 ab	72 ab
JB2P3	53 a	55 a	63 abc	63 a	63 ab	69 a	70 a
JB2P4	55 a	56 a	65 abc	65 ab	65 a	69 a	74 ab
BNJ 5 %	6.021	6.35	6.98	7.046	7.24	7.62	7.93
	**	**	**	**	**	**	**

Note: Values followed by the same letter in the same column in the Tukey's test at a level of 5 % show very significant different

TR1: Isolate *Trichoderma* sp. Trenggalek Regency in Suruh District, East Java, Indonesia; TR2: Isolate *Trichoderma* sp. Trenggalek Regency in Karangan District, East Java, Indonesia; JB1: Isolate *Trichoderma* sp. Jember Regency in PUSLITKAKAO Jember, East Java, Indonesia; JB2: *Trichoderma* sp. Jember Regency in the District of Banjarsari, East Java, Indonesia; P1: *P. palmivora* from the Suruh cacao plantation in Trenggalek Regency, East Java, Indonesia; P2: *P. palmivora* from Karangan cacao plantation in Trenggalek Regency, East Java, Indonesia; P3: *P. palmivora* from PUSLITKAKAO cacao plantation in Jember Regency, East Java, Indonesia; P4: *P. palmivora* from Banjarsari cacao plantation in Jember Regency, East Java, Indonesia.

Control mechanisms with biological agents against plant pathogenic fungi are generally divided into three types, firstly competition for growth sites and nutrition, secondly antibiosis, and thirdly parasitism [8]. The mechanism of parasitism is an interesting phenomenon that plays an essential role in the process of biological control. *Trichoderma* spp. usually use this mechanism with other, namely competition and antibiosis.

4 Conclusions

Antagonistic test results, isolates of *Trichoderma* sp. very effective in inhibiting the growth of *P. palmivora*. Isolates from Trenggalek district, Suruh sub–district (TR1P1) were the most effective isolates because they can inhibit the growth of *P. palmivora* by 78 %, and a significant difference from *Trichoderma* sp. JB2P3 of 70 %

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