Annals of Biology **38** (2) : 226-230, 2022

Impact Score : 0.32 (Scopus)

Ability Test of *Trichoderma harzianum* in Inhibiting the Growth of *Phytophthora palmivora* Causing Black Pod Disease of Cocoa

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(Received : April 15, 2022; Accepted : May 20, 2022)

ABSTRACT

The ability test of *Trichoderma harzianum* in inhibiting the growth of *Phytophthora palmivora* causing black pod disease of cocoa was done both in the field and in the laboratory. The test was performed *in vitro* to determine the antagonistic mechanism of *T. harzianum* in suppressing the growth of *P. palmivora*, a pathogenic fungus causing black pod disease of cocoa and to obtain the most effective formulation of *T. harzianum* for inhibiting the growth of the pathogenic fungus. Exploration method was pathogenic fungus exploration, and laboratory testings : Koch's postulates test, antagonism test and effectiveness test were carried out. The results revealed that the antagonistic mechanism of *T. harzianum* against *P. palmivora* was its surpassing at competition for nutrients, thus developing faster in occupying space and causing inhibited growth of the pathogenic fungus. The antagonism test results showed that *T. harzianum* was able to inhibit the growth of *P. palmivora* by 54.39% (good). Based on the results of the effectiveness test, the formulation of *T. harzianum* with kaolin was considered the best formulation to inhibit the growth of the pathogenic fungus in the tested fruit by 15.3%.

Key words : Black pod disease, effectiveness, *Trichoderma harzianum*, *Phytophthora palmivora*

INTRODUCTION

Phytophthora palmivora is a pathogenic fungus that causes fruit rot of *Theobroma cacao* L. (cacao). This pathogen attacks various parts of the cocoa tree : leaves, trunks, stems, branches, shoots, receptacles and fruits (Akrofi *et al.*, 2015; Pakora *et al.*, 2017; Linaldeddu *et al.*, 2020). *P. palmivora* can attack the plant at various ages, from seedlings to productive plants. Intensity of this pathogen attack can reach 85% in high rainfall areas. Economically, the attack resulted in a decline of 10-30% in world cocoa production, while it caused yield loss of 15-53% in Indonesia (Balittri, 2019). Based on the 2019's monitoring data of the Pontianak Plantation Crop Protection Center, the attack of black pot disease caused by the pathogenic fungus reached 282 ha of areas in West Kalimantan with estimated monthly losses of IDR 11,359,700.

As generally *P. palmivora* survives in the form of mycelium and chlamydospores on infected plant parts or in the soil, it is hard to control. Thus, an integrated implementation of controlling the disease is needed. A biological control of plant pathogens is highly preferred due to its minimum damage to the environment. The use of *T. harzianum* as antagonist agent is one of the biocontrol techniques. Pesticides could be effective, but are costly and pose health hazards to farmers (Sriwati *et al.*, 2015).

Trichoderma spp. was previously shown to reduce the growth of cacao pathogens and subsequent diseases through antibiosis, antagonism, mycoparasitism, many plant pathogens, competing with microbial pests, and induced resistance (Jaber and Ownley, 2017). In the field tests, *Trichoderma* spp. was successfully used to control black pod rot of cacao. *T virens* reduced *P. palmivora* lesion expansion in a detached pod assay, reducing severity by 71% and also reduced *P. palmivora* infection on seedlings when applied aerially (Sriwati *et al.*, 2015). Application of *T. amazonicum* and *T. hamatum* with potassium fertilizer prolonged the incubation period and reduced the number of *P. palmivora* spots on cacao leaves (Harni *et al.*, 2020). In the field, the application of *T. harzianum* suspension reduced the percentage of cacao fruit infection and disease intensity caused by *P. palmivora* (Sriwati *et al.*, 2019). In accordance with the previous successful utilizations of *Trichoderma* spp. to control the fungal diseases of cacao trees, this study aimed at determining the potential of endophytic *T. harzianum* as a biological control agent against *P. palmivora*.

MATERIALS AND METHODS

This study was carried out for approximately three months (May-July 2018), at the Pontianak Plantation Crop Protection Center Laboratory. The materials used were : *Trichoderma harzianum* isolates, *Phytopthora palmivora* isolates, potato dextrose agar (PDA), V8 juice agar (V8A), water agar, rice, bran, kaolin, antibiotics, methylated spirit, tissue rolls, 96% alcohol, disinfectants, sticker papers, aluminum foil and cotton. The tools used included : electric oven, isolation box, bunsen burner, microscope, object glass, cover glass, petri dish, erlenmeyer flask, reaction tube, dissecting set, scale, hand sprayer, toolkit, hand counter, haemocytometer, cork drill, ruler, camera and stationery. The method applied in this study included antagonism test and effectiveness test. The research stages were : exploration of *P. palmivora*, purification of pathogenic fungi, and multiplication of *T. harzianum*.

The infected cacao fruits from field were taken into the laboratory and were isolated. The isolation was performed by planting the tissues of diseased and healthy fruits on the water agar media and incubating them for 5-7 days at room temperature under lamp light. The growing colonies were observed from the bottom of the petri dishes using a binocular microscope to confirm the presence of pathogenic sporangia (*P. palmivora)*. The exploration sites were : (1) Karimunting Village, Sungai Raya Kepulauan Subdistrict of Bengkayang Regency; (2) Sungai Raya Village, Sungai Raya Kepulauan Subdistrict of Bengkayang Regency; (3) Mendalok Village, Sungai Kunyit Subdistrict of Mempawah Regency; and (4) Sengkubang Village, Mempawah Hilir Subdistrict of Mempawah Regency.

To ensure that the *P. palmivora* obtained from the exploration was the pathogenic fungi that primarily causes cacao black pod disease, the Koch's postulates test was performed in the laboratory. The test results showed that the pathogenic fungi were only found on the diseased fruits. The fungi resulted from the isolation of the diseased fruits were cultivated in pure cultures. The cultured *P. palmivora* were able to infect the healthy fruits both in the laboratory and in the field.

Based on the observation results during the Koch's postulates test, each isolate of *P. palmivora* had a time difference of initial infection in the test fruit. The isolates of *P. palmivora* obtained from Karimunting Village were the fastest isolates : they caused the initial infection in cacao pods a day after infection at room temperature, while the other isolates were at two days after infection on average. Then the isolates from Karimunting Village became the tested isolates.

The pathogenic colonies were reisolated by implanting them on the V8A media to obtain pure cultures which were then identified. The pathogenic isolates were multiplied with the same media for the test purposes. *T. harzianum* isolates were taken from the collection of the Pontianak Plantation Crop Protection Center Laboratory. The isolates were obtained from Bengkayang Village, and tested for their ability against *Rigidoporus lignosus* causing white root rot (WRR) disease and for their persistence in the field. The multiplication of *T. harzianum* involved multiplication of starter for the *in vitro* tests on rice media and formulation with bran and kaolin for the effectiveness tests of the fungi against *P. palmivora* in the laboratory.

The antagonism test was carried out in the laboratory using the dual culture method which aimed at determining the inhibition mechanism of *T. harzianum* against *P. palmivora in vitro*. The percentage of inhibition was calculated as :

$$Z = \frac{R_1 - R_2}{R_1} \times 100\%$$

Where,

Z is the per cent inhibition. R_1 is the radii of the pathogens without antagonist in the control, and R_2 is the radii of the pathogens with antagonist. The test was performed four times.

The test was carried out in the laboratory to determine the effectiveness level of *T. harzianum* in suppressing the infection rate of *P. palmivora*. A completely randomized design (CRD) with five repeats. The replicates were the treated cocoa fruits. Each treatment used four furits, so the total fruits observed were

100. The treatments of the biological disease control using *Trichoderma* fungus were :

P_1=Control (disstilled water + Tween 80)
P_2=*Trichoderma* spp. formulation with bran
P_3=*Trichoderma* spp. formulation with kaolin
P_4=*Trichoderma* spp. formulation on rice media
P_5=Recommended fungicide for *Phytophthora* sp.

The conidial density of *Trichoderma* spp. of each treatment was 10^7/ml. The healthy cacao fruits were taken of the same age and size (±10 cm length) from the field and were brought to the laboratory. To create suspensions of the *Trichoderma* spp. resulted from the multiplication, the fungi were formulated with carrier material (bran and kaolin) at 1 : 5 ratio of *T. harzianum* and the carriers were mixed with 0.1% Tween 80. Then the suspensions were sprayed evenly on the healthy fruits from the field. After dried (± 1 h), *P. palmivora* fungi were inoculated. The inoculation was performed by making holes in the cacao fruits with a 5 mm borer. Then the holes were filled in with sterile cottons; the suspensions of *P. palmivora* were injected into, and the holes were then covered with tape. The inoculated fruits were placed on plastic trays then put into large plastic bags which have been given wet tissue. Observations for the presence or absence of symptoms of infection on the treated fruits were done. Diameter of the infected pods was measured every two days until the ten-day post-treatment.
Statistik 8 was used to analyze the data. An F-test was applied for the analysis. When it showed significant differences, a Tukey's HSD (honestly significant difference) test with a 5% significance level was then performed.

RESULTS AND DISCUSSION

Based on the observation results during the antagonism test, *T. harzianum* had a good ability in inhibiting the growth of the pathogenic fungus, *P. palmivora* (Table 1).

Table 1. Per cent inhibition of *Trichoderma harzianum* against *Phytophthora palmivora*

Repeats	Inhibition (%)	Description
I	58.82	Dual culture method
II	51.42	
III	52.94	
IV	54.38	
Average	54.39	

The observations were carried out for eight days after the inoculation (dai) because the growth of the antagonist fungus, *T. harzianum*, inhibited the growth of the pathogenic fungus, *P. palmivora*. This was due to ability of the antagonist fungus to grow faster than the colony of pathogenic one. This clearly revealed that the antagonistic mechanism of *T. harzianum* in inhibiting the growth of *P. palmivora* causing the black pod disease of cacao is its surpassing at competition for nutrients (Fig. 1). Thus, developing faster in occupying space and causing inhibited growth of the pathogenic fungus (Sriwati *et al.*, 2019). Effectiveness test was aimed at determining the effectiveness of *T harzianum* in suppressing the infection rate of pathogens causing the black pod disease. The

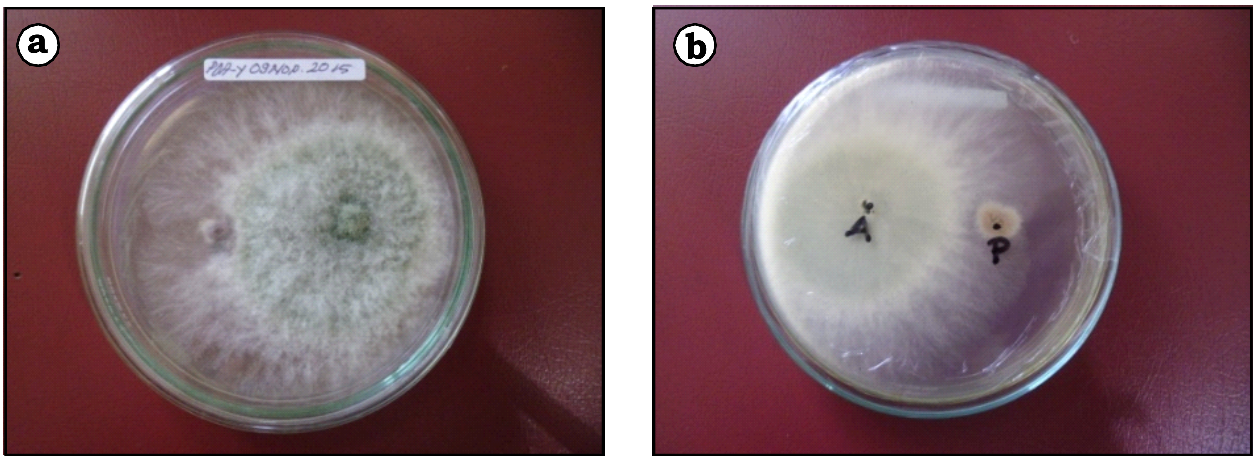

Fig. 1. Ability of *Trichoderma harzianum* fungus to inhibit the growth of *Phytophthora palmivora* on 7 dai in PDA media : (a) the top of the petri dish and (b) the bottom of the petri dish.

Table 2. Average percentage inhibition of *T. harzianum* against *P. Palmivora*

Treatments of *T. harzianum*	Average percentage of infection on fruits (%)				
	Observation 1	Observation 2	Observation 3	Observation 4	Observation 5
P_1	3.42	24.02	40.12	63.65	99.4^{b}
P_2	3.42	19.97	36.12	56.87	91.01^{ab}
P_3	1.55	14.6	34.78	54.76	84.7^{a}
P_4	1.72	17.16	34.29	58.21	88.8^{a}
P_5	1.92	20.14	37.17	64.28	89.7^{a}

Values followed by the same superscript in the same column are not significantly different at P=0.05.

observations were done in the laboratory until the ten-day post-treatment (Tabel 2).

Based on the results, the *T. harzianum* formulated with kaolin had better inhibitory ability than the other treatments. It is assumed that kaolin contains important micronutrients to enhance the fungus growth ability. This is in accordance with the results of an analysis conducted showing that kaolin contained silicone dioxide (SiO_2) 46.48%, aluminum oxide (Al_2O_3) 42.49%, titanium dioxide (TiO_2) 0.84%, iron oxide (Fe_2O_3) 2.79% and moisture 0.41%. Alex *et al.* (2018) stated that important micronutrients were usually found in raw materials such as soil including kaolin as the natural habitat of pathogenic fungi, and they were needed by *Trichoderma* for growth processes and vitamin sources. In addition to being a support for the formulation, kaolin constituted growth support for biopesticides.

It is assumed that *Trichoderma* directly and indirectly reduces the percentage of infections on the fruits. The direct inhibitory mechanism was through competition and antibiosis, while the indirect one was by enhancing plant resistance to pathogens (Latz *et al.*, 2018). *Trichoderma* sp. also had ability to remove hydrolytic enzymes; a class of hydrolytic enzymes playing very important role in the process of mycoparasitism of some pathogenic fungi was chitinolytic enzymes, which consisted of chitinase (Sriwati *et al.*, 2019). Fig. 2 shows the spots area of the infected cacao pods of each treatment.

Compared to *T. virens* and *T. asperellum*, *T. harzianum* had the highest colonization level on cocoa fruits (Sriwati *et al.*, 2015). The cacao plants that were given a *T. harzianum* suspension colonized by the fungus, and this resulted in the reduced infected spot area on the fruits. In addition, *Trichoderma* was able to produce secondary metabolites : viridine, trichomidine and alkaloid compounds that were able to produce hydrolytic enzymes such as 1,3-gluconase, chitinase and cellulase that actively degraded pathogenic fungal cells (Saravanakumar *et al.*, 2015). Plants had a specific response to pathogen attacks (Sriwati *et al.*, 2019). The use of the right formulation ingredients increased conidial germination and survival of *T. hypha.* In this study, kaolin had the potential to be a formulation material mixed with *T. harzianum* due to its better inhibitory rate.

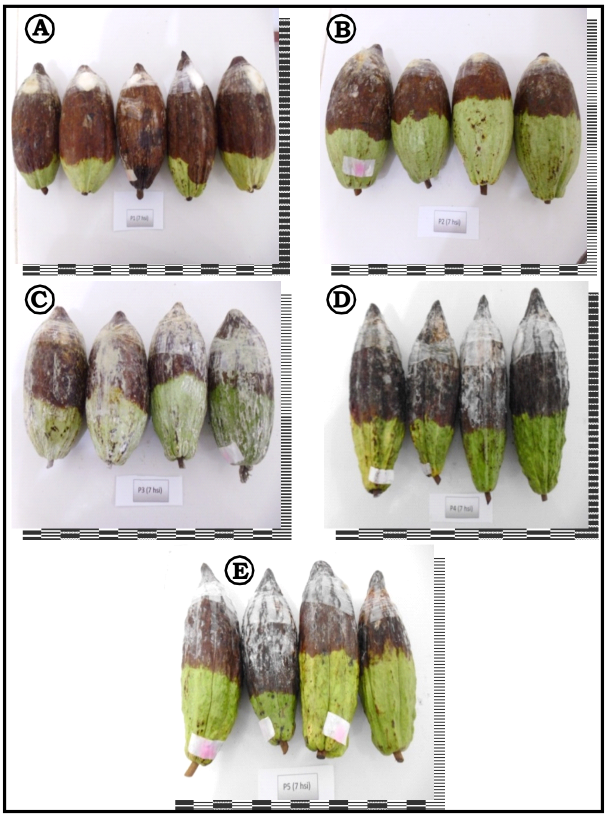

Fig. 2. Spots of cocoa fruit rot of all treatments on 10 dai : A=P_1, B=P_2, C=P_3, D=P_4 and E=P_5 (*Source* : Test photos).

CONCLUSION

Based on the antagonism test results, *Trichoderma* spp. was able to inhibit the growth of pathogenic fungus, *P. palmivora,* by 54.39% (good). The effectiveness test showed that the formulation of *T. harzianum* with kaolin was considered the best formulation and able to inhibit the growth of the pathogenic fungus in the tested fruit by 15.3%.

ACKNOWLEDGEMENT

The authors are grateful to the Faculty of Agriculture, Panca Bhakti University and Pontianak Plantation Protection Center Laboratory for their support and assistance.

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