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Effectiveness of Liquid *Trichoderma* spp. Formulation as Biopesticides Against *Phytophthora palmivora* and *Oncobasidium theobromae* in Cocoa Plants (*Theobroma cocoa* L.)

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ABSTRACT

Trichoderma sp. has antifungal activity. In nature, Trichoderma is found in forest and agricultural soils or on woody substrates. Trichoderma was gained maximum attention as bio control agent due to the fact that it's effective against a huge number of soil-borne plant pathogenic funguses. There are two main methods on production of biopesticide that containing *Trichoderma* spp., either by solid state fermentation or by liquid state fermentation. *Trichoderma* sp., after being formulated as conidia mass, potentially could be used as biopesticide. This form of biopesticide will be produced by cell and spore propagation, until produce viable propagules and having high survival rate even after spread out on to the surface of target (*leaf, plant etc.*). This study was aimed to know the effectiveness of liquid *Trichoderma* spp. formulation as biopesticides against *Phytophthora palmivora* and *Oncobasidium theobromae* in Cocoa plants (*Theobroma cocoa* L.). The experiment was designed in a completely randomized design (CRD), by applying series of treatments consisting mix of extract of soybean (ES) and coconut water (CW). Tested treatments were: T0 (sterile distilled water 100% as control); T1 (100% ES); T2 (80% ES + 20% CW); T3 (60% ES + 40% CW); T4 (40% ES + 60% CW); T5 (20% ES + 80% CW); and T6 (100% CW). The results showed that the best treatment was T4 formula, which indicated by the highest number of spores density (9.76×10^8) during 48 hours incubation. Furthermore, this T4 formula treatment also gives the best effect as a biopesticide agent during application test in the cocoa pods. Biopesticides made from *Trichoderma* sp. are effective in controlling the pathogenic fungus *Phytophthora palmivora* that causes fruit rot and *Oncobasidium theobromae* that causes Vascular Streak Dieback (VSD) disease in cocoa plants, and more consequently the biopesticides are more effective in controlling *Oncobasidium theobromae* disease. The application of Trichoderma and its propagules as a biological control method can help reduce the use of pesticides and chemical fertilizers in agriculture in the future.

1. INTRODUCTION

Cocoa *(Theobroma cocoa* L.) is one of the most productive crops that contribute to increasing Indonesia's foreign exchange. Indonesia is currently the third major producer of cocoa after Ghana and Ivory Coast. This was a very important contribution to the Indonesian economy [1]. One of the causes of low cocoa crop production is fruit rot disease caused by the fungus *P. palmivora* [2]. *P. palmivora* causes serious losses to cocoa in Indonesia and around the world. This pathogen affects decrease the production of cocoa by 44% [3].

According to Hamdi and Lakani [4], the symptoms on infected cocoa pod caused by attack of *P. palmivora* fungus is brownish black skin around the fruit and fruit spotting finally happened overall. Infection with *P. palmivora* on cocoa pods directly through the skin tissue cocoa growing hyphae usually interselluler and forming Haustoria the host cells, or indirectly through the degradation of the cell wall of cocoa as an artificial wound.

In addition to *P. palmivora*, *Oncobasidium theobromae* also causes a reduction in fruit production as it causes Vascular Streak Dieback (VSD) disease. Because this pathogen attacks shoots and branches as well as xylem tissue, it causes the cocoa plant to die completely [5]. This pathogen attack develops throughout the leaf surface which is brownish yellow in color and will eventually fall off so that it looks like rotting twigs [6]. Diseased branches will split longitudinally when brown lines are visible in the xylem tissue [7]. Furthermore, tissue death will spread to branches or to the main stem, so that the plant dies [8].

The control that has been done recently was mostly using chemical pesticide. The implementation of agricultural system prioritized more on the use of pesticide and chemical fertilizers, whereas by using such materials will only decrease the production temporarily. Beside, the negative impacts of the use of such materials is very substantial. It could lead to enviromental pollution and the death of other useful organisms [9].

Biopesticides are one method in place to reduce the impact caused by chemical pesticides [10]. Natural pesticide or biopesticide is a material that is easy to decompose in the environment and environmentally friendly containing microbes as the active ingredient biopesticides such as fungi, bacteria or viruses that are antagonistic to other microbes or produce specific compounds that are toxic to both insects (pests) and nematodes (cause plant diseases) [11]. One of the microbes used in the manufacture of a biopesticide is a fungus *Trichoderma* sp. because *Trichoderma* sp. antagonism against plant pathogens.

The results of previous studies reported that *Trichoderma* sp. fungi are able to control pathogenic fungi in plants such as *Phytopthora* sp. [12], and *Fusarium oxysporum* [13] and *Phytophthora nicotianae* [14]. *Trichoderma* sp. controls pathogenic fungi in three ways, namely by antibiosis (clear zone as an inhibitor of pathogenic fungal growth), competition (fungi grow and take food and interfere with the growth of other fungi) [15], *Trichoderma* sp. also produces cellulase enzymes (degrading cellulose), chitinase (degrading chitin) [16], hemicellulase (degrading hemicellulose) [17] and glucanase [13] which can lyse the cell walls of plant pathogenic fungi [18].

Trichoderma sp. also functions as a decomposer, plant growth stimulator and as a biodecomposer, breaking down organic waste into quality compost [19]. The proliferation of *Trichoderma* sp. fungi will occur if the fungus is in an environment that contains organic matter as a food source [15].

Some organic wastes can be used as media for *Trichoderma* sp. propagation, such as coconut water and soybean extract. Coconut water is one of the abundant liquid organic wastes, in its utilization has not been maximized. Disposal of coconut water can cause acetic acid pollution, due to fermentation of coconut water waste, the acetic acid produced can affect soil acidity and have a negative effect on plants [20]. Therefore, it can be used as a medium for making biopesticides, because it contains complete nutrients, namely 4% carbohydrates, 0.1% fat, 0.02% calcium, 0.01% phosphorus, iron and 0.5% total protein (9 g per L) [21]. The carbohydrate content contained in coconut water is sucrose, glucose, fructose, mannitol, surbitol, and minositol [22], various hormones such as auxins and cytokinins.

Furthermore, soybean extract processing liquid waste contains dissolved organic matter which contains many protein compounds [23]. Soybean extract liquid waste has been used in the manufacture of animal feed ingredients, but generally soybean extract processing waste is still disposed of without being reprocessed so that it has the potential to become a source of environmental pollution [24].

The role of *Trichoderma* sp. very large in suppressing fungal pathogen population, and able to live in wastes containing organic materials. Resistance of cocoa plants to fruit rot disease is commonly evaluated and tested by inoculating beneficial microorganisms such as Trichoderma sp. wowever, so far these methods have yielded inconsistent results. These research aims were: 1) Observation of symptoms of attacks by *P. palmivora* which causes fruit rot disease and *O. theobromae* which causes Vascular Streak Dieback on cocoa plants in the field, 2) Determine the effectiveness of biopesticides made from *Trichoderma* sp. which are propagated using basic substrates from soybean extract and coconut water and its application in *P. palmivora* (cause fruit rot on cocoa) and *O. theobromae* (causing vascular streak dieback).

2. MATERIAL AND METHODS

2.1 Research design

This research was divided in two stages, namely:

1. Observation of symptoms of attacks by *Phytophthora palmivora* which causes fruit rot disease and *Oncobasidium theobromae* which causes Vascular Streak Dieback on cocoa plants in the field.

This research was conducted by direct observation to cocoa plantations in five villages, i.e ; Langaleso Village-Dolo Sub-district, Sidondo I Village, Sigi Biromaru Sub-district, Petimbe Village, Makmur Village and Rahmat Village, Palolo Sub-District in Sigi Regency, Central Sulawesi, Indonesia.

Collection of pod and infected plants obtained from the results of observation and directly retrieval in local farm. Any pod affected by pod rot disease is caused by *P. palmivora* and VSD-infected plants caused by *O. theobroma*, sampling required for further analysis of the causing factors.

2. The second research stages was conducted in the Biotechnology Laboratory of Department of Biology, Faculty of Mathematic and Natural Sciences, Tadulako University.

The materials used in this study were PSA (Potato Sucrose Agar) media, distilled water, *Trichoderma* spp. inoculum (local isolate of Sigi Regency, Central Sulawesi), coconut water/CW (taken from fresh coconut fruits in the traditional market), soybean extract/SE (soybean water taken is liquid waste from the soybean industry), alcohol, and filter paper. *O. theobromae* fungus isolates were collected from infected cocoa plant twigs, *P. palmivora* isolates from infested cocoa fruits, sterile distilled water, tissue paper, sterile cotton, and methylene blue, and healthy cocoa fruits.

Biopesticide formulation study was designed in Completely Randomized Design (CRD) (RAL) consisting of seven treatments and three replications. Composition of following treatments was : P0 (100% distilled water), P1 (100% ES); P2 (80% ES + 20% CW); P3 (60% ES + 40% CW); P4 (40% ES + 60% CW); P5 (20% ES + 80% CW); and P6 (100% CW). Implementation stages of this study: Formulation biopesticide with activated *Trichoderma* sp.

a. Preparation of *Trichoderma* sp. inoculums

Pure culture of fungus *Trichoderma* sp., was propagated by culturing *Trichoderma* spp in a PSA medium in a petri, and then incubated at 30°C for 5 days. Grown *Trichoderma* on the PSA, in a petri was harvested by adding 10 ml sterile distilled water, then the mycelium was stirred to separate the mycelium from the growth medium. Furthermore, stir until become formed suspense, then dilution spores (conidia) using haematocytometer and observation under a microscope.

b. The calculation of the density of spores (conidia)

- Conidia calculated by using haemacytometer. Haemacytometer used was *NeubauerImproved* type. Which have large main box that divided into 25 squares and subdivided into 16 small boxes 1 small box with an area of 0.0025 mm^2 and a depth of 0.1 mm [25]. Conidia density was calculated of by taking 1 ml of spore suspension and placed slowly on the count area to fill the canal by using a micro pipette. Next put in stable position and closed with a cover glass. Calculating conidia density in the box count on 5 fields of view with a magnification of 100x.

- Culture of *Trichoderma* sp. aged 4 days was harvested, then filtered using a filter paper (dry weight known). Filtering Using a funnel and erlenmeyer to obtain filtrate containing conidia.Conidia produced in the filtrate was calculated using a hemacytometer.
- According to Herlinda et al. [26], conidia density calculated using the method by Gabriel and Riyatno [27] as follows:

$$C = t/(n \times 0.25) \times 10^6$$

where,
C = density of conidia per ml of solution
t = total number of conidia in a box the observed sample
n = number of samples box
0.25 = correction factor use box small scale samples hemocytometer

c. Application of media formulation and *Trichoderma* sp. inoculums

Culture media used in this study was an extra soy in the form of liquid waste industries soybean oil and waste of coconut water. The culture is done in a culture bottle with media formula volume of 250ml per bottle. The composition of the treatment formula as follows: P0 (100% distilled water), P1 (100% ES); P2 (80% ES + 20% CW); P3 (60% ES + 40% CW); P4 (40% ES + 60% CW); P5 (20% ES + 80% CW); and P6 (100% CW). That formulated media according to treatment, was added to the culture bottles each of 250 ml. Media formula sterilized using an autoclave for 15 minutes at 121°C. Conidia suspension *Trichoderma* sp 2.5 ml inoculated on media formula with a concentration of 10^{-6} conidia per ml^{-1}. The cultures were incubated at 30°C shaker 150 rpm for 4 days.

Furthermore, 0.5 ml biopesticides liquid formula that was spilled on cotton in the cocoa fruit. Then cotton covered with transparent tape. Next the fruit wrapped with tissue paper and transparent plastic, the development of *P. palmivora* were observed until day 7. Furthermore, the infected twigs *O. theobromae* dipped into the liquid formula biopesticides, dipped twigs placed in petri dishes with cotton in it. Each petri dish contains 5 pieces of twig or 5 spot. Four spot position within 2 cm from the edge of the Petri dish and 1 spot in the midst of a petri dish [28]. All petridishes were incubated for 5×24 hours in a room temperature.

d. Data analysis

Data were quantitatively analyzed variation (ANOVA), one-way ANOVA using the "Statistics Software Version 7". In the event of significant differences among treatments, then proceed to Test Multiple "Duncan".

3. RESULTS AND DISCUSSIONS

3.1 Symptoms of attacks by *Phytophthora palmivora*

The symptoms of *P. palmivora* attack on cocoa pod can be seen in Figures 1 and 2.

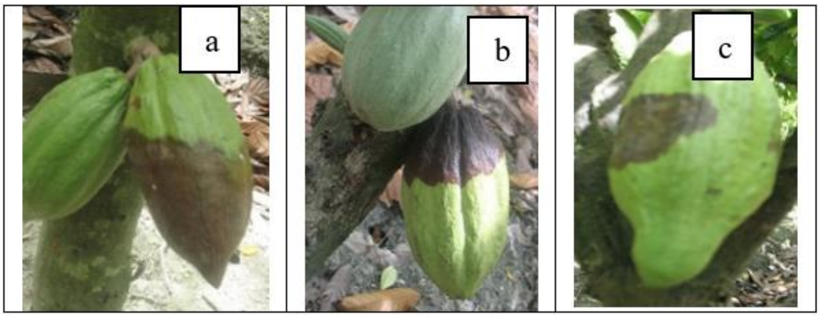

Figure 1. (a) *P. palmivora* attacking begins from the tip of the cocoa pod (b) *P. palmivora* attacking begins from the base of the cocoa pod (c) *P. palmivora* attacking begins from the middle of the cocoa pod

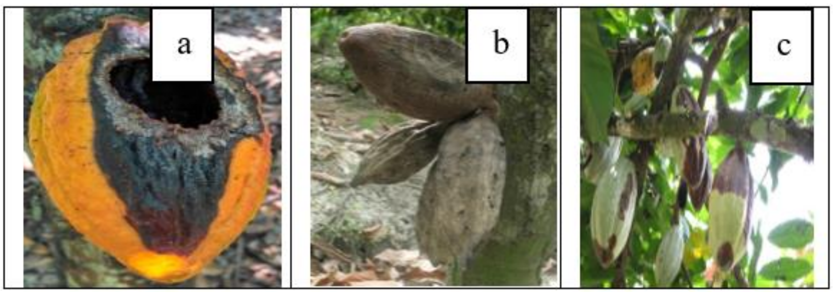

Figure 2. (a) *P. palmivora* has grown and progressed from the opening pit of rats, (b) *P. palmivora* has grown and progressed to meet the pod, (c) Pathogen attack starts from one pod, then spreads to another pod

The results of observation of cocoa fruit attacked *P. palmivora* can be seen in Figures 1 and 2. Initial infection can occur through wounds, both wounds caused by mechanical friction between the fruit to one another, as well as small wounds by pest bites as well as large wounds hole of rat pest attack. Position of initial infection, can occur at the base of the fruit caused by the presence of a momentary puddle of zoospores can sprout and at the tip of the pod caused by hanging raindrops. *P. palmivora* is a water fungus that can stimulate the acceleration of zoospora release from sporangium, followed by zoospores sprout, then instantly invade pod tissue, after incubated 2-5 days can seen the symptoms of brown spots to blackish until finally decay cocoa pod as it knows as cocoa rot pod disease. Hyphae and sporangium of *P. palmivora* can be seen in the Figures 3 and 4.

Figure 3. *P. palmivora* attack on healthy pod

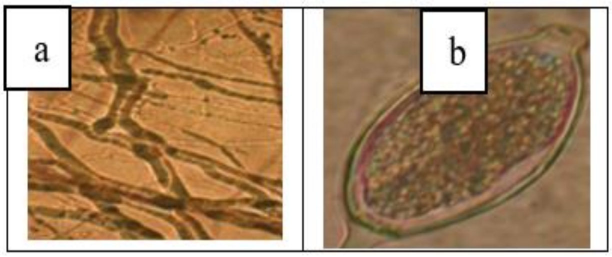

Figure 4. Microscopic observation of *P. palmivora* from affected cocoa (a) Hyphae, (b) Sporangium

P. palmivora attack on healthy pod and microscopic observation can be seen in Figure 3 and 4.

Raindrops can release the mycelium on the infected part of the pod and when accompanied by wind the loose spores will be spread and infections others pod. In addition to rain water droplets, the disease can also be spread through insects i.e.: ants and termites from the soil to cocoa pod. Ants can carry *Phytophthora palmivora* fungus for making a ground nest. This is in accordance with research [29].

Infected cocoa pod have symptoms of dark brown spots that can appear on the base, middle or end of pod. Infected cocoa pod collected from the plantation showed blackish brown spots. The *P. palmivora* spreading is caused by environmental humidity in the rainy season, high humidity helps the spores forming and increased infection in cocoa [30]. Fungus in the ant species *Iridomyrmex cordatus* has the ability to carry many fungus *P. palmivora*, characterized by the appearance of black spots on the surface of the cocoa pod and *P. palmivora* can be isolated back in that pod.

3.2 Symptoms of attacks by *Oncobasidium theobromae*

The symptomps of *O. theobromae* attacks on cocoa plants can be seen in Figures 5 and 6.

Figure 6 shows that symptoms of *O. theobromae* attacks that appear are yellowing and drying leaves and bald ends of twigs. VSD can be caused by the lack of sanitation, because vector insects are commonly found in cocoa waste under the trees, too tight plant spacing and random trimming can incrase spreading of VSD. The branches contact between a healthy tree and a sick tree, or that too close trees faster the transmission, both by wind and friction directly.

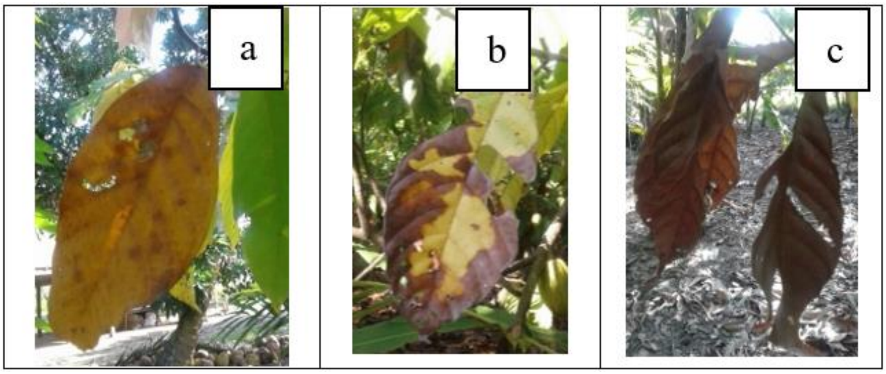

Figure 5. (a-c) Attacks of *O. theobromae* on cocoa leaf

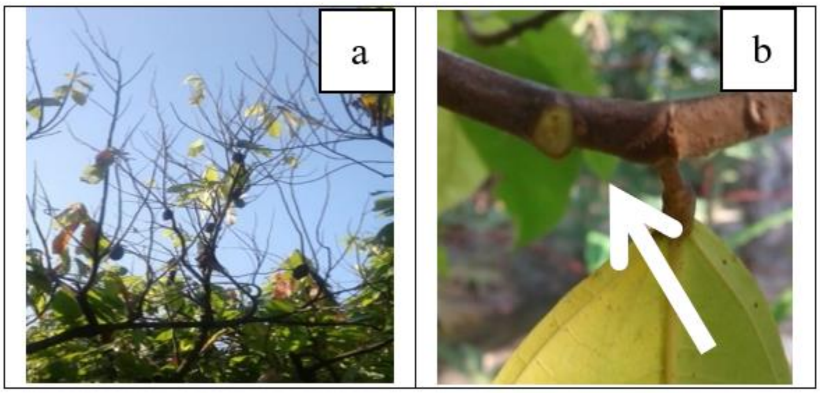

Figure 6. (a, b) Attacks of *O. theobromae* on cocoa twigs

O. theobromae spread by the wind at night during high humidity. The presence of rain followed by moisture is more helpful during the disease spreading. Early symptoms attack on young leaves then spreading infection following xylem vessel tissue [31]. High rainfall conducive to the pathogens development.

The leaves look yellow, drying and then fall off, leaving a bare twig, shown in Figure 4. *O. theobromae* develops in the xylem vein, obstructing the transport of water, nutrients and minerals from the root system to the leaf. Lacking nutrient in leaves, causing metabolic processes disturbed, eventually leaves begin yellowing, dry and fall off. The bare twig will dry out, and this happens systemically until the tree totally dead.

VSD attacks characterized by the first symptoms of yellowing leaves, especially on the first and second leaves, then the disease will enter into the xylem from the tip and base of twigs. Then the leaves will fall off from the branches followed by the drying of twigs and branches of plants [31].

Lacking of sanitation in cocoa farm will cause the pod rot disease causing by *P. palmivora* and Vascular Streak Dieback causing by *O. theobromae* [4].

3.3 The density of conidia of *Trichoderma* sp.

The results showed that the highest conidia density of *Trichoderma* sp on 4 days aftre incubation was achieved on the treatment media P4 (9.76×10^6 conidia ml^{-1}) and it was significantly different with another treatments (P1, P2, P3, P5, and P6). While in the control treatment (P0), there was not found conidia (Figure 7).

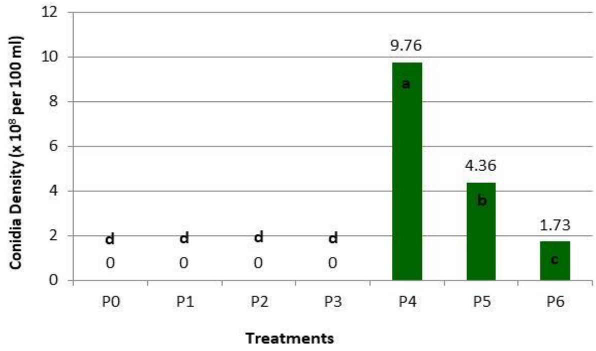

Figure 7. The conidia density of *Trichoderma* sp on various different media compositions

3.4 Morphology cocoa organ infected by fungi *Phytoptohora palmivora* and *Oncobasidium theobromae*

Morphology cocoa organ infected by fungi *P. palmivora* and *O. theobromae* can be seen in Figure 8.

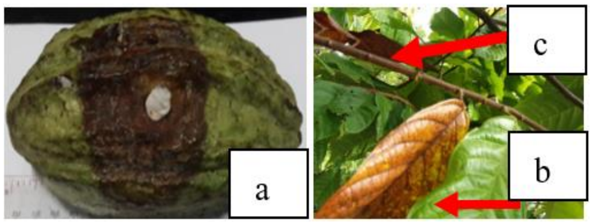

Figure 8. Cocoa pods infected *P. palmivora*.(a), Surface colored leaves tawny (b) roten branch (c)

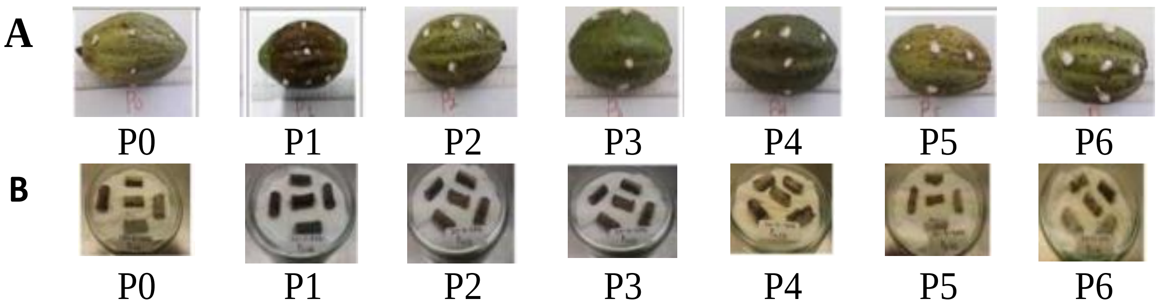

Figure 9. *P. palmivora* on cocoa pods (A), *Oncobasidium theobromae* on cocoa twigs (B)

3.5 The effectiveness of biopesticide to control and inhibit pathogen growth on cocoa pods

Effectiveness of biopesticide to control and inhibit pathogen *P. palmivora* on cocoa pods and *Oncobasidium theobromae* twigs cocoa can be seen in Figure 9.

3.6 Test fungal biopesticides in *Phytoptohora palmivora* and *Oncobasidium theobromae*

1. The percentage of colonization of *Trichoderma* sp. Against *Phytophthora palmivora*

Percentage of *P. palmivora* infection can be seen in Figure 10.

Figure 10 shows that the average percentage of infection with *P. palmivora* in treatment T1 (*P. palmivora* without biopesticides) is 90%, while seven another treatment P0, P2, P3, P4, P5, P6 and P7 treatment showed no colonization.

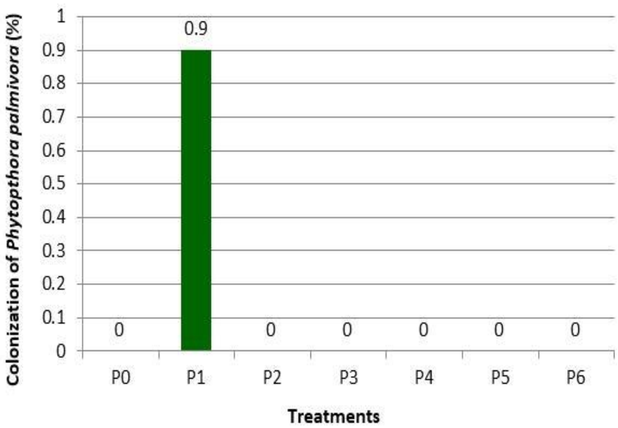

Figure 10. The average colonization percentage of *Trichoderma* sp. against *P. palmivora*

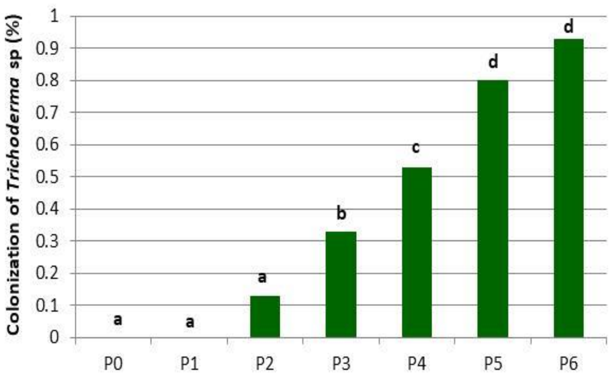

Figure 11. The average colonization percentage of *Trichoderma* sp. against *O. theobromae*

2. The percentage of colonization of *Trichoderma* sp. against *Oncobasidium theobromae*

Results in Figure 11 shows that the highest percentage of colonization of *Trichoderma* sp. against *O. theobromae* after 5 days of incubation was achieved by treatment P6 (100% CW) with an average of 0.93 or 93.33% and the lowest in the treatment P0 (control) with an average of 0%.

Trichoderma sp. produces three types of propagules that can be used as an ingredient in the formula controlling pathogenic fungi on plants consisting of hyphae,clamidiospora and conidia. Hyphae rarely used because hyphae not withstand the drying process. Klamidiospora initially thought to be used as a biopesticide active ingredient because of its ability to survive long enough period of time, but the capacity is very low germination. While conidia have walls thick and fast that the process of germination of conidia most developed in the mass production of a biological control agent [32].

The results showed that soybean industrial wastewater and coconut water have the potential as a growing medium for *Trichoderma* sp. to be used as a biopesticide. Conidia production in the P4 treatment (40% ES + 60% CW) of 9.76×10^6 ml^{-1} was higher than the 100% coconut water waste treatment of 4.56×10^8 per 100 ml and 100%, while in the treatment of 100% soybean industrial waste no conidia were found. This is confirmed by the results of Syahnen's [31], that the *Trichoderma* sp. fungus applied as much as 10^6-10^8 conidia g^{-1} did not show any symptoms of *P. palmivora* infection which can be seen in Figure 3. The presence of biopesticides (*Trichoderma* sp. active ingredients) in cocoa tissue causes *P. palmivora* to be unable to colonize the tissue so that there are no visible symptoms of *P. palmivora* development infection. According Efendi et al [33], that *Trichoderma* sp. able to grow rapidly, compete, and produce antibiotics that broken host pathogen hyphae so as to inhibit the pathogen on the development of broad patches of cocoa fruit rot caused by the fungus *P. palmivora*. Furthermore, Sukorini et al. [34] stated that the suppression mechanism of *Trichoderma* sp. through mikoparasitisme which can inhibit the growth of pathogenic fungi with parasitism and cause lysis of the hyphae *P. palmivora* were able to remodel the cell walls of pathogens because the growth rate is faster than most pathogens.

The results showed that treatment P6 (100% CW) exhibited the highest Trichoderma sp. colonization, with an average percentage of 93.33% more effective against O. theobromae than the other treatments. Notably, treatment P0 (100% distilled water), used as the control, had the lowest effectiveness with an average colonization percentage of 0%. The more conidia contained in a liquid formula, the more hyphae biopesticides that will be generated to find food and a place to live so that there will be competition with mushrooms *O. theobromae* to get a place to live. This is in accordance with the opinion of Berlian et al. [35], that one way the fungus *Trichoderma* sp. to control fungal pathogens in plants is to compete for nutrients and a place to live. Once the fungus *Trichoderma* sp. get a life then *Trichoderma* sp. This will broken pathogenic fungi to take up the nutrients contained in the fungal pathogen [15].

The results of this research confirm the success of *Trichoderma* strains as biocontrol agents against pathogenic microorganisms, which are also expected to improve plant resistance, plant growth and development, leading to increased crop production and promising a clean environment to achieve sustainable agriculture in the future.

4. CONCLUSIONS

Based on the results, it can be concluded that the results showed that treatment P4 (40% soybean extract/ES + 60% coconut water/CW) can be used as a biopesticide because it can maintain and increase the growth of *Trichoderma* sp. until it reaches a density of 10^6 conidia ml^{-1} and produces high conidia, namely 9.76×10^6 conidia ml^{-1}. Furthermore, statistically, the treatment P6 (100% CW). showed the highest percentage of *Trichoderma* sp. colonization against *O. theobromae* colonization with an average percentage of 93.33% higher than the other treatments. Biopesticides made from *Trichoderma* sp. are effective in controlling *P. palmivora*, which causes cocoa pod rot, and *O. theobromae*, which causes Vascular Streak Dieback (VSD). This study confirms that *Trichoderma* sp. can replace the role of fungicides in controlling important cocoa diseases, maintaining soil health and sustainable agriculture.

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REFERENCES

[1] Defitri, Y. (2018). Penyakit Vaskular Streak Dieback (VSD) pada tanaman kakao (*Theobroma cocoa* L.) serta oersentase serangannya. Jurnal Media Pertanian, 3(2): 99-106. https://doi.org/10.33087/jagro.v3i2.70

[2] Harni, R., Amariah, W., Supriadi. (2013). Keefektivan beberapa formula fungisida nabati eugenol dan sitronella terhadap *Phytophthora palmivora* Bult. Asal kakao (The effectiveness of botanical containing eugenol and citronella on *Phytophthora palmivora* Bult. Of cocoa). Bulletin RISTRI, 4(1): 11-18.

[3] Rezita D., Syamsuddin, S., Hasanuddin, H. (2021). Resistance induction of cocoa seeds against fruit rot

disease (*Phytophthora palmivora* Butl.) using rhizobacteria-threated seed. Jurnal Agrista, 25(1): 60-72.

[4] Hamdi, I., Lakani, I. (2021). Tingkat keparahan penyakit vascular streak dieback (*Ceratobasidium theobromae*) pada tanaman kakao (*Theobroma cocoa* L.) setelah pemberian perlakuan infus akar. Agrotekbis, 9(1): 188-196.

[5] Rosmana, A. (2005). Vascular streak dieback (VSD): a new disease on cocoa in Sulawesi. In Proceedings of the Scientific Seminar and Annual Meeting of the PEI and PFI XVI Komda South Sulawesi, pp. 1-7.

[6] Harni, R., Baharuddin, B. (2014). Keefektifan minyak cengkeh, serai wangi, dan ekstrak bawang putih terhadap penyakit vascular streak dieback (*Ceratobasidium theobromae*) pada kakao. Journal of Industrial and Beverage Crops, 1(3):167-174.

[7] Keane, P.J., Flentje, N.T., Lamb, K.P. (1972). Investigation of vascular-streak dieback of cocoa in Papua New Guinea. Australian Journal of Biological Sciences, 25(3): 553-564. https://doi.org/10.1071/BI9720553

[8] Guest, D., Keane, P. (2007). Vascular-streak dieback: A new encounter disease of cocoa in Papua New Guinea and Southeast Asia caused by the obligate basidiomycete *Oncobasidium theobromae*. Phytopathology, 97(12): 1654-1657. https://doi.org/10.1094/PHYTO-97-12-1654

[9] Upara, M., Umrah, U. (2012). Formulasi *Aspergillus* sp. sebagai agen pengendali hayati terhadap *phytopthora palmivora* butler. penyebab busuk buah pada kakao (*Theobroma cocoa* L.) dalam bentuk sediaan tablet. Biocelebes, 6(2): 113-119.

[10] Lengai, G.M.W., Muthomi, J.W. (2018) Biopesticides and their role in sustainable agricultural production. Journal of Biosciences and Medicines, 6(6): 7-41. https://doi.org/10.4236/jbm.2018.66002

[11] Valdez-Ramirez, A., Flores-Macias, A., Figueroa-Brito, R., Torre-Hernandez, M.E., Ramos-Lopez, M.A., Beltran-Ontiveros, S.A., Becerril-Camacho, D.M., Diaz, D. (2023). A systematic review of the bioactivity of *Jatropha curcas* L. (Euphorbiaceae) extracts in the control of insect *pests.* Sustainability, 15(15): 11637. https://doi.org/10.3390/su151511637

[12] Sriwati, R., Chamzum, T., Soesanto, L., Munazhirah, M. (2019). Field application of *Trichoderma suspension* to control cocoa pod rot (*Phytopthora palmivora*), AGRIVITA Journal of Agricultural Science, 41(1): 175-182. http://doi.org/10.17503/agrivita.v41i1.2146

[13] Herlina, L. (2009). Potensi *Trichoderma harzianum* sebagai Biofungisida pada Tanaman Tomat (*Trichoderma harzianum* Potency as a Biofungicide on Tomato Plant). Biosantifika, 1(1): 1-7. https://doi.org/10.15294/biosaintifika.v1i1.35

[14] Agustina, I., Pinem, M.I., Zahara, F. (2013). Uji efektivitas jamur antagonis *Trichoderma* sp. dan Gliocladium sp. untuk mengendalikan penyakit lanas (*Phytophthora nicotianae*) pada tanaman tembakau deli (*Nicotiana Tabaccum* L.). Jurnal Agroekoteknologi Universitas Sumatera Utara, 1(4): 95809.

[15] Purwantisari, S., Hastuti, R.B. (2009). Uji antagonisme jamur patogen *Phytophthora infestans* penyebab penyakit busuk daun dan umbi tanaman kentang dengan menggunakan *Trichoderma* spp. isolat lokal [Test of pathogenic fungus *Phytophthora infestans* antagonism cause of disease rotting leaves and tubers of potato plants by use of *Trichoderma* spp. local isolates]. BIOMA, 11(1): 24-32.

[16] Minarseh, L., Kuswinanti, T. (2023). Organic rice rhizosphere fungi produce cellulase and chitinase as a biological control agent. IOP Conference Series: Earth and Environmental Science, 1230: 012104. https://doi.org/10.1088/1755-1315/1230/1/012104

[17] Zaki, M., Suhendrayatna., Hadi, M., Adha, S. (2017). Optimization of glucose production of cocopeat using whole cell Trichoderma reesei. MATEC Web of Conferences, 156: 01016. https://doi.org/10.1051/matecconf/201815601016

[18] Herman, H., Lakani, I., Yunus, M. (2014). Potensi Trichoderma sp. dalam mengendalikan penyakit vascular streak dieback (*Oncobasidium theobroma*) pada tanaman kakao (*Theobroma cocoa*). Doctoral Dissertation. Tadulako University.

[19] Fitria, E., Kesumawaty, E., Basyah, B., Asis. (2021). Peran *Trichoderma harzianum* sebagai Penghasil Zat Pengatur Tumbuh terhadap Pertumbuhan dan Produktivitas Varietas Cabai (*Capsicum annuum* L.). Indonesian Journal of Agronomy, 49(1): 45-52. https://doi.org/10.24831/jai.v49i1.34341

[20] Hafsan, H., Mustami, M.K., Masriany, M., Aziz, I.R., Mustakim, M. (2018). The utilization of coconut water waste as a growth media of the in-vitro potato cutting. Scientiae Educatia: Jurnal Pendidikan Sains, 7(2): 108-116.

[21] Vigliar, R., Sdepanian, V.L., Fagundes-Neto, U. (2006). Biochemical profile of coconut water from coconut palms planted in an inland region. Jornal de Pediatria, 82(4): 308-312. https://doi.org/10.2223/JPED.1508

[22] Radenahmad, N., Sereemaspun, A., Bueraheng, N., Hutapea, A.M. (2022). Beneficial effects of young coconut juice on increasing skin thickness, enhancing skin whitening, and reducing skin wrinkles in ovariectomized rats. Applied Sciences, 12(3): 1584. https://doi.org/10.3390/app12031584

[23] Bintari, S.H., Purnama, D.F.E., Saputro, D.D., Sunyoto, S., Dewi, P., Mubarok, I. (2022). Microbiological and biochemical tests on tempe production using tempe mold innovation. Biosaintifika: Journal of Biology & Biology Education, 14(2). https://doi.org/10.15294/biosaintifika.v14i2.37700

[24] Mahdi, S.A., Astawan, M., Wulandari, N., Muhandri, T., Wresdiyati, T., Febrinda, A.E. (2022). Formula optimization and physicochemical characterization of tempe drink powder. Current Research in Nutrition and Food Science Journal, 10(3): 1178-1195. https://doi.org/10.12944/CRNFSJ.10.3.31

[25] Nega, A. (2014). Review on concepts in biological control of plant pathogens. Journal of Biology, Agriculture and Healthcare, 4(27): 33-54.

[26] Herlinda, S., Utama, M.D., Pujiastuti, Y., Suwandi dan, S. (2006). Density and viability of spores of *Beauveria bassiana* (Bals.) Vuill. due to sub-cultures and media enriched, and its virulence against larvae of *Plutella xylostella* (Linn.). Jurnal Hama dan Penyakit Tumbuhan Tropika, 6: 70-78.

[27] Gabriel, B., Riyanto, P. (1989). *Metarhizium anisopliae* (Metch) sor: Taksonomi, patologi, produksi dan aplikasinya. Direktorat Perlindungan Tanaman Perkebunan, Departemen Pertanian, Jakarta.

[28] Khalimi, K., Wirya, G.N.A.S. (2009). Utilization of plant

growth promotion rhizobacteria for biostimulants and bioprotectants. Ecotrophic, 4(2): 131-135.

[29] Rosmana, A., Waniada, C., Junaid, M., Gassa, A. (2010). Peranan semut *Iridomirmex cordatus* (Hyminoptera: Formicidae) dalam menularkan patogen busuk buah *Phytophthora palmivora*. Pelita Perkebunan, 26(3): 169-176.

[30] Purwantara, A., McMahon, P., Susilo, A.W., Sukamto, S., Mulia, S., Nurlaila, Saftar, A., Purung, H., Lambert, S., Keane, P., Guest, D. (2015). Testing local cocoa selections in Sulawesi: (ii) resistance to stem canker and pod rot (black pod) caused by *Phytophthora palmivora*. Crop Protection, 77: 18-26. https://doi.org/10.1016/j.cropro.2015.07.005

[31] Syarif, M. (2016). Identifikasi penyakit vascular streak dieback (VSD) dan tingkat serangan serta pengaruhnya pada pertumbuhan kakao di tiga desa kec. Palolo kab. Sigi. Jurnal Sains dan Teknologi Tadulako, 5(2).

[32] Valeria, G.B., Gabriela D.B., Gustavo, R. (2024). Trichoderma spp.: Characteristics and applications. Journal of Applied Biotechnology & Bioengineering, 11(1): 18-22. https://doi.org/10.15406/jabb.2024.11.00354

[33] Efendi, S., Sulistyowati, L., Cholil, A. (2014). Potensi jamur antagonis dari seresah kulit buah kakao untuk menekan perkembangan *Phytophthora palmivora* (Pythiales : Phythiaceae) Pada buah dan kompos kulit kakao. Jurnal HPT (Hama Penyakit Tumbuhan), 2(3): 121-130.

[34] Sukorini, H., Aigahayunindy, F.W., Septia, E.D., Khewkhom, N. (2021). Exploration and effectiveness of *Trichoderma* sp. from Jember and Trenggalek, East Java, Indonesia Cocoa plantation as a biological control of *Phytophthora palmivora*. E3S Web of Conferences, 226: 00022. https://doi.org/10.1051/e3sconf/202122600022

[35] Berlian, I., Setyawan, B., Hadi, H. (2013). Antagonism mechanism of *Trichoderma* Spp. against some soil contagious pathogens. Warta Perkaretan, 32(2): 74-82.