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Assessment of a Formulation of Locally Available Organic Recycling Materials from Indigenous Trichoderma sp. from West Sumatra, Indonesia for Suppressing Vascular Streak Dieback (VSD) Symptoms at Cocoa Plants in the Field

Wulan Komala Sari[1]*, Periadnadi Periadnadi[1], Nurmiati Nurmiati[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University,

Padang 25163, West Sumatra, Indonesia.

*email: 2220422008_wulan@student.unand.ac.id

Abstract—The cocoa industry, a promising commodity in tropical regions, is experiencing a decline in production in West Sumatra, Indonesia, due to plant diseases such as vascular streak dieback. This disease can persist for years within the vascular system, causing damage to the xylem tissue and resulting in declining cocoa yields. For numerous developing countries, the most realistic strategy to attain integrated pest management and unlock maximum crop yield potential involves intensifying initiatives aimed at swiftly restoring the land to its former productivity levels by traditionally utilizing various kinds of organic materials locally available to support the farmer's employed organic recycling practices in plant disease control. The use of indigenous Trichoderma species from West Sumatra involving a locally available organic recycling approach was evaluated in this study to reveal the effectiveness of several methods that are antagonistic to Ceratobasidium theobromae as the cause of VSD. The study was conducted in the field and was conducted in accordance with a completely randomized design. This study demonstrated that indigenous Trichoderma strains, which were isolated and propagated using locally available organic materials, are viable and suitable for future biocontrol. The organic substrates used in this study, including rice, crushed corn, and rice bran, exhibited spore densities of 20.41 x 10^9, 12,36x$10^{9,}$ and 10,85x10^9 spores/mL, respectively. The indigenous Trichoderma with organic formulations, accessible to farmers through techniques such as biopore infiltration and root infusion, yielded the most substantial decrease in vascular streak dieback (VSD) intensity, showing reductions of 18% and 14%, respectively, after 3 months of application. In comparison, samples treated solely with fungicide displayed a reduction of 17.6%. Importantly, combining bioformulations of indigenous Trichoderma with chemical fungicides significantly suppressed the intensity of VSDs by 13%. The current study demonstrated that the application of indigenous Trichoderma species formulated with locally available organic materials is effective in managing vascular streak dieback (VSD) in cocoa plants. Furthermore, the organic materials used for isolating and propagating Trichoderma from soils were found to be viable and confirmed for future biocontrol.

Keywords—Trichoderma, Organic Agriculture, Natural Agent, C. Theobromae

I. INTRODUCTION

The productivity of cocoa in Indonesia is influenced by several factors, primarily the lack of maintenance by farmers and the presence of disturbances from plant pests and diseases. Two significant diseases affecting cocoa in Indonesia are Phytophthora pod rot (PPR) and vascular streak dieback (VSD). Vascular streak dieback (VSD) symptoms in several cacao plantations in West Sumatera were initially reported to be caused by *Ceratobasidium theobromae* and affected cocoa plantations across West Sumatra, with disease incidences between 59% and 100%, and disease severities ranging from 24.29%

Corresponding Author: Wulan Komala Sari

to 44.71% (Trisno et al., 2016). Symptoms of vascular streak dieback disease in cocoa plants in West Sumatra include chlorosis on leaves with green spots on a single leaf, necrosis on leaf tips or leaf margins, but the leaves remain attached to the branches, bare branches due to leaf shedding, three dark brown spots on leaf stalks, brown-colored xylem tissue, and overall defoliation of the plants. These diseases can result in deciduous leaves, barren plants, and cessation of production. Conversely, inadequate garden maintenance can expedite disease transmission in the field. These diseases can result in deciduous leaves, barren plants, and cessation of production. It has been noted that controlling the disease is challenging due to its presence within the vessel network. Hence, there is a need to explore technologies that harness the natural pesticidal properties of plants, which might offer effective control measures (Noveriza et al., 2018). Cocoa cultivation in Indonesia is primarily carried out in smallholder plantations, where management and maintenance are often lacking. Rapidly rising production expenses linked to the growing costs and unpredictable accessibility of energy sources, such as pesticides and fertilizers, have sparked significant attention to cheaper and more environmentally friendly production options such as organic farming.

Utilizing microorganisms that boost plant growth and protect against pests and pathogens, such as Trichoderma fungi, offers a cost-effective and eco-friendly solution. The use of bioinoculants containing *Trichoderma* as an antagonistic agent is widely recognized as a highly efficient biological control method (Guzmán-Guzmán et al., 2023). The use of biological agents for controlling plant diseases has specific properties. With respect to local biological control (indigenous), the ability to control disease in plants is improved by the isolation of *Trichoderma*. A profound understanding of the properties of Trichoderma, including its metabolic activity and interactions with plants and other microbes, can supp ort its effective use in agriculture (Joo & Hussein, 2022). Secondary metabolites have the potential to be used for controlling VSD in cocoa plants. Among them, T. amazonicum LP3 and T. virens LP1 exhibited the greatest potential, with disease suppression rates reaching up to 81.8% and 63.2%, respectively, surpassing or equaling those of chemical fungicides (63.6%). Furthermore, these metabolites were found to increase plant height, leaf count, and girth diameter (Harni et al., 2017). The raw secondary metabolites of two *T. harzianum* isolates in a liquid formula, composed of washed rice residual water, coconut water, and sugar inoculated with *T. harzianum* derived from corn were assessed for their impact on growth, as were the phenolic compounds in cocoa seedlings. The findings showed that the raw secondary metabolites of *T. harzianum* T10 could suppress disease intensity and delay the incubation period by 62.17 and 24.97%, respectively, and were able to increase the phenolic compounds (saponins, tannins, and glycosides) in cocoa seedlings qualitatively (Soesanto et al., 2019). Hence, the present study was conducted to assess the bioformulation of indigenous *Trichoderma* species involving a traditional organic recycling approach from West Sumatra with various application methods in the field.

II. MATERIALS AND METHODS

2.1 Isolation of Indigenous Trichoderma sp.

The isolates were obtained from cultures grown on half sections of mature coconut flesh planted downward in excavated holes measuring 20 cm × 20 cm × 20 cm for 7 days in the soil surrounding fertile cocoa plant trees. A colony with a scattered blue–green or yellow–green pigment (Siddiquee, 2017) became observable when conidia formed on the surface of the coconut flesh and was subsequently inoculated onto PDA media to obtain single colonies of green mold. Subsequently, they were regenerated on a PDA slant medium and incubated at a temperature of 25°C for 5–7 days maintain a pure culture (Soesanto et al., 2019).

2.2 Multiplication of Indigenous Trichoderma sp. Using Locally Available Organic Substrates

The multiplication of Trichoderma sp. using three kinds of locally available organic substrates, rice, corn, and rice husk, was performed by inoculating the isolate into half-cooked rice, corn, and rice husk. Half of the substrates cooled into a prepared plastic container, approximately 200 - 300 grams, were cooled with a spoon while lighting a candle to neutralize the surroundings during packaging. Then, the packaged substrates were steamed in a plastic bag for 1 hour. After cooling, the bacteria were mixed with *Trichoderma* sp. isolates using a spoon that had been sterilized with alcohol and heated over the candle (the candle should remain lit during the mixing process). The Trichoderma sp. isolates (1/3 spoonful) were added to each plastic bag, and the bag was shaken to ensure the even distribution of the medium and *Trichoderma* sp. isolates. The open end of the plastic bag was folded, and only the edges and center were stowed, leaving the other edge slightly loosened for easy opening when *Trichoderma* sp. became active. Then, the samples were stored in a dimly lit place at a slightly humid room

temperature. The process is considered successful when the substrates change evenly to a green color (Anwar et al., 2021). The viability of indigenous *Trichoderma* in every substrate was determined by measuring the additional weight on the third and fifth days and then calculating the average spore density/conidia density of *Trichoderma* sp. on various multiplication media. To calculate the spore density, a dilution of 10-2 was used to facilitate the observation and calculation process. Some support tools, such as a Neubauer Improve type hemocytometer, a light microscope at 400x magnification, and a hand counter, were used to calculate spore density. The provision for calculating spore density refers to the protocol of SNI 8027.3:2014 Appendix C pages 6-9 concerning the Conidium Density Test. The spore count was carried out on two hemocytometer fields, 5 squares were counted in each field diagonally, and three replications were carried out. The number of spores in each replicate was counted and averaged. An illustration of the spore count according to the Indonesian Ministry of Agriculture (2014) is shown in Figure 1 (a-b).

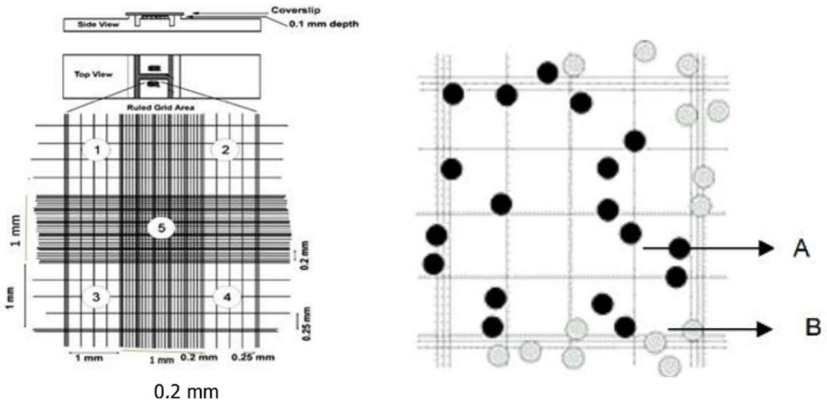

Figure 1. Procedure for calculating spore density. (a.) Five diagonal squares for spore counting. Box number 5 has an area of 1 mm^2 divided into 25 squares, so boxes a, b, c, d, and e each have an area of 0.04 mm2 (b.) Spores to count (A) and not count (B). The borders of the right and bottom are not counted.

The spore densities were determined according to the methods of the Indonesia Ministry of Agriculture (2014).

$$S = \frac{X}{L(mm^2) \times t(mm) x d} \times 10^3$$

where S is the abbreviation for spore density (spores/mL); X is the number of spores in boxes a, b, c, d, and e; L is the area of the calculated box; t is the calculated depth of field; d is the dilution factor; and 10^3 is the calculated suspension volume.

2.3 Bioformulation Preparation of Indigenous Trichoderma sp.

The bioformulation of indigenous *Trichoderma* underwent processing to extract its raw secondary metabolites. This process involved combining 8 liters of washed rice residual water, 2 liters of coconut water, and 10 grams of sugar per liter, which were then sterilized and transferred into a sterile jerry can (Soesanto et al., 2015). Subsequently, the cooled medium was inoculated with 150 grams of *Trichoderma* sp. obtained from crushed corn medium mixed with 250 millilitres of sterile water. After thorough stirring to achieve homogeneity, the mixture was filtered and transferred to jerry cans. These cans were then subjected to shaking for 7 days at room temperature while maintaining a speed of 150 rpm. Following this incubation period, the density of conidia per milliliter of solution was determined to be $10^{7,}$ and the conidia were filtered using Whatman no. 42 filter paper.

2.4 Field applications and biocontrol treatments

The application of *Trichoderma* sp. bioformulation and fungicides was carried out twice a week, with 4 application methods performed using *Trichoderma* sp. bioformulation via root infusion by cutting the lateral roots of the cocoa plant (diameter ± 1 cm) at 2 points, namely, North and South. Next, the roots were put into a plastic bag, which was composed of a secondary metabolite suspension of 1 liter/plastic bag with a concentration of 1:10 (1 liter of metabolite suspension: 10 liter of water)

Trichoderma sp. Bioformulation through a biopore infiltration hole was performed by stringing 3 plastic bottles with the bottom part removed and the cross-section of the bottle perforated using a sharp object and a larger bottle cut in half to cover the biopore pipe, which was then planted down to the soil near the root system at a depth of 40 cm and placed between 2 trees at least 50 cm from the main tree of the cocoa plant. Then, 1 liter of *Trichoderma* sp was added to the bioformulation solution in the morning or afternoon. A single systemic difenoconazole fungicide was applied at one-month intervals by spraying all parts of the plant, each with a concentration of 2 ml/l and a volume of ±250 ml/tree (Harni & Baharudin, 2014), and 10 ml of *Trichoderma* sp. bioformulation was supplemented with fungicide according to the recommended dose by directly pouring 1 liter per tree at the base of the cocoa stem.

2.5 Field Observations Parameters

Observations for three months were conducted to assess disease symptoms and progression. This involved monitoring the percentage and intensity of diseases each month as well as evaluating the efficacy of the formula after the observation period. Observation of disease intensity was performed on each tree by counting the number of leaves and branches

affected by the disease based on the attack category in Table 1. The intensity of the disease was calculated by the formula of Strange (Noveriza, 2018):

$$I = \left(\frac{\Sigma(ni \times vi)}{z \times n}\right) \times 100\%$$

I = intensity of disease; ni = number of plants in each attack category; vi = scale values of each attack category; Z = the scale value of the highest attack category; N = number of plants observed.

Table 1. Scoring of VSD symptoms in cocoa plants

Scoring	Attack category	Symptom
0	Healthy	0% are infected
1	Light Mild	1-10% of leaves are infected.
2	Mild	11-50% of leaves are infected, chlorosis, necrosis, deciduous leaves, and existing lenticell swelling.
3	Light Heavy	51-75% of leaves are infected, chlorosis, necrosis, deciduous leaves, lenticell swelling, and there is a fruit body.
4	Heavy	>75% of leaves are infected, chlorosis, necrosis, deciduous leaves, lenticel swelling, there is a fruiting body and there are dead branches.

2.6 Data analysis

1. Quantitative data such as disease intensity data were subjected to ANOVA followed by Duncan's multiple range test to determine the significance of differences between the treatments and the control, and a significance level of $p < 0.05$ was applied, while fungal viability was descriptively analyzed. ANOVA was performed using IBM SPSS Statistics 25.0.

III. RESULTS AND DISCUSSION

3.1 Indigenous Trichoderma isolated from coconut traps

The isolates were obtained from cultures grown on half sections of mature coconut flesh, as shown in Figure 2. After reisolation in PDA medium, a pure culture was obtained and maintained for viability testing.

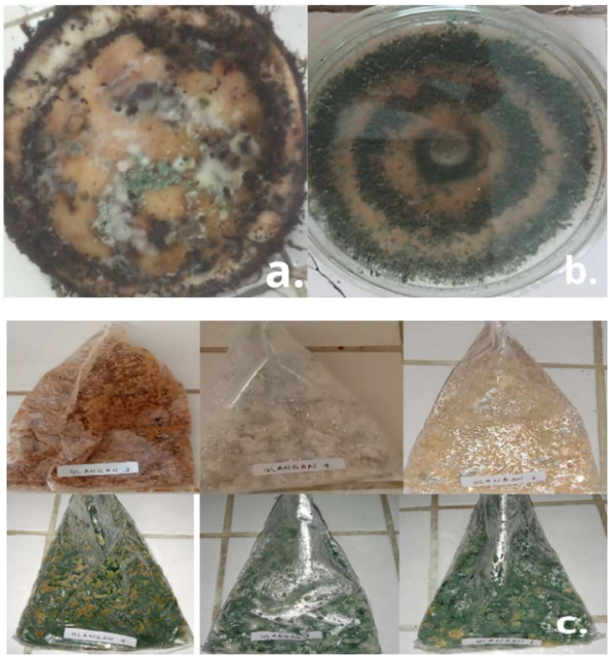

Figure 2. a. Greenish moldy surface of coconut flesh after 4 days of incubation, b. a pure culture obtained after several reisolations to PDA media, c. multiplication of Trichoderma in rice bran, rice, and crushed corn.

IV. CONCLUSION

The current study concluded that isolating indigenous Trichoderma sp. from healthy plant soils using split coconut and propagating it with locally available organic substrates, such as rice, crushed corn, and rice bran, effectively generates a viable propagation medium. This medium adheres to established quality control standards, ensuring its suitability for future field applications. Additionally, this study demonstrated the biocontrol potential of indigenous Trichoderma formulated with locally available organic materials, both individually and in combination with fungicides, against vascular streak dieback (VSD) for three months. This research confirmed that indigenous Trichoderma organic material formulations can serve as alternatives to fungicides for biocontrol purposes. Therefore, for intensive VSD control, the application of indigenous Trichoderma sp. formulated with locally available organic materials through various practical techniques in the field is recommended as an economically viable and efficient practice.

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