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The Potential of Local Isolated of Entomopathogenic Fungusas BioinsecticideAgainst Termite Pests on Cocoa (*Theobroma cacao* L).

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Abstract: *Cocoa (Theobroma cacao L) is an essential commodity in the Indonesian plantation sector. However, the recent cocoa production, especially in Aceh, is decreasing. Plant-disturbing organisms, such as termites, trigger this condition. Then, the control has been carried out using chemical pesticides that can pollute the environment. It is necessary to use eco-friendly pesticides such as the use of entomopathogenic fungus. Therefore, this study determines the potential of local isolates of entomopathogenic fungus originating from cocoa plantations to be bioinsecticides. The fungus was explored by soil sampling and dead insects from cocoa plantations. The isolates from each sample were purified and identified. Then it is tested on termites. The isolates application was applied by suspending entomopathogenic fungus to termites and observed until 1-5 DAS. The research results showed that the highest mortality was found in fungus from dead insects (C3), 95%. Then fungus in the roots of the second block of cocoa plants (C2) is 87.5%, and fungus on the roots of the first block of cocoa plants (C1) is 85%.*
Keywords: *cocoa, termites, entomopathogenic fungus.*

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I. Introduction

Cocoa (*Theobroma cacao* L) is a plantation commodity that plays an essential role in the Indonesian economy. Indonesia's widespread cocoa plantations area is proved by the widespread cocoa plantations area, 1,704,982 hectares (Directorate General of Plantations, 2016). However, productivity is inhibited due to termites.

Termites are pests that attack the plant stem base. Then, it causes the plant to porous, then died. The attack is getting wider to the stem of the plant. Sometimes, they form a travelling tube around the plant stem to attack the plant (Sayuthi, 2012). This pest can cause direct physical damage to plants and decrease harvest yield. Correspondingly it affects substantial economic losses (Nandika *et al*., 2003).

Currently, the use of pesticides is the main pest-control technique. However, unconsciously it triggers a negative impact on the environment. Indeed, alternative pest control is needed that is eco-friendly. Integrated pest control can be used as biological agents such as entomopathogenic fungus (Oka, 1998).

The most widely used entomopathogenic fungus for biological control of insect pests is *Beauveria bassiana* Balsamo (Trizelia, 2005), *Metarhizium anisopliae* Metch (Prayogo *et al*., 2005), *Aspergillus* sp. (Nur, 2005). The fungus has a pathogenic character to various types of insects with a wide organism range. The ability of the entomopathogenic fungus to kill insect pests varies and is strongly influenced by the physiological and genetic characteristics of the fungus (Trizelia 2005).

Entomopathogenic fungi are known to exist in nature. Therefore, exploration is necessary to obtain local isolates of entomopathogenic fungi, which can then be used as bioinsecticides to control termites.

II. Material and Methods

This research was carried out in 2 stages. The first stage was exploring entomopathogenic fungus from cocoa farmers' land, Treinggadeng District, Pidie Jaya Regency. The second stage was testing the potential of isolated fungus against termites at the Plant Protection Laboratory, Plant Protection Study Program, Agriculture Faculty, Syiah Kuala University.

Taking fungal isolates in cocoa plantations

Isolation of the fungus in the field was carried out at 2 points using the diagonal method (Nurudin, 2018), namely:

1. Soil sampling around cocoa plants that were attacked by pests and healthy cocoa plants. The soil sampling was diluted to obtain a dilution level of 10^{-7}. Then the suspension is poured on PDA media to determine the fungus that grows from the suspension of the soil sampling.
2. Taking the dead insects infected with fungus around the cocoa plant. Then the infected insects by the fungus were sterilized. It is distilled water and placed in damp tissue, then planted on PDA media to determine the growth of the fungus infected the insect.

Identification of fungus from cocoa plantation

Identification was conducted by characterizing each fungus colony that grew in colour, shape, and pattern of colony distribution. It was taken using an ose needle and then re-grown on new PDA media. Macroscopic observations included colony colour, colony texture, colony diameter, and colony shape. Microscopic observations were conducted by taking some selected isolates using a needle and observing with a microscope. The microscopic observation parameters included hyphae morphology, conidiophores shape, size and shape of conidia, and unique characteristics to determine the type of fungus using the Watanabe (2002), Barnett & Hunter (1972), and Gandjar & Indrawati (2000) manuals.

Fungal applications on test pests

The testing for termites was carried out by placing the termites in a sterile petridish that had been given a moistened straw paper using distilled water as food. The termites used consisted of 10 pests/petridishes. According to the treatment, the termites were dripped with fungal suspension into the petridish, while the control termites just treated by giving aquadest. Observations were made from 1-5 DAS by recording the number of termites that died in each treatment. Observation of test termite mortality was carried out by counting the dead and living termites. Calculating the percentage of termite mortality using the formula (Abbot, 1925 in Prijono, 1999):

$$Po = \frac{r}{n} x\ 100\%$$

Information:

Po : Termite mortality
r : Number of dead termites
n : Total number of termites

III. Results and Discussion

Exploration and identification result of fungus.

The exploration resulted in 3 fungi from 2 sampling points from cocoa fields. The results of the identification of entomopathogenic fungus showed almost the same macroscopic characteristics. For microscopic characteristics, the shape of the conidiophores and conidia of each fungus was observed. The identification of entomopathogenic fungus can be described as follows.

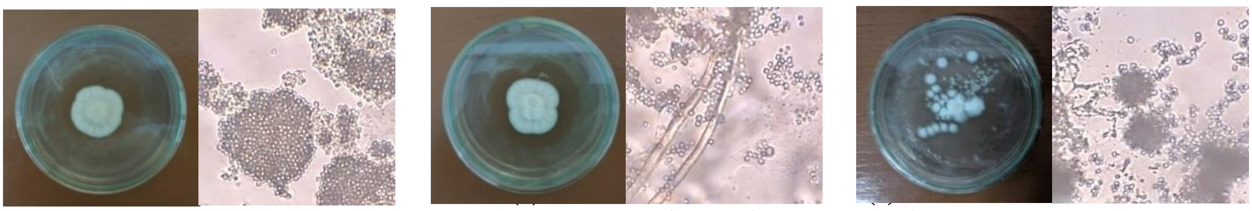

Figure 1. Macroscopic and microscopic forms of local isolates fungus.

Notes: (a) fungus from the roots of the first block of cocoa plants. (b) the fungus from the roots of the second block of cocoa plants. (c) the fungus from dead insects on cocoa plants.

Table 1. Macroscopic morphological characterization of an entomopathogenic fungus after 7 Days After Isolation

Fungus Source	Location	Fungus Macrocopic Characteristic			Fungus MicroscopicCharacteristic		
		Colony Color	Colony Shape	Colony Growing Direction	Hyphae density	Conidia	Conidiophores
Cacao Root (C1)	The first block, sloping ground.	White	Rounded	To the side and up	Solid	Present, Hyaline Colored	Present

Cacao Root (C2)	The second block, flat land.	White	Rounded	To the side and up	Solid	Present, Hyaline Colored	Present
Dead Termites (C3)	The first block, sloping ground.	White	Spread	To the side	Solid	Present, Hyaline Colored	Present

Those three fungi have characteristics such as the *Beauveria* fungus, white like cotton, and lumps of flour. It also states by Barnet & Hunter (1972) states that the *Beauveria* fungus is white with an appearance like being covered with powder. According to Trizelia *et al*., (2015), the characteristics of the *Beauveria* fungus are microscopical. The fungus conidiophores erect and single with tapered conidiophores. At the tip of the conidiophores, there are conidia which are round, single-celled, and hyaline in colour.

The identification results also show that entomopathogenic fungus can be found in the soil or plant roots and dead insects in the plantation area. According to Desyanti (2007), isolates of entomopathogenic fungus result from various hosts (organism) or inoculum sources in nature. It indicated that the diversity of entomopathogenic species was higher than in hosts (organism) from plant pests (81%) compared to those from soil (13%) and sand (6 %). An entomopathogenic fungus from soil and sand inoculum sources had lower species diversity. It is possible because entomopathogenic fungi live as obligate pathogens, and many also live as facultative pathogens. It means that besides, they can continue their life cycle as pathogens to host insects. They can also survive as saprophytes on various media in nature.

The diversity of entomopathogenic fungus in the soil can be influenced by several factors, such as soil water content, organic matter content, and temperature (Sosa-Gomez *et al*., 2001). Sutanto (2002), states that organic matter will increase the energy needed for the life of soil microorganisms. Soil rich in organic matter will accelerate fungus, bacteria, microflora, and another microfauna. According to Rao (1994), the type and amount of compounds released by plant roots depend on the plant species, age, and environmental conditions in which the plant grows.

Fungus application on test pests

Testing fungi against termites is done by calculating termite mortality to determine the level of fungal infection in termites. The results of observations made showed that the average mortality of termites due to the application of several fungi from cocoa plantations generally influenced by the treatment. The highest average termite mortality was found in fungi from dead insects (C3), which reached 95%, then fungi on the roots of the second block of cocoa plants (C2) which was 87.5% and fungi on the roots of the first block of cocoa plants (C1) which was 85%. Some of the dead termites have fungi that grow on the termites to cover their entire body with fungal mycelium. According to Steinhaus (1963), fungi that can be categorized as bioinsecticides are fungi that successfully control insects by 72-95%.

Table 2. Average Mortality of Termites Due to Application of Fungi from Cocoa Plants.

Fungus	Termite Mortality				
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS
Cacao root first block (C1)	15	27.5	45	72.5	85
Cacao root second block (C2)	12.5	30	50	80	87.5
Dead Termites (C3)	22.5	40	65	90	95

(a) (b) (c) (d) (e)

Figure 2. Termites infected with fungus from the roots of the first block of cocoa plants (C1).
(a) 1 DAS (b) 2 DAS (c) 3 DAS (d) 4 DAS (e) 5 DAS.

(a) (b) (c) (d) (e)

Figure 3. Termites infected with fungus from the roots of the second block of cocoa plants(C2).
(a) 1 DAS (b) 2 DAS (c) 3 DAS (d) 4 DAS (e) 5 DAS.

(a) (b) (c) (d) (e)

Figure 4. Termites infected with fungus from dead insects in cocoa plantations (C3).
(a) 1 DAS (b) 2 DAS (c) 3 DAS (d) 4 DAS (e) 5 DAS.

After applying the fungus, the infected termites showed early symptoms with reduced activity,and the abdomen turned to pale white. Then it slowly turned black hardened, and mycelium began to grow from the head to the abdomen. Tanada & Kaya (1993) stated that the host (organism) became less active and stressed after infection. When it has entered the final stage of infection, termites become weak, paralyzed, and die. Boucias & Pendland (1998) said that entomopathogenic fungi are characterized by their ability to adhere and penetrate the cuticle of the host and grow to the internal part of the host (organism) and consume them. So that the rapid growth depletes the nutrients in the hemolymph of the fungus, then it causes the host (organism) to die, and it bossy hardened.

Transmission of entomopathogenic fungus to their hosts (organism) may happen through grooming behaviour (touching), namely friction or touching between individual termites when they gather in their colonies. Then, it causes the transmission of fungal conidia from sick individuals to healthy individuals (Strack, 2003). Surtikanti & Yasin (2009) argue that the increase in mortality occurs when there is contact between insects and fungal spores. Upon contact, the spores form a germination tube and secrete enzymes to soften the insect's cuticle so that the spores can enter the insect's body. The growth of spores in the body of insects will disrupt all organ activities and result in the death of insects.

IV. Conclusion

The Exploration results that three fungi were found from cocoa plantations. Isolates resulting from exploration from natural sources had characteristics such as the Beauveria fungus. The test results on termites showed that the highest termite mortality was found in fungus from dead insects (C3), which was 95%, then fungus on the roots of the second block of cocoa plants (C2), which was 87.5%, and fungus on the roots of the first block of cocoa plants (C1), which was 85%.

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