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Investigation of Entomopathogenic fungi of Soil Source in Plantation Cultivation Areas of North Kalimantan

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Abstract: Exploration and investigation in various locations are essential for identifying biological agent fungi that can be developed for pest control. This study determined identifying and comparing potential entomopathogenic fungi from different plantation areas. Soil samples were collected from oil palm (Sebuku), cocoa (Sebatik), and coffee (Malinau) plantations. We used *T. mollitor* insects to isolate and select the entomopathogenic fungi. We compared the mortality rates of insects exposed to each fungal isolate against a control group during the selection process. *t-tests* and descriptive analysis were utilized to evaluate the data. This study identified fifteen different fungal isolates. Most of these isolates were obtained from coffee plantations or areas in Malinau. However, only four isolates showed potential as entomopathogens. Furthermore, isolate ST16^{-3}, when diluted to a concentration of 10^{-1}, exhibited higher virulence than the other isolates. The virulence is reached 50.5%. The findings of this study suggested that plantations areas may potentially source of fungi entomopathogens.

Keywords: Fungi; Entomopathogenic; Plantation; North Kalimantan.

Introduction

North Kalimantan Province has high area of plantation crop cultivation. The area of cultivated land for oil palm plantations in 2022 reached 38,061.3 ha, while the coffee plantations reached 852.8 ha and the cocoa plantations reached 2,572 ha (Badan Pusat Statistik, 2023). Fluctuating prices, weather, and plant pests (OPT) are factors that affect the increase in plantation crop production.

The pest group always causes high-yield losses. cocoa pod borer pests decreased around 95.7% production in Indonesia (Hayata, 2016). Meanwhile, the species that was most frequently identified was *Setothosea asigna*. The extent of plant damage differed, with an average of 27.46%, classified as moderate damage. Insects that attack oil palm plants can significantly decrease their productivity, highlighting the need for effective pest control measures to lessen their effects (Sapria et al., 2025), and coffee berry borer pests loss of yield reach 30% - 50% in coffee cultivation areas (Venkatesha & Kiran, 2024). Therefore, one of the important factors that must prevent a decrease in production. Its carry out pest control or suppress the development of pest populations.

The synthetic chemicals are the main choice in pest control in Indonesia. However, this control is driving pest resistance and resurgence, as well as environmental pollution. Then, that it causes residues in plants of agricultural production (Torres & Bueno, 2018). To reduce these negative effects, biological control is applied in the planting area. The principles of biological control are the control of plant pests by utilizing their natural enemies and using biological agents, such as predators, parasitoids, and entomopathogens (Stenberg et al., 2021)

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Biological control is an alternative of pest control which are using the biological agents. The agents consisted of entomopathogens (Souza & Marucci, 2021) and microorganism antagonists (Maake & Sibisi, 2025). There are several groups of microbes as biological agents that is bacteria, viruses, fungi, protozoa, mycoplasma, and nematodes (Gaur & Sharma, 2021). For Example, the species *Metharizium anisopliae*, *Beauveria bassiana*, and *Lecanicillium lecanii*, are often biological agents or entomopathogenic fungi (Bamisile et al., 2021). Entomopathogenic fungi and other biological agents from microbes has an impact on improving the environment for a long-term effect on their control (Khun et al., 2020).

Entomopathogens have an essential role in crop cultivation areas which is to control insect populations and remain safe for non-target insects (Khun et al., 2020). However, the success rate of using entomopathogens is largely determined by the ability of microbial biological agents to adapt to the environment (Bardin et al., 2015). For instance, compared to the usage of synthetic pesticides, study of three biological pesticides revealed a weak effect on lowering bug populations (Rahim et al., 2024).

The result discovered the potential for entomopathogenic fungi originating from crop cultivation areas. It has a high mortality rate, and the potential to develop as a local biological agent in the area (Nelly et al., 2019).

This study aims to find that plantations in North Kalimantan also have the potential as a source of biological agents, so that they can overcome the lack of more adaptive biological agents in that location.

Method

Location of Soil Sampling

Soil sampling in the coffee plantation area in North Malinau sub-districts in Malinau regency. Also, Sebatik and Sebuku sub-districts in Nunukan Regency for coffee and cocoa, respectively. Soil samples were taken at 20 locations per location with the weight of each sample (± 100 g).

Isolation of the fungus in the soil

Isolation of the fungus was carried out by mixing 10 g of soil sample with 100 mL of sterile distilled water. The suspension was shaken evenly and heated in a water bath at 80°C for 10 minutes. Subsequently, serial dilutions were performed up to 10^{-5}. The serial dilution results were taken 0.1 mL, and speeded on *Potato Dextrose Agar* (PDA) media. Then the petri dishes were wrapped in plastic wrapping and incubated for 3 days.

Purification aims to obtain the desired pure culture without any contaminants from other microbes. The selection of purified microbial colonies was based on differences in the morphological appearance of the colonies. The characteristics were examined in color, elevation, and surface texture so that pure isolates were obtained in each colony. In the process of transferring into PDA, media Purification of the fungus isolate was carried out by moving the fungus using the point method.

Selection of fungal isolates as potential entomopathogens

Selection were used the insect bait methods. We were put soil + 50 gr into the container, then put too the bait insect *Tenebrio mollitor* (Coleoptera: Tenebrionidae). Furthermore, the soil was inoculated with a fungus isolate that had gone through a 10^{-1} dilution process, at a rate of 3 ml per container. Thus, a selection test will be obtained using control, 10^{-1} dilution, and without dilution or stock. Incubation was carried out for 14 days at room temperature (23°C – 25°C).

We were analyzed the mortality rates of the test insects and described the macroscopic characteristics of the isolates in each of research site. Meanwhile, we compared mortality between isolates potentially as entomopathogenic fungi. The value of the test statistic calculated a small sample with the form of a hypothesis, namely H_1: $\mu \neq \mu_0$ or there is no difference between the control and the isolate (Putri et al., 2023), with;

$$t_h = \frac{\bar{x} - \mu_0}{s / \sqrt{n}} \quad ; \; | \, t_h \, | > t_{(\alpha/2;\, db=n-1)}; \qquad (1)$$

where, $\bar{x}$, he mean of the observation; μ, population mean; σ, population variance; n, sample.

Result and Discussion

Isolate fungi from plantation soil

From 60 soil samples in the plantation, we were found 15 isolate the fungus PDA media. There were differences number of isolates from one location to another. In coffee cultivation at North Malinau, we were found nine isolates, and it is the biggest among another location. Then, the cocoa cultivation had four isolates, and the oil palm plantation in had two isolates (Table 1).

The soil of the coffee plantation area, the characteristics of colonies were dominated by white, yellowish-white, and green colors. The edges of the colony were flat and convex, and the surface was flat and convex, then there are fungi that form mycelium. Furthermore, isolate from soil in the cocoa cultivation area showed white, yellowish-white, greenish-yellow, and green colors. The edges of the colony were flat with surfaces predominantly convex, and some isolates were flat. Then, isolation from the soil of the oil palm cultivation area showed white, yellowish-white, greenish-yellow, and green colors colonies. Meanwhile, the edges of the colony were flat but were predominantly convex. In addition, the surfaces were convex, and some formed mycelium (Table 1).

There were fifteen fungal isolates from 60 observation points in plantation cultivation areas. This is due to the environmental conditions in the plantation area that support the presence of fungi that grow and develop. Several studies on the exploration of entomopathogenic fungi also used PDA media, for example in the rhizosphere area of horticultural plants (Permadi et al., 2018), vegetable cultivation area (Trizelia et al., 2015), peatlands in Kalimantan (Sebayang et al., 2022), and forest areas in West Kalimantan (Halimah et al., 2018). However, the fifteen isolates from sixty soil sampling points obtained in this study were still very low. It is suspected that not all fungi grow well using PDA media or require other selective media. Other exploratory research using SDAY media (*Saboraud Dextrose Agar with Yeast Extract*) (Nelly et al., 2019), media containing *Potato Dextrose Agar Yeast Extract* 0,5% (PDAY 0,5%), able to increase the number of isolates obtained from the results of exploration (Wawan et al., 2018).

This study also showed that there were differences in the number of isolates between one region and another. This is presumably because there are different cultivation practices, including pest control efforts. Application of pesticides are determining the potential of the fungal isolates in soil. In addition, the organic fertilizers potentially add microbes to the soil and cause microbes to survive and thrive (Lori et al., 2017). For example, the results of other studies found only one genus of entomopathogenic fungi in the cultivation area of horticultural crops, due to the low content of soil organic matter from the rhizosphere, as well as the intensive use of pesticides (Permadi et al., 2018). Further investigation into entomopathogenic bacteria revealed that among the 13 isolates, only 7 had the potential to serve as biological agents (Rahim et al., 2022).

The another study explained that pH, soil type, altitude, habitat, soil temperature and plant types affect the distribution of entomopathogenic fungi (Zheng et al., 2024). The content of organic matter has a considerable impact on soil microbial communities, as it provides essential building materials and energy for these microbes (Afandhi et al., 2020). The limited number of isolates found in these three ecosystems suggests that organic matter levels are relatively low.

Potential isolates of an entomopathogenic fungi.

The selection overall showed a corrected mortality rate between 0–80%. The selection for isolates from the coffee plantation were corrected the mortality rate between 0–55%. MT18^{-3} isolate without dilution (stock) had the highest mortality percentage. Meanwhile, the lowest corrected mortality rate was in isolate MT7^{-1} stock and isolate MT15^{-1} with dilution 10^{-1} (Table 1).

We were checked the mortality rate between 13.3%–80% in soil of cocoa plantations. Furthermore, SBT6^{-1} isolate without dilution had the highest mortality percentage. Meanwhile, the lowest corrected mortality rate was in SBT10^{-1} and SBT14^{-1} isolates without 10^{-1} dilution of 13.3%. Furthermore, the corrected mortality rate of isolates from soil of oil palm plantations were between 20%–63%, then the highest corrected mortality rate was isolate ST12^{-2} without dilution (stock) of 63%. Then, the lowest corrected mortality rate was in isolate ST12^{-2} at 10^{-1} dilution of 20% (Table 1).

Table 1. The percentage (%) mortality rate of larvae in the entomopathogenic potency test of soil-origin isolates in plantation areas.

Origin Location/ Isolate Code	MC	MS	MD 10^{-1}	MCS*	MC* D10^{-1}
Malinau (Coffee)					
MT11-1	0.00	13.33	20.00	13.33	20.00
MT15-1	0.00	20.00	13.33	20.00	13.33
MT15-5	0.00	20.00	6.67	20.00	6.67
MT18-2	0.00	33.33	0.00	33.33	0.00
MT18-3	13.33	60.00	26.7	53.83	15.38
MT18-4	0.00	20.00	20.0	20.00	20.00
MT7-1	0.00	13.33	40.0	13.33	40.00
MT7-5	0.00	0.00	40.0	0.00	40.00
MT9-4	0.00	13.33	20.0	13.33	20.00
Sebatik (Cocoa)					
SBT10-1	0.00	46.67	13.33	46.67	13.33
SBT11-1	6.67	33.33	40.00	28.57	33.33
SBT14-1	0.00	33.33	13.33	33.33	13.33
SBT6-1	0.00	80.00	40.00	80.00	40.00
Sebuku (Oil Palm)					
ST12-2	6.67	66.67	26.67	63.33	20.00
ST16-3	0.00	46.67	53.33	46.67	53.33

Notes: **Formula Abbot; MC, mortality of control; MS, mortality of stock, MD, mortality of dilution, MCS, mortality corrected of stock, MCD, mortality corrected of dilution*

The selection showed that the control treatment or bait insects were still alive and carrying out their activities, such as molting (Figure 1). Meanwhile, bait insects that experience physiological disorders were shown with a weak body until they die. The symptoms of larvae death where the color of the hyphae was white, yellow and colorless. While the body of the insect is orange-black and black (Figure 1).

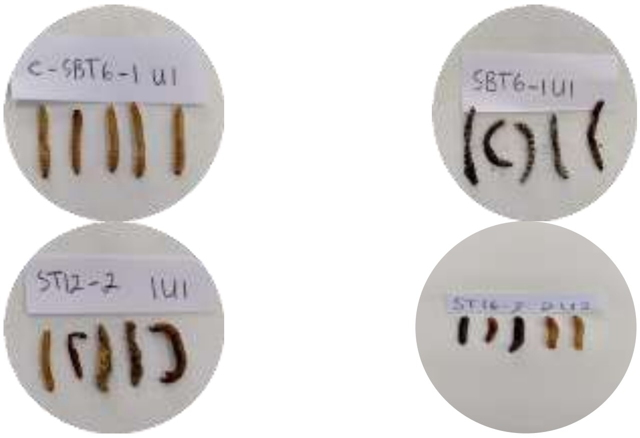

Figure 1. Characteristics of insect death selection of fungal isolates

Furthermore, statistical analysis was showed that there were differences with the controls. Statistical analysis was performed on all isolates except SBT5^{-1}. The analysis was compared the two corrected control mean mortality rates with all isolates using the *t-test*. The results showed that there were 3 (three) isolates without dilution (stock) which had different median values than the control isolates, namely isolates SBT6^{-1}, SBT14^{-1}, and ST12^{-2}. Further investigation revealed that isolate ST16^{-3} was the only 10^{-1} dilution isolate whose mean value was different from the control isolate (Figure 2).

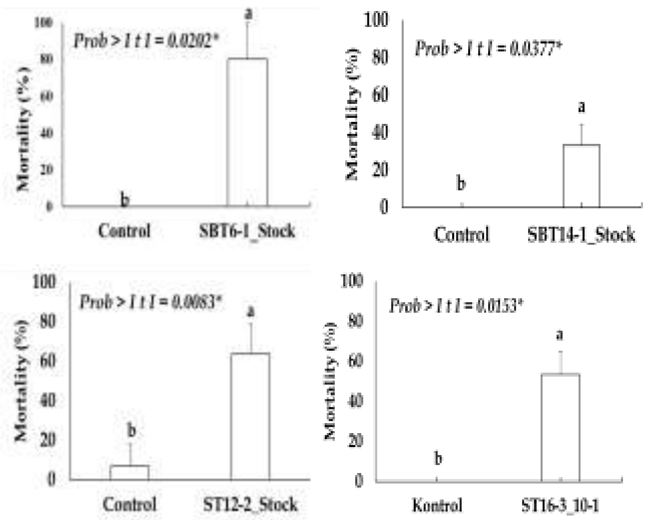

Figure 2. Comparative test (*t-test*) of four isolates against the control.

The selection results showed that out of fifteen isolates there were only four isolates. There were several isolates or had the potential to be entomopathogenic fungi. These results indicate that not all fungi present in the soil in plantation areas act as entomopathogens. The results of research on the identification and inventory of soil-borne pathogens found soil-borne pathogenic fungi that differ from one location to another or a different distribution of microbes in cultivation areas (Soesanto et al., 2016). In addition, there are also groups of arbuscular mycorrhizal fungi (CMA) in several rhizospheres of cocoa, coffee, rubber and oil palm plantations (Yuliyanto et al., 2016). Therefore, the results of the study also showed that non-entomopathogenic fungi were higher in coffee cultivation areas in Malinau than in Sebatik and Sebuku areas. This is thought to have a close connection with observations at sampling locations where the gardens were damp or the pruning was insufficient and the shade trees were too heavy, as well as areas with high rainfall.

Based on the selection of four isolates suspected of being entomopathogenic fungi. The highest mortality rate was 80% using insect bait methods. The Research showed the percentage mortality of *T. molitor* larvae by *Metarhizium* sp and *Beauveria* sp reached 100% of (Nelly et al., 2019). Its concluded entomopathogenic fungal isolates obtained from the three types of land have the potential to control insect pests. However, in this study the mortality rate of most of the isolates was generally below 50%. This was suspected to be the host and the selection environment were not suitable for these fungi to grow and develop. The age of the host and the storage location of the entomopathogenic fungi affect the infection rate of the entomopathogenic fungi (Prayogo et al., 2017). In addition, The culture media also affects the virulence level of the entomopathogenic fungi against the test insects used (Kusnara & Rahmat, 2019).

Isolates SBT6^{-1} and SBT14^{-1} were mortality rate of between 33% - 80%. These isolates have the potential to become entomopathogenic fungi, but generally were undiluted (stock). Fungus population density has a significant effect on insect mortality rates (Prayogo et al., 2017). On the other hand, isolate ST16^{-3} has a higher potential, due of with a dilution of 10^{-1} or a lower density of the three, it can cause a mortality rate of up to 53%. reported the fungus *Lecanicillium* sp at a density of 10^7 conidia/ml caused 41.25% with values $LT_{25,\ 50,\ 75}$, and 12.82 (days), and higher than densities of 10^5 and 10^6 conidia/ml (Ginting et al., 2019).

The success of investigating and developing entomopathogenic fungi depends on the location of soil collection to obtain isolates, the density of the fungal mycelium, and the pathogen's virulence. Therefore, selecting a location, especially in plantation regions, is highly crucial in generating entomopathogenic fungi that are adaptive and have a significant ability to infect insects.

Conclusion

The research examined 15 isolates from cultivated plantation regions. The coffee growing area in Malinau had the greatest number of isolates. However, the selection process revealed only four fungal isolates with potential entomopathogenic properties due to their mortality rate differences compared to controls. The isolate exhibiting the highest entomopathogenic potential was ST16^{-3}, which came from an oil palm plantation in the Sebuku area at a 10^{-1} dilution. Its virulence percentage was recorded at 53.3%. This study demonstrates that plantations in North Kalimantan possess a substantial potential as a reservoir for biological control agents, implying that these indigenous isolates could be harnessed and developed into more ecologically adaptive biological agents compared to those originating from other agricultural regions.

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Author Contributions
Conceptualization, A.R.; methodology, A.R., and S.; formal analysis, A. R.; investigation, A. R., and S; resources, A.R, and S.; data curation, S; writing—original draft preparation, A.R; writing—review and editing, A.R., and S.: visualization.

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Conflicts of Interest
The authors declare no conflict of interest.

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