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Influence of the Entomopathogenic Fungus, *Verticillium lecanii* (Zimm.) on the Cocoa Pod Borer, *Conopomorpha cramerella* (Snellen) Under in-Vitro Conditions

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ABSTRACT
Conopomorpha cramerella (Snellen) (Lepidoptera: Gracillariidae), known as the cocoa pod borer (CPB), is the main pest on cocoa plantation mainly in the Central Sulawesi Province-Indonesia. This pest significantly reduces cocoa production and the farmers' income. Entomopathogenic fungi such as *Verticillium lecanii* (Zimm) Viegas have potential that used as a biological control agent. Pathogenicity of *V. lecanii* Central Sulawesi strain against CPB has been studied under in-vitro conditions. This strain was isolated from the prepupae stadium of CPB. Testing of four *V. lecanii* conidia density (10^4, 10^6, 10^8, 10^{10} ml^{-1}) showed a significant effect on the mortality of prepupae, pupae and imago of CPB. Mortality effects of *V. lecanii* were different among the treatments in which the 10^8 and 10^{10} ml^{-1} conidia density were more effective on prepupae, pupae, and imago of CPB compared to other densities. Prepupae was more susceptible than pupae and imago stadia. *V. lecanii* showed efficacy towards the prepupae, pupae and imago stages of CPB. The results of this study indicated that *V. lecanii* has a high potential as a biological agent for CPB.

***Keywords**:* Conopomorpha cramerella, Verticillium lecanii, *entomopathogen, biological control*

1. INTRODUCTION

In Central Sulawesi, Indonesia, cocoa pod borer (CPB) is still a problem in the cocoa cultivation because it has causes low production and quality of cocoa beans since 2008. In a high attack rate, CPB can reduce around 80% - 90% of cacao yields [1]. The majority of the cocoa farmers in this region used synthetic insecticides to control CPB. However, the application of synthetic insecticides increases environmental pollution and insect resistance in cocoa plantations in Africa [2, 3]. Therefore, integrated pest control is needed in a friendly way.

Agricultural development policies that maintain environmental sustainability and minimize the adverse effects of the use of inorganic chemical pesticides need to be supported by developing and application of pest control that considers ecological, economic and social aspects in the context of the ecologically based pest management [4]. Various methods and tactics, as well as environmentally friendly agents, have been developed to meet these needs. One alternative control is using biological agents such as *Verticillium lecanii* (Zim). Several entomopathogenic fungi that infect cocoa pod borer have been recorded in Central Sulawesi [5]. The potential of *V. tricorpus* as biological agents for CPB has also been reported by Gomies [6] and Setiawaty [7].

Bioassay and effectiveness studies of the *V. lecanii* have been reported against several pests such as aphids [8], *Riptortus linearis* [9], larvae of *Anopheles* [10] and *Spodoptera litura* [11]. Karthikeyan and Selvanarayanan [12] found that conidia concentrations of *V. lecanii* at the 0.25% resulted in the mortality of 100%, and 93.3% of *Bemisia tabacci* and *Amrasca devastans*, respectively. All studies showed that *V. lecanii* has the potential to be used as a biological control agent for insect pests. However, the efficacy of *V. lecanii* on CPB was rarely tested. Therefore, this study aims to evaluate the effect of various densities of *V. lecanii* conidia on CPB mortality in vitro conditions.

1.1. *Materials and Methods*

1.1.1. *Insect sampling and rearing*

Insect samples (prepupae, pupae, and imago of CPB) were obtained from cocoa fruits which showed symptoms of CPB attack. These samples were reared and propagated in the laboratory as described by Anshary [1].

1.1.2. Inoculum propagation

Propagation of *V. lecanii* inoculum was modified from Gomies [6]. Verticillium culture used for propagation was obtained from CPB larvae infected by *V. lecanii* in the field and purified in the Laboratory. Inoculation of *V. lecanii* conidia on rice media was carried out in laminar cabinet. Rice + *V. lecanii* was put in an incubator for 10 days.
Inoculum of *V. lecanii* was dried for 3 days. To make a conidia formula, the conidia were taken by filtering it from growing media. The number of conidia was calculated using a haemocytometer

1.1.3. Pathogenicity test

The pathogenicity test was conducted in February 2019 until May 2019 in the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, University of Tadulako. *V. lecanii* obtained from the cocoa plantation in Makmur village, Palolo sub-district, Sigi district, Central Sulawesi in January 2017. The study was arranged in a Completely Randomized Design. The treatments of conidia density were as follows: P_0 = control (distilled water), $P_1 = 10^4$ ml^{-1}, $P_2 = 10^6$ ml^{-}1, $P_3 = 10^8$ ml^{-1}, and $P_4 = 10^{10}$ ml^{-1}. Each treatment had four replications. The conidia suspension of *V. lecanii* was used for the pathogenicity test. Twenty of CPB prepupae were put into a petri dish layered with moist filter paper, then the prepupae sprayed by 0.53 ml conidia suspension for two seconds. The same procedure was used for the pupae and imago stage of CPB. Percentage of mortality of prepupae, pupae and imago were used as research parameters and observed 6 times (2, 3, 4, 5, 6 and 7 days after the application). Mortality data were analysed using ANOVA followed by HSD test [13].

1.2. Our Contribution

This study provides basic data regarding the capability of *V.lecanii* for controlling the CPB and therefore will contribute to the development of the biological control agents of CPB and supports the integrated pest management of this cacao pest.

1.3. Paper Structure

The rest of the paper is structured as follows. Section 2 presents the mortality of three stadia of CPB at a different density of *V.lecanii* applied. Discussion of the results and the direction for future research are also provided in this section. The results of this study conclude in the section 3.

2. RESULTS AND DISCUSSION

The effect of *V. lecanii* conidia density on the mortality of prepupae, pupae and imago was shown in Table 1, Table 2, and Table 3. Mortality of all CPB stadia tested increased with increasing of conidia density. However, the highest mortality occurred at the prepupae stage followed by pupae and adult stages. Mortality of the pupae and imago at 10^8 and 10^{10} ml^{-1} significantly higher than 10^4 and 10^6 ml^{-1} (Table 2 & Table 3). In all CPB stadia tested, the highest mortality was obtained at the 7 days after the application of *V. lecanii.*

Entomopathogenic fungi have been known as biological agents to control insect pests. In general, they have a similar mode of action in attacking their host. The fungi enter through the insect's cuticle directly. This process occurs partly physic-

Table 1 The average mortality of *Conopomorpha cramerella* prepupae at a different density of *Verticillium lecanii* conidia

Days after application	**Spore density of *V.lecanii* (number of conidia/ml^{-1})**			
	10^4	**10^6**	**10^8**	**10^{10}**
2	9.50	10.25	16.50	16.5
3	9.50	10.25	17.50	16.25
4	10.50	20.75	22.25	26.25
5	10.50	22.75	22.75	26.5
6	13.75	22.75	23.25	27.25
7	15.75	24.25	23.50	27.5
Total	**69.5**	**111**	**125.75**	**140.25**
Average*	**11.58**a	**18.5**b	**20.9b**	**23.37b**

*Numbers with the same letter in the same line are not significantly different at the HSD test (α = 0.05)

Table 2 The average mortality of *Conopomorpha cramerella* pupae at a different density of *Verticillium lecanii* conidia

Days after application	**Spore density of *V.lecanii* (number of conidia/ml^{-1})**			
	10^4	**10^6**	**10^8**	**10^{10}**
2	4.50	6.50	11.25	15.50
3	4.50	6.50	11.25	15.75
4	8.50	10.75	21.25	27.75
5	9.50	13.75	25.75	27.75
6	10.50	16.25	25.25	36.50
7	11.75	16.75	26.25	36.75
Total	**49.25**	**71.25**	**121**	**160**
Average*	**8.21a**	**11.87a**	**20.16b**	**26.66bc**

*Numbers with the same letter in the same line are not significantly different at the HSD test ($\alpha = 0.05$).

Table 3 The average mortality of *Conopomorpha cramerella* imago at a different density of *Verticillium lecanii* conidia

Days after application	**Spore density of *V.lecanii* (number of conidia/ml^{-1})**			
	10^4	**10^6**	**10^8**	**10^{10}**
2	0.25	1.50	6,25	6,25
3	3.50	5.50	10.25	15,5
4	7.50	9.25	16.00	20.50
5	13,50	16,25	26.75	27.50
6	13.25	16,50	26.75	27.50
7	13,25	16,50	26.75	27.50
Total	**50.25**	**64.25**	**112.75**	**124.25**
Average*	**8.37a**	**10.71a**	**18.79b**	**20.71bc**

*Numbers with the same letter in the same line are not significantly different at the HSD test ($\alpha = 0.05$)

ally and enzymatically. Their mode of action as follows; firstly, the fungus spores settle on the insect cuticle, then the spores germinate and enter the cuticle by forming appressorium. Hyphae develop in hypodermis and they continue to multiply in insect body and blood cells and cause the death of the insect [14].

The pathogenicity of entomopathogen depends on several factors including the concentration of the spores inoculum that infecting or penetrates the insect body [15]. In this study, the mortality of CPB increases with increasing density of conidia (Table 1, Table 2, and Table 3). The insect's mortality due to the fungal infection is greatly influenced by virulence, conidia density, and host size [16]. The size and morphological structure of CPB imago are bigger and more complex than the prepupae and pupae stage. Therefore, they are less sensitive to *V. lecanii* infection compared to other stages.

The use of biological agents such as parasitoids, predators and insect pathogens were needs to reduce the ecological impact of pest control by synthetic pesticide [4]. By using a local isolate of *V. lecanii* this study presents laboratory evidence regarding the potency of *V.lecanii* as biological tools against the CPB. The previous study noted the effectiveness of *V. tricorpus* in controlling CPB in the field particularly the prepupae stage. [6]. Such study was in line with this study showed that the average mortality of the prepupae was higher than those of pupae and imago (Tables 2 and 3).

The pathogenicity of *V. lecanii* in other pest insect imago had been reported by researchers in Indonesia. Pasaru *et al.* [5] conducted a pathogenicity test of *V. lecanii* isolates under laboratory conditions showing the average mortality of

Helopeltis sp. by 77% on the 7th day. All of this study shows that *V. lecanii* is pathogenic and deadly to various types of insect imago. However, persistence and pathogenicity of fungal entomopathogens in the filed depend on several ecological factors [17]. Therefore, the efficacy of *V.lecanii* for controlling the CPB should be tested at the cacao plantation to evaluated its potency as a biological control agent of this cacao pest.

3. CONCLUSION

Entomopathogenic fungi *V.lecanii* had high potential as a bioinsecticide to control prepupae, pupae, and imago of the CPB. This study showed that the mortality of CPB increased due to an increase of *V. lecanii* conidia density. The prepupae were more susceptible than the pupae and imago stages.

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