Efficacy of entomopathogenic fungi *Beauveria bassiana* and *Cordyceps* sp against *Crocidolomia pavonana* (FABR.) Lepidoptera: Pyralidae) as possible pest management strategy on pepper multi-cropping plantation

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**Abstract.** Black and white pepper have been considered as the most important perennial crops in some regions of Indonesia yet it took about 3-5 years before the plant start producing. One of the possible approaches to maintain the income of the farmer in the perennial plantation is multi-cropping with high-value annual crops, such as vegetables. However, many pests of vegetables already showed resistance to the common synthetic pesticide applied on the black pepper plant. Thus, it is necessary to apply another strategy for pest control, by applying entomopathogenic fungi. In this study, bioassays and field efficacy of two local isolates of entomopathogenic fungi (*Beauveria bassiana* and *Cordyceps* sp.) against one of the most insecticide-resistant pests, larvae of *Crocidolomia pavonana* (Fabr.) was conducted. In this study, exposure to 1 x 10⁴ conidia mL⁻¹ to 1 x 10⁷ conidia mL⁻¹ resulted in mean larval mortalities from 23.33 to 100% in two days. The probit analysis showed that the LC₅₀ of *B. bassiana* was estimated at 4.26 x 10⁴ conidia mL⁻¹ which significantly more toxic than *Cordyceps* sp. (1.57 x 10⁵ conidia mL⁻¹) although the lethal time was similar. From the field tests, cabbage was sprayed with suspension contained 10⁷ conidia mL⁻¹. Means percent of mortality for all treatments was more than 75% and significantly higher (P < 0.05) than the control (tween 80). Conidia of *Cordyceps* sp. in palm oil cooking oil offered the most promising result against the *C. pavonana*.

**Keywords:** Entomopathogenic fungi, *Beauveria bassiana*, *Cordyceps* sp. *Crocidolomia pavonana*

1. Introduction

Pepper (black and white) is one of the important spices products of Indonesia and is considered the most important and most widely used spice crop. Some regions in Indonesia had been known for major pepper such as Lampung black pepper from Lampung province and Munthok white pepper from Bangka province. This perennial crop, however, requires about 3-5 years of growth period before production. To maintain a steady income, some farmers integrated their pepper plantation with vegetable production. Although there is a limited report on the pest outbreak, there is the possibility of the development of insecticide resistance as most pepper farmers (more than 90% of all farmers) applied synthetic insecticide regularly [1]. Heavy insecticide resistance will lead to higher
production costs and in the end significantly reduce the profit from vegetables as an alternative income. Furthermore, the insecticide residue on the agricultural products may lead to reducing economic value due to price reduction, especially for export products like black pepper. One of the alternatives to solve this problem is by using microbial control as a method of controlling the pest. The role of entomopathogens as natural enemies for several horticulture pests has recently been explored. Several isolates of entomopathogenic fungi have been identified and developed such as Beauveria bassiana, Metarhizium anisopliae, Paecilomyces sp., Verticillium sp. and Spicaria sp. [2-5].

In this study, the cabbage caterpillar, Crocidolomia pavonana was applied as the study object. This insect is considered one of the most significant pests of cabbage in Indonesia [6,7]. Long-term application of synthetic insecticide to control this insect has to lead the insecticide resistance [8]. On the other hand, the information on the effectiveness of the entomopathogenic fungi as a biological control agent for this insect was hardly found. Thus, this study was conducted to assess the potential of B. bassiana and Cordyceps sp. for managing C. pavonana population as a first step before application on the pepper plant.

2. Research methods

2.1 Insect Culture
Laboratory culture of C. pavonana was established from cabbage crops in Lembang, West Jawa, Indonesia. Adults insects were reared on cabbage plants in a screen cage (40 x 40 x 40 cm) made of fine net cloth. Local cabbage was grown in 1 L polybag in a screen house to meet the continuous supply for this study. The plant was not sprayed with any insecticide. The bioassay and rearing were conducted in the laboratory (T 18-29°C, RH 65-85%, 12L:12D period). Insects used in this study were reared for 4-6 generations under the above condition.

2.2 Fungal Culture
Six (6) strains of Cordyceps sp. were isolated from the dead ants isolated from the forest of Dipterocarpaceae in South Kalimantan. Beauveria bassiana was reisolated from the Laboratory of Microbiology, Life Sciences Center Institut Teknologi Bandung. Both fungal were cultured and maintained at room temperature on sterilized potato dextrose agar (PDA) medium as well as on sterilized rice flour medium stored in autoclaved polybags. To prepare the fungal inocula, conidia from 9 days old PDA cultures were scraped from the surface of the plates with a sterile scalpel and suspended in 0.05% Tween 80 in sterile distilled water. A Neubauer hemocytometer was used to estimate the conidial concentration and subsequent appropriate serial dilutions were prepared from 1 x 10^7 to 1 x 10^4 conidia mL^-1. A hand sprayer was used to deliver approximately 2 mL of each treatment on a cabbage leaf preparation containing the test larvae. The leaf preparation consists of Chinese cabbage leaf, Brassica juncea, of ca. 120 cm^2 were kept fresh with its stalk wrapped in wet cotton enveloped in aluminum foil. This way, the leaf freshness could be maintained for approximately 5-7 days. Each treatment was also sprayed on clear bacteriological agar (Bacto®) plate for CFU (germinated conidia) counts under a microscope 24 h after treatment. [4,5,9].

2.3 Dosage-Mortality Bioassay
Twenty-third instar larvae of C. pavonana were transferred to each cabbage leaf preparation. Five such leaf preparations (replicates) were assigned randomly to each dosage-response assay. The assays included four inoculum dosages of each fungal plus control, which consisted of sterile distilled water containing 0.01% Tween 80. This bioassay was conducted in the same laboratory as insect rearing. Ten to fifteen minutes after inoculation with a hand sprayer, the treated leaf preparations were each placed in cylindrical plastic vials (Ø 12 cm and 11 cm in height). Larval mortality, including moribund individuals, was recorded daily for 6 days. The LC50 and LT50 from the regression line with 95% fiducial limits were obtained through a probit program (Polo PC) based on probit analysis [5,9].

2.4 Field Trials
Four weeks old cabbages grown in polybags in the glasshouse were placed in an open field spaced 0.4 m within and 0.45 m between rows. There were three treatments of each fungal and untreated control, each consisted of five
plants arranged in a row. The plants were set in a randomized completely block design with five replications. Ten third instar *C. pavonana* larvae were transferred to each cabbage plants. Three formulations of *Cordyceps* sp. were prepared with conidia from PDA in oil palm cooking oil (40% mono-unsaturated, Kunci Mas®), conidia from rice flour culture (powder), and conidia from PDA in sterile distilled water containing 0.01% Tween 80. Sterile distilled water containing 0.01% Tween 80 was the spray carrier which also served as the control. A concentration of 1 x 10^7 conidia mL^-1 was used in this program.

Oil palm cooking oil formulations were prepared from conidia (0.03 g) from 9-days old cultures scraped from the surface of the plates with a sterile scalpel mixed with 30 mL Kunci Mas®. Conidia from rice flour medium were collected from 9 days old cultures suspended in sterile distilled water containing 0.01% Tween 80. Those solutions were shaken vigorously and the conidia were sieved using a muslin cloth to separate the rice flour. Sterile distilled water containing 0.01% Tween 80 was used to dilute the conidial suspension which was standardized to 1 x 10^5 conidia mL^-1. Ten mL of each treatment were sprayed using a hand sprayer to each cabbage plant infested with 10 third instar larvae. All treatments were sprayed in the morning, two days after treatment, each group of 10 larvae from the five plants were transferred to a petri dish with fresh cabbage leaves which were replaced after five days. Larval mortality was recorded daily for 6 days to determine the cumulative percent mortality. All dead and moribund larvae were individually surface-sterilized in 0.5% sodium hypochlorite for three minutes, rinsed in sterilized distilled water, and then placed on a PDA medium to prove the presence of *Cordyceps* sp.

2.4 Data analysis

Data for ANOVA were transformed by Arc Sine ½ to stabilize the variance before the analysis. Treatments means were subjected to two-way ANOVA and subsequently compared using LSD at a 5% level of probability [9].

3. Result and Discussion

3.1 Dosage-Mortality Bioassay

The isolates of *Cordyceps* sp. and *B. bassiana* were pathogenic for third instar larvae of *C. pavonana* (Table 1). Almost all of the larvae became moribund within two days after treatment. Both fungal species were sporulated on the surface of larvae which were all mummified.

| Dosage (conidia mL^-1) | Percent mortality ± standard error |
|------------------------|-----------------------------------|
|            | *Cordyceps* sp. | *B. bassiana* |
| Control     | 0               | 0              |
| 10^4        | 35.33 ± 1.67    | 38.33 ± 1.67   |
| 10^5        | 51.67 ± 3.33    | 58.33 ± 4.41   |
| 10^6        | 78.33 ± 3.33    | 83.33 ± 1.67   |
| 10^7        | 100 ± 0.00      | 98.33 ± 1.67   |

The death symptom of *C. pavonana* similar to symptom on the other insect species infected by *Cordyceps* sp. and *B. bassiana* [4,10,11]. Larval mortality was positively correlated with the dose rate range from 35 to 100%. At concentration exceeds 1 x 10^6 conidia mL^-1, larval mortality after 4 days was more than 75%, while a 100% mortality was observed at 1 x 10^7 conidia mL^-1 only for *Cordyceps* sp (Table 1).

The probit analysis showed that the LC\textsubscript{50} of *B. bassiana* was estimated at 4.26 x 10^4 conidia mL^-1, and was significantly higher than that of *Cordyceps* sp. at 1.57 x 10^4 conidia mL^-1(Table 2). This result agrees with the result of Ou et al. [11] but disagree with Hashim et al. [4] which indicated some factors affect the effectiveness which should be addressed in the future study.

| Fungi            | LC\textsubscript{50} conidia mL^-1 |
|------------------|-----------------------------------|
| *Cordyceps* sp. | 4.26 x 10^4                      |
| *B. bassiana*   | 1.02 x 10^5                      |

Table 1. Mean percentage of mortality on third instar larvae of *C. pavonana* after 4 days exposure to *Cordyceps* sp. and *B. bassiana*.

Table 2. Effect of *Cordyceps* sp. and *B. bassiana* on third instar larvae of *C. pavonana*.
The virulence of both fungal, as displayed by the decreasing LT$_{50}$ and LT$_{90}$ values, demonstrated a common trend of generally increasing potential (i.e. the rate and speed of mortality) with increasing concentration (Table 3). Increasing concentration of conidia improves the possibilities of material to be colonized.

**Table 3.** Median lethal time of third instar larvae of *C. pavonana* after 4 days exposure to *Cordyceps* sp. and *B. bassiana*.

| Dosage (conidia mL$^{-1}$) | *Cordyceps* sp. LT$_{90}$ (days) | *Cordyceps* sp. LT$_{50}$ (days) | *B. bassiana* LT$_{90}$ (days) | *B. bassiana* LT$_{50}$ (days) |
|---------------------------|----------------------------------|----------------------------------|---------------------------------|---------------------------------|
| 10$^4$                    | 11.78                            | 4.71                             | 9.96                            | 4.97                            |
| 10$^5$                    | 7.66                             | 3.68                             | 6.54                            | 3.93                            |
| 10$^6$                    | 5.06                             | 2.80                             | 4.60                            | 3.01                            |
| 10$^7$                    | 3.57                             | 2.40                             | 3.76                            | 2.50                            |

**3.2 Field Trial with Cordyceps sp.**

The mean percent mortalities for all the treatments were more than 70% except for the control. On the other hand, the application of conidia combined with oil palm cooking oil significantly improved the effectiveness of the conidia as an insecticide (Table 4).

**Table 4.** Mean percentage of mortality amongst the treatments of *Cordyceps* sp. on third instar larvae of *C. pavonana*.

| Treatments                                      | Mean % mortality |
|------------------------------------------------|------------------|
| 1. Conidia from PDA in oil palm cooking oil    | 86.7 a           |
| 2. Conidia from PDA only                       | 74.3 ab          |
| 3. Conidia from rice flour                     | 71.0 b           |
| 4. Control (Tween 80)                          | 12.5 c           |
| LSD                                            | 9.08             |
| MSE                                            | 305.5            |
| CV                                             | 17.87            |

Means followed by the same letter are not significantly different at P=0.05 as determined by 2-way ANOVA and LSD. The analysis was performed on Arc Sine $\sqrt{x}$ values. a sterile aqueous Tween 80 (0.05%) was the spray carrier.

Conidia from PDA medium in palm cooking oil was the most effective to kill the larvae. The prevailing high humidity of the surroundings, typical weather conditions in Bandung throughout the year, could have positively influenced the effectiveness. Conidia from rice flour culture resulted in 71.0% mortality, which did not differ significantly from conidia in PDA medium. However, this formulation caused significantly lesser mortality than the conidia in palm oil formulation. Oil may have helped in spreading the conidia on the surface of a hydrophobic surface such as an insect cuticle [12]. (Ingles et al. 1996).

**4. Conclusion**

Both *Cordyceps* sp. and *B. bassiana* were effective in controlling the population of *C. pavonana*. Field application could be effectively done by mixing the conidia with oil palm cooking oil, an abundant resource in Sumatera, on major pepper plantation areas. This study provides baseline information for future study on the application of these fungi for major pepper pests and the possibility of establishing a natural population of entomopathogenic fungi in pepper plantation.

**5. Acknowledgments**

This research was partly funded by the Penelitian Dasar Unggulan Perguruan Tinggi 2020 fund granted to the corresponding author.
References

[1] Wiratno, Taniwiryono, Brink, Rietjens, and Murk 2007 Environmental Toxicology 22(4) 405.
[2] Shah and Pell 2003 Applied Microbiology and Biotechnology 61(5-6) 413.
[3] Prayogo, Tengkano, dan Marwoto 2005 Jurnal Litbang Pertanian 24(1) 19.
[4] Hasyim, Setiawati, Murtiningsih, Hilam, and Sofiar 2011 Proceedings The 6th Inter. Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pest, AVRDC, 77-86.
[5] Duarte, Gonvalces, Espinosa, Moreira, De Bortoli, Humber, and Polanczyk 2016 Journal of Economic Entomology 109(5) 594.
[6] Kalshoven 1981. The Pest of Crops in Indonesia.
[7] Sastrosiswojo and Setiawati 1993. Biology and Control of Crocidolomia binotalis in Indonesia.
[8] Dono, Ismayana, Idar, Prijono, and Muslikha. Jurnal Entomologi Indonesia 7 9.
[9] Liu, Skinner, Parker, and Brownbridge 2002 Journal of Economic Entomology 95(4) 675.
[10] Priwiratama 2014 Journal of Agricultural Science and Technology A 4 103.
[11] Ou, Ren, Liu, Ali, Wang, Ahmed, Qiu 2019 Insects 10(12) 425.
[12] Inglis, Johnson, and Goethel 1996 Biocontrol Science and Technology 6 35.