Effectiveness of *Cordyceps* sp. and *Beauveria bassiana* against large cabbage-heart caterpillar, *Crocidolomia pavonana* Fabricius (Lepidoptera: Crambidae)

Agus Dana Permana[1*], Sally Marlinda[1], Agus Susanto[2], Amin Setyo Leksono[3]
[1]School of Life Sciences and Technology – Institut Teknologi Bandung.
Jl. Ganesha No. 10 Bandung 40132, Indonesia. Phone: +62222500258; Fax. +62222534107.
[2]Department of Pest and Plant Disease, Faculty of Agriculture,
Universitas Padjadjaran, Jatinangor, 45363, Indonesia.
[3]Departments of Biology, Faculty of Mathematics and Natural Sciences,
Universitas Brawijaya, Indonesia
*corresponding author: agus@sith.itb.ac.id

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Abstract—*Crocidolomia pavonana* Fabr. (Lepidoptera: Crambidae) is a major pest on various cabbage crops in Indonesia. Various insecticides have been used to control this insect pest including several entomopathogenic fungi, and this cabbage caterpillar has become resistant to various insecticides. In order to develop insecticides from entomopathogenic fungi, species or varieties from natural area such as forest are needed. A bioassays of *Cordyceps* sp. and *Beauveria bassiana* has been carried out in the laboratory. Inoculum of *Cordyceps* sp. obtained from the Dipterocarpaceae forest in South Kalimantan, Indonesia recovered from dead ants by the mycelium of *Cordyceps* sp. Four concentration of *Cordyceps* sp. and *Beauveria bassiana* inoculum and tween^{80} as a control were tested against 3rd instar larvae of *C. pavonana* with five replications. Field efficacy trial was carried out in a screen house (8 x 12 M^2) using Chinese cabbage plants (*Brassica juncea*) aged 28 days after sowing, with a spacing of 0.4 M between rows. Three formulation of *Cordyceps* sp. and tween^{80} as a control were used with 6 replications. The result of the bioassays in the laboratory showed that *Cordyceps* sp. with concentration of 1 x 10^7 conidia mL^{-1} was able to give 100% mortality od *C. pavonana* larvae, while *B. bassiana* with the same concentration caused morality of *C. pavonana* larvae of 98.33%, although statistically not significantly different. The results of probit analysis also showed that LC$_{90}$ *Cordyceps* sp. against cabbage caterpillar larvae was 1.57 x 10^6 conidia mL^{-1}, while *B. bassiana* required a concentration of 2.6 x 10^6 conidia mL^{-1} to reach the LC$_{90}$ value. The results of filed trial showed that the formulation of *Cordyceps* sp. 1 x 107 conidia mL^{-1} using palm oil cooking oil was able to give an average mortality of 86.7%, and significantly higher (P<0.05) than the other treatment. The results of this study indicate that the inoculum of *Cordyceps* sp. from Dipterocarpaceae forest is very promising to be developed as an insecticide to control *C. pavonana* in cabbage

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

I. INTRODUCTION

The large cabbage-heart caterpillar, *Crocidolomia pavonana* Fabricius (Lepidoptera: Crambidae), is an insect pest of cabbage plants in many countries of Africa, the Pacific and Asia, including in Indonesia [1-3]. *C. pavonana* often attacks cabbage plants grown in areas hot, humid, and at the altitude of more than 600 M above sea level [4]. The early instar larvae od *C. pavonana* will live on the underside of the leaves, the larger ones will attack all parts of the cabbage plant [2,4]. The cabbage caterpillar populations often cause economic losses.

Synthetic insecticides are often used to control *C. pavonana*. In Indonesia, cabbage farmers can apply synthetic insecticides 2 to 3 time in 1 week [4].The continuous and inappropriate use of synthetic insecticides can lead environmental pollution, the occurrence of insecticides resistance, the destruction of natural enemies, disruption of human health, and excess chemical residues in crops, which can also affect consumers to obtain organic product [2-5].

Pests control efforts can be carried out with more environmentally friendly pesticides such as using botanical and microbial insecticides. Most of the studies that have been conducted have tested the effectiveness of botanical insecticides [4, 6-8]. Another alternative in controlling insect pests is the use of microbes as pest control agents. Various types of entomopathogens as insect pest control have been developed. Several isolates of entomopathogenic fungi have been studied and developed, such as *Paecilomyces sp., B.*

bassiana, Verticillium sp., Spicaria sp. and *Metharhizium anisopliae* [9-11]. A study on molecular identification showed that seven genera of entomopathogenic fungi, namely *Beauveria, Clonostachys, Cordyceps, Fusarium, Metarhizium, Penicillium,* and *Purpureocillium,* had very high virulence [12]. Furthermore, the screening results from isolates of the genus *Beauveria,* and *Metarhizium* had high pathogenicity against *Spodoptera litura* [12]. The results of another study showed that isolates of *Beauveria bassiana, Isaria fumosorosea, Isaria sinclairii*, *Lecanicillium muscarium, Metarhizium rileyi,* and at a concentration of 1 x 10^7 conidia mL^{-1} were very effective in controlling the larvae of *Plutela xylostella* [11]. Application of entomopathogenic fungi for insect control must pay attention to management cost, such as the results of applying mathematical models in control of *Bemisia tabaci*, an insect that carries yellow viral disease of red chili using the entomopathogenic fungi, *Verticillium lecanii,* is only requires time for 15 days and a dose of 90% of the recommended dose [13].

Research on the effect of entomopathogenic fungi to control *C. pavonana* has not been widely carried out, although there have been several research results on the effect of *M. anisopliase* and *B. bassiana* on *C. pavonana* [14,15]. Study on the effect of *Cordyceps* sp. (especially those from natural forests) against *C. pavonana* larvae has never been done. Therefore, the objective of this study was to evaluate the potential of *Cordyceps* sp. from the Dipterocarpaceae forest in an effort to control C. *pavonana* in Chinese cabbage, and ultimately reduce the use of synthetic insecticides.

II. MATERIALS AND METHODS

A. Fungal Culture.

Cordyceps sp. were isolated from the dead ants isolated from the forest of Dipterocarpaceae in South Kalimantan. *B. bassiana* were reisolated from Laboratory of Microbiology Life Sciences Center, Institut Teknologi Bandung. *Cordyceps* sp. was isolated and re-cultured from mycelium collected on dead ants covered with fungi from Dipterocarp forest in South Kalimantan, Indonesia. Then identified and maintained using potato dextrose agar (PDA) media. *B. bassiana* was cultured using PDA media, and both fungi were maintained at room temperature. The 9-days-old fungal conidia from PDA media were scraped using a sterile scalpel from the plate surface, then suspended in distilled water containing 0.05% $tween^{80}$. Conidia concentration of both entomopathogenic fungi were calculated using a Neubauer haemocytometer, and serial dilutions were carried out from 1 x 10^7 to 1 x 10^4 conidia mL^{-1}.

C. pavonana was reared in the laboratory. The larvae were fed with Chinese cabbage (*Brassica juncea*) leaf which were planted in a screen house (8 x 12 M^2) and without insecticides treatment. Adult insect were kept in gauze cages (0.7 x 0.6 x 1.0 M^2), polybags containing Chinese cabbage were placed in the cage, 10% honey diluted with distilled water was used as adult insect feed [2-4]. Third instar larvae od *C. pavonana* were used for bioassays in the laboratory and field efficacy tests.

B. Dosage-Mortality Bioassay.

Twenty 3rd instar larvae of *C. pavonana* were transformed to each 5 sets of Chinese cabbage leaf (120 Cm^2 per leaf). The leaf stalks were wrapped in wet cotton with by aluminium foil cover, this method can keep the cabbage leaves fresh for up to 5-7 days. The two entomopathogenic fungi with concentration of 1 x 10^4, 1 x 10^5, 1 x 10^6, 1 x 10^7 conidia mL^{-1}, and 0.05% $tween^{80}$ as a control were sprayed on cabbages leaves using a hand sprayer (volume 1 L). Each treatment was repeated 5 times and every treatment were also sprayed on PDA plate using bacteriological agar (Bacto®). After 24-96 hours, germinated conidia were counted under the microscope [10-12,14].

Bioassays were carried out in the same laboratory as insect rearing. Fifteen to twenty minutes after treatment, all cabbages leaves were transferred into cylindrical plastic bottles (height 11 Cm x ∅ 12 Cm) and covered with gauze.

Bioassays were conducted in the same laboratory as insect rearing. Ten to fifteen minutes after inoculation with hand sprayer, each treated leaf was placed in a sylindrical plastic bottle (Ø 12 cm and 11 cm in height). The numer of dead larvae was counted up to 6 days. Probit analysis to determine the LC_{50} and LT_{50} values were carried out using the Polo PC program [11,15].

C. Field Trial.

Twenty eight days Chinese cabbage (insecticides free) in polybag (height 14 Cm x ∅ 20 Cm) was placed in a screen house with the distance of 0.4 x 0.45 M. The treatment in the field efficacy test used *Cordyceps* sp. with concentration of 1 x 10^7 conidia mL^{-1}, and made into a formulation of PDA, oil palm cooking oil (40% mono-unsaturated; Kunci Mas®), and rice flour. The formulation of *Cordyceps* sp. with palm oil cooking oil was made from 0.03 g of 9-days old conidia of *Cordyceps* sp. cultured mixed with 30 mL of Kunci Mas®. The same amount and age of *Cordyceps* sp. conidia were mixed with 30 mg of rice flour, then suspended with 0.01% $tween^{80}$ in sterile distilled water, and this $tween^{80}$ solution was used as a control [9,-12].

All formulation solution were shaken vigorously, then the conidia solution filtered using cotton gauze, so there was a separation between the conidia liquid and the solid phase. Conidia solution from each treatment was sprayed as much as 10 mL with a hand sprayer (volume 1 L) for each cabbage plant that had been infested with ten 3rd instar larvae of *C. pavonana*. Each treatment was repeated 6 times, and spraying was carried out in the morning between 07.00 – 09.00.Observation of dead larvae were carried out after 24 hours, and recorded for 6 days to calculated the percentage of cumulative mortality [12,14]. To prove that the dead *C. pavonana* larvae were caused by the fungus *Cordyceps sp,* the dead larvae were sterilized with 0.5% sodium hypochlorite for 5 minutes, then rinsed using sterile distilled water and inoculated on PDA media.

III. Results and Discussion

A. Bioassays

The results of the study showed that all concentration of enromopathogenic fungi used has caused the death of 3rd instar larvae of *C. pavonana* (Table 1), with the higher concentration of treatment used, the percentage of *C. pavonana* larvae mortality increased. From laboratory bioassays, the *Cordyceps* sp. concentration of 1 x 10^6 conidia mL^{-1} has caused more than 75% mortality, and mortality of the *C. pavonana* larvae has reached 100% mortality with the *Cordyceps* sp. concentration of 1 x 10^7 conidia mL^{-1}.

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

Dosage (conidia mL^{-1})	Percent mortality ± standard error	
	Cordyceps sp.	*B. bassiana*
10^4	35 .33 ± 1.67[a]	38.33 ± 1.67[a]
10^5	51.67 ± 3.33[b]	58.33 ± 4.41[c]
10^6	78.33 ± 3.33[d]	83.33 ± 1.67[e]
10^7	100 ± 0.00[f]	98.33 ± 1.67f[g]

The results of probit analysis showed that the LC90 of *Cordyceps* sp. was estimated at 1.57 x 10^5 conidia mL-1, and statistically, it was lower than LC90 of *B. bassiana* (2.6 x 10^6 conidia mL1) (Table 2). The calculation of probit analysis predicted that between log-dosage and probit mortality has significant correlation ($P<0.05$) for the two type of entomopathogenic fungi.

Table 2. Effect of Cordyceps sp. and B. bassiana on third instar larvae of C. Pavonana

Fungi	LC_{50} conidia mL^{-1}	LC_{90} conidia mL^{-1}
Cordyceps sp.	4.26 x 10^4	1.57 x 10^6
B. bassiana	1.02 x 10^5	2.6 x 10^6

From the observation of the lethal concentration test, it has been shown that many *C. pavonana* larvae stay away from feed on the second day after treatment, this is because both entomopathogenic fungi started to produce a lot of spores on the surface of the larvae. The results of this test also showed that there was a decrease in pathogenicity over time. In line with the increasing concentration of the fungi, the time needed to control *C. pavonana* larvae was getting faster (Table 3).

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.		*B. bassiana*	
	LT_{90} (days)	LT_{50} (days)	LT_{90} (days)	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

B. Field Trial with Cordyceps sp.

The results of the efficacy test of the formulation of *Cordyceps sp.* showed that the formulation using cooking oil from palm oil resulted in average mortality of 86.7% (Table 4), and was significantly different from the formulation using rice flour and control, but slightly different from the formulation using only PDA (74.3%).

The high mortality of the formulation using cooking oil from palm oil was probably due to the increased adhesion of the solution to cabbage leaves compared to the formulation that only used PDA and rice flour. The relative humidity at the study site in the city of Bandung, which is located at an altitude of 800 M above sea level, and surrounded by mountains, may also increase the pathogenicity of the fungi.

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

Treatments[a]	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

A sterile aqueous Tween[80] (0.05%) was the spray carrier.
Mortality data was transformed by X root arc sine.
The same letter in the mean mortality are not significantly different (2-way Anova and LSD, P=0.05).

Discussion

Beauveria bassiana, Nomuraea, Metarhizium, Paecilomyces, and *Fusarium* are some fungal genera that have high pathogenicity against insect pest species [17-22]. Bioassay of *B. bassiana* and *Cordyceps* sp. on *Sitophilus oryzae* showed that the death of *S. oryzae* was caused by

sporulation of these fungi on the insect body surface [18], the result of this study also showed the same symptoms. *Paecilomyces fumosoroseus* (teleomorph: *Cordyceps* sp.) has high pathogenicity against *Plutella xylostella* (Diamondback moth) larvae [19], as well as egg and larvae of *C. Pavonana* [20]. *P. fumosoroseus* can caused ca. 2.5 times than *B. Bassiana* against *P. xylostella* larvae. The sporulation of *P. fumosoroseus* (teleomorph: *Cordyceps* sp.) and *B. Bassiana* also occured on the surface of dead *P. xylostella* larvae, as well as the case of this study, however, the body of *P. xylostella* larvae infected by *M. anisopliae* var. majus was not completely covered with fungal mycelium [22]. Several different results occurred in the pathogenicity of entomopathogenic fungi other than *Cordyceps* and *B. bassiana*on *C. pavonana* eggs, although, the virulence of these fungi remained high on *C. pavonana* larvae [20].

The use of the fungus *M. anisopliae* has the potential to control *P. xylostella* and *C. binotalis* (*C. pavonana*), Concentration of 200 g/L water showed the highest net weight (1,583 g/crop) [14]. Meanwhile, the best bioactivity of *M. anisopliae* was found in the 10^{th} week after storage, namely in the pellet formulation with a concentration of 10^7 conidia mL^{-1}.

The results of the field efficacy test indicated that *M. anisopliae* formula can still live in the field, beside its presence in the field, is still able to control *C. pavonana* larvae until the 4^{th} day after application, both pellet formulations and flour formulations [14]. The results of bioassays of the suspension of *M. anisopliase* in $tween^{80}$ showed that from the day 3, *M. anisopliase* could produces mycotoxins, the amount of mycotoxins production was directly proportional to the growth period of the fungi, and optimal production occurs on day 7, these mycotoxins include myroridins, destruxins, hydroxyfungerins A dan B, metacytophilin, fusarin C, cytochalasins C and D [23]. Destruxin known to be the most abundant most abundant mycotoxins produced by *Metharhizium,* however, its virulence varies depend on the variety of species *Metharhizium* [24], Until now, destruxin, a mycotoxins from *M. anisopliase* is known to have insecticidal activity, destruxin can reduce insect immunity, however, its mode of action is not widely known [25]. The results of other study indicated that destruxin A can bind to transmembrane protein 214 and the transport protein (BmSEC23) from the larvae of *Bombyx mori,* and this binding has been predicted to increase the mortality of *B. mori* [26].

The study of the virulence level of 41 entomopathogenic isolates from Thailand against western flower thrips (*Frankliniella occidentalis*), showed that there were 14 isolates that were virulent. Among these isolates, two isolates of *B. Bassiana* had an LC50 level below 6.61×10^4, while the isolate *Cordyceps* sp. had an LC50 of 4.47×10^5 [27]. Our results showed the opposite results. LC50 level *Cordyceps* sp. against *C. pavonana* was lower than *B. bassiana,* meaning that *Cordyceps* sp. was more virulent than *B. Bassiana*. Therefore, the results of the field test are important to evaluate the success of the application. Another study showed that the results of mathematical modeling of infected red chili plant populations (in the vegetative and generative phases) decreased due to the application of *V. lecanii*, which drastically reduced the population of *B. tabaci* of the yellow virus [13].

Several types of oil may help spread the conidia of the fungi on hydrophobic surface such as on insect cuticles [23,24].This results of this research may also the theory of conidia dispersal with the help of certain oils on the body surface of insect larvae. Research on mass production of spore suspensions of *B. bassiana* and *P. fumosoroseus* in Malaysia showed that the two fungi with concentration of 1×10^8 spores/mL, equivalent to 3.75×10^{13} spores/ha. could reduce population of *P. xylostella* larvae [21]. The results also showed that the rice and coconut water formulation were suitable media for the growth and sporulation of *B. bassiana* and *P. fumosoroseus*. The concentration of entomopathogenic fungi should be considered in the cost of pest control, such as the results of research on controlling *B. tabaci* in red chilli plants [13]. The results of *Cordyceps* sp. formulation using rice flour on *C. pavonana* larvae in this research, are in line with the results of research [21], where the rice flour formulation can produce *C. pavonana* mortality of 71%. However, further effort are still needed to develop the management strategies for *C. pavonana* in Indonesia.

IV. Conclusion

The results of this present study suggest entomopathogenic fungi, *Cordyceps* sp. originating from Dipterocarpaceae forest in Central Kalimantan Indonesia was able to provide better mortality against *C. pavonana* larvae than *B. bassiana*. Field efficacy test of *Cordyceps* sp. suspension with a mixture of cooking oil from oil palm offers promising results for further development in the control strategy of the large cabbage-heart caterpillar, *C. pavonana.*

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