

Virulence of conidia *Beauveria bassiana* (Bals.) as a bioinsecticide against *Crociodolomia pavonana* (F.) (Lepidoptera: Pyralidae) on broccoli plants

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Abstract. *Crociodolomia pavonana* is an important pest of broccoli plants. Pest control is currently still using chemical pesticides that negatively impact the environment. It is hoped that *Beauveria bassiana* will become one of the environmentally friendly bioinsecticides. The research aimed to study the virulence level of conidia density of *B. bassiana* as a bioinsecticide against *C. pavonana* pests on broccoli plants. This experiment used a completely randomized design consisting of 5 treatments and four replications so that 20 experimental units were obtained. The Basic Plant Protection Laboratory and Plant Pest Science Laboratory Faculty of Agriculture, Syiah Kuala University as the research location, which was carried out from January to September 2021. The parameters observed were larval mortality, pupae formed, imago appeared, an incubation period of *B. bassiana* conidia, and average time of *C. pavonana*. The results showed that *B. bassiana* with conidia density of 10^8 /mL distilled water was effective as a bioinsecticide against *C. pavonana*. The highest percentage of *C. pavonana* mortality observed at 6 Days After Application (DAA) occurred at a density of 10^8 conidia/mL of water (97.50%), and the lowest was at a density of 102 conidia/mL of distilled water (62.50%). The percentage of pupae formed was observed. At 7, DAA was 15% using a density of 10^2 conidia /mL aquadest. Produce 5%, a density of 10^4 conidia/mL of distilled water was used. The percentage of imago that appeared with a conidia density of 10^2 /mL of distilled water killed at 14 DAA yielded 15%, and 5% resulted from a density of 10^4 conidia/mL of distilled water. The fastest incubation occurred at a density of 10^8 conidia/mL of distilled water with a time of 3.50 days. Death of *C. pavonana* at 3.03 days required conidia density of *B. bassiana* 10^2 /mL of distilled water.

Keywords: Bioinsecticide, *Beauveria bassiana*, Pest, *Crociodolomia pavonana*, broccoli.

INTRODUCTION

Crociodolomia pavonana is an important pest on vegetable crops, such as broccoli and other vegetables [2]. This pest destroys broccoli plants in groups. The initial attack on the growing point of the leaf, then all the leaves are eaten, leaving only the leaf bones. Heavy attacks from this pest result in economic losses [15]. Pest control is still using synthetic insecticides, which have a negative impact on the environment [18]. Therefore, it is necessary to control other effective and environmentally friendly methods, one of which is through the use of biological agents [13]. The fungus *B. bassiana* is a biological agent that can be used as a bioinsecticide against insect pests from several insect orders, such as Lepidoptera,

Coleoptera, Isoptera, Hemiptera, Orthoptera, and other orders [17]. Effectiveness *B. bassiana* as a bioinsecticide is determined by the density of conidia and environmental conditions. Application of conidia *B. bassiana* with a density of 10^8 /mL aquadest and 10^9 /mL aquadest against the insect pest *H. antoni* by spraying method can produce mortality up to 100% [4]. The higher the density of *B. bassiana* conidia applied to insect pests, the faster and higher mortality will be produced. Conidia *B. bassiana* to produce mortality against insect pests is also influenced by the activity of secondary metabolites such as enzymes and toxins that cause disturbances in hemolymph function, and the insect body becomes swollen and hard [19].

The research aimed to study the virulence level of conidia density of *B. bassiana* as a bioinsecticide against *C. pavonana* pests on broccoli plants.

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METHODOLOGY

This research was conducted at the Basic Plant Protection Laboratory and Plant Pest Science Laboratory Faculty of Agriculture, Syiah Kuala University, from January to September 2021.

Equipment

The tools used in this study were Petri dish, Erlenmeyer, beaker glass, test tube, needle loop, stirring rod, scalpel, microscope, tweezers, analytical balance, Bunsen lamp, hemocytometer, laminar airflow, autoclave, cover glass, gas stoves, pans, vortexes, jars, gauze, stationery, and documentation tools. At the same time, the materials used were PDA flour, aluminum foil, aquadest, spiritus, 70% alcohol, chloramphenicol, wrap paper, sawdust, straw paper, cotton, mustard greens, honey, thread, insect pests *C. pavonana* instar II. Experimental design. This study used a non-factorial Completely Randomized Design (CRD) with five treatments and four replications. Thus, 20 experimental units were obtained (Table 1).

Table 1. Treatment of Conidia Density of *B. bassiana* against *C. pavonana*

Number	Treatment
1	B ₀ (control)
2	B ₁ (10 ²)
3	B ₂ (10 ⁴)
4	B ₃ (10 ⁶)
5	B ₄ (10 ⁸)

If the F hit test analysis shows a significant difference, then the data analysis is continued with the smallest significant difference test at the 5% level (LSD 0.05).

Research implementation test insect Breeding

Propagation of the test insects by collecting *C. pavonana* larvae from mustard plants from the East Sector, Darussalam, Aceh Besar. The collection of *C. pavonana* larvae was then brought to the Plant Pest Science Laboratory using plastic jars and covered with gauze. The food for *C. pavonana* larvae given was fresh mustard greens which were changed every day. Larvae that will enter the pupae stage are transferred into jars that have been filled with sawdust. In the container hung cotton that has been dipped in 10% honey liquid as feed for imago *C. pavonana*. Eggs of *C. pavonana* placed on mustard leaves, then transferred to a jar and left until the eggs hatch. The larvae that have come out of their eggs are then put in fresh mustard leaves as feed which is changed every day until the larvae enter the second instar phase (test insects).

PDA media preparation

PDA was weighed using an analytical balance in the amount of 3.9 g. The material is then dissolved into 100 mL of distilled water in an Erlenmeyer as a mixture; the solution is stirred until homogeneous and heated. Next, the erlenmeyer was covered with cotton covered with aluminum foil, and sealed using the paper wrap. The PDA was then sterilized in an autoclave as well as the tools to be used for research such as petridish, use needles, and scalpel, which had been wrapped in HVS paper at 121°C for 15 minutes. The sterile PDA solution was added with 125 mg of Chloramphenicol then cooled in Laminar airflow, and then put into a sterilized petridish.

Breeding of *B. bassiana* on PDA

The isolates of the fungus *B. bassiana* used in this study came from the Integrated Pest Control Laboratory of the UPTD Aceh Plantation Plant Protection Center. The isolates were rejuvenated in the Plant Protection Basic Laboratory of the Plant Protection Study Program. *B. bassiana* isolates were propagated in laminar airflow using a loop needle, then inoculated into the prepared sterile PDA media. The fungal culture was incubated at 25°C in the laboratory. Propagation of *B. bassiana* was carried out with the aim of obtaining research materials and pure cultures as well as getting the fungus age not too old. The *B. bassiana* inoculum that had been propagated was then incubated at room temperature for 14 days. The propagation of this fungus was carried out in a laminar airflow cabinet to avoid contamination.

Preparation of suspension of conidia *B. bassiana*

The conidia suspension of *B. bassiana* was obtained by adding 10 mL of aquadest to a petridish containing 14 days of *B. bassiana* culture. The conidia were taken using a loop needle until they were released from the PDA media; 1 mL of the conidia suspension was taken using a dropper and diluted into 9 mL of distilled water and homogenized. Then the conidia were counted using a haemocytometer [5]

$$C = \frac{t}{N \times 0,25} 10^6$$

Notes:

C = Density of spores per mL of solution

t = Total number of observed samples

N = Number of sample boxes (5 large boxes x 16 small boxes)

0.25 = Correction factor for the use of a small-scale sample box on the Hemacytometer

Application of *B. bassiana* to *C. pavonana* larvae.

Prepared suspension of *B. bassiana*, and 10 individual *C. pavonana* second instar larvae. Application of conidia *B. bassiana* on *C. pavonana* larvae was carried out by the contamination method by spraying a suspension of *B. bassiana* conidia on *C. pavonana* larvae for 5 seconds. Then air-dried for a few seconds and put in each jar. The mustard leaves were cut in a rectangular shape, measuring 5x5 cm, a total of 3 pieces in each treatment; then the mustard leaves were put into a jar that had been lined with straw paper. Then 10 larvae of *C. pavonana* instar II were added. After 24 hours, the feed was replaced with fresh leaves, as well as for other treatments.

Observed variables mortality of *C. pavonana* larvae

Mortality of *C. pavonana* larvae was carried out by counting the number of dead larvae from one day after application to each treatment unit until all larvae became pupa. Larvae mortality was calculated using the formula [1].

$$Po = r/n \times 100\%$$

notes:

Po = Percentage of mortality

r = Number of dead larvae

n = total number of larvae

C. pavonana pupa formed

The percentage of pupa was calculated from the period the larva entered the pre-pupae stage until the pupae were formed. The percentage of pupa formed is calculated using the following formula:

$$\text{Percentage of pupa formed} = (\text{number of pupa formed}) / (\text{number of early larvae}) \times 100\%$$

Imago *C. pavonana* appearance

The percentage of imago was calculated using the following formula:

$$\text{Percentage of imago that appeared} = (\text{number of imago that appeared}) / (\text{number of early larvae}) \times 100\%$$

Average period to death of *C. pavonana*

The rate of death was calculated with an interval of 1 day after application until there are treatment units that die 100%; the calculation is carried out using the following formula:

$$\text{Rate of death period} = (\Sigma(\text{period of death} \times \text{number of dead larvae}) / (\text{Number of early larvae})).$$

The percentage of pupa was calculated from the period the larva entered the pre-pupae stage until the pupae were formed. The percentage of

pupa formed is calculated using the following formula:

$$\text{Percentage of pupa formed} = (\text{number of pupa formed}) / (\text{number of early larvae}) \times 100\%$$

Imago *C. pavonana* Appears.

Incubation period of the fungus *B. Bassiana*

The incubation period is the period of period between the inoculation of the fungus *B. bassiana* until it causes symptoms of infection in the larvae of *C. pavonana*. This observation was made based on the incubation period of the fungus, whether there were any changes observed visually in the larvae. Calculations were made on the average incubation period of the fungus from one day after application until the appearance of symptoms of infection in the larvae.

RESULTS AND DISCUSSION

The results of observations on the mortality of *C. pavonana* larvae due to the application of *B. bassiana* conidia with different density levels in each treatment, and observed at 3 to 6 days after application (DAA) showed that the effect was very significant at each observation time, namely 3, 4, 5 and 6 days after application of conidia *B. bassiana*. The average mortality of *C. pavonana* larvae can be observed in Table 2.

Table 2. Average mortality of *C. pavonana* larvae due to application of *B. bassiana* conidia

Treatment (Conidia Density/mL aquadest)	Mortality Larvae (%)			
	3 DAA	4 DAA	5 DAA	6 DAA
B0 (Control)	0.00 c	0.00 b	0.00 c	0.00 d
B1 (10 ²)	22.50 b	37.50 a	50.00 b	62.50 c
B2 (10 ⁴)	30.00 a	50.00 a	57.50 b	75.00 b
B3 (10 ⁶)	30.00 a	37.50 a	60.00 b	82.50 b
B4 (10 ⁸)	37.50 a	60.00 a	80.00 a	97.50 a
Score LSD	12.65	9.28	11.77	10.17

The numbers followed by the same letter in each column were not significantly different at 0.05 test LSD.

Table 2 shows that the average mortality of *C. pavonana* larvae due to the application of *B. bassiana* conidia experienced a very significant change at 3,4,5 and 6 DAA observations. In general, the mortality of *C. pavonana* larvae occurred from 3 to 6 DAA in each treatment. Observations on 6 DAA that the highest mean mortality was found in the treatment of conidia density 10⁸ (97.50%), followed by 10⁶ (82.50%) and 10⁴ (75%). while the lowest was found in treatment 10², (62.50%). This is thought to be related to the application level of conidia density. The higher the conidia density given, the higher the

mortality of *C. pavonana*. This is related to the ability of conidia to invade and spread evenly on parts of the insect's body. The conidia of *B. bassiana* can penetrate into the host's body quickly, and influenced by the density level of conidia which are infective propagules of *B. bassiana*[14].

That the application of conidia *B. bassiana* to *Plutella xylostella* larvae resulted in the death of larvae up to 55.00% using a density of 108 conidia/mL aquadest which was observed 4 days after application [3]. and application of *B. bassiana* conidia to *Spodoptera litura* larvae caused 72.50% mortality with a density of 10⁸ conidia / mL of distilled water and was observed 7 days after application[11].

Conidia *B. bassiana* can infect *C. pavonana* larvae, presumably due to the activity of the enzymes it products [8], when infecting the host, the conidia of the fungus *B. bassiana* produce enzyme compounds such as proteases, lipotics, amylase and chitinase [15]. If there is contact between the conidia and their host, the conidia will form a sprout tube and secrete enzymes to penetrate the cuticle of the larvae so that it can enter the larva's body. The fungus *B. bassiana* produces toxins, such as beauvericin, beauverolite, bassianolite, isorolite and oxalic acid, then *B. bassiana* hyphae enter the host's body through natural holes [19].

The infection entomopathogen fungi cause death in the host by absorbing nutrients and spreading toxins to the hemolymph so that it can affect the development and physiology and reproduction of insects. Visually, early symptoms of infected *C. pavonana* larvae were characterized by reduced feeding activity and weaker physical characteristics of the larvae and slower larval movement[7]. The larvae will experience a change in skin color to pale white then the larvae will die and harden like a mummy. Dead larvae will have black spots before the growth of mycelium on the outer skin of the insect [21]. The initial symptoms due to infection from entomopathogenic fungi are slow, silent, or inactive movements until they eventually die [10]

The result of *C. pavonana* pupae

The results of observations of *C. pavonana* pupae produced due to the application of *B. bassiana* conidia with various conidia density levels were calculated on observations 7 days after application. The results of the F test on analysis of variance showed that the treatment of conidia density of the fungus *B. bassiana* had a very significant effect on the percentage of pupae production at 7 days after application.

The average of *C. pavonana* pupae produced can be seen in table 3.

Table 3. Average Pupae of *C. pavonana* Formed by Application of the Fungus *B. bassiana*

Treatment (Conidia Density/mL aquadest)	Produces pupae (%) at 7 days after application
B0 (Control)	100.00 a
B1 (10 ²)	15.00 b
B2 (10 ⁴)	5.00 c
B3 (10 ⁶)	0.00 c
B4 (10 ⁸)	0.00 c
LSD	8,45

The numbers followed by the same letter in each column were not significantly different at 0.05 test (LSD).

Table 3 shows that the larvae of *C. pavonana* that successfully pupae after the application of *B. bassiana* conidia were significantly different in each treatment. The highest percentage of pupae up to 100% occurred at control. 15% produce pupae occurred in the treatment of conidia 10²/ mL aquadest of distilled water. 5% produced *C. pavonana* pupae after application of *B. basiana* conidia with conidia density of 10⁴ conidia/mL of distilled water. The occurrence of differences in the quantity of individual larvae to turn into pupae is thought to be strongly related to the size of the application of different *B. bassiana* conidia density. At the period of application, it was suspected that not all of the conidia were attached to the cuticle of the host. so it takes a period to infect the host. The ability of *B. bassiana* conidia to infect the host is influenced by concentration, viability and virulence [6]. Thus, the application of low conidia density of *B. bassiana* took longer to infect the host.

Generally, the larvae applied to *B. bassiana* conidia died, but there were still larvae that became pupae and imago. The change of skin at each instar is one of the inhibiting factors for *B. bassiana* conidia in penetrating the cuticle of *C. pavonana*. That conidia density affects the percentage of mortality and the process of pupae formation. Different application of *B. bassiana* conidia resulted in different number of conidia that were able to attach to the pupae [22].

C. pavonana larvae that successfully become Imago

Observation of *C. pavonana* larvae into imago. The application of *B. bassiana* conidia with different conidia densities and observed

at 14 days after application, showed that it had a very significant effect on the change from successful *C. pavonana* larvae to imago. The average imago of *C. pavonana* that appeared can be observed in Table 4.

Table 4. Average Imago of *C. pavonana* that appeared due to the application of conidia density of *B. bassiana*

Treatment (Conidia Density/mL aquadest)	Imago generated (%) at 14 Days After Application
B0 (No Treatment)	100.00 d
B1 (10^2)	15.00 c
B2 (10^4)	5.00 b
B3 (10^6)	0.00 a
B4 (10^8)	0.00 a
LSD	8.45

The numbers followed by the same letter in each column were not significantly different in the 0.05 test LSD

Table 4 shows that the imago of *C. pavonana* that was successfully transformed into imago after the application of *B. bassiana* conidia was significantly different between each treatment. The highest percentage of *C. pavonana* larvae that managed to become imago using a density of 10^2 conidia/mL aquadest, which was 15.00%, compared to the use of a density of 10^4 conidia/mL aquadest, which was 5%. and control reaches 100% to imago. The percentage of *C. pavonana* larvae that managed to become imago was closely related to the conidia density of *B. bassiana* applied. The density of the applied *B. bassiana* conidia is related to the opportunity for the conidia to adhere to the host cuticle. The higher the density of conidia attached to the host, the greater the amount of toxins and enzymes produced that enter the host's body. This resulted in the mortality rate of *C. pavonana* increasing.

The application of conidia of *B. bassiana* 10^8 mL/10 mL of distilled water to *Bactrocera carambolae* pupae showed that the percentage of pupae that succeeded in becoming imagos reached 52% [21]. If the larvae that have been infected by the fungus *B. bassiana* and cause symptoms of infection at the pupa stage, it is possible that symptoms will appear when entering the imago stage, marked by not being to produce eggs. This is a result of the fungus that has entered the insect's body will grow and taking nutrients from the host [4].

Incubation period of the fungus *B. bassiana*

The results of observations of the incubation period of the fungus *B. bassiana* on *C. pavonana* larvae with several sizes of conidia

density were calculated based on the presence or absence of changes observed visually in the larvae. The results of the F test on analysis of variance showed that the incubation period of the fungus *B. bassiana* had a very significant effect on the infection activity of *C. pavonana* larvae. The average incubation period of the *B. bassiana* against *C. pavonana* can be observed in Table 5.

Table 5. Average incubation period of *B. bassiana* against *C. pavonana* larvae

Treatment (Conidia Density/mL aquadest)	Incubation period (days)
B1 (10^2)	5.75a
B2 (10^4)	4.75b
B3 (10^6)	4.50c
B4 (10^8)	3.50d
LSD	0.244

The numbers followed by the same letter in each column were not significantly different in the 0.05 test LSD

Table 5 shows that the fastest incubation period for the fungus *B. bassiana* occurred in the conidia 10^8 /mL Aquades treatment, which was 3.50 days, and the 10^4 /mL aquadest and 10^6 /mL aquadest treatments showed that the average incubation period was 4.75 and 4, respectively. 50 days and the longest incubation period occurred in treatment 10^2 , 5.75 days. The difference in incubation period in each treatment was thought to be related to the level of the different conidia density in each treatment. This is because the higher the conidia density of the fungus, the easier it is for the fungus to infect the host. Likewise, if the conidia density is low, the host infection process takes long.

Visual observation of *C. pavonana* larvae infected with the fungus *B. bassiana* was indicated by decreased feeding activity of larvae, slow movement of larvae. Larvae infected with the fungus *B. bassiana* will experience a process of changing the color of the insect's body to become pale white and harden, then the larva's body will change color to black-brown, then the mycelia will appear out through natural holes that will penetrate the integument of the *C. pavonana* larvae. The fungus will absorb the body fluids of the host, so that the integumental surface of the infected host's body looks dry, wrinkled, and dark in color and hardens like a mummy [14].

B. bassiana conidia inoculation process is strongly influenced by insect body structure. The fungus *B. bassiana* on larvae with a thin cuticle layer and relatively soft larvae body

structure causes the fungus to more easily infect and penetrate. The results of research by stated that the growth of *B. bassiana* colonies showed a faster growth rate from the second day to the fourth day, on the sixth day the colonies grew the most, in addition to genetic factors produced was also influenced by external factors, namely temperature and pH [17].



Figure 1. Healthy *C. pavonana* (A) and (B). *C. pavonana* infected with *B. bassiana* conidia. (Using a microscope 40x magnification).

Mean Time to Death of *C. pavonana*.

The results of observations of the average time of death of *C. pavonana* due to the application of *B. bassiana* with different conidia density levels significantly affected the time of death of *C. pavonana* larvae and can be observed in Table 6.

Table 6. Average time of death of *C. pavonana* due to application of conidia *B. bassiana*

Treatment (Conidia Density/mL aquadest)	Average Time of Death (Days)
B1 (10^2)	3.03a
B2 (10^4)	4.20b
B3 (10^6)	5.05b
B4 (10^8)	4.08b
LSD	0.25

The numbers followed by the same letter in each column were not significantly different at 0.05 test LSD

Table 6 shows that the application of *B. bassiana* conidia to *C. pavonana* larvae had very significant differences in each treatment. The fastest average time of death occurred in treatment 10^2 , which was 3.03 days. While the longest occurred in treatment 10^6 conidia/mL aquadest (5.05 days). This is presumably because the time required to cause insect death varies depending on the virulence and resistance properties of the host. In treatment 10^2 conidia/mL aquadest the average time of death was found to be faster this is because there are individual larvae that have successfully entered the pupae stage. The longest average death time

occurred in the 10^6 conidia/mL aquadest treatments because the highest average larvae mortality occurred at Treatment of conidia density of 10^2 /mL aquadest to produce larval death of *C. pavonana* took 4.20 days while 10^8 conidia/mL aquadest was needed to produce larvae death it took 4.08 days. The difference in mean time of death required for *C. pavonana* larvae is thought to be strongly related to time and application and conidia density of *B. bassiana*. Conidia from *B. bassiana* to infect the host takes 2 to 14 days after application, and its effectiveness is strongly influenced by environmental factors such as temperature, humidity and light. [9]. The morphological condition of the host affects the process of conidia infection in the host body until it enters the host's body [12].

CONCLUSION

The results showed that the density of conidia aquadest *B. bassiana* 10^8 /mL had a positive effect on its virulence against *C. pavonana* pests. The highest mortality of *C. pavonana* larvae was found in the density of *B. bassiana* conidia 10^8 /mL of distilled water, which was 97.50% at observation 6 days after application, compared to the density of 10^2 conidia/mL of distilled water. that is 62.50%. *C. pavonana* larvae successfully pupated 15% at a conidia density of *B. bassiana* 10^2 /mL and 5% at a density of 10^4 conidia/mL aquadest which were observed 7 days after application. The percentage of *C. pavonana* larvae that were successfully transformed into imago at a conidia density of 10^2 /mL was 15%, and 5% at a conidia density of 10^4 /mL of distilled water, which were observed 14 days after application. The fastest incubation time occurred at a density of 10^8 conidia/mL aquadest, which was 3.50 days. The fastest average time of death occurred at a density of 10^2 /mL aquadest, which was 3.03 days

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