AGRIVITA VOLUME 32 No. 3 *OCTOBER-2010* *ISSN : 0126-0537*

VIRULENCE OF ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* ISOLATES TO *CROCIDOLOMIA PAVONANA* F (LEPIDOPTERA: CRAMBIDAE)

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Received : March 12, 2009 / Accepted : February 12, 2010

ABSTRACT

The purpose of this research was to identify the virulent isolates of *Beauveria bassiana* (Bals.) Vuill. to larvae of *Crocidolomia pavonana* (F.) and also to investigate the effects of conidial concentrations on larval mortality of *C. pavonana*. This experiment was conducted at Laboratory of Biological Control, Plant pest and diseases Department, Faculty of Agriculture, Andalas University from April to October 2008. *B. bassiana* were collected from insects and soils then cultured using selective medium. Thirteen isolates and four different concentration conidia(10^6-10^9 conidia/ml) were tested in the experiment. Larvae directly inoculated with conidial suspensions of entomopathogenic fungus. The results showed at a concentration of 10^8 conidia/ml, isolate HhTK9 was the most virulent caused 82.50% mortality of 2nd instar larvae, with LT_{50} 3.39 days. The mortality *C. pavonana* larvae was positively correlated with by fungal isolate and conidial concentration.

Keywords: Entomopathogenic, *Beauveria bassiana*, virulence, isolates, *Crocidolomia pavonana*

INTRODUCTION

Crocidolomia pavonana (F.) (Lepidoptera: Crambidae) is one of the important pests on crucifer crops such as cabbage, broccoli, cauliflower, an other cruciferous crops in Indonesia. Larvae damage by eating leaves, especially young leaves and toward growing point of plants and can cause death in plants. They often cause heavily damage on cabbage crops particularly in dry season (Kalshoven, 1981; Dadang *et al.,* 2009).

Until now, pest control of *C. pavonana* still very dependent on synthetic pesticides, because it is easily implemented and can reduce pest populations quickly. Applications of pesticide conducted intensively and sometimes farmers are spraying on the plants ready for harvest without considering its impact on consumers. The use of chemical insecticides that very intensive will disrupt the life of even natural enemies and pollute the environment. This is unfortunate given to the era of environmentally Indonesia's agricultural development, so the use of synthetic chemical insecticides should be minimized.

Environmental concerns and health risks associated with the use of synthetic chemical insecticides have stimulated efforts to develop biological control agents for integrated pest management (Mancebo *et al.,* 2005). Reduction of pesticide inputs will also reduce pesticide residues, so that the product can be more competitive in the market.

In IPM, using of natural enemies and other biological potential is the main component, because natural enemies have an important role in the suppression of pest populations and maintain the ecosystem balance. Among the natural enemies that can be used for pest control *C.pavonana* biologically is entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill.

In general, *B. bassiana* fungus infect the insect through the integuments of insect. Other than through the integuments, this fungi may also infect through the buccal cavity, spiracles and injury (Broome *et al.,* 1976). Infection through insect integuments began after

integuments are contaminated by conidia of fungi. Conidia will germinate and form germ tubes and produce enzyme such as proteinase, lipase, and chitinase are useful to soften the integuments of insects. After successfully penetrating into the body of insects, mycelium will follow the blood stream and spread throughout the insect body parts, hyphae will multiply and produce beauvericin toxins. The presence of biochemical changes in the protein content of hemolimfa especially, nutritional deficiency, the formation of toxins released by fungi, and destruction of tissues of insects that may cause paralysis and death in insects. If the insect host has died, hyphae will penetrate out and form spores on the outer surface of the body (Tanada and Kaya, 1993).

Various information about the use of *B. bassiana* fungus for pest control has been widely reported. Use of *B. bassiana* can reduce populations of potato beetle larvae, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) up to 76.6% in potato growing (Poprowski *et al.,* 1997), on *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) reduced the population up to 72% (Nankinga and Moore. 2000), and in *Ceratitis capitata* (Diptera:: Tephritidae) effective in reducing adult population and protecting orange fruits in the field (Ortu *et al.,* 2009). In Indonesia, *B. bassiana* had been widely used to control coffee fruit borer, *Hypothenemus hampei* (Ferr.) (Coleoptera: Scolytidae) (Indonesians Ornamental Crops Research Institute, 2006).

Utilization of *B. bassiana* to control *C. pavonana* pests not been widely reported. Results of preliminary research showed that *B. bassiana* isolated from *H. hampei* pests effective in controlling pests of *C. pavonana* (Trizelia 2000) and *Spodoptera litura* (F.) (Huri, 2009).

To support the successful use of *B. bassiana* to control *C. pavonana* pests biologically, the choice of high virulence isolates is needed. This study aimed to identify the virulent isolates of *Beauveria bassiana* (Bals.) Vuill. to larvae of *Crocidolomia pavonana* (F.) and also to investigate the effects of conidial concentrations on larval mortality of *C. pavonana*.

MATERIALS AND METHODS

Fungal Isolates

B. bassiana isolates used in this study were obtained from the collection of Biological Control Laboratory of Department Plant Pest and Diseases of Agricultural Faculty Andalas University and collections directly from the infected insects in the field and from soil from different host and locations (Table 1).

Isolates were collected from two districts in West Sumatra (Solok and Agam) and one district in Jambi (Sarolangun). All the isolates were grown on Sabouraud dextrose Agar with yeast extract (SDAY) (dextrose 10 g, peptone 2.5 g, 2.5 g yeast extract, to 20 g, chloramphenicol 0.5 g and distilled water, 1 l) (Samuels *et al.,* 2002).

Table 1. *B. bassiana* isolates and geographic origins

Isolates	Original Host	Geographic Origins
HhTK1	*Hypothenemus hampei*	Teluk Kecimbung (Sarolangun)
HhTK9	*Hypothenemus hampei*	Teluk Kecimbung (Sarolangun)
HhKA1	*Hypothenemus hampei*	Kayu Aro (Solok)
HhKA2	*Hypothenemus hampei*	Kayu Aro (Solok)
HhKA3	*Hypothenemus hampei*	Kayu Aro (Solok)
HhKA4	*Hypothenemus hampei*	Kayu Aro (Solok)
HhKA5	*Hypothenemus hampei*	Kayu Aro (Solok)
HhKA7	*Hypothenemus hampei*	Kayu Aro (Solok)
HhKA11	*Hypothenemus hampei*	Kayu Aro (Solok)
HhKA12	*Hypothenemus hampei*	Kayu Aro (Solok)
HhKA14	*Hypothenemus hampei*	Kayu Aro (Solok)
TKA	Tanah Kopi	Kayu Aro (Solok)
TPLB2	Tanah Kubis	Padang Luar (Agam)

Isolation of entomopathogenic fungi from soil using selective medium DOC2 (Bacto-peptone 3 g, 0.2 g CuCl2, 2 mg of crystal violet, to 15 g, water 1000 ml) (Shimazu *et al.,* 2002). From each soil sample taken as many as 10g, was dissolved in 90 ml of sterile distilled water, which has been given a 0.05% Tween 80 and was vortexed for 2 minutes. Soil suspension was diluted up to three times and 0.1 ml of suspension poured in Petri dishes that contained DOC2 medium for growth of B. bassiana. Petri dishes were incubated for 8 days and colonies of B. bassiana were isolated again and purified on SDAY medium (Sabouraud dextrose with yeast extract in order).

Cabbage Plants

Cabbage plants used as food of *C. pavonana* larvae planted in polybags. Seedlings that have been aged for one month is transferred into polybags containing a mixture of soil and manure. Plants fertilized with NPK (15:15:15) a week after planting as much as 0.5 g / polybag. Fertilization is done back at the time the plant has aged one and two months. Plants watered every day and the plants are not sprayed with pesticides.

Rearing of *C. pavonana* Larvae

Larvae of *C. pavonana* were collected from cabbage crops in the Padang Laweh, Agam regency, West Sumatra. These larvae were reared in plastic boxes and fed with cabbage leaves. Cabbage leaves were changed everyday. Larvae were reared until forming pupa and adult . Adults kept in cages containing cabbage leaves as the laying of eggs. As adult food used honey with in concentration 10%. Eggs cluster transferred to another plastic box and kept until hatching. Second instar larvae which is used for testing.

Preparation of Conidial Suspension

All the isolates were grown on SDAY medium and incubated in a petri dish at 25^{o} C for 15 days. Fungal suspensions were prepared by scrapping conidia from the surface of 15 day-old cultures using fine brushes and added 5 ml sterilized distilled water containing 0.05% Tween 80. This suspension was filtered and the conidial concentration was determined using the hemocytometer.

Screening of *B. bassiana* Isolates

Second instar larvae of *C. pavonana* were used in this experiment. Concentration of conidia of each fungus used adalah10^{8} conidia / ml. Fungi inoculated by spraying conidial suspension (0.2 cc/20 larvae) in the dorsal part body of larvae using handsprayer. Larvae that had been sprayed fed with fresh cabbage.

The treatment were repeated four times and each treatment unit consisted of 20 larvae. Mortality of larvae was observed every day until seven days after the application of fungi. The experiment was arranged in completely randomized design (CRD). Results were analysed using Analysis of Variance followed by followed by Duncan's New Multiple Range Test (DNMRT).

Effect of Conidia Concentration of *B. bassiana*

Second instar larvae of *C. pavonana* were used in this tests.. Concentration of conidia of the isolates used were 10^{9}, 10^{8}, 10^{7}, 10^{6} conidia / ml and 0 (control). Inoculation of fungi carried by spraying conidial suspension (0.2 cc/20 larvae) in the dorsal part of the larvae using handsprayer, and the larvae fed with fresh cabbage leaves.

The treatment was repeated four times and each treatment unit consisted of 20 larvae. Mortality of larvae was observed every day until seven days after the application of fungus The experiment was arranged in completely randomized design (CRD). Results were analysed using Analysis of Variance followed by followed by Duncan's New Multiple Range Test (DNMRT).

RESULTS AND DISCUSSION

Screening Isolates

The results showed that the virulence of isolates of *B. bassiana* against second instar larvae of *C. pavonana* differs according to host or geographic origin. HhTk9 isolates are most virulent isolates, because it causes mortality of larvae of *C. pavonana* highest up to 82.50% (on the observation of the seventh day after application). Virulence of HhKA3 isolates derived from the Kayu Aro, Solok very low with an average mortality of 41.25% (Table 2).

Virulence differences between isolates are common things that have happened in entomophatogenic fungus (Wekesa *et al.,*2005).

Results of previous studies showed that isolates originating from the same insect host and region (Bb-Cp) only cause mortality of larvae of 61.25% (Trizelia, 2005). This indicates that *B. bassiana* isolates that are virulent to *C. pavonana* can be obtained from other sources. Results of previous studies also showed that the entomopathogenic fungus B. bassiana originating from the same insect with test insects do not always have a higher pathogenicity of the test insect, so it needs to do more testing of isolates to obtain a more virulent isolates (Kreutz *et al.,* 2004).

Table 2. Mortality of second instar larvae of *C. pavonana* seven days after the application of *B. bassiana* isolates isolates at a concentration of 10^8 conidia / ml

Isolat	Mortalitas (%) ± SD
HhTK9	82.50 ± 9.57 a
HhTK1	80.00 ± 4.08 ab
HhKA14	78.75 ± 4.79 ab
HhKA7	71.25 ± 2.50 bc
HhKA1	66.25 ± 7.50 cd
HhKA11	63.75 ± 4.79 cde
HhKA4	62.50 ± 11.90 cde
HhKA2	57.50 ± 10.41de
HhKA5	57.50 ± 6.45 de
TKA	56.25 ± 4.79 de
HhKA12	56.25 ± 4.79 de
TPLB2	55.00 ± 4.08 e
HhKA3	41.25 ± 8.54 f
Kontrol	2.50 ± 2.89 g

Remarks: Means within column bearing the same letter are not significantly different by Duncan test(DNMRT) ($p< 0.05\%$).

The results of this study differ from the results of research that has been reported by other researchers suggested that the isolates or strains of entomopathogenic fungi were isolated from the same host or adjacent to the test host more virulent than strains isolated from other hosts (Cottrell and ShapiroIlan 2003; Ansari *et al.,*2004; Samuels and Coracini 2004).

The differences in the virulence of *B. bassiana* isolates tested were due to physiological characters differences between isolates such as germination of conidia, growth of colonies, the sporulation and secondary metabolism that is produced such as the ability to produce enzymes and toxins. Mahdneshin *et al.,*. (2009) reported that virulence differences of *B. bassiana* isolates against *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) is caused by differences in ability of conidia germination from each isolates.Germination of conidia is one of the determinants of virulence. Virulent isolates had conidia germination was higher than that avirulen isolates.

Tanada and Kaya (1993) suggested that the differences in virulence between isolates was due to differences in the ability to produce enzymes and mycotoxins during the passage of an infection process in insects such as contact with the cuticle and within hemosoel. Virulent isolates had a higher enzyme activity compared with avirulen isolates.

To determine the virulence LT_{50} values also be observed, ie the time required to kill a population of at least 50%. Based on the LT_{50} values there is a difference between isolates (Table 3). LT_{50} values *B. bassiana* ranging from 3:39 - 8:46 day. HhTK9 isolate had LT_{50} values shortest 3:39 day compared with other isolates. This means that the time required to kill 50% of second instar larvae of *C. pavonana* shorter than the other isolates.

Table 3. LT_{50} values of *B. bassiana* isolates

Isolat	LT_{50} (SK 95%) (days)
HhTK9	3.39 (3.09-3.68)
HhTK1	5.13 (4.45-6.14)
HhKA14	5.39 (5.07-5.74)
HhKA7	5.14 (4.77-5.59)
HhKA1	5.97 (5.62-6.43)
HhKA11	5.77 (5.25-6.53)
HhKA4	6.14 (5.64-6.87)
HhKA2	6.04 (5.48-6.85)
HhKA5	6.78 (6.08-7.98)
TKA	6.52 (5.90-7.53)
HhKA12	6.75 (6.15-7.70)
TPLB2	7.40 (6.60-8.93)
HhKA3	8.46 (7.28-11.08)

LT50 values differences between *B. bassiana* isolates was also reported by Junianto and Sulistyowati (1994) that suggests that the isolates of *B. bassiana* virulent against H. hampei (Bb-704) adults had LT_{50} value of 4.6 days shorter than the avirulen isolates (Bb-706) which has the LT_{50} value of 7.1 days. Lengther time of the death of the *C. pavonana* larvae infected by *B.bass*iana caused by the fungus requires several steps for the process to infect

and kill insects, which is from attachment of conidia on the insect body, germination, penetration, invasion and colonization in hemosul, tissues and organs. Time for each of these stages varies depending on the type of fungus, host and environment (Neves and Alves, 2004).

The results Neves and Alves (2004) showed that conidia attachment of *B. bassiana* on the *Cornitermes cumulans* (Kollar) (Isoptera: Termitidae) cuticle occur until 6 hours after application and germination began to occur between 6-12 hours after application. Penetration occurs 12-24 hours after inoculation and insect death occurs between 48-72 hours after inoculation. Hashim (2000) reported that the attachment and germination of conidia of B. bassiana on the cuticle of *Crocidolomia binotalis* Zell. larvae began to occur 4-6 hours after application and larval mortality occurred between 24-48 hours after inoculation.

Variation of insect mortality speed among *B. bassiana* isolates also reported by Kassa *et al.,* (2002) who stated that different *B. bassiana* isolate had a different deadly speed against *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). For isolate PPRC-HH, mortality occured at the beginning of the second day and on the fourth day of the test insect mortality reached 100% so MST value (*Median Survival Time*) was shorter. Another isolates(PPRC-9609 and PPRC-9614), mortality of *S. zeamais* occured on the fourth day.

Effect of Conidia Concentration of *B. bassiana* against Mortality of *C. pavonana*

Results showed that the concentration of conidia *B.bassiana* significant effect on mortality of second instar larvae of *C. pavonana*. There is a strong correlation (R2 = 0.9761) between the concentration of conidia with mortality. Larval mortality of *C. pavonana* increased with increasing concentration of conidia *B.bassiana* (Figure 1). At a concentration of 106 conidia / ml caused 55% mortality of larvae, and at a concentration of 10^9 conidia / ml of larval mortality increased to 100%. This means that the higher number of conidia, giving better opportunities for conidia to attachment, germinate and penetrate into the body of the target pest insects that are infected or die bigger. Roberts and Yendol (1971) suggested that one factor to guarantee the occurrence of entomopathogenic fungi infection in insects is the amount of inoculum.

Yoon *et al.,* (1999) suggested that the increased mortality of larvae of *P. xylostella* from infections caused by *B. bassiana* conidia related with the increasing concentration caused by an increase in the number of conidia attached to the body of larvae. At a concentration of 107 conidia / ml. number of conidia attached to the body larvae of about 1813.89 while the at a concentration conidia 108 conidia / ml, the number of conidia attached to the body of the larvae was 9861.11 conidia.

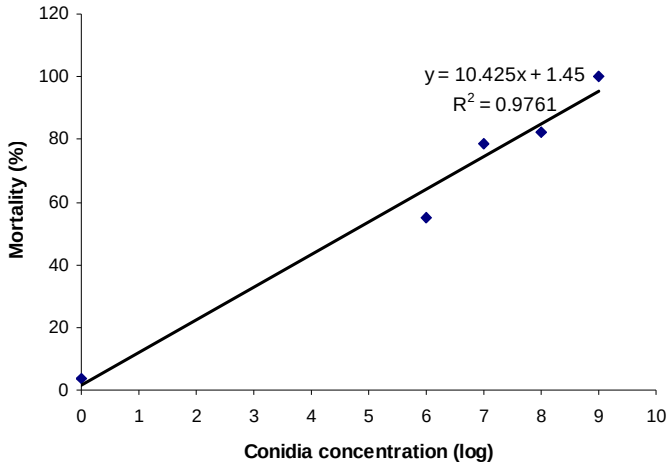

Figure 1. Relationship between concentration of conidia of *B. bassiana* with mortality of *C. pavonana*

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The results of this study was similar with experiment results reported by several researchers. There was a positive correlation between the concentration of conidia of B. bassiana with mortality of insects. This positive correlation indicates that with increasing concentration of the conidia of fungi can enhance and accelerate the death of the larvae. The higher the concentration of conidia have higher mortality of the insect (Eken *et al.,* 2006). For pest control C. pavonana concentration of conidia of B. bassiana is used at least 10^8 conidia / ml.

CONCLUSIONS AND SUGESSTION

Pathogenicity or virulence of *B. bassiana* isolates against *C. pavonana* larvae vary greatly depending on the source of isolates and conidia concentrations. HhTK9 most virulent isolates compared with other isolates. Larval mortality of *C. pavonana* influenced by the concentration of conidia. The higher the concentration of conidia, the higher the mortality of larvae of C. pavonana. Therefore, in controlling *C. pavonana* with entomopathogenic fungi both of these factors (isolates and conidia concentrations) should be considered.

For the development of *B. bassiana* as bioinsecticides need to study more about the propagation of fungus, formulations and test its effectiveness on cabbage crops in the field.

ACKNOWLEDGEMENTS

Thanks to Directorate General of Higher Education in accordance with the Imple-mentation Agreement of Employment Research Number 005/SP2H/PP/DP2M/III/2008 March 6, 2008 which has helped fund this research.

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