J. HPT Tropika. ISSN 1411-7525
Vol. 9, No. 2: 99 - 108, September 2009

POTENTIAL IMPACT OF *METARHIZIUM ANISOPLIAE* ON THE DIAMONDBACK MOTH (LEPIDOPTERA: PLUTELLIDAE) AND ITS PARASITOID *DIADEGMA SEMICLAUSUM* (HYMENOPTERA: ICHNEUMONIDAE)

Rosma Hasibuan[1], Nilly Christalia[2], F.X. Susilo[1], and Nur Yasin[1]

ABSTRACT

***Potential Impact of* Metarhizium anisopliae *on the Diamondback Moth (Lepidoptera: Plutellidae) and Its Parasitoid* Diadegma semiclausum *(Hymenoptera: Ichneumonidae)*.** Laboratory studies were conducted to evaluate the effect of the *Metarhizium anisopliae* against the diamondback moth, *Plutella xylostella* and its parasitoid, *Diadegma semiclausum*. A completely randomized design consisted of 5 treatments (4 concentrations of conidial suspension: 5 x 10^4, 3.5 x 10^5, 2.5 x 10^6, 1.2 x 10^7 conidia/ml and control) was used. The results indicated that the mortality of *P. xylostella* larvae were significantly induced by the fungal treatments. A significant reduction in pupation and adult emergence of *P. xylostella* was also detected in all treatments when compared with that in the control. The fungus might also result in a male-biased sex ratio of the surviving *P. xylostella*. When applied at a concentration of 1.2 x 10^7 conidia/ml, *M. anisopliae* might significantly reduce the survival of the parasitoid, *D. semiclausum*. Thus, despite its potential as a biological control agent against *P. xylostella*, the entomomogenous fungus *M. anisopliae* was also detrimental to the larvae parasitoid *D. semiclausum.*

Key words : *Metarhizium anisopliae, Plutella xylostella, Diadegma semiclausum,* Entomopathogen, target effect, nontarget effect

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.), is one of the most destructive pests of cruciferous vegetables worldwide including Indonesia. This insect pest attacks only plants in the family Cruciferae (Chua & Lim, 1979). Virtually all cruciferous vegetable crops are eaten by diamondback moth, including cabbage, Chinese cabbage, cauliflower, mustard, and false pak choi. Plant damage is caused by larval feeding. Although the larvae are very small, they can be quite numerous, resulting in complete removal of foliar tissues except for the leaf veins (Talekar & Griggs, 1986; Talekar & Shelton, 1993).

To control this pest, growers apply various chemical pesticides regularly such as profenofos 500 gr/L, deltametrin 25gr/L, and formetanat 25%, sometimes as frequently as twice per week (Pracaya, 2005). Heavy reliance on pesticides has been known to cause widespread damage to the environment, including development of insecticide resistant insects and secondary pest outbreaks (Croft, 1990; Tabashnik *et al*., 1987; Dent, 2000; Koul & Cuperus, 2007). One way to reduce the reliance on pesticide application is to maximize the role of natural control agents. The entomomogenous fungus, *Metarhizium anisopliae* (Metschnikoff)Sorokin(Deuteromycotina: Hyphomycetes) is a promising alternative control agent for the diamondback moth. Fungus *M. anisopliae* is well known for its ability to control insect pests. This fungus has been reported infecting various insect pests, including the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Garcia *et al*., 1984), coconut leaf beetle, *Brontispa longissima* (Liu *et al.*, 1989), the house fly, *Musca domestica* L. (Barson *et al.,*1994), the Japanese beetle, *Popillia japonica* (Re Lacey *et al*., 1994); black vine weevil, *Otiorhynchus sulcatus* (Moorhouse *et al.*, 1993), the coffee berry borer, *Hypothenemus hampei*

[1] Department of Plant protection, Faculty of Agriculture, University of Lampung,
Jl. Prof. Sumantri Brojonegoro No. 1 Bandar Lampung, 35145. Email : rosma@unila.ac.id
[2] Alumnus from Department of Plant protection, Faculty of Agriculture, University of Lampung,
Jl. Prof. Sumantri Brojonegoro No. 1 Bandar Lampung, 35145

(Ferrari) (De La Rosa *et al.,* 2000); tsetse flies, *Glossina* spp., (Kaaya & Munyinyi, 1995), stink bugs, *Nezara viridula, Piezodorus guildinii*, and *Euschistus heros* (Sosa-Gomez & Moscardi, 1998). This pathogenic fungus has been used widely for biological control of various agricultural pests and other soil inhabiting insects (Moore & Prior, 1993; Zimmermann, 1993).

More than 90 hymenopterous parasitoids are associated with diamondback moth, *P. xylostella* worldwide (Goodwin, 1979). Among them, *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) is the major endoparasitoid. This larval parasitoid of *P. xylostella* is widespread in many regions around the world (Ho, 1965; Chua & Lim, 1979; Mustata, 1992) and is believed to be one of the parasitoids that are keeping *P. xylostella* population under control. This parasitoid has been introduced into Indonesia from New Zealand in the early 1950s, and it is now well established in many vegetable production areas of this country (Sastrosiswojo, 1990; Sastrosiswojo & Sastrodihardjo, 1986). In Lampung Province, this parasitoid was introduced into many vegetable production areas including Gisting (Tanggamus District) and Sekincau (West Lampung District) as biological control agent of *P. xylostella* (Swibawa, 2002).

Because *M. anisopliae* has been isolated from a wide variety of insect species and is pathogenic to diverse arthropods, it is important to understand if this pathogenic fungus has adverse effects on nontarget insects including decomposers, pollinators, predators, or parasitoids. Therefore, even though *M. anisopliae* is well known as microbial control agents for insect pests, the potential for negative effects on nontarget insects needs to be assessed. Therefore, this laboratory study was conducted to evaluate the effect of the fungus *M. anisopliae* against both the pest species (diamondback moth, *P. xylostella)* and biological control agent (larva1 parasitoid, *D. semiclausum*).

MATERIALS AND METHODS

All experiments were conducted at the Laboratory of Arthropod Pests, College of Agriculture, University of Lampung from February through April in 2009.

Host Plants. False pak choi (syn. ci xin and choi sum), *Brassica rapa var. parachinensis* L. (syn. *Brassica parachinensis* (L.H.Bailey) was used for the experiment. The seeds were sown and germinated in a plastic tray that had been filled with soil and maintained under room conditions. After 15 days, the seedlings were carefully transferred into individual pots (27 x 48 cm) and watered daily with tap water.

Insect Rearing. Larvae of diamondback moths used in this study were taken from a laboratory colony originated from field-collected larvae. Diamondback moths larvae were collected from cabbage fields located in Gisting, Tanggamus District, Lampung Province. Larvae were reared on natural diet (false pak choi leaves) in plastic containers (14 cm diameter) at the Laboratory of Arthropod Pests, College of Agriculture, University of Lampung. Diamondback moth larvae were kept in the plastic containers until adult emergence. Four female and two male adults were then placed inside the transparant plastic cage (14 cm diameter and 30 cm high) with its top and sides cut off and covered with fine mesh nylon net for air ventilation. There was an opening at one side for inserting and removing plant leaves, moths, and their food. The oviposition substrate was to 3-wk-old host plant that was planted in individual pot (14 x 30 cm). Long cotton wick soaked with diluted honey was hung from the top of the rearing cage to feed adult diamondback moths. Host plant leaves with diamondback moth eggs were transferred to 14-cm diameter plastic containers containing fresh leaves and maintained until third instar larvae (used for the diamondback moth bioassay).

All parasitoid *D. semiclausum* individuals used in this study were obtained from the parasitized diamondback moth larvae and pupae collected from the cabbage growing areas around Gisting, Tanggamus District. Initially, pupae of *P. xylostella* and those parasitized by *D. semiclausum* looked alike. However, as the pupal period progressed, the parasitized pupae darken, whereas normal pupae of *P. xylostella* remained light brown. In addition, the parasitized pupae are short with a blunt abdominal end, compared to the pointed abdominal end of the normal pupae that were slightly longer (Figure 1).

All newly emerged *D. semiclausum* adults were transferred and released into a clear plastic cage (14 cm diameter and 30 cm high) consisted of a 3-wk potted host plant that had been infested with *P. xylostella* larvae. All parasitized diamondback moth larvae were provided with fresh host leaves and kept in the cage until pupation or parasitoid cocoons formed.

Figure 1. Pupae of *P. xylostella* : (a) normal pupa (b) pupa parasitized by *D. semiclausum*

Fungal Preparation. The fungus *Metarhizium anisopliae* was initially isolated from rhinoceros beetle larvae, *Oryctes rhinoceros* provided by Biological Control Laboratory in Tegineneng, Central Lampung. The fungus was cultured on a medium of ground maize. The medium was autoclaved for 1 h at 121^{0}C and transfered to plastic trays. When the sterilized ground maize reached room temperature, the medium was inoculated with *M. anisopliae* culture and covered with sterile alumunium foil. After the inoculation, the cultures were maintained at room condition (27 ± 2°C, 80 ± 5% RH) for 2 weeks to induce growth and sporulation of the entomopathogens. A mixture of conidia and hyphae were harvested from the surface of substrate with a sterile blade (± 10 gr) and suspended with 100 ml distilled water. The hyphal debris was removed by filtering the mixture through sterile nylon-chiffon mesh. The conidial concentration of the final suspension was determined and counted using haemocytometer. A series of conidial suspensions with concentration of 5 x 10^4, 3.5 x 10^5, 2.5 x 10^6, 1.2 x 10^7 conidia/ml was prepared for laboratory bioassays.

Fungal Application on Insects. Two sets of experiments : bioassay for insect pest (diamondback moth, *P. xylostella)* and bioassay for biological control agent (larval parasitoid, *D. semiclausum*) were conducted in laboratory. For each treatment, there were four concentration treatments (5 x 10^4, 3.5 x 10^5, 2.5 x 10^6, 1.2 x 10^7 conidia/ml) and an untreated control. Each treatment was replicated three times. All treatments for each experiment were arranged in a completely randomized design. All experiments were conducted at room conditions.

The first experiment, the host insect pest assay, was conducted by preparing fifteen detached leaves (false pak choi) for test arena. Ten third instars of *P. xylostella* larvae were placed on each leaf and held in plastic containers (13 cm diameter). The top of each container was covered with nylon mesh, for air ventilation. Using hand sprayer, all infested leaves were sprayed evenly with conidia of *M. anisopliae* suspension (at each concentration treatment). The control was sprayed by water. Insect tests were maintained in the containers until pupation and adult emergence. Numbers of infected larvae and pupae, number of survived larvae and pupae, and number and sex of adults were counted daily for 14 d. Dead insects were incubated in sterile petri dishes on filter paper moistened with sterile distilled water at room conditions for 4 d to allow growth of fungus. The infected insect tests were confirmed by examining the specimens with a stereo microscope.

The second experiment, parasitoid assay, was initially conducted by placing five parasitoid cocoons, *D. semiclausum,* in a plastic container (13 cm diameter). The group tests were performed by spraying each of conidial suspension at each concentration treatment on parasitoid cococons evenly. Control group was treated by spraying water. All insect tests were maintained in the containers until adult emergence. On emergence, the parasitoid *D. semiclausum* adults were counted and sexed. The parasitoid adults were provided with dilute honey.

Statistical Analysis. The significance of treatments were determined by analysis of variance (ANOVA). All treatment means were separated by least significant difference (LSD) at P < 0.05. All analyses were conducted with standard program (general linear model procedure; PROG GLM procedure) of the Statistical Analysis System (SAS) (SAS *Institute* Inc., 1998). Linear Regression Analysis using SAS (PROG REG procedure) was used to determine whether the increasing concentrations of the fungus *M. anisopliae* related to the number of infected *P. xylostella* larvae and the number of parasitoid *D. semiclausum* adult emergence (SAS *Institute* Inc., 1998). In addition, a standard normal statistic (Z) at P < 0.05 was used (Walpole, 1982) to test whether the sex ratio (female : male) of the surviving adult insects (*P. xylostella* or *D. semiclausum*) in each treatment departed from 1. In other words, the sex ratio was < 1, > 1, or = 1 when the relative number of females to the total survivors was < 0.5, > 0.5, or = 0.5, respectively.

RESULTS AND DISCUSSION

Larval *P. xylostella* mortality. Results of this study that the mean percentage of mortality of the host insect (*P. xylostella* larvae) in all treatments ranged from 6.67% to 30%. In the highest concentration (1.2×10^7 conidia/ml), mortality of *P. xylostella* attributable to the fungal infection was first manifested at 36 h after treatment and increased sharply thereafter. At lower concentrations, initial mortality occurred later (48 h after conidial application) and increased slowly. The mean percentage of mortality of *P. xylostella* larvae were significantly influenced by the fungal treatments at 36 h (F = 4.00; df = 4,10; *P* < 0.0343), at 48 h (F = 6.08; df = 4,10; P < 0.0095); and at 60 h (F = 10.58; df = 4,10; P <0.0013). Although the significant difference results, in all treatments, the mortality of *P. xylostella* from *M. anisopliae* infection did not exceed 50%. Even at the highest conidia concentration, the percentages of mortality of *P. xylostella* caused by the fungal infection were low, i.e. 6.67%, 23.33%, and 30% at 36 h, 48 h, and at 60 h after conidial application, respectively. While, mortality in the control was always zero througout observation hours. At 60 h after conidial application, low level of host mortality from fungus infection were observed in larvae exposed to the lowest conidia concentration (5×10^4 conidia/ml), however, this mortality (16.67%) was significantly higher than that in the control (0.00%) (Table 1).

The low percentage of infection of *P. xylostella* by entomophatogen fungus *M. anisopliae* may be partly due to the source of inoculums (different isolates or varieties). The fungus was isolated from rhinoceros beetle larvae, *Oryctes rhinoceros.* This result is in agreement with reports by St.Leger *et al.* (1992) and Ekesi *et al.* (2001) who found that the pathogenicity of *Metarhizium anisopliae* varied among the isolates. Furthermore, Moorhouse *et al.* (1993) tested vine weevil larval, *Otiorhynchus sulcatus* with two strains of *M. anisopliae* and found that var. *majus* were more pathogenic but less virulent than the var. *anisopliae.*

At first, the symptom of slugishness (slow movement) of infected *P.xylostella* larvae were observed after they were exposed with fungus *M. anisopliae.* There was no visible external fungal development on the infected larvae prior to the host death. Accoding to St. Leger *et al.* (1992), *M. anisopliae* generally enters insects through spiracles and pores in the sense organs. When the conidia of the fungus come into contact with the body of an insect host, they germinate and penetrate the cuticle (St. Leger *et al*, 1991; Chamley & St. Leger, 1991). Once inside the insect, the fungus produces a lateral extension of hyphae, which eventually proliferate and consume the internal contents of the insect. Hyphal growth continues until the insect is filled with mycelia (St. Leger *et al*, 1992). The fungus continues to develop inside the body and eventually kills the insect (Chamley, 1989; and Fresimoser *et al.* 2003). According to Freimoser *et al.* (2003), the fungus can also produce secondary metabolites, such as destruxin, which have insecticidal properties on insects. Similar to the other results, this study also confirmed that on dead larvae of *P. xylostella* the fungus *M. anisopliae* changed in color from white (mycelia) to green (conidia) (Figure 2). Because of the green color of its spores, the disease caused by the fungus is called green muscardine (Zimmermann, 1993; Re Lacey *et al*., 1994).

A linear regression analysis between the conidial concentration of the entomopathogenic fungus, *M. anisopliae,* and percentage larval mortality of *P. xylostella* was performed at 48 h and 60 h after treatments. The results indicated that the regression coefficient (slopes) represented the positive effect, indicating that the mortality of *P. xylostella* increased

Table 1. Mean percentage larval mortality (+ SE) of *P. xylostella* following treatment with different conidial concentration of the entomopathogenic fungus, *M. anisopliae*

Concentrations (conidia/ml)	Mean percentage mortality after			
	24 h	36 h	48 h	60 h
1.2×10^7	0.00	6.67 $\pm$ 3,33a[1)]	23.33 $\pm$ 3,33a	30.00 $\pm$ 3,33a
2.5×10^6	0.00	0.00 $\pm$ 0,00b	10.00 $\pm$ 5,77cb	26.67 $\pm$ 3,33ba
3.5×10^5	0.00	0.00 $\pm$ 0,00b	10.00 $\pm$ 3,33 cb	23.33 $\pm$ 3,33ba
5×10^4	0.00	0.00 $\pm$ 0,00 b	6.67 $\pm$ 3,33 cb	16.67 $\pm$ 5,77 b
0	0.00	0.00 $\pm$ 0,00b	0.00 $\pm$ 0,00 c	0.00 $\pm$ 0,00 c
F Value	0.00	4.00	6.08	10.58
LSD Value	-	4.6973	11.506	11.506

[1)] Means followed by the same letter within a column are not significantly different ($P < 0.05$; LSD) using PROG GLM Procedure of SAS (SAS *Institute* Inc., 1998).

Table 2. Linear regressions[1)] of percentage of *P. xylostella* versus fungal concentrations[2)] of *M. anisopliae*

Time after application	Variable	Parameter estimate	SE	t-Value	Prob>T
48 h	Intercept	6.9287	2.2225	3.118	0.0082
	Slope	0.0145	0.0040	3.647	0.0030
60 h	Intercept	15.1828	3.2779	4.632	0.0005
	Slope	0.0139	0.0059	2.330	0.0366

[1)] Regression models were statistically significant at 48 h after application ($F = 13.301$; $P = 0.003$) and 60 h after application ($F = 5.430$; $P = 0.037$) using PROG REG Procedure of SAS (SAS *Institute* Inc., 1998).
[2)] Conidial concentration transformed to log 10

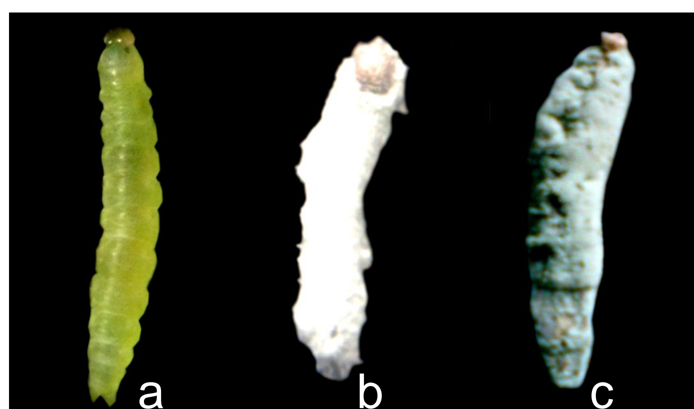

Figure 2. The larvae of *P. xylostella*: (a) uninfected larvae, (b) larvae cadaver covered with white mycelia of *M. anisopliae,* (c) larvae cadaver covered with green conidia of *M. anisopliae*

Table 3. Mean percentage *P. xylostella* (+ SE) larvae survived to pupation and adult emergence following treatment with different conidial concentration of the entomopathogenic fungus, *M. anisopliae*

Concentrations (conidia/ml)	Mean percentage *P. xylostella* larvae surviving to			
	Pupation after		Adult emergence after	
	60 h	70 h	144 h	168 h
1.2 x 10^7	3.33 ± 3.34[1)] c	60.00 ± 5.78 c	10.00 ± 5.78d	33.33 ± 3.34 d
2.5 x 10^6	16.67 ± 3.34 c	63.33 ± 3.34 c	16.67± 3.34 cd	46.67 ± 3.34 c
3.5 x 10^5	23.33 ± 3.34c	66.67 ± 3.34cb	23.33 ± 3.34cb	53.33 ± 6.67 c
5 x 10^4	36.67 ± 3.34b	76.67 ± 3.34 b	33.33 ± 3.34 b	66.67 ± 3.34 b
0	50.00 ± 5.78a	100.00 ± 0.00a	46.67 ± 3.34a	100.0 ± 0.00 a
F Value	14.86	19.58	13.36	41.43
LSD Value	12.428	11.506	12.428	12.428

[1)] Means followed by the same letter within a column are not significantly different (P < 0.05; LSD test preceded by a completely randomized design (CRD ANOVA))

Table 4. Mean percentage of parasitoid *D. semiclausum* (+ SE) larvae survived to adult emergence following treatment with different conidial concentration of the entomopathogenic fungus, *M. anisopliae*

Concentrations (conidia/ml)	Parasitoid *D. semiclausum* adult emergence (%) after		
	6 d	7 d	8 d
1.2 x 10^7	6.67 ± 6,67 c	26.67 ± 6,67 c	33.33 ± 6,67 d
2.5 x 10^6	13.33 ± 6,67c	33.33 ± 6,67cb	40.00 ± 11.56dc
3.5 x 10^5	26.67 ± 11.56 cb	40.00 ± 0.00 cb	60.00 ± 11.56cb
5 x 10^4	40.00 ± 11.56b	46.67 ± 6,67 b	66.67 ± 6,67 b
0	53.33 ± 6,67 a	66.67 ± 6,67a	100.00 ± 0.00a
F Value	5.86	6.62	9.69
LSD Value	24.856	18.789	26.572

[1)] Means followed by the same letter within a column are not significantly different (P < 0.05; LSD test preceded by a completely randomized design (CRD ANOVA))

with the increase of conidial concentrations of *M. anisopliae.* In line with this positive effect, the value of regression coefficients at 48 h and 60 h were 0.0145 ± 0.004 and 00.0139 ± 0.0059 respectively, and these values were significantly different from zero at 48 h (t = 3.647, P <0.003) and 60 h (t = 2.330, P <0.0366) (Table 2). The significant values of slopes confirmed that larval mortality of *P. xylostella* was dependent on conidial concentrations of entomophatogenic fungus *M. anisopliae.*

Larval *P. xylostella* survival. The results of this study have also shown that the percentage *P. xylostella* larvae survived to pupation were significantly different between *M. anisopliae* concentrations of the entomophatogen fungus *M. anisopliae* at 60 h (F = 14.86; df = 4,10; P < 0.0003) and at 70 h (F = 19.58; df = 4,10; P < 0.0001). In other words, fungal concentrations of *M. anisopliae* was able to reduce significantly the pupation of *P. xylostella* at 60 h (LSD = 12.428; P < 0.05) and at 70 h (LSD = 11.506*;* P < 0.05) after

Table 5. Linear regressions[1)] of *Diadegma semiclausum* pupation versus fungal concentrations[2)] of *M. anisopliae*

Time after application	Variable	Parameter estimate	SE	t-Value	Prob>T
6 d[1)]	Intercept	36.2261	5.2705	6.673	0.0001
	Slope	-0.0276	0.0096	-2.872	0.0131
7 d	Intercept	48.7425	4.3259	11.267	0.0001
	Slope	-0.0203	0.0079	-2.585	0.0227
8 d	Intercept	70.3951	8.9410	10.211	0.0001
	Slope	-0.0348	0.0126	-2.775	0.0158

[1)] Regression models were statistically significant at 6 d after application (F = 13.301; P= 0.003), 7 d after application (F =6.861; P= 0.0227), and 8 d after application (F =7.700; P= 0.0158) using PROG REG Procedure of SAS

[2)] Conidial concentration transformed to log 10

Table 6. Sex ratio of the surviving the diamondback moth *P. xylostella* and their parasitoid *D. semiclausum* following tretments of entomopathogenic fungus *M. anisopliae* of with various concentration

Concentrations (conidia/ml)	*P. xylostella*		*D. semiclausum*	
	p	Z	p	Z
1.2×10^7	0.10	-2.50*	0.60	0.45^{tn}
2.5×10^6	0.43	-0.50^{tn}	0.83	1.63^{tn}
3.5×10^5	0.50	0.00^{tn}	0.33	-1.00^{tn}
5×10^4	0.40	-0.90^{tn}	0.50	0.00^{tn}
0	0.57	0.73^{tn}	0.60	0.77^{tn}

Notes : p = proportion of females over total number of survivors, Z = standard normal statistic, * = p significantly different from 0.5 (sex ratio different from 1, P < 0.05), ^{tn} = p not significantly different from 0.5 (sex ratio not different from 1, P > 0.05)

conidial application (Table 3). At 70 h after treatment, pupation of *P. xylostella* treated with the highest conidial concentration (1.2×10^7 conidia/ml) was 60.00% and in the control was 100%, resulting a significant reduction (40%). Moreover, even at the lowest concentration (5.4×10^4 conidia/ml), there was significant reduction in pupation (23.33%) when compared with that in the control.

Similar with the pupation, the results clearly demonstrated that the application of *M.anisopliae* conidia reduced significantly the percentage of *P. xylostella* larvae survived to adult emergence at 144 h (F = 13,36; df = 4,10; P < 0.0005) and 168 h (F = 41.43; df = 4,10; P < 0.0001) after conidial teratments. A significant difference in mean percentage of *P. xylostella* adult emergence were detected in all treatments (33.33%, 46.67%, 53.33%, 66,67%) as compared with that in the control (100%), at 168 h after treatments (LSD = 12.428; P < 0.05) (Table 3).

The reduction of surviving number of the tested insects treated with *M. anisopliae* conidial suspension as shown in this study is in agreement with Chandler & Davidson (2005) who found that application of *M. anisopliae* reduced the pupation and adult emergence of cabbage root fly, *Delia radicum* (L.). Moreover, Bonifacio *et al.* (2005) found that the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* was able to reduce the survival of adult female of the false spider mite *Brevipalpus phoenicis*.

Parasitoid *D. semiclausum* survival. The results clearly demonstrated that the application of *M. anisopliae* conidia reduced significantly the percentage of parasitoid *D. semiclausum* adult emergence at 6 d ($F = 5.86$; $P < 0.0108$), at 7 d ($F = 6.62$; $P < 0.0071$), and 8 d ($F = 9.69$; $P < 0.0018$) after conidial teratments. In all observation days, significant reductions in parasitoid adult emergence were detected in all treatments when compared with that in the control (Table 4).

Unlike the host test results, the regression coefficient (slopes) between the conidial concentration of *M. anisopliae* and percentage parasitoid *D. semiclausum* pupation resulted the negative effect indicating that the mortality of *P. xylostella* decreased with the increase of conidial concentrations of *M. anisopliae.* Furthermore, the value of regression coefficients at 6 d, 7 d, and 8 d were -0.0276 ± 0.0096, -0.0203 ± 0.0079, and -0.0348 ± 0.0126 respectively, and these values were significantly different from zero at 6 d ($t = -2.82$, $P < 0.0131$), 7 d ($t = -2.585$, $P < 0.0227$), and 8 d ($t = -2.775$, $P < 0.0158$), (Table 5). The significant values of slopes indicated the significant negative effects of *M. anisopliae* conidial application on the percentage parasitoid *D. semiclausum* pupation.

This laboratory bioassay demonstrated that the parasitoid *Diadegma semiclausum* was also susceptible to entomophatogen fungus *Metarhizium anisopliae*. Similar results were reported by Thungrabeab and Tongma (2007) who found that *Metarhizium anisopliae* (Metsch) (isolate Ma.7965) had pathogenicity to natural enemies, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae).

Sex Ratio of the Surviving Insects. The sex ratio of the surviving adults of *P. xylostella* and *D. semiclausum,* expressed in the proportion of females over the total number (p), is depicted in Table 6. It shows in general that the application of *M. anisopliae* did not affect the sex ratio of the surviving diamondback moth and that of its parasitoid *D. semiclausum.* However, a male-biased sex ratio of the surviving *P. xylostella* was resulted from the application of the fungus highest rate (1.2×10^7 conidia/ml). Does this indicate the susceptibility of the female as compared with male of the pest ? Similar phenomenon has been observed by Susilo (1991) in the *Tetranychus – Neozygites* pathosystem. Compared with the females, the two-spotted spider mite males are more resistant to *Neozygites fungal* infection.

CONCLUSION

The results of this study have shown that all tested conidial concentrations of entomophatogen fungus *M. anisopliae* were able to infect diamondback, *P. xylostella* in the laboratory and resulted in a male-biased sex ratio of the surviving pest. The overall mean percentage of mortality from *M. anisopliae* in test larvae (*P. xylostella*) were considered to be low. All treated larvae did not reach 50% mortality levels when infected by fungus. In contrast, the fungal infection from application rate of 1.2×10^7 conidia/ml might significantly reduce the survival of the parasitoid, *D. semiclausum.*

REFERENCES

Barson, G., N. Renn, & A. F. Bywater. 1994. Laboratory evaluation of six species of entomopathogenic fungi for the control of the house fly (*Musca domestica* L.), a pest of intensive animal units. *J. Invertebr. Pathol.* 64 : 107–113.

Bonifácio, P. J., M. José, C. V. Rodrigues, D. G. Boucias, & C. C. Childers. 2005. Pathogenicity of *Metarhizium anisopliae* var. acridum to the false spider mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae). *Florida Entomologist* 88 : 195–198.

Chamley, A. K. 1989. Mechanisms of fungal pathogenesis in insects. Pages 85-125 *in:* J. M. Whipps & R. D. Lumsden, Eds. *The Biotechnology of Fungi for Improving Plant Growth*. Cambridge University, London.

Chamley, A. K. & R.J. St. Leger. 1991. The role of cuticle-degrading enzymes in fungal pathogenesis in insects. Pages 267-286 *in*: G. T. Cole & H.C. Hoch, Eds. *The Fungal Spore and Disease Initiation in Plants and Animals*. Plenum, New York.

Chandler, D. & G. Davidson. 2005. Evaluation of Entomopathogenic Fungus *Metarhizium anisopliae* Against Soil-Dwelling Stages of Cabbage Maggot (Diptera: Anthomyiidae) in Glasshouse and Field Experiments and Effect of Fungicides on Fungal Activity. *J. Econ. Entomol.* 98 (6) :1856-1862.

Chua, T. H. & B. H. Lim. 1979. Distribution Pattern of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on choy-sum plants. *Z. Ang. Ent.* 88 : 170-175.

Croft, F.A. 1990. *Arthropod Biological Control Agents And Pesticides*. Wiley, New York.

Dent, D. 2000. *Insect Pest Management* 2nd Edition. CABI Publishing New York.

De La Rosa, W., R. Alatorre, J. F. Barrera, & C. Toriello. 2000. Effect of *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycetes) upon the Coffee Berry Borer (Coleoptera: Scolytidae) Under Field Conditions. *J. Econ. Entomol*. 93 : 1409–1414.

Ekesi, S. E., A. Egwurube, A. D. Akpa, & I. Onu. 2001. Laboratory evaluation of the entomopathogenic fungus, *Metarhizium anisopliae* for the control of the groundnut bruchid, *Caryedon serratus* on groundnut. *J. Stored Products Res*. 37: 313-321.

Freimoser, F. M., S. Screen, S. Bagga, G. Hu, & R. J. St. Leger. 2003. EST analysis of two subspecies of *Metarhizium anisopliae* reveals a plethora of secreted proteins with potential activity in insect hosts. *Microbiology* 149: 239–247.

Goodwin, S. 1979. Changes in numbers in the parasitoid complex associated with the diamondback moth *Plutella xylostella* (L.) (Lepidoptera) in Victoria. *Australian J. Zool*. 27 : 981-989.

Harcourt, D.G. 1957. Biology of the diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae), in Eastern Ontario. II. Life-history, behaviour, and host relationships. *Canadian Entomologist* 89: 554-564.

Ho, T. H. 1965. The Life-History and Control of the Diamond-back moth in Malaya. *Bull. Div of Agriculture Malaysia*. No. 118.

Kaaya, G. P. & D. M. Munyinyi. 1995. Biocontrol potential of the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* for Tsetse flies (*Glossina* spp.) at development sites. *J. Invertebr. Pathol* 66:237–241.

Koul, O. & G.W. Cuperus. 2007. *Ecologically Based Integrated Pest Management*. CABI Publishing New York, NY.

Liu, S.D., S.C. Lin, & J.F. Shiau. 1989. Microbial control of coconut leaf beetle (*Brontispa longissima*) with green muscardine fungus, *Metarhizium anisopliae* varanisopliae. *J. Invertebr. Pathol*. 53: 307-314.

Moore, D. & C. Prior. 1993. The potential of mycoinsecticides. *Biocontrol News Inf*. 14:31N-40N.

Moorhouse, E. R., A.T. Gillespie, & A. K. Charnley. 1993. Laboratory selection of *Metarhizium* sp. Isolates for control of vine weevill larvae (*Otiorhynchus sulcatus*). *J. Invertebr. Pathol*. 62: 15-21.

Mustata, G. 1992. Role of parasitoid complex in limiting the population of diamondback moth in Moldavia, Romania. Pages 201-212 *in*: Talekar, N. S., ed. Diamondback Moth and Other Crucifers Pests. *Proceedings of the Second International Workshop. Agricultural Vegetable Research and Development Center*, Shanhua, Taiwan, 10-14 Dec. 1990.

Re Lacey, L. A., A. Martins, & C. Ribeiro. 1994. The pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* for adults of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). Eur. *J. Entomol*. 91 : 313–319.

Moorhouse, E. R., A.T. Gillespie, & A.K. Charnley. 1993. Selection of virulent and persistent *Metarhizium anisopliae* isolates to control black vine weevil (*Otiorhynchus sulcatus*) larvae on glasshouse begonia. *J. Invertebr. Pathol*. 62:67–52.

Pracaya. 2005. *Kol alias Kubis*. Penebar Swadaya. Jakarta.

Sastrosiswojo, S. 1990. Pest Management of Selected Vegetable Crops for Indonesia *The Regional Workshop of Vegetables*, Cameroon Highlands, Malaysia.

Sastrosiswojo, S. & S. Sastrodihardjo. 1986. Status of biological control of diamondback moth by introduction of parasitoid *Diadegma eucerophaga* in Indonesia. Pages: 185-194 *in* Talekar, N. S. & T. D. Griggs, eds., Diamondbanck Moth Management. *Proceedings of the first International Workshop. Asian Vegetable Research and Development Center*, Shanhua, Taiwan. 11-15 March, 1985.

SAS Institute Inc. 1998. *SAS/SAT User's Guide*. Version 6. Fourth Edition. SAS Institute, Cary, N.C.

Sosa-Gomez, D. R. & F. Moscardi. 1998. Laboratory and field studies on the infection of stink bugs, *Nezara viridula, Piezodorus guildinii*, and *Euschistus heros* (Hemiptera: Pentatomidae) *with Metarhizium anisopliae* and *Beauveria bassiana* in Brazil. *J. Invertebr. Pathol.* 71 : 115–120.

St. Leger, R. J., M. Goettel, D. W. Roberts, & R.C. Staples. 1991. Pre-penetration events during infection of host cuticle by *Metarhizium anisopliae. J. Invertebr. Pathol*. 58: 168-179.

St. Leger, R.J., B. May, L.L. Alle, D.C. Frank, R.C. Stables, & D.W. Roberts. 1992. Genetic differences in allozymes and information of infection structures among isolates of the entomopathogenic fungus *Metarhizium anisopliae*. *J. Invertebr. Pathol*. 60: 89-101.

Susilo, F.X. 1991. Epizootiological studies on *Neozygites floridana* (Entomophthorales: Neozygitaceae) infectious to the two-spotted spider mite, *Tetranychus urticae*. Ph.D. *Dissertation*. University of Kentucky, Lexington.

Swibawa, I, G. 2002. Introduksi parasit *Diadegma semiclausum* untuk Pengendalian Hayati Hama Kubis *Plutella xylostella* di Lampung. *Research Report*. University of Lampung, Bandar Lampung.

Tabashnik, B. E., N. L. Kushing, & M. W. Johnson. 1987. Diamondback Moth (Lepidoptera: Plutellidae) Resistance to Insecticides in Hawaii: Intra-island Variation and Cross Resistance. *J. Econ. Entomol*. 80: 1091-1099.

Talekar, N. S. & T.D. Griggs. 1986. Diamonback Moth Management. *Proceeding of the First International Workshop, Asian Vegetable Research and Development Center (AVRDC)*, Shanhua, Taiwan, 11-15 March, 1985. AVRDC Publication No. 86-248.

Talekar, N.S. & Shelton A.M. 1993. Biology, ecology, and management of the diamondback moth. *An. Rev. Entomol*. 38 : 275-301.

Talekar, N.S., H.C. Yang, S.T. Lee, B.S. Chen, & L.Y. Sun. 1985. *Annotated Bibliography of Diamondback Moth*. Asian Vegetable Research and Development Center, Taipei, Taiwan.

Thungrabeab, M. & S. Lampang. 2007. Effect of Entomopathogenic Fungi, *Beauveria bassiana* (Balsam) and *Metarhizium anisopliae* (Metsch.*)* on Non Target Insects. KMITL *Sci. Tech. J*. 7 : 1-12.

Walpole, R.E. 1982. *Pengantar Statistika*. 3rd ed. PT Gramedia Pustaka Utama. Jakarta.

Zimmermann, G. 1993. The entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol. *Pestic. Sci*. 37 : 375–379.