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: \(5 \times 10^4\), \(3.5 \times 10^5\), \(2.5 \times 10^6\), \(1.2 \times 10^7\) 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 \(1.2 \times 10^7\) 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 (Deuteromycota: 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, Otiorrhynchus sulcatus (Moorhouse et al., 1993), the coffee berry borer, Hypothenemus hampei.
(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 (larval 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 moth 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 transparent 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 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.
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°C and transferred to plastic trays. When the sterilized ground maize reached room temperature, the medium was inoculated with *M. anisopliae* culture and covered with sterile aluminum 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 hemocytometer. 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 cocoons 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.

Figure 1. Pupae of *P. xylostella*: (a) normal pupa (b) pupa parasitized by *D. semiclausum*
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 x 10^4 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 differences 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 60 h after conidial application, respectively. While, mortality in the control was always zero throughout 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 x 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 entomopathogen 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 sluggishness (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. According 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 x 10^7                 | 0.00   | 6.67 ± 3.33a | 23.33 ± 3.33a | 30.00 ± 3.33a |
| 2.5 x 10^6                 | 0.00   | 0.00 ± 0,00b  | 10.00 ± 5,77cb | 26.67 ± 3,33ba |
| 3.5 x 10^5                 | 0.00   | 0.00 ± 0,00b  | 10.00 ± 3,33 cb | 23.33 ± 3,33ba |
| 5 x 10^4                   | 0.00   | 0.00 ± 0,00 b | 6.67 ± 3,33 cb  | 16.67 ± 5,77 b  |
| 0                          | 0.00   | 0.00 ± 0,00 b | 0.00 ± 0,00 c    | 0.00 ± 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 regressions1) of percentage of *P. xylostella* versus fungal concentrations2) 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 and adult emergence after | 60 h | 70 h | 144 h | 168 h |
|-----------------------------|--------------------------------------------------------------------------------------|------|------|-------|-------|
| 1.2 x 10^7                  | 3.33 ± 3.34 supplement                                                                 | 60.00 ± 5.78 c | 10.00 ± 5.78 d | 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 | 100.00 ± 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 ± 6.67 c                                       | 33.33 ± 6.67 cb | 40.00 ± 11.56cb |
| 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 ± 11.56 cb                                     | 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 entomopathogenic 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 entomopathogen 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
At 70 h after treatment, pupation of *P. xylostella* treated with the highest conidial concentration (1.2 x 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 x 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 treatments. 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*.

### Table 5. Linear regressions of *Diadegma semiclausum* pupation versus fungal concentrations 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 treatment of entomopathogenic fungus *M. anisopliae* with various concentration

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

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), ^m = p not significantly different from 0.5 (sex ratio not different from 1, P > 0.05)
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 treatments. 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 *Diaegma semiclausum* was also susceptible to entomopathogen 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 x 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 entomopathogen 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 x 10^7 conidia/ml might significantly reduce the survival of the parasitoid, *D. semiclausum*.

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