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Pathogenicity of entomopathogenic fungus *Metarhizium* spp. against predators *Menochilus sexmaculatus* Fabricius (Coleoptera: Coccinellidae)

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Abstract. *Trizelia, Busniah M, Agung Permadi A. 2017. Pathogenicity of entomopathogenic fungus* Metarhizium *spp. against predators Menochilus sexmaculatus Fabricius (Coleoptera: Coccinellidae). Asian J Agric 1: 1-5. Metarhizium* spp is an entomopathogenic fungus that has wide host range. *Metarhizium* spp can not only infect insect pests, but can also infect beneficial insects such as predators. The pathogenicity of four isolates of *Metarhizium* spp. was investigated against *Menochilus sexmaculatus* predators. Isolates of *Metarhizium* spp were isolated from rhizosphere of cacao, cabbage, chili and rubber crops. The experiment was conducted by treating the fourth instar larvae with an appropriate conidial suspension of 10^8 conidia mL^{-1}. The results showed that all isolates of *Metarhizium* spp were pathogenic toward beetle predator *M. sexmaculatus*. Mortality of larvae within 7 days after application of conidial suspension varied between 27.50 to 67.50% and there were statistically significant differences among the tested isolates. *Metarhizium* spp. had also a significant effect in reducing pupation and adult emergence of *M. sexmaculatus* to below 30% and 3%, respectively. These studies indicate that entomopathogenic fungus *Metarhizium* spp was pathogenic to beetle predators, *M. sexmaculatus*

Keywords: Beetle predators, entomopathogenic fungus, *Menochilus sexmaculatus*, *Metarhizium*

INTRODUCTION

Entomopathogenic fungi have been used in control of several insect pests as alternative to chemical insecticides (Hajek and Leger, 1994). Among these entomopathogenic fungi, *Metarhizium* spp have great potential as biological control agents against insects pest and as a component within integrated pest management systems. Utilization of *Metarhizium* spp. for pest control has been widely reported. Trizelia et al (2011) reported that fungus *Metarhizium* spp can kill *Spodoptera litura* eggs. Mortality of *S. litura* eggs depends on the fungal isolates, ranging between 19.79%-75.70%. First instar larvae also die 3 days after eclosion. The maximum mortality of first instar larvae was 58.65%. Nunilahwati (2012) reported that *Metarhizium* spp can also infect *Plutella xylostella* larvae and the mortality of larvae up to 82% with LT_{50} value 2.26 days. Saranya et al (2010) reported that mortality of aphids, *Aphis craccivora* was 80.76% observed in 168 h, at the highest concentration (10^8 spores mL^{-1}) of *Metarhizium anisopliae*.

One of the most important aspects that should be considered in the use of *Metarhizium* spp as biological control agents, is the wide compatibility with other biological control agents such as insect predator *Menochilus sexmaculatus*. This is due to the fact that this fungus can infect many kinds of insects, both predators and pests. Although some entomopathogenic fungi are known to have narrow host ranges, especially some of those belonging to Deuteromycotina, they have a rather wide range of hosts from many insect orders, including natural enemies (Inglis et al. 2001). Compatibility between entomopathogenic fungi and predators is required to minimize risk to the environment.

Menochilus sexmaculatus beetle predators (Coleoptera: Coccinellidae) are one of the biological control agents. These predators feed on *Lipaphis erysimi* nymphs (Ali and Rizvi, 2009). Adults of *M. sexmaculatus* were able to prey on *Aphis gossypii* on an average rate of 44.50 individuals per hour (Nelly et al.2012). Solangi et al. (2007) reported that the mean number of prey consumed by *M. sexmaculatus* during entire adult life was 80.08, 69.95 and 68.96 of *R. maidis*, *A. gosypii* and *T. trifolii* respectively. This beetle has great potential to be a biological control of these three aphid species. Radiyanto et al (2011) reported that the maximum number of prey consumed by adult *M. sexmaculatus* females, was 300 individuals at various stages of *Rhopalosiphum maidis* Fitch (Homoptera: Aphididae) per 24 h. Ali et al (2012) found that the predatory potential of male *M. sexmaculatus* on *Rhopalosiphum padi* was 2294 to 2422 and female was 2912 to 3085 aphids.

An understanding of the interaction between entomopathogenic fungi *Metarhizium* spp and predator *M. sexmaculatus* in agricultural ecosystems is needed to predict the environmental impact of *Metarhizium* spp application to the development of predator populations. In addition to direct contact with entomopathogenic fungi, predators can also be threatened by consuming fungus infected prey. Er et al (2008) reported that *Metarhizium anisopliae* (Metschnikoff) Sorokin. was generally found to be pathogenic to *Coccinella septempunctata* and the mortality of adults was 47.61%. While Wu et al (2014) reported that *B. bassiana* strain SZ-26 showed high toxicity

against *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) but not a detrimental effect to predatory mite *Neoseiulus (Amblyseius) barkeri* (Hughes) (Acarina: Phytoseiidae).

The purpose of this research was to study the pathogenicity of several isolates of *Metarhizium* spp against a predatory beetle *Menochilus sexmaculatus*.

MATERIALS AND METHODS

Fungal isolates

The isolates of *Metarhizium* spp. used was from the collection of the Laboratory of Biological Control, Department of Plant Pests and Diseases, Faculty of Agriculture, University of Andalas, Padang, Indonesia. Isolates were isolated from rhizosphere of cacao, rubber, pepper and coffee crops from various locations (Table 1). All isolates were grown on SDAY (*Sabouraud dextrose agar + yeast extract*) medium.

Aphids and *M. sexmaculatus* culture

Aphids (*Aphis maydis* and *A. craccivora*) and *M. sexmaculatus* predators were collected from the corn and long bean fields in the city of Padang and brought to the laboratory. Predators were reared in the laboratory on this aphid species in wide-mouth plastic jars. Fresh stock of leaves respective for aphids hosts were provided daily as oviposition substrate for female beetles. The eggs laid by female beetles on aphids-host leaves were transferred to plastic box for further rearing. The newly hatched larvae were reared to the fourth instar to be used as test insects.

Preparation of conidial suspension

Isolates of *Metarhizium* spp. were grown using autoclaved SDAY media. The fungal cultures were incubated at 25ºC for 15 days. Cultures with fully developed conidiospores were washed by 5 mL sterilized distilled water mixed with 0.05% Tween 80 to obtain the stock of conidial suspension. The conidial suspensions were passed through two layers of sterile muslin to remove any agar pieces and hyphae from the conidial suspensions. Conidia were counted in a compound microscope using a Neubauer hemocytometer.

Pathogenicity test

The conidial density was used 10^8 conidia mL^{-1} for all isolates. Two mL of the suspension was sprayed against the fourth instar larvae of *M. sexmaculatus* tested. For the control, larvae were sprayed with the same volume of distilled water mixed with 0.05% Tween 80. Then the fourth instar larvae of *M. sexmaculatus* were kept in the box provided. Each box contained only one of the fourth instar larvae of *M. sexmaculatus*. Each experimental unit consisted of 10 fourth instar larvae of *M. sexmaculatus*. The experiment was repeated four times. The mortality of larvae was recorded at 24 h intervals until adult emergence. Parameters observed were larval mortality, pupation, adult emergence and the ability of *M. sexmaculatus* to prey on aphids.

Data analysis

This experiment was arranged in the Completely Randomized Design (CRD) and data were analyzed by means of the analysis of variance (ANOVA) and Duncans Multiple Range Test (DNMRT) (P=0.05%).

RESULTS AND DISCUSSION

The results of pathogenicity test showed that all of the *Metarhizium* spp. isolates were pathogenic on the larvae of *Menochilus sexmaculatus*. Mortality of *M. sexmaculatus* larvae after application of Metarhizium spp., depended on the isolates. *Metarhizium* spp isolates tested induced cumulative mortalities between 27.50 to 67.50% in fourth instar larvae of *M. sexmaculatus*. ANOVA test revealed statistically significant differences amongst the treatments ($F=16.85$; $P<0.05$). All the *Metarhizium* spp isolates applied gave significant higher mortality than the control unit (Table 2). Three isolates (MetAKi, MetLKt and MetPKo) caused higher mortality (62.5-67.5%) on the fourth instar larvae, while MetKbCi isolates showed the lowest degree of pathogenicity (27.50%).

The fourth instar larvae of *M. sexmaculatus* infected by entomopathogenic fungi *Metarhizium* spp. was covered by fungus mycelia or conidia having green in color around the body surface *M. sexmaculatus* larvae (Figure 1).

The mortality of *M. sexmaculatus* larvae after *Metarhizium* spp application was due to the fact that *Metarhizium* spp was able to kill not only the pest insect but also predators. This is caused by the body structure of both being almost the same. Thungrabeab and Tongma (2007) reported that *Metarhizium anisopliae* could kill *Dicyphus tamanii* (Hymenoptera; Miridae) up to 10% and *Carnea chrysoperla* (Neuroptera; Chrysopidae) up to 4% at density of conidia 10^8 conidia mL^{-1}. The same result was also found by Ibrahim et al (2011) that isolates of *Metarhizium* spp. caused mortality for *Cryptolaemus montrouzieri* (Coleoptera; Coccinellidae) larvae about 7.7 %. Er et al (2008) also reported that *Metarhizium anisopliae* caused mortality of *Coccinella septempunctata* (Coleoptera; Coccinellidae) adults up to 47.61%. Sahayaraj et al (2008) reported that the survival rate and the particle carrying capacity of reduviid predator, *Acanthaspis pedestris* were reduced after application of *Metarhizium anisopliae* compared to the control. While Huang et al (2012) reported that there are no adverse effects of entomopathogenic fungus *Beauveria bassiana* application on the survival and reproduction of predator *Prynocaria congener* (Coleoptera; Coccinellidae).

Difference in pathogenicity among isolates is common for entomopathogenic fungi. Differences in pathogenicity among isolates were attributed to the differences in the ability to produce enzymes and mycotoxins during the infection process in insects such as the time of contact with the cuticle and hemocoel (Tanada and Kaya, 1993; Balachander et al. 2012). A toxin called destruxin which is produced by *Metarhizium* spp during infection was thought to be the cause of *M. sexmaculatus* death.

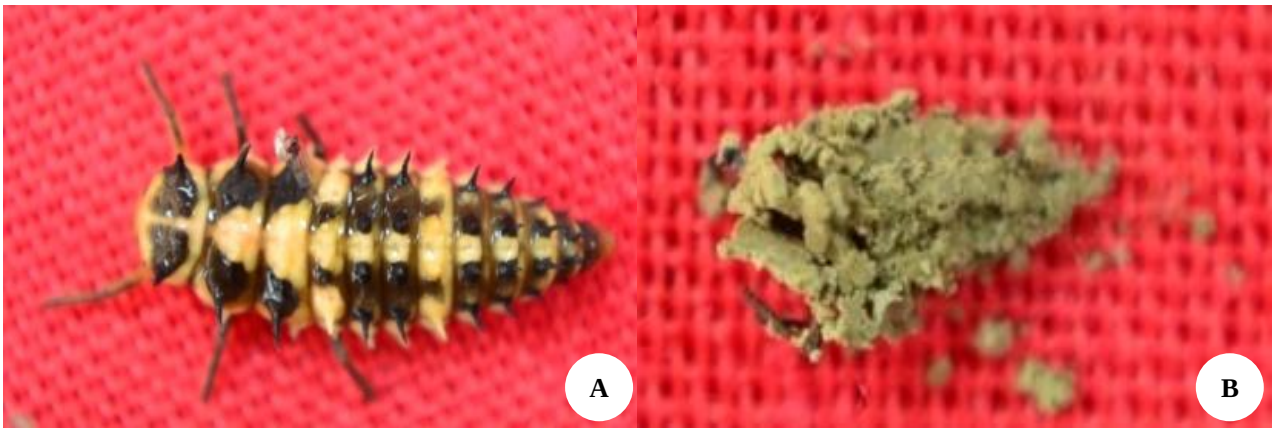

Figure 1. The fourth instar larvae of *M. sexmaculatus* in form of normal (A) and infected by entomopathogenic fungi *Metarhizium* spp. (B)

Table 1. The source of *Metarhizium* spp. isolates

Isolate	Host	Origin
MetPKo	Cacao	Pariaman
MetLKt	Rubber	Lintau (Tanah Datar)
MetKbCi	Chili	Koto Baru (Tanah Datar)
MetAKi	Coffee	Matur (Agam)

Table 2. Mortality of the fourth instar larvae of *M. sexmaculatus* after application of four isolates of *Metarhizium* spp with density conidia 10^8 conidia/mL.

Isolates	Mortality ± SD (%)
MetAKi	67.50 ± 9.57 a
MetLKt	65.00 ± 17.32 a
MetPKo	62.50 ± 9.57 a
MetKbCi	27.50 ± 18.93 b
Control	7.50 ± 5.00 c

Note: Different letters indicate a statistically significant difference according to DNMRT test, P<0.05

Besides killing larvae, fungi infections also affected the development of pupae. The amount of pupae formed (pupation) is inversely related to the mortality of larvae. Based on the analysis of variance, the percentage of pupae formed was significantly different among isolates (F=17.20; P<0.05), (Table 3). The highest percentage of pupa formed (70%) were found under application of isolate MetKbCi, while the lowest was under MetPKo, MetLKt and MetAKi.

As for larvae, pupae *M. sexmaculatus* infected by entomopathogenic fungi *Metarhizium* spp. were also covered by the fungal mycelia or conidia having green color at the body surface (Figure 2).

Percentage of adult emergence from larvae infected by *Metarhizium* spp. also reduced significantly. Application of *Metarhizium* spp. could only generate adults about 2.5% - 27.5%, while the control could reach 90% (Table 4).

Metarhizium spp. had also a significant effect in reducing pupation of *M. sexmaculatus* and adult emergence to below 30% and 3%, respectively. The low percentage of pupation and adult emergence was due to the fact that a high number of larvae and pupae were killed before becoming adult. This proves that the influence of *Metarhizium* spp. was not only active and destructive in certain stadia treated, but also had an impact on subsequent stadium. Malarvannan et al (2010) reported that entomopathogenic fungi, *Beauveria bassiana* can reduce pupation and adult emergence of *Spodoptera litura*, due to phagodepression and difficulty in molting.

The ability to prey on aphids of unapplied *M. sexmaculatus* larvae by *Metarhizium* spp. was also significantly different from those applied *M. sexmaculatus* (P <0.0001) (Table 5). In control, the predation ability of *M. sexmaculatus* on aphids reached 10.63 individuals per hour, while the predation ability of predators sprayed with entomopathogenic fungi, decreased significantly (from 6.93 to 1.08 individuals per hour).

The ability of the fourth instar larvae of *M. sexmaculatus* to prey on aphids was generally decreased following fungal infection. The reduction in preying insects was assumed due to production of toxic substances by fungi or mechanical disruption of the insect structural integrity by hyphal growth. Destruction affected target cell organelles (mitochondria, endoplasmic reticulum and nuclear membrane) and caused paralysis of cells. It also affected the mesenteron, Malpighian tubes, and tissue larval hemocyte dysfunction (Tanada and Kaya, 1993).

Based on the research conducted, it can be concluded that all isolates of *Metarhizium* spp tested were pathogenic to predators *M. sexmaculatus*. The lowest pathogenicity (27.5%) isolates were found at MetKbCi, while the isolates MetPKo, MetLKt and MetAKi had high pathogenicity (62.50%, 65%, and 67.50% respectively). Further research is required to determine how direct effects observed in laboratory play out in a field environment.

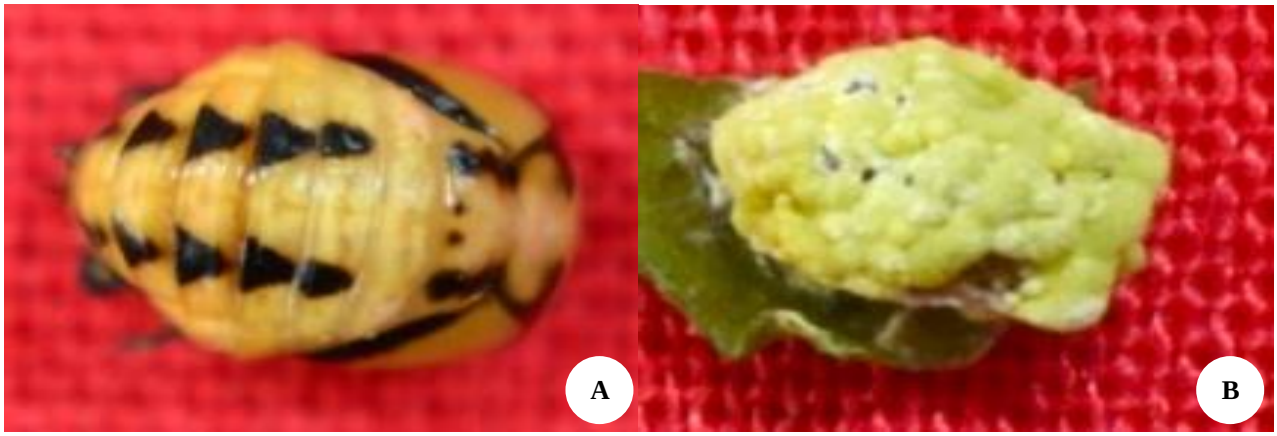

Figure 2. Pupae of *M. sexmaculatus* in form of normal (A) and infected by entomopathogenic fungi *Metarhizium* spp. (B)

Table 3. Formation of *M. sexmaculatus* pupae

Isolates	Pupae formed ± SD (%)
Control	92.50 ± 5.00 a
MetKbCi	70.00 ± 20.00 b
MetPKo	32.50 ± 8.16 c
MetLKt	30.00 ± 17.08 c
MetAKi	27.50 ± 9.58 c

Note: Different letters indicate a statistically significant difference according to DNMRT test, P<0.05

Table 4. Percentage of adult emergence of *M. sexmaculatus*

Isolates	Adult emergence ± SD (%)
Control	90.00 ± 0.00 a
MetAKi	27.50 ± 5.00 b
MetLKt	15.00 ± 12.91 b c
MetKbCi	15.00 ± 23.81 b c
MetPKo	2.50 ± 5.00 c

Note: Different letters indicate a statistically significant difference according to DNMRT test, P<0.05

Table 5. The predation ability on aphids of *M. sexmaculatus* larvae for 1 h after 24 h application of *Metarhizium* spp.

Isolates	Ability of predation (ind./hour) ± SD
Control	10.63 ± 1.56 a
MetPKo	6.93 ± 0.41 b
MetLKt	4.35 ± 0.79 c
MetAKi	1.48 ± 0.22 d
MetKbCi	1.08 ± 0.21 d

Note: Different letters indicate a statistically significant difference according to DNMRT test, P<0.05

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