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EFFECT OF ENTOMOPATHOGENIC FUNGI, *Beauveria bassiana* (BALSAM) AND *Metarhizium anisopliae* (METSCH) ON NON TARGET INSECTS

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ABSTRACT

The effect of entomopathogenic fungi, *Beauveria bassiana* (Balsamo) (isolate Bb.5335) and *Metarhizium anisopliae* (Metsch) (isolate Ma.7965) on non target insects, such as natural enemies, *Coccinella septempunctata* L. (Col., Coccinellidae), *Chrysoperla carnea* (Stephens) (Neur., Chrysopidae) and *Dicyphus tamaninii* Wagner (Him., Miridae) as well as beneficial soil insect, *Heteromurus nitidus* Templeton (Collembola: Entomobryidae) were studied. The experiments were conducted on conidial suspensions at a concentration of 1×10^8 conidia/ml. The 1^{st} larval instars of *C. septempunctata*, *C. carnea* and adults of *D. tamaninii* and *H. nitidus* were tested. Mortality was recorded daily till the next generation.The results showed that *B. bassiana* was found to be non-pathogenic to natural enemies and beneficial soil insect. While *M. anisopliae* had pathogenicity to natural enemies, *C. carnea* and *D. tamaninii*, in which *D. tamaninii* was more susceptible than *C. carnea* with corrected mortalities of 10 and 4%, respectively.

KEYWORDS: entomopathogenic fungi, side effect, non target insect

1. INTRODUCTION

Entomopathogenic hyphomycete fungi have great potential as biological control agents against insects and as one component within integrated pest management systems. They are being developed worldwide for the control of many pests of agricultural importance [1] and some are already available commercially for the control of various species of thrips and aphids [2-3]. Previous research reported that among 41 isolates of entomopathogenic fungi from Thailand, *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 were virulent against *Myzus persicae* Sulzer, *Macrosiphum euphobia* (Thomas) (Hom., Aphididae), *Thrips tabaci* Lindeman and *Frakliniella occidentalis* Pergande (Thys., Thripidae) [4 -7]. However, the success of fungal entomopathogens as biological control agents depends not only on high efficacy against insect pests, but also on low virulence against non target insects. Moreover, *B. bassiana* and *M. anisopliae* are known to have broad host ranges [8], and use of such biological control agents to

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control insect pests might have effect on beneficial insects, such as natural enemies of insect pests. In addition, most guidelines for the registration of biopesticides require laboratory testing for infectivity to non target organism. Thus, before considering fungi isolates from Thailand as biological control agents, it is necessary to investigate their effects on non target insects prior to their release. Lady beetles (Coccinellidae), green lacewings (Chrysopidae) and predatory bugs are common predator of aphids and thrips. Likewise, Collembolan represents one of the most abundant arthropod groups in soil. They contribute to the decomposition of organic matter, mineralization of nutrients as well as distribution and control soil microflora. The present study was aimed to investigate the effect of *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 on natural enemies, *Coccinella septempunctata* Linnaeus (Col., Coccinellidae), *Chrysoperla carnea* (Stephens) (Neur., Chrysopidae) and *Dicyphus tamaninii* Wagner (Hom., Miridae) as well as beneficial soil insect, collembolan, *Heteromurus nitidus* Templeton (Collembola: Entomobryidae).

2. MATERIALS AND METHODS

2.1 Insects

The non target insects used in the present study originated from Bayer Crop Science® laboratory. The stock culture of *Heteromurus nitidus* Templeton (Collembola: Entomobryidae) was reared in plastic cups (9-cm in diameter, 7-cm in height) containing peat substrate as rearing substrate. Cups were covered to maintain approximately 100% relative humidity. Commercial nutritional yeast, *Saccharomyces cerevisiae* (Ascomycetes: Saccharomycetaceae), was used as the standard diet, and was added to rearing cups approximately twice each week. *Dicyphus tamaninii* Wagner (Hom., Miridae) was reared in cages (60×60×40 cm) sealed with gauze from four sides in order to allow aeration, and the broad bean leaves, *Vicia fabae* L. cv. Scirocco infested with pea aphid, *Acyrthosiphon pisum* (Harris) (Hom., Aphididae) was used for rearing. The stock culture of *Chrysoperla carnea* (Stephens) (Neur., Chrysopidae) was held on eggs of grain moth, *Sitotroga cerealella* (Olivier) (Lep; Gelechiidae) in small Plexiglas cages (3.5 cm in diameter and 1 cm in height). *Coccinella septempunctata* L. (Col., Coccinellidae) was maintained on broad bean leaves in round Plexiglas cages (11 cm in diameter and 3 cm in height), which had been infested with pea aphid, *A. pisum* as prey.

2.2 Fungus

Two entomopathogenic fungi, *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma. 7965 were obtained from the culture collection of the National Centre for Genetic Engineering and Biotechnology, Thailand. *B. bassiana* was originally isolated from ant (Hymenoptera) while, *M. anisopliae* Ma. 7965 was obtained from Homoptera. Stock cultures of the isolates were stored at–80°C. The fungi were cultured on malt extract peptone agar (MEA, Merck, Darmstadt,Germany) and incubated at 25 ± 1°C under continuous light condition.

2.3 Assay methods

The conidia were harvested from 1 to 3- week-old surface cultures by flooding the plates with sterile 0.05% Tween® 80 (Sigma USA) water solution. The concentration of conidia was determined with an improved haemocytometer and adjusted to a concentration of $1×10^8$ conidia/ml with sterile 0.05% Tween® 80 water solution.

For the experiment on *H. nitidus*, 3 ml of the conidial suspensions were mixed with 5 grams of rearing substrate in each plastic cup. After that, 30 adults of collembolans were added into each individual cup and dried yeast was supplied as food source. The cups were closed with lid and incubated for 14 days at room temperature. The control was treated in a similar way, except that substrate was treated with 0.05% Tween® 80 water solution. Mortality was recorded daily for 14 days and then dead collembolans were transferred to plates with moist filter paper in order to allow fungi to sporulate.

For the experiment on *D. tamaninii*, broad bean leaves infested with *A. pisum* were sprayed with conidial suspensions. Leaves were allowed to air dry and then placed individually into a vial (2.5 cm in diameter and 7.5 cm in height). Thirty adults of *D. tamaninii* were sprayed with 1 ml conidial suspensions through mesh sheet using the sprayer (Eco spray; Labo chimic France) and were then transferred individually into each vial. The vials were covered with a mesh-hole in the lid. The control was processed as previously described, except conidial suspension was replaced by 0.05% Tween® 80 water solution. Mortality was recorded daily until next generation.

To determine the effect on *C. carnea*, experiment was carried out on 1st larval instars. For each replicate, 30 larvae were transferred into 90-mm diameter petri dish with Whatman no. 1 filter paper, and then 1 ml of conidial suspensions of *B. bassiana* or *M. anisopliae* were sprayed onto the larvae using the sprayer as described above. The treated larvae were transferred singly to small round Plexiglas cages, 3.5 cm in diameter and 1 cm in height. The larvae were fed with egg of grain moth as prey. Control was sprayed with 0.05% Tween 80® water solution. Mortality was recorded daily until the next generation and cadavers were conducted similarly as the test collembolans. The above procedure was repeated with 1st larval instars of *C. septempunctata* to investigate the effects on Coleoptera, except that the small round Plexiglas cages contained broad bean leaf infested with *A. pisum* as prey sprayed with conidial suspensions earlier. *C. septempunctata* was fortnightly offered fresh prey. In addition, mortality was recorded daily till the next generation. The cadavers were transferred to plates with moist filter paper in order to allow fungi to sporulate. Each experiment was replicated 3 times and the entire experiment was repeated twice.

2.4 Statistical analysis

Percentage of mortality was transformed by arcsine square root to normalize the mean percentage [9] after correcting for natural mortality [10]. The means were separated by Duncan multiple rang test ($P \leq 0.05$) using the ANOVA procedure of SAS [11].

3. RESULTS AND DISCUSSION

B. bassiana was found to be non-pathogenic to both natural enemies and beneficial soil insect. However, *M. anisopliae* had pathogenicity to *D. tamaninii* and *C. carnea*, in which *D. tamaninii* was more susceptible than *C. carnea*. Mortalities caused by *M. anisopliae* were 10 % on *D. tamaninii* and 4% on *C. carnea*, while mortality in control was 9.3% on *D. tamaninii* and 5.7% on *C. carnea* (Figure 1). The results reveal that different genera or species of fungi had different pathogenicity and virulence. Entomopathogenic fungus could be quite specific and might infect only certain type of host. These results were supported by Broza *et al*. [12], Dromph and Vestergaard [13], who noted that *B. bassiana*, *B. brongniartii*, *Hirsutella* spp. *M. anisopliae* and *V. lecanii* did not affect the mortalities of three collembolan species, *Folsomia fimetaria* L. *Proisotoma minuta* (TULLBERG) (Collembola: Isotomidae) and *Hypogastrura assimilis* (KRAUSBAUER) (Collembola: Hypogastruidae). In addition, *C. septempuntata* showed resistance to *B. bassiana* and *M. anisopliae*. Previously, James and Lighthart [14] reported that *M. anisopliae*, *B. bassiana* and *P. fumosoroseus* have the potential to infect *Hippodamia convergens* Guérin Méneville (Col., Coccinellidae), whereas *Nomuraea rileyi* (Farlow) did not. *Serangium parcesetosum* Sicard (Col., Coccinellidae) had significantly lower survival when sprayed with *B. bassiana* than with *P. fumosoroseus* [15]. Magalhães *et al.* [16] noted that *B. bassiana* caused mycosis in 60% of adult *Coleomegilla maculata lengi* Timberlake (Col., Coccinellidae) and in 35% of adult *Eriopis connexa* (Col., Coccinellidae), when conidia were applied directly to the insects. Todorova and colleagues [17] remarked that different strains of *B. bassiana* showed different efficacies on larvae of *C. maculata lengi*. The different ecological host ranges of different entomopathogenic fungus isolates, e.g. co-evolution between hosts and pathogens could partially explain the different susceptibilities found in this study and in previously report.

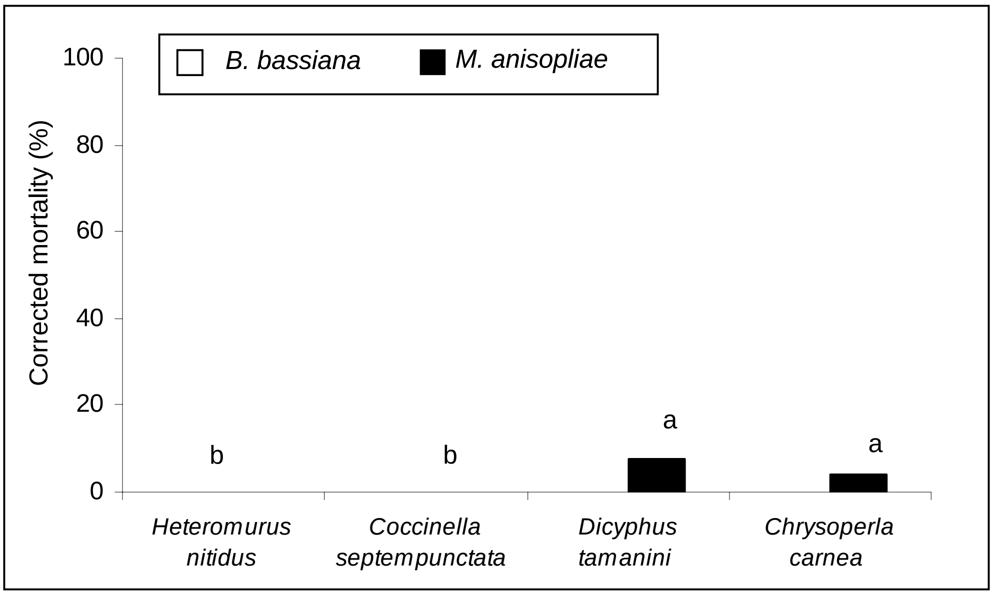

Figure 1 Corrected mortalities of non target insects treated with *Beauveria bassiana* and *Metarhizium anisopliae* at a concentration of $1x10^8$ conidia/ml (Bar with different letters indicates significant differences among different non target insects (Duncan's multiple range test at P≤0.05)

4. CONCLUSIONS

B. bassiana Bb. 5335 and *M. anisopliae* Ma.7965 have the potential use as biological control agents against insect pests because they were relatively safe on non target insects, such as natural enemies and beneficial soil insects.

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