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# Programs and Abstracts

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# Development of entomopathogenic microbial in Thailand

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## INTRODUCTION

Biological control with pathogenic agents is a good alternative to chemical control against the insect pest on vegetable. Recently, the need to reduce the use of pesticides in the control of insect pest is more and more urgent. Entomopathogenic fungi are ideal candidates for integrated pest management (IPM) in forests and agricultural uses (1). Biocontrol is the use of living organisms such as entomopathogens or natural enemies to kill or suppress the harmful pest. Entomopathogenic fungi that are highly pathogenic to insects are valuable weapons for biocontrol and play an important role in promoting integrated pest management. The aim of the present study was to evaluate the virulence of the fungus and select entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria bassiana* that are highly pathogenic to *Thrips tabaci*(2) and *Spodoptera litura* (cutworm).

## RESULTS AND DISCUSSIONS

Isolates of *Metarhizium anisopliae* (Metschnikoff) (BCC 1964, 4810, 4849 and 5797) and four isolates of *Beauveria bassiana* (Balsamo) Vuillemin (BCC 2637, 6241 and 6966) were evaluated for their pathogenicity to the first instar of *Thrips tabaci* and 3 isolates of *Metarhizium anisopliae* fungus, BCC1858, BCC4849 and Khon Kaen were tested on cutworm. For thrips larvae were tested by micro sprayer with conidial suspension at a concentration of  $10^8$  spores/ml under laboratory conditions. *Metarhizium anisopliae* isolates caused 50% mortality rates ranged from 20.85-97.61% at 5 days post-inoculation. The LT90 values in all the isolates did not differ. The highest mortality of 97.61% was obtained by using *Metarhizium anisopliae* BCC 4849. Mortality rates more than 82% were observed in all isolates of *Beauveria bassiana*. For cutworm control, isolate 4849 at a concentration of  $6 \times 10^8$  spores/ml was the most effective one. Microbial control with fungal pathogens offers promising new avenues for control of insect pest and could be a useful component of an integrated pest management program for the organic crops and ornamental

Table 1. Percent mortality of thrips on vary stain of *Metarhizium anisopliae* and *Beauveria bassiana*

Fungi	No.	Strain	% mortality of 1st larvae at 5 days
<i>Metarhizium anisopliae</i>	1	BCC 5797	46.27
	2	BCC 4810	20.85
	3	BCC 4849	97.61
	4	BCC 1964	95.00
<i>Beauveria bassiana</i>	5	BCC 6241	82.75
	6	BCC 2637	98.85
	7	BCC 5436	97.75
	8	BCC 6966	84.78

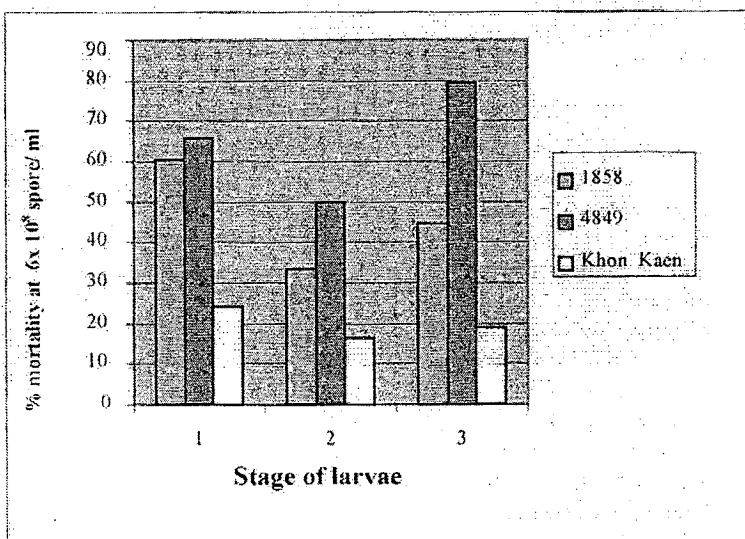


Fig. 1. Percent mortality of larvae of *Spodoptera litula* that treated with  $6 \times 10^8$  spore/ml of 3 isolates of *Metarhizium anisopliae*

## ACKNOWLEDGMENT

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## Aflp Characterisation of the Entomopathogenic Fungus *Metarhizium Anisopliae* Strains from Thailand

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### Abstract

*Metarhizium anisopliae* is a mitosporic entomopathogenic fungus that has been exploited extensively as biological control agent (BCA) against several pests. *Metarhizium anisopliae* isolates from several insect hosts and from various sugar cane growing areas of Thailand, were examined for genetic diversity using polymerase chain reaction (PCR)-based technology, involving amplified fragment length polymorphism (AFLP) was used to assess the genomic variability between 4 isolates of *Metarhizium* spp strains. Amplified fragment length polymorphism (AFLP) analysis of entomopathogenic fungus evidence provides a means of obtaining a reproducible DNA profile in a relatively short period of time in species for which no sequence information is available. Genomic DNA from mycelium of each strain was optimised and the use of cetyl trimethyl ammonium bromide (CTAB) and sodium chloride (NaCl) was incorporated. All strains could be typed in these conditions. DNA were double-digested by two restriction endonucleases (*EcoRI* and *MseI*) and ligated to oligonucleotide adapters. Two consecutive PCR reactions (pre-amplification and selective amplification) were performed using a modification of the AFLP protocol described by Gibco (Invitrogen, Rockville, MD). The DNA fragments were separated by electrophoresis using silver staining for band visualisation. Based on 23 AFLP primer combinations, a total of 1504 bands were detected. An average of approx. 65 bands were scored for each primer pair. Among of which 3 polymorphic fragments (obtained from E-AGG/M-CAA, E-AGG/M-CAA, E-AGG/M-CAA) were identified as potentially a strain specific. DNA fragments of between 0.26 and 0.38 kp were obtained. These markers have practical utility for (1) establishing conspiracy in the cultivation and distribution of *Metarhizium* sp (2) identifying geographic sources. The results also suggest that AFLP markers may be useful for the tracking of specific biocontrol strains in the field.

**Keywords:** AFLP, Biological control agent (BCA), Entomopathogenic fungus, *Metarhizium anisopliae*.



Tropentag, October 7-9, 2008, Hohenheim

“Competition for Resources in a Changing World:  
New Drive for Rural Development”

Laboratory Evaluation of Entomopathogenic Fungi *Metarhizium*  
*Anisopliae* and *Beauveria Bassiana* Against Thrips *Tabaci*  
(Thysanoptera: Thripidae)

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**Abstract**

Thrips *tabaci* is a polyphagous pest that attacks many different horticultural crop and ornamental plant species. Recently, the need to reduce the use of insecticides in the control of thrips is more and more increasing. On that account, the possibility to make use of the entomopathogenic fungus was tested to Thrips *tabaci*. Four isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin (BCC 1964, 4810, 4849 and 5797) and four isolates of *Beauveria bassiana* (Balsamo) Vuillemin (BCC 2637, 5436, 6241 and 6966) were evaluated for their pathogenicity against the first instar of Thrips *tabaci*. Larvae were exposed by micro sprayer with conidial suspension at a single concentration of 10(8) spores/ml under laboratory conditions. (16L: 8D photoperiod, 30 +/- 1 degrees C temperature, 96% RH). *Metarhizium anisopliae* isolates caused cumulative mortality rates ranged from 20.85–97.61% at 5 days post-inoculation. The LT90 values in all the isolates did not exceed 5 days. The highest mortality of 97.61% was achieved by using *Metarhizium anisopliae* BCC 4849. Mortality rates more than 82% were observed in all isolates of *Beauveria bassiana*. Isolates of both fungal causing more than 80% mortality will be subjected to dose-response mortality bioassays, 1 x 10(8), 10(7), 10(6), and 10(5) conidia/ml. However, the implications of above results in relation to thrips control in greenhouse and to future research are discussed. Microbial control with fungal pathogens provides promising new avenues for control of Thrips *tabaci* and related species and could be a useful component of an integrated pest management programme for the organic crops and ornamental industry.

**Keywords:** Thrips *tabaci*, *Beauveria bassiana*, entomopathogenic fungi, *Metarhizium anisopliae*



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## Selection of Entomopathogenic Fungi for *Spodoptera litura* Control

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### Abstract

Biological control with pathogenic fungi is a promising alternative to chemical control against the insect pest of vegetable. Ten isolates of green muscardine fungus, *Metarhizium anisopliae* as entomopathogenic fungus were used to test for pathogenicity on second instar of common cutworm, *Spodoptera litura* under the laboratory conditions. The tested larvae were placed in Petri dishes containing green muscardine fungus and they were allowed to make a direct contact with the particular entomogenous fungus. It was revealed that 3 isolates of green muscardine fungus, BCC1858, BCC4849 and Khon Kaen were effectively killed 100% of the cutworm larvae within 2 days. Subsequently, *M. anisopliae* isolates were brought to examine with 8 different media for physiological properties. The result showed that mungbean agar (MU) was the best for mycelial growth and sporulation. Moreover, the optimum temperature for growth was ranged around 30–35 °C. When the isolates were kept in the room with 12 hours light alternated with 12 hours dark, they were produced more green spores than the other. [The best conditions for sporulation were observed when the isolates were kept at 30–35 °C with 12 hours light alternated with 12 hours dark.] When the 3 most effective isolates were tested with the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instars of cutworm at 4 concentration levels included of 10<sup>7</sup>, 10<sup>8</sup>, 10<sup>9</sup> and 10<sup>10</sup> spores/ml. The result indicated that the isolate 4849 with the concentration of 6 × 10<sup>8</sup> spores/ml was the most effective one. It was observed to cease the 3<sup>rd</sup> instar of cutworm by 79.49% within 7 days.

**Keywords:** *Spodoptera litura*, Biological control, Entomopathogenic fungus, *metarhizium anisopliae*