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Efficacy of Entomopathogenic Fungi, *Paecilomyces fumosoroseus*, *Beauveria bassiana* and *Metarhizium anisopliae* var. *majus* Against *Crocidolomia binotalis* (Lepidoptera; Pyralidae)

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ABSTRAK

Bioasai di makmal tiga pencilan tempatan entomopatogen hyphomycete ke atas ulat hati kubis, Crocidolomia binotalis *Zeller (Lepidoptera: Pyralidae) dan efikasi lapangan beberapa persediaan formulasi* Paecilomyces fumosoroseus *(Wise) Brown & Smith telah dinilai. Bioasai dos-kematian menunjukkan kesemua pencilan berupaya menyebabkan maut ke atas larva instar kedua. Kebanyakan larva menjadi moribund dalam masa dua hari selepas rawatan. Pendedahan kepada dos mulai 2 x 10^1 konidia mL^{-1} hingga 2 x 10^7 konidia mL^{-1} mengakibatkan purata kematian larva dari 10.3 hingga 100%. Pada kepekatan melebihi 2 x 10^6 konidia ml^{-1} kematian larva adalah melebihi 80%, dan 100% kematian telah didapati pada 2 x 10^7 konidia mL^{-1} bagi ketiga-tiga spesies kulat. Perhubungan signifikan ($P<0.005$) telah diperolehi di antara log dos dan kematian probit bagi kesemua pencilan. Nilai EC_{50} bagi* P. fumosoroseus *telah dianggarkan pada 1.926 x 10^3 konidia mL^{-1}, dan didapati lebih rendah dan signifikan daripada* Beauveria bassiana *(Bals) Vuill pada 5.038 x 10^3 konidia mL^{-1}. Nilai EC_{50} bagi* Metarhizium anisopliae *var.* majus *(Metsch) Sorokin adalah yang tertinggi dan signifikan pada 2.0 x 10^4 konidia mL^{-1}. Pencilan yang terbaik sekali ialah* P. fumosoroseus *dengan LT_{50} hampir setengah hari lebih singkat pada dos setanding 2 x 10^7 konidia mL^{-1}, dengan nilai kecerunan 4.656 berbanding 4.356 bagi* B. bassiana *dan 4.193 bagi* M. anisopliae *var.* majus. *Pada rawatan lapangan, tanaman telah disembur dengan ampaian mengandungi 10^7 konidia mL^{-1}. Purata peratus kematian bagi kesemua rawatan didapati melebihi 70% dan lebih signifikan ($P<0.05$) daripada kawalan. Konidia yang diampai dalam minyak kelapa sawit Vesawit® telah memberi keputusan yang paling menggalakkan ke atas ulat hati kubis.*

ABSTRACT

Laboratory bioassays of three local isolates of entomopathogenic hypomycetes against the cabbage-heart caterpillar, Crocidolomia binotalis *Zeller (Lepidoptera: Pyralidae) and field efficacy of several formulations of* Paecilomyces fumosoroseus *(Wise) Brown and Smith were evaluated. Dosage-mortality bioassays revealed that all the isolates were able to cause mortality to second instar larvae. Majority of the larvae became moribund within two days after treatment. Exposures to doses varying from 2 x 10^1 conidia mL^{-1} to 2 x 10^7 conidia mL^{-1} resulted in mean larval mortalities from 10.3 to 100%. At concentrations exceeding 2 x 10^6 conidia mL^{-1} larval mortality was in excess of 80% and 100% mortality was observed at 2 x 10^7 conidia mL^{-1} for all three fungal species. Significant relationships ($P<0.05$) were obtained between log dosage and probit mortality for all the isolates. The EC_{50} for* P. fumosoroseus *was estimated at 1.926 x 10^3 conidia mL^{-1}, and was significantly lower than that of* Beauveria bassiana *(Bals) Vuill at 5.038 x 10^3 conidia mL^{-1}. The EC_{50} for* Metarhizium anisopliae *var* majus *(Metsch) Sorokin was significantly the highest, at 2.0 x 10^4 conidia mL^{-1}. The best isolate was* P.fumosoroseus *which had LT_{50} almost half a day lower at a comparable dosage of 2 x10^7 conidia mL^{-1}, with a gradient of 4.656 as compared to 4.356 for* B. bassiana *and 4.193 for* M. anisopliae *var* majus. *In field treatments, plants were sprayed with suspensions containing 10^7 conidia mL^{-1}. Mean percent mortality for all the treatments were in excess of 70% and significantly higher ($P<0.05$) than the control. Conidia in palm oil Vesawit® gave the most promising result against the cabbage-heart caterpillar.*

INTRODUCTION

Cruciferous vegetables are economically important throughout the world. However, their production has been seriously affected by a steady increase in insect pest damage. The cabbage-heart caterpillar (CHC), *Crocidolomia binotalis* Zeller, is currently considered the second most important insect pest of cabbage in the Cameron Highlands of Malaysia (Ooi and Kelderman 1979). It is almost exclusively found in hot humid highland tropics, and constitutes a more serious pest problem during the drier months. Even a single mature larva is capable of causing economic loss to head cabbage, *Brassica oleracea* L. (Peter *et al.* 1988). This pest is not reported in Europe and the Americas (Waterhouse and Norris 1987). The larvae live gregariously feeding at first on the underside of cabbage leaves which may eventually be eaten completely. Damage to the heart at preheading leads to abortion or production of multiple heads which then are unmarketable. By and large, farmers use large quantities of insecticides, often spraying tank mixes of several chemicals to control the pest. As reported by Ooi and Sudderuddin (1978) and Fauziah *et al.* (1992), these practices have resulted in many problems such as development of insecticide resistance, pest resurgence, excessive chemical residues and environmental contamination.

The concept of integrated pest management (IPM) in vegetables is beginning to be accepted by farmers, but so far no parasitoids of CHC have been reported in Malaysia. Consequently, microbial control could play an important role in the IPM strategy. The role of fungal pathogens as natural enemies for cruciferous insect pests has recently been explored and several isolates of hyphomycetous fungi have been identified. Besides being infective against the bagworms, the cocoa mirids and cocoa podborer (Lim *et al.* 1988), *Paecilomyces fumosoroseus* (Wise) Brown and Smith and *Beauveria bassiana* (Bals.) Vuill have been reported effective against the cosmopolitan diamondback moth, *Plutella xylostella* L (Lepidoptera: Yponomeutidae) (Ibrahim and Low 1993; Ibrahim and Hashim 1998), while *Metarhizium anisopliae* var. *majus* (Metsch) Sorokin has been shown to be infective against the diamondback moth (Hashim *et al.* 1999).

Against this background, studies were undertaken to evaluate the IPM potential of microbial control of CHC. Three endemic fungal species, *P. fumosoroseus, B. bassiana,* and *M. anisopliae* var. *majus* were compared in bioassays against the CHC. The efficacy of several formulations of the best fungal species was tested in a field trial.

MATERIAL AND METHODS

Insect Culture

Cultures of CHC were maintained in the insectary under a controlled environment of 27±2°C, 80±10% RH and with 12:12 day:night photoperiod on hybrid cabbage leaves, *Brassica oleracea* var. *capitata,* obtained from greenhouse-grown plants. Distilled water and 5% (w/v) honey were provided separately as food for the moths. To obtain larvae of standardised age, plants with 1-day old eggs from oviposition cages were transferred daily into new cages. Only second instar larvae were used in the study.

Fungal Culture

For the purpose of this study, fungal isolates originated from heterologous hosts (Table 1) were passaged through *P. xylostella* larvae and then re-isolated as single spore isolates by means of a micromanipulator under a microscope, cultured and maintained at an ambient environment of 27±2°C on sterilised potato dextrose agar (PDA) as well as on sterilised rice flour medium maintained in autoclaved polybags.

TABLE 1
Origin of fungal isolates

Species	Insect host	Plant host	Location
Paecilomyces fumosoroseus	*Pteroma pendula* (Psychidae, larva & pupa)	*Acacia mangium*	Selangor
Beauveria bassiana	*Glenia celia* (Cerambycidae, pupa)	*Theobroma cacao*	Sabah
Metarhizium anisopliae var *majus*	*Oryctes rhinoceros* (Scarabaeidae, larva)	*Elaeis guineensis*	Selangor

To prepare fungal inocula, conidia from 3-week old PDA cultures were scraped from the surface of the plates with a sterile scalpel and suspended in 0.05% Tween 80 in sterile distilled water. A Neubauer haemocytometer was used to estimate the conidial concentration and subsequent appropriate serial dilutions were prepared from 2×10^7 to 2×10^1 conidia mL^{-1}. A Sigma hand altomiser was used to deliver approximately 2 mL of each treatment on a cabbage leaf preparation containing the test larvae. The leaf preparation consists of Chinese cabbage leaf, *Brassica juncea*, of ca. 120 cm^2 kept fresh with its stalk wrapped in wet cotton enveloped in aluminium foil. In this way the leaf freshness could be maintained for about 5-7 days. Each treatment was also sprayed on clear bacteriological agar (Bacto®) plate for CFU (germinated conidia) counts under a microscope 24 h after treatment.

Dosage-Mortality Bioassay

Ten second instar larvae of CHC were transferred onto each cabbage leaf preparation and left to acclimatise for about 10 min. Six such leaf preparations (replicates) were assigned randomly to each dosage-response assay. The assay included seven inoculum dosages of each fungal species plus a sterile control which consisted of 0.05% aqueous Tween 80. It was conducted in an ambient environment of 27 ± 2°C, 80 ± 10% RH and with 12:12 day:night photoperiod. Immediately after inoculation with the Sigma hand atomiser, the treated leaf preparations were each placed in an unsealed plastic container (15.5 x 8.5 x 9 cm) which was covered with a clear plastic sheet to maintain 100% RH for 24 h. Thereafter, the sheet was removed and water trays were used to maintain high air humidity in the containers.

Larval mortality, including moribund individuals, was recorded daily for 12 days. The EC_{50s} and LT_{50s} from the regression line with 95% fiducial limits were obtained through a probit programme (S103, Statistical Research Service, Canada DOA, unpublished) based on probit analysis (Finney 1971).

Field Trial with P. fumosoroseus

Five-week-old cabbages grown in polybags in the glasshouse were placed in an open field spaced 0.5 m within and 0.5 m between rows. There were four treatments and an untreated control, each consisted of six plants arranged in a row. Only the middle four plants were used for the purpose of evaluation. The plants were arranged in a randomised completed block design with five replications. Ten second instar CHC larvae were transferred to each cabbage plant, and to prevent ants from predating on the larvae, gamma-HCH (Lindacide®) powder was scattered over the area. Four formulations of *P. fumosoroseus* were prepared with conidia from PDA in oil palm cooking oil (40% mono-unsaturated, Vesawit®), conidia from PDA in kaolin (fine dust), conidia from rice flour culture (powder) and conidia from PDA in sterile distilled water containing 0.05% Tween 80. Sterile distilled water containing 0.05% Tween 80 was the spray carrier which also served as the control. A concentration of 2×10^7 conidia mL^{-1} was used in this spray programme. Oil and kaolin formulations were prepared from conidia (0.03 g) from 3-week old cultures scraped from the surface of the plates with a sterile scalpel mixed with either 30 mL Vesawit® or 30 g kaolin. Conidia from rice flour medium were collected from 3-week old cultures suspended in sterile distilled water containing 0.05% Tween 80. This was then shaken vigorously and the conidia were sieved using muslin cloth in order to separate the rice flour. Sterile distilled water containing 0.05% Tween 80 was used to dilute the conidial suspension which was standardised to 2×10^7 conidia mL^{-1}.

Four plants in the centre of each treatment row were designated as the experimental units. Four mL of each treatment were sprayed using a Sigma hand atomoser on each cabbage plant infested with 10 second instar CHC larvae. All treatments were sprayed late in the evening. Twenty-four hours after treatment, each group of 10 larvae from the four plants were transferred to a Petri dish with fresh cabbage leaves which were replaced after five days. Larval mortality was recorded daily for 12 days to determine the cumulative percent mortality. All dead and moribund larvae suspected of being were individually surface-sterilised in 0.5% sodium hypochlorite for three minutes, rinsed in sterilised distilled water and then placed on PDA tro ascertain the presence of *P. fumosoroseus.*

Data for ANOVA were transformed by Arc Sine $\sqrt{x}$ to stabilise the variance prior to the analysis. Treatment means were subjected to two-way ANOVA and subsequently compared using LSD at 5% level of probability.

RESULTS AND DISCUSSION

Dosage-Mortality Biasssay

All the fungal isolates were pathogenic for second instar larvae of *C. binotalis* (Table 2). The majority of the larvae became moribund within two days after treatment. All the fungal species were observed to sporulate on the surface of cadavers which were all mummified. Hashim *et al.* (1999) had previously observed *P. fumosoroseus* sporulation on the surface of dead diamondback moth larvae. The cadavers infected with *M. anisopliae* var. *majus* were not completely overgrown with the fungal mycelium unlike those larvae infected with *P. fumosoroseus* or *B. bassiana.*

Larval mortality was positively correlated with dose rate. Exposures to conidial doses varying from 2 x 10^1 conidia mL^{-1} to 2 x 10^7 conidia mL^{-1} resulted in larval mortality from 10.3 to 100%. At concentrations exceeding 2 x10^6 conidia mL^{-1}, larval mortality after 12 days was in excess of 80%, and a 100% mortality was observed at 2 x 10^7 conidia mL^{-1} for all the three species. The isolates of *P. fumosoroseus* and *B. bassiana* have also been found to be pathogenic against diamondback moth (DBM) larvae (Ibrahim and Hashim 1998). Tulloch (1976) had previously reported that *M. anisopliae* var. *majus* appeared to be restricted to the rhinoceros beetle *Oryctes* spp. However, Hashim *et al.* (1999) found that this isolate of *M. anisopliae* var *majus* caused over 80% mortality on DBM larvae when exposed to 2 x 10^6 conidia mL^{-1}.

Results of probit analyses indicated that there was significant relationship ($P< 0.05$) between log-dosage and probit mortality for the three fungal species (Table 3). Estimates of the median effective concentration (EC_{50}) computed for *P. fumosoroseus* were 1.926 x 10^3 conidia mL^{-1} with 95% fiducial limits between 7.0 x 10^2 – 4.58 x 10^3 conidia mL^{-1}. This was significantly lower than that of *B. bassiana* which was 5.038 x 10^3 conidia mL^{-1} with 95% fiducial limit between 1.907 x 10^3 – 1.198 x 10^4 conidial mL^{-1}. The EC_{50} for *M. anisopliae* var *majus* was much higher than that of the earlier mentioned fungal species. Suffice to say that *P. fumosoroseus* was the most virulent against the CHC, being ca. 2.5 times more virulent than *B. bassiana* and ca. 10 times more virulent than *M. anisopliae* var. *majus.*

The virulence by these three fungal species, as displayed by the decreasing LT_{50} values, demonstrated a common trend of generally increasing potency (i.e. the rate and speed of mortality) with increasing concentration (Table

TABLE 2
Mean percent mortality of second instar larvae of *Crocidolomia binotalis* after 12 days exposure to *Paecilomyces fumosoroseus, Beauveria bassiana* and *Metarhizium anisopliae* var *majus*

Dosage (Conidia mL^{-1})	*Paecilomyces fumosoroseus*	*Beauveria bassiana*	*Metarhizium anisopliae var majus*
2 x 10^7	100.0 (1248.09)[a]	100.0 (44.750)	100.0 (318.13)
2 x 10^6	89.5 (295.84)	83.9 (125.84)	81.0 (133.32)
2 x 10^5	70.2 (79.52)	62.5 (33.43)	56.9 (17.29)
2 x 10^4	57.9 (9.10)	53.6 (11.19)	44.8 (4.44)
2 x 10^3	47.4 (1.33)	42.9 (10.15)	29.3 (0.78)
2 x 10^2	38.6 (1.25)	28.6 (7.24)	25.9 (0.34)
2 x 10^1	28.1 (0.73)	26.8 (4.62)	10.3 (0.25)

Control = zero mortality
[a] CFU / mm^2 equivalent

TABLE 3
Effect of *Paecilomyces fumosoroseus* (Pf), *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* var *majus* (Mam) on second instar larvae of *Crocodolomia binotalis*

Species	a (intercept)	b ± SE (slope)	ED_{50} (conidia mL^{-1})	95% fiducial limit
Pf	3.735	0.3852±0.0404	1926	700.2 – 4580
Bb	3.592	0.3838±0.0407	5038	1907 – 11980
Mam	3.021	0.4602±0.0430	20000	9443 – 41530

4). The most virulent isolate was *P. fumosoroseus*. This is shown by the lower LT_{50} for *P. fumosoroseus*, which was almost half day lower than that of the other two species.

Field Trial with P. fumosoroseus

Mean percent mortalities for all the treatments were in excess of 75% except for the control (Table 5). Conidia from PDA in palm oil Vesawit® significantly resulted in the highest larval mortality. The prevailing high humidity of the surrounding environment, typical of the weather conditions in Malaysia throughout the year, could have positively influenced the infectivity. Conidia from rice flour culture achieved 76.0% mortality, which did not differ significantly from conidia in kaolin. However, this formulation caused significantly lesser mortality than the conidia in palm oil formulation. Oil may have helped in spreading the conidia on the surface of a hydrophobic surface such as insect cuticle (Inglis *et al.* 1996). Ibrahim and Low (1993) reported *P. fumosoroseus* to be highly efficacious in the cabbage field against the diamondback moth when applied at the rate of 10^8 conidia mL^{-1} in 375 L water ha^{-1}, however, further effort is necessary in order to develop management strategies for CHC in the highlands.

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TABLE 4
Median lethal time for varying dosages of *Paecilomyces fumosoroseus* (Pf), *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* var. *majus* (Mam) on second instar larvae of *Crocidolomia binotalis*

Dosage (conidia mL^{-1})	Pf				Bb				Mam			
	a	b±SE (slope)	LT_{50} (days)	95% FL	a	b±SE (slope)	LT_{50} (days)	95% FL	a	b±SE (slope)	LT_{50} (days)	95% FL
2×10^7	3.93	4.656± 0.402	1.70	1.518-1.876	3.57	4.356± 0.330	2.13	0.415-3.006	3.58	4.193± 0.310	2.18	1.952-2.388
2×10^6	4.92	2.229± 0.336	2.29	1.440-3.037	3.65	2.427± 0.187	3.61	2.356-3.737	2.61	3.368± 0.241	5.11	4.716-5.505
2×10^5	3.52	2.164± 0.185	4.83	4.308-5.365	3.54	1.904± 0.182	5.87	3.267-5.191	3.06	2.310± 0.294	6.93	5.887-8.299
2×10^4	3.60	1.700± 0.179	6.62	5.814-7.631	3.32	1.943± 0.193	7.34	7.926-11.49			na	

na LT_{50} is not available since mean % mortality was below 50%

TABLE 5
Mean percent mortality for varying treatments of *Paecilomyces fumosoroseus* on second instar larvae of *Crocidolomia binotalis*

Treatments [a]	Mean % mortality
1. Conidia from PDA in Vesawit® oil	88.5 a
2. Conidia from PDA only	78.0 ab
3. Conidia from rice flour	76.0 b
4. Conidia from PDA in kaolin	75.0 b
5. Control (Tween 80)	15.5 c
LSD	13.48
MSE	403.5
CV	21.97

Means followed by the same letter are not significantly different at P=0.05 as determined by 2-way ANOVA and LSD.
Analysis was performed on Arc Sine $\sqrt{x}$ values.
[a] sterile aqueous Tween 80 (0.05%) was the spray carrier.

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