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SHORT COMMUNICATION

Pathogenicity of *Paecilomyces fumosoroseus* (Wise) Brown & Smith, *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin on the Striped Flea Beetle *Phyllotreta striolata* F. (Coleoptera: Chrysomelidae)

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ABSTRAK

Keupayaan tiga spesies kulat entomopatogen, Paecilomyces fumosoroseus *(Wise) Brown & Smith,* Beauveria bassiana *(Bals.) Vuill. dan* Metarhizium anisopliae *(Metsch.) Sorokin menyebabkan jangkitan ke atas dewasa, telur dan larva kumbang lenting berjalur* Phyllotreta striolata F. *telah diuji. Hanya satu pencilan* M. anisopliae *dan tiada bagi* B. bassiana *dan* P. fumosoroseus *yang menyebabkan kematian peringkat dewasa melebihi 50% pada kepekatan 2 x 10^7 conidium mL^{-1}. Satu pencilan* P. fumosoroseus *(Pf), satu bagi* B. bassiana *(Wls) dan dua bagi* M. anisopliae *(MPs dan Cy3) didapati patogenik ke atas larva instar pertama* P. striolata *menyebabkan lebih daripada 50% kematian pada kepekatan 2 x 10^6 conidium mL^{-1}. Kadar kematian larva meningkat mengikut peningkatan kepekatan. Nilai anggaran LT_{50} untuk Pf, Wls, MPs dan Cy3 masing-masing bagi larva pada kadar 2 x 10^6 conidium mL^{-1} ialah 2.9, 3.5, 3.0 dan 3.0 hari. Kedua-dua pencilan* M. anisopliae *ini juga didapati amat patogenik ke atas telur mengakibatkan kerencatan penetasan yang signifikan, manakala* B. bassiana *dan* P. fumosoroseus *didapati kurang patogenik. Anggaran median kepekatan maut masing-masing bagi Cy3 dan MPs ialah 13.0 x 10^5 dan 5.03 x 10^5 conidium mL^{-1}.*

ABSTRAK

The ability of three species of entomopathogenic fungi, Paecilomyces fumosoroseus *(Wise) Brown & Smith,* Beauveria bassiana *(Bals.) Vuill. and* Metarhizium anisopliae *(Metsch.) Sorokin to cause infection on the adults, eggs and larvae of the striped flea beetle* Phyllotreta striolata F. *was tested. Only one isolate of* M. anisopliae *and none of the* B. bassiana *and* P. fumosoroseus *caused adult mortality in excess of 50% at a concentration of 2 x 10^7 conidia mL^{-1}. One isolate of* P. fumosoroseus *(Pf), one* B. bassiana *(Wls) and two of* M. anisopliae *(MPs and Cy3) were pathogenic to the first instar larvae of* P. striolata *causing more than 50% mortality at a concentration of 2 x 10^6 conidia mL^{-1}. The rate of larval mortality increased with increase in conidia concentration. The respective estimated LT_{50} values for Pf, Wls, MPs and Cy3 for the larvae at 2 x 10^6 conidia mL^{-1} were2.9, 3.5, 3.0 and 3.0 days. The two isolates of* M. anisopliae *were also highly pathogenic to the eggs causing significant inhibition of hatching, while* B. bassiana *and* P. fumosoroseus *were less pathogenic. Estimates of the median lethal concentration for Cy3 and MPs were 13.0 x 10^5 and 5.03 x 10^5 conidia mL^{-1} respectively.*

INTRODUCTION

The striped flea beetle, *Phyllotreta striolata* F., is not only a serious pest of canola and mustard but also feeds on a wide range of other brassicas (Ibrahim and Khoo 1989; Bartlet and Williams 1991). Injury to plants is caused by beetles and larvae feeding on leaves, or by larvae mining within stems or leaves, or feeding on the roots. They can kill plants directly by severing the hypocotyls or by eating the newly emerged meristem (Soroka and Pitchard 1987). The implementation of effective IPM in crop systems as the extension of biological control in a much more effective way is not yet established because to date the effectiveness of biocontrol agents such as predators, parasites or *Bacillus thuringiensis*

seems rather limited (Burgess 1982; Wylie 1984; Hazzard and Ferro 1991). Consequently, majority of the growers preferred applying broad spectrum chemical insecticides instead. For a strongly bio-based IPM to be successful, dependence upon chemical insecticides must be minimized and some suitable and safe alternative control measures are needed.

Entomopathogenic fungi, the common pathogen of soil-associating coleopterans such as the flea beetle, are promising agents for biological control and are gaining increasing attention worldwide as mycoinsecticides. The use of fungus as a biological control agent against flea beetle was first reported by Butt *et al.*(1992) when *M. anisopliae* isolate V90 was found to be pathogenic to the cabbage stem flea beetle, *Psylliodes chrysochephala*. Miranpuri and Kachatourians (1995) also reported the pathogenicity of *B. bassiana* against the cabbage flea beetle, *Phyllotreta cruciferae*. Similarly, *M. anisopliae* isolate with high pathogenicity against *P. cochleariae* demonstrated little or no pathogenicity for *P. chrysocephala* (Butt *et al.* 1992).

The aim of this study was to determine the susceptibility of the striped flea beetle adults, eggs and larvae to three species of entomopathogenic fungi and to evaluate the relative potency of these fungi against the beetles.

MATERIALS AND METHODS

Adult flea beetles collected using an aspirator from Chinese mustard, *Brassica juncea*, grown in the vegetable plot at the Universiti Putra Malaysia (UPM) Agricultural Park were used in the study. The beetles were maintained in plastic containers (38 x 23 x 38 cm) provided with fresh Chinese mustard leaves in a room at an ambient environment of 28 ± 2°C, a 12 h photoperiod and 85 ± 15% RH. To obtain eggs, gravid females were confined overnight in plastic containers (38 x 23x 38 cm) each lined with moist filter paper and provided with fresh Chinese mustard leaves. The eggs were then collected from these filter papers and left to incubate for 24 h in separate plastic cups (6 cm diameter). The first instar larvae were obtained the following day.

The original hosts and countries of origin for the fungal isolates used in this study are listed in Table 1. All isolates were maintained at room temperature of 28 ± 2°C on potato dextrose agar (PDA, Oxoid, Basingstoke, UK) containing 0.5% yeast extract (Difco) which had been sterilised for 20 minutes and 121°C and 1.05 kg/cm^2. To prepare fungal inocula, conidia from 15 day-old sporulating cultures were scraped from the surface of the plates with a sterile scalpel and suspended in a 0.05% aqueous Tween 80. A Neubauer haemocytometer was used to estimate the conidial concentration and subsequent appropriate dilutions were made.

A preliminary test on adult flea beetles was made using a dose of 2 x 10^7 conidia mL^{-1}. Inoculation was done by spraying 0.5 mL of the conidial suspension with a Sigma hand atomiser. Control insects were treated similarly with 0.05% aqueous Tween 80. After inoculation, the insects were transferred to a Petri dish (15 cm diameter) lined with moist filter paper and supplied with a fresh Chinese mustard leaf. Mortality was recorded 10 days after inoculation. Each assay consisted of five replicates with 10 adults per replicate.

The preliminary assays identified isolate *M. anisopliae* (Mps) (see Table 1) as worthy of further investigation, and it was passaged through and reisolated from the host onto PDA. Further bioassays were conducted using conidial concentrations ranging from 2 x 10^3 to 2 x 10^9 conidia mL^{-1} to determine the LC_{50} and LT_{50} values. Adult flea beetle mortalities were recorded daily for 10 days. Each assay was replicated five times with 10 adults per replicate.

The isolates tested for pathogenicity against the flea beetle eggs and first instar larvae were *M. anisopliae* (Cy3 and MPs), *B. bassiana* (Wls) and *P. fumosoroseus* (Pf). Bioassays were using conidial concentrations ranging from 2 x 10^3 to 2 x 10^8 conidia mL^{-1}. A 24-h old egg cluster (10-15 eggs) placed on a leaf section was inoculated by a drop (5 ml) of conidial suspension using a Gilson micropipette (P20). Larvae were inoculated by dipping them into conidial suspension for five seconds. The eggs or the larvae were transferred to Petri dishes (9 cm diameter) lined with moist filter paper and fresh Chinese mustard seedlings were supplied for the larvae. Number of eggs and larvae infected were recorded for five days. Each assay consisted of five replicates with 10 eggs or larvae per replicate. The final proportions of dead adults, larvae and infected eggs for each concentration were analysed using probit analysis (S103, Statistical Research Service, Canada Department of Agriculture, unpublished) based on Finney (1971).

TABLE 1
Isolates of *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces fumosoroseus,* their original hosts and countries of origin

Species	Code	Insect host	Country of origin
M. anisopliae	*Cy3*	*Cylas formicarius* (Curculionidae)	Indonesia
	MPs	*Phyllotreta striolata* (Chrysomelidae)	Malaysia
B. bassiana	*Wls*	*Leptocorisa oratorius* (Alydidae)	Indonesia
P. fumosoroseus	*Pf*	*Pteroma pendula* (Psychidae)	Malaysia

TABLE 2
Pathogenicity[a] of *M. anisopliae, B. bassiana* and *P. fumosoroseus* isolates on adult *P. striolata*

Species	Code	%Mortality
M. anisopliae	Cy3	8
	PPs	58
B. bassiana	Wls	0
P. fumosoroseus	Pf	26

[a] at a dose of 2 x 10^7 conidia mL^{-1}

RESULT AND DISCUSSION

The pathogenicity of *M. anisopliae* on the flea beetles differed between isolates. Only one isolate of *M. anisopliae* caused mortality in excess of 50% (Table 2). The isolate of MPs was found to be the most pathogenic. No mortality was observed in the control.

The mortality of *P. striolata* was dose-dependent using the *M. anisopliae* (MPs) isolate. The estimated LC_{50} value was 6.77 x 10^7 conidia mL^{-1} while the LT_{50} values of *M. anisopliae* (MPs) at 2 x 10^9 and 2 x 10^8 conidia mL^{-1} were 4.7 and 5.8 days respectively. A 10-fold increase in dose did not result in any marked decrease in median lethal time. The LT_{50} values were not obtained at doses 2 x 10^7 conidia mL^{-1} and below because less than 50% mortality of the beetles was observed within the 10-days period (Table 3). The earliest death at the higher dose (2 x 10^9 conidia mL^{-1}) occurred on the third day. Fungal development was observed for at least 60% of the cadavers within 4-6 days. Sporulation occurred by the 7th day. Butt *et al.* (1992) also reported over 70% fungal growth within 2-5 days after the cabbage stem flea beetle, *P. chroscephala,* was exposed with 1 x 10^{10} conidia mL^{-1} of a highly pathogenic isolate of *M. anisopliae.*

Two isolates of *M. anisopliae* (MPs and Cy3), one *B. bassiana* (Wls) and one *P. fumosoroseus* (Pf) were pathogenic to the first instar larvae of *P. striolata.* All isolates caused more than 50% mortality at a dose of 2 x 10^6 mL^{-1}. No mortality was recorded in the control (Table 4). This indicated that the flea beetle larvae were more susceptible than the adult. Butt *et al.*(1992) also reported that adult flea beetles were less susceptible than the aphids *Myzus persicae* and *Lipaphis erydimi* which have softer bodies. The heavily sclerotised beetle cuticle, as opposed to a thinner integument for the larvae, could be the probable factor affording resistance to infection by entomopathogenic fungi.

Dose-related mortality was evident in all the isolates tested. The values of LC_{50s} and LT_{50s} for

TABLE 3
Median lethal concentration an median lethal time for varying dosages of *M. anisopliae* on adult *P. striolata*

Isolate	LC_{50} (95% FL) x 10^7 (conidia mL^{-1})	LT_{50}(95%FL) (days)	
		2 x 10^9 (conidia mL^{-1})	2 x 10^8 (conidia mL^{-1})
MPs	6.77 (3.25 – 18.16)	4.7(4.1 – 5.2)	5.8(3.9 – 13.1)

TABLE 4
Mean percent infection on first instar larvae of *P. striolata* upon treatment with varying dosages of *M. anisopliae*, *B. bassiana* and *P. fumosoroseus*

Doses (conidia mL^{-1})	*M. anisopliae*		*B. bassiana* (Wls)	*P.fumosoroseus* (Pf)
	(Cy3)	(MPs)		
2 x 10^8	74	86	82	80
2 x 10^7	64	78	68	64
2 x 10^6	66	72	54	70
2 x 10^5	18	20	22	26
2 x 10^4	8	12	12	10
2 x 10^3	2	0	0	4
Control	0	0	0	0

TABLE 5
Median lethal concentration of *M. anisopliae* (Cy3, MPs), *B bassiana* (Wls) and *P. fumosoroseus* (Pf) on first instar larvae of *P. striolata*

Isolate	Intercept	Slope ± SE	LC_{50}(95% FL) x 10^6 (conidia m^L1)
Cy3	1.408	0.545 ± 0.126	3.92 (0.22 – 57.29)
MPs	0.851	0.673 ± 0.138	1.47 (0.88 – 6.91)
W ls	1.222	0.579 ± 0.059	3.32 (1.70 – 6.91)
Pf	1.517	0.544 ± 0.111	2.20 (0.15 ± 74.93)

isolates MPs, Cy3, Wls and Pf are presented in Table 5. Dose-dependent relationship have similarly been reported for flea beetles in crucifers (Butt *et al.* 1992). The LT_{50} values of MPs, Cy3, Wls and Pf for *P. striolata* larvae at 2 x 106 conidia mL^{-1} were 3.0, 3.0, 3.5 and 2.9 days. A 10- to 100-fold increase in dosage significantly reduced the LT_{50} values of MPs and Wls, while for Cy3 and Pf a 100-fold increase occurred (Table 6).

Both the isolates of *M. anisopliae* were also highly pathogenic to flea beetle eggs, while *B. bassiana* and *P. fumosoroseus* were less pathogenic. *M. anisopliae* achieved infection in excess of 50% with the highest being a 97.03% and 100% infection by Cy3 and MPs respectively at a dose of 2 x 10^8 conidia mL^{-1}. Fungal infection to eggs significantly inhibited hatching (Table 7). Estimates of median lethal dose for isolates Cy3 and MPs are presented in Table 8. Zimmermann (1982) reported that *M. anisopliae* could infect the eggs or *Otiorhincus sulcatus* F. (Coleoptera: Curculionidae) only at the early stage before the eggs were melanised. Species reported to have

TABLE 6
Median lethal time of *M. anisopliae* (Cy3, MPs), *B. bassiana* (Wls) and *P. fumosoroseus* (Pf) on first instar larvae of *P. striolata*

Isolate	Dose (conidia mL^{-1})	Intercept	Slope ± SE	LT50(95%FL) days
Cy3	2 x 10^6	2.947	4.261 ± 0.899	3.0 (2.7 – 3.5)
	2 x 10^7	3.139	3.791 ± 0.887	3.1 (2.7 – 3.7)
	2 x 10^8	2.042	5.804 ± 1.397	3.2 (2.9 – 3.7)
MPs	2 x 10^6	3.569	3.003 ± 0.632	3.0 (2.5 – 3.4)
	2 x 10^7	3.318	3.861±0.659	2.7 (2.3 – 3.1)
	2 x 10^8	2.836	4.858 ± 0.699	2.8 (2.5 – 3.1)
Wls	2 x 10^6	2.991	3.639 ± 0.905	3.5 (3.1 – 4.5)
	2 x 10^7	2.802	4.620 ± 0.916	3.1(2.6 –3.5)
	2 x 10^8	2.679	6.317 ± 0.983	2.7 (2.4 – 2.9)
Pf	2 x 10^6	3.003	4.388 ± 0.898	2.9 (2.4 – 3.3)
	2 x 10^7	2.748	4.539 ± 1.842	3.1 (2.8 – 3.5)
	2 x 10^8	2.084	5.452 ± 0.935	2.9 (2.7 – 3.2)

TABLE 7
Mean percent infection of *P. striolata* eggs against doses used in assays of isolates of *M. anisopliae, B. bassiana* and *P. fumosoroseus*

Doses (conidia mL^{-1})	*M.anisopliae*		*B. bassiana* (Wls)	*P.fumosoroseus* (Pf)
	(Cy3)	(MPs)		
2 x 10^8	97.0 (0)	100.0 (0)	48.8 (46.8)	35.9 (40.5)
2 x 10^7	83.2 (2.4)	97.4 (4.2)	13.9 (76.6)	9.3 (63.9)
2 x 10^6	59.2 (20.3)	92.7 (3.0)	0 (75.3)	0 (82.3)
2 x 10^5	34.9 (38.9)	85.6 (5.6)	0 (84.0)	0 (95.5)
2 x 10^4	18.7 (74.5)	47.4 (42.5)	0 (80.7)	0 (82.6)
2 x 10^3	0 (85.5)	0 (91.1)	0 (83.1)	0 (86.3)
Control	0 (81.0)	0 (94.3)	0 (87.5)	0 (87.3)

( ) % eggs hatched.

TABLE 8
Median lethal concentration of isolates of *M. anisopliae* on the eggs of *P. striolata*

Isolate	Intercept	Slope ± SE	LC_{50} (95% FL) x 10^5 (conidia mL^{-1})
Cy3	1.564	0.562±0.045	13.00 (7.62 – 22.76)
MPs	0.558	0.945+0.209	5.03 (0.46 – 8.38)

caused egg mycosis were *Oospora ovorum* on locust and others that were limited to *Aspergillus, Fusarium,* and *Penicillium* (Madelin 1963; Fransen 1987).

Treatment at the egg stage could be advantageous because newly emerged larvae could quickly become infected by the persisting conidia in the immediate vicinity. Hence, for

soil-borne pest such as the larvae and eggs of the flea beetles treated with fungal spores, either prophylactically or curatively, could be applied as suggested by Charnley (1997). Moorhouse *et al.* (1993) reported that prior incorporation of *M. anisopliae* conidia into compost gave year-long protection to impatiens plant *(Impatients wallerana)* against the vine weevil. By direct drilling of *M. anisopliae* conidia using existing crop-sowing machinery to a depth of 20-25 cm, a long-term control of the redheaded cockchafer *Adophorus couloni* in the pasture was achieved (Rath 1992). Application of dried mycelial pellets were also particularly suitable as soil treatment (Stenzel *et al. 1992).* Krueger *et al.* (1991) used standard and lyophilized mycelial particles of *M. anisopliae* against scarab grubs. They concluded that the grub mortality occurred significantly quicker in mycelium-inoculated compared with conidia-inoculated soil.

CONCLUSION

This study has demonstrated that the three fungal isolates were infective against the larval stage and one of these isolates was highly pathogenic against the eggs. Further tests using formulated mycelial particles of these three fungi isolates are being planned against the flea beetle larvae in the field with the rational that the ambient soil moisture and temperature in Malaysia would promote profuse sporulation of the mycelium.

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