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Short communication

BIOLOGICAL CONTROL OF THE SPOTTED STEM BORER *CHILO PARTELLUS* (SWINHOE) (LEPIDOPTERA: CRAMBIDAE) WITH THE ENTOMOPATHOGENIC FUNGI, *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE*

Tadele Tefera [1] and K.L. Pringle [2]

[1] Department of Plant Sciences, Haramaya University, PO Box 42, Ethiopia.
E-mail: tadeletefera@yahoo.com
[2] Department of Entomology & Nematology, Private Bag X1, 7602 Matieland, South Africa

ABSTRACT: The pathogenicity of ten fungal isolates of *Beauveria bassiana* and *Metarhizium anisopliae* to second, third, fourth, fifth and sixth instar *Chilo partellus* larvae was studied. A single concentration ($1x10^8$ conidia/ml) of each isolate was assayed against second instar larvae. Of the ten isolates tested, *B. bassiana* (BB-01) and *M. anisopliae* (PPRC-4, PPRC-19, PPRC-61, EE-01) were the most pathogenic inducing 93 to 100% mortality. A single concentration of the five pathogenic isolates was further tested against third, fourth, fifth and sixth instar larvae. Second and sixth instar larvae were the most susceptible stages to the pathogenic isolates suffering 97% and 98% mortality, respectively. The LT_{50} values were low for second instar (2 days) and sixth (4.8 days) instar larvae. Multiple concentration assays ($1.25x10^6$, $2.5x10^7$, $5x10^7$, and $1x10^8$ conidia /ml) were conducted against second instar larvae with three of the most pathogenic isolates (PPRC-4, BB-01, EE-01). The LC_{50} was $1.44x10^3$, $1.53x10^4$, and $3.12x10^4$ for BB-01, PPRC-4 and EE-01, respectively. In a greenhouse trial, treatment of 3-week old maize seedlings with the virulent isolates considerably reduced stem tunneling and deadheart formation by *C. partellus* second instar larvae. The isolates, PPRC-4, PPRC-19 and PPRC-61 seemed to be the best candidates for further development and testing under field conditions.

Key words/phrases: *Beauveria bassiana*, biological control, *Chilo partellus*, entomopathogenic fungi, *Metarhizium anisopliae*

INTRODUCTION

In preliminary studies, Tefera and Pringle (2003 a & b) reported the pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin against second instar *C. partellus* at different temperature and exposure methods. An important consideration in developing the use of entomopathogenic fungi as mycoinsecticides is the selection of effective isolates (Ekesi *et al.*, 2002). The pathogenicity of *M. anisopliae* and *B. bassiana* was affected by host age (Butt and Goettel, 2000). *C. partellus* has six larval instars (Kfir, 1988). However, the effects of *M. anisopliae* and *B. bassiana* against these developmental stages have not been studied. Thus, the goals of the present study were first to determine the mortality of second, third, fourth, fifth and sixth instar larvae of *C. partellus* treated with a range of isolates of *B. bassiana* and *M. anisopliae*; second to quantify the concentration-mortality response of the three most pathogenic isolates against second instar larvae; and third to evaluate *B. bassiana* and *M. anisopliae* using artificially infested maize plant in greenhouse.

MATERIALS AND METHODS

Laboratory experiment

Insects

A laboratory colony of *C. partellus* larvae reared on an artificial diet was used. In order to condition the larvae to natural diet, they were allowed to feed on 4-week old maize leaves for 2 days before application of the fungi.

Fungi

Four *B. bassiana* and six *M. anisopliae* isolates were used (Table 1). Most of the isolates were isolated between 1995 and 2001.

Table 1. Host and country of origin of *Beauveria bassiana* and *Metarhizium anisopliae* isolates used in bioassays against *C. partellus*.

Fungal species	Isolate	Origin	Host
B. bassiana	BB-01	Ethiopia	*Heliothis armigera* (Lepidoptera: Noctuidae)
	BCP-01	South Africa	Unknown (commercial product)
	POCH-01	South Africa	*Chilo partellus* (Lepidoptera: Crambidae)
	POCH-02	South Africa	*Busseola fusca* (Lepidoptera: Noctuidae)
M. anisopliae	PPRC-4	Ethiopia	*Pachnoda interrupta* (Coleoptera: Scarabaeidae)
	PPRC-19	Ethiopia	*P. interrupta* (Coleoptera: Scarabaeidae)
	PPRC-61	Ethiopia	*P. interrupta* (Coleoptera: Scarabaeidae)
	EE-01	Ethiopia	Unidentified Crustacea (Isopoda?)
	MA-01	Ethiopia	*Heliothis armigera* (Lepidoptera: Noctuidae)
	MA-02	Ethiopia	Unknown

Conidia preparation

Conidia were obtained from 3 week old sporulating cultures at 25°C on Sabouraud Dextrose Agar (SDA). The conidia were harvested by scrapping the surface of the culture with a sterile camel hair-brush into a 500 ml glass beaker containing 50 ml sterile distilled water with Tween 80 (0.1% v/v) (Difco™). The conidial suspension was prepared by mixing the solution with a magnetic stirrer for five minutes. It was then adjusted to the desired concentration using an improved heamocytometer. The conidia germination rate was over 90.

Bioassay

Single concentration assays

Twenty second instar *C. partellus* larvae were placed in a sterile Petri dish 9 cm in diameter. The larvae were then treated with one of the fungal suspensions at 1x 10^8 conidia /ml using a Potter's precision laboratory spray tower. Three ml of each fungal isolate was used. Initially, ten fungal isolates (Table 2) were tested against second instar larvae. From the results of this assay, five pathogenic isolates (BB-01, PPRC-4, PPRC-19, PPRC-61 and EE-01) were selected for further assays. The selected isolates were tested against twenty, third, fourth, fifth and sixth instar larvae following the same procedure as described above for the second instar larvae. Twenty larvae of each instar treated with distilled water containing Tween 80 (0.1% v/v) served as controls. First instar larvae were not included due to high mortality encountered.

Petri dishes containing treated and control insects were sealed with masking tape and incubated at 25°C. All treatments and their controls were replicated four times with 20 larvae per replicate. They were arranged in a completely randomized design. The treated insects and controls were provided with maize leaves daily after frass and leaf debris had been removed. Mortality was recorded daily. Dead insects were removed and placed in Petri dishes lined with moist filter paper. Fungal infection was confirmed after observing mycosed cadavers under stereo-microscope.

Statistical analysis

Mortality data were corrected for control mortality (Abbott, 1925). The data were then angular-transformed in order to stabilize the variances. Mortality data for second instar larvae were subjected to a one-way analysis of variance. Student-Newman-Keuls test was used to separate the means. A factorial analysis with five fungal isolates (PPRC-4, PPRC-19, PPRC-61, EE-01 & BB-01) and four larval instars (third, fourth, fifth & sixth) as main effects was performed on the angular transformed mortality data. Student-Newman-Keuls test was used to separate the means. The LT_{50} (lethal time required to kill 50% of the treated insect population) was determined using probit analysis (Throne *et al.*, 1995). A factorial analysis with the five fungal isolates and four larval instars as main effects was performed for LT_{50} data. Student-Newman-Keuls test was used to separate the means.

Multiple concentration assays

An experiment was conducted to determine the concentration-mortality response of some of the most pathogenic isolates, BB-01, PPRC-4 and EE-01. Concentrations of 1.25x10^6, 2.5x10^7, 5x10^7, and 1x10^8 conidia ml^{-1} were applied to second instar larvae following the same procedure as described above for single concentration assays. Twenty larvae were used for each concentration of each isolate and the control, which was treated with distilled water containing Tween 80 (0.1% v/v).

Petri dishes containing treated and control insects were sealed with masking tape and

incubated at 25°C. All treatments and their controls were replicated four times with 20 larvae per replication. They were arranged in a completely randomized design. The treated insects and controls were provided with maize leaves daily after frass and leaf debris had been removed. Mortality was recorded daily. Dead insects were removed and placed in Petri dishes lined with moist filter paper. Fungal infection was confirmed after observing mycosed cadavers under stereo-microscope.

Statistical analysis

The LC_{50} (lethal concentration of conidia required to kill 50% of the treated insect population) was determined using probit analysis with the POLO-PC (LeOra software, Berkeley, CA, USA).

Greenhouse experiment

Seeds of the *C. partellus* susceptible maize variety 'Katumani' were used. Two seeds were planted in plastic pots (16 cm by 30 cm) filled with approximately 5 kg of topsoil. The pots containing the seedlings were kept on greenhouse benches at a photoperiod of 12 h : 12 h light and dark. No fertilizers were added. The plants were watered as needed. Three weeks after plant emergence, the seedlings were thinned to one plant per pot and infested with 20 second instar larvae per plant. Twenty-four hours after infestation, a 5 ml conidia suspension ($2x10^8$ conidia/ml) of each isolate was applied to the leaf whorl of each seedling using a hand sprayer (MATABI 1.5 L). There were six treatments consisting of five fungal isolates and a control. The fungal isolates were PPRC-4, PPRC-19, PPRC-61, EE-01 and BB-01. The control was infested with the larvae but untreated with the fungal isolates. The pots containing the seedlings were arranged in a randomized complete block design with seven replicates per treatment. The plants were sprayed only once. Temperature and humidity in the greenhouse were measured using a thermo-hygrograph. Tunnel length, plants with dead heart and number of larva and pupae, were recorded 6 weeks after plants were treated. After dissecting the stems with a knife tunnel length (cm) was measured, and the number of larvae and pupae per plant were recorded.

Statistical analysis

Tunnel length was expressed as proportion of stem height, and dead heart as percentage of a total sample. Percent reduction in tunnel length, dead heart and number of larvae and pupae were expressed as a percentage of control. Percent dead heart and percent tunnel length were angular-transformed ($\arcsin\sqrt{proportion}$) in order to stabilize the variances. Number of larvae and pupae per plant were $\log_{10}$-transformed. Student-Newman-Keuls Test was used.

RESULTS

Laboratory experiment

Single concentration assays

B. bassiana (BB-01) and *M. anisopliae* (PPRC-4, PPRC-19, PPRC-61 & EE-01) induced the highest mortality (93 to 100%) (Table 2). The LT_{50} values for BB-01, PPRC-4, PPRC-19, PPRC-61 and EE-01 were also shorter (1.7 to 2.6) than for the other isolates.

Table 2. Percent corrected mortality after 7 days and lethal time for 50 % mortality (LT_{50}) of second instar *Chilo partellus* larvae treated with isolates of *Beauveria bassiana* and *Metarhizium anisopliae* at the rate of $1x10^8$ conidia/ml. The chi-square (X^2) and P-values indicate the goodness of fit of the regression model.

Isolate	%mortality ± SE* (after 7 days)	LT_{50} ± SE* (days)	Intercept ± SE	Slope ± SE	X^2	P-value
PPRC-4	100 ± 0.00c	1.70 ± 0.20 a	-1.04 ± 0.33	4.75 ± 1.04	3.93	0.25
BB-01	98.3 ± 1.67 c	1.99 ± 0.20 a	-0.83 ± 0.32	2.94 ± 0.71	9.99	0.01
PPRC-19	98.3 ± 1.67 c	2.31 ± 0.64 a	-1.07 ± 0.34	2.95 ± 0.66	6.82	0.11
PPRC-61	96.7 ± 3.33 c	2.03 ± 0.17 a	-0.52 ± 0.29	1.77 ± 0.50	15.2	0.33
EE-01	93.3 ± 6.67 c	2.61± 0.44 a	-1.26 ± 0.371	3.02 ± 0.73	12.4	0.56
MA-02	50.4 ± 15.3 b	10.23 ± 3.33 b	-2.34 ± 0.64	0.64 ± 2.42	6.41	0.30
POCH-01	23.3 ± 9.83 a	-**	-	-	-	-
BCP-01	12.5 ± 5.81 a	-	-	-	-	-
POCH-02	15.4 ± 2.57 a	-	-	-	-	-
MA-01	13.3 ± 9.43 a	-	-	-	-	-

*Mean ± SE followed by the same letter with in a column are not significant at $P > 0.05$ using Student-Newman-Keuls Test. ** LT_{50} was not determined when mortality was less than 50%.

There were no interaction between isolate and instar for mortality (F = 1.0; DF = 16; P = 0.45). However, there were differences in mortality between third, fourth, fifth and sixth instar larvae (Table 3). Sixth instar larvae suffered the highest mortality (97.5%), while fifth instar larvae suffered the least (41.2%). PPRC-4 and BB-01 induced higher mortality (83%) than the other isolates (64 to 66%).

The LT_{50} values were shortest in third and sixth instar larvae (Table 3). The LT_{50} values for PPRC-4, PPRC-19 and BB-01 were shorter (6.9 to 7.5 days) than those for PPRC-61 and EE-01 (9.1 to 9.2). There was an increase in the LT_{50} from the third instar to the fifth instar and a decrease in the LT_{50} in sixth instar larvae for PPRC-4 and EE-01 (Table 3). However, in the case of PPRC-19, PPRC-61 and BB-01, the LT_{50} increased in the fifth instar. This discrepancy in sensitivity of the fifth instar larvae to the different isolates resulted in the interactions between isolate and instar (F = 4.1; df = 12; P < 0.01).

Multiple concentration assays

The common probit regression line was described by Y = 3.45 + 0.412 (x) (X^2 = 16.4; DF = 11; P = 0.12). The LC_{50} for BB-01 (1443 conidia/ml; 95% fiducial limit = $6.5685 \times 10^{-5} - 9.5794 \times 10^{4}$) was lower than for PPRC-4 (15293 conidia/ml; 95% fiducial limit = $1.0669 \times 10^{-1} - 3.8204 \times 10^{5}$) and EE-01 (31241 conidia/ml; 95% fiducial limit = $8.6707 \times 10^{-1} - 6.0107 \times 10^{5}$). However, there was overlap in fiducial limits between BB-01, PPRC-4 and EE-01. Therefore, the differences in the LC_{50} values were not significant.

Greenhouse experiment

The mean daily temperature and relative humidity in the greenhouse ranged from 10 to 35°C and 30 to 90%, respectively. Fungal treatments caused considerable reduction in stem tunnelling (85-100%), dead heart formation (83-100%) and number of larvae and pupae per plant (78-100%) over the infested control plants. However, there were no differences between the fungal treatments in causing reduction in stem tunnelling, dead heart formation and number of larvae and pupae per plant.

DISCUSSION

The isolates originating from *C. partellus* and *Busseola fusca* were less pathogenic than isolates from *Heliothis armigera* and *Pachnoda interrupta*. In most cases, however, fungal isolates are highly pathogenic to their original host or to a closely related species (Samuels *et al.*, 1989). The fact that this fungal species have wide host ranges might made them to be pathogenic to *C. partellus*. Maniania (1992) reported that *B. bassiana* and *M. anisopliae* infect over 200 species of insects in nine orders.

Table 3. Mean mortality (±SE) and LT_{50} (±SE) of *Chilo partellus* third, fourth, fifth and sixth instar larvae treated with *Beauveria bassiana* and *Metarhizium anisopliae* at the rate of $1x10^8$ conidia/ml 15 days after treatment.

	Mortality				
Isolate	Third	Fourth	Fifth	Sixth	Mean
PPRC-4	91.8 ± 4.8*	82.5 ± 10.3	62.2 ± 9.1	97.5 ± 5.0	83.5 ± 7.5 b
PPRC-19	77.9 ± 7.5	58.2 ± 11.2	33.2 ± 9.4	95.0 ± 5.7	66.1 ± 7.1 a
PPRC-61	82.2 ± 11	45.1 ± 10.6	35.8 ± 13.1	100 ± 0.00	65.7 ± 8.2 a
EE-01	78.0 ± 7.7	62.6 ± 9.2	21.7 ± 7.8	97.5 ± 5.0	64.9 ± 7.8 a
BB-01	94.4 ± 5.5	85.6 ± 5.7	53.1 ± 7.7	97.5 ± 5.0	82.6 ± 5.2 b
Mean	84.9 ± 8.5 c	66.8 ± 6.2 b	41.2 ± 9.3 a	97.5 ± 6.3 d	
			LT_{50}		
PPRC-4	5.4 ± 0.8*	8.4 ± 0.7	8.9 ± 1.2	5.2 ± 1.4	6.9 ± 1.3 a
PPRC-19	6.8 ± 0.6	10.8 ± 3.2	7.8 ± 2.9	4.7 ± 0.2	7.5 ± 2.1 a
PPRC-61	6.0 ± 1.5	15.1 ± 2.3	11.3 ± 4.2	4.6 ± 0.9	9.2 ± 1.1 b
EE-01	7.7 ± 0.6	10.2 ± 3.7	13.5 ± 1.5	4.9 ± 0.2	9.1 ± 2.3 b
BB-01	6.1 ± 1.5	5.6 ± 0.9	11.2 ± 1.6	4.8 ± 0.1	6.9 ± 1.4 a
Mean	6.4 ± 0.3 b	10.0 ± 1.9 c	10.2 ± 0.6 c	4.8 ± 0.3 a	

*Mean ± SE followed by the same letter within a column (isolate mean) and a raw (instar mean) are not significant at P > 0.05 using Student-Newman-Keuls Test.

Another possible explanation could be attributed to the habitat of the host insects. Both *C. partellus* and *P. interrupta* prefer similar habitat, warm and dry environments. Therefore, fungal isolates, from insect species occupying similar habitat might be pathogenic to both species. There is increasing evidence that habitat selection, not insect host selection, drives the virulence of *B. bassiana* and *M. anisopliae* (Bidochka *et al.*, 2001). Thus, results from this study indicate that screening of potential isolates should not be limited to those isolated from the original host.

Second and sixth instar larvae of *C. partellus* were the most susceptible stages to infection by *B. bassiana* and *M. anisopliae* isolates. Early instars of *Spodoptera littoralis* were more susceptible than late instar larvae to infection by *Nomurae riley* and *Paecilomyces fumosoroseus* (Fargues and Rodriguez-Rueda, 1980), while first and fifth instar larvae were the most susceptible stages of European corn borer *Ostrinia nubilalis* to *B. bassiana* infection (Feng *et al.*, 1985). In contrast, first and second instar larvae of *Heliothis zea* were less susceptible to *Nomurae riley* than the third and fourth instars (Mohamed *et al.*, 1977). The sixth instar larvae of *C. partellus* are the diapausing stage (Kfir, 1988). Thus, the sixth instar will not cast the conidia through molting before infection occurs. Van Den Berg *et al.* (1998) reported that Colorado potato beetle (*Lepitinotarsa decemlineata*) escaped *B. bassiana* infection by casting the conidia with their exoskeleton at the time of molting.

Application of aqueous suspension of the fungal isolates using conventional insecticide application methods may play a strategic role in the management of *C. partellus*, provided applications are made sufficiently early to target young larvae feeding in the leaf whorl. The critical period for control of *C. partellus* is the first two weeks after hatching when the first, second and third instar larvae feed externally on maize leaves. Once inside the stem (fourth, fifth and sixth instar larvae) the larvae may not be infected by pathogen applications. However, further mortality may occur through previous infection by pathogens consumed on the leaves, or dispersal of the pathogen from infected cadavers. Thus, adult and larval activity will require intensive scouting in maize plantings for timing spray applications.

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