academicJournals

Vol. 8(30), pp. 4157-4161, 8 August, 2013
DOI: 10.5897/AJAR11.2296
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Studies on the virulence of different isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metcsn.) Sorokin against Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

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Accepted 8 August, 2013

Virulence of ten fungal isolates of entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metcsn.) Sorokin against third instar larvae of Mediterranean flour moth, *Ephestia kuehniella* Zeller, was tested under laboratory conditions. These isolates were originated from soil by using *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) as a live insect bait. All isolates were inoculated by immersing the insects in 10 ml of a fungal suspension for 5 s. The experiments carried out with 3 replications and 10 larvae were used for each replication.Cumulative mortality after treatment varied from 11 to 92% in *M. anisopliae* and in *B. bassiana* from 17 to 88%. The increase of dose and exposure interval increased mortality. LC_{50} values ranged from 8.3×10^5 to 6.5×10^6 for *B. bassiana* and from 5.4×10^7 to 3.4×10^8 for *M. anisopliae*. Among the isolates evaluated in the study, C-IIA7 isolate of *B. bassiana* had the lowest LC_{50}.The LT_{50} values for these isolates varied from 107 to 154 h for *B. bassiana* and from 93 to 162 h for *M. anisopliae*, whereas, B-VM1 isolate of *M. anisopliae*, recorded the least LT_{50} of 93 h. Our results demonstrated that the entomopathogenic fungi could be used as an alternative for the control of stored products pests in IPM programs.

Key words: Virulence, *Beauveria bassiana*, *Metarhizium anisopliae*, *Ephestia kuehniella*, immersion bioassay.

INTRODUCTION

Storage of grains is part of the post-harvest system through which food material passes on its way from field to consumer. It is generally accepted that 5 to 15% of the total weight of all cereals is lost after harvest (Padin et al., 2002). Mediterranean flour moth, *Ephestia kuehniella* is one of the most important insect pests infesting stored grain of many cereals throughout the world. The continuous use of chemical insecticides such as pirimiphos- Methyl for control of pests has resulted in serious problems such as resistance, pest resurgence, elimination of beneficial insects and toxicity to humans and wildlife (Hendrawan and Ibrahim, 2006). These problems and the demand for pesticide-free foods have triggered efforts to find alternative management options.

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Table 1. Source and germination percent of *B. bassiana* and *M. anisopliae* isolates used in the study.

Isolates	Source	Location	Country	Germination (%)
B. bassiana				
C-IIIA8	Soil	Maragheh-Tabriz	Iran	93
C-IIA7	Soil	Maragheh-Tabriz	Iran	90
B-IVA8	Soil	Maragheh-Tabriz	Iran	90
B-VA6	Soil	Maragheh-Tabriz	Iran	86
C-IA2	Soil	Maragheh-Tabriz	Iran	93
M. anisopliae				
C-IIIM14	Soil	Maragheh-Tabriz	Iran	91
C-IM5	Soil	Maragheh-Tabriz	Iran	85
B-IIM9	Soil	Maragheh-Tabriz	Iran	87
C-IVM4	Soil	Maragheh-Tabriz	Iran	86
B-VM1	Soil	Maragheh-Tabriz	Iran	87

Entomopathogenic fungi offer an alternative management strategy to chemical control. The potential of entomopathogenic fungi to control insect pests of stored products has been evaluated in several studies in recent years (Adane et al., 1996; Bischoff and Reichmuth, 1997; Kassa et al., 2002; Cherry et al., 2005; Wakefield et al., 2005; Draganova and Markova, 2006). In this study the potential ten indigenous isolates of *B. bassiana* and *M. anisopliae* for the control of *E. kuehniella* were investigated under laboratory conditions.

MATERIALS AND METHODS

Insect rearing

The initial *E. kuehniella* eggs were obtained from the laboratory of Plant Protection Department, University of Tabriz. Insects were reared on wheat meal. Meal was incubated at 60°C for 24 h to eliminate unwanted organisms. Boxes (25×10×7 cm) containing 0.2 g eggs and 1 kg of meal were maintained under the laboratory conditions (28±2°C, 65±5% RH) with a natural photoperiod. After 2-3 weeks of (incubation) third instar larvae were used in the experiments.

Fungal isolates

Ten isolates of *B. bassiana* and *M. anisopliae* were used in this study. All isolates were obtained from soil by using *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) as a live insect bait (Bedding and Akhurst 1975; Zimmermann, 1986) (Table 1).

Preparation of conidial suspensions and conidial germination

In order to complete fungal sporulation, all the isolates were cultured on Sabouraud Dextrose Agar (10% neopeptone, 40% dextrose, 15% agar) with 0.25% wt:vol yeast extract (SDAY) and incubated at 24°C for 14 days. Fungal conidia were harvested in 2.5% Tween 80 and suspended in 5 ml sterile distilled water. Number of conidia was determined using a haemocytometer (Liu et al., 2002). Serial dilutions (seven spore concentrations) of each fungal isolate were performed to obtain the desired concentrations for each experiment. Viability of conidia was determined by spreading a drop of conidial suspensions onto the surface of glass slides held in Petri dishes lined with moistened sterile filter paper. Three glass slides per isolate representing three replicates were used and scored for germination after 24 h at 25±2°C. Conidia with germ tubes equal or greater than the width were considered to have germinated.

Bioassay

Ten third instar *E. kuehniella* larvae were inoculated by immersion for 5 s in 10 ml of conidial suspension of each isolate as described by Butt et al. (1994). Control insects were treated as above, but without fungus to confirm spore viability and virulence. The fungal suspension was drained off through a tea strainer (4 cm diameter) and treated insects were transferred to a Petri dish (9 cm) using a hair brush. Each assay replicated thrice. The treated larvae were starved for 24 h and then were fed with little wheat meal as food. All treated insects were kept at 26°C and 70±5% R.H. for 10 days. Mortality was noted daily and dead insects were kept separately in humid sterile Petri dishes for another 10 days to examine the evidence of fungal infection.

Statistical analysis

Cumulative mortality counts obtained from experiments were corrected for natural mortality using Abbott's formula (Abbott, 1925). Data were analyzed using a Genstat statistical package (Genstat 2002) and means were separated using the Duncan's Multiple range test at $p = 0.05$. From the mortality data, 50% lethal time (LT_{50}) and 50% lethality concentration (LC_{50}) values were calculated by probit analysis test (SAS, 2000).

RESULTS

The virulence of *B. bassiana* and *M. anisopliae* isolates against third instar larvae of *E. kuehniella* under laboratory conditions were statistically different ($p = 0.002$, $F = 9.43$, $gl = 1$) (Tables 2 and 3). Mortality rates

Table 2. Cumulative percent mortality (corrected mortality* ± SE) of *E. kuehniella* larvae 10 days after treatment by *B. bassiana.*

Isolates	Concentrations (Conidia/ml)						
B. bassiana	**1×108**	**5×107**	**1×107**	**5×106**	**1×106**	**5×105**	**1×105**
C-IIIA8	88±3.3[a]	82±2.2[a]	70±3.1[a]	63±1.8[b]	58±2.2[a]	47±1.8[a]	35±1.6[a]
C-IIA7	88±1.6[a]	82±3.3[a]	70±3.1[a]	58±1.6[c]	41±1.8[c]	29±1.6[c]	17±1.4[d]
B-IVA8	82±1.8[b]	64±2.4[c]	58±2.2[b]	47±1.6[d]	41±1.8[c]	35±1.6[b]	29±1.6[b]
B-VA6	82±1.6[b]	70±1.6[b]	58±2.2[b]	47±1.6[d]	35±1.6[d]	23±1.3[d]	17±1.3[d]
C-IA2	82±2.5[b]	82±1.6[a]	70±2.1[a]	64±1.8[a]	47±1.8[b]	35±1.6[b]	23±1.3[c]

*Corrected mortality by Abbott's method (1925); [#]Means followed by the same letter in a column are not significantly different (P<0.05) by DMRT (Duncan's Multiple range tested).

Table 3. Cumulative percent mortality (corrected mortality* ± SE) of *E. kuehniella* larvae 10 days after treatment by *M. anisopliae.*

Isolates	5×10^9	1×10^9	5×10^8	1×10^8	5×10^7	1×10^7	5×10^6
M. anisopliae							
C-IIIM14	92±3.1[a]	76±2.2[a]	64±1.8[a]	44±1.6[b]	40±1.3[b]	32±1.1[b]	20±1.1[b]
C-IM5	76±2.2[d]	70±1.8[b]	58±1.8[b]	47±1.6[a]	35±1.3e	17±1.1e	11±1.1e
B-IIM9	82±2.2[c]	76.2±[a]	58±1.8[b]	47±1.6[a]	41±1.6[a]	35±1.3[a]	29±1.3[a]
C-IVM4	72±2.2e	61±1.6[c]	55±1.8[c]	44±1.6[b]	38.3±[d]	27±1.3[d]	16±1.1[d]
B-VM1	88±2.2[b]	76±1.6[a]	58±1.8[b]	41.6±[c]	35±1.3[c]	29±1.1[c]	17±1.1[c]

*Corrected mortality by Abbott's method (1925); [#] Means followed by the same letter in a column are not significantly different (P<0.05) by DMRT (Duncan's Multiple range tested).

Table 4. Probit analyses of concentration-mortality responses of third instar larvae of *E. kuehniella* to *B. bassiana.*

Isolates	LC_{50} (Conidia/ ml)	Intercept(a) ±SE	Slope (b) ± SE	χ^2	LC_{90} (Conidia/ ml)	LC_{30} (Conidia/ ml)
B. bassiana						
C-IIIA8	8.3×10^5 (4.4×10^5-1.3×10^6)	2.8±0.35	0.5±0.05	1.69	2.7×10^8	7.8×10^4
C-IIA7	2.8×10^6 ($2\times.10^6$-4×10^6)	4.9±0.4	0.7±0.06	0.88	1.3×10^8	5.9×10^5
B-IVA8	4.6×10^6 (2.7×10^6-7.7×10^6)	3.1±0.3	0.46±0.05	6.7	2.6×10^9	3.4×10^5
B-VA6	6.5×10^6 (4.4×10^6-9.6×10^6)	4.5±0.4	0.6±0.06	2.3	5.5×10^8	1×10^6
C-IA2	2.2×10^6 (1.4×10^6-3.3×10^6)	3.7±0.3	0.58±0.05	3.2	3.4×10^8	2.7×10^5

*All lines are insignificant at p<0.05. [#]Figures in parenthesis shows upper and lower fiducial limits.

increased according conidial concentration. Germination of all the *B. bassiana* and *M. anisopliae* isolates tested in the study ranged from 86 to 93% (Table 1). No fungal infection was detected in the control treatments. The larvae were highly susceptible to all the isolates in higher concentrations (Tables 2 and 3). The first mortalities recorded at first day post-infection in higher concentrations. *B. bassiana* isolates caused more than 80% mortality and LT_{50} values of 125 to 135 hours. The isolates of *M. anisopliae* showed more than 70% mortality and LT_{50} values varied from 93 to 160 h (Tables 2 to 5). Besides, among the isolates of *B. bassiana* evaluated in the study, C-IIA7 had the lowest LT_{50} of 107 hours whereas, B-VM1 isolate of *M. anisopliae*, recorded the least LT_{50} of 93 h (Table 6).

DISCUSSION

One of the key elements of Insect Pest Management (IPM) in stored-products is the combination of several, reduced risk control methods, because storage pests are not always effectively controlled by the application of only one measure (Kavallieratos et al., 2006). Besides, several studies documented that entomopathogenic fungi *B. bassiana* and *M. anisopliae*can be used with success against stored product insect pests and some products are already available commercially (Ekesi et al., 2001).

Table 5. Probit analyses of concentration-mortality responses of third instar larvae of *E. kuehniella* to *M. anisopliae.*

Isolates	LC_{50} (Conidia/ ml)	Intercept(a) ± SE	Slope(b) ± SE	χ^2	LC_{90} (Conidia/ ml)	LC_{30} (Conidia /ml)
M. anisopliae						
C-IIIM14	5.4×10^7 (5.3×10^6-3.2×10^7)	3.9±0.7	0.6±0.07	2.2	2.9×10^9	7.8×10^6
C-IM5	3×10^8 (1.4×10^8-6.7×10^8)	6.2±0.8	0.7±0.09	12.1	1×10^{10}	5.8×10^7
B-IIM9	7.8×10^7 (1.2×10^7-4.7×10^8)	4.1±0.4	0.5±0.05	1.9	2.1×10^{10}	7.8×10^6
C-IVM4	3.4×10^8 (2.1×10^8-5.9×10^8)	4.3±0.4	0.5±0.05	2.1	1×10^{11}	3.2×10^7
B-VM1	1.7×10^8 (1.2×10^8-2.5×10^8)	5.8±0.5	0.7±0.06	6.6	1×10^{10}	3.2×10^6

*All lines are insignificant at $p<0.05$; #Figures in parenthesis shows upper and lower fiducial limits.

Table 6. Probit analyses of time-mortality responses of third instar larvae of *E. kuehniella* to *B. bassiana* and *M. anisopliae**

Isolates	LT_{50} (h)	Fiducial limits (95%)		χ^2	Slope(b) ± SE	Intercept(a) ± SE
		Upper (h)	Lower (h)			
B. bassiana						
C-IIIA8	135.16	113.06	158.26	158.26	4.2±0.6	8.9± 1.4
C-IIA7	107.07	96.44	117.94	117.94	2.1±0.2	4.2±0.3
B-IVA8	128.59	117.29	140.99	140.99	2.2± 0.2	4.7±0.4
B-VA6	154.26	132.09	184.98	184.98	2.1±0.3	5.1±0.6
C-IA2	151.67	138.08	168.04	168.04	2.2±0.2	4.8±0.4
M. anisopliae						
C-IIIM14	98.73	79.64	117.65	117.65	2.5±0.3	4.9±0.6
C-IM5	131.26	119.2	144.68	144.68	2.1±0.2	4.5±0.4
B-IIM9	135.32	123.09	149.2	149.2	2.1±0.2	4.6±0.4
C-IVM4	162.72	149.34	179	179	2.4±0.2	5.5±0.4
B-VM1	93.92	65.27	120.61	120.61	1.7±0.3	3.3±0.6

*The fungal concentration for *B. bassiana* was 1×10^8 Conidia/ml and for *M. anisopliae* was 5×10^9 Conidia/ml. #All lines are insignificant at $p<0.05$.

Other studies on the effects of fungi on storage pests have been equally encouraging Various isolates of *M. anisopliae* and *B. bassiana* were found to give good control of *Plodia interpunctella* (Hübner), and *E. kuehniella* (Zeller) (Bischoff and Reichmuth, 1997). Rice and Cogburn (1999) reported up to 80 to 100% mortality at 21 days post-treatment in *S. oryzae*, *R. dominica* and *Triboliumcastaneum* (Herbst) following treatment with *B. bassiana*. In the present study, isolates of *B. bassiana* and *M. anisopliae*, showed variations in their virulence. Our results indicated that all the isolates were pathogenic to *E. kuehniella* larvae. Wakefeild et al. (2005) reported that some *B. bassiana* isolates provided 100% mortality in *E. kuehniella* 10 days after treatment at 1×10^8 conidia ml^{-1}. Another study with four isolates of *B. bassiana*, two *M. anisopliae* isolates, and one isolate of *Verticillium lecanii* have been proved pathogenecity against larvae of *E. kuehniella* (Draganova and Markova, 2006). Isolates 15 and 31 Ma of *M. anisopliae* and the isolate 32Vl of *V. lecanii* showed less lethal effect in comparison with the other tested isolates. Our results showed high susceptibility of *E. kuehniella* larvae to *M. anisopliae* and *B. bassiana*. Our results are according with those which suggested that dry conidia of *B. bassiana* and *M. anisopliae* could provide a novel alternative to chemical insecticides for the management of pests. However, further experiments are needed to screen more virulent isolates for biocontrol of storage pests.

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