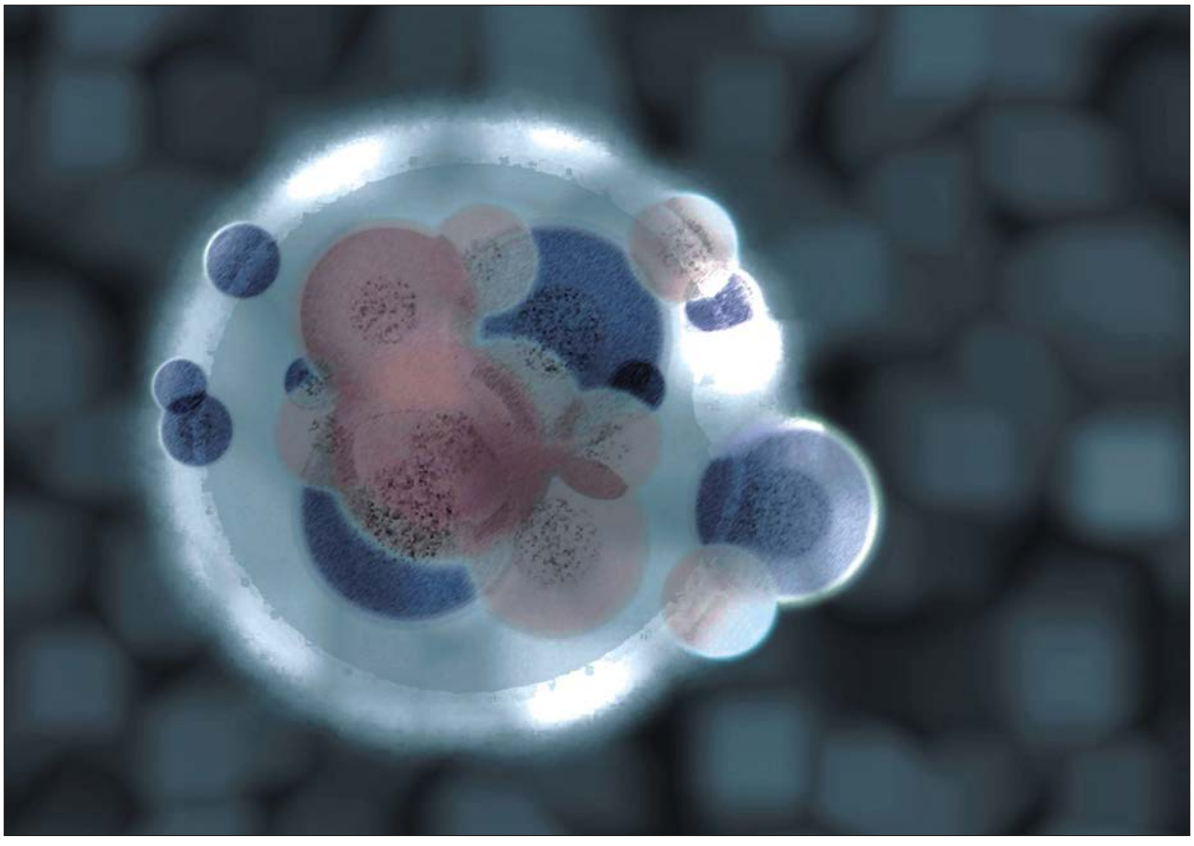

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Study on the Efficacy of Iranian Isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin Against *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae)

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Abstract: Laboratory bioassays were conducted in order to evaluate the efficacy of Iranian isolates of entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin against adults of the lesser grain borer, *Rhyzopertha dominica* (F.) on stored wheat. All the isolates tested were pathogenic to the beetle although mortality rates were different between them. The cumulative mortality after treatment varied from 14.78% in *M. anisopliae* DEMI001 at low concentration (1.5×10^4 conidia mL^{-1}) to 89.35% in *B. bassiana* Iran 441C at the highest concentration (1×10^{10} conidia mL^{-1}). Probit analysis showed that the lowest LC_{50} values were 9.6×10^5 and 1.9×10^7 (conidia mL^{-1}) for *B. bassiana* Iran 187C and *M. anisopliae* DEMI001, respectively. The values of LT_{50} varied from 6.77 to 9.28 days for *B. bassiana* isolates, and from 7.48 to 8.25 days for *M. anisopliae* isolates.

Key words: Biological control, *Beauveria bassiana*, *Metarhizium anisopliae*, *Rhyzopertha dominica*, 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 5-15% of the total weight of all cereals, oilseeds and pulses is lost after harvest (Padin *et al*., 2002). The lesser grain borer, *Rhyzopertha dominica* F. is one of the most important insect pests infesting stored grain of many cereals and legumes throughout the world. Both adults and larvae attack grain and can cause substantial damage to unprotected grain. Losses due to this pest have been estimated at 15% or more of total grains stored each year (Batta, 2005).

Control of *R. dominica* and other stored-grain insect pests mostly relies on the use of chemical insecticides such as chlordane, lindane and resmethrin and the fumigants methyl bromide and phosphine (Lorini and Galley, 1999; Zettler and Arthur, 2000; Batta, 2005). The continuous use of chemical insecticides for control of pests has resulted in serious problems such as resistance to the insecticides, pest resurgence, elimination of economically 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. Entomopathogenic fungi offer an alternative management strategy to chemical control. The potential of entomopathogenic fungi to control insect pests of stored products particularly Coleopteran insects has been evaluated in several studies in recent years (Adane *et al*., 1996; Kassa *et al*., 2002; Cherry *et al*., 2005). Mixtures of *Metarhizium anisopliae* conidial suspensions with those of *Beauveria bassiana* have been reported to be effective against *S. oryzae* on wheat grains (Batta and Abu Safieh, 2005).

In this study the potential of five indigenous isolates of *B. bassiana* and *M. anisopliae* for the control of *R. dominica* were investigated under laboratory conditions.

MATERIALS AND METHODS

Insect rearing: The initial stock of *R. dominica* was obtained from the Department of Plant Protection, Faculty of Agriculture, Urmia University. Adults of *R. dominica* were reared on dried, healthy and mature wheat grains, Zarin variety. Grains of Zarin variety were stored at -5°C for 1 week to eliminate natural unwanted infestations. Two hundred adults of *R. dominica* of mixed sexes and different ages were introduced in each jar (15 cm diameter and 20 cm height) containing 400 g of wheat grain and were maintained under the laboratory conditions (28±2°C, 65±5% R.H.) with a natural photoperiod. The jars were

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Table 1: Host, origin and germination of *Beauveria bassiana* and *Metarhizium anisopliae* isolates used in the present study

Isolates	Host (Order: Family)	Location	Country	Germination (%)
B. bassiana				
Iran 187C	*Leptinotarsa decemlineata* (Col.: Chrysomelidae)	Ardebil	Iran	80.00±3.62
Iran 429C	*Chilo supperssalis* (Lep.: Pyralidae)	Gilan-Rasht	Iran	86.97±2.26
Iran 441C	*Rhynchophorus ferrugineus* (Col.: Curculionidae)	Saravan	Iran	90.14±0.69
M. anisopliae				
Iran 715 C	Locust (Orthoptera: Acrididae)	Ahvaz	Iran	81.15±6.35
DEMI001	*Rhynchophorus ferrugineus* (Col.: Curculionidae)	Saravan-Balochestan	Iran	94.00±2.20

covered with cheese cloth fastened by rubber bands. The original adults were removed from the jars after two weeks of infestation. Newly emerged adults (males and females) were used in the experiments.

Fungal isolates: Three Iranian isolates of *B. bassiana* and two isolates of *M. anisopliae* were obtained from the fungal culture collection maintained by the Plant Pest and Diseases Research Institute, Tehran, Iran. Detail information's about the isolates are given in Table 1.

Preparation of conidial suspensions: Fungal isolates were cultured on Potato Dextrose Agar (PDA) in 8 cm diameter Petri dishes and were incubated under dark conditions at 25°C for 14 days for complete sporulation. A mixture of conidia and hyphae was harvested by flooding the Petri dishes with sterile distilled water containing 0.05% (v/v) Tween 80 and agitating with a glass rod. All samples were vortexed for 3 min to break up the conidial chains or clumps. Conidia were separated from hyphae and substrate materials by filtration of the suspension through five layers of cheese-cloth. The concentrations of fungal conidia in suspension were determined using a haemocytometer (Improved Neubauer, 0.1 mm depth). 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: After preliminary assays, 5 different conidial concentrations were prepared in sterile distilled water containing Tween 80 (0.05% v/v) based on the logarithmic series. For each replicate, thirty (7-14 day old) *R. dominica* adults were inoculated by immersing them for 5 sec in 5 mL of conidial suspension. Control insects were treated using sterile distilled water containing Tween 80 (0.05% v/v).

The treated insects were transferred into petri dishes containing sterile filter paper (9 cm diameter) and sealed with parafilm to prevent them from escaping. The filter paper helped to absorb excess moisture and increased conidial load in each insect by allowing secondary conidia growth (Adane *et al*., 1996). The treated insects were starved for 24 h then transferred into glass pots (7 cm diameter and 8.5 cm height) with perforated lids containing 30 g wheat grains and kept at 27±1°C and 70±5% R.H. for 14 days. The experiment was arranged in a completely randomized design with four replications. Mortality was counted for 14 days. Dead insects from each treatment were washed three times in sterile distilled water and kept separately in Petri dishes. The dishes were then incubated in a plastic box with high R.H. (approximately 100%) to observe the outgrowth of fungus.

Statistical analysis: Cumulative mortality counts obtained from experiments were corrected for natural mortality using Abbott's formula (Abbott, 1925) and were normalized using arcsine transformation prior to analysis. Data were statistically analyzed using ANOVA (SAS, 2000) and means were separated using the Duncan's Multiple range test at $p = 0.05$. Probit analysis was used to estimate both LC_{50} and LC_{95} of the isolates with 95% Confidence Limits (CL) as well as LT_{50} values (SPSS, 1999).

RESULTS

Germination of all *B. bassiana* and *M. anisopliae* isolates tested varied from 80 to 94% (Table 1). All the isolates tested were pathogenic to adults of *R. dominica* in immersion bioassays, but they had different virulence. Mortality rates increased with increasing conidial concentration (*B. bassiana* Iran 187C: $F = 66.54$, $p<0.0001$), (*B. bassiana* Iran 429C: $F = 64.95$, $p<0.0001$), (*B. bassiana* Iran 441C: $F = 80.67$, $p<0.0001$), (*M. anisopliae* DEMI001: $F = 79.40$, $p<0.0001$) (*M. anisopliae* Iran 715C: $F = 63.66$, $p<0.0001$). The *B. bassiana* 441C and *M. anisopliae* DEMI001 isolates were the most virulent isolates (Table 2). The maximum and minimum percentage of mortality observed in *M. anisopliae* DEMI001 and *B. bassiana* Iran 441C isolates, respectively. The lowest LC_{50} value was observed in *B. bassiana* Iran 187C isolate. The LT_{50}

Table 2: Cumulative percentage mortality (corrected ± SE) of *Rhyzopertha dominica* adults 14 days after immersion in aqueous conidial suspensions of *Beauveria bassiana* and *Metarhizium anisopliae* isolates

Isolates	Concentration (conidia mL^{-1})				
B. bassiana					
Iran 187C	1.4×10^{3}	3.3×10^{4}	7.9×10^{5}	1.8×10^{7}	4.4×10^{8}
	19.82±1.65^{e}	29.30±2.22^{d}	39.65±2.22^{c}	47.40±1.65^{b}	69.82±2.94^{a}
Iran 429C	19.82±1.65^{e}	29.30±2.22^{d}	39.65±2.22^{c}	47.40±1.65^{b}	69.82±2.94^{a}
	29.05±1.63^{e}	34.18±3.13^{d}	52.98±3.56^{c}	69.22±3.11^{b}	84.61±2.96^{a}
Iran 441C	5.6×10^{6}	3.7×10^{7}	2.3×10^{8}	1.5×10^{9}	1.1×10^{10}
	30.97±2.28^{e}	46.01±2.22^{d}	61.94±2.22^{c}	72.56±3.64^{b}	89.35±1.44^{a}
M. anisopliae					
Iran 715C	3.3×10^{4}	7.9×10^{5}	1.8×10^{7}	2×10^{8}	4.4×10^{9}
	21.92±2.20^{e}	31.57± 2.26^{d}	48.23± 2.20^{c}	59.99± 2.43^{b}	75.43 ±3.20^{a}
DEMI001	1.5×10^{4}	3.5×10^{5}	8.3×10^{6}	2×10^{8}	4.6×10^{9}
	14.78±2.24^{e}	32.17±2.24^{d}	44.34±2.45^{c}	58.25±3.17^{b}	79.99±2.96^{a}

Means followed by the same letter in the row are not significantly different (Duncan's Multiple range test at p = 0.05). Cumulative mortality data were corrected for natural mortality using Abbott's formula and normalized using arcsine transformation before analysis

Table 3: LC_{50} and LC_{95} (conidia mL^{-1}) values with 95 fiducial limits and probit analysis parameters for adults of *Rhyzopertha dominica*

			Probit parameters±SE			
Isolates	LC_{50} (95% CL)	LC_{95} (95% CL)	Intercept (a)	Slope (b)	χ^2-value	p-value
B. bassiana						
Iran 187C	9.6×10^{5}	1×10^{14}	3.42±0.189	0.220±0.0227	1.790	0.616
	(3×10^{6}-3.9×10^{7})	(3.3×10^{12}-1×10^{16})				
Iran 429C	2.2×10^{7}	1.1×10^{11}	1.75±0.373	0.442±0.048	0.787	0.852
	(1.2-3.9×10^{7})	(2.6×10^{10}-1.1×10^{12})				
Iran 441C	5.7×10^{7}	1.2×10^{11}	1.16±0.448	0.490±0.053	2.230	0.525
	(3-9.9×10^{7})	(3.5×10^{10}-7.6×10^{11})				
M. anisopliae						
Iran 715C	3.2×10^{7}	4×10^{13}	2.98±0.236	0.269±0.026	0.305	0.959
	(1.2-8.8×10^{7})	(2.5×10^{12}-2×10^{15})				
DEM I001	1.9×10^{7}	2.8×10^{12}	2.68±0.233	0.310±0.031	2.010	0.570
	(8.6×10^{6}-4.6×10^{7})	(3.3×10^{11}-6.5×10^{13})				

Table 4: LT_{50} values in days with 95 fiducial limits following immersion of *Rhyzopertha dominica* adults in aqueous suspensions of *Beauveria bassiana* and *Metarhizium anisopliae* isolates

			95% fiducial limits	
Isolates	Concentration (conidia mL^{-1})	LT_{50} (days)	Lower	Upper
B. bassiana				
Iran 187C	4.4×10^{8}	9.28	8.47	10.33
Iran 429C	2.2×10^{9}	7.27	6.74	7.87
Iran 441C	1.1×10^{10}	6.77	4.98	9.32
M. anisopliae				
Iran 715C	4.4×10^{9}	8.25	6.71	10.77
DEM I001	4.6×10^{9}	7.48	6.84	8.24

values for *B. bassiana* isolates varied from 6.77 to 9.28 days, with an average of 7.77 days, while those for *M. anisopliae* isolates varied from 7.48 to 8.25 days with an average of 7.86 days (Table 3). Among *B. bassiana* isolates, Iran 441C had the shortest LT_{50} of 6.77 days and among *M. anisopliae* isolates, DEMI001 had the shortest LT_{50} of 7.48 days (Table 4).

DISCUSSION

On the other hand, 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). However, 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; Ferron *et al*., 1991).

The results of the present study indicated that although all tested isolates were pathogenic and caused mortality in *R. dominica*, they had different virulence. *B. bassiana* Iran 441C caused significantly higher mortality (89.35%) than the other isolates while *M. anisopliae*, DEMI001 isolate caused high mortality (79.99%) at the highest conidia concentration (4.6×10^{9} conidia mL^{-1}).

Our results for LT_{50} indicated that the present isolates are not effective as much as other isolates cited in literatures. For instance, Batta (2005) reported that high mortalities of adult *R. dominica* were obtained 7 days after treatment of newly emerged adults with *M. anisopliae* conidia. Wakefield *et al*. (2005) reported that some *B. bassiana* isolates provided 100% mortality in *Oryzaephilus surinamensis* L., *Ephestia kuehniella* (Zeller) and *Acarus siro* L. 10 days after treatment in 1×10^{8} conidia mL^{-1}. Unformulated conidia of

M. anisopliae isolate MaPs and *B. bassiana* isolates BbPs and BbGc at the rate of 0.15 g a.i. to 50 g a.i. rice grain caused mortality ranging from 77.5- 90% in adults of *Sitophilus oryzae* (Hendrawan and Ibrahim, 2006). Rodrigues and Pratissoli (1990) reported 6 months protection of maize and bean grains from damage by *Sitophilus zeamais* (Motsch) and *Acanthoscelides obtectus* (Say) following treatment with *B. brongniartii* (Sacc.) Petch. and *M. anisopliae* at a dose of 1×10^{8} conidia mL^{-1}. Cherry *et al.* (2005) also have demonstrated that different isolates from *M. anisopliae* and *B. bassiana* can provide good control of *Callosobruchus maculatus* F. by immersion bioassay. On the contrary, other investigators have reported that treatment of *S. oryzae* on wheat grains with *M. anisopliae* alone was not effective, but that 50% adult mortality of the pest was achieved 30 days after treatment with a conidial suspension mixture of *M. anisopliae* and *B. bassiana* (Dal-Bello *et al.*, 2001). The difference in *M. anisopliae* effectiveness obtained in the above studies may be attributed to differences in aggression of the fungal strains used, host susceptibility, experimental conditions and food substrate used (Batta, 2005). Among the isolates tested, *M. anisopliae* Iran 715C and *B. bassiana* Iran 187C caused lower mortality that may be attributed to their low percentage of conidia germination (Table 1).

In conclusion, our research showed high susceptibility of adult of *R. dominica* to *B. bassiana* and *M. anisopliae*. However, further experiments are needed to be carried out for screening for more virulent isolates for biocontrol of storage pest.

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