

Virulence of *Metarhizium anisopliae* on *Callosobruchus maculatus* (F.) (Col.: Bruchidae) larvae in stored cowpea

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Abstract

Virulence of the entomopathogenic fungus; *Metarhizium anisopliae* (isolates: M14 and DEMI001) was studied against third instar larvae of *Callosobruchus maculatus* (F.) (Col.: Bruchidae) by immersion method. For each isolates, 5 aqueous suspensions (10^4 - 10^8 conidia/ml) were prepared using Tween 80 (0.02% v/v). Results revealed that third instar larvae of this bruchid pest were susceptible to both isolates of *M. anisopliae*. The mortality percents of larvae increased with increasing conidial concentration. The cumulative mortality percent of *C. maculatus* larvae ranged from 6.67-96.67% and 3.33-93.33% for M14 and DEMI001 isolates at different conidial concentration, respectively, 10 days after inoculation. Probit analysis demonstrated overlapping of 95% confidence limits of LC_{50} and LC_{95} and significant differences weren't observed among two isolates. The corresponding LC_{50} values were 1.15×10^6 and 1.63×10^6 (conidia/ml) for M14 and DEMI001 isolates, respectively, while the LT_{50} values were 5.45 and 6.68 days. The mean comparisons for cumulative mortality percent at different conidial concentrations indicated that the mortality rates were not affected significantly by different fungus isolate at any conidial concentration. The results of this study highlighted the importance of these M14 and DEMI001 isolates for the biological control of *C. maculatus*.

Key words: virulence, *Metarhizium anisopliae*, *Callosobruchus maculatus*, biological control, LC_{50}

چکیده

قدرت بیماری‌زایی قارچ *Metarhizium anisopliae* روی لاروهای *Callosobruchus maculatus* (F.) (Col.: Bruchidae) در لویبای چشم‌بلی انبارشده آزاده جراحی و سید علی صفوی

قدرت بیماری‌زایی دو جدایه M14 و DEMI001 از قارچ *Metarhizium anisopliae* روی لاروهای سن سوم سوسک چهار نقطه‌ای حیوانات به روش غوطه‌وری مورد مطالعه قرار گرفت. سوسپانسیون‌های قارچی در ۵ غلظت (10^4 - 10^8 کنیدی بر میلی لیتر) با استفاده از توئین ۸۰ (۰/۰۲ درصد) تهیه گردید. نتایج حاکی از حساسیت لاروهای سن سوم سوسک چهار نقطه‌ای حیوانات به دو جدایه قارچی مورد استفاده بود. افزایش غلظت سوسپانسیون قارچی موجب افزایش درصد مرگ و میر لاروها گردید. درصد مرگ و میر تجمعی لاروها در غلظت‌های مختلف از سوسپانسیون قارچی، ۱۰ روز پس از آلوده‌سازی به ترتیب برابر با ۶/۶۷-۹۶/۶۷ درصد و ۳/۳۳-۹۳/۳۳ درصد برای جدایه‌های M14 و DEMI001 بود. نتایج تجزیه پروبیت داده‌ها نشان داد که بر اساس هم‌پوشانی حدود اطمینان ۹۵ درصد مقادیر LC_{50} و LC_{95} در دو جدایه قارچی مورد مطالعه تفاوت معنی‌داری با یکدیگر نداشتند. مقدار LC_{50} برای جدایه‌های M14 و DEMI001 به ترتیب برابر با 1.15×10^6 و 1.63×10^6 کنیدی بر میلی‌لیتر و مقدار LT_{50} برابر با ۵/۴۵ و ۶/۶۸ روز بود. مقایسه میانگین درصد مرگ و میر در هر غلظت از سوسپانسیون قارچی نشان داد که میزان مرگ و میر به طور معنی‌داری تحت تأثیر دو جدایه مختلف قرار نگرفت. نتایج این تحقیق حاکی از اهمیت دو جدایه M14 و DEMI001 برای کنترل بیولوژیک سوسک چهار نقطه‌ای حیوانات می‌باشد.

واژگان کلیدی: بیماری‌زایی، *Metarhizium anisopliae*، *Callosobruchus maculatus*، کنترل بیولوژی، LC_{50}

Introduction

The cowpea beetle, *Callosobruchus maculatus* (F.) (Col.: Bruchidae), is an important pest of cowpea, *Vigna unguiculata* (L.) Walps worldwide. The field Infestation level of cowpea by this bruchid pest is very low at harvest and may sometimes be undetectable. The cowpea weevil multiplies rapidly in storage, produces a new generation every month, and may cause losses up to 30% in 3 months of storage. Complete loss of cowpea could occur within 6 months of storage if this pest is not controlled (Cherry *et al.*, 2005). Chemical control with protectant synthetic insecticides (organophosphates and pyrethroids) and fumigants (phosphine) is a common practice used to control pests of stored grains. However, due to the accumulation of residues in grains, the selection of resistant insect population and other side effects, alternative approaches in Integrated Pest Management (IPM) have been considered (Gusmão *et al.*, 2013). In this context, biological control, including the use of entomopathogenic fungi considered promising for the control of stored product pests (Mohapatra *et al.*, 2015). The entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) is a valuable biocontrol agent worldwide with relatively wide host range (Zimmermann, 2007). The capacity of entomopathogenic fungi to control stored grain pests, particularly Coleoptera, has been investigated in several studies in recent years. Emphasis has been on the evaluation of *Beauveria bassiana* (Balsamo) Vuillemin and *M. anisopliae* against pests of stored maize (Cherry *et al.*, 2005). Few studies have

evaluated fungal pathogens for control of *C. maculatus* in cowpea (Cherry *et al.*, 2005; Murad *et al.*, 2006; Cherry *et al.*, 2007; Vanmathi *et al.*, 2011). Virulence of *B. bassiana* and *M. anisopliae* has been studied on *C. maculatus* in Iran (Mahdneshtin *et al.*, 2011; Nabaei, 2011). In this research, we investigated the lethal effect of two isolates of *M. anisopliae* on third instar larvae of *C. maculatus*.

Material and methods

Insect rearing

Callosobruchus maculatus was reared in 1-liter jars containing cowpea seeds, which were covered by a fine mesh cloth for ventilation. The cultures were maintained in the dark in a growth chamber set at $27\pm 2^\circ\text{C}$ and $65\pm 5\%$ R.h. Third instar larvae were used for bioassays. All experimental procedures were carried out under the same environmental conditions as the cultures.

Fungal entomopathogen

Metarhizium anisopliae isolates M14 and DEMI001 used in bioassays were isolated by Dr. M. Ghazavi from soil (Garmsar-Iran), and *Rhynchophorus ferrugineus* (Col.: Curculionidae): from Saravan-Iran, respectively (Iranian Research Institute of Plant Protection, Tehran-Iran). They were cultured on Sabouraud Dextrose Agar (SDA) for two weeks. Then, conidia were used to make aqueous suspension (10^4 - 10^8 conidia/ml) with 0.02% Tween-80. Conidial concentrations were estimated using a Neubauer haemocytometer (Weber Scientific International Ltd, UK).

Bioassay

Third instar larvae of *C. maculatus* ($n=50$) were treated with different concentrations of fungus isolates by immersion method. Control insects were immersed at 0.02% Tween-80 for 10 seconds. The experiment was conducted with 3 replications for each conidial concentration and mortality of insects was recorded daily up to 10 days.

Statistical analysis

Probit analysis (Finney, 1971) was used to estimate lethal concentration and lethal time values. Statistical differences among means at each fungus concentration were evaluated using t-test ($P < 0.05$) by SPSS. 22. All charts were plotted using Excel 2013 software.

Results and Discussion

The results indicated that the mortality percent increased with increase in both conidial concentration and exposure times in a linear relationship (figs. 1 and 2). The highest mortality percents were 96.67% and 93.33% for isolate M14 and DEMI001 respectively, at 10^8 conidial concentration (conidia/ml). Moreover, the lowest mortality were 6.67% and 3.33% for isolate M14 and DEMI001, respectively, at 10^4 conidial concentration (conidia/ml). Probit analysis showed that both *M. anisopliae* isolates were virulent to *C. maculatus*. The corresponding LC_{50} and LC_{95} values are shown in Table 1. There were no significant differences among the LC_{50} and LC_{95} values of two fungus isolates but *M. anisopliae* isolate M14 demonstrated shorter LT_{50} and it was faster-acting than isolate DEMI001 (Table 2). There were significant differences among LT_{50} values at two fungus treatments. The mean comparisons for cumulative mortality percent at different conidial concentrations are shown in Table 3. The results indicated that the mortality rates weren't affected significantly by different fungus isolates at any conidial concentration.

Our results is consistent to Cherry *et al.* (2005) revealed that *M. anisopliae* isolate 0351 is virulent to the adults of *C. maculatus*. The LC_{50} values were estimated 2.6×10^8 and 1.2×10^8 (conidia/ml) for *M. anisopliae* isolate DEMI001 and IRAN 715C, respectively, against the adults of *C. maculatus* (Mahdneshtin *et al.*, 2011). The LT_{50} values were 7.7 and 7.8

days for DEMI001 and IRAN 715C isolates respectively. Similar to our results, two isolate of *M. anisopliae* (CG34 and CG100) showed remarkable mortality rates on the adults of *C. maculatus*. Our study revealed that two isolates of *M. anisopliae* caused considerable mortality on third instar larvae of *C. maculatus*. After conducting further experiments on lethal effects of *M. anisopliae* isolate M14 and DEMI001 in warehouses they may be used as potential biocontrol agents for the control of this important bruchid pest.

Table 1. Lethal concentration values of two fungal-isolates of *M. anisopliae* on *C. maculatus* larvae.

Fungus-isolate	LC ₅₀ (conidia/ml)	LC ₉₅ (conidia/ml)	Slope ± SE	Intercept ± SE	χ ² (df=3)	P-value
M14	1.15×10 ⁶ (5.36×10 ⁵ -2.48×10 ⁶) ^a	1.78×10 ⁸ (4.96×10 ⁷ -1.42×10 ⁹)	0.75±0.10	-4.55±0.63	1.40	0.71
DEMI001	1.63×10 ⁶ (7.69×10 ⁵ -3.53×10 ⁶)	2.36×10 ⁸ (6.59×10 ⁷ -1.90×10 ⁹)	0.76±0.10	-4.72±0.65	1.00	0.80

^a 95% confidence limit

Table 2. Lethal time values of two fungal-isolates of *M. anisopliae* on *C. maculatus* larvae.

Fungus-isolate	LT ₅₀ (days)	LT ₉₅ (days)	Slope ± SE	Intercept ± SE	χ ² (df=2)	P-value
M14	5.45 (5.15-5.70) ^a	6.93 (6.51-7.76)	15.77 ± 2.75	-6.62 ± 2.09	0.59	0.74
DEMI001	6.68 (6.37-6.94)	8.23 (7.76-9.19)	17.17 ± 2.81	-9.16 ± 2.36	2.51	0.28

^a 95% confidence limit

Table 3. Mean (±SE) larval mortality (%) of *C. maculatus* exposed to different concentrations of *M. anisopliae*.

Concentration (conidia/ml)	Fungus isolate	Mean ± SE	F	T (df=4)	Sig. (T)
10 ⁴	M14	0.67 ± 0.33	0.000	0.707	0.519
	DEMI001	0.33 ± 0.33			
10 ⁵	M14	2.33 ± 0.33	0.400	0.5	0.643
	DEMI001	2 ± 0.58			
10 ⁶	M14	4.67 ± 0.67	0.235	0.000	1.000
	DEMI001	4.67 ± 0.88			
10 ⁷	M14	7 ± 0.58	0.308	0.378	0.725
	DEMI001	6.67 ± 0.67			
10 ⁸	M14	9.67 ± 0.33	3.2	0.447	0.678
	DEMI001	9.33 ± 0.67			

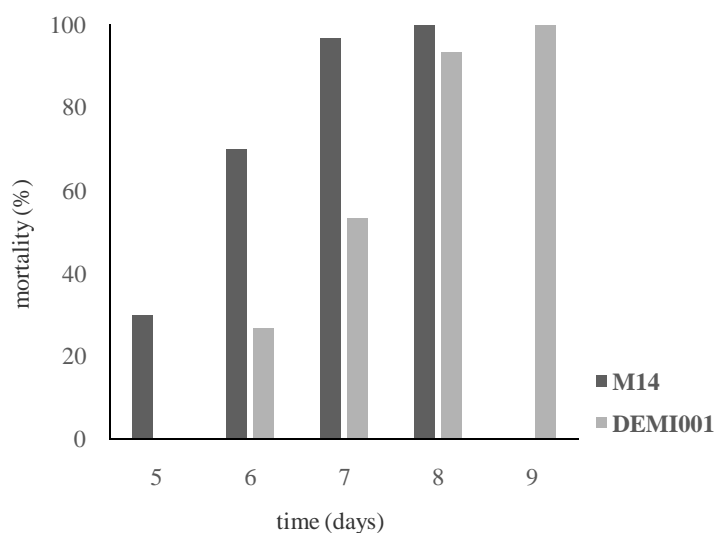


Fig 1. Cumulative mortality percent of *C. maculatus* larvae exposed to two fungal-isolates of *M. anisopliae* for various periods.

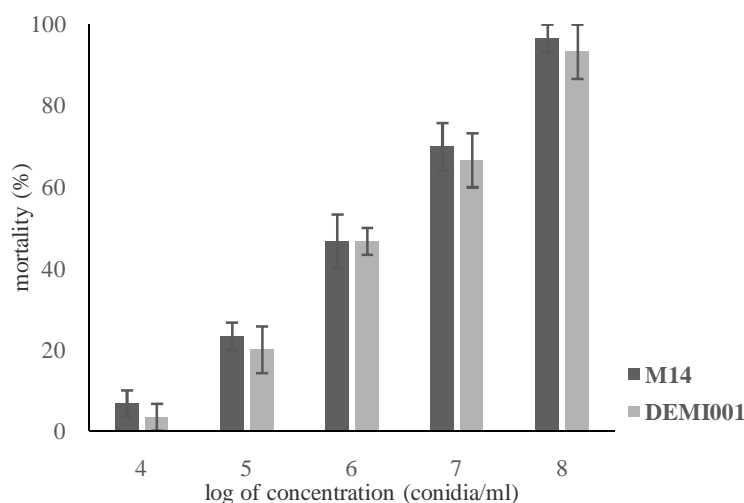


Fig 2. Mortality of *C. maculatus* larvae exposed to different conidial concentrations of two fungal-isolate of *M. anisopliae*

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