African Journal of Microbiology Research Vol. 5(10), pp. 1192-1196, 18 May, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.011
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

A laboratory assessment of the potential of the entomopathogenic fungi *Beauveria bassiana* (Beauvarin®) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)

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Accepted 1March, 2011

This research was carried out to evaluate the suppressive efficacy of entomopathogenic fungi *Beauveria bassiana* against adults of *Callosobruchus maculatus* (F.) and *Sitophilus granarius* (L.) on stored grains in darkness (27±2°C and 65±5% RH). The insects were inoculated by following immersion in five aqueous suspensions with concentrations determined with preliminary tests and compared with untreated wheat and cowpea as control. Probit analysis showed that the lowest LT_{50} values in suspensions with highest concentrations (2.3×10^7 conidia ml^{-1}) were 6.63 and 10.45 days for *C. maculatus* and *S. granarius*, respectively. On the other hand, the LC_{50} values on day 9 post-treatment were 3.17×10^6 and 6.08×10^7 con.ml^{-1} for *C. maculatus* and *S. granarius*, respectively. Comparison of Lc_{50}, LT_{50} values and mortalities indicated that in both assays, *B. bassiana* was consistently more virulent for *C. maculatus* than *S. granarius.* The overall results showed that these two pests were controlled successfully with entomopathogenic fungi *Beauveria bassiana*.

Key words: Callosobruchus maculatus, Beauveria bassiana, Sitophilus granarius, stored grain.

INTRODUCTION

Grains produced may be stored for periods of a few weeks to a few years before they are fed or processed. The profitability of such storage depends, not only upon marketing concerns, but also upon maintaining grain quality. The harvest and storage of grain do not signal an end to the possibility of losses caused by insects and pathogens (Weinzierl, 2008). A serious post-harvest pest of black-eyed peas (cowpeas) is the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae), a worldwide pest which infests pods in the fields as well as stored seeds. Infection rate of cowpea is very low in harvest time and may sometimes be undetectable (Huignard et al., 1985). The pest multiplies rapidly in stored condition, giving rise to a new generation every month (Ouedraogo et al., 1998). Therefore, it is essential to reduce such losses by controlling the pest on stored grains (Tapondjou et al., 2002). The granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), is one of the most destructive insects of stored grains worldwide, that cause both quantitative and qualitative damage to grain. Quantitative damage is due to grain weight loss caused by insect feeding (Steffan, 1963; Golebiowska, 1969). Qualitative damage is due to product alterations such as loss of nutritional and aesthetic value, increased levels of rejects in the grain mass and loss of industrial (baking) characteristics (Moino et al., 1998).

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Grain losses due to insect pests are a serious problem throughout the world (Subramanyam and Hagstrum, 1995). Residual insecticides are the most common agents for protection of stored products against stored products pests. They have several negative properties such as mammal toxicity, residues on grain, as well as increased resistance of pests (Arthur, 1996). The increased public awareness and concern for environmental safety has directed research to the development of alternative control strategies (Inglis et al., 1997; Lomer et al., 2001).

A promising strategy with good potential to minimize the adverse effects of insecticides is the use of entomopathogenic fungi and other microbial control agents. The possibility of using fungal pathogens to control insects has been studied for many years but little attention has been paid to the use of fungi as control agents against storage pests (Khan and Selman, 1988; Rodrigues and Pratissoli, 1990; Adane, 1994; Adane et al., 1996; Padı´n et al., 1997; Hidalgo et al., 1998; Moino et al., 1998).

As part of our project on the development and use of entomopathogenic fungi in integrated pest management (IPM)-based control of stored pests, we are investigating the potential of *B. bassiana* as a biological control agent against two important and destructive stored pests' *C. maculatus* and *S. granarius* adults. The main purpose of present study is, introducing a safe alternative method for pest's control.

MATERIALS AND METHODS

Test insects

The insects tested were cowpea weevil, *C. maculatus* and granary weevil, *S. granarius*. Insects were collected from the breeding stock of Faculty of Agriculture, Urmia University, Iran. They were reared in darkness under controlled temperature and humidity (27±2°C and 65±5% RH) on cowpea and clean wheat + cracked wheat (3:1 w/w), respectively. For obtaining 1-day-old adults of *C. maculatus*, seeds with pupa window were separated and after one day, emerged adults were collected with an aspirator.

The fungus

Commercially produced conidia of *B. bassiana* (Beauvarin®) were used in all experiments. The *B. bassiana* technical aqueous conidial suspension contained 2.3×10^7 conidia/ ml. This product was obtained from Semnan-Iran Biological products Co., Ltd.

Laboratory bioassay

Five aquenous conidial concentrations were determined with preliminary tests.

Inoculation was made by immersing twenty adults of both insects for 5 s in 1 ml suspension containing 2.3×10^4, 1.2×10^5, 7.2×10^5, 4.07×10^6 and 2.3×10^7 conidia ml^{-1} for *C. maculatus* and 2.3×10^6, 4.7×10^6, 7.2×10^6, 1.2×10^7 and 2.3×10^7 conidia ml^{-1} for *S. granarius*. The control insects were treated in de-ionised water Tween80 (0.1% v/v). The insects and the suspension were subsequently poured into plastic plates (8.8 cm diameter, 60.7 cm^2) containing sterile filter paper (8.8 cm diameter) and 20 g cowpea and wheat for *C. maculatus* and *S. granarius* tests, respectively. Plates were sealed with parafilm to prevent insects from escaping. There were 3 replicates per treatment. Insects were kept in incubator (27±2°C and 65±5% RH). Total numbers of living or dead adults were recorded for 9 and 13 days for *C. maculatus* and *S. granarius*, respectively (Cherry, 2005). To observe the outgrowth of fungus, dead insects from each plate were washed in sterile distilled water and transferred to new plates with high RH (approximately 100%).

Data analysis

All of the data were transformed into arcsine scale followed by correction of cumulative mortality percentage for the corresponding control mortality (Abbott, 1925). Analysis of variance was carried out to assess the effect of formulation, time of exposure and their interaction on the mortality of the insects (Nissen, 1989). Concentrations required to kill 50% (Lc_{50}) and 95% (Lc_{95}) and also times needed to kill 50% (LT_{50}) and 95% (LT_{95}) of insects were calculated using probit analysis (SPSS, 1999).

RESULTS

The evaluation of mortality among *C. maculatus* and *S. granarius* adults following immersion in aqueous conidial suspensions of *B bassiana* indicates the great efficacy for *B. bassiana*.

In the case of *C. maculatus*, the main effects for adults: Concentration, exposure time and their interactions; concentration × exposure time were significant ($P < 0.01$) (Table1). The mortality percentage for adults of *C. maculatus* (3, 5, 7 and 9 d) against different conidial concentrations is indicated in Figure 1.

To achieve 50 and 95% mortality after 7-day, 1.99×10^7 and 1.21×10^{10} $con.ml^{-1}$ *B. bassiana* was needed, respectively. As exposure time increased, the LC_{50} and LC_{95} values decreased. Table 2 shows that after 9 d exposure, the LC_{50} and LC_{95} were 3.17×10^6 and 4.9×10^8 $con.ml^{-1}$, respectively. The lowest LC_{50} value observed for *C. maculatus* was 3.17×10^6 $con.ml^{-1}$ (Table 2). After 9 days, the highest mortality (85%) was at *B. bassiana* at the highest concentration rates. Also, the lowest LT_{50} values in highest conidial concentration applied was 6.63 days.

In other case regarding *S. granaries,* it was observed that exposure time of insects to different *B. bassiana* concentrations had significant effects ($P < 0.01$) (Table1). The mortality percentage for adults of *S. granarius* (7, 9, 11 and 13 d), against different conidial concentrations is shown in Figure 2. After 7 d, LC_{50} and LC_{95} values were 1.35×10^8 and 4.75×10^9, respectively. The mortality in initial exposure day was low. To get 50 and 95% mortality after 9 days, 6.08×10^7 and 3.25×10^9 $con.ml^{-1}$ of *B. bassiana* was needed, respectively (Table 3). After 13 days, the highest mortality (75%) was at *B. bassiana* at

Table 1. Analysis of variance on mortality of *C. maculatus* and *S. granarius* adults exposed to different concentrations of *B. bassiana* with different time of exposure.

	C. maculatus			*S. granarius*		
Source	**df**	**Mean square**	**F value**	**df**	**Mean square**	**F value**
Time	3	2033.645	184.05**	3	1040.38	105.68**
Dose	5	2143.645	174.56**	5	2227.25	226.24**
Time × dose	15	71.263	6.11**	15	59.47	6.04**
Error	48	11.647		48	9.84	
Total	71			71		

**Indicate significant difference at P≤0.01.

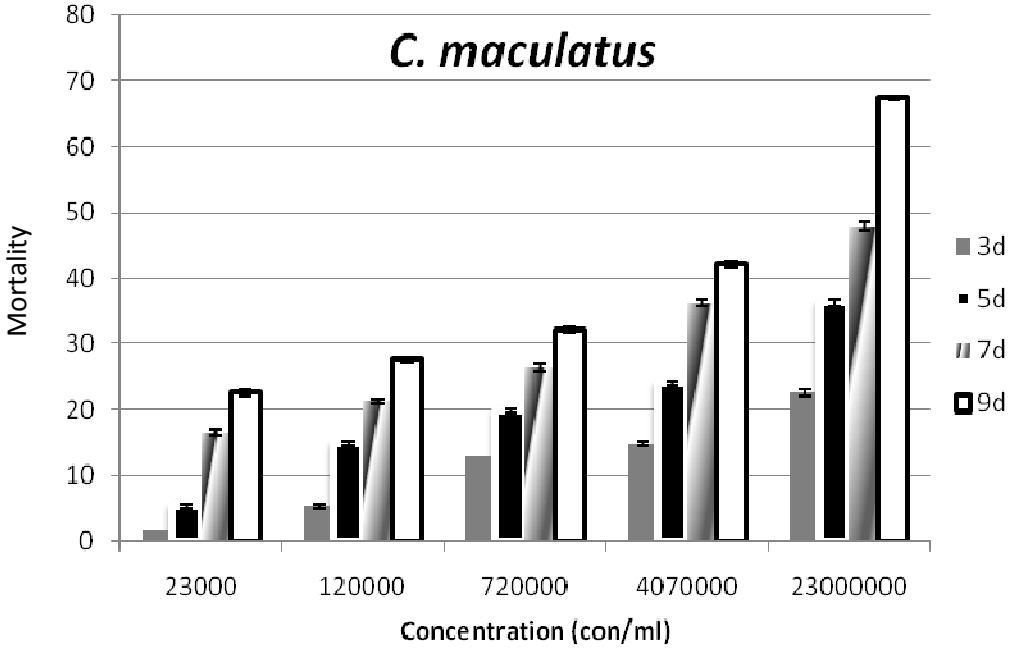

Figure 1. Mean mortality (%) ± SE of *C. maculatus* adults exposed to different concentrations of *B. bassiana* after 3, 5, 7 and 9 days.

Table 2. Lc_{50} and Lc_{95} (con.ml^{-1}) values with 95 fiducial limits and probit analysis parameters for adult of *C. maculatus*.

Exposure time (d)			95% confidence limits	Intercept + SE	Slope ± SE	P-value	χ2 (df = 3)
7	LC_{50}	1.99×10^{7}	8.5×10^{6} - 8.35×10^{7}	-4.31+0.67	0.59+0.1	0.92	0.46^{NS}
7	LC_{95}	1.21×10^{10}	1.23×10^{9} -1.22×10^{12}				
9	LC_{50}	3.17×10^{6}	4.9×10^{5} -1.03×10^{8}	-4.88+0.68	0.75+0.1	0.032	8.77*
9	LC_{95}	4.9×10^{8}	3.26×10^{7}- 2.44×10^{17}				

*: Indicate significant difference at P≤0.05.NS; no significant difference.

the highest concentration rates. Thus, the lowest LT_{50} values in highest conidial concentration (2.3×10^{7} con.ml^{-1}) were 10.45 days.

In result, it is noticeable that, *B. bassiana* was pathogenic to adults of *C. maculatus* and *S. granarius* in immersion bioassays, but there were different virulence. Comparison of Lc_{50}, Lc_{95} values and mortalities indicates that *B. bassiana* was more efficacious and caused in general, better mortality for *C. maculatus* rather than *S. granarius*.

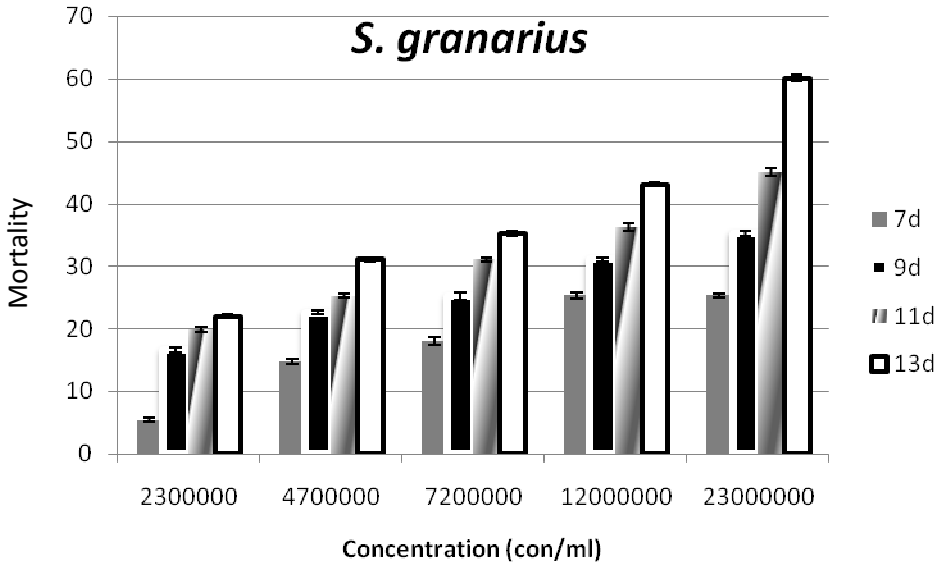

Figure 2. Mean mortality (%) ± SE of *S. granarius* adults exposed to different concentrations of *B. bassiana* after 7, 9, 11 and 13 days.

Table 3. Lc_{50} and Lc_{95} (con.ml^{-1}) values with 95 fiducial limits and probit analysis parameters for adult of *S. granarius*.

Exposure time (d)			95% confidence limits	Intercept ± SE	Slope ± SE	P-value	χ2 (df = 3)
7	LC_{50}	1.35×10^{8}	8.5×10^{6}-8.35×10^{7}	-8.64+2.24	1.06+0.32	0.82	0.89^{NS}
7	LC_{95}	4.75×10^{9}	1.23×10^{9}-1.22×10^{12}				
9	LC_{50}	6.08×10^{7}	4.9×10^{5}-1.03×10^{8}	-7.4+1.77	0.95+0.25	0.98	0.14^{NS}
9	LC_{95}	3.25×10^{9}	3.26×10^{7}-2.44×10^{17}				
11	LC_{50}	2.39×10^{7}	1.62×10^{7}-5.14×10^{7}	-8.95+1.68	1.21+0.24	0.98	0.13^{NS}
11	LC_{95}	5.42×10^{8}	1.64×10^{8}-8.06×10^{9}				
13	LC_{50}	1.16×10^{7}	9.3×10^{6}-1.54×10^{7}	-11.82+1.74	1.67+0.25	0.48	2.45^{NS}
13	LC_{95}	1.11×10^{8}	5.98×10^{7}-3.41×10^{8}				

NS: No significant difference.

DISCUSION

Several studies documented the high potential of entomopathogenic fungi for the control of insect pests in stored products as promising alternatives to fumigants (Moore et al., 2000).

The insecticidal efficacy of *B. bassiana* is highly influenced by several factors such as insect's behavior, population density, age, nutrition and genetic information, also, physiology and morphology of host effect on their sensitivity to biological control agents such as entomopathogenic fungi (Fargues et al., 1996). So, the differences in storage beetle susceptibility to *B. bassiana* could not be explained as a function of the concentration of conidia used (Cox et al., 2004).

In general, host physiology and fungi physiology (their enzymes and toxins) have great impact in virulence of entomopathogenic fungi. Among environmental condition, temperature and humidity play important role in fungi function. The optimum temperature for *B. bassiana* conidial germination and growth frequency is around 25℃ (Walstad et al., 1970; Ekesi et al., 1999). Also, James et al. (1998) reported that *B. bassiana* germinated most rapidly at 25 to 32℃. In present study, all bioassays were done at 27±2℃, which, according to the previous studies, was suitable for growth and conidial germination.

It is clear that, entomopathogenic fungi, germinate at relative humidity above 90% (Roberts and Campbell, 1977) although, it has been shown that fungi work also at lower humidity levels, probably due to favourable microclimates surrounding the hosts (Ramoska, 1984; Akbar et al., 2004). The other studies have reported that the longevity of *B. bassiana* conidia is best when dry (Wraight et al., 2001; Lord, 2005).

The results of current study documented that, the lower humidity (65±5% RH) conditions of stored-product environments are not an impediment to the use of *B. bassiana* for management of insect pests. In fact, such conditions are advantageous.

The results of the present study extend the findings of Cherry et al. (2005) which stated that different isolates from *M. anisopliae* and *B. bassiana* can provide good control of *C. maculatus* by immersion bioassay.

Hidalgo et al. (1998) pointed out that it is possible to achieve a useful level of *S. zeamais* by using formulated *B. bassiana* conidia.

Evidence from these experiments indicates that *B. bassiana* is able to significantly reduce *C. maculatus* and *S. granarius* in stored cowpea and wheat, respectively. Comparison of Lc_{50}, LT_{50} values and other results showed clearly that adults of *C. maculatus* are more sensitive to *B. bassiana* formulation rather than *S. granarius*.

In conclusion, microbial pest control as a safe and non-chemical method can potentially benefit the environment and lead to more effective protection of stored grains.

ACKNOWLEDGEMENTS

We thank Dr. Amir Sobhanmanesh for his comments in manuscript preparation and Dr. Masoud Shams for the assistance with his insect bioassay.

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