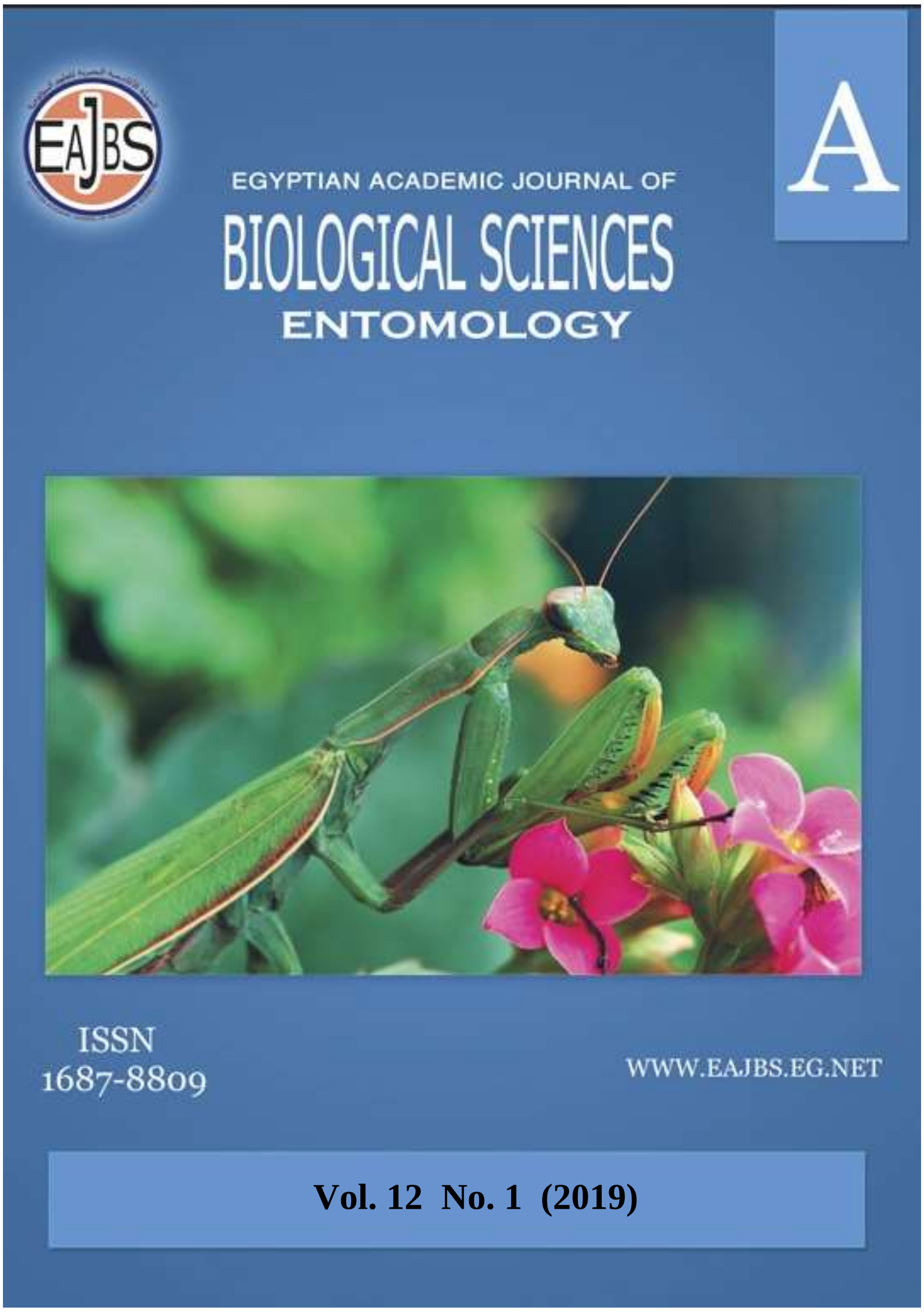

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Application of Wheat Cultivars and Pathogenic Fungus Combination against the Lesser Grain Borer, *Rhyzopertha dominica* (Col.: Bostrichidae) and the Red Flour Beetle *Tribolium castaneum* (Col.: Tenebrionidae)

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ABSTRACT

The objective of the present study was to investigate the effect of two wheat cultivars (the Sirvan and Mihan) on the interaction between *Beauveria bassiana* strain EUT116, *Rhyzopertha dominica* and *Tribolium castaneum* at 25± 1°C, 85±5% RH and darkness. The results of bioassay by submersion method showed that the lethal concentrations and times of the fungus were significantly different between the Sirvan and Mihan. The mortality of *R. dominica* was significantly lower on the Mihan infected with a dose rate of 1 g/kg than the Sirvan. The mortality of *Rhyzopertha dominica* was remarkably higher for the adults exposed to the dose rate of 1 g/kg than the adults exposed to 0.25 g/kg. The mortality of *Tribolium castaneum* was remarkably higher for the adults were exposed to the dose rate of 1 g/kg than the adults were exposed to 0.25 g/kg. The highest mortality occurred for *R. dominica* infected with 1 g of *B. bassiana* per kg of the Sirvan. The pathogenicity of dose rate 1 g/kg on the Sirvan was higher against *R. dominica* than *T. castaneum*. The 0.25 g of *B. bassiana* per kg of the Mihan differentially caused higher mortality on *R. dominica* than *T. castaneum.* It seems that planting the Sirvan cultivar in the field can be more helpful to decrease the population of stored grain beetles rather than the Mihan in post-harvest conditions. According to our results, *B. bassiana* strain EUT116 was a promising candidate as a biocontrol agent to suppress the storage grain beetles, especially, *R. dominica*.

INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (Col.: Bostrichidae) (Fabricius) is an important stored grain pest of cereals such as wheat seeds in both temperate and tropical regions. Feeding by the adults and their larvae cause enormous losses to stored grains. The adults make holes in grains and it is resulted in weight loss due to flour products as well as their larvae (Rees, 2004). Also, the red flour beetle, *Tribolium castaneum* (Herbst) (Col.: Tenebrionidae) is the most serious pest of stored cereals worldwide and benzoquinone secretions by the adults cause unpleasant odors in grains. The larvae like their adults are grain feeders and produce damages to the commodity. The red flour beetles have a long lifespan and adapted well to the different storage conditions (Rees, 2004).

Dependence on persisted usage of fumigant insecticides such as methyl bromide and phosphine for several decades to control stored grain pest population (White and Leesch, 1995) has led to develop the pest resistance and threaten non-target organisms, environmental and human health (White and Leesch, 1995). To prevent these serious effects of chemical insecticides, biological control methods should be developed and applied as an alternative by applying entomopathogenic fungi, especially for integrated pest management program (IPM).

Up to now, different strains of entomopathogenic fungi have been used for insect pest control including *Beauveria bassiana, Lecanicillium sp., Metarhizium anisopliae, Isaria fumosorosea and Nomuraea rileyi (*Vu *et al.,* 2007*). B.* bassiana is the most important pathogenic fungi that its conidia attach to insect cuticle and germinate under optimal conditions. After degradation of the insect cuticle, the fungus enters the hemocoel and reproduces. The death of insect hosts will occur by losing body nutrition, tissue degradation and poisoning with secreted toxins by the entomopathogenic fungus. Fortunately, *B. bassiana* is compatible with other natural enemies, insect pathogens, different types of pesticides and pollinators (Inglis *et al.,* 2001).

B. bassiana is able to colonize inside the wide plant ranges as an endophyte and significantly causes to decrease pest populations (Vega, 2008). The entomopathogen infects a broad insect range belonging to more than 200 species related to 9 orders (Feng *et al.,* 1994). The products based on the entomopathogenic fungi including *B. bassiana* and *M. anisopliae* are the most insecticides and acaricides (33.9%) in the whole world (Faria and Wraight, 2007).

The pathogenicity of *B. bassiana* is higher than *M. anisopliae* and *Nomuraea rileyi* against different kinds of stored product pests such as *T. castaneum*, *Sitophilus oryzae* (L.) (Col.: Curculionidae) and *Acanthoscelides obtectus* (Say) (Col.: Chrysomelidae) (Padin *et al*., 2002) and results in 50 % mortality to *T. castaneum* 14 days after treatment (Padin *et al*., 1997). *B.bassiana* is a potential biocontrol agent against the lesser grain borer, *R. dominica* when mixed with diatomaceous earth (Vassilakos *et al*., 2006). Also, the eggs of *R. dominica* and *T. castaneum* are influenced by *B. bassiana* with dose of 1.1×10^5 conidia/mm^2 at 92% relative humidity and their hatchability will be reduced by penetrating the pathogenic fungus into the eggs (Lord, 2009).

The adults and larvae of sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Col.: Silvanidae) feed on date palm fruits as stored product pest. The adults are so sensitive to *B. bassiana* strain GHA and the fungus can control the pest population under store conditions (Lord, 2001). Surprisingly, *B. bassiana* (*Bb*Weevil™) was able to influence progeny production of the beetles (Khashaveh *et al.*, 2012)

Quality of host plants affects the upper trophic levels (Zvereva and Rank 2003; Kagata et al. 2005) such as development, survival and reproduction of herbivorous insects (Liu et al., 2004; Hasan and Ansari, 2010; Hasan and Ansari, 2011). For instance, survey of biological parameters of Russian wheat aphid, *Schizaphis graminum* (Rondani) (Hem.: Aphididae) has been declared that different genotypes of wheat can affect the reproductive of the aphid and cause to elongate the period of a generation, because of different level of antibiosis (Barkhordar et **al., *2012).***

Moreover, host plants in different ways, such as inadequate nutrition, affect the growth rates and sensitivity of the herbivorous insects to their pathogenic microorganisms (Santiago-Alvarez *et al.*, 1992). Plants affect the growth rate of herbivorous insects by creating nutrient stress (Mayer *et al*., 2002) and finally, through the production of antimicrobial compounds, the pathogenicity of entomopathogenic microorganisms will be affected (Jones, 1991).Cory and Hoover (2006) argue that the chemical compounds produced by host plants reduce the effect of entomopathogenic fungi against their insect hosts.

For example, according to a research, the efficiency of the B. bassiana strain GHA against *Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae) on the Impatiens walleriana (Hook) F. (Balsaminaceae) plant was low compared to the bean plant (Ugine et al., 2005; Ugine et al., 2006). Also, the host plant can affect the mortality of Acyrthosiphon pisum (Harris) (Hem.: Aphididae) infected with Pandora neoaphidis (Remaudière & Hennebert) Humber (Zygomycetes: Entomophthorales) (Duetting et al., 2003).*

Also, it was proved that the entomopathogenic fungus *B. bassiana* had various impacts on different species of stored grain beetle (Wakefield, 2006). The study showed that *B. bassiana* was more pathogenic to the saw-toothed grain beetle, *O. surinamensis* than the confused flour beetle, *T. confusum* (Wakefield, 2006).

Therefore, the objective of the present study is to investigate the effect of two different wheat cultivars on interaction between the entomopathogenic fungus *B. bassiana strain EUT116, the lesser grain borer, R. dominica and* the red flour beetle, *T. castaneum* to increase the efficiency of integrated pest management programs.

MATERIALS AND METHODS

Insect Cultures:

The insects used in this study were the red flour beetle, *T. castaneum* and the lesser grain borer *R. dominica* that were collected from stored grains and flour in Kerman province, Iran. Then, the adults were reared on two native wheat cultivars including, Sirvan and Mihan in plastic containers (13 cm in diameters and 26 cm height) and incubated in a controlled environment room (at 25 ± 1 °C, 60 ± 5 % RH and darkness). In all experiments, 1-7 day-old adults were used.

Fungus:

After the passage of the fungus *B. bassiana* strain EUT116 through each beetle pest of stored product (e.i., *T. castaneum and R. dominica*), it was cultured on Sabouraud dextrose agar with yeast extract (SDAY) for 15 days at 25 °C. The spores of the fungus were harvested and stored at 4 °C over silica gel (Hansen and Steenberg 2007). To estimate the viability of the fungus, the number of germinated conidia per 100 conidia was counted in four plates. All conidia were germinated.

Bioassay by Submersion Method:

To determine the different concentrations of the fungus *B. bassiana* including LC_{10}, LC_{50} and LC_{90}, firstly, a fungal suspension of 0.02% Tween 80 was prepared in sterile distilled water and then, glass beads and shakers were used to separate conidia from the mycelia. After passing the suspension through a fine mesh net, the conidial concentration was calculated using a hemocytometer and $5x \times 10^4$ formula. By using the $C_1V_1 = C_2V_2$ equation, the other concentrations, namely, 10^4, 10^5, 10^6, 10^7 and 10^8conidia/ml were prepared and 0.02% of Tween 80 was used as control. After infecting with the fungus by submerging the 1-7 day-old adults into each suspension for six seconds, 15 adult beetles were put in a Petri dish (6 cm diameter) as an experimental unit and incubated in a controlled environment room (at 25 ± 1

°C, 85 ± 5 % RH and darkness). After 24 h, the lids of Petri dish were replaced with holed ones containing wheat seeds of each cultivar (the Sirvan and Mihan). The hole was covered with a fine mesh net for ventilation. Each treatment had three replicates. The death of storage grain beetles was recorded daily till 15 days. Subsequently, the fungal infection was confirmed by placing the beetle corpuses onto a distilled filter paper and incubating at 25 ± 1 °C.

Bioassay by Infecting The Wheat Seeds:

Two different amounts of the pathogenic fungus *B. bassiana* were prepared at a rate of 0.5 and 1 g per kg of wheat. Ten lots of each wheat cultivar, the Sirvan and Mihan, were poured into cylindrical plastic containers (18 cm diameter, 15 cm height) and infected with two amounts of the fungus. All plastic containers were shaken for two minutes to uniformly spread the fungal powder in all the wheat masses. After one day, four 50 g-samples were taken from each container and placed in smaller plastic containers (5 cm diameter, 8 cm height) as experimental units. Thirty 1-7-day old adults were put into each unit and covered the lid with fine mesh. The experiment was carried out in a controlled environment room (at 25 ± 1 °C, 85 ± 5 % RH and darkness). The number of grain beetles cadavers was recorded daily till 7 days. Afterward, the fungal infection was confirmed.

Data Analysis:

The lethal concentrations (LC_{10}, LC_{50} and LC_{90}) and lethal times (LT_{10}, LT_{50} and LT_{90}) were calculated using the POLO-PC 2002 software. The bioassay by infecting the wheat seeds was carried out as a completely randomized design. After analysis of variance (ANOVA), comparison of the averages was done using the Tukey's test at the 0.05 level. The Statistical Analysis System (SAS, 1989) was used for calculations.

RESULTS

Bioassay by Submersion Method:

The results of bioassay by submersion method showed that the LC_{50} of the entomopathogenic fungus, *B. bassiana* strain EUT116 for the lesser grain borer, *R. dominica* was significantly different between two wheat cultivars the Sirvan and Mihan (Table 1). In addition, the type of wheat cultivars affected the two other calculated lethal concentrations (e.i., LC_{10} and LC_{90}) (Table 1). All calculated lethal concentrations were lower on the Sirvan than Mihan cultivar.

According to Table 2, all the calculated lethal times (e.i., LT_{10}, LT_{50} and LT_{90}) for *R. dominica* treated with 10^8 conidia/ml of *B. bassiana* strain EUT116 were shorter on the Sirvan than Mihan cultivar.

The results of bioassay by submersion method for the red flour beetle, *T. castaneum* showed that the LC_{50} of the entomopathogenic fungus, *B. bassiana* strain EUT116 was higher on the Mihan than Sirvan cultivar (Table 3). The type of wheat cultivars influenced the two other calculated lethal concentrations (e.i., LC_{10} and LC_{90}) as well (Table 3). All calculated lethal concentrations were higher on the Mihan than Sirvan cultivar.

The Table 4 indicated that all the calculated lethal times (e.i., LT_{10}, LT_{50} and LT_{90}) for *T. castaneum* treated with 10^8 conidia/ml of *B. bassiana* strain EUT116 were longer on the Mihan than Sirvan cultivar.According to the lethal concentration and time results, it could be concluded that B. bassiana strain EUT116 was more pathogenic to R. dominica than T. castaneum fed on both cultivars.

Table 1. Results of the probit analyses for, *Rhyzopertha dominica* treated with *Beauveria bassiana* strain EUT116 on two wheat cultivars.

Cultivars	n	LC_{10} (CL) (conidia/ml)	LC_{50} (CL) (conidia/ml)	LC_{90} (CL) (conidia/ml)	Slope±SE	χ^2
Sirvan	225	3.1×10^{-2} (0.0–8.2)	3.8×10^{2} (2.83×10^{-1}–5.11×10^{3})	4.52×10^{6} (8.96×10^{5}–1.32×10^{8})	0.31±0.08	0.3
Mihan	225	2.8×10^{1} (1.19×10^{-1}–4.32×10^{2})	0.7×10^{5} (1.24×10^{4}–2.17×10^{5})	1.7×10^{8} (3.02×10^{7}–4.7×10^{9})	0.38±0.07	0.4

n= number of treated nymphs; CL= confidence limits (95% probability)

Table 2. Different lethal times (day) for, *Rhyzopertha dominica* treated with 10^8 conidia/ml of *Beauveria bassiana* strain EUT116 on two wheat cultivars.

Cultivars	LT_{10}	LT_{50}	LT_{90}
Sirvan	1.3	2.9	6.5
Mihan	2.1	6.9	7.22

Table 3. Results of the probit analyses for, *Tribolium castaneum* treated with *Beauveria bassiana* strain EUT116 on two wheat cultivars.

Cultivars	n	LC_{10} (CL) (conidia/ml)	LC_{50} (CL) (conidia/ml)	LC_{90} (CL) (conidia/ml)	Slope±SE	χ^2
Sirvan	225	6.30×10^{5} (2.0×10^{4}–5.5×10^{6})	2.6×10^{6} (5.3×10^{5}–7.2×10^{6})	1.10×10^{7} (8.1×10^{6}–1.7×10^{8})	2.06±0.35	0.66
Mihan	225	9.32×10^{5} (2.21×10^{5}–2.01×10^{6})	7.23×10^{6} (3.92×10^{6}–1.18×10^{7})	5.6×10^{7} (3.10×10^{7}–1.55×10^{8})	1.44±0.25	1.74

n= number of treated nymphs; CI= confidence limits (95% probability)

Table 4. Different lethal times (day) for, *Tribolium castaneum* treated with 10^8 conidia/ml of *Beauveria bassiana* strain EUT116 on two wheat cultivars

Cultivars	LT_{10}	LT_{50}	LT_{90}
Sirvan	0.7	2.5	5.1
Mihan	1.3	3.0	6.8

Bioassay by Infecting The Wheat Seeds:

There were 6.22×10^{10} conidia in each gram of the fungal spore. The analyses of variation declared that there were significant interactions between the studied parameters including the fungal treatment, the lesser grain borer, *R. dominica*, the red flour beetle, *T. castaneum* and the wheat cultivars (Table 5).

The results presented in Table 6 showed that the entomopathogenic fungus, *B. bassiana* strain EUT116 (both fungal dose rates: 0.25 and 1 g per kg) significantly influenced the lesser grain borer, *R. dominica* on the both tested cultivars (e. i., the Sirvan and Mihan). Therefore, the mean mortality of *R. dominica* was significantly higher when fed on fungus-treated wheat seeds compared to the control.

The mean mortality of *R. dominica* was significantly lower on the Mihan cultivar infected with 1 g of the pathogenic fungus per kg than the Sirvan cultivar (Table 6).The mean mortality of the lesser grain borer was remarkably higher for the adults were feeding on wheat seeds infected with 1 g of the pathogenic fungus per kg than the adults were feeding on seeds treated with 0.25 g of the fungus per kg of each wheat cultivar (Table 6). However, on each cultivar, there was no marked different between the mean mortality of the lesser grain borer affected with 0.25 g of the fungus per kg and the control.

Mean mortality of the red flour beetle, *T. castaneum* were significantly affected by all fungal treatments (0.25 and 1 g of *B. bassiana* strain EUT116 per kg) on two different cultivars of wheat compared to the control (Table 6). The mean mortality of *T. castaneum* affected with 1 and 0.25g of the pathogenic fungus per kg showed no significant difference between the Sirvan and Mihan cultivars (Table 6). However, the mean mortality of the red flour beetle was remarkably higher for the adults were feeding on wheat seeds infected with 1 g of the pathogenic fungus per kg than the adults were feeding on seeds treated with 0.25 g of the fungus per kg of each wheat cultivar.

The mean mortality of *T. castaneum* on the Sirvan cultivar was not significantly different from that of the Mihan in the control as well as *R. dominica*. The results of Table 6 reflected that the highest mean mortality was occurred for *R. dominica* (13.33 ± 0.57 adults) infected with 1 g of *B. bassiana* per kg of the Sirvan cultivar.
The pathogenicity of 1 g of *B. bassiana* per kg of the Sirvan cultivar was higher against *R. dominica* than *T. castaneum* (Table 6). Nevertheless, there was no significantly various between *R. dominica* than *T. castaneumin* in the pathogenicity of 0.25 g of *B. bassiana* per kg of the Sirvan cultivar. The 0.25 g of *B. bassiana* per kg of the Mihan cultivar differentially caused higher mortality on *R. dominica* than *T. castaneum* (Table 6). Nevertheless, there was no significantly various between *R. dominica* than *T. castaneumin* in the mortality of 1 g of *B. bassiana* per kg of the Mihan cultivar.

Table 5. ANOVA parameters for major effects and related interactions on counting the mortality (±SE) of *Rhyzopertha dominica* (*Rd*) and *Tribolium castaneum* (*Tc*) affected with *Beauveria bassiana* strain EUT116 on two wheat cultivars

Source	df	F	*P*
Treatment	2	60.47	<0.0001
Cultivar	1	1974.36	<0.0001
Insect	1	115.08	<0.0001
Treatment× Cultivar	2	30.69	<0.0001
Treatment× Insect	2	14.90	<0.0001
Cultivar× Insect	1	14.66	<0.0001
Treatment×Cultivar×Insect	2	22.42	<0.0001

df: degree of freedom; F: calculated F value; *P*: coefficient of confidence ($P < 0.05$).

Table 6. Mean mortality (±SE) of the adults of *Rhyzopertha dominica* (*Rd*) and *Tribolium castaneum* (*Tc*) affected with 0.25 and 1 g of *Beauveria bassiana* strain EUT116 per kg of each wheat cultivar

Cultivar	Treatment	Insect	Mortality
Sirvan	control	*Tc*	0.73 ± 0.07e
		Rd	1.90 ± 0.11e
	0.25	*Tc*	5.98 ± 0.20 cd
		Rd	7.40 ± 0.10 c
	1	*Tc*	13.45 ± 0.45 b
		Rd	19.23 ± 0.43 a
Mihan	control	*Tc*	0.48 ± 0.08 e
		Rd	1.13 ± 0.13 e
	0.25	*Tc*	5.53 ± 0.17 d
		Rd	7.28 ± 0.10 c
	1	*Tc*	12.65 ± 0.43 b
		Rd	13.33 ±0.57 b

Means followed by the same letter in the same column are not significantly different (Tukey's test, $P < 0.05$).

DISCUSSION

The present research elicited that the entomopathogenic fungus, *B. bassiana* strain EUT116 had a high potential for control of storage grain beetles including the lesser grain borer, *R. dominica* and the red flour beetle, *T. castaneum.* Also, there are further evidence in previous studies that illustrate the susceptibility of stored product pests to the pathogenic fungus, *B. bassiana*.

Several strains of *B. bassiana* were tested against *Sitophilus zaemais* Motschulsky (Col.: Curculionidae) and *Prostephanus truncatus* (Horn) (Col.: Bostrichidae) using submersion method (Kassa *et al.*, 2002). It clarified that some strains caused more than 90 % mortality on both storage grain beetles. The LT_{50} ranged from 3.58-6.28 and 2.85-4.05 days for *S. zaemais* and *P. truncates,* respectively that was similar to the present findings for *R. dominica* and *T. castaneum*. Santoro *et al*. (2008) showed that four isolates of *B. bassiana* had favorable lethal effect against the lesser mealworm *Alphitobius diaperinus* (Panzer) (Col.: Tenebrionidae). The fungus could even affect the eggs of *R. dominica* and *T. castaneum* by penetrating and decreased the hatchability of the eggs (Lord, 2009).

The outcomes obtained by Khashaveh et al. (2012) was in accordance with our results and explained that *B. bassiana* (BbWeevil™) was an appropriate candidate to control grain storage beetle pests such as *T. castaneum*. Surprisingly, BbWeevil™ was able to influence progeny production of the beetles (Khashaveh et al., 2012). Likewise, the mortality of *T. castaneum*, *S. granarius* and *Oryzaephilus surinamensis* (L.) (Col.: Silvanidae) were elevated by increasing the dose rate of the entomopathogenic fungus as well as the result of present study for *R. dominica* and *T. castaneum*.

A laboratory bioassay revealed that Iranian strains of *B. bassiana* had effective impact against adults of *R. dominica* (Mahdneshin et al., 2009) like *B. bassiana* strain EUT116 in this research. The values of LT_{50} of the previous bioassay (Mahdneshin et al., 2009) were higher than those of the present bioassay and it exhibited that *B. bassiana* strain EUT116 was more pathogenic to *R. dominica* than those Iranian strains. Also, it could be concluded from the value of LC_{50}. Because, the values of LC_{50} obtained for strain EUT116 were significantly lower on both tested wheat cultivars than those of Iranian strains of *B. bassiana* reported by Mahdneshin et al. (2009).

As a corollary of the present study, the type of wheat cultivar had influence on susceptibility of *R. dominica* and *T. castaneum* to *B. bassiana* strain EUT116. Plants could influence the entomopathogens by changing the contact rates between the pathogen and its insect host, as well as, altering the resistance of the pathogen and insect host (Elliot *et al*., 2000). Furthermore, the secondary metabolites existed in host plants could be toxic and might decrease the reproduction of herbivores (Rosenthal and Berenbaum, 1991). Consistent with our results, different host plants affected the interaction between the entomopathogenic fungus *B. bassiana* strain EUT106 and the fall armyworm, *Hyphantria cunea* (Drury) (Lep.: Arctiidae) (Zibaee *et al*., 2013).

In addition, Shikano *et al*. (2010) demonstrated that the susceptibility of the cabbage looper, *Trichoplusia ni* (Hübner) (Lep.: Noctuidae) to entomopathogenic virus, *T. ni* SNPV was increased when the larvae fed on cucumber than on broccoli. Because, the immune response and disease resistance of larvae to the pathogen were promoted by feeding on the cucumber.

The present study showed that *R. dominica* and *T. castaneum* had different susceptibility to *B. bassiana* strain EUT106 at the same fungal dose rate which was in accordance with the result obtained by Wakefield (2006) for *O. surinamensis* and *T. confusum* and it was related to the adherence and germination of the fungal conidia on the beetle cuticles.

According to our results, the entomopathogenic fungus, *B. bassiana* strain EUT116 was a promising candidate as a biocontrol agent to suppress the storage grain beetles, especially, *R. dominica*. But, further studies should be carried out to treat the beetles with the pathogenic fungus under store conditions. In addition, bioassay by submersion method was predicted that the Sirvan cultivar could increase the pathogenicity of the fungus against *R. dominica* and *T. castaneum*. Moreover, the highest mortality was obtained on the Sirvan cultivar by infecting the wheat seeds. It seems that planting the Sirvan cultivar in field can be more helpful to decrease the population of stored grain beetles rather than the Mihan in post-harvest conditions.

Overall, our findings were made obvious that there were multitrophic interactions among *B. bassiana* strain EUT116, the beetle pests, *R. dominica* and *T. castaneum* and the two cultivars, Sirvan and Mihan. To promote the efficacy of the biological control agent, focusing on these interactions should be undertaken.

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