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LABORATORY ASSESSMENT OF ENTOMOPATHOGENIC FUNGI *Beauveria bassiana* (Balsamo) TO CONTROL THE LESSER GRAIN BORER *Rhyzopertha dominica* (F.) ON WHEAT KERNELS.

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ABESTRACT

Effect of *Beauveria bassiana* on *Rhyzopertha dominica* parental adult mortality, F1 emerged adults, wheat kernels weight loss and damage were evaluated. Lesser grain borer, *R. dominica* adults were exposed to *B. bassiana* (BIOVER®) conidia powder admixed with wheat kernels in different six rates w/w (weight of *B. bassiana* conidial powder to weight of wheat kernels) and compared to untreated (check). Mortality percentage of *R. dominica* adults increased with increasing rates of *B. bassiana* after seven days. Significant differences were observed between all tested *B. bassiana* rates (P=0.0001). The maximum percentage of adult mortality after seven days of exposure was achieved at the highest rate compared to the untreated. Same trend was recorded after 14 days of exposure (P= 0.0001) at the previously mentioned rates compared to the untreated check. Negative relationship between *B. bassiana* rate and number of emerged *R. dominica* adults was recorded. Significant difference (P= 0.0001) was recorded between the untreated and the treated mean numbers of adult progeny emerged. The maximum adult progeny emerged was observed in the lowest rate (0.1% w/w) averaged 100.33 adult, while the minimum adult progeny emerged was recorded in the highest rate (1.0% w/w) averaged 16 adult compare to the untreated (225.33 adult). Weight loss means varied significantly among the tested rates of *B. bassiana*. Increasing *B. bassiana* rates led to significant decrease in weight loss on wheat treated. The minimum percentage of weight loss was recorded in the highest rate (1.0% w/w) compared to the untreated wheat which showed more feeding activities of the *R. dominica* beetles. In addition, the untreated wheat grains infested with *R. dominica* was significantly more damaged by the feeding of beetles than grains treated with *B. bassiana*. Generally, *B. bassiana* treated wheat kernels significantly decrease in mean numbers of F1 emerged adult, weight loss and damage compared to untreated wheat kernels. As an alternative control method of stored product insect pests, *B. bassiana* could be implicated as a part of integrated pest management (IPM) strategies.

Keywords: Lesser grain borer, *Beauveria bassiana,* wheat kernels, adult mortality, weight loss, damage

INTRODUCTION

Lesser grain borer *Rhyzopertha dominica* (F.), is a major insect pest of many stored grains, including wheat (Pedersen, 1992; Toews *et al.*, 2000; Jood *et al.*, 1993; Bashir, 2002), corn (Demianyk and Sinha, 1987; Jood and Kapoor, 1993), rice (Jilani *et al.*, 1989; Arthur *et al.*, 2007), sorghum (Jood and Kapoor, 1992; Jood *et al.*, 1993, 1996), as well as tubers such as cassava chip (Kumar *et al.,* 1996). Infestations of *R. dominica* cause loss of biomass (Brower and Tilton, 1973; Swaminathan, 1977) and decrease grain quality through feeding damage (Williams *et al.*, 1981) or contamination with

insect fragments and uric acid (Swaminathan, 1977) (Jood and Kapoor, 1993). Lesser grain borer, *R. dominica* infestation also reduces the essential amino acid content of wheat, maize, and sorghum (Jood *et al.,* 1995), and depresses the germination and vigor of seeds (Jilani *et al.*, 1989), and the infested grains is then vulnerable to further damage caused by secondary pests and fungi (Mukherjee and Nandi, 1993), and change dough properties of wheat and negatively affect the final bread quality through offensive odors and low loaf volume (Sa´nches-Marin˜ez *et al.*, 1997). Lesser grain borer is often difficult to be killed by using insecticides application directly to grains because the majority of the life cycle is spent inside the kernel (Arthur, 1992; Lorini and Galley, 1996; Huang and Subramanyam, 2005). 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 using chemical insecticides (Lorini and Galley; 1999; Zettler and Arthur 2000, and Batta 2005). Application of insecticides is one mean of preventing some losses during storage period. However, the choice of insecticides for storage pest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food (Padin *et al.,* 2002). The continuous use of chemical insecticides for control of storage grain pests has also resulted in serious problems such as resistance to the insecticides, pest resurgence, elimination of economically beneficial insects, and toxicity to humans and wildlife (Khan and Selman 1989; Adane *et al.* 1996; Padin *et al.,* 2002, and Hendrawan and Ibrahim 2006). These problems and the demand for pesticide free foods have triggered efforts to find alternative management options (Padin *et al.* 2002). Entomopathogenic fungi offer an alternative management strategy to chemical control. The potential of entompathogenic 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). Isolates of *B. bassiana* and *M. anisopliae* are potential microbial control agents against some stored product pests. Mixtures of *Metarhizium anisopliae* conidial suspensions with those of *B. bassiana* have been reported to be effective against *Sitophilus oryzae* (L.) of wheat grains (Batta and Abu Safieh, 2005). *Beauveria bassiana* application at lower rate achieved 80% mortality to rice weevil, *Sitophilus oryze* (Hendrwan and Ibrahim, 2006). *Beauveria bassiana* has proven highly effective against the major stored grain insects: *S. oryze*, *R. dominica*, and *Tribolium castaneum* (H.) (Dal-Bello *et al*., 2001).

The objectives of this study was to evaluate the efficacy of the entomopathogenic fungi *B. bassiana* on the biological aspects (parental adult mortality, F1 emerged adults, wheat kernels damage and weight loss) of *R. dominica*.

MATERIALS AND METHODS

This study was carried out in the Entomology Research Laboratory in Department of Plant Protection, Faculty of Agriculture, Al-Azhar University (Cairo, Egypt).

Insect rearing

Adult of *R. dominica* obtained from entomology research laboratory in the Department of Biological Control, Plant Protection Institute, Agriculture Research Center (ARC), Dokki, Giza, Egypt. Grains were stored at -5°c for one week to eliminate natural unwanted infestations before inoculation with adult beetles. Rearing of incubated *R. dominica* in an environmental controlled condition at temperature 25±1°C and 60±5% relative humidity (RH) at the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University. The adults of *R. dominica* were reared on dried healthy and matured wheat grains. Reared insects were placed in plastic pots (15 cm diameter and 20 cm deep). The pots were then covered with muslin fastened by a rubber-band to prevent the escape of insects and to ensure the proper ventilation.

Fungal treatments

Beauveria bassiana (BIOVER®) were obtained from the fungal culture produced by the bioinsecticides poroduction unit, Plant Protection Institute, ARC, Dokki, Giza, Egypt. BIOVER® Biological insecticide produced in a rate of 32000 viabk spore/mg (10 % active ingredient and 90 inert ingredients). *Beauveria bassiana* was applied in rates of 0, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.0% w/w (weight of *B. bassiana* conidial powder to 50 g weight of wheat kernels).

Bioassay

The six different fungal conidia rates of BIOVER® were admixed with 50 g wheat kernels. Twenty new emerged adults were introduced to each plastic pot. The experiment was replicated three times, and pots were kept in an environmental condition 25°±1C and 60±5 RH%. The bioassay was carried out to verify the adult mortality% after 7 and 14 days of exposure. Mortality assessment was then made by counting dead and living adult insects and percentage of adult mortality was calculated for treated and untreated wheat kernels. Adult emergence (Progeny emergence %) was determined by counting the number of all visible F1 adults found in wheat. The effect of the *B. bassiana* on the weight loss percentage of wheat kerenls was also determined after excluding all insect stages, frass and dust from the kernels. Percentage of weight loss was calculated using weight loss percentage equation by Khare and Johari (1984). Wheat kernels damage percentages also determined by inspect the damaged and undamaged kernels of 100 wheat kernel in each treatment.

Statistical analysis

The effect *B. bassiana* on *R. dominica* parental adult mortality, F1 emerged adults, wheat kernels damage and weight loss were subjected to statistical analysis by Analysis of variance (ANOVA) test and using a computer software SAS (SAS Institute, 2000). Means were determined and compared by Duncan multiple range test at 0.05% probability level (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of fungal entomopathogen *B. bassiana* on parental adult mortality:

Mortality rates of *R. dominica* adults increased with increasing rate of *B. bassiana* after seven days (F=96.15, P=0.0001). The maximum percentage of adult mortality after seven days of exposure observed at the highest rate (1.0% w/w) averaged 33.33%. While, the lowest was observed at the lowest rate (0.01% w/w) averaged 3.33% compared to the untreated (1.66%). The same trend was recorded after 14 days of exposure (P= 0.0001, F= 165.23) by 81.66 and 20.0%, respectively at the previously two mentioned rates compared to the untreated (check) (8.33%). The increased mortality percentage after 14 days may due to the latent effect of the *B. bassiana* fungal conidia which takes time to penetrate through the cuticle of the insect. The current results agree with Mahdneshin *et al.* (2009), who cleared that the application of *B. bassiana* had a great impact on the adult of *R. dominica* that achieved a significantly high cumulative mortality percentage averaged 89.35%. Application of *B. bassiana* at the lowest rate of 0.05 g thoroughly mixed with long grain rice resulted in excess of 80% mortality to the adult rice weevils by the 7^{th} day of exposure (Hendrawan and Ibrahim 2006).

Table (1): Mortality percentage of *R. dominica* adults exposed to wheat treated with *B. bassiana*.

B. bassiana **rate (w/w)**	**Mean Mortality %**	
	7d	**14d**
0	1.66±1.15a	8.33±2.08a
0.1	3.33±1.52a	20.0±3.60b
0.3	13.33±1.52b	31.33±3.51c
0.5	16.66±3.05b	36.66±2.51c
0.7	21.66±2.08c	53.33±4.16d
0.9	24.0±0.20c	68.33±3.05e
1	33.33±2.1d	81.66±5.03f
P=0.05	0.0001	0.0001
F-Value	96.15	165.23
LSD	3.50	6.20

Means followed by the same letter in a column are not significantly different at 5% level of probability (Duncan's Multiple Rang Test)

Effect of fungal entomopathogen *B. bassiana* on adult progeny emergence:

Negative relationship between *B. bassiana* rate and number of emerged *R. dominica* adults was recorded. The maximum adult progeny emerged was observed in the lowest rate (0.1% w/w) averaged 100.33 adult, while the minimum adult progeny emerged was recorded in the highest rate (1.0% w/w) averaged 16 adult compared to untreated (225.33 adult). Significant difference (P= 0.0001) was recorded between untreated and the treated mean numbers of adult progeny emerged (Table 2). In addition,

regardless to the rate, a significant difference of F1 emerged adults between the untreated and treated wheat kernels was observed averaged 225.00 and 50.56, respectively (Table 3 and Figure 1). Results agree with Batta (2005) who reported that treated wheat kernels with the entomopathogenic fungi, *Metarhizium anisopliae* reduced the emergence of F1 adults. The fungus thus delayed adult emergence of *R. dominica* by 8-12 days and gives promise for control with *M. anisopliae* mixed with wheat flour. Similarly, Cherry *et al.* (2005) indicated that *Callosobruchus maculates* (L.) exposed to wheat grains treated with *B. bassiana* reduced F1 emergence relative the untreated population. Wakefield (2006) illustrated that *B. bassiana* have been shown to produce germ tubes that grow over the surface of the insect cuticle until they contact an area of relative weakness where penetration can easily be achieved. This study has shown that the early stages of fungal infection may play a key role in the susceptibility of storage beetles to some isolates of *B. bassiana* (Pekrul and Grula, 1979; Butt et al, 1995).

Table (2): Effect of *B. bassiana* on the mean number of F1 progeny, percentage of wheat kernels weight loss and damage.

***B. bassiana* Rate (w/w)**	**F1 emerged Adults**	**Wight Loss %**	**Wheat kernels damage %**
0	225.33±8.0a	56.33±6.85a	58.66±10.61a
0.1	100.33±6.24b	43.80±6.09b	45.6±8.97b
0.3	96.33±13.0b	37.77±5.61b	37.07±3.26bc
0.5	41.67±14.22c	28.43±2.91c	26.80±7.76cd
0.7	27.0±6.24cd	24.40±2.63c	22.9±2.72d
0.9	22.33±3.06d	15.97±3.02d	17.13±3.98de
1	16.00±9.64d	11.90±1.18d	12.13±5.41e
P=0.05	0.0001	0.0001	0.0001
F-Value	188.61	36.92	19.59
LSD	16.44	7.87	10.54

Means followed by the same letter in a column are not significantly different at 5% level of probability (Duncan's Multiple Rang Test)

Effect of fungal entomopathogen *B. bassiana* on weight loss:

The insecticidal effect of *B. bassiana* was tested by measuring the weight loss of wheat kernels exposed to *R. dominica*. The untreated wheat grains infested with *R. dominica* was significantly more damaged by the feeding of beetles than grains treated with *B. bassiana*. In addition, weight loss means varied significantly (P=0.0001) among the tested rates of *B. bassiana*. Increasing *B. bassiana* rates led to significant decrease in weight loss on wheat treated. The maximum percentage of weight loss observed in the lowest rate (0.1% w/w) averaged 43.8%, whereas, the minimum percentage of weight loss was recorded in the highest rate (1.0% w/w) averaged 11.90% compare to untreated wheat (56.33%) which showed more feeding activites of the *R. dominica* beetles. Regardless to the *B. bassiana* rate a significant difference between the untreated and treated wheat kernels was observed averaged 56.33 and 27.04%, respectively (Table 3 and Figure

1). In this respect, Rodrigues and Pratissoli (1990) reported that 6 months protection of maize and beans from damages from *Sitophilus zeamais* (Motsch.). Cherry *et al.* (2005) also have demonstrated that different isolates from *B. bassiana* can provide a good control of *C. maculatus.* On the other hand, Hendrawan and Ibrahim (2006) recorded that admixtures *B. bassiana* with long grain rice significantly reduced grain weight loss caused by *S. oryzae* infestation after four months of storage.

Effect of fungal entomopathogen *B. bassiana* on kernel damage:

Results in table (2) revealed that the insecticidal influence of *B. bassiana* on damage percentage caused by the feeding activities of *R. dominica* insects were clearly noticed in the highest rate of *B. bassiana* (1.0% w/w) that showed the greatest protection to wheat kernels with low percent of feeding damage percentage averaged 12.13%. Results also showed that feeding damage percentage increased gradually as rate decreased. Regardless to the rate significant difference (P=0.0015) of damage percentage was recorded between treated wheat kernels (26.94%) by *B. bassiana* and untreated kernels (58.66%) (Table 3 and Figure 1). Results are in agreement with the findings of Hendrawan and Ibrahim (2006) who indicated that fungal formulations of *B. bassiana* in kaolin and talc provided better protection against the rice weevil by giving a reduced damage percentage significantly compared to the unformulated control. Percentage of wheat durum weight loss caused by *Tribolium castaneum* (H.) feeding decreased by 81.5% and was significantly smaller than the loss from the untreated grain (Padin *et al.* 2002).

In conclusion**,** *B. bassiana* showed a promising alternative control method that can be implicated in the Integrated Pest Management (IPM) of lesser grain borer and generally for stored product insect pests.

Table (3): General effect of *B. bassiana* on the wheat kernels F1 emerged adults (a), weight loss percentage, (b), damage % (C) of *R. dominica* compared to the untreated.

Treatment	F1 emerged adults	Wight Loss %	Wheat kernel damage %
Untreated	225.00a	56.33a	58.66a
Treated	50.56b	27.04b	26.94b
P=0.05	0.0001	0.0003	0.0015
LSD	30.75	7.15	10.47

Means followed by the same letter in a column are not significantly different at 5% level of probability (Duncan's Multiple Rang Test)

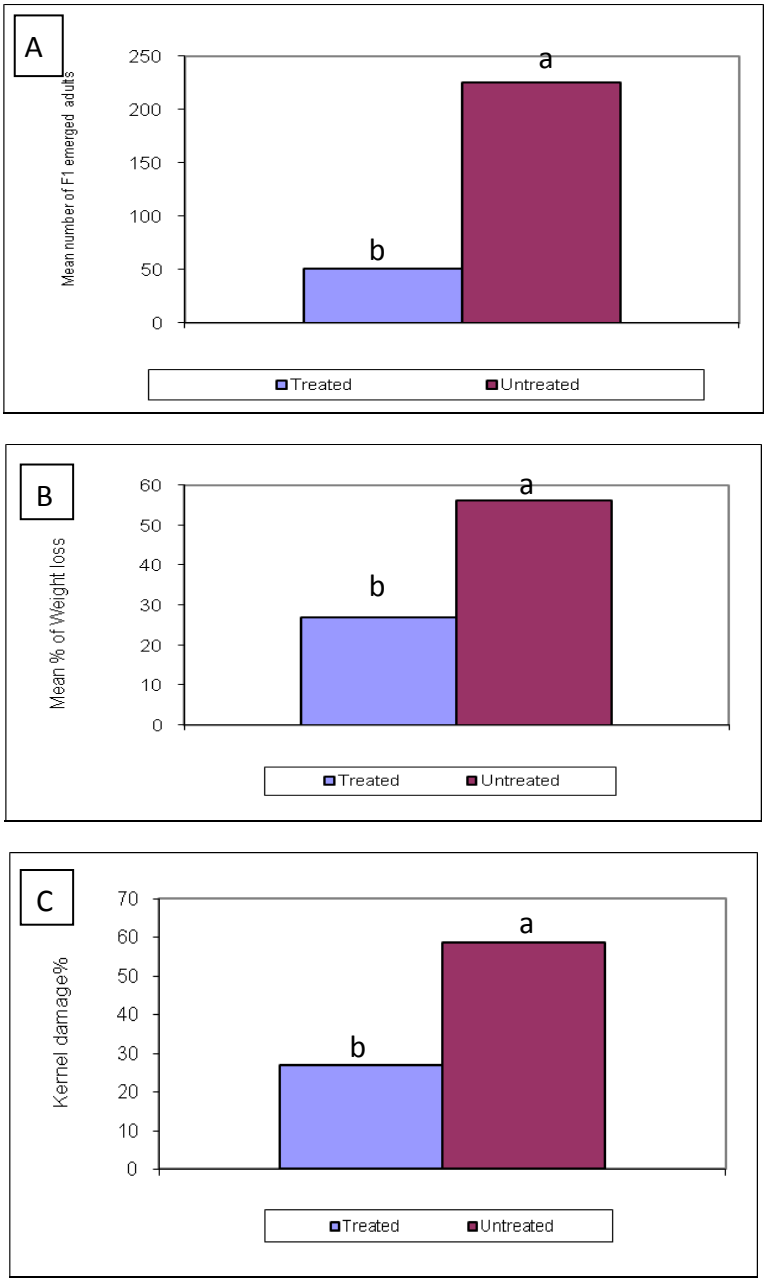

Figure (1): Overall effect of *B. bassiana* on the F1 emerged adults (A), kernels weight loss percentage (B) and damage percentage (C) of *R. dominica*.

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التقييم المعملى لفطر *Beauveria bassiana* (Balsamo) فى مكافحة حشرة ثاقبة الحبوب الصغرى *Rhyzopertha dominica* (F.) على حبوب القمح

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أجريت الدراسة لتقييم التأثيرات المختلفة لفطر *Beauveria bassiana* على حشرة ثاقبة الحبوب الصغرى من حيث نسبة الموت فى الآباء وعدد حشرات الجيل الأول الناتجة والفقد فى وزن حبوب القمح وكذلك الضرر اللاحق بالحبوب. ثم تعريض الحشرات البالغة لفطر *B. bassiana* والمنتج تجارياً باسم BIOVER® فى شكل مسحوق تم خلطه بحبوب القمح فى معدلات مختلفة (وزن من مسحوق كونيديا الفطر إلى وزن من حبوب القمح) بالمقارنة بغير المعامل . أظهرت النتائج أنه بزيادة معدلات الفطر أدى إلى زيادة فى نسبة الموت للآباء من الحشرات بعد ٧ أيام من المعاملة وقد كان هناك فروقاً معنوية بين جميع معدلات الفطر المختبرة حيث كانت أعلى نسبة موت للآباء عند أعلى معدل للفطر المميت مقارنة بغير المعامل وكذلك بالنسبة لنسبة الموت بعد ١٤ يوماً. دلت النتائج على وجود علاقة عكسية بين معدل الفطر وعدد الحشرات البالغة الناتجة فى الجيل الأول ووجدت فروقاً معنوية بين الحشرات المعاملة وغير المعاملة بغض النظر عن معدلات الفطر وكانت زيادة الأعداد الناتجة عند أقل معدل للفطر وذلك بمتوسط ١٠٠.٣٣ حشرة بالغة بينما كان العدد الأقل الناتج عند المعدل الأعلى للفطر وذلك بمتوسط ١٦ حشرة بالغة مقارنة بغير المعامل ٢٢٥.٣٣ حشرة بالغة . إن نسبة الفقد فى الحبوب قد تباينت بشكل معنوى باختلاف معدلات الفطر، وقد دلت الدراسة على أنه بزيادة معدلات الفطر أدت إلى انخفاض معنوى فى نسبة الفقد فى وزن حبوب القمح المعامل وكانت أقل نسبة الفقد فى وزن الحبوب تم تسجيلها عند أعلى معدل للفطر مقارنة بغير المعامل والذى لوحظ فيه نشاط تغذية زائد لحشرات ثاقبة الحبوب الصغرى وكذلك لوحظ ضرراً أعلى لحبوب القمح الغير معاملة مقارنة بغير المعامل وعموماً قد بينت النتائج أن معاملة حبوب القمح بالفطر الممرض أدى إلى خفض أعداد الحشرات البالغة الناتجة للجيل الأول ونسبة الفقد فى الوزن للحبوب وكذلك الضرر الناتج من تغذية الحشرات على حبوب القمح مقارنة بغير المعامل وعلى ذلك فإن الفطر الممرض تحت الاختبار *B. bassiana*. يوصى باستخدامه كأحد الطرق البديلة فى مكافحة آفات الحبوب المخزونة الحشرية وإدراجه ضمن استراتيجيات المكافحة المتكاملة.

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