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EFFICACY OF THE ENTOMOPATHOGENIC FUNGUS *Beauveria bassiana* (Balsamo) AGAINST *Tribolium confusum* (Duval) ON STORED WHEAT FLOUR

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ABSTRACT

The red flour beetle, *Tribolium confusum* (Duval) adults were exposed to *Beauveria bassiana* (BIOVER®) conidia in six rates w/w (weight of *B. bassiana* conidial powder to weight of wheat flour) and compared to untreated. The effectiveness of *B. bassiana* on *T. confusum* parental adult mortality, F1 and F2 emerged adults, duration of life cycle and weight loss of wheat flour were evaluated. Results showed that gradual positive lethal effect on *T. confusum* adults (mortality increased as the rates of *B. bassiana* increased). There was a significant difference between the untreated and the treated in 7 and 14 days after exposure (P=0.041 and P=0.0015), respectively. There was an inverse relationship between progeny production of *T. confusum* and *B. bassiana* rate. Regardless to the *B. bassiana* rate a significant difference of F1 and F2 emerged adults was observed between the untreated and treated wheat flour averaged 204.33 & 126.33 and 57.28 & 24.22, respectively. Prolongation of *T. confusum* life cycle duration increased gradually as the rate of *B. bassiana* increased. The treatment with highest *B. bassiana* rate (1.0% w/w) significantly prolonged duration of the insect life-cycle (48.67 days) with about 7 days delay of duration compared to the duration of the insect life-cycle in the untreated averaged 41.0 days. There was a significant drop (P=0.0001) of the weight loss of treated wheat flour with highest *B. bassiana* rate compared to the untreated. Generally, *B. bassiana* may applied as an alternative to chemical control method in the strategy of Integrated Pest Management (IPM) of stored product insect pests.
Keywords: *Beauveria bassiana,* wheat flour, *Tribolium confusum*, biocontrol

INTRODUCTION

The red flour beetle, *Tribolium confusum* (Duval) is cosmopolitan and considered one of the major stored-product insects of sound and processed grains with high rate of movement among food patches (Ziegler 1976), (Campbell and Hagstrum, 2002). Significant losses were reported to be caused by this insect on grains and its by products throughout the world**.** The most frequent control measure of this insect and other stored-product insects is usually by fumigation of cereal-grain processing facilities (Tebbets *et al.* 1986) and (Zettler & Arthur 2000). Although of its effectiveness, some of the fumigants are considered by EPA (1993) as ozone-layer depleting substance so it is subjected to be prohibited or substituted with other substances. Insecticidal treatments with malathion (Arthur and Zettler 1991 and 1992), deltamethrin (Arthur, 1997), cyfluthrin (Arthur, 1994 and 1999), bioresmethrin (Ardley, 1976), and chlorpyrifos-methyl (LaHue, 1997) also gave good control of red flour beetles and other stored-product insects. However, the resistance to repeatedly applied insecticides and some fumigants such as phosphine has developed, in addition to appearance of environmental and human health concerns as a result of insecticide application (Zettler, 2000; Arthur and Zettler, 1992). Heat treatment, controlled atmospheres, aeration and cooling technologies could be offered as alternative control measures of stored grain insects (Donahaye, 2000).

Until present, limited number of published articles on biocontrol of stored graininsects using entomopathogenic fungi are available. Entomopathogenic fungus; *Beauveria bassiana*, for example, has proven highly effective against the major stored grain insects: *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Tribolium castaneum* (Padin *et al.* 1996 and Dal-Bello *et al.* 2001). In contrast, *Metarhizium anisopliae* has been less frequently reported for control of stored-grain insects although it has been used effectively to control other insects especially termites (Quarles, 1995). Mixtures of *M. anisopliae* conidial suspensions with those of *B. bassiana* have been reported to be used against *S. oryzae* on wheat grains. Various combinations of *M. anisopliae* conidia with four types of dust carriers were used effectively against *S. oryzae* infesting stored wheat grains (Batta, 2004). The aim of this study was to assess the efficacy of the entomopathogenic fungus *B. bassiana* on the different biological of *T. confusum*.

MATERIALS AND METHODS

Culturing of insects

Adults of *Tribolium confusum* were obtained from Plant Protection Research Institute, Agriculture research center, Dokky, Giza, Egypt. The adult beetles were introduced into wheat flour to which 5% yeast according to the rearing method of Park and Frank (1984). The emerging adults were transferred into another jar and reared in an environmental controlled condition at temperature 25^{o}±1C and 60±5 RH%. Insect reared were placed in plastic cups (15 cm diameter and 20 cm deep). The cups were then covered with muslin or cheese-cloth fastened by a rubber-band to prevent the escape of insects and to ensure the proper ventilation. Newly emerged adult insects (males and females) obtained from the culture were used in the experiments.

Fungal treatments

The entomopathogenic fungus *B. bassiana* (BIOVER®) were obtained from the fungal culture produced by the bioinsecticides poroduction unit, Plant Protection Research Institute, Agriculture research center (ARC), Dokky, Giza, Egypt. The BIOVER® Biological insecticide produced in a rate of 32000 viabk spore/mg (10 % active ingredient and 90% inert ingredients). *B. bassiana* applied in rates of 0, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.0% w/w (weight of *B. bassiana* and weight of wheat flour).

Bioassay

Six different fungal conidia rates of (BIOVER®) were mixed with 50 g wheat flour for each rate. Twenty newly emerged adults were introduced to each cup. The experiment was replicated three times, cups were kept under laboratory conditions of 25^{o}±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 flour. Adult emergence (Progeny emergence %) was determined by counting the number of all visible F1 and F2 adults found in wheat flour. Duration period evaluation of the treatment effect on life cycle of *T. confusum* was done by removing the surviving adult insects from cups in all replicates 21 days after the treatment. This time period is enough for egg deposition in all replicates. The plastic cups which represent the treatment replicates were then incubated under the laboratory conditions previously mentioned until adults emergence of the new generation. The duration of life cycle or time period needed for production of one generation of *T. confusum* for each treatment was calculated. The effect of the *B. bassiana* on the weight loss percentage of wheat flour was also determined after excluding all insect stages, frass and dust from the wheat flour. Percent of weight loss was calculated using weight loss% equation by Khare and Johari (1984).

Statistical analysis

The effects *B. bassiana* on *T. confusum* parental adult mortality, F1, F2 emerged adults, duration period and wheat flour 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

Adult mortality:

Results in table (1) showed that *B. bassiana* caused a gradual positive lethal effect on *T. confusum* adults (mortality increased as rate of *B. bassiana* increased). There was a significant difference between the untreated and the treated in 7 and 14 days after exposure (P=0.041 and P=0.0015, respectively). The maximum percentage of adult mortality was achieved in the highest rate (1.0% w/w) averaged 30 and 58.33%, while the minimum percentage mortality was recorded in the lowest rate (0.1% w/w) averaged 6.66 and 16.66%, respectively, compare to 1.66 and 5% in the untreated. From these results it is obvious that the highest degree of mortality (58.33%) was achieved at the highest *B. bassiana* rate (1.0% w/w) after 14 days of exposure, this may be due to the latent effect of *B. bassiana* because conidia takes period to penetrate through spiracles and insect cuticle. Wakefield (2006) illustrated the mode of action of *B. bassiana,* and reported that contact of *B. bassiana* conidia on the insect surface 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. Results agreed with Batta (2008) who cleared that adults with the formulated fungi resulted in a significantly higher mean mortality percentage compared to the treatment with the unformulated fungi. Treatments with a combination of fungal conidia mixture caused a significant high percentage of *T. castaneum* adult mortality averaged 53.3% (Batta and Abu Safieh 2005). 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% (Mahdneshin *et al.* 2009).

Table (1): Mortality percentage of *T. confusum* adults exposed to wheat flour treated with *B. bassiana*.

B. bassiana rate (w/w)	Mortality %	
	7d	14d
0	1.66±0.58a	5.0±1c
0.1	6.66±0.50ab	16.66±1.53bc
0.3	6.66±0.58ab	20.0±2bc
0.5	10.0±1.73ab	25.0±.2bc
0.7	16.66±1.53abc	30.0±2abc
0.9	23.33±2.08ab	38.33±2.08ab
1	30.0±4.0d	58.33±2.65a
P=0.05	0.041	0.0015
F-Value	3.05	6.87
LSD	3.54	3.42

Means in a column with the same letter are not significantly different

Influence of *B. bassiana* on *T. confusum* progeny production:

Highly significant impact of *B. bassiana* was clearly effective in suppressing progeny production of *T. confusum* (P = 0.0001). A relatively high mean of 204.33 progeny of *T. confusum* produced on untreated wheat flour, while few numbers of F1 progeny emerged (23.33) were produced at the highest *B. bassiana* rate (1.0% w/ w). Whereas, 103.0 adult progeny were observed at the lowest rate (0.1% w/w). There was an inverse relationship between progeny production and *B. bassiana* rate for *T. confusum* (number of F1 emerged adults were reduced as rate increased). Regardless to the *B. bassiana* rate a significant difference (P=0001) between the untreated and treated wheat flour was observed averaged 204.33 and 57.28, respectively (Table 3 and Figure 1). On the other hand, the second generation F2 of *T. confusum* progeny extremely affected by *B. bassiana* application compared to F1 progeny. Significant reduction (P=0.0001) was resulted in the highest *B. bassiana* rate (6.33 adult) compared to the untreated 126.33 adult (Table 2).

Table (2): Effect of *B. bassiana* on the mean number of F1, F2 progeny, duration period and percentage of wheat flour weight loss of *T. confusum*.

B. bassiana rate (w/w)	F1 Emerged adult	Duration Period	Weight loss %	F2 Emerged adult
0	204.33±7.77a	41.0±1.0a	50.15±4.36a	126.33±34.56a
0.1	103.0±12.04b	41.67±1.52a	39.07±4.25b	54.0±7.54b
0.3	80.673±5.51c	42.33±1.53ab	37.24±3.09b	36.33±11.50bc
0.5	53.33±11.59d	43.67±1.15ab	36.41±3.27b	29.33±4.69bcd
0.7	55.0±7.0cd	45.33±2.08bc	29.68±3.06c	11.33±4.0cd
0.9	28.33±3.5e	47.0±2.64cd	22.91±2.41d	8.0±2.41d
1	23.33±7.57e	48.67±1.53d	16.03±4.31e	6.33±2.08d
P=0.05	0.0001	0.0005	0.0001	0.0001
F-Value	166.39	8.38	28.46	25.89
LSD	14.61	3.01	6.39	25.22

Means in a column with the same letter are not significantly different

Furthermore, the reduction of F2 progeny may also due to the shortage of food in each container and the competition between the *T. confusum* individuals that represent a double impact on suppression of *T. confusum* population. This results are in agreement with the findings of Cherry *et al*. (2005) who indicated that *C. maculatus* exposed to wheat grains treated with *B. bassiana* reduced F1 emergence relative the untreated population. Batta (2005) reported that treated wheat kernels with the entomopathogenic fungi *M. anisopliae* reduced the emergence of F1 adults.

Influence of *B. bassiana* on duration period:

The treatment with *B. bassiana* showed a significant (P=0.0005) effect on duration of *T. confusum* life-cycle. As shown in table (2), the treatment with highest *B. bassiana* rate (1.0% w/w) significantly prolonged duration of the insect life-cycle (48.67 days) with about 7 days delay of duration compared to the duration of the insect life-cycle in the untreated averaged 41.0 days. Prolongation of *T. confusum* increased gradually as the rate of *B. bassiana* increased. Generally, duration period of *T. confusum* regardless to the *B. bassiana* rate was different in treated flour to that in untreated one (41.0 and 44.78 days), respectively (Table 3 and Figure 1). Results are in agreement with Batta and Abu Safieh (2005) who stated that significant longer durations of *T. castaneum* life-cycle were obtained after treatment with charcoal + fungal conidia (54.7 days) and chalk powder + fungal conidia (60.0 days) compared with treatments with charcoal and chalk powder alone (48.0 and 53.7 days, respectively). This demonstrates clearly the effect of treatment on retardation of the insect development by > 6 days. Also results strongly agreed with Batta (2005) who reported that the fungus delayed adult emergence of *R. dominica* by 8-12 days and gives promise for control with *M. anisopliae* mixed with wheat flour

Influence of *B. bassiana* on wheat flour weight loss percentage:

After seven weeks of *T. confusum* adults introduction the treatment with fungus led to decreasing in weight loss (P=0.0001) of the wheat flour compared to that untreated one. Wheat flour treated with highest *B. bassiana* rate (1.0% w/w) averaged 16.03% compared to (50.15%) in the untreated (Table 2). However, the lowest rate *B. bassiana* resulted in weight loss was (39.07%).

Table (3): General effect of *B. bassiana* on the F1 emerged adults (a), Duration period (b), wheat flour weight loss percentage (C) and F2 emerged adults (d) of *T. confusum* compared to the untreated.

Treatment	F1 emerged adults	Duration period (days)	Weight Loss %	F2 emerged adults
Untreated	204.33a	41.0a	50.15a	126.33a
Treated	57.278b	44.78a	30.22b	24.22b
P=0.05	0.0001	0.48	0.0057	0.0001
LSD	6.84	13.41	10.26	9.35

Means in a column with the same letter are not significantly different

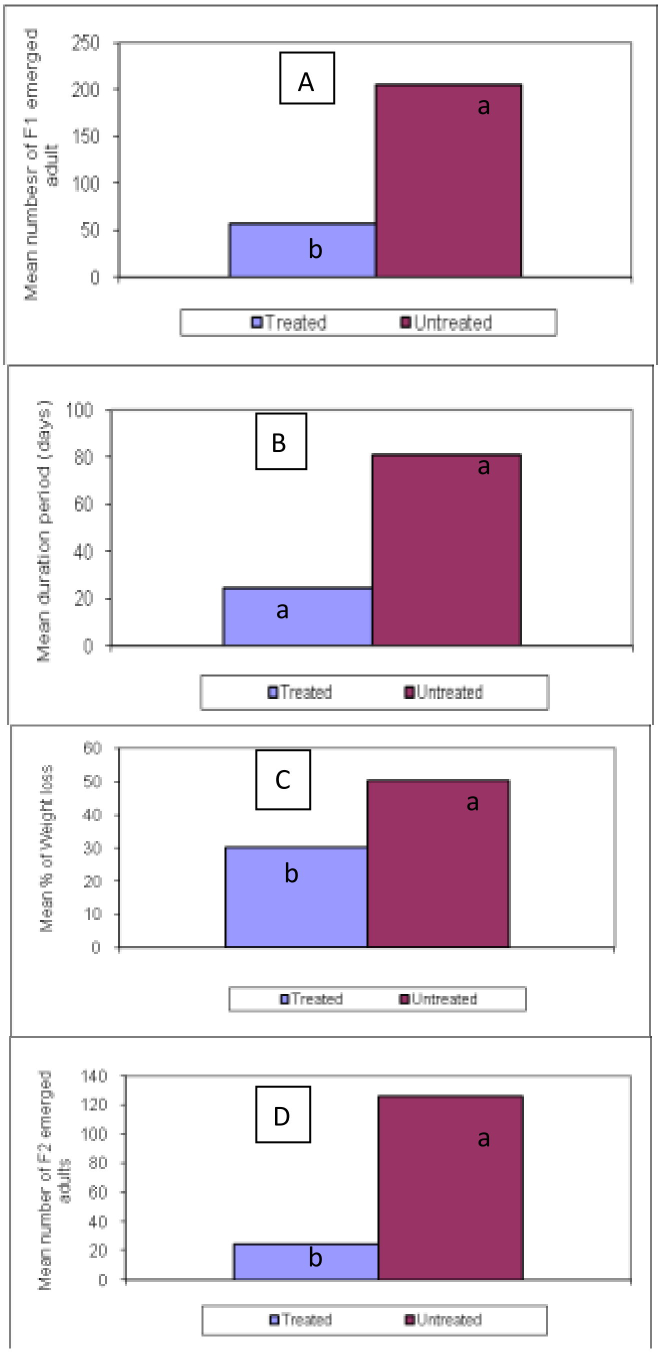

Figure (1): Overall effect of *B. bassiana* on the F1 emerged adults (A), Duration period (B), wheat flour weight loss percentage (C) and F2 emerged adults (D) of *T. confusum.*

These results indicated that *B. bassiana* has a potential effect as a bioagent on *T. confusum* feeding activity that resulted in reduction of wheat flour weight loss. In general, significant difference between the untreated and treated wheat flour was observed (P=0.0057) regardless to the *B. bassiana* rate averaged 50.15 and 30.22%, respectively (Table 3 and Figure 1).

Our results are in agreement with the finding of Padin *et al.* (2002) who indicated that percentage of treated wheat durum weight loss caused by *T. castaneum* feeding decreased by 81.5% and was significantly smaller than the loss from the untreated grain. Hendrawan and Ibrahim (2006) 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.

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كفاءة الفطرالممرض ***Beauveria bassiana* (Balsamo)** فى مكافحة خنفساء الدقيق المتشابهة ***Tribolium confusum* (Duval)**

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تم تعريض الحشرات البالغة لخنفساء الدقيق المتشابهة لست معدلات من مسحوق كونيديا الفطر والمنتج تجارياً باسم ®BIOVER (وزن/وزن) وزن من مسحوق كونيديا الفطر إلى وزن من دقيق القمح مقارنة بالغير المعامل . وقد أجريت الدراسة لتقييم فاعلية الفطر الممرض على حشرة خنفساء الدقيق المتشابهة من حيث نسبة الموت فى الآباء من الحشرات البالغة وعدد الحشرات البالغة الناتجة فى الجيلين الأول والثانى وطول فترة دورة الحياة وكذلك الفقد فى وزن الدقيق ، وقد أظهرت النتائج أنه توجد علاقة طردية موجبة تدريجية بين التأثير القاتل للحشرات البالغة وزيادة معدلات الفطر المختبرة . وكانت هناك فروقاً معنوية فى نسبة الموت فى الحشرات البالغة للآباء بين المعامل وغير المعامل بعد 7 ، 14 يوم من التعريض للفطر الممرض . وجدت علاقة عكسية بين الذرية الناتجة من الحشرات البالغة ومعدل الفطر المميت، وبينت النتائج أن هناك فرقاً معنوياً بين أعداد حشرات الجيلين الأول والثاني فى غير المعامل (204.33 ، 126.33) والمعامل (57.28 ، 24.22) لكلاً من الجيلين الأول والثاني على التوالى ، وزيادة فترة دورة الحياة بشكل تدريجى بزيادة معدلات الفطر المختبرة وكان أطول فترة لدورة الحياة قد تم تسجيلها عند أعلى معدل للفطر بمتوسط 48.76 يوم وذلك بزيادة حوالى 7 أيام فى المعامل مقارنة بغير المعامل (41 يوم) . كان هناك انخفاضاً معنوياً فى نسبة الفقد بالدقيق المعامل فى المعدل الأعلى للفطر المميت مقارنة بغير المعامل ، وبوجه عام ، توصى تلك الدراسة بإمكانية استخدام الفطر *B. bassiana* كإحدى البدائل الآمنة بيئياً عن المكافحة الكيميائية وذلك بإدراجها ضمن استراتيجية المكافحة المتكاملة لآفات المنتجات المخزونة .

قام بتحكيم البحث

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