

SHORT ARTICLES / INVESTIGACIÓN

Evaluation of the efficacy of *Beauveria bassiana* filtrate in controlling the beetle *Everts TragoDerma granarium* (Coleoptera: Dermestidae) *in vitro*

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Abstract: The present study was performed to test the impacts of the fungus *B. bassiana* filtrate, at 25, 50, and 75% concentrations, on the larvae of the second and fourth instars of *T. granarium*. The study showed the superiority of the fungus filtrate at the concentration of 75% when treating the larvae of the second and fourth instars, with mortality rate values of 45.0% and 33.3%, respectively, after 7 days of treatment and diagnosis of several chemical compounds of different chemical groups for the filtrate fungus *B. bassiana* using the GC mass.

Key words: *TragoDerma granarium*, *Beauveria bassiana*, GC mass.

Introduction

Stored materials, particularly those composed of cereal grains and their products, are of major importance to the food security of the entire global population, providing the necessary calories for daily consumption. Stored materials are among the main protein sources essential for human nutrition¹. The wheat species *Triticum aestivum* L. is among these crops, constituting one-third of the world's grain production. The importance of wheat lies in its nutritional value, being the main source of carbohydrates, including starch. One of the problems facing cultivating different crops in developing countries is the losses that occur after the harvest and during the storage period. These crops are exposed to damage due to infections with various warehouse pests². One of the most important pests that attack crops are the hairy grain beetle *T. granarium Everts* (Khabra), considered one of the most dangerous and pesticide-resistant insects³. It attacks the stored grains and their products, which suffered tremendous damage due to their usage as food by the larvae. The infection is usually determined by the presence of the pest, whether adults or larvae and molting skins⁴. To reduce the damages caused by this insect, several means were used, the chemical pesticides were the most common. But several problems have been encountered upon applying these pesticides, including the threats resulting from the residuals of pesticides or used chemicals and their economic cost. Therefore, Researchers have developed Modern methods, the latest of which was nano pesticides, to control stored pests⁵. And among the other ways, biological control by entomopathogenic, one of the best alternatives to chemical pesticides, is safe, environmentally friendly, and has equal efficacy. The fungus *Beauveria bassiana* is a biological control agent used on a large scale to control insect stored such as *Sitophilus granarius*⁶. This study aims to test the effects of crude filtrate of *B. bassiana* on some aspects of the biological performance of *T. granarium* beetle *in vitro* and to detect the active compounds in the filtrate by using the GC-mass technique.

Materials and methods

Collection and breeding of *T. granarium*

The hairy grain beetles were obtained from the laboratories of the Ministry of Science and Technology, Baghdad, Iraq. Healthy wheat grains were cleaned of impurities and placed in the freezer for 72 hours at -20 °C to ensure they were internally free from other insect infections. Ten adult beetle pairs of males and females were placed on 1 kg of healthy wheat grains with 5 g of dry yeast in plastic bottles of 100 ml volume and 10 cm diameter. The bottle opening was covered with organza fabric and sealed with a rubber band to prevent adults' escape. The renewal of the culture was occasionally ensured⁷. The insect was identified by an expert in the Natural History Research Center and Museum / the University of Baghdad.

Preparation of Fungal Filtrates

The entomopathogenic fungus *B. bassiana* was obtained from the Laboratory of Insects, Department of biological control, Al-Furat Al-Awsat University; the fungus were cultured on potato dextrose agar at 25 ± 1 °C for 15 days before the initiation of the experiment. Two discs (7 mm diameter) were cut from the growing pathogen, placed in a sterile flask containing 500 mL of potato dextrose broth, and incubated at 27 °C for 21 days. The filtrates were sterilized using the Millipore sterile filter (Millipore 0.45 µm syringe filter). The filtrate was considered a stock solution (100%) and then diluted to other concentrations by sterile distilled water. The control treatment was sterile distilled water only.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

To identify compounds of the filtrate of *B. bassiana* This analysis was performed by Instrument of Gas Chromatography-Mass spectrometry Shimadzu's in laboratories of the Ministry of Science and Technology – Baghdad.

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Effects of *B. bassiana* filtrate on the mortality rate of the second and fourth larval instars

Three concentrations of the filtrate (25, 50, and 75%) were prepared by withdrawing a fixed volume of the filtrate through a sterile medical syringe and diluting it with different volumes of sterile distilled water. Twenty larvae from each stage were tested for each concentration, with three replicates, and compared to distilled water as control. For each replicate, 5 larvae were placed in a Petri dish, treated with the filtrate, and sprayed from a distance of 5 cm to ensure complete coverage. After spraying, the larvae were supplied with feeding, a wheat powder and a yeast mixture, as prepared previously. The dishes were incubated at a temperature of 30 ± 2 °C. The mortality rate was calculated after 1, 3, 5, and 7 days.

Statistical analysis

The results of the laboratory experiments were analyzed by applying the Complete Randomized Design (CRD) according to the factorial experiments. The Least Significant Difference (LSD) test was used under the probability level of 0.05 to test the significance of the differences⁸.

Results

Figure 1 shows the most important active compounds separated from the filtrate of the fungus *B. bassiana* using the GC mass technique. Abdel-Wareth The results presented in this study are consistent with the findings of Abdel-Wareth^{9,10}, who indicated that the filtrate of *B. bassiana* contains the compounds 9- octadecenoic acid, nonacosane, nonanedioic acid, and dibutyl ester, as identified by using the GC mass technique.

Table 2 shows the superiority of *B. bassiana* filtrate at the concentration of 75%, which exerted the highest mortality rate of 45.0% for the second larval stage. In contrast, the lowest was 15.0% at the lowest concentration 25%. The most increased mortality of the fourth larval stage was 33.3% at the concentration of 75%, whereas the lowest was 13.2% at the lowest concentration 25%. It is noticed that the longer the period after the treatment with the filtrate, the higher the mortality rate, which reached values of 5.8, 15.0,

25.8, and 36.6% after 1, 3, 5, and 7 days, respectively. It was also noticed that the higher the concentration, the higher the mortality rate, which reached 30.0, 18.3, and 45.0% at concentrations of 25, 50, and 75%, respectively, for the second larval instar. For the fourth instar, the mortality rate values reached 13.2, 26.7, and 33.3% at 25, 50, and 75% concentrations, respectively. As for the concentration/period interaction treatment, the highest mortality rate for the second larval instar (66.7%) was recorded at the concentration of 75%, showing highly significant differences with the remainder of the treatments, where the lowest was 33.3% at the concentration of 25% concentration. As for the fourth larval instar, the highest mortality rate was 53.3% at 75% concentration, and the lowest was 26.7% at 25% concentration. However, the control treatment did not record any mortality. These results agree with the reported effectiveness of the fungal filtrate of *B. bassiana*, which is conferred by the active compounds shown in Figure 1 and Table 1.

Discussion

These compounds are like those found in manufactured pesticides but with no effects on the environment. The cause of death is also attributed to the fact that the secondary metabolites of the fungus can interfere with the immune system, thus causing changes in the host's behavior. These changes include for example, reduced feeding, reduced activity, and paralysis of the insect, as well as changes in tissue structures and, thus, the rapid death of the host¹¹. Al-Shuwaili¹² utilized the filtrate of *B. bassiana* against the black bean aphid *Aphis fabae*, where the concentration of 100% of the filtrate caused mortality rates of 52.17 and 54.10% of nymphs and adults, respectively. These results agree with the study of Ghaylan¹³, where the filtrate of *B. bassiana* caused the highest mortality rate. The mortality rate of *Spodoptera litura* larvae was 73.33% at 100% concentration. (14,15), used *Trichoderma harzianum* filtrate against *Aphis fabae* nymphs, which showed the highest mortality rate of 71.81% at 100% concentration.

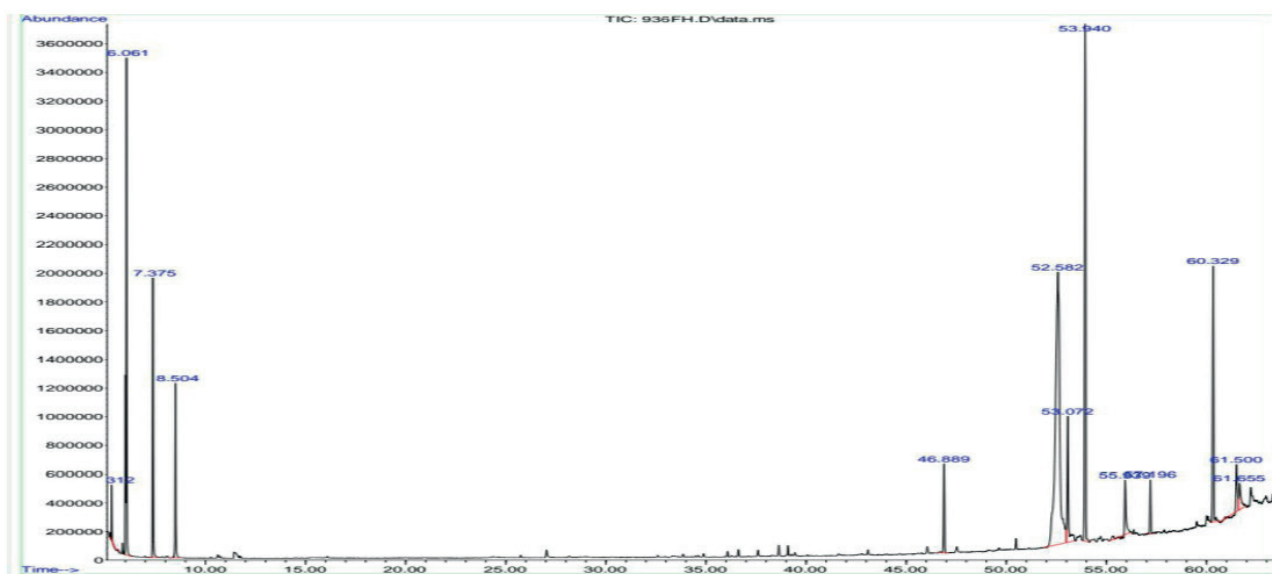


Figure 1. Identification of the active compounds in the crude filtrate of the fungus *B. bassiana* using the GG mass technique.

Peak number	Name of compound	Area	Detention number
1	1-Butanol	0.77	5.310
2	Acetic acid, propyl ester	11.10	6.059
3	Acetic acid, 2-methyl propyl ester	5.52	7.374
4	Nonanedioic acid, dibutyl ester	3.80	8.505
5	Heptadecane	2.70	46.889
6	Hexanedioic acid, bis (2-ethylhexyl)	38.25	52.582
7	Z-5-Nonadecane	5.15	53.073
8	Nonacosane	16.92	53.942
9	n- Hexadecanoic acid	2.53	55.937
10	Eicosane	1.61	57.194
11	Heneicosane	8.11	60.332
12	9-Octadecenoic acid	1.76	61.498
13	9-Tricosene	1.78	61.658

Table 1. Effects of *B. bassiana* filtrate on the second and fourth instar larvae of *T. granarium*.

Larval instar	Period (days) Conc. (%)	1	3	5	7	Mean mortality rate for concentrations.
Second larval instar	0	0.0	0.0	0.0	0.0	0.0
	25	0.0	6.7	20.0	33.3	15.0
	50	6.7	26.7	33.3	53.3	30.0
	75	20.0	40.0	53.3	66.7	45.0
Fourth larval instar	0	0.0	0.0	0.0	0.0	0.0
	25	0.0	0.0	20.0	26.7	13.2
	50	6.7	20.0	33.3	46.7	26.7
	75	13.3	26.7	40.0	53.3	33.3
Mean mortality rate for period		5.8	15.0	25.0		36.6
LSD (0.05) for concentration						2.83
LSD (0.05) for period						3.22
LSD (0.05) for conc./period interaction						5.88

Table 2. Effects of *B. bassiana* filtrate concentration on the mortality rate of the second and fourth larval instars of *T. granarium*.

Conclusions

The results of the laboratory experiments were analyzed by applying the Complete Randomized Design (CRD) according to the factorial experiments. The Least Significant Difference (LSD) test was used under the probability level of 0.05 to test the significance of the differences.

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