

The efficacy of local isolate of Entomopathogenic fungus *Beauveria bassiana* against potato tuber moth *Phthorimaea operculella* (Zeller) Lepidoptera: Gelechiidae under laboratory conditions

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Abstract

In this laboratory study, the effect of different concentrations of local isolate of entomopathogenic fungus *Beauveria bassiana* against potato tuber moth *Phthorimaea opercullella* was evaluated. In addition, GC Mass technique was used to identify the chemical compounds in the crude filtrate, the most important of which are Phenol, Methane, Ethane of *B. bassiana*. The results showed that *B. bassiana* was significantly the highest efficacy against compared to fourth instar larvae of *P. opercullella*. Where the mortality rate was 41.67 and 32.96% when at a concentration of 7.5 g/L, respectively. The overall results indicated that the fungus has a high efficiency in affecting the eggs and larvae of the fourth instar of the insect, which can be used efficiently to reduce the infection rate in the insect and can be a promising method for insect control and plant protection.

Keywords: Potato tuber moth *P. operculella* , *Beauveria bassiana*. GC.Mass



Introduction

Potato *Solanum tuberosum* L. is one of the important vegetable crops in the world (11). It is exposed to many insect pests such as potato tuber moth, *Phthorimaea operculella* (Zeller) Lepidoptera: Gelechiidae whose larvae infect the buds and tubers in the field and store, causing great economic damage (12). The use of chemical insecticides has caused harm to the environment and humans, biodiversity and environmental pollution, as well as the development of insect resistance as a result of excessive use of pesticides, therefore we have to search for effective and safe alternatives has become a necessity for researchers and one of the most important alternative is use Entomopathogenic in control (9). For example, *Beauveria bassiana* fungi is one of the most effective insect pathogens against insect pests on many crops and throughout countries (5 and 7). Entomopathogenics were used in several forms such as sporangial suspensions and secondary metabolites as insecticides. (3and 4). The current study aims to test the concentrations of *B. bassiana* and diagnose the secondary metabolites of the crude filtrate of the fungus by GC Mass technology, also to test the effectiveness of the fungus *B. bassiana* in affecting the eggs and larvae of the fourth stage of the insect *P. operculella*.

Materials and methods

Preparing of the insect

The study experiments were carried out in the Atomic Energy Laboratory, Department of Integrated Control /Baghdad 2020-2021. The larvae and pupae of the potato tuber moth insect were collected from infested potato tubers from the Entomology Laboratory of the Plant Protection Department / College of Agriculture / University of Baghdad on 11/5/2020. It was identified by the Natural History Museum/ Baghdad. It was prepared inside wooden cages with dimensions (30 x 30 x 30) cm. The four fronts are surrounded by a boring cloth in the form of a cone that wraps over each other when not in use. The base was made of plywood on which sterilized sawdust was placed for the purpose of preventing the larvae. Eight healthy potato tubers from one of the potato cultivars (Arizona, poteen) were added to the cages and placed in specialized incubators at a temperature of (27) C°, relative humidity of $70 \pm 1\%$, with an 8:16 h (L:D). The small petri dish was placed inside each cage containing a cotton swab moistened with a sugar solution of 5 % for adult feeding. (10) Adults were left to mate for several generations to obtain eggs and larvae used in the study Source and preparation of *B. bassiana* isolate.

A local isolate of the entomopathogenic fungus was obtained from the Ministry of Agriculture(Iraqi) / the Department of Plant Protection, where it is used



according to the recommendation 5 gm/L, two additional concentrations were tested experimentally to find out the effectiveness 2.5 gm/L (0.5×10^5) and 7.5 gm/L (1.5×10^5).

Preparation crude filtrate of *B. bassiana*
Isolation of pure \ fungi was obtained from the Department of Plant Protection / Baghdad and cultured on P.D.A. medium growth, then it was incubated at a temperature of 25 ± 3 C° for a period of 4-7 days. To prepare the fungus crude filtrate, the liquid media P.D.B. was prepared, and the fungal colonies' growth was purified, the flasks were then incubated at a temperature of (25 ± 3) C°, for (28) days. The inoculum was then filtered by Whatman No. 1 and re-filtered using a Millipore sterile filter 0.45 μ m according to methods described by Mohamed (9).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis technique

The gas chromatography-mass spectrometer technique (GC-MS) was used to diagnose compounds of the crude filtrate *B. bassiana*. by Gas Chromatography–Mass spectrometry Shimadzu's in Ministry of Sciences and Technology in Baghdad, Iraq.

Effect of *B. bassiana* on the eggs of *P. operculella* in laboratory condition

Three concentrations of the fungal extract (2.5, 5 and 7.5 g. L⁻¹) in. Eggs were sprayed with 1 ml of each concentration and each concentration

was replicated three times (20 eggs for each replicate) using a small sprinkler with a capacity of (100) ml. Eggs in the control treatment were sprayed with sterile water.

Effect of *B. bassiana* on the fourth larval stage of *P. operculella* in laboratory condition

The same concentrations and replications, by taking (11) larvae from the fourth larval instar and then calculating the corrected mortality rates using Abbott's equation after (3, 5, 7, 10) days.

Statistical analysis

Factorial experiments were carried out according to the complete random design (CRD) and the differences between the means of the interactions were compared according to the least significant difference test (L.S.D) at the probability level of 0.05 to show the significant differences and the results were analyzed by according to the statistical program Genstat 12.

Results and Discussion

Identification of the compounds of the fungus *B. bassiana* using the GC. Mass technique:

Table 1 and Figure 1 show the most important active compounds separated from the filtrate crude of *B. bassiana* using the GC mass technique. where appeared 9 secondary metabolic



compounds of the fungus *B. bassiana* such as Carbazol-1-one, 1,2,3, 4-tetrahydro, 1,3-Dihydrobenzimidazol-2-

one, N, N, and Oleic Acid. The results presented in this study are consistent

Table 1. Diagnosis of secondary metabolites of *B. bassiana* using GC. Mass technique

Peak Number	Compound name	Retention time/min	%Area
1	Carbazol-1-one, 1,2,3,4-tetrahydro	16.872	3.75
2	1,3-Dihydrobenzimidazol-2-one, N,N	21.779	10.44
3	Oleic Acid	23.972	7.02
4	3-Eicosene	25.939	2.99
5	Phenol, 2,2'-methylenebis(6-(1,1-d	27.868	10.96
6	Silane, chlorotripropyl	28.738	6.33
7	Steric Acid	29.003	4.71
8	2-Piperidinone, N-(4-bromo-n-butyl	30.865	3.65
9	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,	31.348	9.78

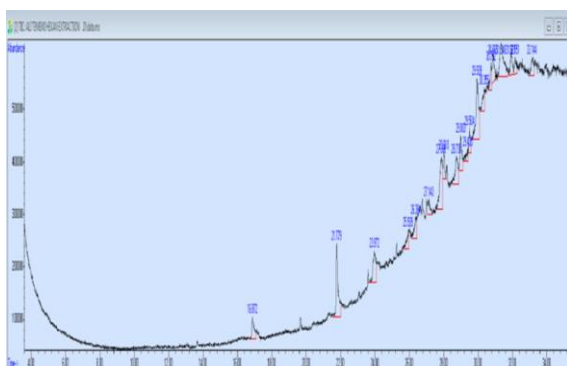


Figure 1. Identification of secondary metabolites of *B. bassiana* using GC. Mass Technique

with the findings of Abdel Warwth *et al.*

(10) who indicated in their study that the fungus filtrates *B.bassiana* contained Octasiloxane, Oleic Acid, Methyl and Phenol compounds, which were identified using GC mass technology. It agrees with Vivkanandhan *et al.* (13) who found several compounds in the fungus *B. bassiana*, including Octasiloxane and Eicosene, and also agrees with what was mentioned by Al-Bayaati (1) who indicated in her study that the fungus fungus *B.bassiana*

contains an Eicosene compound that was identified using the technique G.C. mass

Table 2. Effect of *B. bassiana* concentrations on the percentage of eggs mortality of *P. operculella*

Fungal concentrations (g/L)	Spore number/ml	Mortality %
Control	0	15.00
2.5	0.5×10^5	20.71
5	1×10^5	39.58
7.5	1.5×10^5	41.67
LSD 0.05=5.94*		

Effect of the fungus *B. bassiana* on the rates of eggs mortality

The result presented in Table (2) shown a significant differences in the effect of the fungus *B. bassiana* in the rates of eggs mortality, as the highest effect was when using the concentration of 7.5 g/L, where the mortality rate was 41.67% compared to with control 15% in the control treatment. . The reasons for this effect were attributed to the ability of the fungus to parasitize the eggs through the secretion of many toxins, the most important of which is the chitinase enzyme, which plays a key role in the

process of breaking down the chitin in the egg wall, allowing the fungal hyphae to penetrate the egg wall and break down its contents (6).

Effect of the fungus *B. bassiana* on the rates of mortality of the fourth larval stage

Effect of the fungus *B. bassiana* on the mortality rate of the fourth instar larvae of *P. operculella* of potato tuber moth was 32.96% at a concentration of 7.5 g/L of water compared to the control treatment, which was 6.67%. As for the effect of the interaction between the concentration and the time factor, the highest effect of the fungus was 50% when using the

Table 3. Effect of the highest rate of the effect of *B. bassiana* concentrations on the fourth larval stage mortality rates

Mortality %					
Days					
concentration	spore number	3	5	7	means
Control	0	0	6.67	13.33	6.67
2.5	0.5×10^5	6.67	10.74	23.15	13.52
5	1×10^5	13.33	28.52	38.42	26.76
7.5	1.5×10^5	13.33	35.55	50	32.96
means		8.33	20.37	31.23	
L.S.D 0.05	days=4.06* concentrations $\times$ days= 4.69*				

concentration of 7.5 g / L on the seventh day compared with (0)% in the control treatment. The larvae infected with the



fungus were characterized by slow movement and looseness of their body compared to the bodies of healthy larvae. This is due to the invasion of the fungal yarn to its body parts, as well as the appearance of black spots in most parts of the body, which is an indication of the places where the fungus threads penetrated the body of the larva. (15). The effect of the fungus *B. bassiana* may be due to interference with the melting process, as the larva is not able to transform from one stage to another or it cannot transform into the pupa, as the melting process depends mainly on food to form the new cuticle, so the infection with the fungus may cause a nutritional imbalance in the blood (14).

Conclusion

The study concluded that the chemical analysis of the extract of the fungus *B. bassiana* by using GC mass showed the presence of many active chemical compounds. The effectiveness of the fungus *B. bassiana* in affecting the eggs and larvae of the fourth stage of the insect and therefore it can be a good method for insect control. In addition, the best concentration in which highest effectiveness on insect mortality was 7.5 g/L.

Conflict of interest

The authors have no conflict of interest.

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