Testing the Portability Trichoderma harzianum fungus filtrate and hot aqueous extract of eucalyptus leaves on the mortality of second and fourth instar larvae of Everts Trogoderma granarium in the laboratory

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Abstract

A laboratory experiment was conducted in the postgraduate insect laborator at Al-Musayyab Technical College/Al-Furat Al-Awsat University, to test the effect of different concentrations of hot water extract of eucalyptus leaves and filtrate of the fungus Trichoderma harzianum on the death of the larval stages of the Khabra grain beetle, Trogoderma granarium Everts. Which is considered one of the most pestsilent that attack stored grains in the world, the results showed that treatment with concentrations of (5, 10, 20) mg/ml of the aqueous extract of eucalyptus leaves, where the mortality rate reached (60.00), (43.33), (30.67) in the second stage, while for the fourth stage it reached (30.68), (38.68), (56.32) ,respectively, after 7 days of treatment. As for the filtrate of the fungus T. harzianum, it gave treatment with concentrations of 50, 75, 100% (53.33, 55.33, 66.00) for the second larval stage, while for the fourth stage 43.20, 53.16 and 61.33), respectively. After 7 days of treatment, the results showed the effectiveness of the aqueous extract. Eucalyptus leaves and T. harzianum fungus filtrate and the possibility of introducing them in the future into integrated management programms to control Trogoderma granarium.

Key words : Coleoptera insect: Dermestidae , Trichoderma, Eucalyptus leaves.

.1Introduction

The Khabra beetle (Trogoderma granarium Everts) feeds on more than 100 types of grains in different regions of the world, making it one of the most important pests of stored grains and posing a threat to global food security[24]. The Khabra beetle (T. granarium Everts) is considered . Which belongs to the order (Coleoptera: Dermestidae) is one of the most important primary insect pests that cause great damage to stored grains and other grain crops, especially in tropical and subtropical regions. The larvae feed on whole and broken grains, causing significant loss in weight, germination, and quality of the infested grains in severe cases [12] [19]and [22] [11.[

The continuous and irrational use of chemical pesticides to control stored insects has led to major problems affecting the environment and human and animal health. [18]Therefore, plant extracts have been used as alternatives to the use of chemical pesticides because they are environmentally safe[3]. Trichoderma spp. is also considered among the biological control agents (BCAs) that produce active volatile organic compounds, and is considered one of the most studied fungal genera for its effectiveness and impact[15]. To expand the use of the concept of integrated management, the current study aimed to use the aqueous extract of eucalyptus and the filtrate of the fungus Trichoderma harzianum in the laboratory killing of some stages of the Trogoderma granarium Everts insect.

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.2Materials and methods

.2.1Collection, diagnosis and rearing of the grain beetle (Khabra) Trogoderma granarium Everts.

The grain beetle was obtained from the Agricultural Research Department/Abu Ghraib and identified at the Natural History Research Center and Museum/University of Baghdad by Prof. Dr. Razzaq Shaalan Akl and Asst. M. Fatima Hassan Breij. Whole wheat grains were used after being cleaned from impurities and placed in the freezer for 72 hours at -20°C to ensure that they were internally free from any other insect infestation.

Adults of 10 pairs of males and females of the hairy grain beetle were taken and placed on 1 kg of healthy wheat grains and 5 grams of dry yeast in 100 ml plastic bottles with a diameter of 20 cm. The mouths were then covered with tulle and tightly closed with a rubber band to prevent the adults from escaping, taking into account the farm's renewal from time to time.[7.[

.2.1Preparation of hot aqueous of extract eucalyptus leaves L.eucalyptus

The preparation was carried out according to the method of [6] adapted from[16] for the aqueous extract of the eucalyptus plant, where the dried leaves were ground at laboratory temperature using an electric grinder, then 10 g of eucalyptus leaf powder were taken and placed in a 500 ml glass beaker containing 200 ml of lukewarm boiled distilled water. The plant material was mixed with a magnetic mixer for 15 minutes, and then the solution was left for 24 hours (to obtain better extraction) after covering it tightly to avoid the entry of impurities. The solution was filtered with a piece of cloth several times, and the filtrate was taken. After that, foreign materials were precipitated using a centrifuge at a speed of 3000 rpm for ten minutes. The filtrate was concentrated using a rotary evaporator at a temperature of 40-45°C to obtain the dregs, which were stored in small, tightly sealed glass bottles after recording their weight when empty and kept in the refrigerator until use.

In order to prepare the required concentrations, the method of [9] was followed by taking (5) grams of the extracted material and completing the volume to 100 ml with distilled water. Thus, the concentration of the basic solution, stock solution, becomes 5%, i.e., equivalent to (50) mg/ml. From this, the concentrations (5, 10, 20) mg/ml were prepared with the addition of a ml of the spreading material (20) Tween[4.[

.2.3The effect of different concentrations of boiled aqueous extract of eucalyptus leaves on the death of the second and fourth larval stages of the Khabra grain beetle T. granarium.

40insects were taken for each concentration, with three replicates, with a comparison treatment for each of the larval stages (second and fourth). 10 insects were placed in each plastic box, and sprayed with the extract at concentrations of (5, 10, and 20) mg/ml. The food consisting of a mixture of yeast and ground wheat prepared in advance was placed, while the comparison treatment was distilled water only, and the mortality rate was calculated after 1, 3, 5, and 7 days of treatment.

.2.4Preparation of T. harzianum fungus filtrate

To prepare the Potato Dextrose Broth (PDB) medium, the method of [17] was followed, where 200 g of peeled potatoes cut into small pieces were boiled in 500 ml of distilled water for 20 minutes and filtered with a clean gauze cloth. The filtrate was taken and 20 g of dextrose was added to it, and the volume was completed to 1 liter by adding distilled water.

The filtrate was distributed in 250 ml glass bottles at a rate of 150 ml and sterilized with an autoclave at a temperature of 121 °C and a pressure of 15 pounds / inch for 15 minutes.

The antibiotic Tetracycline was added to it at a rate of 250 mg/liter, and each bottle was inoculated with three discs with a diameter of (5) mm using a cork piercer from the edge of the purified fungal colony on PDA culture medium and diagnosed at the age of 7 days. The glass vessels were incubated at a temperature of 2 ± 25 °C, taking into account shaking the vessels every 3-4 days to distribute the fungal growth. After 28 days, the filtrate was filtered using Whatman No. 1 filter paper. Then, it was filtered again using a Millpore microfilter[23]. Three concentrations of the filtrate were prepared, namely (50, 75, and 100%), by withdrawing an amount of the filtrate using a sterile medical syringe and completing it by adding sterile distilled water to 100 ml.

.2.5The effect of different concentrations of T. harzianum fungus filtrate on the mortality of the second and fourth instar larvae of the Khabra grain beetle T. granarium.

40insects were taken for each concentration, with three replicates, with a comparison treatment for each of the larval stages (second and fourth). 10 insects were placed in each plastic box, and sprayed with filtrate concentrations (50, 75, 100%), and food consisting of a mixture of yeast and ground wheat prepared in advance was placed.

Either the control treatment was with distilled water only, and the food consisting of yeast and previously prepared ground wheat was placed, and the larvae of the second and fourth stages were treated, and the mortality rate was calculated after 1, 3, 5 and 7 days of treatment.

.2.6Statistical analysis

The percentage of insect mortality was calculated and the percentages of mortality were corrected using Abbott's equation[1]. The data were analyzed using analysis of variance (ANOVA) using GenStat package 3 (version 3 rh). Significant differences were observe8d between treatments. Mean values were significantly separated using the least significant difference (LSD) test at the 5% level.

.3 Results and discussion

-1Testing the effect of concentrations of boiled water extract of eucalyptus leaves on the death of the second and fourth instar larvae of the Khabra grain beetle T. granarium.

The results of the statistical analysis of Table (1) show the significance of the differences, as the table indicates the effect of the interaction between the concentrations of the aqueous extract of eucalyptus leaves and the time period on the percentage of mortality rate of the second and fourth instar larvae of the hairy grain beetle (Khabra). For the interaction, the highest percentage reached 60.00 at a concentration of 20 mg/ml for the second instar, while for the fourth instar, it reached 56.32 at a concentration of 20 mg/ml on the seventh day, while the comparison treatment did not cause any mortality.

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Table (1) shows the effect of different concentrations of the aqueous extract of eucalyptus leaves on the death of the larval stages of the hairy grain beetle (Khabra).

Larval stages	Concentrations mg/ml	destruction and Time(days)				Average concentrations
The second phase		1	3	5	7	
	0	0	0	0	0	0
	5	3.33	19.33	23.33	30.67	19.17
	10	10.00	21.33	36.67	43.33	27.83
	20	28.33	33.33	40.00	60.00	40.42
	Average periods	10.42	18.50	25.00	33.50	
	L.S.D value 0.05	For periods = 5.182 For concentrations = 5.182 For overlap = 10.370				
The fourth phase	0	0	0	0	0	0
	5	0	12.67	20.25	30.68	15.9
	10	8.13	16.42	26.67	38.68	22.30
	20	10.64	28.67	40.00	56.32	33.90
	Average Periods	4.69	14.44	21.73	31.25	
	L.S.D value 0.05	For periods = 4.8 For concentrations = 4.8 For overlap = 9.6				

Some of the active compounds extracted from plants, including medicinal plants, affect the enzyme Protase in the midgut, which affects the level of protein and sugar in the blood of insects [10]. This study agrees with[21], who explained that the high mortality rate of larvae is due to the weakness of the defensive mechanisms in the sugar beet worm or due to the small number of plasma cells responsible for the encapsulation process in its body. In a study conducted by[5]. the effect of Aloe Vera plant on the southern bean beetle Callosbruchus maculatus, which is raised on pea seeds, as the mortality rate reached (80.0, 73.3, 66.7)% at concentrations (7.0, 5.0, 3.0), respectively.

-1 The effect of different concentrations of T. harzianum fungus filtrate on the mortality of larval stages of the hairy grain beetle (Khabra.(

The results of the statistical analysis of Table (2) show the significance of the differences, as the table indicates the effect of the interaction between the concentrations of the fungal filtrate and the time period on the percentage of the mortality rate of the second and fourth instar larvae of the hairy grain beetle (Khabra). For the interaction, the highest percentage reached 66.00 at a concentration of 100% for the second instar, while for the fourth instar, it reached 61.33 at a concentration of 100% on the seventh day, while no mortality occurred in the comparison treatment.

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Table (2) shows the effect of concentrations of T. harzianum fungus filtrate on the de ath of larval stages of the hairy grain beetle (Khabra).

Larval stages	Concentrations mg/ml	destruction and Time(days)				Average concentrations
The second phase		1	3	5	7	
	0	0	0	0	0	0
	50	8.13	24.67	33.25	53.33	29.84
	75	13.33	29.33	40.33	55.33	34.58
	100	16.67	31.20	46.67	66.00	40.13
	Average periods	9.53	21.83	30.06	43.66	
	L.S.D value 0.05	For periods = 4.744 For concentrations = 4.744 For overlap = 9.487				
The fourth phase	0	0	0	0	0	0
	50	0.00	7.25	23.20	43.20	18.41
	75	6.67	23.42	33.13	53.16	29.09
	100	14.67	28.33	40.00	61.33	36.08
	Average Periods	5.33	14.75	24.08	39.42	
	L.S.D value 0.05	For periods = 5.207 For concentrations =5.207 For overlap =10.414				

The study agrees with what was reached by[20], where the fungus filtrate Trichoderma harzianum gave a mortality rate of wheat nymphs of (31.49, 41.44, 46.30)% for concentrations of 25, 50, 75)%, respectively. As for adults, the mortality rate reached 28.05, 37.00, and 42.30% for concentrations of (25, 50, and 75)%, respectively. Increasing the concentrations of mycotoxins leads to an increase in the mortality rate after these toxins enter through contact with the body wall, or through the mouth or respiratory pores, or through the penetration of the fungus into the body wall. The areas that it can penetrate the most are the areas of ring joints, which leads to the insect's weak ability to defend itself, thus increasing its susceptibility to infection with the disease[17.[

In a study conducted by[2], the effect of the T. harzianum fungus filtrate gave the lowest killing percentage, reaching 42.45% and 52.64%, respectively, for nymphs and adults of the green peach aphid, compared to the rest of the filtrates used in the study at a concentration of 100% and 50% after 24 hours, while the killing percentage after 72 hours for the T. harzianum filtrate was 65.18% and 61.48% for the same concentrations.

.4Coclusions

The study showed that the second instar larvae were more sensitive to control than the fourth instar larvae, whether in the aqueous extract of eucalyptus or in the filtrate of the fungus T. harzianum.

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-2The fungus filtrate has an effective effect compared to the aqueous extract of Eucalyptus leaves on the second and fourth instar larvae.

- 3The results showed that the aqueous extract of Eucalyptus leaves had an effect on the

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second instar larvae compared to the fourth instar.

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