

Evaluation of the efficiency of aqueous and alcoholic extracts of *Taraxacum officinale* leaves and Cress (*Lepidium sativum*) seeds in killing the second and fourth instar larvae of *Callosobruchus maculatus* (F.) in the laboratory

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Abstract

A series of laboratory experiments were conducted in the postgraduate insect laboratory/Al-Musayyab Technical College, Al-Furat Al-Awsat University. The study included extracting two types of alcoholic and aqueous extracts of *Taraxacum officinale* leaves and Cress seeds. The results showed the aqueous extract of *Taraxacum officinale* leaves, it gave the highest mortality rate of 100% at a concentration of 10 mg/ml after (7) days in a row for the second larvae, and the lowest mortality rate of 0.00% at a concentration of 2.5 mg/ml for the fourth larvae after one day the highest mortality rate of 100% for the alcoholic extract of Cress seeds at a concentration of 10 mg/ml for the second larval stage after (7) days in a row, and the lowest mortality rate of 46.60% at a concentration of 2.5 mg/ml for the fourth larvae after one day..

Introduction

Callosobruchus maculatus is considered *Callosobruchus maculatus* is one of the most important insect pests belonging to the Bruchidae family of the Coleoptera order and infects legume seeds all over the world, including Iraq. The infection occurs in the field and in storage. The importance of the insect is due to its feeding and the development of its larvae inside the seeds and consuming their contents, thus increasing the percentage of seed damage and reducing the nutritional value and germination rate as well [8]. The *Callosobruchus maculatus* insect has a wide host range that includes stored legume seeds such as lentils, soybeans, peas and broad beans, in addition to its main host, which is the seeds of cowpea plants. Plants differ in the intensity of repelling and attracting the insect and can be divided into severe when used, including its damage to the environment and its negative impact on natural enemies, and medium and weak in repelling and attracting

[10]. Due to the great damage caused by chemical pesticides and the emergence of a resistance trait in pests against the action of pesticides[3,13] For this reason, researchers have resorted to using environmentally safe alternatives, the most important of which are plant extracts, because many plants contain secondary compounds and their products are compounds similar in effect to those found in chemical pesticides. For this reason, plant extracts are among the most important pesticides of plant origin because they have good features and characteristics, such as being rapidly decomposed and leaving no residues in the environment [14]. Therefore, the current study aimed to use the aqueous and alcoholic extracts of *Taraxacum officinale* leaves and Cress seeds to kill some stages of the insect *Callosobruchus maculatus* in the laboratory.

Materials and methods

1- Collection, identification and rearing of *Callosobruchus maculatus*

Callosobruchus maculatus was obtained from infected seeds in grain stores in the study area and its classification and distinction between male and female were confirmed as *C. maculatus*, and it was diagnosed at the Natural History Research Center and Museum / University of Baghdad by Prof. Dr. Razzaq Shaalan Akl. Healthy cowpea seeds were used and placed in the freezer for 48 hours to ensure that they were free from insect infestations. Cowpea seeds were obtained from local markets and placed in the freezer at -20°C for 72 hours to ensure that they were internally free from any insect infestations [2]. Then the seeds were taken out of the freezer and 10 adult males and females of *Callosobruchus maculatus* were transferred to 100 ml plastic bottles with a diameter of 20 cm. The mouths were then covered with a cloth and tightly closed with a rubber band and placed in an incubator with a temperature of $30 \pm 2^{\circ}\text{C}$ and a humidity of $70 \pm 5\%$ [9].

2- Preparation of the aqueous extract of *Taraxacum officinale* leaves and *Cress l. sativum* seeds

The cold aqueous extract of each of *Taraxacum officinale* and *Cress* plants was prepared according to the method [5] adapted from [11] 10 g of *Taraxacum officinale* powder and *Cress* seeds were taken separately and placed in a 500 ml glass flask containing 200 ml of cold distilled water, and the plant material was mixed in a magnetic mixer for 15 minutes, then left for 24 hours (to obtain better extraction) after tightly covering it to avoid the entry of impurities, and the solution was filtered using Whatman No. 1 filter paper and the filtrate was taken and placed in a centrifuge at a speed of 3000 rpm for 10 minutes to get rid of the suspended matter and

the filtrate was taken and placed in the oven at a temperature of 45°C To obtain the dry dregs, which were stored in small, tightly sealed glass bottles after recording their weight when empty, and stored in the refrigerator until use.

3- Preparation of alcoholic extract of *T. officinale* leaves and *Cress l. sativum* seeds

The method of (Harborn, 1984) was followed in the preparation of the alcoholic extract by taking a weight of 10 g of the ground dry powder of each of the leaves of *Taraxacum officinale* and *Cress* seeds separately and then placing them in the Soxhlet apparatus and adding 200 ml of ethyl alcohol and extracting for 24 hours at a temperature of 45°C . After that, the extracted sample containing the raw extracted materials of the plants was concentrated. The process was repeated several times to obtain the sufficient quantity. The materials were dried in an electric oven at a temperature of $40\text{--}45^{\circ}\text{C}$. After that, the dry residue was taken and placed in tightly sealed glass containers with a known weight and kept in the refrigerator until use. In order to determine the biological effectiveness of the crude alcoholic extract of *Taraxacum officinale* and *Cress* plants, 5 g of dry dregs were taken for each extract separately, dissolved in 5 ml of ethyl alcohol and 3 ml of the diffuser, then the volume was completed to 100 ml with distilled water, and the concentration of the basic solution became 5% or equivalent to 50 mg/ml, and concentrations of (1, 0.75, 0.50, 0.25) mg/ml were prepared from it.

4- Effect of different concentrations of the aqueous extract of *Taraxacum officinale* leaves and *Cress* seeds on the death of the second and fourth larval stages of *Callosobruchus maculatus*

20 larvae were taken for each concentration in 3 replicates with a control treatment for each

of the second and fourth larval stages separately. 5 larvae were placed in each replicate of the plastic boxes and then sprayed with the extract with concentrations of (0.25, 5, 10 and 20) mg/ml and food consisting of powdered bean seeds prepared in advance was placed. As for the control treatment, it was distilled water only, and then the percentage of death was calculated after 1, 3, 5 and 7 days of treatment.

5- Effect of different concentrations of the alcoholic extract of *Taraxacum officinale* leaves and Cress seeds on the death of the second and fourth larval stages of *Callosobruchus maculatus*

(20) larvae were taken for each concentration in 3 replicates with a control treatment for each of the second and fourth larval stages separately. (5) larvae were placed in each replicate of the plastic boxes and then sprayed with the extract with concentrations of (0.25, 5, 10 and 20) mg/ml and food consisting of powdered bean seeds prepared in advance was placed. As for the control treatment, it was (5 ml distilled water + 5 ml ethyl alcohol), after which the percentage of death was calculated after (1, 3, 5 and 7) days of treatment.

6- Statistical analysis

The laboratory experiment was carried out according to the Complete Randomized Design (C.R.D) according to the factorial

experiments and the Less Significant Difference (LSD) test was used at the probability level of 0.05 to test the significance of the results [6] The percentage of mortality was corrected according to the Abbott equation [1].

Corrected mortality percentage%=(mortality in treatment %-mortality in control treatment%)/(100-%mortality in control treatment)×100

Results and discussion

1- Testing Effect of the concentrations of the aqueous extract of *Taraxacum officinale* leaves on the death of the second and fourth instar larvae of *Callosobruchus maculatus*

The results of the statistical analysis of Table (1) show significant differences between the concentrations of the aqueous extract of *Taraxacum officinale* leaves and the time period on the percentage of the death rate of the second and fourth instar larvae of *Callosobruchus maculatus*, as the highest percentage reached 100% at a concentration of 10 mg/ml for the second instar, while for the fourth instar it reached 80% at a concentration of 10 mg/ml on the seventh day. The reason for the increase in the percentage of the second and fourth instar larvae may be attributed to the extract containing toxic compounds that enter through food.

Table (1) shows Effect of different concentrations of the aqueous extract of *Taraxacum officinale* leaves on the death of the larval stages of *Callosobruchus maculatus*.

second phase						fourth phase					
Concentration Mg/ml	Period				average Period	Concentration Mg/ml	Period				average Period
	1	3	5	7			1	3	5	7	
0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
2.5	20.0	40.0	60.0	80.0	50.0	2.5	0.0	6.7	46.7	60.0	28.3
5	26.7	46.7	66.7	86.7	56.7	5	6.7	26.7	53.3	73.3	40.0
7.5	33.3	60.0	73.3	93.3	65.0	7.5	13.3	33.3	53.3	73.3	43.3
10	53.3	73.3	93.3	100.0	80.0	10	46.7	53.3	73.3	80.0	63.3
average	26.7	44.0	58.7	72.0		average	13.3	24.0	45.3	57.3	
LSD0.05		Concentration	Period	interaction		LSD0.05		Concentration	Period	interaction	
		6.73	6.03	13.74				9.99	8.93	19.98	

The study agrees with what [4] found that Aloe Vera plants affected *Callosobruchus maculatus* insects that were reared on pea seeds, and the mortality rates reached (66.7, 73.3, 80.0)% at concentrations (3.0, 5.0, 7.0), respectively. The study also agrees with (Mahdi et al., 2005), who showed that the high mortality rates in larvae are due to weakness in the defense mechanisms of the sugar beet worm, as well as weakness in the plasma cells responsible for the encapsulation processes in its body.

Effect of concentrations of alcoholic extract of Cress seeds on the death of second and fourth instar larvae of *Callosobruchus maculatus*.
Effect of concentrations of alcoholic extract of *L. sativum* seeds on the death of second and fourth instar larvae of *Callosobruchus*

maculatus. Table (2) indicates Effect of different concentrations of alcoholic extract of Cress seeds on the death of second and fourth instar larvae of *Callosobruchus maculatus*. The results showed Effect of the interaction between concentrations and time period on the percentage of mortality rate of the second and fourth instar larvae of *Callosobruchus maculatus*, as the highest mortality rate reached 100% at a concentration of 10 mg/ml for the second instar, while the fourth instar reached the highest mortality rate of 86.67% at a concentration of 10 mg/ml on the seventh day, while the lowest mortality rate reached 46.60 and 26.67 for the second and fourth instar larvae respectively at a concentration of 2.5 mg/ml after 24 hours of treatment. The results in the table

above indicated that the mortality rate increases with increasing concentration, and the reason for the mortality rates of the larvae of the *Callosobruchus maculatus* insect C.

maculatus may be due to the fact that the alcoholic extract of Cress seeds contains toxic compounds that lead to killing, preventing or [inhibiting feeding [15

Table (2) shows Effect of different concentrations of the alcoholic extract of Cress seeds on the death of the larval stages of *Callosobruchus maculatus*.

second phase						fourth phase					
Concentration Mg/ml	Period				average	Concentration Mg/ml	Period				average
	1	3	5	7			1	3	5	7	
0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00	0.00
2.5	46.6	73.33	80.00	86.67	71.67	2.5	26.67	46.67	66.67	73.33	53.33
5	60.0	73.33	86.67	93.33	78.33	5	46.67	53.33	66.67	73.33	60.00
7.5	66.6	86.67	93.33	100.0	86.65	7.5	46.67	60.00	73.33	80.00	65.00
10	73.3	93.33	100.0	100.0	91.65	10	53.33	73.33	80.00	86.67	73.33
average	49.3	65.33	72.00	76.00		average	34.67	46.67	57.33	62.67	
LSD0.05		Concentration	Period	interaction		LSD0.05		Concentration	Period	interaction	
		7.97	7.13	15.94				7.68	6.86	15.36	

This study agrees with [12,15] who explained that the reason for the death of the larvae is Effect of the toxic substances present in the extracts on their nervous system. The work of the extracts is to activate the enzyme Ache)) Acetylcholinesterase found in the nervous system, which works to break down the

Conclusions

The results showed that the aqueous and alcoholic extracts had an effect on the rate of death of the second and fourth stage larvae of the *Callosobruchus maculatus* insect. The study showed that the second stage larvae were more

substance acetylcholine necessary in transmitting nerve signals that are at the end of the nerves, and ultimately causes paralysis and then death [7] showed that the active compounds extracted from plants affect the enzyme Protase found in the middle digestive tract and also affect the levels of sugar and protein in the blood of insects

sensitive in the mortality rate of the fourth stage larvae in both the aqueous and alcoholic extracts. The alcoholic extract of Cress seeds was superior in mortality rate to the aqueous extract of *Taraxacum officinale* leaves. The

mortality rate increases with increasing

concentration

Recommendations

chemical pesticides. Test the studied extracts against other pests. Isolation and identification of active compounds and conduct biological tests on them.

-1 Use alcoholic extracts of Cress seeds in integrated management programs for stored pests in the future and reduce the use of

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