

## SHORT ARTICLE / INVESTIGACIÓN

## Effects of crude alkaloid compounds extract of *Ammi majus* leaves and flowers on some aspects of biological performance *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)

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**Abstract:** This *in vitro* study evaluated the effects of crude alkaloid compounds extract of plant *A. majus* leaves and flowers on some biological performance aspects of the insect. The results showed that the alkaloid extract of flowers at concentrations of 10, 20 and 30 mg/ml significantly affected cumulative mortality at the different stages. The concentration (30) mg/ml had the most noticeable effects, causing the highest mortality rate in the second and fourth larval instars (86.7 and 73.3 %, respectively) after 72 hours. At the same time, the mortality rates upon applying the same concentrations but of the alkaloid extract of the leaves were 73.3, and 53.3 %, respectively, following 72 hours of treatment.

**Key words:** *Oryzaephilus surinamensis*; *Ammi majus*; crude alkaloid compounds extract.

### Introduction

Grain crops are usually exposed to many issues, from harvesting and storing to their marketing and consumption. The global population has suffered and is still suffering, from many damages caused by harmful insects, both in terms of economy and health. Grains and other materials, during their storage, are exposed to attacks by many insects that cause severe damage, whether in quantity or quality<sup>1</sup>. The sawtoothed grain beetle *Oryzaephilus surinamensis* (Family, Silvanidae; order, Coleoptera) is an economically important insect species, as it infects grains of various plant types of the family Gramineae in Iraq and various countries of the world. Its small size, flat body shape, and fast movement helped the insect hide in cracks and small and narrow places and reach grain bags and stored materials<sup>2</sup>. What increases the danger of the insect is its ability to smash food and build tunnels inside it, leading to an increase in its humidity. In addition, *O. surinamensis* leaves fecal residues and molting skins from dead larvae and adults, which leads to poor taste and food spoilage<sup>3</sup>. Many methods have been used to combat the sawtoothed grain beetle, among which chemical control is ranked first, as it is the fastest approach to control this pest.

Nevertheless, issues related to the resistance to chemical pesticides and the threat resulting from pesticide residues on human health and the environment have been emerging. Hence, researchers in insect pest control have been prompted to search for alternative and safe means to control and reduce the economic losses resulting from this critical insect pest on cereal crops and their products<sup>4,5</sup>. These means included pesticides of plant origin, considered one of the available alternatives that have shown their effectiveness in controlling insect pests<sup>6</sup>. Therefore, *A. majus* was selected to invest in it as a safe alternative to chemical pesticides. In the absence of similar studies on this plant,

the present research aimed at investigating its biological effectiveness in some aspects of the biological performance of the insect by using naturally effective products against the INSECT.

### Materials and methods

#### Collect the insect and raise them

The insects were collected from palm date and walnut fruits from a production factory's warehouses. The insects were reared and reproduced on different foodstuffs for 11-30/4/2021 after ensuring the safety of these materials from the presence of other storing pests. The purpose was to maintain the permanence and survival of the studied insects by treating the food material with a temperature of -20 °C for 60-90 minutes to ensure the killing of other insect stages, if present<sup>7,8</sup>. The insects were grown on oats and flour. The adult males and females were placed in glass breeding bottles (20 pairs per bottle) with a capacity of 250 gm, each containing 100 gm of oats feed. The opening of each bottle was closed by a piece of cloth that allowed ventilation, which was tied with a rubber band. The bottles were then placed in an incubator at a temperature (of 32 ± 2) °C and relative humidity of (65 ± 5)% for the growth of insects before subsequent experiments. Samples of the larvae and adults were collected for identification at the Natural History Museum / University of Baghdad, where it was confirmed that they belong to *Oryzaephilus surinamensis* L. of the family Silvanidae family.

#### Collection and identification of plant samples

The leaves and flowers of the plant were collected in

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the middle of March 2021, during the flowering phase in the spring season, from Babylon Governorate / Al-Syehi area. The samples were identified in the herbarium of the College of Science / University of Babylon. The samples were dried in laboratory conditions and ground to obtain the powder and put into a refrigerator until use.

#### Preparation of crude alkaloid compounds extract from the plant leaves

Modified from an earlier study<sup>9,10</sup>, was applied in preparing the leaves extract. A weight from (10) g of dry material to the leaves was weighed and extracted with 200 ml of ethyl alcohol in a Soxhlet extractor for (24) hours at a temperature (of 45 °C). The extracted sample was concentrated using a rotary evaporator, then dried in an electric oven at 40-45 °C temperature and saved in the refrigerator until use.

To estimate the vitality of the crude alkaloid compounds extract of the leaves, 12 g of the leaf-dry crude alkaloids extract was dissolved, onto which 5 ml of ethyl alcohol and 3 ml of Tween 20 were added. The volume was completed to 100 ml with distilled water. The stock solution concentration became 12%, or the equivalent of 120 mg/ml, from which a concentration of 3.2.1% or 10,20,30 mg/ml was prepared. As for the control treatment, 5 ml of ethyl alcohol was employed, brought up to 100 ml with distilled water.

#### Preparation of the crude alkaloid compounds extract from the plant flowers

The same preparation method described above was applied, except for replacing leaves with flowers.

#### Effects of alkaloid compounds extract of the plant leaves on mortality in the insect the non-cumulative

To test the biological activity of the extract on the mortality of the various stages of the insect, including the second larval stage, five larvae were isolated and separately transferred to Petri dishes. They were treated with all extract concentrations (30, 20, 10 mg/ml) at 3 duplicates/concentration rates. Feed (1 g of oats and yeast) was added, and the treated larvae were incubated at a temperature of (32 ± 2) °C and a relative humidity of (65 ± 5)%<sup>19</sup>. The mortality rate was recorded at 24, 48, and 72 hours post-treatment.

The same method was followed for the fourth-instar larvae.

#### Statistical analysis

The experiments are designed according to the factorial experiment model with a completely randomized design (CRD). The least significant difference (LSD) test under the probability level (50.0) was applied to show the significance of the existing differences. The mortality rate was corrected according to (11).

## Results

#### Effects of the extract of *A. majus* leaves and flowers on the non-cumulative mortality rate of different stages of *O. surinamensis*

Table 1 shows the superiority of the crude alkaloid extract of flowers at a concentration of 30 mg/ml, where the highest mortality rate was 73.33%. In contrast, the lowest was 44.43% at the concentration of 10 mg/ml. However, the crude alkaloid extract of leaves had the highest mortality rate (62.2%) at a concentration of 30 mg/ml. In contrast,

the lowest was 33.33% at a concentration (10 mg/ml). That reached (44.43, 57.76, 73.33)% using the concentrations of (10, 20, 30) mg/ml after 24, 48 and 72 hours. Also, the mortality rates in response to the alkaloid extract of leaves reached (33.33, 46.66, 62.2)% at concentrations of (10, 20, 30) mg/ml after 24, 48 and 72 hours, whereas the control treatment demonstrated no mortality. As for the effect of the type of plant part extracted, the flower extract outperformed the leaves extract through its effect on increasing the mortality rate, which reached 43.88% in the flowers and (35.55)% in the leaves. As for the concentration/time interaction experiments, the highest mortality rate was (86.7)% at the concentration (30) mg/ml after (72) hours for both types of extracts, as compared to the control treatment. The results of the statistical analysis indicated significant differences.

As shown in Table 2, the crude alkaloid extract of flowers was superior at a concentration of 30 mg/ml, where the highest mortality rate was 62.2%, whereas the lowest was (33.33)% while (10) mg/ml. As for the crude leaf extract, the highest mortality rate was recorded at the concentration of (30) mg/ml, reaching 46.66%, whereas the lowest was 26.66% while (10) mg/ml. It is noticeable that the higher the concentration and the period, the higher the mortality rate, which reached (33.33, 46.66, 62.2)% at concentrations of (10, 20, 30) mg/ml after (24, 48, 72) hours of treatment.

Concerning the effect of extract type, the flower extract was superior to the leaf extract in increasing the mortality rate, which reached 35.55% by using flowers and 27.22% by using leaves.

As for the interaction between the factors of concentration and period, the highest mortality rate was 73.3% at the concentration of 30 mg/ml after 72 hours, for both types of extracts, in comparison with the control treatment, which showed no mortality. The results of the statistical analysis indicated the presence of significant differences.

Another reason for the high rate of larval mortality might be the decrease in metabolism due to the interaction of alkaloid compounds with digestive enzymes, which led to the poisoning of the digestive tract and, consequently, the death of the larvae. Moreover, these compounds have severe toxic effects, resulting in their action as nutrition inhibitors. Thus, the larvae fail to utilize the food and die after a short feeding period. Their growth and survival are also affected. In addition, these compounds affect the gastrointestinal tract, especially the epithelial cells, leading to incidences of insect poisoning. The highest mortality rate for all insect larval stages reached 90%, achieved at a concentration of 10%. This agrees with The results of the current study in terms of the impacts, despite the difference in the types of plants and insects.

## Discussion

More severe nutritional effects of the treated larvae appear in the early larval stages because they are more efficient in converting food, demonstrating these compounds' toxic or physiological effects on insects<sup>12,13</sup>. Also, the interaction of these compounds might lead to their excretion without benefiting from them. This, in turn, causes the death of the larvae or damages their nervous system leading to paralysis, along with failure to continue growing<sup>14-16</sup>. The study of (17) showed that the alkaloid extracts of the leaves of *Moringa oleifera*, *Conyza dioscoridis* and *Cymbopogon citratus* had significant effects rate of different stages of the

Extract type	Time/ hour Conc. mg/ml	24	48	72	Mean for concentra- tions	Mean for extract type
Leaf extract	0	0.0	0.0	0.0	0.00	35.55
	10	26.7	33.3	40.0	33.33	
	20	40.0	46.7	53.3	46.66	
	30	53.3	60.0	73.3	62.2	
Flower extract	0	0.0	0.0	0.0	0.00	43.88
	10	33.3	46.7	53.3	44.43	
	20	46.7	53.3	73.3	57.76	
	30	60.0	73.3	86.7	73.33	
Mean for period		32.5	39.16	47.48		
I.s.d (0.05) extract type				1.863		
I.s.d (0.05) extract concentration				3.475		
I.s.d (0.05) period of treatment				2.734		
I.s.d (0.05) interaction				6.707		

**Table 1.** Effects of concentrations of crude alkaloid compounds extract of leaves and flowers of *A. majus* on the mortality rate of the second larval stage of the sawtoothed grain beetle *O. surinamensis*.

insect, where the highest mortality rate was 73.18%. Also, (18) reported that extracting crude alkaloid compounds from the residues of the tobacco plant *Nicotiana tabacum* caused the death of the date moth *Ephestia cautella* Walker.

### Conclusions

The study showed that the second larval stage was more sensitive to the action of the extracted compounds. This might be attributed to several factors, including the presence of active compounds that have toxic effects when they enter the digestive system of the larva, as well as the thickness of the cuticle layer in the insect's body. The toxic compounds in the extract penetrate into the larva's body and show effects on food conversion efficiency.

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Extract type	Time/ hour Conc. mg/ml	24	48	72	Mean for concentration	Mean for extract
Leaf extract	0	0.0	0.0	0.0	0.00	27.22
	10	20.0	26.7	33.3	26.66	
	20	26.7	33.3	46.7	35.56	
	30	40.0	46.7	53.3	46.66	
Flower extract	0	0.0	0.0	0.0	0.00	35.55
	10	26.7	33.3	40.0	33.33	
	20	40.0	46.7	53.3	46.66	
	30	53.3	60.0	73.3	62.2	
Mean for period		25.83	30.83	37.48		
l.s.d (0.05) extraction type				2.474		
l.s.d (0.05) concentration				3.326		
l.s.d (0.05) period				2.738		
l.s.d (0.05) interaction				5.879		

**Table 2.** Effects of concentrations of crude alkaloid compounds extract of *A. majus* leaves and flowers on the mortality rate of the fourth larval stage of the sawtoothed grain beetle *O. surinamensis*.

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