

Schemical Detection by GC-MS Technology of Asclepias Curassavica and the Effect of Chloroform Extract on the Stages of Tribolium Castaneum (Coleoptera Tenebrionidae)

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Abstract

The active compounds were detected using gas chromatography-mass spectrometry GC-MS of Turkish oleander plant *Asclepias curassavica* and their effect on the life stages of the red flour beetle *Tribolium castaneum*. The results showed that Turkish oleander contains 19 active compounds, including Phytol and Hexadecane, which it has an insecticide effect and many therapeutic properties. The effect of chloroform extract of oleander leaves was tested using direct and indirect spraying methods on the percentage of death of red flour beetle phases in concentrations (10%, 20%, and 30%). The study also showed the superiority of the direct method on The indirect method where the killing rate reached 84.8% and 81.9%, respectively, for the stages of the insect, and the concentration of 30% gave the highest killing rate of 94.4%. The results also showed that the most affected by the chloroform extract is the adult insect.

Keywords: active compounds, red flour beetle (*Tribolium castaneum*), medicinal plants, plant extracts

1. Introduction

Cereals and their products are the main source of human food in various parts of the world, and they are exposed to various damages as a result of being infected with insect pests during the harvest and storage process, as many of these pests feed on those grains, and thus lead to a decrease in their nutritional value, and it is considered an insect The red flour beetle, *Tribolium castaneum*, which belongs to the family Coleoptera Tenebrionidae, is the most important insect pest and causes many problems to it and its products such as flour, nuts, pasta and beans, causing great economic losses due to its rapid multiplication and high adaptability [1], and the difficulty Controlling these pests to their presence with foodstuffs, as the use of chemical pesticides leads to contamination of these materials, and the repeated use of them leads to the emergence of new strains that are resistant to these pesticides and thus affect the environment in addition to their harmful effect on non-target biological enemies [2], In recent years, the use of biological control, such as plant pesticides, has been directed to the plant it is an alternative source for Botanical Pesticides because it contains many active compounds that can be separated using gas chromatography-mass spectrometry (GC-MS) device and is safer for humans and the environment and highly effective against these insects [3, 4]. Of plants as nutritional or repellent inhibitors or used for the purpose of preventing grains from being infected with these pests.

The use of biological control with plant extracts, whether aqueous, phenolic or chloroform, has increased to control the red flour beetle *T.castaneum*. The extracts of rue were used to control the rusty flour beetle *Tribolium castaneum*. The use of water extracts of 27 plants had an effect on the percentage of larval stage mortality of the rusty flour beetle *T.castaneum* [5], and in a study conducted by Suleiman [6] it

proved the efficiency of three plant powders: *Quercus infectoria*, *Zingiber officinale* and pomegranate *Punica granatum* against adults of the rusty flour beetle *T.castaneum* at concentrations of 0.0, 3, 5, 10 and 15, as the percentage was 8.616, 7.122 and 4.912% for powdered ginger, tannins and pomegranate plants, respectively, and the results were shown after 6 days of The treatment was superior to the ginger plant powder over the rest of the plant powders with a concentration of 15% for the repellent effect, an average of 9.024%, and the same concentration of tannins and pomegranate powders, and an average of 7.899% and 6.711%, respectively, as Youssef [7] indicated in a study to control the rusty flour beetle by using extracts of the rusty flour beetle. *Rosmarinus officinalis* leaves, *Zizyphus lotus* fruits and *Trigonella foenum graecum* seeds in four concentrations for all stages of the insect. The killing is very high, as it reached 100% at the concentration of 20 mg/ml for the larval stage, while the killing rate was 25% for the adults.

2. Materials and Methods

1. Sample collection: the red flour beetle, *Tribolium castaneum*, obtained from flour infested with the bug from one of the shops in Ramadi, which was placed in plastic containers and the container mouth was covered with a boring cloth for the purpose of ventilation and preservation of the insect from destruction. The Turkish oleander plant *A. curassavica* was collected from one of the nurseries in The city of Ramadi during its flowering season in the summer, the leaves were carefully and meticulously collected for the purpose of obtaining clean leaves free from diseases, insects and not torn, after that clean the plant and spread it in a well-ventilated place away from direct sunlight, taking

into account the constant stirring to avoid rotting and after making sure of the Dry the plant completely, grind it using an electric grinder and keep it in clean, sterile glass containers until use.

2. Preparation of the chloroform extract of the plant: the chloroform extract was prepared according to the method [8], where (50) grams of plant powder was placed in a volumetric flask with a capacity of 1000 ml and 500 ml of chloroform was added to it at a concentration of 80%, then placed in a vibrator for half an hour, after which it was placed in a bath Water at 37°C for 24 hours. The extract was filtered using a funnel and several layers of medical gauze. The filtrate was taken from it and placed in the electric oven at a temperature of 50°C. After obtaining the powder, it was kept in the refrigerator until use.
3. Gas chromatography-mass spectrometry GC-MS analysis: a sample of the plant powder was taken to prepare the 99% alcoholic extract according to the method [9] by soaking 4 g of the plant in 100 ml of ethyl alcohol for 24 hours in a tabletop vibrating incubator. Central at a speed of 2000 rpm and the filtrate was taken and placed at room temperature until a dry powder was obtained, and the powder was kept in dark containers until use. The alcohol-extracted filtered plant samples were placed in a GC-MS device of the type GC-MS-QP2010 plus helium gas, its purity (9), the injection port temperature is kept within 280°C, the flow rate of helium gas is 1ml per minute, and the ionization energy value is Electron 70 volt ev. Separation was accomplished by heating a 5MS Column for 30 minutes. The quadrupole mass detector was used to detect compounds through a hole in the column. The temperature of the detector

3. Testing the effectiveness of different concentrations of chloroform extract on insect mortality rates

direct method: effect of different concentrations of plant extract on the mortality rates of the first and late larval stages of the red flour beetle.

10 larvae of the first larval instars were placed in Petri dishes with three replicates for each concentration of the prepared concentrations 10%, 20%, and 30% g/ml. A small hand sprayer sprayed the dishes containing insects with the plant extracts that were prepared previously, in addition to placing 10 larvae were placed in a special dish for control treatment, sprinkled with distilled water, left the dishes to dry a little, and 5 g of semolina was added to each dish, then the dish was closed and left for 10 days. Insect mortality was followed up daily and the results were recorded.

4. Effect of different concentrations of plant extract on the mortality rates of red flour beetle adults

The same steps mentioned in the two paragraphs above were followed, except for the replacement of larval stages

with adults.

Indirect method

Effect of different concentrations of plant extract on the mortality rates of the first and late larval stages of the red flour beetle *T. castaneum*.

In this method of treatment, the empty dishes are sprayed by a small hand sprayer with the prepared plant extract. After spraying the dishes, we put 10 larvae of the primitive stage for each replicate and three replicates in the extract-treated dishes for each concentration of the prepared concentrations 10%, 20% and 30% g/ml, in addition to placing 10 larvae in a special dish for control treatment and spraying with distilled water, then leave the dishes to dry a little, then put 5 g of semolina in each dish, then close the dish and leave for 10 days with daily follow-up to the destruction of insects, and the results are recorded.

Effect of different concentrations of plant extract on the mortality rates of red flour beetle adults: the same steps mentioned in the two paragraphs above were followed, except for the replacement of larval stages with adults.

5. Statistical Analysis

The results were analyzed according to a complete randomized design factorial (CRDF) design.

6. Results and Discussion

Qualitative detection using gas chromatography (GC-MS) technique for *A. curassavica*

The results of the gas chromatography technique in [Table \(1\)](#) and [Figure \(1\)](#) showed that the leaves of Turkish oleander plant *A. curassavica* contain 19 chemical compounds, the highest retention time was 29.159 for Germanicol, followed by Olean -12-ene, whose retention time was 28.735. the minimum retention time was 7.708 for Silane, cyclohexyldimethoxymethyl and Dodecane, whose retention time was 8.220. In terms of the percentage of presence, the compound Germanicol had the highest percentage, which amounted to 22.838%, followed by the compound Phenol, 2,4-bis(1,1-dimethylethyl) - which amounted to 19.833%, and the lowest percentage is Silane, cyclohexyldimethoxyme-thyl, which had a percentage of 0.407%.

The presence of many chemical components in the plant gives it importance in the field of plant medicine, including glycosides, phenols, saponins, amino acid activators, proteins and terpenoids. Alkaloids and flavonoids were absent in this plant [10], where the plant has many pharmacological activities. As an anti-microbial, antipyretic, analgesic, anti-cancer, and many other pharmacological activities because it contains a wide range of chemical components of therapeutic importance [11], Insects and other organisms.

In a study conducted by [12] to detect the active compounds in the *Cassia italic* plant, it was found that the plant contains 17 compounds, as these compounds have many biological properties, e.g. Phytol and Hexadecane, which have an insecticide effect and many therapeutic properties. Where found that Hexadecane possesses an antioxidant, hypocholesterolemic, nematocidal, and pesticide effect, and that Phytol has an insecticidal effect as it is a larvicide, insect

repellent and stops the growth of eggs [13]. Confirmed that Phytol extracted from Essential oils of *Artemisia argyi* against malaria vector *Anopheles sinensis* showed the strongest larvicide activity among all the components of the tested essential oils, and the cause of insect death is due to the

active substances present in the plants. Phytol is also one of the natural substances that are extracted from the plant and is highly effective against insects, especially aphids, which resist most pesticides, and this compound is considered an effective green insecticide against their growth [14, 15].

Table (1): Active compounds separated from the extract of Turkish oleander leaves *A. curassavica* using GC-MS technique

Chemical formula	Compounds	R.T Min	Peak height	Of total %
	Silane, cyclohexyldimethoxymethyl-	7.708	57547	0.407%
	Dodecane	8.220	237812	1.591%
	Hexadecane	9.117	82519	0.508%
	Tetradecane	10.938	401470	1.943%
	Heptadecane, 2,6,10,15-tetramethyl-	12.169	342455	1.750%
	Phenol, 2,4-bis(1,1-dimethylethyl)-	12.408	3367295	19.833%
	Hexadecane	13.392	179176	0.886%
	Heneicosane	14.667	192343	1.080%
	Pentadecanal-	16.032	190027	1.078%
	Oxirane, tetradecyl-	16.288	75384	0.511%
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	16.474	146789	0.756%
	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	16.964	157340	0.890%
	Phytol	18.642	120453	0.684%
	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	21.174	255670	3.129%
	Olean-12-ene	24.386	251086	8.161%
	Germanicol	24.655	113600	3.087%
	Lupeol	26.146	334836	13.996%
	Olean-12-ene	28.735	438562	16.871%
	Germanicol	29.159	610369	22.838%

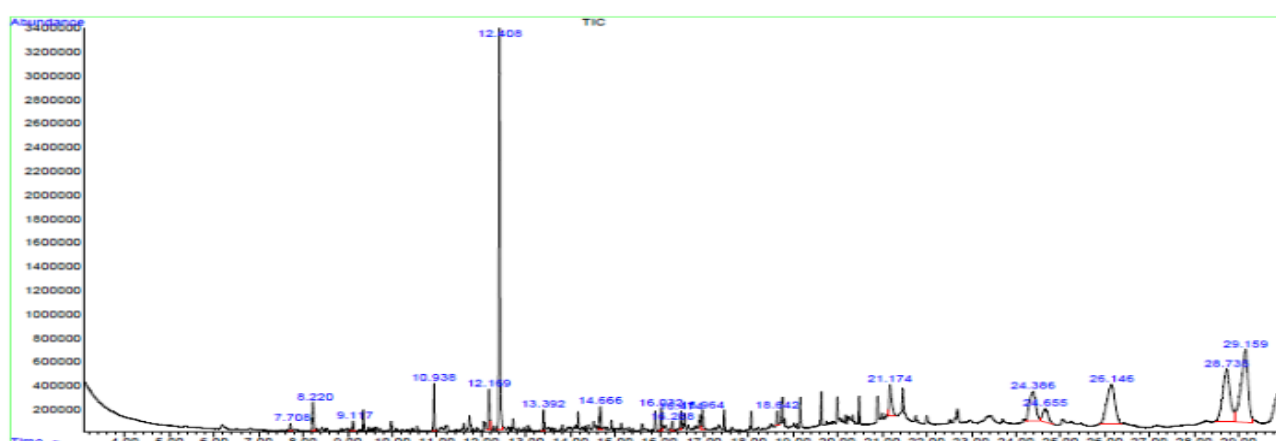


Figure (1): Analysis curve of the active compounds separated from the leaves of *A. curassavica*

The effect of chloroform extract of *A. curassavica* by two methods of direct and indirect spraying on the life stages of *T. castaneum*

The results are shown in Table (2) for treating the insect with chloroform extract of Turkish oleander plant *A. curassavica* to determine the effect of the extract on the red flour beetle *T. castaneum* with three concentrations of 0.100, 0.200, and 0.300 mg/ml, by

using indirect and direct spraying methods and for the three stages, the first larval stage, the late larval stage and the adult insect. There were no significant differences at the level ($p < 0.05$) between the two spraying methods, but the first indirect method had less effect than the second direct method, as the killing rate reached 81.9% and 84.8%, respectively, after 10 days of treatment. It was also found through the study

that the most effective stage of the chloroform extract is the adult stage, as the killing rate in this stage was 83.9%, which is higher than the killing rate of the first and second larval instars, which reached (82.8%, 83.3) respectively after 10 days of treatment.

As for the concentrations used in the killing, the results showed that the high concentration is more effective than the low concentration, as the highest killing rate was in the third concentration, reaching 94.4%, while the killing rate decreased to 70% in the first concentration. These differences are considered significant at the level of ($p < 0.05$) after 10 days of treatment, and the results of the above table indicate that there are no significant differences at the level ($p < 0.05$) of the binary interactions between concentrations and the killing phases and the binary interaction between the killing method and the concentrations and the binary interaction between the killing method and the phases, as the results of the table appeared Same, there were no significant differences at the level ($p < 0.05$) of the triple interaction method of killing, phases and concentrations after 10 days of treatment.

Through the results of the study, it was found that plant extracts have a clear effect on the killing rate of all insect stages of the red flour beetle *T. castaneum*. It is clear from the above that the higher the concentration used, the higher the rates of killing larvae and adults, and this agrees with the result of [Khatib et al. \[16\]](#) that higher concentrations of plant extracts of the leaves of *Acacia cyanophylla*, *Nerium oleander* and *Eucalyptus gomphocephala* increased the percentage of mortality of *Oryzaephilus surinamensis* at the same concentration, whether they were larvae or adults, where the extract of oleander extract and adults outperformed *Eucalyptus* larvae. Similar to what [Nirjara et al. \[17\]](#) mentioned, high concentrations of plant extracts of pomegranate peel and curry leaves led to a higher rate of mortality of the same insect, and this is consistent with what [Al-Nasiri et al. \[18\]](#) found about the effect of chloroform extracts of red tea leaves *Hibiscus sabdariffa* L, peppermint leaves, *Mentha piperita* and *Quercus infectoria* leaves, and the results are close to the results of [Farhana et al. \[19\]](#) on the repellent and lethal effect of chloroform extracts for the three plants, *Zingiber officinale*, *Coriandrum sativum* L and pomegranate *Punica granatum* against the red flour beetle *T. castaneum*, where coriander was less lethal to other plants. Also, these results were close to what was indicated by [Al-Janabi \[20\]](#) about the effectiveness of three types of plant extracts, *Thymus vulgaris*, Dill,

Anethum graveolens and *Trigonella foenum*, with four concentrations of each type, 1, 5, 10 and 15% against similar flour beetle adults *T. confusum* where the extract of thyme gave the highest killing rate of 60% at 15% concentration, followed by dill and fenugreek at the concentration and the percentage of killing increases with increasing concentration. This is due to the insect's sensitivity to the chloroform extract of the Turkish oleander plant *T. castaneum*, as the death rates were very high for all stages, and this gives preference to choosing this extract in the fight against the insect. The results of [Khamis et al. \[21\]](#) showed that The extract of chloroform from the fruits of the locust plant has a fatal effect on the adults of the rusty flour beetle, and the percentage of killing increases with the increase in the concentration of the extract. These results are in agreement with the results of our study.

And our results do not agree with what [Youssef \[7\]](#) mentioned that the plant extract affects the larvae at higher rates than the whole, and the reason may be due to the nature of the extract and the solvent used in the GC-MS test that was conducted in paragraph (1-3) on the leaves of the plant between containing two compounds. Phytol and Hexadecane, which are pesticides and harmful to insects [Ramos et al. \[22\]](#), as they work to poison cells in the gastrointestinal tract and reduce the efficiency of food transformation and thus the insect dies.

The plant extracts have the ability to enter the egg through the opening of the hilum, which negatively affects the fetus, and the soapy compounds, phenols, alkaloids and resins have an effective effect in hatching eggs, and the effect of chloroform extract on these stages is due to the presence of a large group of The active substances that appear during the extraction of the plant with this alcohol [23], in addition to the efficiency of chloroform in dissolving many types of crude secondary compounds that are present in this plant, which gives it great effectiveness in biological control of insects [24] and [25].

The plant extracts have an effective effect against pests *officinalis* through their effect on the synaptic areas and the formation of acetylcholine, which gives symptoms of poisoning when using industrial pesticides. Those compounds or these toxic compounds affect the nervous tissues of the larva and thus lead to the insect's paralysis and failure to grow [26], or through the respiratory stomata of laboratory insects, so the insect suffocates and leads to its death [27].

Table (2): The effect of chloroform extract of *A. curassavica* on the percentages of the first, late and adult larval stages of *T. castaneum* using direct and indirect spraying method

Method of killing A	Phases B	Kill rate% C			Kill rate%	
		Concentration first	Concentration second	Concentration third	rate A	
					A1	A2
Method first	First	70.0%	80.0%	93.3%	81.9%	84.8%
	Second	66.7%	80.0%	90.0%		
	Third	70.0%	90.0%	96.7%	L.S.D. 0.05 = N.S	
Method second	First	70.0%	90.0%	93.3%	S. E= 0.153	
	Second	73.3%	90.0%	100.0%		
	Third	70.0%	83.3%	93.3%		
L.S.D. 0.05 = N.S S.E=0.458						
A x B		B1	B2	B3	Kill rate%	

	A1	81.1%	78.9%	85.6%	B1	B2	B3
	A2	84.4%	87.8%	82.2%			
L.S.D. 0.05 = N.S S.E=0.264					82.8%	83.3%	83.9%
A x C		C1	C2	C3	L.S.D. 0.05 = 0.043 S.E= 0.187		
	A1	68.9%	83.3%	93.3%			
	A2	71.1%	87.8%	95.6%			
L.S.D. 0.05 = N.S S.E=0.264							
B x C		C1	C2	C3	Kill rate%		
	B1	70.0%	85.0%	93.3%	C1	C2	C3
	B2	70.0%	85.0%	95.0%			
	B3	70.0%	86.7%	95.0%	70.0%	85.6%	94.4%
L.S.D. 0.05 = N.S S.E=0.324					L.S.D. 0.05 = 0.536 S.E= 0.187		

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