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Comparing the Effects of Ethyl Elcoholic and Aquatic Extracts and Alkaline Compounds of Some Plants on the Bioefficacy of *Culex pipiens* (Diptera: Culicidae)

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Abstract

The results of the present study show the biological effects of ethyl alcoholic and aquatic extracts as well as alkaloids compounds of *Atriplex helmius* and *Capparis spinosa* roots. The results demonstrated the superiority of the cold aqueous extract of *C. capparis* at the highest concentration of 20 mg / ml, as the killing rate was 81.16% for the second larval instar of *Cx. pipiens* after 72 hours of exposure. While, the killing rate caused by the same treatment was 79.52% for the third larval instar. The results also showed the superiority of the alcoholic extract against *C. spinosa*, as the killing rate reached 98.55% for the second larval instar after 72 hours of treatment at a concentration of 20 mg/ml. As for *A. halimus* extract, the same treatment caused the lowest killing rate of 89.27% for the third instar of *Cx. pipiens*. The study also showed significant effects of the raw alkaline compounds' extracts of *A. halimus* roots on the killing of non-adult instars of *Cx. pipiens* L. The highest rate of mortality observed was 88.54% at the highest concentration of 20 mg /ml at the second instar. Also, significant effects of raw alkaline compounds' extracts of *C. spinosa* roots were observed. The highest mortality was 69.21% at the highest concentration of 20 mg/ml for the third larval instar,

Keywords: *Culex* mosquito, alkaloids compounds, plant extracts

مقارنة تأثير مستخلصي الكحول الأثيلي والمائي والمركبات القلوانية لبعض النباتات في الأداء الحياتي لبعوض الكيولكس (*Culex pipiens*. (Diptera: Culicidae)

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الخلاصة

تم خلال هذه الدراسة تقييم فعالية المستخلص الكحولي والمائي والمركبات القلوانية لجذور نباتي الرغل الملحي *Atriplex halimus* والكبر *Capparis spinosa* في الأداء الحياتي لبعوض الكيولكس *Cx. pipiens*. بينت الدراسة الحالية تفوق المستخلص المائي البارد لجذور نبات الكبر *C. spinosa* إذ حقق أعلى نسبة قتل للعمر اليرقي الثاني عند أعلى تركيز مستخدم 20 ملغرام / مل إذ بلغت نسبة القتل

81.16% بعد مرور 72 ساعة من المعاملة . بينما بلغت نسبة القتل 79.52% بعد مرور 72 ساعة من المعاملة للعمر اليرقي الثالث عند اعلى تركيز 20 ملغم / مل لنفس المستخلص . أما المستخلصات الكحولية فقد بينت تفوق المستخلص الكحولي لنبات الكبر *C. spinosa* . إذ بلغت نسبة القتل 98.55% للعمر اليرقي الثاني بعد 72 ساعة من المعاملة عند التركيز 20 ملغم / مل من المعاملة أما مستخلص نبات الرغل الملحي فقد حقق نسبة قتل بلغت ، 89.27% عند اعلى تركيز مستخدم 20 ملغم / مل، بعد مرور 72 ساعة من المعاملة للعمر اليرقي الثالث لبعوض *Cx. pipiens* وكذلك بينت الدراسة وجود تأثير معنوي لتراكيز مستخلص المركبات القلوانية الخام لجذور نبات الرغل الملحي *A. halimus* في الهلاك الادوار غير البالغة لبعوض الكيولكس *Cx. pipiens* . إذ بلغ اعلى نسبة هلاك عند العمر اليرقي الثاني 88.54% عند اعلى تركيز مستخدم 20 ملغم / مل . كذلك وجود تأثير معنوي لتراكيز مستخلص المركبات القلوانية الخام لجذور نبات الكبر *C. spinosa* . للعمر اليرقي الثالث إذ بلغ اعلى نسبة هلاك 69.21% عند اعلى تركيز مستخدم 20 ملغم / مل.

1. Introduction

There is still a great need for studies on *Culex* mosquitoes to determine the other species present in Iraq; there may be other types that can be detected and recorded in the future [1]. That more hidden sites in plants and weeds are more attractive to female mosquitoes to lay eggs than open sites [2]. *Cx. pipiens* is one of the species known to be associated with humans [3]. The home mosquito *Cx. pipiens* is the vectors of many human and animal pathogens, including West Nile virus, meningitis viruses, Dengue fever virus [4] and Rift Valley Fever virus, in addition to the transmission of Filariasis [5]. There have been continuous searches for other alternatives, including the use of plant extracts, to combat insects, given the active substances they contain against insects and their desired characteristics, the most important of which is the absence of resistance to them. One of the most important modern methods of control Are those that utilize alkaloid compounds, which are found freely or in the form of salts containing carbon, hydrogen, oxygen, and nitrogen. These compounds are usually crystalline and colorless, but contain low oxygen levels in the liquid forms in the leaves of the plant [6]. The reason for choosing alternative plants is because they contain desirable traits that are not present in chemical pesticides.

The random, sometimes excessive and non-programmed, use of pesticides in the control of agricultural and medical pests has led to environmental pollution in addition to the side effects of toxins within the pesticide industry on humans [7]. There is a growing environmental awareness and learning about the dangers of pesticides, in parallel to the loss of effectiveness of many pesticides and the acquisition of resistance by most insect pests. . Thus, the search for ways to control pests, rather than exterminating them, is currently considered as the correct way of thinking for better environmental protection. This can be reached by using several biological means, including biological control [8], which encouraged researchers to reduce dependence on the pests and chemicals and look for other alternatives, including the use of plant extracts for insect control. This is due to the discoveries that plants contain effective substances against insects and have desired characteristics. Most importantly, insects are unable to develop resistance to plant materials [9]. This research aims to set another alternative to chemical control by assessing the effectiveness of alcoholic and aqueous extracts as well as alkaloid compounds of *A. halimus* and *C. spinosa* in the life performance of *Cx. pipiens*.

2. Materials and methods

2.1 Plant specimen collection

The roots of *A. halimus* and *C. spinosa* were collected from Ishaqi region, Saladin Governorate, Iraq, in January 2020. The samples were then dried and ground to obtain a fine vegetable powder, which was kept in a sealed bottle and placed in the refrigerator until use.

2.2 Insect collection and breeding

The immature instars (eggs and larvae) were collected from a water drainage site in in the sampling areas by using a long-arm net placed in a plastic container with a lid. The samples were transferred to the laboratory and placed in plastic tanks filled with chlorine - free water. Proportions of wheat, maize, rice, and protein (0.25: 1: 1: 1) were added (2 gm) in breeding ponds to feed the larvae [10]. For the purpose of obtaining a pure permanent culture, the newly emerging pupae were

transported to plastic containers deposited in an aluminum cage (1m × 1m × 2.20 m) with very fine wired sides. Inside the cage, petri dishes were placed containing cotton saturated with 10% sugar solution [11]. Also, a small water container was placed inside the cage to be utilized as a place for laying eggs. Egg-boats placed in a small brush were transferred to new water containers containing food for the larvae; water was added every three days or removing the surface layer. This method was repeated until the fourth generation of adults. Samples from the larvae of the second and third instar and adults for this generation were prepared on slides for the purpose of diagnosis, according to the classification characteristics mentioned in the classification keys [12]. Insect breeding was carried out under controlled laboratory conditions of $28 \pm 2^\circ\text{C}$, $60 \pm 5\%$ relative humidity, and 12 hours rate of illumination.

2.3 Preparing aqueous extracts

Harbone method [13] was followed in preparing aqueous extracts of plant roots. In brief, 20 g of plant root was placed in 30 ml of cold distilled water, mixed using an electric mixer for 15 minutes, left for 30 minutes, and filtered by filter paper [14] to separate the large plankton. The filtrate was taken and evaporated with a rotary evaporator at a temperature of 40°C . Then, the filtrate was dried in an electric oven at $40\text{-}50^\circ\text{C}$, after which the dry raw material at a weight of 8 g was obtained. For the purpose of testing the effectiveness of the cold aqueous extracts of the plant roots on the second and third larval stages of *Cx. pipines*, 2 g of the dry raw material from each extract was separately dissolved in 100 ml distilled water to obtain a concentration of 20%, or the equivalent of 20 mg / ml. Then, the preparations were made into four concentrations (5, 10, 15, 20 mg / ml) of root extracts. The control sample involved distilled water only.

2.4 Ethyl alcoholic extract

An amount of 20 g of the ground plant portion was placed in Reflex Extractor with 100 ml of 95% ethyl alcohol. Extraction was carried out at a temperature of 30°C for 30 hours. The extracts were dried by a rotary evaporator at a temperature of 40°C . Then, the leachate was dried in an electric oven at $40\text{-}50^\circ\text{C}$, yielding a dry raw material at a weight of 8 g, which was preserved until use [15]. Dry raw material (2 g) from each extract was separately dissolves in 100 ml ethyl alcohol (95% concentration) to obtain a concentration of 20%, or the equivalent of 20 g / ml. Then, concentrations of 5, 10, 15, and 20 mg / ml of the extracts were used, whereas distilled water was employed as control.

2.5 Preparing raw alkaloid compounds extracted from roots

Harbone method [13] was followed in preparing the extracts of roots of the plant by using raw alkaline compounds. 5 g of dry matter powder was weighed for each root and extracted with 100 ml ethyl alcohol for a period of 24 hours in a soxhlet apparatus at a temperature 45°C . The extracted substance was concentrated in the rotary evaporator, then this substance was dissolved in 5 ml of ethyl alcohol and 30 ml of sulfuric acid 2% was added to the alcoholic extract. Meyer test was performed to this solution to confirm the presence of alkaloids, where the test showed a cloudy white precipitate when adding a drop of the reagent to a drop of H_2SO_4 [16]. An adequate amount of ammonium hydroxide with a concentration of 10% to was added to this solution to bring become pH to 8. The base solution was placed in the separating funnel and 5 ml of chloroform was added, followed by shaking for several times. Thereafter, the mixture was left to separate in two layers. The bottom layer (containing chlorophyll soluble alkaloids) was taken and the process was repeated three times, taking the bottom layer each time, so that the combined solution volume became about 20 ml. Then, the sample was dried in the oven at a temperature of $40\text{-}45^\circ\text{C}$. The dry matter was kept in a sealed glass container in the refrigerator until use. The extraction process was repeated several times in order to obtain an adequate amount of alkaloids.

For the purpose of estimating the biological efficacy of the extract of dry raw alkaloids, 1 g of dry alkaline extract (roots) was dissolved in 3 ml of 96% ethyl alcohol and the volume was completed to 100 ml with distilled water. Hence, the stock solution concentration became 1%, or the equivalent of 10 mg / ml, from which the four concentrations of 5, 10, 15, 20 mg /ml were prepared.

2.6 Effects of the aqueous and alcoholic extracts on the larval instar

Four concentrations of aqueous and alcoholic extracts of the plants were used in the study (5, 10, 15, 20 mg / ml). Insects from the second and third larval stages were placed in a container with distilled water or chlorine-free water, The toxicity of the extracts prepared on the larvae were tested using four replicates per concentration at 50 ml. 20 larvae were placed per a replicate. The

experimental pots were left at room temperature and the number of dead larvae was counted after 24 and 72 hours of exposure.

2.7 Effects of extracted alkaloids from *A. hilmus* roots on the larval instars

20 larvae were taken from the second instar larvae for each replicate, with 4 replicates for each concentration. The larvae were transferred to plastic containers with 100 ml of the extract and 0.2 g of larval food. The percentage of loss in the second larval phase was counted after 24 and 72 hours of exposure. The same process was repeated with respect to the third larval instar, and the percentage of loss was adjusted according to its equivalent [12].

2.8 Effects of extracted alkaloids from *C. spinosa* roots on larval instars

20 larvae were taken from the second larval instar for each replicate, with 4 replicates for each concentration. The larvae were transferred to plastic containers with 100 ml of the extract and 0.2 g of larval food. The percentage of loss in the second larval phase was recorded after 24 and 72 hours of exposure. The same process was repeated with respect to the third larval instar, and the percentage of loss was adjusted according to its equivalent [12].

3. Results and Discussion

The superiority of alcoholic extracts in general over aqueous extracts in killing mosquito larvae was clear. Ethanol solvent was more effective in dissolving effective compounds than water. The results confirm that the toxicity of alcoholic extracts is higher than that of water extracts for all plants used. This might be due to the difference in the polarity degree of the solvents used, as the polarization coefficient is 5.2, ethanol alcohol 5.2 and water 9 [17].

3.1 Effects of cold extract of roots on second larval instar of *Cx. pipiens*.

The results of the effects of the aqueous extracts in killing mosquito larvae are shown in Table-1. Water extract of *A. halimus* caused the highest killing rate of 68.48% at the highest used concentration of 20 mg /ml. *C. spinosa* root called extract caused the highest rate of killing at the highest user concentration of 20 mg /ml, with a killing rate of 81.16% after 72 hours of treatment. It is clear that the killing rates increased with increasing the duration of the treatment, on one hand, and with increasing concentrations used, on the other hand. The statistical analysis showed that there are significant differences in the percentage of killing.

Table 1- Effects of the cold extract of plant roots on the survival of the second larval instar of *Cx. pipiens*.

plant	Time / h	instar	concentration, mg /ml					killing rate
			5	10	15	20	control	
<i>A .halimus</i>	24	second	The Percentage dead					50.43 A
			11.12	40.14	61.64	67.19	0.00	
	72		31.62	45.62	59.71	68.48	0.00	
killing rate			67.82 d	60.67 c	42.87 b	21.19 a	0.00 e	
<i>C .spinosa</i>	24	second	16.51	53.61	68.27	79.39	0.00	60.65 B
			72	34.62	69.68	73.52	81.16	
	killing rate		25.57 a	61.64 b	71.32 c	80.27 d	0.00 e	

* Horizontally similar small letters mean no significant differences

3.2 Effects of the root cold extracts on the third larval instar

The results of the effects of root aqueous extracts on the killing of mosquito larvae are shown in Table-2. The aqueous extract of *A. halimus* caused the highest killing rate of 74.34 % at the highest used concentration of 20 mg /ml. *C. spinosa* root aqueous extract caused the highest rate of killing at the highest user concentration of 20 mg / ml, with a killing rate of 79.25 % after 72 hours of treatment. It appears that the killing rates increased with increasing the duration of the exposure.

Table 2 Effects of cold extract of plant roots on the second larval instar of *Cx. pipiens*.

plant	Time / h	instar	concentration, mg / ml					killing rate
			5	10	15	20	control	
<i>A .halimus</i>	24	third	The Percentage dead					53.51 B
			13.42	56.47	64.43	70.29	0.00	
	72		32.12	50.12	70.83	74.34	0.00	
killing rate			22.77 a	53.29 b	67.33 c	72.31 d	0.00 e	
<i>C .spinosa</i>	24	third	19.42	66.43	70.13	76.59	0.00	65.33 A
			72	45.94	76.32	72.26	79.52	
	killing rate			67.36 a	71.37 b	71.37 b	78.23 c	

* Horizontally similar small letters mean no significant differences .

3.3 Effects of root alcoholic extracts on the second larval instar

The results of the effects of the root alcoholic extracts on killing mosquito larvae are shown in Table-3. The alcoholic extract of *A. halimus* caused the highest rate of killing of the second stage of 87.39% at the highest concentration of 20 mg /ml, while *C. spinosa* caused the highest rate of killing of the second larval instar at the same concentration, with a killing rate that reached 98.55% after 72 hours of treatment . It appears that the killing rates increase with the increase of the treatment period. The statistical analysis showed significant differences in the ratio of killing for the second instar.

Table 3-Effects of roots alcoholic extract on the second larval instar of *Cx .pipiens*.

plant	Time / h	instar	concentration, mg /ml					killing rate
			5	10	15	20	control	
<i>A .halimus</i>	24	second	The Percentage dead					61.81 B
			19.63	50.56	70.32	86.21	0.00	
	72		37.85	62.89	79.66	87.39	0.00	
killing rate			28.74 a	56.72 b	74.98 c	86.88 d	0.00 e	
<i>C .spinosa</i>	24	second	23.42	69.71	82.86	93.91	0.00	70.21 A
			72	27.67	78.58	85.32	98.55	
	killing rate			25.54 a	74.14 b	86.39 c	95.37 d	

* Horizontally similar small letters mean no significant differences.

3.4 Effects of roots alcoholic extract on the third larval instar of *Cx. pipiens*

The results of the effect of alcoholic extracts on the killing of mosquito larvae are shown in Table-4. The extract of *A. halimus* caused the highest killing rate of 79.23% at the highest used concentration of 20 mg /ml, while *C. spinosa* caused the highest rate of killing of the third larval instar at the same concentration with killing rate of 89.27% after 72 hours of treatment . It appears that the killing rates increase with the increase of the treatment period, on one hand, and with the increase in the concentrations used, on the other hand.

Table 4-Effects of root alcoholic extracts on the third larval instar of *Cx. pipiens*.

plant	Time / h	instar	Concentration mg /ml					killing rate
			5	10	15	20	control	
<i>A .halimus</i>	24	third	The Percentage dead					58.92
			27.93	45.61	76.25	78.45	0.00	
	37.18 c		48.74 b	78.65 a	79.23 a	0.00 d		
killing rate			32.05	47.17	77.45	78.93	0.00	
<i>C .spinosa</i>	24	third	29.82	62.21	76.72	83.72	0.00	63.79
			36.49	78.53	78.18	89.27	0.00	
	killing rate			33.14 c	70.37 b	78.45 b	86.39 a	

* Horizontally similar small letters mean no significant differences.

The results show that the alcoholic extracts generally outperformed the aqueous extracts of plants in killing mosquito larvae. Ethanol solvent was more effective in dissolving active compounds than water.

The possible cause for the emergence of the distortions is that the toxic compounds in the plant could have effects similar to those of the anti-insect hormones, especially the juvenile, molting, and ecdysis hormones, thereby preventing the occurrence of alienation. This toxicity is due to the fact that the active compounds of the plant act as infectious agents, which leads to the obstruction of bowel movement and influences on the course of the activity of digestion and absorption.

This study is consistent with that of Alkazrzi [18], which revealed that the alcoholic extract of the datura plant had a significant effect on the ratios of larval and pupae instars of *Cx .pipeins*. The results also showed the appearance of larval and pupae deformities, as adults were discouraged from laying eggs. The current study also agrees with that of Alkafagi[19], which demonstrated that the alcoholic extract of *Peganum harmala* increased significantly the destruction of *Cx .pipeins* larvae. This study also agrees with that of Alkafaji[20] in which a cold water extract of *Schangina aegyptica* leaves had a significant effect on mortality rate of the larvae and virgins of *Cx .pipeins*.

Table-5 shows the effects of the extract concentration on the mortality of the larvae, where the highest mortality rates were recorded at 88.54% for the second instar and 65.42% for the third instar using the concentration of 20 mg / ml. These results confirm that the extract of the roots of *A. halimus* significantly affected the different larval instars of the insect. While the mortality rate decreased to its lowest levels using the concentration of 5 mg / ml, as the mortality rates were 5.43% for the second instar and 4.64% for the third instar. This is an indication of the existence of a correlation relationship; the rates of loss increase with increasing concentrations of root extract, where the results of the statistical analysis indicated the presence of significant differences.

Table 5 Effects of the alkaloid compounds from *A. halimus* roots on *Cx. pipiens*.

Concentration of extract mg/ ml	Mortality rate of second larval instar	Mortality rate of third larval instar
5	5.43	4.61
10	38.61	19.22
15	42.13	40.31
20	88.54	65.42
Killing Mortality rate	43.76	23.35

*Similar small letters in a row mean that there are no significant differences

Table-6 shows the effects of the concentrations of the extract of alkaloids compounds on the mortality of the larval stages . The highest mortality rates were recorded at 69.21% for the second instar and 42.29% for the third instar using the concentration of 20 mg /ml .These results confirm that

C. spinosa root extract significantly affected the different larval stages of the insect. While the mortality rate decreased to its lowest levels using the concentration of 5 mg /ml, as the mortality rates were 3.23% for the second instar and 3.28% for the third instar. This is an indication of the existence of a correlation relationship, so that the rates of loss increase with increasing concentrations of leaf extract, where the results of the statistical analysis indicated the presence of significant differences.

This study is consistent with a previous report [21] which established that the extract of raw alkaloids of *Ricinus communis* roots affected the different larval instars of *Cx. pipiens*, where the highest mortality was 100% as a result of treatment with a concentration of 20 mg / ml. This study is also consistent with another study [22], which revealed that the raw coliform compounds of leaves and roots of the licorice plant *Glycyrrhiza glabra* L. resulted in the killing of the mosquito *Cx. pipiens* at a rates of 75.8 and 83.4% using concentrations of 1 and 20 gl/ l.

Table 6 Effects of alkaloid compounds' concentrations from *C.spinosa* roots on *Cx. pipienes*.

Concentration of extract mg / ml	Mortality rate of second larval instar	Mortality rate of third larval instar
5	3.23	3.28
10	41.57	28.57
15	48.65	33.24
20	69.21	42.29
Killing Mortality rate	40.68 b	26.48 a

*Similar small letters in a row mean that there are no significant differences.

4. Conclusions

The results showed that the aqueous and alcoholic extracts and alkaloid compounds of *A. halimus* and *C. spinosa* roots had significant differences between the different concentrations of the extracts. The alcoholic extract showed the highest killing rate in the second and third instars of mosquito larvae. According to the results , the roots of these plants can be used as an alternative to the chemical control of this insect due to the presence of active substances.

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