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Effect of some plant extracts on the *Culex pipiens molestus* Forskal larvae

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Abstract

A preliminary study was conducted to investigate the effects of the extracts of eight plant species collected from Ninavah governorate on the second instar of larval stage of *Culex pipiens molestus* Forskal. Three out of the eight plant extracts *Azadirachta excelsa* Jack, *Cleome glaucescens* Dc. and *Quercus infectoria* DL. caused 100% mortality of larvae at a concentration of 200 µg/mL after 3days of treatment. The LC50 values were less than 150µg/mL (62.5µg/mL-140µg/mL). The *A. excelsa* leave extract showed mortality on larval and pupal at low concentrations 40µg/mL-10µg/mL also affected in delaying larval development. The extracts of *Achillea santolina* L., *Ammi majus* L. and *Ricinus communis* L. caused high mortality to the larvae after 7 days of treatment, but the *Datura stramonium* L. and *Carum petroselinum* Benth extracts did not cause any mortality to the larvae at the same date.

Keywords: Plant extracts; *Culex molestus*; Instar

تأثير بعض المستخلصات النباتية في يرقات البعوض *Culex pipiens molestus* Forskal

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الخلاصة

تضمنت الدراسة الحالية تأثير مستخلصات ثمان أنواع من النباتات التي جمعت من محافظة نينوى في يرقات العمر الثاني للبعوض *Culex pipiens molestus* Forskal. إذ سببت مستخلصات كل من *Azadirachta excelsa* Jack و *Cleome glaucescens* Dc. و *Quercus infectoria* DL. موتاً لليرقات بنسبة ١٠٠% عند التركيز 200 مايكروغرام/ملليتر بعد ثلاثة أيام من المعاملة. وكانت قيم LC50 أقل من ١٥٠ مايكروغرام/ملليتر (٦٢,٥ مايكروغرام/ملليتر-١٤٠ مايكروغرام/ملليتر). وقد أظهر مستخلص أوراق *A. excelsa* تأثيراً مميتاً لليرقات والعذارى عند التراكيز المنخفضة ٤٠ مايكروغرام/ملليتر-١٠ مايكروغرام/ملليتر، كما سبب المستخلص تأخراً في نمو اليرقات. وكان لمستخلصات *Achillea santolina* L و *Ammi majus* L. و *Ricinus communis* L تأثيراً مميتاً لليرقات عند اليوم السابع من المعاملة، ولكن مستخلصات *Datura stramonium* L. و *Carum petroselinum* Benth لم تؤد إلى موت اليرقات في نفس الفترة المذكورة.

Introduction

Since the discovery of the synthetic insecticides for the control of pests as well as human disease vectors has led to concerns about their toxicity and environmental impact (1), and control of pests is becoming increasingly difficult because of increasing resistance to pesticides (2). Because of this, the search for new environmentally safe, target–specific insecticides in active throughout the world. To find new modes of action and to develop active agents based on natural plant products, efforts are being made of isolate,

screen and develop phytochemicals possessing pesticidal activity.

In Mosul university, one early study on the use of plant extracts against mosquito larvae was that of (3), where it was found that thirty medicinal plants were effective against to fourth instar larvae of *Culex molestus* F., (4) found that acetone extracts from *Melia volkensii* and *Melia azedarach* seeds exhibit growth inhibitory activity against *C. pipiens molestus*. (7) reported that the seeds extract of *Pimpinella anisum* L. has high toxicity to second instar larvae of *C. pipiens molestus*. Also, many studies conducted in around the world have shown that chemicals from plants have insecticidal effects on mosquitoes (5,6,8,9). The aim of the present study was to screen the plants found in Ninavah governorate for test the effects on the second instar larvae of the *Culex pipiens molestus* Forskal.

Materials and Methods

Plant collection and extraction

Samples of seven plant species, *Azadirachta excelsa* Jack (leaves), *Cleome glaucescens* Dc. (leaves and twig), *Achillea santolina* L. (flowers), *Ammi majus* L. (seeds), *Ricinus communis* L. (leaves), *Datura stramonium* L. (seeds) and *Carum petroselinum* Benth (leaves and twig) were collected from the Ninavah governorate from November 2004 to March 2005, except the *Quercus infectoria* DL. (fruits) were getting from the local market. All parts of the plants were dried and powdered using a mechanical grinder.

The method of plant extraction was modified from (6). One hundred grams of each plant dried was macerated with 300 mL of 80% methanol solution and left to stand at room temperature for 2 days. The mixture was filtered through a whatman no. 1 filter paper by suction and the filtrate was evaporated under vacuum at 40°C until completely dried, and kept at a constant 4°C until needed for test.

Rearing of *Culex pipiens molestus*

C. pipiens molestus larvae were obtained from a colony maintained at Department of Biology, College of Science. They were reared indoors at 27 ± 2°C, 70 % ± 10% relative humidity and a 14:10 light: dark photo-period and they were fed daily with ground mouse feed until such time as they molt to become pupae. They were moved into mosquito cage where the emergent adults were fed with a 10 % sucrose solution.

Larvicidal test

Second instar larvae of laboratory – reared strain of *C. pipiens molestus* were tested with different concent-rations of plant extracts (50, 100 and 200 µg/mL). Plant extract, which produced more than 50% larval mortality after three days in preliminary screening, were serially diluted at concentrations of 40, 20 and 10 µg/mL. Fifty milliliters of each test solution was placed in a plastic cup along with 10 second instar larvae. Each experiment was conducted with five replicates and a concurrent control group. Dead larvae, pupae and adults were counted every 24 hours after exposure untile the test was terminated (when all the adults had emerged) and preserved in a 70 % ethanol solution.

Mortality due to treatment, was corrected by (10) formula. The relative development stage (rDS) was calculated using the following formula (11).

$$\frac{\sum(\text{ntSp}\times\text{Fp})}{\text{NtS}} = \text{rDS}$$

Where ntSp the number of a particular development stage living at time t of observation, NtS means the total of all stage living on the day of observation t and Fp means the particular arbitrary multiplication factor.

Results

Three out of eight plant species. *Azadirachta excelsa, Cleome glaucescens* and *Quercus infectoria* at a concentration of 200 µg/mL showed 100% larval mortality after an exposure of 3 days (Table 1). Five remaining plant species, *Achillea santolina, Ammi majus, Ricinus communis, Datura stramonium* and *Carum petroselinum* at a concentration 200 µg/mL caused very low mortality rates of larvae after 3 days of treatment but *A. santolina, A. majus* and *R. communis* extracts showed 70% - 50% mortality of a larvae at a concentration 200 µg/mL after 7 days from exposure, but *D. stramonium* and *C. petroselinum* were not effect to larvae at all concent-rations of used until 7 days of exposure.

Table 1: Toxicity of eight plant extracts on second instar larvae of *Culex pipiens molestus*.

Scientific name	3 days			7 days		
	Concentration (µg/mL)			Concentration (µg/mL)		
	200	100	50	200	100	50
	% Mortality			% Mortality		
A. excelsa	100	100	100	100	100	100
C. glaucescens	100	80	40	100	90	70
Q. infectoria	100	60	20	100	90	40
A. santolina	30	20	10	70	60	40
A. majus	40	10	0	50	20	0
R. communis	30	0	0	50	30	0
D. stramonium	0	0	0	0	0	0
C. petroselinum	0	0	0	0	0	0

Details of the comparative effects of first three plant extracts on mortality of *C. pipiens molestus* are shown in (Table 2). It was found that *A. excelsa* caused high

percentage mortality of larvae (90%) at a concentration 50 µg/mL, the LC50 was 62.5 µg/mL after 1 day and 27.5 µg/mL after 3 days. But the plant extract of *C. glaucescens* caused 80% mortality of larvae at a concentration 100 µg/mL, the LC50 was 72.5 µg/mL after 3 days. The *Q. infectoria* extracts showed 90 % larval mortality after 7 days at a concentration 100 µg/mL, the LC50 was 110.0 µg/mL after 3 days. The means mortality of three plant extracts, *A. excelsa, C. glaucescens* and *Q. infectoria* were 100, 66.6 and 56.6% at a concentration 100 µg/mL respectively.

The larvae took 21 days to develop to pupae, compared to control, when the second instar larvae treated with *A. excelsa* extract, the relative develop-mental stage (rDs) was 3.6, 4.4 and 3.2 in a concentra-tions 40, 20 and 10 µg/mL respectively (Table 3). The dead larvae were in the second instar larval exuvium at a concentraion 40 µg/mL, the pupae which have died in 20 and 10 µg/mL concentrations could not release the pupal exuvium when the abdomen retracted, and observed the pupae dead with unmelanized.

Table 2: Effect of three plant extracts on *Culex pipiens molestus*.

Plant	Days	Concentration (µg/mL) 200	100	50	40	20	10	LC50 (µg/mL)
		% Mortality						
A. excelsa	1	100	100	90	20	0	0	62.5
	3	100	100	100	70	20	10	27.5
	7	100	100	100	80	30	10	
	Mean	100	100	96.6	56.6	16.6	6.6	
C. glaucescens	1	100	30	0	0	0	0	127.5
	3	100	80	40	0	0	0	72.5
	7	100	90	70	70	20	0	
	Mean	100	66.6	36.6	23.3	6.6	0	
Q. infectoria	1	70	20	20	10	0	0	140.0
	3	100	60	20	0	0	0	110.0
	7	100	90	40	0	0	0	
	Mean	90	56.6	26.6	3.3	0	0	

Table 3: Effect of *A. excelsa* extract on immature stages mortality of *Culex pipiens molestus* after 21 days.

Concentration (µg/mL)	Larvae dead	Pupae dead	Adults dead	Mortality %	rDs
40	40	10	0	100	3.6
20	15	20	5	80	4.4
10	5	20	0	50	3.2

Discussion

Three species from eight of plant collected from Ninavah governorate, showed high mortality for the second instar larvae of *C. pipiens molestus*. *A. excelsa*, *C. glaucescens* and *Q. infectoria*, showed larvicidal properties against larvae, the LC50 value at 1 day were 62.5, 127.5 and 140.0 µg/mL respectively.

Extract of *A. excelsa* produced larvae mortality rates after 1 day and 7 days of more than extracts of *C. glaucescens* and *Q. infectoria*, Some of the larvae did not die within the 2 days, but instead they died at the pupal or at the adult stage, due to the chronic effects of chemical compounds attributable to the plant extract (12).

There was a delay in the development of larvae to the pupal stage when the second instar larvae was exposed to concentrations 40 and 20 µg/mL of *A. excelsa*. This may be due to the presence of high juvenile hormone levels in the larvae or due to chemical compounds in the plant, preventing normal pupation and preventing adult emergence from occurring. Alcoholic extract of leave *A. excelsa* have been shown to effect on growth and development of *Epilachna varivestis* larvae at a concentraion 200µg/mL (13). Also (11) reports that azadirachtin may act as an anti–ecdysteroid or else otherwise affected the neuroendocrine control of ecdysteroids such that growth is inhibited and that the developmental period is prolonged. (9) who investigated

the effect of the extracts of eight medicinal plant species on *Aedes aegypti*, showed 100% mosquito larvae mortality, the LC50 values were less than 100 µg/mL, and found the some plant extracts caused delayed larval development and inhibited emergence. (4) found that acetone extracts from *Melia volkensii* and *Melia azedarach* seeds exhibit growth inhibitory activity against *C. pipiens molestus* larvae.

A. santolina, A. majus and *R. communis* caused low mortality to the second instar larvae after 3 days of exposure, but after 7 days caused high mortality in a concentration 200 µg/mL. (3) reports similar effect for the flower extract of *A. micrantha* M.B., which had lethal effects on fourth instar larvae of *C. pipiens molestus* at a concentration 200 µg/mL. While aqueous extracts of *R. communis* leaved strong activity against 2nd and 4th instar larvae of *C. pipiens* L. (14).

No mortality rates of larvae were observed after 3 and 7 days exposure to the extracts of *D. stramonium* and *C. petroselinum* .

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