The Cumulative Effects of Stinging Nettle Plant Extract (*Urtica pilulifera*) on Some Biological Aspects of Mosquito (*Culex pipiens* L.)

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Abstract: The present study was conducted in Duhok city to evaluate the cumulative biological effects of aqueous extract for *(Urtica pilulifera*) which is widely abundant in Iraqi Kurdistan region against the mosquito (*Culex pipiens* L.) using different concentrations (4- 16 mg/ml) on all immature stages of mosquito. The cumulative effect of the Plant extract significantly affected the hatchability of eggs, mortality rate and elongation the duration of the larvae and pupae of *C. pipiens*. The eggs mortality was concentration dependent. The highest incubation period of eggs was found to be 5 days at a concentration of 16 mg/ml. Also the total growth period from the egg to adult reached 21.5 days with the concentration 12 mg /ml. The adult emergence and the most vital biological activities of the emerging females including fecundity (fertility) were obviously affected. Complete emergence inhibition (100%) was recorded at concentrations 14 &16mg/ml while the lowest rate (6.48%) was with a concentration of 4 mg /ml. The highest rate of adult emergence was recorded at the concentration 4mg/ml which was 93.52% while the lowest adult emergence rate was recorded at the concentration 14 mg/ml which was 0%.The preoviposition period of the females which developed from the treated eggs was tested, and it was found that at the concentrations 12 & 14 mg/ml the females were sterile. Female fecundity was significantly decreased at all concentrations above 4 mg/ml. The lowest and highest mean scores of deposited eggs were 32% and 58.39% at concentrations of 8 & 4 mg/ml, respectively. The percentage of egg hatching was extremely reduced at all concentrations compared with control group. It was found that the plant extract caused morphological deformations in different developmental stages.

Key words: Mosquito, Culex Pipiens, Stinging Nettle, Plant Extract, Urtica Pilulifera

1. Introduction

The extensive use of synthetic insecticides, create many problems, such as resistant, the appearance of the secondary pests, the effects on the wild life, and environmental contamination with toxic substance (Yang *et al.*, 2002). In present time many species of mosquitoes which they are medically important and responsible for the spreading of many diseases such as malaria, have the resistant to many insecticides. For this reason many workers start to search for new compounds that can be safe, effective, and not dangerous to human and wild life. These compounds are called (plant origin insecticides) (Klocke & Barby, 1989). Many insecticides are used to control and exterminate mosquitoes, but in many cases, the pests would acclimate themselves to the toxins and develop a resistance (Hemingway and Ranson, 2000; Raymond, *et al.*, 2001). Plant extracts may be the alternative sources of mosquito control due to a lot of bioactive compounds i.e. growth regulation, fecundity suppression, male sterility, loss of flying ability, immune depression and enzyme inhibition (Govindarajan, 2010). Many studies have been done using plant extracts against mosquito (Das *et*

al., 2007; Mustafa and Al- khazraji, 2008 and Al-Chalabi *et al*., 2014). This study was conducted to investigate the presence of natural insecticides in the extract of stinging nettle plant (*Urtica pilulifera*) which is naturally abundant in Iraqi Kurdistan Region, and may help in the controlling the mosquito *Culex pipiens* by determination the cumulative effects of the plant extract on all immature stages of Culex mosquitoes and identifying the morphological abnormalities in the larvae, pupae and biological activities of adult mosquito.

2. Materials and Methods

The plant species was collected from Masike in Duhok Governorate, Kurdistan Region, Iraq during April 2014.The plants were dried and the whole plant (except root) was ground into fine powder, the obtained powder was kept in a cloth bag and placed in a refrigerator until the time of use. Plant identification was done at the College of Agricultur, University of Duhok. Stock culture of Culex mosquitoes (which was maintained under insectary condition for three generations) was made by using plastic containers (500 ml) containing tap water, and supplied with rabbit chew as food source. The adults, after their emergence, were provided with a piece of cotton soaked in 10% sugar solution using those (females) for egg desposition (Mohsen & Mehdi. 1989). The culex mosquito species used in this study had been classified by Research Centre and Natural History Museum, Baghdad as *Culex pipienes* L. (Diptra: Culicidae). Immature stages rearing were based on Osgood (1971). Rearing conditions were 28±1ºC, 50-70% relative humidity, and 12 hrs. photoperiod. Aqueous extraction was prepared from *Urtica pilulifera* according to the method described by Rahhal (2000). Concentrations of 4, 6, 8, 12, 14 and 16 mg/ml were prepared from stock solution prepared by dissolving 25 gram of the plant extract in 400 ml of distilled water. After deposition of eggs, the egg rafts were collected and transferred to plastic bowls (100 ml capacity) 4 replicates were used for each concentration of the extract. Biological criteria used in this study were: egg hatchability, developmental period of immature stages, mortality rate, and female fecundity. All mortality rates were corrected according to Abbott formula (Abbott, 1925). Analysis of data was performed by using Graph pad prism 5. Statistical differences were determined by Dunnetts test for multiple comparisons after ANOVA.

3. Results and Discussion

Egg mortality rate of *C. pipiens* was significantly affected by the aqueous extract of *Urtica pilulifera* at all concentrations used (except concentration 4 mg/ml) and the eggs mortality was concentration dependent ranged between 11.58% – 79.85% at concentrations of 4-16 mg/ml respectively (table 1). The eggs mortality might be attributed to the fact that the extract prevent gas exchange, or solidify egg shell that leads to the death of embryos and unhachability of the eggs, or may be due to the capability of some chemical compounds which exist in the aqueous extract to penetrate into the eggs causing the toxic effect and kill the embryo, (Ouda *et al.*, 1998). Mortality rate of *C. pipiens* larvae was also affected strongly, and all the larvae 100% died at a concentration of 14 mg/ml, while the lowest percentage 25% died at a concentration of 4mg/ml (Table 1). In this respect AL-Sharook *et al.* (1991) found that the crude extract of neem *Melia azedrachor* affects the mortality rate of subspecies *Culex pipiens molestus* larvae and pupae. While Mwaiko and Savaeli (1994) mentioned that lemon peel oils have toxic effects on the eggs, larvae and pupae of C. quinquefasciatus. The larval mortality rates may be due to the effect of compounds on the digestive tract, especially the epithelial cells and to the poisoning of the insect (Abdelgaleil *et al.*, 2002). Growth abnormalities of the larvae were observed. This may reflect growth inhibitory effect of the plant extract, and the dead

larvae (Fig. 1&2) which has been developed from treated eggs, has dark and short abdominal segments.The highest percentage 100 % of pupal mortality (Table 1) was found to be at a concentration of 14 mg/ml and the lowest percentage 6.47% mortality was found to be at a concentration of 4 mg/ml.The pupae which have successfully developed from treated larvae were also affected by nettle extract displaying a concentration dependent mortality. The intermediate formation (fig.3) and growth abnormalities of pupae (fig.4) were also observed. Figures (1,2 and.4) indicating that this extract has an inhibitory activity on the growth of larval and pupal stages. The results presented in Figure (3) agree with the findings of Abdulmajeed (2011) when he tested the tavri shrub (*Daphne mucronata*) extract on the *Culex molestus* , and found some abnormalities of larval –pupal intermediate. Development period of immature stages of *C. pipiens* was strongly affected by the aqueous extract especially at concentration 8-16 mg/ml (Table 2), Where it ranged between 14.75 and 21.5 days at concentration of 4-12 mg/ml respectively The prolongation of immature stage period for the insect may be due to the inhibition of normal development for larvae, which leads to elongation of the mosquito larval stages (Al-Chalabi *et al*., 2002). Most of the dead adults were failed to emerge. Present study results revealed that all mortalities were dosage-dependent. The rate of the adult emergence decreased with increasing the concentrations of the plant extract .The highest rate of the adult emergence was 93.52% in the concentration of 4mg/ml and the lowest one was 0% at a concentration of 14 mg/ml (table 3) and the emergence inhibition rate increased with the increasing the plant extract concentrations, where at the highest concentration (14 mg/ml) the inhibition of adult emergence recorded 100%. Most of the adults which have failed to emerge (Table 3) were unable to release their bodies from the pupal integument and partially emerged (Figure 5). Mohsen *et al.* (1989) showed an inhibition percentage of 96.0% in adult emergence of mosquito *C. quinquefasciatus* from larvae treated with 1000 ppm ethanolic extract of *Vinca rosea* . Female productivity significantly dropped from 355 eggs to zero eggs at concentration of 0-12 mg/ml respectively. Egg hatchability reduced from 96.90% to 0.0% at the concentration mentioned above. Al-Rubaai and Al-Zubaidi (1999) found that female fecundity and productivity were reduced when *Musca domestica* was treated with *D. innoxia* alkalons extracte. Ouda *et al*. (1998) found that the *Atriplex canescens* extract significantly affected the fertility of *Culex quinquefasciatus* and it decreased with the increasing concentrations of the extract leading to reduced hatchability percentage. The ethanolic neem extract and Jojoba seeds have been reported to have significant effects on the fecundity of house fly *Musca domestica* (Ghoneim *et al.*, 2007). The results presented in table (4) is relatively similar to that reported by Abdulmajeed (2011) who found that the *Daphne mucronata* and *Urtica dioica* extracts were significantly affected both preoviposition period and fertility of *Culex molestus* and the fertility rate decreased with increasing the concentrations.

Table (1): The cumulative effect of *Urtica pilulifera* aqueous extract on the mortality rates of different immature stages of *Culex pipiens*

Concentration mg/ml	% of egg mortality	% of larval mortality	% of pupal mortality
0	1.93	5.88	2.77
4	11.58	* 25	6.47
6	** 25.50	** 32.43	9.33

8	*** 31.86	*** 52.55	14.45
12	*** 49.82	*** 65.27	18
14	** 57.44	** 100	** 100
16	** 79.85	** 100	** 100

Note:* (p<0.05), ** (p< 0.01), * (p< 0.001)**

Table (2): The cumulative effect of *Urtica pilulifera* extract on the growth duration of different immature stage of *Culex pipiens*.

Concentration mg/ml	Mean Incubation period of egg (days)	Mean Larval duration (days)	Mean Pupal duration (days)
0	1.25	8.75	3.75
4	1.5	9	4.25
6	2	9.75	*** 5.25
8	** 2.75	*10.25	*** 5.75
12	*** 3.5	** 11	*** 7
14	*** 4	** -	*** -
16	*** 5	*** -	*** -

Note: * (p<0.05), ** (p< 0.01) , * (p< 0.001)**

Table (3): The cumulative effect of *Urtica pilulifera* extract on the emergence of adults developed from treated eggs and the inhibition of emergence of *Culex pipien*

Concentration mg/ml	% adult emergency	% emergence inhibition
0	97.23	2.77
4	*** 93.52	6.48
6	*** 90.67	9.33
8	*** 85.55	* 14.45
12	*** 82	** 18
14	*** -	*** 100

Note: * (p<0.05), ** (p< 0.01) , * (p< 0.001)**

Table (4): The cumulative effect of *Urtica pilulifera* extract on the pre-oviposition period, fecundity (fertility) of the adult females developed from treated eggs and the percentage of egg hatching of *Culex pipiens*

Concentration mg/ml	Pre-oviposition period (days)	No. of egg rafts	No. of eggs	%of egg hatching
0	3.25	6	355	96.90
4	5	3	137	58.39
6	*** 7.25	* 2	* 101	*** 53.46
8	*** 8.25	** 1	** 50	*** 32
12	*** 14.75	*** 0	*** -	*** -

Note: * (p<0.05), ** (p< 0.01), * (p< 0.001)**

Figure (1): Upper figure is dead third instar larva with dark body region developed from egg treated with 14mg/ml *Urtica pilulifera* extract compared with lower figure which is normal larva (28x).

Figure (2): Left side is abnormal fourth instar larva with dark body region resulted from eggs treated with 14 mg/ml *Urtica pilulifera* extract compared with normal larva (right side) (28x).

Figure (3): Dead intermediate stage during molting process resulted from egg treated with 8 mg/ml of *Urtica pilulifera* extract (28 x)

Figure (4): Left side is abnormal pupa with dark body region and shortened abdomen region resulted from eggs treated with 8 mg/ml *Urtica pilulifera* extract compared with right side normal larva (28 x)

Figure (5): Partially emerged mosquito resulted from egg treated with (14mg/ml) *Urtica pilulifera* extract (28 x)

4. Conclusion

It appears that the extract of *Urtica pilulifera* affected the growth survival rate, developmental period, female fecundity and productivity of *C. pipiens*. Also growth abnormalities, the appearance of intermediates and the adults failed to emerged, were observed as well.

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