



EFFECT OF CRUDE ALKALOID COMPOUNDS EXTRACT OF DAWUDI FLOWERS *CHRYSANTHEMUM CINERARIIFOLIUM* VIS MOSQUITO ON DIFFERENT STAGES OF *CULEX PIPIENS* I. (DIPTERA : CULICIDAE)

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Abstract

Mosquito considers as one of the causative agents for different diseases to humans such as malaria, filariasis, and dengue fever. The use of synthetic chemicals have harmful effect as, resurgence and resistance insects, human toxicity, and residual effect, therefore, plant extracts have been used instead of synthetic chemicals. This study aimed to assess the biological activity of the crude alkaloid compound extract from Dawudi flowers *Chrysanthemum cinerariifolium* Vis on some biological aspects of the Mosquito *Culex pipiens* by using (0%, 1%, 1.5%, and 2.0%) to evaluate its effect.

These results showed that crude alkaloid had a significant effect on different stages Mosquito *Culex pipiens* as following: the average rate of mortality of the larval stages (1st, 2nd, 3rd, 4th) were (90.0%, 90.0%, 73.3%, 60%) respectively when treated with crude alkaloid compound extracted at 2.0% concentration as compared with (20.3%, 17.2%, 14.3%, 7.9%) respectively to the control treatment. Also, that the mortality percentages of the different stages increase as the concentration increased. Also, the first larval instar was more susceptible than 2nd, 3rd and 4th larval instar, it was showed the highest mortality at different concentrations. The present result showed that the effect 2% percentage crude alkaloid compound extracts on larval were highly significant in the 1st, 2nd stage.

Key words: Mosquito, extracted, alkaloid, and dawodi flowers.

Introduction

Mosquitoes are among the world wide medical insects in the world, as they caused horrific discomfort as a result of pinching human and animal skin causing itching and allergies in the skin, in addition to that mosquitoes are the carrier of many parasitic diseases and viral infections such as malaria, Dengue Fever, Filariasis or elephantiasis, encephalitis or meningitis, Encephalitis haemorrhagic, Chikungunya fever, Yellow fever.

(Savage and Miller; 1995; Twatsin *et al.*, 2001; Abdulhakim A. Elmaghrbi and Mohammed M. Hosn, 2014; Ziaullah *et al.*, 2018).

In Egypt, a *Culex pipiens* I. species is considered as the main carrier to Filarial worm *Wuchereria bancrofti*, it also causes rift valley fever virus (Hanemfathykhater and Afaf Abdel Salamshalaby,

2008), the type *Culex quinquefasciatus* is also considered as an important vector of Filarial worms that cause elephantiasis in humans (Whitney *et al.*, 2000 and Hanemfathykhater and Afaf Abdel Salamshalaby, 2008).

The excessive and unprogrammed use of chemical pesticides, especially organic chlorine, Organophosphorus and Pirethroids led to the occurrence of a significant imbalance in the system of environmental destruction through killing the natural enemies of pests like predators, parasites and pathogens and this leads to the emergence of pests are widely and previously unknown Epidemics occur and the emergence of resistant strains, as well as high toxicity to humans and mammals moreover lead to the occurrence of cancerous tumors, genetic mutations, deformities, environmental pollution, toxic to mammals and the Residual effect. (Zahodiakin *et al.*, 2004; W. Millener 2011; K. Lundbreg 2014; ziaullah *et al.*, 2018).

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For these reasons, researchers have tended to search for new Pesticides of plant origin where they are effective, safe, more environmentally friendly and low toxicity to humans and mammals. (Al-Adil and Abed, 1979 and Al-Zubaidi, 1992 and Begnini, 2001). the appearance of the trait of resistance was not recorded in treated insects (Shaaban and Al-Mallah, 1993). Dawudi flowers were chosen *Chrysanthemum cinerariifolium* Vis against the *Culex pipiens* L., as it contains secondary compounds that are effective against insects such as crude alkaloids and volatile oils (Chakravarty, 1979).

Materials and Methods

Plant Collection & Diagnosis:

Dawud flowers were collected from one of the nurseries in Najaf City during October 2019, the sample was diagnosed in lushly College of Science/ University of Babylon, and then flowers samples dried at room temperature and grinded in the electric grinder for flower powder, the powder saved in a sealed bottle and stored in the refrigerator until use.

Insect collection, breeding, and diagnosis:

The immature stages (eggs and larvae) were obtained from water drainage in the housing complex at the Alforat Alawsat Technical University in 2019, the immature stages were collected by a long-arm net and placed in vials with a lid, transported to the laboratory, and placed in plastic basins filled with tap water which was collected before a day or more to get rid of the chlorine, then stages fed on mice food by 2g per basin to feed larvae. (Al-Ghazali, 1999).

To obtain the adults, the modern pupa transferred by a plastic dropper to a 500-cm³ plastic container, and then placed in wooden breeding crates; the length of its side is 50 cm. covered with towel, containing a small crates 20x20 cm, a pigeon placed in it to feed mosquitoes. Inside the crate, Petri dish containing cotton saturated with a 10% sugar solution added to feed the adults.

To obtain egg boats, used the (Mehdi and Mohsen, 1989) method, the female mosquitoes were fed three days after their release on the blood of a feathered pigeon in the chest and abdomen area, then their wings and legs were tied and placed over the breeding crate at night and after 3-4 days of feeding on the blood of the pigeon, a small plastic container with tap water was placed to receive egg boats, and then transferred boats where transferred by a small brush to new water container containing the larval food, followed up until the appearance of adults taking into account replacing the water every 4 days to maintain the vitality of larvae

(Al-sharook and *et al.*, 1991).

This process repeats until the emergence of the third generation of complete. Samples of the fourth stage larvae and adults taken for diagnosis at the Natural History Museum/the University of Baghdad and make sure it is *Culex pipiens* I. and it is from the Culicidae family. These experiments conducted in the laboratory conditions of 26°C \pm 2 and relative humidity of 50-70% and the lighting period of 12 hours.

Preparing crude alkaline compounds extract from Dawudi flowers:

(Al-Samarai 1983) the method used to extract crude alkaloids for the Dawudi flowers *Chrysanthemum cinerariifolium* Vis. then weigh 10g of flower powder putted in the extract container (Thimble) and inserted into Soxhlet extractor, then add a 200 ml of ethyl alcohol and extraction lasts for 24 hours at 40°C. After the completion of the extraction process, the extract was dried using a rotary evaporator, then the dry matter was dissolved in 5 ml of ethyl alcohol and using a magnetic mixer and 30ml of sulfuric acid 2% was added to the alcohol extract, the rotary evaporator was used again to get rid of the ethyl alcohol so that only the acid solution remains. Then 10% ammonium hydroxide was added to the acid solution in an appropriate amount to make the pH of the solution a base (PH = 9). Then, apply the base solution to the separating funnel, add 10 ml of chloroform were added and shake several times, then leave the mixture were left to settle and separate into two layers. The lower layer containing chloroform alkaloids was taken, the last step was repeated three times and the lower layer was taken each time so that the solution amount was approximately 40 ml. The solution was placed in a rotary evaporator to get rid of chloroform. The sample was dried in the oven at 40 – 45°C and the dry matter was stored in a sealed glass bottle and stored in the refrigerator until use. The extraction process repeated several times to obtain a sufficient amount of alkaloid compounds.

Two grams (2 grams) of extracted alkaloid extraction by this method and this quantity was taken and dissolved in 5ml ethyl alcohol and then the volume was supplemented to 100ml with distilled water whose concentration of the stock solution becomes 2%, or the equivalent (20 mg/ml) and from this solution, Concentrations to work in the experiment (0.5, 1, 1.5, 2) was prepared. The control was prepared as 95 ml distilled water with 5 ml ethyl alcohol added.

And to test the biological effectiveness of the extract in influencing the different larval stages of the *Culex*

pipiens I., 10 larvae were taken for each repeater from the first, second, third and fourth phase larvae and by 3 replicates for each concentration and placed in Petri dishes container on the natural food medium, then the dishes were treated with the concentrations of the extract, then the treated were transferred to the incubator at 30°C 2 ± relative humidity 60 - 70% and mortality rate recorded after 24 hours.

All experiments were carried out as Completed Random Design (C.R.D) with practical experiments; the rates were compared according to the method of Least Significant Difference L.S.D and under the 5% level of morale (Al-Rawi and Khalafullah, 2000). Then, the corrected percentages of mortality rates were converted according to Abbott formula to angle values for inclusion in statistical analysis (Al-Rawi and Khalafullah, 2000) and Abbott, 1925).

Results and Discussion

The results of the current study showed that the extract of crude alkaline compounds for the Dawudi flowers *Chrysanthemum cinerariifolium* Vis have significant moral effect in mortality of different stages of *Culex pipiens* I., as the mortality rate for the first, second, third and fourth larval stages reached (90.0%, 90.0%, 73.3%, 60%) respectively and in the concentration, 2% compared to (20.3%, 17.2%, 14.3%, 7.9%) respectively in the control treatment table 1.

The results of the experiment also indicated that there is a direct relationship between the concentration of the extract and the percentage of mortality, that is, the rate of mortality increases with increasing the concentration

Table 1: Shows the effect of the crude alkaloids of Dawudi flowers *Chrysanthemum cinerariifolium* Vis in Mortality the different stages of *Culex pipiens* I.

Concentration %	Different Stages of Larvae				Average of concentrations
	1 st	2 nd	3 rd	4 th	
0	20.3	17.2	15.3	7.9	14.9
0.5	47.9	40.4	35.5	32.0	38.95
1.0	58.1	50.2	44.5	37.3	47.5
1.5	90.0	75.8	59.6	50.1	68.8
2.. 0	90.0	90.0	73.3	60.0	78.3
Average of stages	61.26	54.72	45.44	37.46	

Least Significant Difference (L.S.D.) under the 5% level of significance for concentrations = 6.12%.

Least Significant Difference (L.S.D.) under the 5% level of significance for different stages = 5.33%.

* 1st= The first larval stage, * 2nd= The second larval stage.

* 3rd= The third larval stage, * 4th= The fourth larval stage.

of the extract Fig. 1. The results of the study also showed that the first larval stage is more sensitive than other stages Fig. 2, (Al-Aaraji, 2013) and the reason may be attributed to the increase in the thickness of the Cuticle, with the progress of larval age (Abdel Hamid and Abdel Majid, 1988a), or the reason may be due to the ability of the larval stages of advanced age to convert some toxic compounds present in plant extracts into non-toxic compounds (Detoxification) by enzymes called Mixed Function Oxidation (M.F.O), while the small stages cannot do this due to its lack of this enzymatic system (Al-Adil and Abed, 1979).

Harbone etal reported as indicated that the death occur in the different stages of the insects may be dueto : i) the toxic effect of the crude alkaline compounds, ii) a physiological effect in the insects, iii) the alkaloids have repellents or antifeedants effect, iv) or have a severe toxic effect (Harbone,1978; Mahfus and Khanam, 2007).

Tuuble *et al.*, reported that Pyrethrin extracted from the Dawudi Flowers *Chrysanthemum* spp. protect the plant from various insect pests, and used to control many insect pests. In this regard (Tuubel and *et al.*, 2002). The study by Chalabi referred to the inhibitory action of the crude alkaloid extract from *Euphorbia granulate* on *Culex pipiens* I. larvae, the mortality rate for the first,

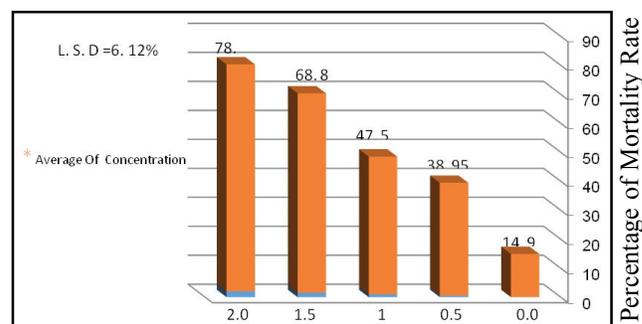


Fig. 1: Shows the Effect of the Crude Alkaloids of Dawudi Flowers *Chrysanthemum cinerariifolium* Vis in a Mortality rate of different stages of *Culex pipiens* I.

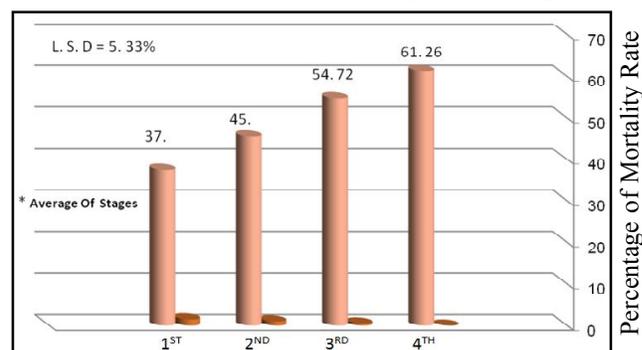


Fig. 2: Shows the Sensitivity of the Different Stages of the *Culex pipiens* I. For crude alkaloids of Dawudi Flowers *Chrysanthemum cinerariifolium*.

second, third and fourth larval stages (92.5%, 91.3%, 88.8%, 86.3%), respectively and in the concentration of 2% (Chalabi, 1988).

The study conducted by (Yousif and *et al.*, 2015) mentioned the effect of alkaloid extract of *Cordia myxa* in the mortality of the larval stages of the *Culex pipiens* I., the mortality rate for the first, second, third and fourth larval stages (90%, 90%, 90% and 90%) respectively and in the concentration of 10 mg/ml.

Our previous study showed that the crude alkaline extract for the Dawudi Flowers *Chrysanthemum cinerariifolium* Vis has moral effect in mortality of different stages of Red Flour Beetle *Tribolium castaneum* (Herbst), the death rate for the second and sixth larval stages was (90. 0% and 62. 6%) respectively, and in the concentration of 2%, and this is consistent with the results of the current study in terms of influencing with the difference in insect type or plant type (Al-Aaraji, 2017).

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