Biological Effective of crude secondary compounds of Leaves *Datura innoxia* in the Non-cumulative for mortality of Immature insect *Culex quinquefasciatus* Say (Diptera : Culicidae)

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Abstract

The present research evaluated the efficacy of crude secondary compounds (phenolic, terpenes and alkaline) respectively using concentrations(2000, 1500, 1000, 500, 250, 100 ppm) For plant leaves *Datura innoxia* and their effect on the non-cumulative mortality of the immature stage of *Culex quinquefasciatus* Say mosquitoes where LC50 and LC90 by probit analysis were estimated The phenolic extract of the leaves *D.innoxia* plant was the most affected terpenes and alkaline in the non-cumulative mortality of the immature instar where LC50 for ppm (2089.89) 9.69, 0.01619, egg mortality was (phenolic, terpenes and alkaline) respectively. For the four larva instar, the first larval instar were the most sensitive of the larval instar of all extracts where LC50(50.77, 97.23, 124.6, 164.4), (79.65, 113.9, 146.1, 227.18), (102.8, 133.65, 168.6, 331.07) for phenolic, terpenes and alkaline respectively. In instar pupa, the value of LC50 (186.3, 829.1, 1147.4) ppm for phenolic, terpenes and alkaline respectively.

Keywords: *Datura innoxia*, insect, *Culex quinquefasciatus*, mortality

Introduction

*Culex quinquefasciatus* mosquito is a medical an veterinary insect globally because it is widespread in the world as it affects the health of huma and animals through the mechanical transfer many pathogens to humans and animals such as dengue. It has a role in the transmission of Wuchereria bancrofti nematodes that cause Filaria vector which currently affects about 120 million people in different parts of the world( Wrenchmesingh and Mendis1980), encephalitis virus, West Nile virus (Rutledge et al., 2003) The methods of controlling the insect have
multiplied. Perhaps the main one is the use of synthetic chemical pesticides, but this insect and throughout the world have shown a characteristic of resistance to these pesticides (Shono and Scoot, 2003)), which encouraged the manufacture of new pesticides with a clear impact on the life of the insect, and this process is costly As such( Ka ufman, 2001) Perhaps one of the most important reasons why many people interested in the environment demand the use of pesticides of plant origin is their desirable characteristics such as rapid decomposition, as their toxicity to humans and animals is very low and they are unlike chemical pesticides that are characterized by slow degradation and their high toxicity to mammals (Al-Adil and Abd, 1979). One of these plants is Datura. innoxia is one of the plants widely spread in Iraq, as the D plant D. innoxia is an important plant because it contains many active compounds, including the main alkaline Hyoscine compound (Verporat and AL-efrmann, 2005). And the lack of studies on the effect of the plant above on some aspects of the life of C. quinquefasciatus the present research.

Materials and methods of work

collected samples were of leaves D. innoxia plant in November 2018 from private nurseries in Al-Qadisiyah Governorate belongs to the Solanaceae family It was cleaned of dust, washed and dried naturally in the shade and at room temperature, then the leaves were ground after completely drying by the electric mill and preserved in dark colored plastic containers until use Preparation of the permanent farm Cx. quinquefasciatus.

The immature instar (eggs and larvae) were collected from a water drainage site in Sumer District in Al-Qadisiyah Governorate They were placed in plastic tubs filled with chlorine-free water and added rats ’diet. For the purpose of obtaining a pure permanent farm, the pupa was transported in a plastic cage with a tulle clip and plastic plates containing cotton saturated with a 10% sugar solution to feed the modern insect and to obtain eggs boats fol modern modern insect and to obtain eggs boats fol female mosquitoes were fed three days after dawn ing on the blood of their pigeons. Then the boats were transferred to the eggs of new water contain food larvae were followed up until the emergence of full and taking into account replacement of water every three days

Prepare plant extracts

Preparation crud phenolic compounds

According to the method Riberean-Gayon (1972) phenolic compounds were prepared by mixing 20 gm of dry powder to the leaves of each of the aforementioned plants separately with 400ml of CH3COOH acetic acid at a concentration of 2% and the extraction process was performed by Reflex Condensre in a water bath at a temperature of 70c For 8 hours and after leaving the mixture to cool. Filter with a piece of tulle cloth and filtered with filter paper Whatman No1 then transfer the filter
to a separating funnel (plate 3-4) then add the same volume of n-propanol then add a quantity of sodium chloride (table salt) until it reaches a limit saturation where two organic layers consist of a container on phenolic compounds and an inorganic lower layer. The top layer was taken, the bottom layer neglected, the top layer extract was collected and dried with a rotary evaporator at a temperature of 45 °C. A stock solution foundation solution was prepared where 1 g of dry extract was dissolved by 10 ml of ethyl alcohol with a concentration of 95% and the volume was supplemented to 100 ml distilled water and thus a concentration of 10,000 ppm was obtained by mixing it with a volumetric flask after being closed by magnetic mixer for 10 minutes until the total solubility of the extract and from this solution the concentrations were prepared 2000, 1500, 1000, 500, 250, 100 ppm). As for the control treatment, by adding 10 ml of ethyl alcohol, complete the volume with 100 ml of distilled water.

Preparation of crude alkaloids compounds

According to the method (Samurai (1983)) extracts of raw alkaline compounds were prepared for leaves of D. innoxia plants, where they weighed 10 gm of dry leaf powder for the above plants separately and placed in a paper extract container (Thumble) in the Soxhlet Extractor and placed in the solvent beaker. Capacity of 500 ml where I use 200 ml of ethyl alcohol for 24 hours at a temperature of 40-45 °C. Then the extracted substance was concentrated in the rotary evaporator, then it was dissolved in 5 ml of ethyl alcohol and then added to the extract 30 ml of sulfuric acid with a concentration of 2%. Then the evaporator was used again to get rid of ethyl alcohol to leave the acid solution. An amount of ammonium hydroxide with a concentration of 10% was added to the acid solution to become pH 9). After that, put the solution in the separating funnel and add 10 ml of chloroform and shake several times, then leave the mixture to separate into two layers. I took the lower layer containing the alkaloids. The last step was repeated several times and each time we take the lower layer until the solution becomes 40 ml. Concentrate the collected solution in a rotary evaporator so that chloroform is disposed of and then dried in an electric oven at a temperature of 40 °C. For the purpose of estimating the biological efficacy of the crude turbine compounds extract, I attended the concentrations and control coefficients, as in the previous paragraph.

Preparation of crude turbine compounds

According to the Harborne (1984) method, raw turbine compounds were prepared with a weight of 20 g of powder leaves of each of the plants D. innoxia, R. sanctus, M. Jalapa and placed in a paper extract container (Thumble) in the Soxhlet Extractor and put into the beaker. The solvent has a capacity of 500 ml where I use 200 ml of chloroform for 24 hours at a temperature of 40-45 °C. Then the extracted substance was concentrated in the rotary evaporator and then dried in an electric oven at a temperature of 45 °C. Then it was placed in sealed glass tubes and kept in the refrigerator until use. For the purpose of estimating the biological efficacy of turbine
extracts, 1 g of turbine extract was dissolved in 5 ml of ethyl alcohol and 5 ml of chloroform and completed the volume to 100 ml distilled water. Control as in the paragraph preparing the phenolic compounds.

Determination of the biological activity of crude secondary compounds (phenolic, terpenes and alkaline) Extracts for leaves of D. innoxia of in Non-Cumulative mortality of Immature instar of Cx quinquefasciatus

Determination of biological activity against eggs

To find out the effect of extracts crude secondary compounds phenolic, terpenes and alkaline for D. innoxia leaves, eggs were taken 24 hours old with live replicates per concentration and one egg boat was placed in a 300 ml plastic container with the concentrations ppm(2000,1500,1000,500,250,100) for the three extracts and spray the same concentration that was put in it and after hatching the eggs were calculated

Determination of biological activity against larvae

40 larvae were taken for each repeater of each adult larval phase (first, second, third and fourth) and used 5 plastic containers containing 100 ml of each of the above concentrations of extracts and the sixth container such as the treatment of using distilled water with the solvent used in the extraction of five replicates each Concentration was added 0.5 gm of the rat bush to all pots. Where the phases were prepared by raising them and following them until dissolution to the next phase, the losses were recorded in each concentration after 24 hours, and the percentage of loss was corrected according to the equation Abbott(1925).

Determination of biological activity against pupa

pupa were isolated from the insect's permanent farm by 40 virgin per repeater in addition to the control treatment using distilled water with the solvent used in the extraction and placed in a 300 ml container with 5 replicates and followed the same test method as in the previous paragraph except for adding the feed

Statistical analysis

The results of the research were analyzed statistically according to (Al-Qassas, 2014) method using the well known statistical program (SPSS) version 23 where the percentages of consumption were corrected according to the Abbott formula (1925)

The value of (LC50) the lethal concentration of 50% of the sample and (LC90) the mortality concentration of 90% of the sample were calculated using Probit analysis (Finney, 1971)

Results and discussion

Effect of crude secondary compounds of leaves of D. innoxia on the non- cumulative mortality of immature instar of Cx. Quinquefasciatus Effect in the mortality of eggs
Figure 1 shows the effect of crude secondary compounds concentrations (phenolic, alkaline, and turbine) on the leaves of the *D. innoxia* plant on the mortality percentage eggs of *Cx. quinquefasciatus*. Where the phenolic extract was recorded Obvious superiority On the alkali and turbine extract. The percentage mortality increases with increasing the concentration of extract, as the concentration of 2000 ppm gave the highest rate. As the percentage of re mortality ached (62, 68, 70) for plant *D. innoxia* extracts for phenolic, turbine and alkaline compounds respectively. Table 1 shows the values of LC50 and LC90 for the crude secondary compounds as LC50 ppm(986.42, 1610.39, 2098.89) ppm The value of le90 was (47943.21, 69536.37, 432815.27) for phenolic, turbine and alkaline compounds respectively.

The reason for the mortality of eggs may be explained by the fact that phenolic compounds affect the envelope of the egg and prevent gas exchange, or that it works to harden the egg shell or the effect is internally through the union of these compounds with the egg cytoplasm (Al-Adil and Abd, 1979) or that the phenols work synergistically to form Complex compounds to inhibit the acetylcholine esterase (Hienrich, 2008). Al-Zubaidi and Muhaisen (2009) showed that the concentrations of the phenolic extracts of a plant of the fruits of the capers plant *C. spinosa* affected the destruction of eggs of the mosquito *C. pipiens*, as the percentage of percussion was limited between (9.04 - 18.4) at a concentration of (0.1 -1) mg / ml, respectively, compared to the control treatment. That all hatched. The kream, (2016) showed in its testing of raw secondary compounds (phenolic, alkaline, and turbine) the leaves and flowers of the Davidic *C. cinerariaefolium* in the mortality of eggs of *Cx. quinquefasciatus* Where she showed that the phenolic extract of leaves and flowers gave the highest rate of mortality compared to turbines and alkaloids at a concentration of 20 mg / ml.
Table (1) LC90, LC50 and Bioactivity of crude secondary compounds of leaves of

*D. innoxia* in mortality non-cumulative of egg *Cx. quinquefasciatus*

|        | phenolic |         | IC50 ppm | IC50 value |
|--------|----------|---------|----------|------------|
| alkaline | 2089.89  | 1610.39 | 986.42   |            |
| turbine | 1401.38-6980.67 | 1162.52-2605.16 | 738.53-1428.6 | Limits 95% |
|        | 432815.27 | 69536.37 | 47943.21 | IC50 value ppm |
| phenolic | 06638.35-27300353.25 | 24296.37-438650.94 | 17892.67-269242.37 | Limits 95% |
|        | 1.065    | 4.356   | 3.315    | X2         |
|        | 0.601    | 0.672   | 0.550    | P value    |
|        | Y=2.23+0.64*X | Y=3.13+0.68*X | Y=2.23+0.78*X | Regression equation |

The effect in the mortality of Larval instar

It is clear from the figures (2) the effect of the concentrations of the secondary crude compounds (phenolic, turbine, and alkalinity), respectively, of leaves of *D. innoxia* in the ratio of mortality on the larval pupae *Cx. quinquefasciatus*. Where we note the superiority of phenolic compounds over turbine and alkalinity in the percentage of mortality. In addition, there is a direct relationship between the concentration and the percentage of mortality, as the percentage of loss reached (78, 80, 85, 95) (75, 78, 81, 84) (63, 67, 70, 74) for the for phenolic, turbine and alkaline compounds, respectively. Tables 2, 3, 4 are shown for phenolic, turbine and alkaline compounds, respectively. Where the highest percentage of mortality was achieved for the four larval instar as a result of exposing the phenolic extract and then the turbine, followed by alkali. It may explain the reason for the superiority of phenolic extracts as they cause two types of physiological effects in the larval tissues which are the indirect toxic effect as a disruption in the nervous secretion system, or the direct effect by the spread and penetration of these active compounds into the target tissues (Chapman, 1978) and the reason may be due due to the different active substances in the tested plants and containing tannins, which are compounds produced in the gaps of the plant cell and are toxic to insects, they are associated with saliva and digestive enzymes, including trypsin and chimotropicin, and then inhibit them, thus insects begin to lose weight and then die (Freeman and Beatti, 2008). Where we note through the results that the LC50 of phenolic compounds of *D. innoxia* plant at concentrations ppm (50.77, 97.23, 124.6, 164.4) and the value of LC90 was ppm (1849.6, 4580.9, 11764.2, 21648.2) for the four larval instar.
respectively. The LC50 of the turbine reached (102.8, 113.9, 146.1, 227.18) and the 
LC90 (2761.8, 8218.1, 13359.8, 42043.4) were for the four larval phases, respectively  
as for The LC50 (79.65, 133.65, 168.6, 331.07) has reached the LC90 (2034.9, 8524.1,  
36326.4, 37185.1) for the four larval instar respectively. The results of (Chalabi, 
1998) indicated that the raw phenolic compounds extracted from the granulate E. 
cancer plant had the greatest effect on the loss of the four larval phases of the C. 
pipiens mosquito, where the proportion of pericardium was confined between (33.8  
- 95)% in the concentration of (2-20) mg/ml. Nikkon et al.2009 mentioned that the  
turbine extract for the stem of the D.repes plant affected the fourth larval of the cx. 
in with a value of LC50 ppm (8.51 -10.70) after 24 hours of quinquefasciatus
treatment Darvin et al. 2018 said that Lawsonia phenolic compound extracted from the \textit{Lawsonia inermis} henna planted the third larval larvae of \textit{Cx. quinquefasciatus} when it reached ppm LC50 230.

![Graph A](image)

![Graph B](image)

![Graph C](image)

Figure (2) Effect of A-phenolic B- turbine C- alkalin of the leaves \textit{D. innoxia} in mortality non-cumulative percentage of Larval instar of \textit{Cx. quinquefasciatus}

Table (2) LC90, LC 50 and Bioactivity ofA-phenolic B- turbine C- alkalin of the leaves \textit{D. innoxia} in mortality non-cumulative of Larval instar of \textit{Cx. quinquefasciatus}
### A

| D. innoxia | 3rd | 2nd | 1s | IC5 ppm |
|------------|-----|-----|----|---------|
| 124.6      | 97.23 | 50.77 | iC50 value |
| 1.63-302.1 | 16.88-195.06 | 5.33-118.1 | Limits 95% |
| 11764.2    | 4580.9 | 1849.6 | iC00 value |
| 3607.5-505783.5 | 1960.3-44389.91 | 1041.6-6210.5 | Limits 95% |
| 0.273      | 0.819 | 2.524 | X2 |
| 0.991      | 0.936 | 0.400 | P value |
| Y=-1.47+0.6 *X | Y=-1.58+0.07 *X | Y=-2+1.05 *X | Regression equation |

### B

| D. innoxia | 3rd | 2nd | 1s | IC50 Ppm |
|------------|-----|-----|----|---------|
| 227.18     | 146.1 | 113.9 | 79.65 | IC50 value |
| 56.24-414.4 | 25.53-283.63 | 16.53-231.7 | 27.68-188.4 | Limits 95% |
| 42043.40   | 13359.8 | 8218.1 | 2761.8 | IC90 value ppm |
| 3048.9-651112 | 6061.5-1.5084+1 | 2833.8-216848 | 1433.5-11906.6 | Limits 95% |
| 0.432      | 0.344 | 0.270 | 1.096 | X2 |
| 0.980      | 0.987 | 0.992 | 0.895 | P value |
| Y=-1.51+0.68 *X | Y=-1.04+0.49 *X | Y=-1.43+0.7 *X | Y=-1.89+0.93 *X | Regression equation |

### C

| D. innoxia | 3rd | 2nd | 1s | IC5 ppm |
|------------|-----|-----|----|---------|
| 351.07     | 168.6 | 133.65 | 102.8 | IC50 value |
| 108.1-600.9 | 13.83-353.9 | 25.44-257.5 | 19.01-152.21 | Limits 95% |
| 37185.1    | 3632.6 | 8524.1 | 2034.9 | IC90 value ppm |
| 7926.9-10942477.4 | 6384.9-10406645.3 | 2978.9-188872.4 | 996.9-19146.9 | Limits 95% |
| 0.586      | 0.437 | 0.708 | 1.577 | X2 |
| 0.966      | 0.879 | 0.950 | 0.813 | P value |
| Y=-1.58+0.63 *X | Y=-1.23+0.55 *X | 133.65 | Y=-1.58+0.89*X | Regression equation |
The effect in the mortality of pupa instar

Figure (3) shows the effect of the concentrations of the secondary crude compounds on the percentage of *Cx. quinquefasciatus*. The effect of secondary compounds took the same curve with the yields of the larval instar as well as the similarity between the concentration and the percentage of perishing, where the mortality percentage was (70, 60.58)% for phenolic, turbine and alkaline extracts Compared with the control treatment. Table (3) shows the values of LC50 and LC90 for the of the crude secondary compound was lc50 ppm (1004.8 , The reason for virgins may 8.5888 . 73288 (lc90ppm (186.3, 829.1, 1147.4). be due to the mortality of the toxicity of the phenolic compound depending on the number of the helixyl groups associated with the aromatic ring, as the more the number of hydroxyl groups increases the toxicity of the phenolic compound, as the phenolic compounds work on the precipitation of proteins by forming hydrogen bonds between the groups of phenolic hydroxyls and proteins and thus a defect will occur in Function of some important and necessary enzymes for the body (Berkoff 1998,). They found Darvin *et al.* (2018) the Lawson phenolic compound extracted from the *Lawsonia inermis* plant caused the of pupa *C. quinquefasciatus* atLC50 300ppm.

![Figure 3](image-url)  
*Figure (3) Effect of crud secondary compound of the leaves of the D. innoxia in In mortality percentage non-cumulative of pupa. of Cx. quinquefasciatus*

Table (3) LC90, LC 50 and Bioactivity crud secondary compound of leaves *D. innoxia* in mortality non-cumulative of pupa of *Cx. quinquefasciatus*
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