Toxicity of Silybum marianum and Nerium oleander extracts against Mosquito Larvae of Culex quinquefasciatus (Say)

Mohammed Jaber Al-Obaidi
Trop. Biol. Res. Unit, College of Science, University of Baghdad, Baghdad, Iraq
Email: lafta@sc.uobaghdad.edu.iq

Abstract. Toxicity of two plant extracts against Culex quinquefasciatus mosquito larvae was measured in this work. The plants include the species; Silybum marianum and Nerium oleander. Their Lc50 and Lc90 after 4 hrs were evaluated against three instars of the mosquito. S. marianum Lc50 values were: 57, 214, and 379 ppm for the three instars respectively. S. marianum Lc90 values were: 2422, 2936, and 3161 ppm for the three instars respectively. N. oleander Lc50 values were: 161, 194, and 360 for the three instars respectively. N. oleander Lc90 values were: 1968, 2004, and 4203 for the three instars respectively. The results showed that the 1st instar larvae were more sensitive than the 2nd instar larvae, and this was more sensitive than the 3rd instar larvae. The results of recent work showed high correlation rate between logs of S. marianum and N. oleander concentrations and C. quinquefasciatus mortality percentage probits for all three larvae stages with high R values (0.911, 0.914 and 0.997) (0.993, 0.970 and 0.943) respectively. The aim of this study is to screen plant materials sources can be used in mosquito control.

Keywords: Culex quinquefasciatus, mortality, plant extracts, toxicity.

1. Introduction
Mosquito is considered a vector of different pathogens such as bacteria, viruses, protozoa, and nematodes. Also, it is responsible for different important diseases like dengue, malaria, Zika, yellow fever, and filarial fever (Gathany 2016). Culex quinquefasciatus is distributed in Iraq especially in middle and southern regions of the state. It is life in the urban areas because of it like the human so that it may be called house mosquito. Many studies conducted on this species (Al-Gerawi 2012) (Molan, A. R., Munther H. A. 2016). Using of natural products of plants to insect control has some advantages like they contain a bioactive chemicals, which are considered as selective substances, do not risk non-target organisms, safety, biodegradable, and no-residual effects to the environment (Benelli, G. P., Roman M. and P. 2017; Stevenson, P. C. I., Murray B. B. 2017). There are many studies on the subject of mosquito control by natural plant products indicate the ability to make them as alternatives to chemical insecticides. Some of them depending on the strategy which used the plant extracts to kill the mosquito larvae in their habitats and terminate their life cycles. Furthermore, the toxicity tolerance of chemicals and leaf extract has been studied on mosquito larvae (Moore, K. J. Q., Whitney B., Victoria Y. 2017) (Haddi, K. T., Hudson V .V.D., Yuzhe V. 2017) (Curtis 2018). Biologically active components in plants are known to be alkaloids, terpenoids, flavonoids,
phenolic compounds, organic acids or lipids (Saltveit 2017). The medical plant *Nerium oleander* L. (Apocynaceae) is a shrub evergreen flowering grows in Mediterranean tropical and subtropical areas (Jabli, M. T., Najeh R., Khiai S. 2018). The plant has some potential activities as the anti-inflammatory (Balkan, . A. G., Ahmet C. K., Hasan Y. 2018), antibacterial (Chauhan, S. S., Manjeet T., Amit D. 2017), antimicrobial (Liu, H.C., Si-Yu G., Jia-Ying S. 2018), antitumor (Schneider, N. F. Z., Cerella C. S. 2017), antinociceptive (Semiz 2017). As well as the plant *Silybum marianum* is found to have different activities, one of them antibacterial activity of seeds (Abed, I. J., Raad A. 2015). The important plant used in this study was used in other studies for different purposes as control of *Schistosoma mansoni* (Al-Obaidi 2016). The objective of recent work is to evaluate the potential of some medical plant extracts against the mosquito *Culex quinquefasciatus*.

2. **Materials and methods**

**Plants samples**

Leaves of *Silybum marianum* and *Nerium oleander* were collected from the gardens distributed in University of Baghdad, Iraq. The plants were identified by taxonomic keys found in Herbarium of Biology Department, College of Science, University of Baghdad. The samples were marketed and kept in Trop. Biol. Res. Unit laboratories for experiments.

**Mosquito samples**

The eggs and larvae of *Culex quinquefasciatus* were collected from a small pond near human dwellings in (Auiaeeeg city) 10km south of Baghdad. These larvae were transferred to the laboratory by class bottle containing 500 mL of water. Mosquito larvae were kept in the laboratory at 27 ±2 °C, 75–85 % RH and 10:14 (light/day). Larvae were fed with 5 ml rabbit blood. Adults were isolated and transferred to plastic containers with 500 mL of water to breeding.

**Larval toxicity test**

Twenty-five larvae (instars 1–3) were placed in 500 mL of dechlorinated water, and different concentrations of the two extracts were added. Tests of each concentration against each instar were replicated three times. In each case, the control comprised 25 larvae of distilled water. Control mortality were corrected by using Abbott’s formula (Abbott 1925), and mortality percent were calculated as follows:

\[
p = \frac{d}{d + m + \frac{f}{e}} - 100 \text{ (WHO 1981)}
\]

**Statistical analysis**

Data were analyzed by calculation the variance; the means were separated using Duncan’s multiple range tests by Alder and Rossler (1977). Mortality values were handled with probit analysis. In order to calculate LC50 and LC90, we are depending on Finney (1971) method. SPSS software package 24 versions was used. Results with p<0.05 were considered to be statistically significant.

3. **Results**

The results of this work appeared that the larval stages of *C. quinquefasciatus* significantly different in their responses to *S. marianum* extract. The median lethal concentration of *S. marianum* against the 3rd instar larvae of *C. quinquefasciatus* mosquito was 379 ppm while the ninety lethal concentration was 3161 ppm. The Lc50 of *S. marianum* against the 2nd instar of *C. quinquefasciatus* larvae was 214 ppm while the Lc90 was 2936ppm. The Lc50 of *S. marianum* against the 1st instar of *C. quinquefasciatus* larvae was 57 ppm while the Lc90 was 2422ppm. From these results, we show the sensitivity of the 1st instar larvae was more than of 2nd instar larvae, and this was more sensitive than the 3rd instar larvae. That means the toxicity of *S. marianum* plant extracts to the 3rd instar larvae was more than to 2nd instar larvae, and
this was more toxic to the 3rd instar larvae Tab. (1). The results of recent work showed high correlation rate between the log of S. marianum concentration and C. quinquefasciatus mortality percent probit for all three larvae stages under study with high R values (0.911, 0.914 and 0.997) respectively Fig. (1, 2 and 3).

**Table (1):** Toxicity of S. marianum aquatic extracts against C. quinquefasciatus mosquito larvae.

| Larvae stage | T value | Sing(2-tail) | LC50 ppm | Fiducial limits | LC90 ppm | Fiducial limits |
|--------------|---------|--------------|----------|-----------------|----------|-----------------|
| 1st instar   | 3.443   | 0.001*       | 57       | 18              | 180      | 777             |
| 2nd instar   | 3.728   | 0.000*       | 214      | 89              | 510      | 7548            |
| 3rd instar   | 3.755   | 0.000*       | 379      | 164             | 861      | 6994            |

**Figure 1:** Scatter of mortality percentage probit of C. quinquefasciatus with S. marianum log of concentrations for instar 1.
The results of this work appeared that the larval stages of *C. quinquefasciatus* significantly different in their responses to *N. oleander* extract. The median lethal concentration of *N. oleander* against the 3rd instar larvae of *C. quinquefasciatus* mosquito was 360 ppm while the ninety lethal concentration was 4203 ppm. The Lc50 of *N. oleander* against the 2nd instar of *C. quinquefasciatus* larvae was 194 ppm while the Lc90 was 2004 ppm. The Lc50 of *N. oleander* against the 1st instar of *C. quinquefasciatus* larvae was 161 ppm while the Lc90 was 1968 ppm. Also, results of this study show that the sensitivity of the 1st instar larvae was more than of 2nd instar larvae, and this was more sensitive than the 3rd instar larvae. That means the toxicity of *N. oleander* plant extracts to the 3rd instar larvae was more than to 2nd instar larvae, and this was more toxic to the 3rd instar larvae Tab. (3).

Also, the results of recent work showed high correlation rate between the log of *N. oleander* concentration and *C. quinquefasciatus* mortality percent probit for all three larvae stages under study with high R values (0.993, 0.970 and 0.943) Fig. (4, 5 and 6) Respectively. The 1st instar was more sensitive than the sensitivity of 2nd and 3rd instars to the extracts of both *S. marianum* and *N. oleander* because the mortality of the 1st instar was more than the mortality of the 2nd and 3rd instars. The standard deviation of the result recorded for 1st instar was less than the SD recorded for 2nd and 3rd instars Fig. (7). The results of multiple comparisons among larvae stages dependent on mortality have appeared that the mean difference of 1st and 3rd instars is significant at the 0.05 level Tab. (3). The results of this study of ANOVA analysis to the three stages of mosquito have appeared that there are significant differences among them (p-value 0.051) Tab. (4).

### Table (2): Toxicity of *N. oleander* aquatic extracts against *C. quinquefasciatus* mosquito larvae.

| Larvae stage | t-value | Sig(2tail) | Lc50 | Fiducial limits | Le90 | Fiducial limits |
|--------------|---------|------------|------|----------------|------|----------------|
|              |         |            |      | LL             | UL   | LL             | UL   |
| 1st instar   | 3.589   | 0.001*     | 161  | 72             | 363  | 1968           | 876  |
| 2nd instar   | 3.646   | 0.000*     | 194  | 89             | 424  | 2004           | 918  |
| 3rd instar   | 3.947   | 0.000*     | 360  | 147            | 881  | 4203           | 1718 |

Figures:

**Figure 2**: Scatter of mortality percentage probits of *C. quinquefasciatus* with *S. marianum* log of concentrations for instar 2.

**Figure 3**: Scatter of mortality percentage probits of *C. quinquefasciatus* with *S. marianum* log of concentrations for instar 3.

**Figure 4**: LC50 probit analysis

\[
y = 1.3864x + 1.4294 \\
R^2 = 0.9977
\]
Figure (4): Scatter of mortality percentage probits of *C. quinquefasciatus* with *N. oleander* log of concentrations for 1\textsuperscript{st} instar.

![LC50 probit analysis](image)

Figure (5): Scatter of mortality percentage probits of *C. quinquefasciatus* with *N. oleander* log of concentrations for 2\textsuperscript{nd} instar.
Figure (6): Scatter of mortality percentage probits of *C. quinquefasciatus* with *N. oleander* log of concentrations for 3rd instar.

Table (3): Multiple comparisons among larvae stages dependent on mortality as variable.

| (I) stage | (J) stage | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | Lower Bound | Upper Bound |
|-----------|-----------|-----------------------|------------|------|-------------------------|-------------|-------------|
| instar1   | instar2   | 22.40000              | 12.5       | .217 | -11.1529                 | 55.9529     |             |
| instar1   | instar3   | 34.40000              | 12.5       | .044 | -8.471                  | 67.9529     |             |
| instar2   | instar1   | -22.40000             | 12.5       | .217 | -55.9529                 | 11.1529     |             |
| instar2   | instar3   | 12.00000              | 12.5       | .618 | -21.5529                 | 45.5529     |             |
| instar3   | instar1   | -34.40000             | 12.5       | .044 | -67.9529                 | -8.471      |             |
| instar3   | instar2   | -12.00000             | 12.5       | .618 | -45.5529                 | 21.5529     |             |

*. The mean difference is significant at the 0.05 level.
Figure (7): Standard deviation of mortality recorded for three instars of mosquito exposed to two plant extracts.

Table (4): ANOVA analysis of the three stages of mosquito (p-value 0.05).

| Mortality       | Sum of Squares | df  | Mean Square | F     | Sig. |
|-----------------|----------------|-----|-------------|-------|------|
| Between Groups  | 3048.533       | 2   | 1524.267    | 3.855 | .051 |
| Within Groups   | 4745.200       | 12  | 395.433     |       |      |
| Total           | 7793.733       | 14  |             |       |      |

4. Discussion

The results of this study which determine the toxicity of two plant extracts against target vector mosquito showed that the *N. oleander* extract was more toxic than the *S. marianum* extract to the mosquito larvae of *C. quinquefasciatus*. Also, we found that the 1st instar larva was more sensitive than 2nd instar larva and 3rd instar larva for both plant tested extracts. This result was agreed with other studies were found that the sensitivity of 1st and 2nd instars were more than the sensitivity of 3rd instar because they have more efficiency to food metabolism which plays a role to the appearance of toxicity (Mahmmod, E. A. A., Hussam A.A. 2011). The LC50 values recorded in this work were 57, 214 and 379 ppm against *C. quinquefasciatus* for *S. marianum* respectively. Our results were nearest of the study conducted by (Mekhlif 2017) which found LC50 (43 ppm) of *Astagalus annulata* plant extracts to *Culex pipiens molestus* larvae. The LC90 values were 2422, 2936 and 3161 ppm against *C. quinquefasciatus* for *S. marianum* respectively. The LC50 values were 161, 194 and 360 ppm against *C. quinquefasciatus* for *N. oleander* respectively. The LC90 values recorded in recent work were 1968, 2004 and 4203 ppm against *C. quinquefasciatus* for *N. oleander* respectively. Our results were agreed with another study which tested the extracts of *N. oleander* and found they were effective against the larvae of *C. quinquefasciatus* (48h-LC50= 168.84 ppm) (48h-LC90=11011.93ppm) (Raveen, R. K., Deepa K .T. 2014). The extracts of *A. mexicana*, *J. curcus*, *P. extensa*, and *W. sornifera* showed acute toxicity causing 100% mortality at 1,000, 500, and 250 ppm, respectively, against *C. quinquefasciatus* larvae (Karmegam, N. S.,
Anuradha M. 1997). Toxicity of Six ethanol plants extracts were tested against the Green Peach Aphid (GPA) and found that the toxic leaf extracts contain active substances that have the aphidicidal activity, so it was used in the control of the GPA in the greenhouse as an alternative to the insecticides in the IPM programs or in the organic culture (Madanat 2016). Solanum xanthocarpum fruits petroleum ether extract was observed as the most toxic with LC50 values of 62.62 ppm after 24 h and 59.45 ppm after 48 h of exposure period against the larvae of C. quinquefasciatus (Mohan, L. S. and N. 2005). Aqueous extract of Ricinus communis L. showed insecticidal activity against the bug Eurystylus oldi (Ajayi, O. S., Hari C. T. 2001).

We are noticed that the LC50 values recorded in different studies were various comparing with each other. It's clear if we focus on these studies; (Komalamisra, N.T., Yuwadee R., Yupha A. 2005) tested the Nerium oleander larvicidal activity against Aedes aegypti with the LC50 value of 197.97mg/l. A study deal with the larvicidal activity of different plants extracts against Cx. quinquefasciatus and recorded the LC50 values. Of these, Gleoonis coronarium flowers extracts (LC50= 53.0 ppm), Sonchus arvensis stem extracts (LC50= 68.0 ppm), Matricaria maritima flowers extracts (LC50 = 72.0 ppm) (Benelli, G. M., Filippo P., Roman M. 2017). A study has tested the effects of some plants extracts against the larvae of Cx. quinquefasciatus included Tagetes erectes leaf extract (LC50= 100.0 ppm) , Achilea millefolium stem extract (LC50= 120.0 ppm), Tanacetum vulgare flower extract (LC50= 178.0 ppm) and Otanthus maritimus stem extract (LC50 195.0 ppm) (Borah, R. Kalita M. C 2010). The mechanism of extracts effect on mosquito larvae due to digestive system effectiveness through the entrance of phytochemicals and bounded with lipids or cell metabolic effectiveness through moulting or skin effectiveness or cuticle hardness through Tyrosinase enzyme effectiveness or respiratory bores closing (Mahdi 2001).

A big number of plant chemicals has larvicidal activity. Mosquito different responses to these extracts due to many causes such as plant species, phytochemical type, plant part, extraction solvent, and extraction method (Ghosh, A. C., Nandita C. 2012) (Shaalan, E. A. C., Deon Y. 2005).

5. Conclusions
In general, the results of this study may open the possibility of future using of natural product extracts as a safe larvicidal environmentally. Further investigations are needed to evaluate the plant extracts activities against a wide range of mosquito species stages.

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