Science Journal of University of Zakho
Vol. 5, No. 2, pp. 167 –171, June-2017
p-ISSN: 2410-7549
e-ISSN: 2414-6943
journals.uoz.edu.krd

THE CUMULATIVE EFFECTS OF PLANT EXTRACT TAVRI SHRUB (*DAPHNE MUCRONATA*) ON SOME BIOLOGICAL ASPECTS OF MOSQUITO *CULEX MOLESTUS* FORSKAL

Anas Mohammed Abdulmajeed *, Badia'a Mahmoud Al-Chalabi

Dept. of Biology, Faculty of Science, University of Zakho, Zakho, Kurdistan Region, Iraq - (anas_abdulmajeed@yahoo.com, bchalabi48@gmail.com)

Received: Jan. 2017 / Accepted: Apr. 2017 / Published: Jun. 2017 ***https://doi.org/10.25271/2017.5.2.363***

ABSTRACT:
The present study was performed to evaluate the toxicity and biological activity of ethanolic extract of the plant tavri shrub (*Daphne mucronata*) which is widely abundant in Iraqi Kurdistan region against the mosquito (*Culex molestus* Forskal) using different concentrations (0.25- 7.5 mg/ml). The plant extract significantly affected the survival rate of all immature stages, prolonged their developmental period, preoviposition period, reduced egg productivity and hatchability, caused growth abnormalities, led to the appearance of intermediates and the adult failed to emerge. 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. molestus*. The adult emergence and most of the vital biological activities of the emerging females including preoviposition period, fecundity and fertility were obviously affected. Also the extract caused various morphological deformations at different developmental stages.

KEYWORDS: Ethanolic , *Daphne Mucronata*, Mosquito, *Culex molestus* Forskal, Morphology.

1. INTRODUCTION

Insects in general and mosquitoes particularly have an obvious effect on the public health of both human being and animals. Some mosquito genera such as Culex, Aedes and Anopheles are proved to be responsible for transmitting many agents of dangerous diseases such as malaria, filariasis, yellow fever and Dengue fever (Sukulku *et al*., 2009 and Yerpude *et al*., 2013).
The control of these vectors mainly depends on the use of chemical pesticides. However, 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 substances (Yang *et al.*, 2002). Thus, many workers start to search for new compounds that can be safe, effective and not dangerous to human, useful to the species and wild life. These compounds are called Plant-natural products, which are considered as defensive means against the animals and insects which attack the plants (Perveen *et al.*, 2008).
Plant extracts may be the alternative sources of mosquito control due to the presence a lot of bioactive compounds (Govindarajan, 2010). These plant extracts have several bioactivities such as, growth regulation, fecundity suppression, male sterility, loss of flying ability, immune depression and enzyme inhibition (Su and Mulla, 1998). It has also been observed that 344 species of plants showed biological activity against mosquito species (Sukumar *et al*., 1991).
The extracts of some Iraqi plants have shown to be biologically active against the urban mosquitoes and many plants have been shown to possess insecticidal, antifeeding and other properties (Ouda *et al.*, 1998; Das *et al.*, 2007; Mustafa and Al- khazraji, 2008 and Al-Chalabi *et al.*, 2014).
The present study was conducted to investigate the presence of natural insecticides through assessing the toxicity of extract of a plant species available in Iraqi Kurdistan region which is tavri shrub (*Daphne mucronata*). Furthermore, it is also aimed to identify the morphological deformations in the immature stages of this mosquito species.

2. MATERIALS AND METHODS

2.1 Laboratory colony of mosquitoes

Stock culture of *Culex* mosquitoes used in this study was made by using plastic bowl of 500 ml capacity containing tap water, and supplied with rabbit chow as food source. Three rearing cages (1x1x 1 m) were used to maintain the stock culture. Adults were used after emergence, for egg deposition (Mohsen and Mehdi, 1989). Rearing conditions were well controlled with 27+-1°C, 60-75 relative humidity and12 hrs. photoperiod (Promsiri *et al.*, 2006).

2.2 Plant Collection

Daphne mucronata was collected from environs of Maye, sub-district near the border with Turkey. After washing, the plant was dried and milled by electric mill cutter, the powder obtained from grounded plant was kept in the freezer until the time of its use was due.

2.3 Preparation of plant extracts

Plant extract was prepared according to the method of Abdullah (2001) which include 50 grams of *Daphne mucronata* dried powder mixed with 300 ml of 70% ethanol, stirred for 24 hours then after the filtration the yielded extract was concentrated by

* Corresponding author

vacuum rotary evaporator. The dried powder was placed in glass vials and stored in a refrigerator at about 4 °C to be used when needed.

2.4 Preparation of stock solutions and dilutions

The concentrations of 0.25, 0.5, 0.75,1, 2.5, 5 and 7.5 mg/ml were prepared from the stock solution using the dechlorinated tap water.

2.5 Determination of bioactivity of plant extracts against the mosquitoes *Culex molestus* Forskal

Egg rafts were collected and transferred to plastic containers 300 ml, 4 replicates for each concentration were tested. 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).

Statistical analysis of data was based on completely randomize design. Analysis of variance was used at confidence interval of 95% (Snedecor and Cockran, 1976).

3. RESULTS AND DISCUSSIONS

3.1 The effect of *Daphne mucronata* (tavri) ethanolic extract on the eggs of mosquito *Culex molestus* Forskal

Egg mortality rate of *C. molestus* was significantly affected by tavri extract (table 1), it ranged between 12.51-100% at concentrations of 0.25 -7.5 mg/ml respectively. A direct correlation was found between the extract concentration and egg mortality rate.

The egg mortality may be due to the presence of some chemical compounds such as formic acid which may have damaged the egg sheaths (Coile, 1999). The toxic effect of the extract may be also due to the fusion of its compounds with the cytoplasmic component of the egg (Al- Chalabi *et al.,* 2002).

Table 1. Effect of tavri extract on the eggs of *Culex molestus*

Concentrations (mg/ml)	Egg Mortality Rate % (Mean ± SE)
0.25	12.51 ± 3.828
0.5	17.05 ± 4.385
0.75	34.69 ± 9.357
1	51.41 ± 7.239
2.5	71.50 ± 4.278
5	100 ± 0.00
Control	4.41 ± 1.021

3.2 The effect of tavri extract on the larvae

The cumulative effects of *Daphne mucronata* extract on the larvae of *Culex molestus* are illustrated in table (2) where the mortality rate of the larvae was also affected strongly. It varies between 94.60 – 100% at concentrations of 0.25 – 7.5mg/ml respectively, this result may reflect growth inhibitory effect of the tavri extract.

Table 2. Effect of tavri extract on the larvae of *Culex molestus*

Concentrations (mg/ml)	Mortality% (Mean ± SE)
0.25	94.6 ± 3.194
0.5	96.06 ± 1.540
0.75	100 ± 0.000
Control	10.06 ± 0.482

The larval mortalities in the present study are relatively in accordance with those of Bream *et al.* (2009) who continuously exposed the 2nd instar larvae of *Culex pipien*s to different concentrations of *Phragmites australis* ethanolic leaf extract**;** they recorded 100% larval mortality at concentration of 400 ppm. The mortality is may be due to the action of some chemical compounds existed in the tavri extract such as daphnecin, aquillochin and others (Rasool *et al*., 2010) which may affected the epithelial cells of the alimentary canal (Ndione *et al*., 2007). Growth abnormalities of the larvae were observed (Figure 1).

Figure 1. abnormal larva of *Culex molestus* (left) resulted from eggs treated with 0.25 mg/ml of tavri extract compared with normal larva (right) (20 x).

Also many cases of morphological abnormalities as well as the larval-pupal intermediates were found among the dead larvae (Figure 2).

The existence of deformity in the dead larvae confirms the inhibition effect of the extract on the larval development which is similar to the effect of growth regulators. This agree with the findings of Mohsen *et al*. (1990) when they tested the *Callistemon lanceolatus* extract against *Culex quinquefasciatus*. They found cases of larval-pupal intermediate abnormalities including shortage or elongation of the abdomen and darkening of the cuticle with deformation of thoracic segments.

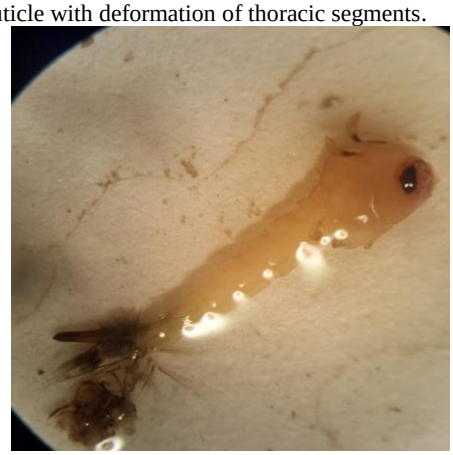

Figure 2. Dead larval- pupal intermediate (25x).

3.3 The effect of tavri extract on the pupae and adult emergence of *Culex molestus*

Since 100% larval mortality was obtained at higher concentration (0.75%mg/ml), no pupae were observed at concentrations above 0.50 mg/ml. The continuous exposure of the larvae to the plant extract until the emergence of the adults has led to enormous effects on the pupae and subsequently on the adult emergence (table, 3).The pupal mortality rate was also significantly affected which increased from 25 to 50% at concentrations of 0.25 to 0.50 mg/ml respectively.

Table 3. Effect of tavri extract on the pupae and adult emergence of *Culex molestus*

Concentration (mg/ml)	% Pupal mortality (Mean ± SE)	% emergence Inhibition(EI) (Mean ± SE)
0.25	25 ± 0.342	93.6 ± 1.020
0.5	50 ± 5.422	97.8 ± 1.101
0.75	-	100 ± 0.00
control	6.4 ± 0.286	26.6 ± 0.898

The present percentage of mortality are higher than those reported by Bream *et al*. (2010) who exposed the second instar larvae of *Culex pipiens* to different concentrations of *Echinochloa stagninum* extract. They obtained the highest pupal mortality rates (25.60 and 33.3%) at concentrations of 4000 and 500 ppm respectively. Some of the pupae had abnormal shape possibly due to the large amounts of fluid in their bodies, (Fig. 2) similarly to those observed by Howard *et al.* (2009).

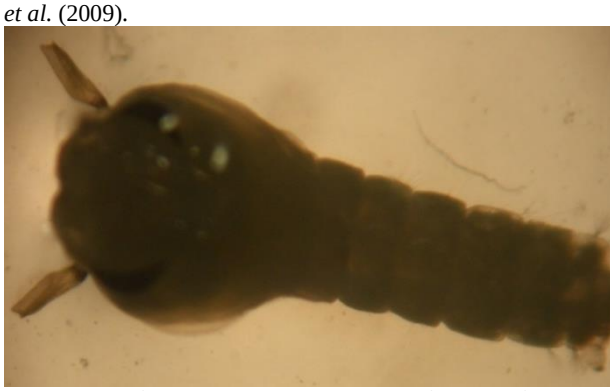

Figure 3. Deformed pupa with large abdomen filled with fluid resulted from egg treated with 0.25 mg/ml (left) compared with a normal pupa (right) (28 x).

Most of the dead adults were failed to emerged (Fig 4), and the present study results revealed that all mortalities were dosage-dependent where, the percentage for the emergence inhibition (EI) increased with the increase of plant extract concentration ranging from 93.67 to 100% at concentrations of 0.25 and all concentrations above 0.5 mg/ml, respectively (Table 3).

Figure 4. Incomplete emerged mosquito resulted from eggs treated with 0.25 mg/ml nettle extract (28 x).

The cumulative effect of tavri extract on the adult emergence is remarkably greater than those reported by Elango *et al*. (2010) when they tested the crude extracts of *Aegle marmelose*, *Andrographis lineate*, *Andrographis paniculata*, *Cocculus hirsutus*, *Eclipta prostrate* and *Tagetes erecta* against *Culex tritaeniorhynchus* inducing only 50 % adult emergence inhibition at highest concentrations.

3.4 The effect of tavri extract on the preoviposition period, female fecundity and fertility of *Culex molestus*

The females which have developed from treated larvae were monitored for their ability to lay eggs and the potential of egg hatching. At concentration of 0.5 mg/ml only a few number of adults has emerged but all of them died before taking any chance for reproduction (table 4). The mean preoviposition period also affected, where it is increased from ran4.33 ± 0.577 days in the control to7 ± 0.333 days at a concentration of 0.24mg/ml. However, few numbers of eggs were laid by females developed from larvae treated with a concentration of 0.25 mg/ml.

Table 4: Effect of tavri extract on the preoviposition period, female fecundity and fertility of *Culex molestus*

Concentration mg/ml	Preoviposition period (days) (Mean ± SE)	Mean of eggs laid (Mean ± SE)	% of egg hatching (Mean ± SE)
control	4.33 ± 0.577	86 ± 3.055	97.6 ± 1.452
0.25	7 ± 0.333	50.6 ± 9.492	76 ± 1.732
0.5	-	-	-

Female productivity significantly dropped from 86 eggs to zero eggs at concentration of 0-0.5 mg/ml respectively (table 4) and Egg hatchability reduced from 97.6% to 0.0% at concentrations of 0-0.5 mg/ml.

The reduction in fecundity of females may be due to the effect of this extract on the ovaries of emerged female, where Al- Jahsany (2004) reported that the alcoholic leaf extracts of *Cupressus sempervirens*, *Datura stramonium*, *Capparis spinosa* and *Melia azedarach* fruit extract resulted in appearance of a variety of abnormalities in the ovaries of females developed from third instar larvae of *Culex pipiens molestus* which have been exposed to different concentrations of these extracts. Sakthivadivel and Thilagavathy (2003) indicated that the petroleum ether seed extract of *Argemone mexicana* was strongly affected the female fecundity of *Aedes aegypti* and completely inhibited the production of eggs at a concentration of 10 ppm.

The present finding is relatively similar to that of Al-Chalabi *et al*. (2002) who confirmed that the hatching percentage of eggs laid by *Culex pipiens* females which have been developed from eggs treated with *Euphorbia granulata* extracts reduced with the increase of plant extract concentration.

3.5 The effect of tavri extract on the developmental periods of immature stages of *Culex molestus*

Developmental period of immature stages of *Culex molestus* was significantly affected by tavri extract. A direct correlation was found between the extract concentration and the developmental periods of *C. molestus* (Table 5).

The continuous exposure of the larvae to low concentrations (0.25 and 0.50 mg/ml) led to significant increase in larval duration as compared with control group. In respect to the larval duration, similar results have been obtained by Promsiri *et al*. (2006) who tested the extracts of three medicinal plants against the larvae of *Aedes aegypti*. They found that the extracts of *Anethum graveolens*, *Mammea siamensis* and *Annona muricata*

prolonged the larval duration in comparison with untreated larvae.

The present results (Table 5) agreed with the findings of Bream *et al.* (2010) who reported that the pupal development period for treated and untreated pupae was monitored, when the second instar larvae of *Culex pipiens* exposed to different concentrations of *Echinochloa stagninum* extract. Moreover, the obvious differences were found between the two groups.

Table 5. Effect of tavri extract on the developmental periods of immature stages, of *Culex molestus*

Concentration (mg/ml)	Egg duration (Days)	Larval duration (Days)	Pupal duration (days)	Total developmental period (days)
control	1	10	3	14
0.25	2	14	5	21
0.5	2	15	11	28
0.75	-	-	-	-

4. CONCLUSIONS

In conclusion it appears that the tavri (*Daphne mucronata*) extract affected the survival rate, developmental period, fecundity, and the productivity of the *Culex molestus* mosquito. Also caused growth abnormalities and led to the appearance of intermediates and the developed adult failed to emerge.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol.,18: 265-267.

Abdullah, A.F. (2001). Isolation and antibacterial evaluation of plant extracts from some medicinal plants in Kurdistan region. M.Sc. Thesis, University of Duhok.

Al-Chalabi, B.M. , Al-Zubaidi, F.S. and Mustafa, A. (2002). Alkaloids of *Euphorbia granulata* Forssk, affecting development of mosquito, *Culex pipiens* L. (Diptera:Culicidae). Journal of Babil University, 7(3): 663-668.

Al- Chalabi, B .M.; Makki, A. M. and Al-Zubaidi, F.SH. (2014). The effect of *Euphorbia granulata* Frossk extract on some biological aspect of the *Culex pipiens* L .(Diptra : Culicidae). Abstracts of the st international scintific conference of Cihan University – Erbil (2014 April), P. 157.

Bream, A.S., Hassan, M.I. , Fouda, M.A. and El-Sheikh, T.M. (2009). Toxicity and repellent activity of *Phragmites australis* extracts against the mosquito vector *Culex pipiens*. Tunisian Journal of Plant Protection, 4(2): 157-172.

Bream, A.S., Ei-Sheikh, T.M.Y., Fouda, M.A. and Hassan, M.I. (2010). Larvicidal and repellent activity of extracts derived from aquatic plant Echinochloa stagninum against Culex pipiens. Tunisian Journal of Plant Protection , 5(1): 107-123.

Coile, N.C. (1999). *Urtica chamaedryoides* Pursh: a Stinging Nettle, or Fireweed and Some Related Species. Botany Circular, 34: 1-6.

Das, N.G., Goswami, D. and Rabha, B. (2007). Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. J. Vet. Borne Dis., 44:145-148.

Elango. G., Abdul Rahuman, A. , Kamaraji, C. , Bagavan, A. and Zahir, A.A. (2010). Adult emergence inhibition and adulticidal activity of leaf crude extracts against Japanese encephalitis vector, *Culex tritaeniorhynchus.* Journal of King Sand University-Science, 8(12): 85-113.

Govindarajan, M. (2010). Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes. Asian Pacific J. Trop. Med., 3: 691-695.

Howard, A.F.V. , Adongo, E.A. , Hassanali, A. , Omlin, F.X. , Wanjoya, A. , Zhou, G. and Vulule, J. (2009).Laboratory evaluation of the aqueous extract of *Azadirachta indica* (neem) wood chippings on *Anopheles gambiae* s.s. (Diptera: Culicidae) mosquitoes. J. Med. Entomol., 46(1): 107-114.

Mohsen, Z.M. and Mehdi, N.S. (1989).Effect of insect growth inhibitor alsystin on *Culex quinquefasciatus* Say. (Diptera: Culicidae). Insect. Sci. Appli.,10(1): 29-33.

Mohsen, Z.H. , Jawad. A. L. M. , Al-Chalabi, B.M. and Al-Naib, A. (1990). Biological activity of *Callistemon lanceolatus* against *Culex quinquefasciatus* Fitoterapia , Vol. LXI- No,3: 270-274.

Mustafa, M.A. and Al-Khazraji, A. (2008). Effect of some plant extracts on the *Culex pipiens molestus* Forskal larvae. Iraqi Journal of Veterinary, 22(1): 9-12.

Ndione, R.D. , Faye, O. , Ndiaye, M. , Dieye, A. and Afoutou, J.M. (2007). Toxic effects of neem products (*Azadirachta indica* A. Juss) on *Aedes aegypti* Linnaeus 1762 larvae. African Journal of Biotechnology, 6(24): 2846-2854.

Ouda, N.A. , Al-Chalabi, B.M. , Al-Charchafchi, F.M.R. and Mohsen, Z.H. (1998). Insecticidal and Ovicidal Effects of the Seed Extract of *Atriplex canescens* Against *Culex quinquefasciatus*. Pharmaceutical Biology, Formerly International Journal of Pharmacognosy, 36 (1):69-71.

Perveen, A.; Sarwar, G. and Hussain, I. (2008). Plant biodiversity and phytosociological attributes. Pak. J. Bot., 40 (1): 17-24.

Promsiri, S. , Naksathit, A. , Kruatrachue, M. , and Thavara, U. (2006). Evaluation of larvicidal activity of medicinal plant extracts to Aedes *aegypti* (Diptera: Culicidae) and other effects on a non-target fish. Insect Science, 13: 179- 188.

Rasool, M.A. , Khan, R. , Malik, A. , Bibi, N. and Kasim, S.U. (2010). Structural determination of daphnecin, a new coumarinolignan from *Daphne mucronata*. J. Asian Nat. Prod. Res., 12(4): 324-327.

Sakthivadivel, M. and Thilagavathy, D. (2003). Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone Mexicana* L. seed. Bioresource technology, 89: 213-216.

Snedecor, M.G.W. and Cochran, W. G. (1976). Statistical methods. Iowa states Univ. Press. 593 pp.

Su, T. and Mulla, M. S., (1998). Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex* quinquefasciatu.) Diptera: Culicidae). J. Am. Mosq. Control Assoc., 14: 204-209

Sukulku, U. , Nuchuchua, O. , Uawongyart, N. , Puttipipatkhachorn, S. , Soottitantawat, A. and Ruktanonchai, U. (2009). Characterization and mosquito repellent activity of citronella oil nanoemulsion. International Journal of Pharmaceutics, 372: 105-111.

Sukumar, K. , Perich, M. J. and Roombar, L. R. (1991). Botanical derivatives in mosquito control: A review. J. Am. Mos. Cont. Assoc., 7: 210-237.

Yang, Y.C.; Lee, S.G.; Kim, H.K. and Lee, H.S. (2002). A piperidine amide extracted from *Piper longum* L. Fruit shows activity against *Aedes aegypti* mosquito larvae. J. Agric. Food Chem., 50: 3765-3767.

Yerpude, P. N.; Jogdand, K.S. and Jogdand, M. (2013). A Study on Awareness and Practice about Preventive Methods against Mosquito Bite among Households in an Urban Slum Area of South India. International Journal of Recent Trends in Science and Technology, 8(2): 69-71.

كورتيا ليكولينێ:

ژبو هەلسەنگاندنا ژەهراتی و كارتێكرنا زیندەییا بۆ شیراڤا ئیسانولییا ڕووەكێ تەڤرێ *Daphnae mucronata* ئەوێ گەلەكێ بەربەلاڤ ل دەڤەرا كوردستانا عێراقێ دژی پێشـــییا توخمێ كیولكس *Culex molestus* Forskal خەســـتییێن جودا جودا (٠,٢٥% - ٠,٧٥%) هاتنە بكارئینان .شـــیراڤا ڤی ڕووەكی كارتێكرنەكا گرنگ هەبوو لسەر هەمی قوناغێن نەپێكهە شتییێن پێشییا كیولكس (هێك و كرمك و پیوپا) ولسەر ماوەییێن گەشەیێ و ماوەیێ بەری هێك دانانێ ژلایێ مێییێن پێشـیانڤە و كێمكرنا بەرهەمئینانا وان و هەروەسـا كارتێكرنەكا گرنگ دیار كر لسـەر گەشـەیا نەسـروشـتییا قوناغێن نەپێگهەشـتی و دیاربوونا ناڤبەرە قوناغان و نەشیانا دەركەفتنا پێشییێن پێگهەشتی ژ پیوپا .كارتێكرنا خرڤەبووی یا شیراڤا ڕووەكی لسەر پەقینا هێكان و تێكرایێ مرنێ بۆ قوناغێن كرمكان و پیوپا و درێژكرنا ماوەیێ گەشەیا ڤان قوناغان یا گرنگ بوو هەروەسا كارتێكرن یا گرنگ بوو لسەر دەركەفتنا پێشییێن پێگهەشتی و لســـەر چالاكییێن زیندەیی وەكی بەرهەنئینانا وان و شـــیانا بەرهەمئینانێ و ماوەیێ بەری هێكدانانێ ژ لایێ وان پێشـــییێن مێڤە ئەوێن ژوان هێكان چێبووین كو ب شـــیراڤا ڕووەكێ تەڤرێ هاتبوونە مامەلەكرن .زێدەباری ڤێ چەندێ شـــیراڤا ڕووەكێ تەڤرییێ بۆ ئەگەرێ دەركەفتنا گەلەك تێكچوونان دشێوەیێ ژدەرڤەدا و دقوناغێن پێشكەفتنێ یێن جودا دا یێن ڤی جورێ پێشیێ.

خلاصة البحث:

لغرض تقييم السمية و الفعالية الحياتية للمستخلص الأيثانولي لنبات عشبة التفري (*Daphne mucronata*) الذي ينتشر بكثرة ويزدهر في منطقة كردستان العراق ضد بعوضة الكيولكس *Culex molestus* Forskal تم استخدام مختلف التراكيز (٠،٢٥ – ٧،٥٠ملغ/مل). ولقد كان لمستخلص النبات تاثيرا معنويا على جميع الاطوار غير الناضـــجة لبعوض الكيولكس (البيوض واليرقات والعذارى) وفترات النمو وفترة ما قبل وضـــع البيض للاناث وتقليل انتاجيتها وكذلك النمو غير الطبيعي للاطوار غير الناضجة وظهور المتوسطات واخفاق في بزوغ او انبثاق الكاملات. وقد كان التاثير التراكمي لمستخلص النبات معنويا على فقس البيوض ومعدل هلاكات الأطوار اليرقية والعذرية وإطالة فترات النمو لهذه الاطوار وكذلك كان التأثير معنويا على بزوغ البالغات. و كذلك لأكثر الفعاليات الحياتية حيوية لاناث البعوض مثل انتاجيتها و خصوبتها وفترة ما قبل وضع البيوض لتلك الاناث الخارجة من بيوض معاملة بمستخلص نبات عشـبة التفري. اضـافة الى ذلك فقد تسـبب مسـتخلص النبات في ظهور تشـوهات مظهرية عديدة و في مختلف المراحل التطورية للنوع آنف الذكر من البعوض.