Available online www.jsaer.com

***Journal of Scientific and Engineering Research*, 2018, 5(4):387-392**

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

The effects of extracted terpenoids, of *Albizia lebbeck* on the biological performance of mosquito, *Culex quinqefasciatus* (Diptera: Culicidae)

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Abstract Crude terpenoids extracts from leaves, flowers and seeds *Albizial ebbeck*. (Benth) were evaluated their biological activities on some biological aspects of *Culex quinqefasciatus*. Accumulative mortality of immature stages were used as biological criteria. Different concentrations (0.10, 0.25, 0.50 and 0.75 mg/ml.) were used for Crude terpenoids. The results of the present study showed that crude terpenoids extract was the effective on all insect stage, egg mortality rate was 45.30% and 50.00% at concentration of 0.75 mg/ml. of flowers and seeds respectively. On the others hand the cumulative mortality of larvae 100% in Crude terpenoids extract of flowers at concentration 0.75 mg/ml., the Crude terpenoids extract of leaves was the most effective on pupal stage at concentration 0.25, 0.50 and 0.75 mg/ml. followed by flowers and seeds extracts at concentration 0.50 and 0.75 mg/ml. Morphological abnormalities in larval and pupal stages were occurred in all extracts compared with control treatments.

Keywords *Albizia lebbeck*, Crude terpenoids, pupal stage and Accumulative mortality

Introduction

It was well documented that medical importance of mosquitoes not derived from its possible transmission of deadly diseases like malaria, filariasis, yellow fever and viral diseases, that cause morbidity, mortality, economic loss and social disruption [1-2]. Since there are no vaccines for these diseases, vector control is the only option available for reducing the morbidity. Therefore insecticides have been used for vector control, mainly organic compounds such as organ chlorides, organophosphates, carbamates and pyrethroids. This method of control has proved to be ineffective and undesirable because of the development of insects resistance and environmental pollution and health hazards due to continued accumulation, and slowly degradable toxic compounds [3]. In addition, these insecticides may affect other beneficial organisms and prove detrimental to animal and human life. Moreover, control of such mosquito- borne diseases is becoming more and more difficult because of lack of effective vaccines and drugs against disease-causing mosquitoes. Hence, an alternative for mosquito control is the use of extracts of plant origin [4]. Botanical insecticides may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, degradable and readily available in many areas of the world. Though several plants from different families have been reported for their mosquitocidal activity [5]. In this respect co-evolution has equipped plant with chemical defenses against phytophagous insect and other organisms. Also, mankind has used plant or plant extracts to control insects since ancient times. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticidal properties [6-7]. In this context extracts of insecticidal activity were obtained from several leguminosae species and were widely used as insecticides against different insect pests. One of these species is *Albizia lebbeck*. Such botanical based insecticides described as environment-friendly compounds viable for field use and for large –scale disease vector pest control [8]. The main constituent of *A. lebbeck* are alkaloids, flavanoids, tannins, proteins and saponins [9].

Study Objectives

Terpenoids extracts from flowers, leaves and seeds of *A. lebbeck*, will be evaluated for their biological activities on some biological aspects of *Culex quinqefasciatus* such as, The accumulative mortality of immature stages.

Collection of Plant Samples

The plant samples (leaves, flowers and seed) were collected from Baghdad University gardens between August and September 2008 at morning hours. These parts air dried at room temperature, then separated and then grinded into powder by using electric grinder.

Mosquitoes collection and rearing:-

Different larval stages of mosquito samples were collected from small pound at botanic garden at Baghdad University on March, 2009. The *Culex* colony was included from one egg raft to ensure the purity of the species, some of the larvae belong to the pure colony were identified by the Natural Historic Museum at Baghdad University which have been identified as *Culex quinqufasciatus* Say .

The larvae were transferred into plastic container (500 ml) which contains (300 ml) of tap water. The larvae were fed on mice chow (0.03 g. for each container) consisting of 21.5% of maize, 20.8% barley, 3% meat extract 12.2% soybean and 6% fish powder [10]. The water was replaced every three days to avoid decay in water. The molted larvae were picked daily and transferred into new plastic container. The cage was used to maintain the colony with dimension of (1x1x1)m. cover with muslin cloth. Another cages with dimension of (30x30x30)cm. were used for breeding and emergence of adults. Adults were fed on sugar solution 10% concentration. Females mosquitoes obtained blood from pigeons in the darkness for 16 hrs according to the method of Mohsen et al [11]. The mosquitoes culture was maintained in a condition of 28 ± 2 ºC. The females start feeding on blood after three days of emergence [12]. After four days the eggs rafts were collected and transferred by small brush to plastic container 500 ml. contained tap water until hatching [13].

Preparation of Plant Extracts

Crude terpenes

The extraction was made at the botany lab. for graduate studies at biology-department college of sciences Baghdad-university**.** Depending on the method of Harborne [14] 15g. of dried material (leaves, seeds and flowers) were successively extracted in a soxhlet extractor for 24 hrs. using chloroform. The solvent was removed by rotary evaporator at 50 ºC. The yield of crude terpenes extract of leaves, seeds and flowers were about: 7.50%, 5.9% and 6.46% respectively.

Preparation of stock solution of extracted allelochemics

Stock solution was prepared for each extract, 2gm. of crud (terpenes, alkaloids and phenols) were dissolved with 5ml. of the appropriate solvent used, then the volume was made up to 100ml. It is equal 2g/100ml. which equal 20 mg/ml. and 0.2 ml. of tween- 20 was added as suffused. Also 0.2 of tween-20 and 5ml. of the appropriate solvent used were added to100 ml. of tap water as control.

Different concentrations have been prepared by using the formula. (C1V1=C2 V2)

Different concentration of (0.10, 0.25, 0.50 and 0.75 mg/ml.), (0.25, 0.50, 0.75 and 1.00) and (0.25, 0.50, 1.00 and 1.5 mg/ml.) were used for crude terpene**s**, extracts respectively as well as control treatment.

Accumulative effects of leaves, seeds and flowers crud terpenes extract of lebbeck plant on immature stages

The mosquitoes were reared in laboratory till third generation to get pure colony. Accumulative effects of terpenes extracts on some biological performance of *C. quinqufasciatus* by using concentrations of 0.00, 0.10, 0.25, 0.50 and 0.75 mg/ml from leaves, seeds and flowers extracts were investigated. The mortality rate was calculated by taking one egg raft (70-110egg) for each replicate (three replicates for each concentration and control treatment for each extract) each egg raft was introduced in 100 ml. plastic beaker containing above mentioned concentrations of the extract then egg rafts sprayed by laboratory spry gun with 5ml. from each

concentrations of extract, the treated egg rafts were kept at incubator with temperature of 28 ± 2 ºC and 70%±5 relative humidity. The egg mortality was calculated after incubation and corrected according to Abott's [15] formula. Larval and pupal stages of *C. quinquefasciatus* also treated with the same concentrations of crud terpenoids extract of the lebbeck plant by using 30 larvae/concentration (three replicates for each concentration and control) were used for all the experiments. For mortality studies, these larvae were kept in 150ml plastic container containing various concentrations of the extract maintained at temperature 28 ± 2 ºC and 70% ±5 relative humidity. The mortality rate was calculated and the mortality rate was corrected according to Abott's [15] formula as follows:

$$\frac{\text{The percentage of treatment mortality - The percentage of controlling mortality}}{100 - \text{The percentage rate of controlling mortality}} \boldsymbol{x}\ 100$$

The effects of terpeniods, of lebbeck plant on developmental period of immature stages of *C. quinquefasciatus* was studied by measuring egg incubation period and, larval and pupal developmental period were determined. Morphological abnormalities observed between the died larvae and pupae were recorded and removed daily by small forceps with wide end to avoid from injury.

Statistical analysis

Statistical analysis was conducted using SAS [16]. Program was used to analysis of data in present study (effect of concentration in all traits). Least significant difference (LSD) test was used to compare the significant difference between treatments.

The cumulative effects of *A. lebbeck* crude terpenes on immature stages of *C. quinqufasciatus:* The effects on eggs

Present study demonstrated that different concentrations of crude terpenes from leaves, seeds and flowers of *A. lebbeck* had different effects on eggs mortality rate. The mortality rate ranged between 0.00 – 33.13, 0.00- 50.53 and 0.00- 45.30 % at concentration ranged between 0.00 – 0.75 mg/ ml. of leaves, seeds and flowers respectively. This indicates a direct correlation between extract concentration and the mortality rate for all treatments (tables 1, 2 and 3).

The effects on larval and pupal stages of *C. quinqefaciatus*

The mortality rate was ranged between 0.00 – 44.03 %, 0.00 – 66.13% and 6.36 – 100% at concentration 0.00 – 0.75 mg/ ml. of leaves, seeds and flowers extracts respectively. The pupal stage was more susceptible than larval stage, the mortality rates reached to 100% at concentration 0.10, 0.25 and 0.50 mg/ml. of leaves extract, of 0.05 and 0.75 mg/ml. seeds and flowers extract respectively. The statistical analysis of the data revealed a significant different between control treatment and extract treatments (tables 1, 2 and 3). Treatment caused morphological abnormalities as well as small size comparing with the control, possible damaged of larval body parts, larval-pupal intermediate and albino pupae were also observed (plate 1).

The effects on adult stage of *C. quinqefaciatus*:

The adults of *C. quinqefaciatus* were affected by a cumulative effect of terpene extract reached 48.03% at concentration 0.10 mg/ml. and reached 47.50% and 49.90% at concentration 0.25 and 0.10 mg/ml. of seeds extract and flowers extract respectively (tables 1, 2 and 3). The adults were failed to emerge because some of the adults died when they were almost completely free from the pupal case, except legs or wing that was still-attached. Also the present study showed that some emerged adults could not fly.

Table 1: The effects of leaves crude terpene mg/ml. of *A. lebbeck* on the cumulative mortality percentage of different developmental stages of *C. quinqefasciatus*

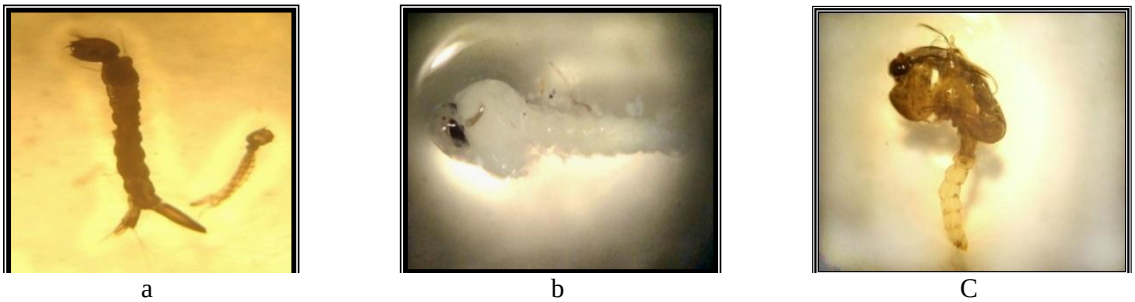
Extract con. Mg/ml	Egg mortality %	Larval stages mortality %	Pupal stages mortality %	adults mortality %
0	0.00 c	0.00 b	5.00 c	4.66 c
0.1	18.50 b	'42.16 a	46.40 b	48.03 b
0.25	19.70 b	46.20 a	100 a	_____
0.50	27.13 a	43.03 a	100 a	_____
0.75	33.13 a	44.03 a	100 a	_____
LSD Value	3.690 *	8.675*	1.633*	4.725 *

Table 2: The effects of seeds crude terpenes mg/ml. of *A. lebbeck* on the cumulative mortality percentage of different developmental stages of *C. quinqefasciatus*

Extract con. Mg/ml	Egg mortality %	Larval stages mortality %	Pupal stages mortality %	Adults mortality %
0	0.00 c	0.00 d	4.00 d	2.00 d
0.1	29.93 b	40.40 c	42.60 c	39.03 c
0.25	25.90 b	42.00 c	48.80 b	47.50 b
0.50	45.16 a	56.03 b	100 a	_____
0.75	50.53 a	66.13 a	100 a	_____
LSD Value	11.134	6.881	1.380	7.094

Table 3: The effects of flowers crude terpenes mg/ml. of *A. lebbeck* on the cumulative mortality percentage of different developmental stages of *C. quinqefasciatus*

Extract con. Mg/ml	Egg mortality %	Larval stages mortality %	Pupal stages mortality %	Adults mortality %
0	0.00 e	6.36 d	2.23 c	4.90 c
0.1	12.50 d	21.73 c	45.76 b	49.90 b
0.25	26.30 c	65.80 b	89.22 a	100 a
0.50	32.90 b	72.83 b	100 a	_____
0.75	45.30 a	100 a	___	_____
LSD Value	1.193	12.651	12.587	2.172



a b C

Plate 1: The morphological abnormalities, of C. quinqefsaciatus due to the effects of crude terpenes extract of A. labbeck

a. Larvae with small size compared with control treatment. B. Albino pupae c. Half emerged adult

The study result revealed that the crude terpenes of leaves, seeds and flowers of *A. lebbeck* significantly affected egg mortality; this effect may be due to the effect of juvenile hormone mimic and other compound which interfere with embryonic development [17]. In this respect Govindarajan et al. [18] mentioned the leaves extract of *Acalypha indica* with different solvents, (chloroform, ethyl acetate and methanol) were tested for ovicidal activity against *A*n. *stephensi*, the highest effective attractancy were 94.20 %, 85.43% and 95.75% respectively at concentration of 100 ppm. AL-Rubaei and AL- Zubiadi [19] found that hot water extract of leaves, flowers and fruits of *D. innoxia* caused 17.6%, 20.0% and 23.2% egg mortality of *M. domastica* respectively at concentration of 50 mg/ ml. On the other hand Yousif [20] studied the effect of crude terpenes of *E. heloscopia* against *C. molestus*, the eggs mortality reached 26.15% at concentration of 1.00 mg/ml. which supported present study findings. Crude terpenes extract of *A. lebbeck* has a role in increasing the cumulative mortality of larval, pupal and adult stages of *C. quinqefasciatus*. This affects insect sensitivity to the poisonous materials that are found in these extract which lead to increase mortality rate and decrease larval efficiency of food conversion. Which affect negatively the growth and increase developmental period or due to the interference with action of endocrine system [21]. In this respect AL- Zubaidi and AL- Taee [22] found that hot water extract of leaves of *D. innoxa* caused cumulative mortality of immature stages of *C. pipiens* 66.2% at concentration of 40 mg/ml. The growth inhibition may be a result to toxicity or feeding deterrent properties of the extract [23]. Many plant chemicals have not only larvicidal property but also pupicidal and adulticidal activity by causing morphological abnormalities, crippling and blocking development of mosquito [25]. The effects of three medicinal plant *Mammea siameusis, Anthum graveolens* and *Annona muricata* extracts induced

several morphological abnormalities in larvae-pupa and adult and cause reduction in adult emergence of *Ae. aegypti* [25]. These findings generally supported present study findings. Al- Zubaidi et al. [26] found that leaves extract of *Lagenaria siceroria* increased mortality rate of adults and immature stages, and reduce egg viability of white fly. On the other hand Saraf and Dixit [27] found that ethanolic extract of flower heads of *Spilanthes acmella* is having potent ovicidal, larvicidal and pupicidal activity. Maximum 7.5 ppm concentration causes 100% mortality of eggs, larvae and pupae of *Anopheles*, *Culex* and *Aedes* mosquito. In another study Sharma et a*l*. [28] examined the effects of crude alcoholic and acetone extracts of *Nerium indicum* leaves against *An. Stephensi* and they found that LC_{50} at 186.00 ppm after 24 h of exposure. The secondary compounds of plants make up a vast repository of compounds with a wide range of biological activities. Most studies reported active compounds as steroidal saponins. Saponins are freely soluble in both organic solvents and water, and they work by interacting with the cuticle membrane of larvae, ultimately disarranging the membrane, which is the most probable reason for larval death [29]. Present findings agree with findings of Wiesman and Chapagain [30] whom reported that saponin extracted from the fruit of *B. aegyptica* showed 100% mortality against larvae of *Ae. aegypti*. Regarding the morphological abnormalities of mosquitoes, several authors recorded similar results when applied different stages of mosquito at sublethal concentrations, Albino pupae were observed after treatment with neem seed kernels extract [31]. The larvicidal activity of a saponin mixture isolated from *Cestrum diurnum* was also applied against *An. stephensi* mosquito [32].

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