Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(2): 483-488

Received: 01-02-2016
Accepted: 04-03-2016

Mohammed M El-Bokl
Department of Zoology, Faculty of Science, Damietta University, Egypt & Zoology Department, faculty of Education, Al-Qubba, Omar Al-Mukhtar University, Libya.

Correspondence
Mohammed M El-Bokl
Department of Zoology, Faculty of Science, Damietta University, Egypt & Zoology Department, faculty of Education, Al-Qubba, Omar Al-Mukhtar University, Libya.

Toxicity and bioefficacy of selected plant extracts against the mosquito vector *Culex pipiens* L. (Diptera: Culicidae)

Mohammed M El-Bokl

Abstract
In this study, the larvicidal activity and bioefficacy of two different extracts (acetone and hexane) of *Artemisia herbalba*, *Lavandula multifida, Peganum harmala* and *Ruta chalepensis* against the 3rd instar larvae of *Culex* pipiens was performed under laboratory evaluation. The toxicity values of acetone extracts based on Lc_{50} values may be arranged in a descending order as follows: *R. chalepensis* > *P. harmala* > *A. herbalba*> *L. multifida*. On the contrary, hexane extracts may be arranged as follows: *L. multifida* > *A. herbalba* > *P. harmala* > *R. chalepensis.* The data also revealed significant decrease in growth index in treated individuals ranged (2.00 - 8.90) when compared to control (11.25). The sublethal concentrations of the acetone extracts induced sterility in the progeny of *C. pipiens* mosquitoes ranged 61(*R. chalepensis*) -73% (*A. herbalba*) whereas, hexane extracts induced sterility ranged 55 (*A. herbalba*) -64% (*R. chalpensis*). There are no negative effects on the hatchability of eggs.

Keywords: Larvicidal activity, Bioefficacy, plant extract, *Culex pipiens*

1 Introduction
Mosquitoes transmit several public health problems, such as malaria, filariasis, and dengue causing millions of deaths every year [1]. Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat. However, the use of synthetic larvicides imposes threats not only to human health but also to the ecosystem because when they are applied into the environment; they may stay on for a very long time or even remain there without end [2]. Synthetic larvicides also disrupt natural biological control systems that sometimes results into a widespread development of resistance [3]. This phenomenon has triggered and urged the development of alternative techniques using natural products.
The use of herbal products is one of the best alternatives for mosquito control [4]. Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against a number of species including specific target-insects and are eco-friendly. Traditionally plant based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as a defense mechanism against insect attacks. These bioactive chemicals may act as insecticides, anti-feedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, anti-moulting hormones as well as attractants. Plant based pesticides are less toxic, delay the development of resistance and are easily biodegradable [5]. Current research trends use plant extracts as alternative larvicides because they contain various phytochemicals that are specific in killing mosquito larvae without harming other organisms and the environment [6, 7].
In view of an increasing interest in developing insecticides of plant origin as alternative to chemical insecticide, in this study acetone and hexane extracts of four native plants in Aljabl Alakhadr region, east Libya *Artemisia herbalba*, *Lavandula multifida, Peganum harmala* and *Ruta chalepensis* were tested against 3rd instar larvae of *Culex pipiens* as larvicides and their bioefficacy on development, growth, fecundity and hatchability.

2. Materials and Methods
2.1 Mosquito culture
The wild species of adult of *Culex* mosquito were collected from houses at Al-Bayda district. The sample (one mother) was reared for several generations during 2014-2015 in the

Department of Zoology, Faculty of science Omar Al-Mukhtar University, Al-Bayda, Libya under controlled conditions (27±2°C, RH 70±10% and 12-12 light-dark regime). Adult mosquitoes were kept in (30 x 30 x 30 cm) wooden cages and daily provided with sponge pieces soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period the females were allowed to take a blood meal from a pigeon host. Plastic cup oviposition (15x15cm) containing dechlorinated tap water was placed in the cage. The resulting egg rafts were picked up from the plastic dish and transferred into plastic pans (25 x 30 x 15 cm) containing 3 liters of tap water left for 24 h. The hatching larvae were provided daily with ground dried bread and dried brewer's yeast (2:1) as a diet [8].

2.2 Selected Plants

The herbal sample consisted of four different plants as listed below in table (1):

Table 1: List of plants and parts used in the study.

Plant species	Common name	Family	Parts used
Artemisia herbalba	Desert wormwood	Asteraceae	Leaves
Lavandula multifida	Egyptian lavender	Lamiaceae	Leaves
Peganum harmala	Harmal	Nitrariaceae	Arial part
Ruta chalepensis	fringed rue	Rutaceae	Arial part

2.3 Preparation of Crude Extract

Fresh plants were dried in the shade at room temperature and ground in a coffee bean grinder. The dried plant material was weighed (50 gm) and then soaked in 200 ml of the solvents used (acetone and hexane) for 7 days with continuous shaking in a shaker at room temperature. At day seven, the plant material was filtered and the filtrate collected. The filtrates were combined and concentrated in air to obtain the crude extract. The dry extracts were weighted and kept in deep freezer (-4ºC) until use. The residues were then used to prepare one per cent stock solution with solvent. From the stock solution, different dilutions were prepared with distilled water.

2.4 Identification of Mosquito Larvae: Larvae of a mosquito can be identified from any other aquatic insects since it has a combination of two characters, they have no legs and the thorax is wider than the head or abdomen. The three divisions of the body part mosquito larvae are head, thorax and abdomen. The structure of three body regions serves as the basis for identifying the mosquito larvae. The mosquito larva was identified according to identification key by Becker *et al.,* [9] using a compound microscope. A small amount of water with a mosquito larvae was drop in a slide to be able to view the specimen in the compound microscope.

2.5 Experimental bioassay

Larvicidal activity: Larvicidal activity of the crude extracts was evaluated as per protocol described earlier [10]. In order to study the toxicity of plant extracts, acetone extracts were dissolved in water while hexane extracts were dissolved in 2 drop of Tween 80 as emulsifier to facilitate the dissolving of material in water. Different concentrations of extracts were prepared. Ten early 3rd instar larvae were put into plastic cups containing different concentrations of extracts prepared in 100 ml of dechlorinated tap water. At least, three replicates were used for each tested concentration. All plastic cups were incubated at a temperature of 27 ± 2°C, relative humidity 70 ± 10% and 12:12 light-dark photoperiod. Controls corresponded to 0.1 ml of acetone or 2 drops of Tween 80 in 100 ml water. After treatment, the larvae were considered dead if, at the end of 24 hrs, they showed no sign of swimming movements even after gentle touching with a glass rod, as described in the World Health Organization's technical report series [10]. The dead larvae in the three replicates were combined and expressed as a sum mortality of each concentration. Mortalities were daily recorded and dead larvae removed. Control mortality was accounted for by the formula of Abbott [11]: Corrected% mortality = (% Mortality Observed -% Mortality Control) / (100 -% Mortality Control) ×100.

Bioefficacy assays: The effects of sublethal concentrations of the crude extracts of the four selected plants on developmental period (time required to pupal formation and adult emergence) of *C. pipiens* mosquito species were evaluated. To investigate the effect of sublethal concentrations of the crude extracts on the hatching performance mosquito species, the filter papers containing eggs collected from the fecundity tests were used.

Growth index was calculated according to Saxena and Sumithra [12]: Growth index = a / b (where: a = % of adult emergence, b = mean developmental period (days).

The number of eggs that hatched into 1st instar larvae in each concentration were counted. Hatchability was calculated as percentage of eggs deposited. Following Saxena *et al.* [13], the sterility index (SI) was calculated as follows:

SI = 100 - {[Fecundity (number of eggs/female) of the treated × hatchability (%)] / [Fecundity (number of eggs/female) of the control × hatchability (%)]} ×100.

2.6 Statistical analysis

The average larval mortality data were subjected according to Finney [14] to probit analysis to calculate LC_{50}, LC_{90} and 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL) to determine lethal concentrations of the plant extracts on *Culex pipiens* mosquito larvae after 24 and 48 hours of treatment, regression equation, Chi-square were estimated by the log concentration probit model using the LdP Line^R Ehabsoft [15]. Analysis of variance (ANOVA) was performed using one-way ANOVA from MSTAT software. All the treatment means were compared for any significant differences using the Duncan's multiple range tests at significant level of $P_{0.05}$.

3. Results and Discussion

Larvicidal activity of acetone and hexane extracts of the four selected medicinal plants native in Aljabl Alakhadr region, east Libya *A. herbalba, L. multifida* and *P. harmala* and *R. chalepensis* were examined against 3rd instar larvae of *C. pipiens.* The LC_{50} and LC_{90} values of the four plant extracts with other associated values such as 95% confidence limits, slope, regression value and chi square were given in table 2. The LC_{50} values of acetone extracts revealed that the larvae of *C. pipiens* after 24 hours was more susceptible to *R. chalepensis* (1.08 ppm) followed by *P. harmala* (18.75 ppm), *A. herbalba* (73.99 ppm) and *L. multifida* (78.55ppm) whereas the LC_{90} values shows that there is a mild variation in the lethality of extracts *R. chalepensis* (5.21 ppm), *L. multifida*(126.70 ppm), *A. herbalba*(177.42 ppm), *P. harmala*(177.93 ppm).The LC_{50} values of hexane extracts showed that the larvae of *C. pipiens* after 24 hours was more susceptible to *L. multifida* (0.15 ppm) followed by *A. herbalba* (1.44 ppm), *P. harmala* (4.07 ppm) and *R. chalepensis* (4.81 ppm) whereas LC_{90} values shows the same lethal hierarchy.

The larval mortality rate of *C. pipiens* after the treatment of the two different extracts of *A. herbalba, L. multifida* and *P. harmala* at LC_{50} and $LC_{90}$was more susceptible to hexane extract followed by acetone and the lethal hierarchy is (hexane >acetone) whereas the LC_{50} and LC_{90} of *R. chalepensis* revealed that the larvae of *C. pipiens* was more susceptible to acetone extract followed by hexane and the lethal hierarchy is (acetone > hexane).

The 48 hrs LC_{50} values were much less as compared to 24 hrs LC_{50} values of all plant extracts applied in this study showing that plant derived insecticides are much slower in their insecticidal action unlike the conventional insecticides.

The results from the study showed the four plants exhibited good larvicidal activities on the mosquito with varying susceptibility. Hexane extracts showed more potent larvicidal activity than acetone extracts except with *R. chalepensis*, indicating the non-polar characteristics of larvicidal components.

Table 2: Probit analysis of larvicidal efficacy of plant extracts against 3rd instar of *Culex pipiens* larvae after 24 and 48 hours of exposur. X^2 = Chi square, r = regression value, SE= standard error.

		24 hrs						
Plant species	**Solvent**	**LC_{50}**	**LCL- UCL***	**LC_{90}**	**LCL- UCL**	**X^2**	**r**	**Slope ± SE**
Artemisia herbalba	Acetone	73.99	62.55 - 86.89	177.42	139.36 - 269.33	1.8448	0.987	3.374 ±0.53
	Hexane	1.44	1.09 -1.83	7.90	5.21 - 16.27	4.8024	0.949	1.7316 ±0.27
Lavandula multifida	Acetone	78.55	72 - 85.62	126.70	107 - 188	2.84	0.935	6.173 ±1.41
	Hexane	0.15	0.12 - 0.19	0.57	0.40 - 1.00	4.7041	0.969	2.1924 ±0.30
Peganum harmala	Acetone	18.75	12.03 - 27.51	177.93	93.37 - 650.97	2.9812	0.949	1.3113 ±0.25
	Hexane	4.07	2.98 - 5.40	25.25	17.11 - 44.16	2.0887	0.989	1.6171 ±0.19
Ruta chalepensis	Acetone	1.08	0.82 - 1.41	5.21	3.24 - 13.57	3.7866	0.981	1.8737 ±0.35
	Hexane	4.81	3.27 - 6.88	36.58	22.96 - 70.74	2.183	0.985	1.4542 ±0.17
		48 hrs						
Plant species	Solvent	LC_{50}	LCL- UCL	LC_{90}	LCL- UCL	X^2	r	Slope ± SE
Artemisia herbalba	Acetone	32.56	14.81 - 45.74	161.32	111.20 - 399.81	1.8993	0.952	1.844 ±0.46
	Hexane	0.88	0.65 - 1.11	3.62	2.63 - 6.20	9.0756	0.976	2.088 ±0.33
Lavandula multifida	Acetone	54.30	38.19 - 62.05	0.83	0.41 - 1.20	0.1906	0.984	6.038 ±1.53
	Hexane	0.08	0.04 - 0.12	3.10	2.46 - 11.36	12.4582	0.904	2.243 ±0.33
Peganum harmala	Acetone	5.04	2.01 - 8.15	2.93	2.12 - 3.90	2.7144	0.947	1.39 ±0.29
	Hexane	2.93	2.12 - 3.90	17.55	11.96 - 30.54	4.1385	0.979	1.648 ±0.20
Ruta chalepensis	Acetone	0.62	0.45 - 0.77	2.25	1.72 - 3.54	7.2415	0.932	2.288 ±0.37
	Hexane	1.79	1.20 - 2.56	11.01	7.07 - 21.17	6.0517	0.980	1.626 ±0.21

Note: * LCL represents the lower confidence limit, UCL represents the upper confidence limit based on a 95% Confidence interval.

Developmental indices

The effects of sublethal concentration of the extracts (acetone and hexane) on still living larvae were monitored continuously after an initial 48-hour period of exposure. Details of the comparative effects of acetone and hexane extracts of *A. herbalba, L. multifida, P. harmala* and *R. chalepensis* on biology, development, growth, fecundity and hatchability of *C. pipiens* are shown in Table 3.

During developmental metamorphosis, time taken for total larval and pupal developmental time (in days). Results revealed that treated individuals took prolonged larval period significantly when compared to control only with acetone extracts of *A. herbalba, L. multifida, P. harmala*. The pupal duration significantly increased in treated individuals only with hexane extract of *L. multifida*. The larval period lasted 7 to 7.7 (control 6 days) and pupal period lasted 2.33 to 3 days (control 2 days) in treated individuals. Total developmental period (larval and pupal development) took 8 to 10 days (control 8 days) in treated individuals while, significantly increased with acetone extract of *L. multifida*. The data also revealed significant decrease in growth index in treated individuals ranged (2.00 - 8.90) when compared to control (11.25).

From the data provided in table 3 decrease in the fecundity of the mosquitoes obtained from the larvae subjected to treatment with sublethal concentration of the different extracts against 3rd instar larvae is evident. *C. pipiens* females in the control series deposited meanly 214 egg/female. Correspondingly, the fecundity of treated mosquitoes with acetone extracts ranged 57.7 (*A. herbalba*) and 83 (*R. chalepensis*) eggs/female. On the other hand, fecundity of treated mosquitoes with hexane extracts ranged 77 (*R. chalepensis*) and 95(*A. herbalba*) eggs/female. The fecundity of the *C. pipiens* was affected more by the acetone extracts of

all tested plants. There are no negative effects of these extracts on the hatchability of eggs of the mosquitoes. Treatment of the larvae with sublethal concentrations of the acetone extracts induced sterility in the progeny of *C. pipiens* mosquitoes ranged 61(*R. chalepensis*) -73% (*A. herbalba*). At this concentration the hexane extracts induced sterility ranged 55 (*A. herbalba*) -64% (*R. chalpensis*).

Table 3. Effect of the plant extracts (LC_{25}) on the development, growth index, fecundity (eggs/female) and hatchability of *Culex pipiens*. The adults were raised from larvae reared at sublethal concentrations of the extracts from 3rd instar to pupation.

		Mean larval period (Days)	Mean pupal period (Days)	Adult emergence % (a)	Mean development days ± SD (b)	Growth index (a/b)	Average number of eggs laid/female	hatched eggs %	Sterility index
	Control	6.00± 0.00 [b]	2.000 [a]	90	8.00± 0.00 [a]	11.25 [a]	214.33[a]	100	-
Artemisia herbalba	Acetone extract	7.00 ± 0.00 [a]	2.333 [a]	50	9.33± 0.58 [a]	5.36 [b]	57.67[b]	100	73.10
	Hexane extract	6.33 ±0.58 [ab]	2.667 [a]	70	9.00± 1.00 [a]	7.78 [b]	95[b]	100	55.7
	LSD	0.7553	1.308		1.772	1.242			
Lavandula multifida	Acetone extract	7.67±1.15 [a]	2.67±0.58 [ab]	40	10.00±1.00 [b]	4.00 [c]	75.33[b]	100	64.9
	Hexane extract	7.00±0.00 [ab]	3.00±0.00 [b]	20	10.00±0.00 [ab]	2.00 [c]	78[b]	100	63.6
	LSD	1.51	0.76		1.308	0.764			
Peganum harmala	Acetone extract	7.00±0.00 [a]	2.33±0.58 [a]	83	9.33±0.58 [a]	8.90 [b]	82.33[b]	100	61.6
	Hexane extract	5.33±0.58 [b]	2.33±0.58 [a]	63	8.00±1.15 [a]	7.88 [c]	90[b]	100	58
	LSD	0.7553	1.195		3.025	2.047			
Ruta chalepensis	Acetone extract	6.00±0.00 [b]	2.00 [a]	40	8.00±0.00 [a]	5.00 [b]	83[b]	100	61.3
	Hexane extract	6.00±0.00 [b]	2.00 [a]	57	8.00±0.00 [a]	7.13 [b]	77[b]	100	64.07
	LSD					0.8309			

Note: Mortality only detected at larval stage

The same treatment and column sharing the same letters are not significantly different at $P<0.05$.

The results of the larvicidal activity of the tested plant extracts against the 3rd larval instar of *C. pipiens* varied according to the extract concentration. The larval mortality percent increased as extract concentration increased for all plant extracts. The toxicity values of acetone extracts based on Lc_{50} values may be arranged in a descending order as follows: *R. chalepensis* > *P. harmala* > *A. herbalba*> *L. multifida*. On the contrary, the toxicity values of hexane extracts based on Lc_{50} values may be arranged in a descending order as follows: *L. multifida* > *A. herbalba* > *P. harmala* > *R. chalepensis.* In the current study, levels of active constituents in each extract may be responsible for the observed differences in their larvicidal potential against *C. pipiens*. The hexane extract contains non-polar chemical compounds and acetone extract contain polar compounds.

The results of the present study confirm previous evidences about the larvicidal activity of plant extracts. Yael *et al*., [16] reported that both ether and methanol extracts of *R. chalepensis* have remarkable larvicidal activities and the lc_{50} values through exposure time 24h were 1.8 and 6.4 µg/ml, respectively against late 3rd instar larvae of *Aedes aegypti*. Essential oils from wild and cultivated *R. chalepensis* plants were able to exert a very good toxic activity against *A. albopictus* larvae (wild plants, LC_{50} = 35.66 ppm; cultivated plants, LC_{50} = 33.18 ppm), and mortality was dosage dependent (Conti *et al.,*) [17]. *Benelli et al.,* [18] proved the high efficacy of *R. chalepensis* essential oil, recognized the most effective larvicidal among ten tested oils against *Aedes albopictus*.

Masotti *et al.*, [19] the larvicidal activity of both ethanolic *Artemisia* extracts is less efficient compared with the dichloromethane extract of *A. borealis*. Both ethanolic extracts of *A. molinieri* and *A. campestris* var *glutinosa* showed larvicidal activity against the mosquito *C. pipiens*. However, extracts of *A. molinieri* revealed a higher larvicidal activity than those of *A. campestris* var *glutinosa*. Botanical extracts offer a melt of many various active components and display a large range of biocide activity. For example, phytoextracts of *Artemisia annua* have effective larvicidal effects and significant influence on hatching and post-hatching development of the mosquito *Anopheles stephensi* [20]. Therefore, phytoextracts of *Artemisia* sp. could be used at different mosquito developmental periods

The larvicidal activity of *Lavandula* was recorded by Pavela [21] with methanolic flower extracts of *Lavandula officinalis* with lc_{50} 59 ppm against the larvae of *C. quinquefasciatus*.

The growth index was considerably reduced at all extracts signifying the anti-juvenile effect of the extract. The negative effect of plant extracts on growth index of mosquitoes reported in previous studies of Arivoli and Tennyson [22] and EL-Sheikh *et al.,* [23].

The larvae which survive the treatment with the sublethal concentrations of the acetone and hexane extracts and manage to emerge as adults ultimately oviposit very few eggs. The effect of the extracts on the fecundity reported in the present study prompt to speculate that the extracts produce these effects through their influence on the endocrine system. However further studies on the mechanism of action of these extracts and their efficacy for control is required for the development of a more potent biocontrol agent for the control of mosquito vectors.

The finding of the current study showed that phytochemicals with insect growth regulator activity can significantly inhibit normal ovarian maturation and egg production in mosquitoes and therefore decrease the vector mosquito population. Possibly, variation in egg production of mosquitoes in response to chemical exposure is due to factors which are involved in the regulation of egg production in mosquitoes such as genetical factors as well as hormonal and nervous system stimulations [24, 25].

Rearing of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* larvae in water at concentrations less than the critical concentration for inhibition of adult emergence in 50% of treated larvae of the ethyl acetate fractions of *Calophyllum inophyllum* seed and leaf, *Solanum surattense* and *Samadera indica* leaf extracts and the petrol ether fraction of *Rhinacanthus nasutus* leaf extract, from hatching to emergence significantly decreased the fecundity of the mosquitoes and the hatchability of their eggs [26].

The effect of the plant extracts on the fecundity of mosquitoes was reported by Kamiabi *et al.,* [27] who observed that the crude extracts of *Cyperus aromaticus* increased the sterility indices in the parental generation females in both *Aedes aegypti* and *Aedes albopictus*. Rearing of *C. quinquefasciatus* larvae in water at concentration less than the critical concentrations for inhibition of adult emergence in 50% of treated larvae of the ethyl acetate fraction of *Croton hirtus* and *Pogostemon quadrifolius* leaf extracts from hatching to emergence significantly decreased the fecundity of the *C. quinquefasciatus* and the hatchability of their eggs (Pushpalatha 2015) [28].

There were no effect of the sublethal concentrations of the plant extracts on the hatching percentage of eggs produced by adults treated in the 3rd instar larvae. These results on the contrary of studies of Arivoli and Tennyson 2011, Muthukrishnan and Pushpalatha 2001, Pushpalatha 2015 [22, 26, 28].

4. Conclusion

The screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system. Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varities and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. The recently developed new isolation techniques and chemical characterization through different types of spectroscopy and chromatography together with new pharmacological testing have led to an interest in plants as the source of new larvicidal compounds [29]. In the present work, the hexane extracts of *L. multifida*, *A. herbalba*, *P. harmala* and *R. chalepensis* displayed good larvicidal activity followed by acetone extracts whereas, in case of *R. chalepensis* were acetone followed by hexane extract against the *C. pipiens* mosquitoes. In the light of this, the effects of the extracts on the growth index and fecundity at sublethal concentrations reported in the present study signifying the anti-juvenile effect of the extracts. Also, it could be concluded that, plant extracts used in the present study act as growth regulators against the mosquito vector, *C. pipiens*. Therefore they may be excellent candidates potential to be used as bioinsecticides. Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species. The active ingredients of the extract responsible for larvicidal and adult emergence inhibition activity in *C. pipiens* should also be identified and utilized, if possible, in preparing a commercial product/formulation to be used as a mosquitocide.

Furthermore, the results of the present study may contribute to a reduction in the application of synthetic insecticides, which increase the opportunity for natural control of various medically important pests by botanical pesticides.

5. References

1. Vatandoost HVM. Larvicidal activity of neem extract *Azadirachta indica* against mosquito larvae in Iran. Pestology, 2001; 25:69-72.
2. Mathivanan T, Govindarajan K, Elumalai K, Ananthan A. Mosquito Larvicidal and Phytochemical Properties of *Ervantania coronaria* Stap f. (Family Apocynaceae). J. Vector Borne Dis. 2000; 44:178-180.
3. Tiwary M, Naik SN, Tewary DK, Mittal PK, Yadav S. Chemical Composition and Larvicidal Activities of the Essential Oil of *Zanthoxylumarmatum* DC (Rutaceae) Against three Mosquito Vectors. J. Vector Borne Dis. 2007; 44:198-204.
4. Nandita Chowdhury AG. Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. BMC Complementary and Alternative Medicine, 2008.
5. Srinivasan R, Shivakumar SM. Natarajan. Larvicidal Potential of Some Indian Medicinal Plant Extracts against *Aedes Aegypti*. Asian journal of pharmecutical and clinical research, 2013, 77-80.
6. Hedlin PA, Holingworth RM, Masler EP, Miyamoto J, Thopson DG. Phytochemicals for Pests Control, American Chemical Society, 1997, 372.
7. Arnason J, Philogene B, Morand P. Insecticides of Plant Origin, American Chemical Society Journal. 1989; 387:213.
8. Kasap M, Demirhan. The effect of various larval foods on the rate of adult emergence and fecundity of mosquitoes. Turkiye Parazitologi Dergisi, 1992; 161:87-97.
9. Becker N, Petric D, Zgomba M, Boase C, Dahl C, Lane J. *et al.* Mosquitoes and their control. Edn 2, Springer-Verlag Berlin Heidelberg, 2010, 577.
10. WHO. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC/81.807,1981.
11. Abbott WS. A method for computing the effectiveness of an insecticide. J. Econ Entomol. 1925; 18:265-277.
12. Saxena SC, Sumithra L. Laboratory evaluation of leaf extract of a new plant to suppress the population of malaria vector *An. stephensi* Liston (Diptera: Culicidae). Curr. Sci, 1985; 54:201-202.
13. Saxena RC, Harshan V, Saxena A, Sukumaran P, Sharma MC, Lakshamana KM. Larvicidal and chemosterillant activity of *Annona squamosa* alkaloids against *An. stephensi*. J. Am. Mosq Control Assoc. 1993; 9(1):84-87.
14. Finney DJ. Probit analysis – A statistical treatment of the sigmoid response curve. Edn 3, Cambridge university press, Cambridge, London, 1971.
15. Ehabsoft [http://www.ehabsoft.com/ldpline]
16. Yael C. DE LA Torre Rodriguez, Francisco Ricardo Marti ´NEZ Estrada, Adriana Elizabeth Flores Suarez, Noemi´Waksman DE Torres, and Ricardo Salazar Aranda Larvicidal and Cytotoxic Activities of Extracts From 11 Native Plants From Northeastern Mexico. J. Med. Entomol. 2013; 50(2):310-313.
17. Conti B, Leonardi M, Pistelli L, Profeti R, Ouerghemmi I, Benelli G. Larvicidal and repellent activity of essential oils from wild and cultivated *Ruta chalepensis* L. (Rutaceae) against *Aedes albopictus* Skuse (Diptera: Culicidae), an arbovirus vector. Parasitol Res, 2013; 112:991-999.
18. Benelli G, Canale A, Conti B. Eco-friendly Control Strategies against the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae): Repellency and Toxic Activity of Plant Essential Oils and Extracts.

Pharmacology On Line, 2014, 44-51.
19. Masotti V, De Jong L, Moreau X, Rabier J, Laffont-Schwob I, Thiery A. Larvicidal activity of extracts from *Artemisia* species against *Culex pipiens* L. mosquito: Comparing endemic versus ubiquist species for effectiveness. C. R. Biologies, 2012; 335:19-25.
20. Sharma P, Mohan L, Srivastava CN. Phytoextract-induced developmental deformities in malaria vector. Bioresource Tech, 2006; 97:1599-604.
21. Pavela R. Larvicidal effects of some Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitology Research, 2008; 102:555-559.
22. Arivoli S, Samuel T. Bioefficacy of *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) whole plant extracts against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae), International Journal of Current Research. 2011; 3(4):296-304.
23. EL-Sheikh MYT, Hassan IM, Moselhy AW, Amer SM, and Shehata ZA. Evaluation of the biological activity of some *Cupressus sempervirens* (Cupressaceae) extracts against the mosquito vector *Culex pipiens* L. (Diptera: Culicidae). Egypt. Acad. J. biolog Sci. 2011; 4(1):33-48.
24. Attardo GM, Hansen IA, Raikhel AS. Nutritional regulation of vitellogenesis in mosquitoes: Implications for anautogeny. Insect Biochem Mol Biol, 2005; 35(7):661-675.
25. Gulia-Nuss M, Robertson AE, Brown MR, Strand MR. Insulin like peptides and the target of rapamycin pathway coordinately regulate blood digestion and egg maturation in the mosquito *Aedes aegypti*. PLoS ONE, 2011. doi: 101371/journalpone0020401.
26. Muthukrishnan J, Pushpalatha E. Effects of plant extracts on fecundity and fertility of mosquitoes. J Appl Entomol. 2001; 125(1-2):31-35.
27. Kamiabi F, Jaal Z, Keng CL. Bioefficacy of crude extract of *Cyperus aromaticus* (Family: Cyperaceae) cultured cells, against *Aedes aegypti* and *Aedes albopictus* mosquitoes. Asian Pac J Trop Biomed. 2013; 3(10):767-775.
28. Pushpalatha E. Acute Toxicity of Two Tropical Plant Extracts on the Fecundity and Fertility of *Culex quinquefasciatus* Say. Advances in Zoology and Botany 2015; 3(3):38-41.
29. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian J Med Res. 2012; 135:581-598.