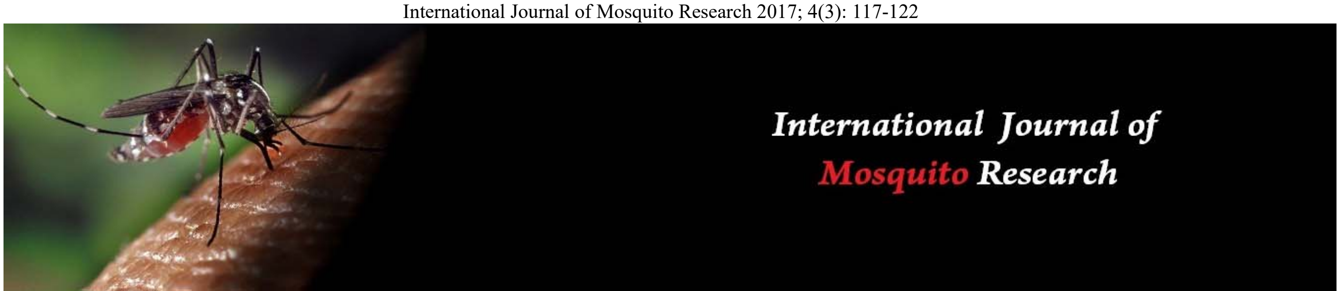
International Journal of Mosquito Research 2017; 4(3): 117-122



ISSN: 2348-5906
CODEN: IJMRK2
IJMR 2017; 4(3): 117-122

Received: 17-03-2017
Accepted: 18-04-2017

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Larvicidal efficacy and residual toxicity of selected xerophyte plants against *Culex pipiens molestus* mosquito

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Abstract
Screening of fourteen xerophyte plants as mosquito larvicides for 4th instar *Culex pipiens molestus*. More effective with high toxicity nine species; *Achelia fragrantissima [Af], Tanacetum cantolinoides [Tc] (Asteraceae), Cakile Arabica [Ca], Horwoodia dicksonia [Hd]* (Brassicaceae), *Astragalus annularis [Aa]* (Fabaceae), *Erodium glaucophyllum [Eg]* (Geraniaceae), *Resida muricata [Rm]* (Residacea) and *Fagonia indica [Fi]* (Zygophyllaceae). Their Lc_{50} and Lc_{90} after 24 and 48 hrs were evaluated. Lc_{50} values after 24 hrs gradually: 12.5, 40.5, 43.0, 70, 86, 103.5, 104, 105.5 and 135 ppm for Fa, Af, Aa, Eg, Ca, Tc, Hd, Rm and Ah respectively. For testing the plant extracts residual toxicity persistence, the test starts with potential concentration causing mortality 100% for the 4th instar larvae, as following ; Fi 25. and Af 100 ppm, Aa, Eg, and Ca 150 ppm, Rm, Tc and Hd 200 ppm and for Ah 250 ppm. Ficum®W was applied as positive control and standard insecticide positive control with 15 ppm and negative control. The extract toxicity declined significantly with plant extract species and its exposure time which becomes zero at day 9 for Rm, day 12 for Aa and Hd,and day 15 for Fi, Ca, Tc and Ah, while continues with 40 and 31.2% mortality at day 15 for Af, and Eg respectively, in comparison with 70.8% mortality for standard insecticide Ficum®W at day 16. On the bases of this study, xerophytic plant could be considered with promising main botanical insecticidal resources which needed more investigation.

Keywords: Xerophytes, plant extracts, *Culex pipiens molestus,* mortality, residual toxicity

1. Introduction
Mosquitoes are responsible for the transmission of many medically important pathogens and parasites such as viruses, bacteria, protozoa and nematodes, which cause serious diseases as malaria, dengue, yellow fever, filariasis (WHO, 2010) [36] and Zica (WHO, 2016) [37]. Of the 3000 species of the mosquitoes recorded worldwide, more than hundred species are capable of transmitting various diseases to human (Reuda *et al*., 2008) [23]. WHO has declared the mosquitoes as "public enemy number one" (WHO, 1996). Vector borne disease are the major concern in the developing countries (Govindarajulu *et al*., 2015) [11].
In the recent years there has been much interest for natural insecticides derived from plants (Basker, *et al.,* 2016) [4], the co-evolution of the plants with insects has equipped item with plethora of chemical defense which can be used against insects (Macedo, *et al*., 1997) [13] which are save to human and ecosystem (Raveen *et al*., 2014) [22]. It has been observed that insecticides of botanical origin be actively toxic to various insects (Browers *et al*., 1976) [5], and reported potentially useful against mosquitoes (Mukhtar *et al*., 2015; Rathy *et al*., 2015) [17, 21]. Some plant extracts were caused 100% mortality and have higher toxicity and potential use for the control of the mosquito larvae (Abdel-Sattar *et al*., 2015) [1]. Recently a considerable work has been done and the use of botanical derivatives against mosquitoes has been reviewed (Arivolie, *et al*., 2012) [3]
The susceptibility of C*ulex pipiens* to plant extract depending on the solvents were used (Mohan *et al.,* 2006) [16], plant part used and plant species. Petroleum ether extract of *Eucalyptus globulus* at a dose of 1000 ppm caused 100% mortality of the larvae C*ulex pipiens* (Sheeren, 2006) [28]. Lc_{50} of *Piper nigrum* seed exteact was 2.6 mg/l. (Shaalan *et al.,* 2007), Volatile oil of *Thymus capitatus* proved high larvicidal potency 49.0 ppm (Mansour *et al*., 2007). Lc_{50} and Lc_{90} of *Solanum xanthocarpum* leaves extract were 41.28 and 111.16 ppm after 24 hrs and 38.48 and 80.83 ppm after 48 hrs respectively (Mohan *et al*., 2006) [16].

Xerophytic plants are unturned stone of natural product as larvicidal agent, Lc_{50} of stem extracts of *Trichodesma africanum* and *Ceolme rupicola* 5.35 and 1.23 and Lc_{90} 8.08 and 7.54 (Al-Mekhlafi *et al*., 2013) [2]. Specification on residual action of a possible alternative insecticide derived from plant materials is important to determine minimum interval time between applications and the environmental persistence of the biopesticides (Mekhlif, 2007; Thiagletchum *et al*., 2014) [15, 31].

In view of promising to find alternative insecticides of botanical origin, this first study in Iraq was carried out on screening xerophyte plants as larvicides of mosquito vectors and their residual action using laboratory tests.

2. Materials and methods

2.1 Collection of plants

The samples of the plant species were collected from western desert about 50 km south Al Kaim town in Al-Anbar province, Iraq. These species were screened for their larvicidal action for chosen more effective them and neglect nonactive ones. In this study, table 1 illustrate these species and their parts used in the test experiments.

2.2 Ethanol plant extracts

The plant materials were washed and dried in shade place completely, then, with hands coarsely crushed and grounded by electric miller. The powdered plant materials sieved through sieve no. 0.250 mm, 100 ml of 96% ethanol alcohol added to 25 gm. of each sample plant in 500 ml conical flask, and kept at 4C°three days for maceration. After that, the flasks contents were stirred for 24 hrs and dual filtrated through wattman filter paper no.1 under low pressure. The filtrates were left overnight for solvent evaporation. To eliminate the chlorophyll contents and extracts enrichment, the extracted plant materials dissolved in v/v of ethanol and petroleum ether in separate funnel for 24 hours. The alcoholic supernatant was evaporated at laboratory temperature and kept in 4c° stock solution of 10.000 ppm was prepared by dissolving one gm of crude extract in 100 ml ethanol solvent.

Table1: The xerophytic plants were tested for larvicidal activity on *Culex pipiens molestus.*

Plant species	Family	Parts used
Achelia fragrantissima	Asteraceae	aired
Aizoon hispamicum	Aizoaceae	aired
Arneba hispidissima	Boraginaceae	aired
Astragalus annularis	Fabaceae	aired
Astragalus sp.	Fabaceae	Leaves, pods
Cakle Arabica	Brassicaceae	aired
Diplatax harra	Brassicaceae	Aired
Erodium glaucophyllum	Geraniaceae	Aired
Fagonia indica	Zygophyllaceae	Leaves, fruits
Gymnarrhena micrantha	Asteraceae	Aired
Horwoodia dicksonia	Brassicaceae	Aired
Koelpinia linearis	Juncaceae	Aired
Reseda muricata	Resedaceae	Leaves, inflorescences
Salvia spinosa	Lamiaceae	Leaves
Tanacetum cantolinoides	Asteraceae	Aired

2.3 Mosquito colony

The larvae the wild *Culex pipiens molestus* were used for establishment the mosquito colony. Larvae were collected after rainfall from open temporary stagnant pools near the buildings of Mosul University, Mosul, City, Iraq. The larvae were kept in enamel trays containing breeding water to obtain in F1 generation. The developed pupae maintained in a mosquito cage, till adults emergence. Adult mosquitoes were fed on 15% honey solution and after 3-4 days periodically blood-feeding within night on naked chest pigeon. After oviposition in the ovitraps, the egg rafts transferred into enamel trays with 3L dechlorinated tap water per tray. The larvae were daily fed with 0.5 gm of powdered mixture of biscuit, yeast and milk at ratio 3:1:1 by weight. Laboratory condition modulated at 12:12 light/dark interval periods, 28±2c° and 70±10% relative humidity.

2.4 Larvieidal bioassay

2.4.1 Mortality

The laboratory study were conducted according to WHO protocol with slight modifications (WHO, 2005), All the experiments were applied in triplicated for each concentration, and control used parallel to each experimental series with adding ethanol solvent equal to that of extract in the applied solution. Batches of healthy five late 3rd and 4th instar larvae were exposed in a 250 ml disposable plastic cup containing treatment solution. Four concentrations were applied after the primary experiments for each plant extract. The effects of the extracts were monitored by counting of dead larvae at the end of 24 and 48 hours, and the mortality values were calculated.

2.4.2 Residual toxicity

The more effective nine plant extracts were selected to elucidate their residual toxicity on the 4th instar larvae, for each extract, the concentration begins at 100% mortality in the end of 24hrs. and continuing for 15 days, the synthetic insecticide Ficum®W were used as standard insecticide, its active constituent Bendiocarb (an anti-choline esterase compound) as well as negative control. 25 larvae were exposed for each extract concentration and tested every three days for counting dead larvae and replaced them and developed pupae with alive larvae.

2.5 Statetical analysis

The data assessed for mean and standard deviation (±SD) using JMP software (SAS, 2000).Duncan test ($p = 0.05$) was applied for mean separation. Lc_{50} and Lc_{90} were used to determine the relative toxicity of the plant extracts to C. *pipines molestus* larvae, probit analysis was carried out by probit line papers (Finney, 1971).

3. Results and discussion

3.1 Larvicidal potency

Table 2 is show the larvicidal activity of the plant extracts group A, which more active ingredient with high toxicity to mosquito fourth instar larvae in comparison with most other studies (Raveen *et al*., 2014) (Okonkwo *et al*., 2014; Sheeren, 2006; Reba *et al.,* 2015). Gomathi *et al*., 2014; Ramanibai and velayutham, L. (2014) [22, 18, 28, 10, 20]

It was evident that all the extracts appeared variable larvicidal effects, also, larval mortality increased with extract concentration and exposure times 24 and 48 hours. The relationship between the plant extract and larval mortality not proportionally as the synthetic insecticide, but were behaved as agonist or antagonist hormones and seemed as sigmoid curves (table2), and that can be revealed through Lc_{50} and Lc_{90} values for the applied plant extracts. Highest effective plant extract was *Fagonia indica* with Lc_{50} and Lc_{90} after 24 hours 12.5 and 20.5 ppm, while, after 48 hrs 4.6 and 13.0 ppm. There was closing of Lc_{50} after 24 and 48 hours for *Achelia fragramtissima* and *Astragalus annularis* (Fig1), also, *Horwoodia dicksonia, Tanacetum cantalinoidas, Resida muricata* with similar Lc_{50}. Lc_{90} values of *Astragalus annularis, Cakle Arabica*, *Erodium glaucophyllum*, with closing activity, and *Horwoodia dicksonia* and *Resida muricata* fellow them, the lowest larvicidal effect represented by Lc_{50} and Lc_{90} was *Arneba hispidissima* (Fig1).

The mortality of the larvae with group B extract application was less than group A efficiency (Fig 1), but are promise in comparison with other studies as (Shivakumar *et al*., 2013; Rahuman *et al*., 2007) [30, 19], *Aizoon hispanicum* and *Astragalus* sp. extracts more promising than them with morality 58% at 200 ppm (Fig 2).

Table2: Mortality, Lc_{50} and Lc_{90} of 4th instar larvae of *Culex pipiens molestus* treated with xerophytic plant extracts.

Plant extract	Conc. (ppm)	Exposure time (hrs)		24 hrs		48 hrs	
				Lc_{50}	Lc_{90}	LC50	Lc_{90}
Fagonia indica	20	22.3±0.6 (89.2)	25.0±0.0 (100)	12.5	20.5	4.6	13.0
	15	20.3±0.6 (81.2)	23.7±7 (94.8)				
	10	10.7±0.6 (42.8)	18.0±1.0 (72.0)				
	5	7.3±1.2 (29.2)	13.3±0.6 (53.2)				
Achelia fragrantissime	80	22.3±1.5 (89.2)	25.0±0.0 (100)	40.5	81.5	60.5	36.0
	60	19.7±0.6 (78.8)	20.0±7 (82.3)				
	40	12.3±2.1 (49.2)	17.0±1 (68.0)				
	20	8.0±1.0 (24.0)	11.3±1.5 (45.2)				
Astragalus annulata	125	22.3±0.6 (89.2)	25.0±0.0 (100)	43.0	126.0	23.0	98.0
	75	20.3±0.6 (81.2)	25.0±0.0 (100)				
	50	14.3 ± 0.6 (57.2)	19.7±0.6 (78.8)				
	25	8.7±0.6 (34.8)	13.7±0.6 (54.8)				
Cakle Arabica	125	22.7±0.6 (90.8)	25.0±0.0 (100)	86.12	124.0	35.0	102.0
	15	14.7±0.6 (58.8)	21.3±1.2 (85.2)				
	50	6.7±0.6 (26.8)	13.7±0.6 (54.8)				
	25	3.7±1.2 (14.8)	11.0±1.0 (44.0)				
Erodium glaucophyllum	125	23.0±1.0 (92)	25±0.0 (100)	70.0	122.0	45.0	101.5
	75	14.3±0.6 (57.2)	20.7±0.6 (82.8)				
	50	6.3±0.6 (25.2)	13.3±0.6 (53.2)				
	25	3.3±0.6 (13.2)	6.7±0.6 (26.8)				
Horwoodia dicksonia	175	22.3±0.6 (89.2)	25±0.0 (100)	104.0	176.0	88.0	132.0
	125	14.7±0.6 (58.8)	19.7±0.6 78.8				
	75	7.3±0.6 (29.2)	10.7±0.6 (42.8)				
	25	3.7±0.6 (14.8)	7.3±0.6 (29.2)				
Tanacetum cantolinoides	175 125	20.7±0.6 (82.8)	25±0.0 (100)	103.0	205.0	50.5	127.0

	75 25	13.7±0.6 (54.8)	20.3±0.6 (81.2)				
		8.3±0.6 (33.2)	15.7±0.6 (62.8)				
		5.7±0.6 (22.8)	9.7±0.6 (38.8)				
Resida muricata	175	22.7±0).6 (90.8	25.0.0 (100)	105.5	172.5	62.0	129.0
	125	11.7±0.6 (46.8)	19.3±1.2 (87.2)				
	75	6.7±0.6 (26.8)	12.7±0.6 (50.8)				
	25	2.7±0.6 (10.8)	8.7±0.6 (34.8)				
Arneba hispidissima	225	22.7±0.6 40.8	25±0.0 100	224.0	135.0	120.0	174.0
	175	16.7±0.6 (66.8)	22.7±0.6 (90.8)				
	125	9.7±0.6 38.8	16.7±0.6 66.8				
	50	2.7±0.6 (10.8)	6.3±0.6 (25.2)				

25 larvae for each treatment.
Mean of killed larvae ±SD standard deviation.
Mortality % is the number between partners.
Lc_{50} and Lc_{90}: Lethal concentrations killing 50 and 90% of 25 4th instar larvae.,

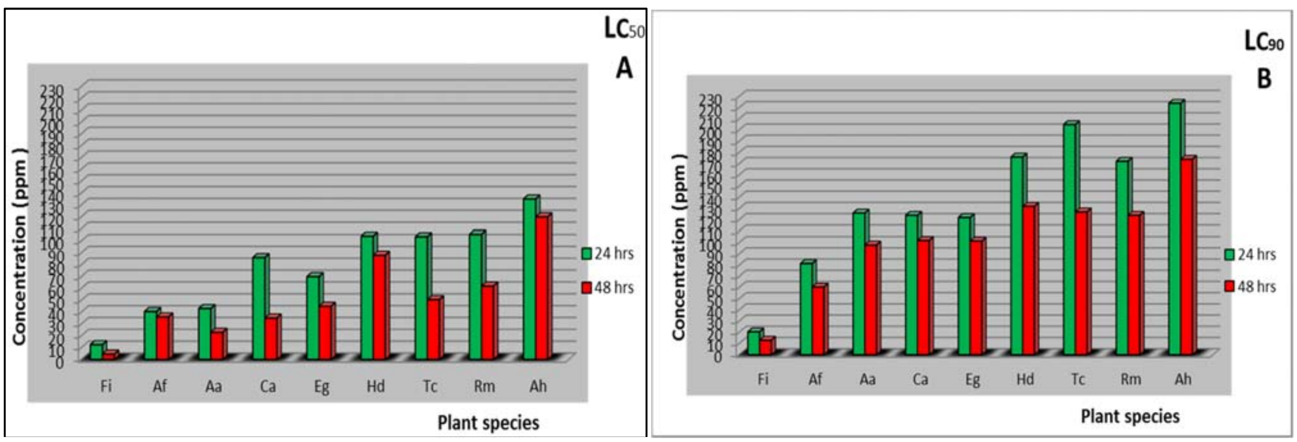

Fig 1: Lethal concentration 50 (A) and 90 (B) at which 50 and 90% 4th instar larvae of *Culex pipens molestus* died. Fi- *Fagonia indica*, Af- *Achelia fragrantissima,* Aa- *Astragalus annularis,* Ca- *Cakle Arabica,* Eg- *Erodium glaucophyllum,* Hd- *Horwoodia dicksonia,* Tc- *Tanacetum cantolinoides,* Rm- *Reseda muricata,* Ah- *Arneba hispidissima.*

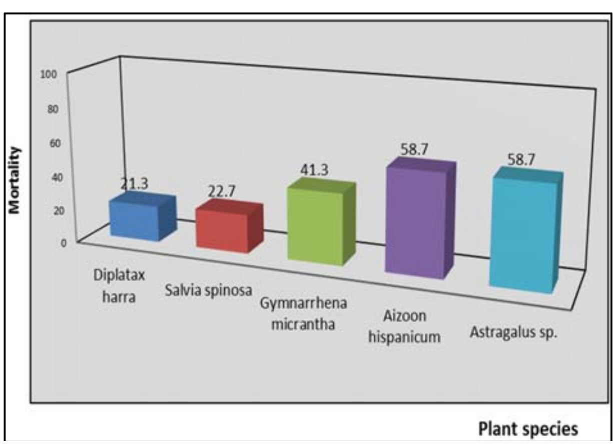

Fig 2: mortality of 4th larvae treated with less effective zerophytic plant extracts at 200 ppm.

3.2 Residual persistence

For test the residual toxicity of *C. pipiens molestus* larvae, The treatment were began at each plant extract concentration causing 100% mortality on day 1 of the treatment, which reduced over time. The larval mortality rates were significantly decreased since day 6 onwards for *A. annularis, C. Arabica* and *R. muricata.* Then, the mortality dropped to zero and the extract lost its efficacy on day 9 for *R. muricata* and day 12 for *A. annularis* and *H. dicksonia.* However, the mortality high significantly decreased or fall down equal to negative control. In comparison with the standard insecticide Ficum®W, its residual toxicity slightly affected on day 15 while it comes zero for 7 applied plant extracts.

The residual toxicity of the applied plant extracts were significantly decreased with increasing residue time (table3), few studies investigated the toxicity of other plant extracts, which significantly decreased with increasing of residue time against other insect pests, commercial extract of neem (Neem

Azal T) protects crop plants for at least three weeks (Seljasen and Meadow, 2006) [26]. Other neem product Neemarin reduced *Anopheles stephensi* and C. *quinquefasciatus* larvae with 7 days residual effect (Vatondoost and Vaziri, 2004) [32], the residual effect may last only a few hours as the essential oil extract from C*henopdium ambrosioides* (Chiasson *et al.,* 2004) [6].

Concisely, The results of this study has been enhanced the application of ecofriendly insecticides, the total world production of biopesticides over 3.000 tons/yr (Gupta and Dirshik, 2010) [12], The xerophytic extracts has found mostly biodegraded in two weeks, as compared to DDT; persists for a long time and accumulated in the food chain and in the tissue living organisms (Vladimir *et al*., 2012) [33].

In the present study, xerophytic extracts far less toxicity persistent compared with Ficum®W standard insecticide with 70% mortality in day 15, and could be entering in the food chain.

Table 3: Residual toxicity of xerophytic extracts on C*ulex pipiens molestus* fourth instar larvae

Plant extract of	Concentration (ppm)	Days after treatment (mortality)				
		3	6	9	12	15
Fagonia indica	25	25±0.0 a (100)	8.7±1.2 i (38.8)	5.3±1.6 j (21.2)	4.3±1.5 j (7.2)	0. ±0.0 m (0)
Achelia fragrantissima	100	25±0.0 a (100)	25±0.0 a (100)	25±0.0 a (100)	25±0.0 a (100)	10.0±1.0h (40)
Astragalus annularis	150	25±0.0 a (100)	19.7±1.5 c (78.8)	4.7±1.2 j (18.8)	0±0.0 m (0)	0. ±0.0 m (0)
Erodium glaucophyllum	150	25±0.0 a (100)	25±0.0 a (100)	22.3±1.5 b (89.2)	18.0±2.0ef (72)	8.7±1.5 I (34.8)
Cakle Arabica	150	25±0.0 a (100)	10.3±1.5 h (41.2)	4.6±1.5 j (18.4)	3.3±0.6 jm (15.2)	0±0.0 m (0)
Resida muricata	200	25±0.0 a (100)	14.7±2.0 efg 58.8	0±0.0 m (0)	0. ±0.0 m (0)	0±0.0 m (0)
Tanacetum cantolinoides	200	25±0.0 a (100)	25±0.0 a (100)	25±0.0 a (100)	25±0.0 a (100)	0±0.0 m (0)
Horwoodia dicksonia	200	25±0.0 a (100)	20.7±1.6 bc (82.8)	8.3±0.6 i (33.2)	0±0.0 m (0)	0±0.0 m (0)
Arneba hispidissima	250	25±0.0 a (100)	25±0.0 a (100)	9.3±0.6 hi 37.2	6.3±0.6 i (25.2)	0±0.0 m (0)
Ficum®W *(+ve)	16	25+0.0 a (100)	25±0.0 a (100)	25±0.0 a (100)	22.3±0.0 b (100)	17.7±0.6 ef (70.8)
Control (-ve)	0	0±0.0 m (0)	0±0.0 m (0)	0±0.0 m (0)	0±0.0 m (0)	0±0.0 m (0)

Means followed by the same latters in the column do not differ significantly at p=0.05 (Duncan's test).
* Standard insecticide.

4. Conclusion

The biogeographic map of the western desert of Iraq is continuing with rich endemic floristic diversity Arabian peninsula (Sher and Aldosari, 2010). In the desert, the plants are endemic and adapted with environmental condition and coevoluted with herbivorous as polyphagous insects through secondary metabolites.

In the present study, high toxicity of nine xerophytes to mosquito larvae may as the synthetic insecticide but are eco-friendly alternatives. Also, relatively short residual persistence can allow use potential plant extracts with high sensitivity and biodegrade easily within 9-15 days. Further work on other *Culex pipiens molestus* stages and the effect of the sub lethal concentration are needed. In the light of promise results if possible, in preparing botanical products -as neem-from the potential extracts to be used as mosquito bioinsecticides.

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