Susceptibility of *Aedes aegypti* (L.) Larvae to Some Non-conventional Insecticides

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Laboratory trials were conducted to determine the larvicidal activity of the bacterial insecticides (spinosad and vectobac), the insect growth regulators, IGRs (sumilarv and dudim) and the plant extracts of neem (*Azadirachta indica*) and usher (*Calotropis procera*) against *Aedes aegypti* (L.). The toxicity curves showed that the bioinsecticide spinosad proved to be more effective than vectobac against *A. aegypti* larvae by about 11.1 times. Such a fact was highly pronounced on the basis of LC_{50} values (concentration which to kill 50% of larvae) which were 0.009 ppm and 0.1 ppm, respectively. According to IC_{50} values (concentration which to inhibit the emergence of 50 % of adults), the results indicated that mosquito larvae of *A. aegypti* were more susceptible to the IGR dudim (0.00038 ppm) than sumilarv (0.004 ppm) by about 10.5 folds. The plant extract of neem was also more effective against the present larvae than usher extract, with IC_{50} of 35 ppm and 66 ppm, respectively. Taking coeffective factor (C.F.) into consideration, the results revealed that the chemical insecticide actellic (pirimiphos-methyl) in combinations with spinosad, dudim and neem extract against the present mosquito larvae produced different levels of potentiation reflected by the inhibition of adult formation.

Key words: *Aedes aegypti*, mosquito larvae, susceptibility, non-conventional insecticides, joint action.

Currently, dengue fever is considered to be the most important epidemic disease, affecting more than 100 million people, especially in tropical countries (WHO, 2002).

The use of chemical insecticide still remains a major component of any control strategy, especially during an outbreak. The extensive application of such conventional insecticides for many years caused environmental pollution and resulted in the development of physiological resistance in mosquito vectors in addition to the increased costs of insecticides. This has necessitated the need for search and development of environmentally safer and low cost replacement methods for vector control (Paul *et al*., 2006; Belinato *et al*., 2009; saleh *et al*., 2013).

The present work was designed to evaluate the larvicidal activity of some non-conventional insecticides against *A. aegypti*, the principal vector of dengue virus in Jeddah, Saudi Arabia. The joint action toxicity resulting from mixing the conventional insecticides with non-conventional ones was also studied.

MATERIAL AND METHODS

Mosquito strain

A field strain of *Aedes aegypti* (L.) was used in the present study. The parental strain was raised from wild larvae, collected from Al-Jamaeh

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District, Jeddah governorate, Saudi Arabia, and maintained under laboratory conditions of 27 ± 1 °C and 70 ± 5 % R.H., with 14 : 10 (L : D). The larvae were reared until pupation and adult emergence took place for maintaining the stock culture.

Compounds tested

The following compounds were used:
1. Tow IGRs: Sumilarv 0.5 % G (pyriproxyfen), 2- [1- methyl -2- (4- phenoxy phenoxy) ethoxy] pyridine, Sumitomo Chem. Co., Japan and Dudim 4% G (Diflubenzuron), 1- (4- chlorophenyl) -3- (2,6 - difluorobenzoyl) - urea, Chemtura Europe limited, UK.
2. Two bacterial insecticides: tracer 24 % SC (spinosad, *Saccharopolyspora spinosa*), Dow Agro Sciences, UK and vectobac 12 % AS (aqueos suspention of *Bacillus thuringiensis* var. *israelensis*, 1200 ITU/ mg, Abbott lab, USA.
3. Two ethanolic leaf extracts of neem (*Azadirachta indica*) and usher (*Calotropis procera*), kindly provided by Dr. Kh. M. Al-Ghamdi, Fac. of Science, King Abdulaziz Univ. the stock solution of each plant extract was prepared by adding 1 ml of it to 99 ml of distilled water containing 0.5 % triton X-100 as an emulsifier to ensure complete solubility of the extract in water. Series of concentrations were prepared in distilled water.

Larval bioassay

Larval susceptibility tests were conducted according to the method of WHO (2005). Treatments were carried out by exposing early fourth instar larvae of *A. aegypti* to various concentrations of the test compounds for 24 hr, in groups of waxed paper cups (400 ml capacity) containing 100 ml of tap water. Five replicates of 20 larvae each per concentration, and so for the control trials were set up. The larvae were given the usual larval food during the tests. Larval mortalities were recorded at 24 hr post-treatment for the bioinsecticides spinosad and vectobac. In the case of larval treatments with both IGRs and plant extracts, cumulative mortalities of larvae and pupae as well as the adult emergence were recorded daily.

Joint action test

The dosage-mortality test was conducted with the organophosphate insecticide actellic 50 % EC (pirimiphos-methyl) against *A. aegypti* larvae as a prelude to the present tests. The larval mortality was recorded after 24 hr of treatment. The effective concentrations ranged from 0.02 - 0.12 ppm, and the corresponding larval mortalities were 17 - 90 % the toxicity line (LC-p line) was drawn using the method of Litchfield and Wilcoxon (1949).

The combinations were conducted using the chemical insecticide actellic at the LC_{25} value (concentration which to kill 25 % of larvae) with the bacterial insecticide spinosad at the values of LC_{30}, LC_{40} and LC_{50} (concentration which to kill 30, 40 and 50 % of larvae, respectively). Another trials were also conducted using the LC_{25} of actellic with the IGR dudim and the plant extract of neem at their values of IC_{30}, IC_{40} and IC_{50} (concentration which to inhibit in respect 30, 40 and 50 % of adult emergence). The concentrations corresponding to these values were obtained from toxicity lines and were prepared. Five replicated of 20 larvae were conducted for each mixture. The joint action of different mixtures was expressed as the coeffective factor (C.F.) according to the equation given by Mansour *et al.* (1966) as follows:

This factor was used to differentiate results into three categories. A positive factor of 20 or more is considered potentiation; a negative factor of 20 or more means antagonism and intermediate values between - 20 and + 20 indicate only additive effects.

Statistical analysis

Percentage of larval mortalities and inhibition of adult emergence were corrected for control mortalities using Abbott's formula (Abbott, 1925). Toxicity lines were drawn for each compound and statistical parameters were also calculated following the method of Litchfield and Wilcoxon (1949).

RESULTS AND DISCUSSION

Susceptibility levels of *A. aegypti* larvae to the bioinsecticides spinosad and vectobac including LC_{50} values and the statistical parameters are shown in Table 1. the effective concentrations of the test insecticides against 4th instar larvae were 0.003-0.025 ppm and 0.04-0.4 ppm, respectively. The corresponding larval mortalities were in respect 13-92 % and 20-91 %. Taking LC_{50} values

(concentration which to kill 50 % of larvae) obtained from toxicity lines into consideration, the records showed that mosquito larvae of *A. aegypti* were more susceptible to spinosad (0.009 ppm) than vectobac (0.1 ppm) by about 11.1 times.

Table 2 shows percentage mortalities of *A. aegypti* larvae and inhibition of adult emergence

following treatments with the IGRs sumilarv and dudim as well as the ethanolic extract of neem *(Azadirachta indica)* and usher (*Calotropis procera*). In general, 10-24 %, 5-18 %, 9-62 % and 18-69 % larval moralities were obtained when the present mosquito larvae were treated with the test IGRs and plant extracts, respectively. This means

Table 1. Susceptibility levels of *A. aegypti* larvae to the bioinsecticides spinosad and vectobac following continuous exposure for 24 hr

Insecticide	Effective concentrations (ppm)	Larval mortality * (%)	LC_{50} (ppm)	Slope
Spinosad	0.003 – 0.025	13 – 92	0.009	3.17
Vectobac	0.04 – 0.4	20 - 91	0.1	1.97

*Five replicates, 20 larvae each; control mortalities ranged from 0.0 – 1.0%

Table 2. The biological effects of IGRs (sumilarv and dudim) and the plant extracts of neem (*Azadirachta indica*) and usher (*Calotropis procera*) on the developmental stages of *A. aegypti*

Compound	Effective concentrations (ppm)	Larval * mortality (%)	Pupae produced (%)	Adult emerged (%)	Inhibition ** (%)	IC_{50} (ppm)	Slope
Sumilarv	0.002 – 0.02	10 – 24	90 – 76	70 – 10	24.7 – 89.2	0.004	1.49
Dudim	0.0002 – 0.004	5 – 18	95 – 82	69 – 7	31 – 93	0.00038	1.46
Neem extract	20 – 90	9 – 62	91 – 38	80 – 7	14.9 – 92.5	35	3.8
Usher extract	40 – 150	18 – 69	82 – 31	79 – 5	21 – 95	66	4.0
control		0.0 – 4	100 – 96	96 – 93	4 – 7		

*Five replicates, 20 larvae each;
**Corrected with Abbott's formula (Abbott, 1925)

Table 3. The joint action of the conventional insecticide actellic with some non-conventional insecticides on the mosquito *A. aegypti*

Compound mixtures	Concentrations used (ppm)	Cumulative mortality (%)		C.F.*	Type of effect
		Expected	Observed		
actellic + spinosad					
LC_{25} + LC_{30}	0.027 + 0.006	55	71	+ 29.1	(XX)
LC_{25} + Lc_{40}	0.027 + 0.0072	65	84	+ 22.6	(XX)
LC_{25} + LC_{50}	0.027 + 0.009	75	91	+ 21.3	(XX)
actellic + dudim					
LC_{25} + IC_{30}	0.027 + 0.00016	55	69	+ 25.4	(XX)
LC_{25} + IC_{40}	0.027 + 0.00025	65	86	+ 32.3	(XX)
LC_{25} + IC_{50}	0.027 + 0.00038	75	92	+ 22.7	(XX)
actellic + neem extract					
LC_{25} + IC_{30}	0.027 + 26	55	67	+ 21.8	(XX)
LC_{25} + IC_{40}	0.027 + 30	65	79	+ 21.5	(XX)
LC_{25} + IC_{50}	0.027 + 35	75	95	+ 26.7	(XX)

*Coeffective factor (Mansour *et al.*, 1966); (XX) Potentiation

that the tested compounds did not appear to give high percentage of mortality against larval stages, although in most cases a clearly delayed inhibition of adult emergence was noted. Therefore, in the present work, cumulative mortality during larval development to pupae and adults has been taken as a criterion for evaluating the efficacy of such compounds as they have more juvenilizing effect than toxic mode of action (WHO, 2005).

The effective concentrations of the IGRs sumilarv (0.002-0.02 ppm) and dudim (0.0002-0.004 ppm) gave 24.7-89.2 % and 31-93 % inhibition of adult emergence respectively. The records indicated that dudim proved to be more effective against *A. aegypti* than sumilarv by about 10.5 times. This was highly pronounced on the basis of IC_{50} values (concentration which to inhibit 50 % of adult emergence) obtained for sumilarv (0.004 ppm) and dudim (0.00038 ppm).

The effective concentrations of the ethanolic plant extracts of neem and usher were in respect 20 - 90 ppm and 40 - 150 ppm. The corresponding percentages of inhibition of adult emergence were 14.9-92.5 % and 21-95 %, respectively. According to IC_{50} values, the results revealed that *A. aegypti* was more susceptible to neem extract (35 ppm) than usher extract (66 ppm) by about 1.9 times. Generally it can be concluded that the response of *A. aegypti* to the test compounds depends entirely on the differential mode of action of these compounds and its effective concentrations (Nazni *et al.,* 2005). Studies in this respect were carried out by several authors to determine the susceptibility levels of mosquito vectors to different bioinsecticides (Nathan *et al.,* 2005; Marina *et al.,* 2012; Kundu *et al.,* 2013), IGRs (Batra *et al.,* 2005; Saleh *et al.,* 2013) and plant extracts (Vatandoost and Vaziri, 2004; Kumar *et al.,* 2012; Tennyson *et al.,* 2013)

Table 3 shows the effect of combinations of the chemical insecticide actellic (pirimiphos-methyl) with the bacterial insecticide spinosad, the IGR dudim and the plant extract of neem on *A. aegypti* larvae. The combinations were applied at the LC_{25} value of actellic (0.027 ppm) and values of LC_{30}, LC_{40} and LC_{50} of spinosad (0.006, 0.0072 and 0.009 ppm, respectively) and IC_{30}, IC_{40} and IC_{50} values of dudim (0.00016, 0.00025 and 0.00038 ppm, respectively) and neem extract (26, 30 and 35 ppm, respectively). Taking values

of coeffective factor (C.F.) into consideration, the records showed that all combinations of actellic with the test compounds produced different levels of potentiation reflected by the inhibition of adult formation. Variations in the level of potentiation among the test mixtures may reflect the differences in their mode of action and the sublethal concentrations tested. Similar trials were carried out by using different combinations of conventional and non-conventional insecticides against *A. aegypti* (Saleh *et al.,* 1990; Darri *et al.,* 2010; Poonia and Kaushik, 2013), *Culex pipiens* (Al-Ghamdi *et al,* 2008) and *Anopheles sundaicus* (Kumar *et al.,* 2012). However, long term follow-up studies were needed to determine how the environmental conditions affect the larvicidal effectivess of such compounds when applied jointly for field control measures.

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