Larvicidal Activity of some Bacterial Insecticides and Insect Growth Regulators against Mosquito Larvae of *Aedes aegypti* (L.)

A. A. Almadiy[1], M.S. Saleh[2] and A.A. Alsagaf[1]

ABSTRACT

The biological effects of the bacterial insecticides Bacilod, VectoLex and Spinosad as well as the insect growth regulators (IGRs) Baycidal, Sumilarv and Dudim against mosquito larvae of *Aedes aegypti* have been evaluated. According to LC_{50} values (concentration which to kill 50% of larvae), the bioinsecticide Spinosad (0.011 ppm) proved to be the most effective compound, followed by Bacilod (0.11 ppm) and VectoLex (0.38 ppm). Taking IC_{50} values (concentration which to inhibit the emergence of 50% of adults) into consideration, mosquito larvae of *A. aegypti* were more susceptible to the IGR Dudim (0.00056 ppm) than Baycidal (0.0007 ppm) and Sumilarv (0.0042 ppm) by about 1.25 and 7.5 folds, respectively. Variations in the susceptibility status of the present mosquito larvae may be attributed to the differential mode of action of the test compounds and its effective concentrations. On the other hand, larval treatments with sublethal concentrations of the above insecticides led to a reduction in the egg production and hatchability of eggs produced by mosquito females that developed from surviving larvae.

Key words: *Aedes aegypti*, bacterial insecticides, insect growth regulators, susceptibility tests, reproductive potential.

INTRODUCTION

Mosquitoes act as vectors for several human diseases like malaria, yellow fever, dengue fever and filariasis. *Aedes aegytpi* (L.) is the principal vector of dengue viruses, causing 50 million cases of infection and 300,000 deaths each year in tropical and subtropical regions (WHO, 2002).

Mosquito control is critical for managing the spread of disease agents and is based primarily on the use of chemical insecticides. Drawbacks associated with widespread use of these conventional insecticides to control mosquitoes have not only resulted in the development of resistance in many species of mosquito vectors, but have also caused environmental pollution. Therefore, more attention has been recently paid to the use of non–conventional insecticides such as bioinsecticides, insect growth regulators (IGRs) and plant extracts for mosquito control in different parts of the world (Bond *et al*., 2004; Seccacini *et al*., 2008; Marina *et al*., 2011; Suman *et al*., 2013; Singh *et al*., 2014).

The present study was planned to evaluate the biological effects of three bacterial insecticides Bacilod, VectoLex and spinosad as well as three IGRs Baycidal, Sumilarv and Dudim against mosquito larvae of *A. aegypti*, the primary vector of dengue fever in Jeddah governorate, Saudi Arabia. Additional trials were also conducted to study the possible delayed effects of larval treatments with the tested compounds on the reproductive potential of mosquito adult survivors.

MATERIALS AND METHODS

Mosquito strain

Tests were performed on a field strain of *A. aegypti* (L.) raised from wild larvae, collected from Al–Ballad district, Jeddah governorate, Saudi Arabia, and had been 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.

Insecticides tested

The following insecticides were used:

1– Three bacterial insecticides : Bacilod WP, 1200 *Bti* ITU/mg (*Bacillus thuringiensis* var. *israelensis*), LOD, Ltd.; VectoLex WG, 50 *Bs* ITU/mg (*Bacillus sphaericus*), Valent Biosciences Corp., Illinois, USA and Spinosad 24% Sc. (*Saccharopolyspora spinosa*), Dow Agro Science, UK.

2– Three IGRs: Baycidal 25 WP (Triflumuron), Benzamide–2–chloro–N– [[[trifluoromethoxy) pheyl] amino] carbonyl], Bayer, Germany; 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–difluorobenzoyl0–urea, Chemtura Europe Limited, UK.

Text experiments

Susceptibility tests of *A. aegypti* larvae were conducted following the method of WHO (2005). Treatments were carried out by exposing early 4th instar

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[1]Dept. of Biological Science, Fac. of Sciences,King Abdul Aziz Univ., Jeddah, KSA.
[2]Present address: Dept. of Applied Entomology, Fac. of Agric., Alexandria Univ., Alex. Egypt.
E-mail: Diptera2012@gmail.com
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larvae to various concentrations of the tested insecticides, in groups of plastic cups containing 100 ml of tap water. Five replicates of 20 larvae each per concentration, and so for control trials were set up. The larvae were given the usual larval food during these experiments. Larval mortalities were recorded at 24 hr post–treatments for the bacterial insecticides Bacilod, VectoLex and Spionsad. In the case of IGRs Baycidal, Sumilarv and Dudim, cumulative mortialities of larvae and pupae were recorded daily. Live pupae were transferred to untreated water in new plastic cups for further observations, i.e. normal emergence, presence of morphologic abnormalities or death. Partially emerged adults or those found completely emerged to unable to leave the water surface were recorded and scored as dead. Therefore, the biological effects of the test IGRs were expressed as the percentage of larvae that do not develop into successfully emerging adults, or the inhibition of adult emergence. Log concentration–probability regression lines were drawn for the tested compounds and statistical parameters were also calculated using the method of Litchfield and Wilcoxon (1949).

Additional trials were also conducted to study the possible delayed effects of larval treatments with the present insecticides on the reproductive potential of mosquito adults that emerged from surviving larvae. Values of LC_{50} (concentration which to kill 50% of mosquito larvae) and IC_{50} were obtained from the toxicity lines of the bacterial insecticides and IGRs, respectively. The concentrations corresponding to these values were prepared and used for treating the early 4th instar larvae of *A. aegypti*. Fifteen replicates of 20 larvae each were conducted for each concentration. Mosquito adults which survived from the above larval treatments were isolated in clean adult cages. Seventy–two hours later, emerged females for both treatment and control groups were fed on a living pigeon for a blood meal. Each engorged female was kept with a male in a small white plastic cup, half–filled with water and covered with muslin cloth. These couples were fed on a 10% sugar solution soaked on cotton pads placed on top of the covered cups. Number of eggs laid per female and hatchability of eggs were recorded for the 1st gonotrophic cycle. Differences between treatments and control ones were compared and analysed using the *t*–test.

RESULTS AND DISCUSSION

Susceptibility levels of *A. aegypti* mosquito larvae following treatment with different concentrations of the bacterial insecticides Bacilod, VectoLex and Spinosad are shown in Table 1 and illustrated by Fig. 1. The effective concentrations of these bioinseciticides against 4th instar larvae ranged from 0.05–0.5 ppm, 0.2–0.8 ppm and 0.004–0.04 ppm, respectively. The corresponding larval mortalities for these compounds were 23–90%, 17–92% and 15–96%. Taking LC_{50} values into consideration, the records showed that the bioinsecticide Spinosad (0.011 ppm) proved to be the most effective compound, followed by Bacilod (0.11 ppm) and vectoLex (0.38 ppm). The results indicate that mosquito larvae of *A. aegypti* were more susceptible to Spinosad than Bacilod and VectoLex by about 10 and 34.5 folds, respectively. However, variations in the susceptibility levels of the present mosquito larvae are possible due to the differential mode of action of the test bioinsecticides and its effective concentrations (Romi *et al*., 2006; Hertlein *et al*., 2010; Kamal and Khater, 2013; Gama and Nakagoshi, 2014).

Table 2 shows the percentage of mortalities of larvae and pupae as well as the inhibition of adult emergence following treatment with different concentrations of the IGRs Baycidal, Sumilarv and Dudim. In general, 10–29%, 8–28%, 7–19% larval mortalities were obtained when the 4th instar larvae of *A. aegypti* were treated with the effective concentrations of Baycidal (0.0003–0.005 ppm), Sumilarv (0.002–0.02 ppm) and Dudim (0.002–0.005 ppm). This means that the tested IGRs did not appear to give high percentages of mortality against larval stages of *A. aegypti*, although in most cases a clearly delayed inhibition of adult emergence was noted. Therefore, in the present work, cumulative mortalities during larval development to pupae and adults have taken as a criterion for evaluating the tested IGRs as they have more juvenilizing effect than toxic mode of action (WHO, 2005).

Generally, larval treatments with the effective concentrations of Baycidal, Sumilarv and Dudim caused 17.2–95.7%, 20.6–92.4% and 22.5–93.5% inhibition of adult emergence, respectively. According to IC_{50} values, the records showed that the IGR Dudim (0.00056 ppm) proved to be more effective against *A. aegypti* than Baycidal (0.0007 ppm) and Sumilarv (0.0042 ppm) by about 1.25 and 7.5 times, respectively (Fig. 2).

However, it can be concluded that the response of 4th instar larvae of *Ae. aegypti* depends entirely on the mode of action of the tested IGR and the concentrations used. The fluctuations in the percentage mortalities obtained for the different concentrations of the tested compounds against the present mosquito strain support this conclusion (Saleh and Wright, 1990). Laboratory and field studies in this respect were carried out by several authors to evaluate the biological effects of bacterial insecticides (Baruah and Das, 1994; Bond *et al*., 2004; Marina *et al*., 2012) and IGRs (Batra *et al*., 2005; Seccacini *et al*., 2008; Chanda *et al*., 2013) against a wide spectrum of mosquito species.

Table 1. Susceptibility levels of *A. aegypti* mosquito larvae to the bacterial insecticides Bacilod, VectoLex and Spinosad following continuous exposure for 24hr

Insecticide	Effective concentrations (ppm)	Larval[a] mortality (%)	Statistical parameters[b]			
			S	LC_{50} (ppm)	fLC_{50}	Slope
Bacilod	0.05 – 0.5	23.– 90	3.6	0.11	1.30	1.8
VectoLex	0.2 – 0.8	17 – 92	1.75	0.38	1.12	4.1
Spinosad	0.004 – 0.04	15 – 96	2.2	0.011	1.16	2.9
Control		0.0–2				

a Five replicates, 20 larvae each.
b Litchfield and Wilcoxon (1949).

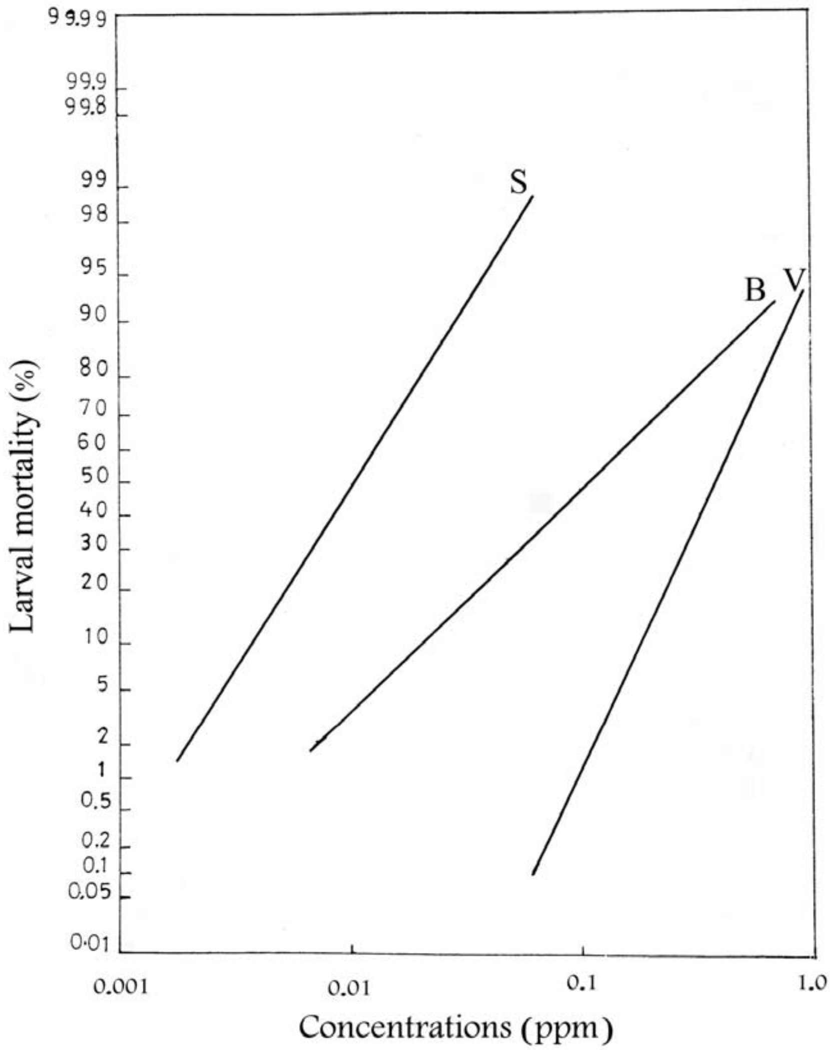

Fig. 1.The relation between concentrations of the bioinsecticides Bacilod (B), VectoLex (V) and Spinosad (S) and the percentage of larval mortality of *A. aegypti* following continuous exposure for 24hr

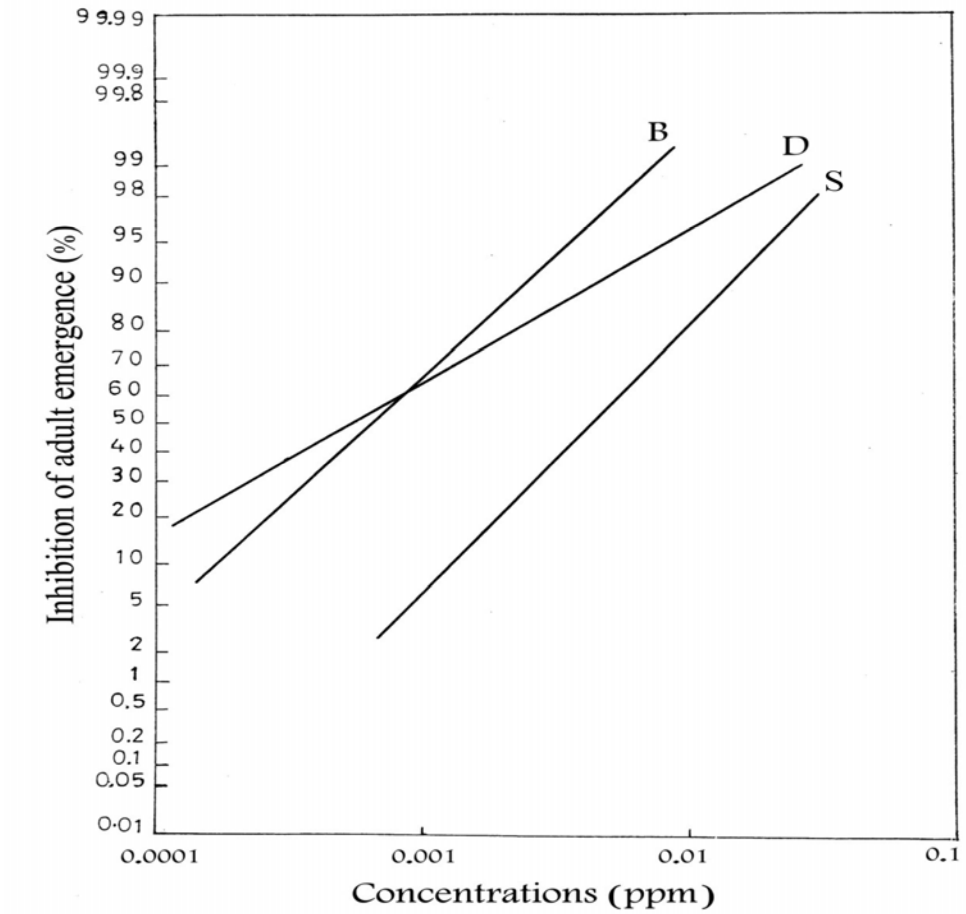

Fig. 2. The effect of larval treatment with the IGRs Baycidal (B), Sumilarv (S) and Dudim (D) on *A. aegypti* adults survived from these treatments

Most of these non–conventional insecticides have been reported to exhibit mosquito larvicidal activity.

Table 3 shows the possible delayed effects of larval treatments with the concentrations corresponding to values of LC_{50} and IC_{50} of bacterial insecticides (Bacilod, VectoLex and Spinosad) and IGRs (Baycidal, Sumilar and Dudim), respectively, on the reproductive potential of mosquito adults of *A. aegypti*. In general, the results showed that larval treatments with these compounds caused a reduction in the egg–laying capacity of mosquito female survivors. The mean number of eggs per female during the 1st gonotrophic cycle was in respect 36.2, 37.2 and 29.8 eggs in bioinsecticide treatments and 26.2, 39.4 and 31.2 eggs in IGR treatments as compared with their control ones,, 39 and 43.6 eggs. The decrease in this mean per female was 7.2, 4.6 and 23.6% in larval treatments with bioinsecticides and 39.9, 9.6 and 28.4% in the case of IGR treatments. Moreover, the same trend of decrease was recorded for the hatchability of eggs produced by *A. aegypti* females that survived from the above larval treatments (Table 3). The results showed that larval treatments with the test bioinsecticides caused in respect 8.8, 10 and 1.6% reduction in the hatchability of eggs while IGR treatments led to a decrease in the hatching levels of eggs by about 31.3, 19.3 and 27.3%, respectively. Such a reducing effect of the non–convential insectidiees was previously recorded by using the bacterial insecticides *Bt* H. 14 (Wang and Jaal, 2005) and Spionsad (Hertlein *et al*., 2010) as well as the IGRs Triflumuron (Belinato *et al*., 2009) and diflubenzuron (Fournet *et al*., 1993; Silva *et al*., 2009) against different species of mosquito vectors. However, it has been suggested that larval treatments with sublethal concentrations of insecticides may be affect the larval gonads and accordingly the reproductive capacity of surviving adults (Vasuki, 1999; Saleh *et al*., 2013). Long term follow–up trials are needed to elucidate the possible delayed effects of larval treatments with non–conventional insecticides on some biological and behavioural aspects of mosquito adult survivors.

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الملخص العربي

النشاط الإبادي اليرقي لبعض المبيدات الحشرية البكتيرية ومنظمات نمو الحشرات ضد يرقات بعوض إيديس إيجيبتاي

عبد الرحمن عبد ا آل معدي، مصطفى سليمان صالح، أحمد إبراهيم السقاف

تم تقييم التأثيرات البيولوجية للمبيدات الحشرية البكتيرية باسيلود، فيكتوليكس، أسبينوساد وكذلك منظمات نمو الحشرات باسيدال، سوميلارف، دوديم ضد يرقات بعوض إيديس إيجيبتاي. طبقاً لقيم LC_{50} (التركيز اللازم لقتل ٥٠% من اليرقات) فإن المبيد الحشري الإحيائي اسبينوساد (٠.٠١١ جزء في المليون) أثبت أنه الأكثر فاعلية يليه باسيلود (٠.١١ جزء في المليون) ثم فيكتوليكس (٠.٣٨ جزء في المليون). وبأخذ قيم IC_{50} في الاعتبار (وهو التركيز الذي يثبط فقس ٥٠% من الحشرات الكاملة) فإن يرقات بعوض إيديس إيجيبتاي كانت أكثر حساسية لمنظم نمو الحشرات دوديم (٠.٠٠٠٥٦ جزء في المليون) عن مركب باسيدال(٠.٠٠٠٧ جزء في المليون) وسوميلارف (٠.٠٠٤٢ جزء في المليون) بحوالي ١.٢٥، ٧.٥ ضعف على الترتيب. إن التغيرات في حالة الحساسية ليرقات البعوض ربما تنسب إلى اختلاف طريقة تأثير مركبات الاختبار وتركيزاتها الفعالة. من ناحية أخرى فإن المعاملات اليرقية بالتركيزات تحت المميتة بالمبيدات الحشرية السابقة قد أدت إلى انخفاض إنتاج البيض وفقس البيض في إناث البعوض الناتجة من اليرقات التي عاشت من تلك المعاملات.