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Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Entomological research doi: 10.1016/S2222-1808(15)61048-9

Effectiveness of seven mosquito larvicides against the West Nile vector *Culex pipiens* (L.) in Saudi Arabia

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ARTICLE INFO

Article history:
Received 14 Mar 2016
Received in revised form 28 Mar, 2nd revised form 30 Mar 2016
Accepted 20 Apr 2016
Available online 4 May 2016

Keywords:
Arbovirus
Culex pipiens
Bacterial insecticides
Filariasis
Insect growth regulators
West Nile virus

ABSTRACT

Objective: To explore the effects of different chemical larvicides, bioinsecticides and insect growth regulators against the West Nile vector *Culex pipiens* (Diptera: Culicidae) (*Cx. pipiens*) in Saudi Arabia.

Methods: We tested seven commercial mosquito larvicides, including classic cyfluthrin, diazinon and propoxur, bioinsecticides Bactilarvae and Tracer 24%, and insect growth regulators Baycidal and Sumilarv. LC_{50} and LC_{90} values were calculated in laboratory conditions using probit analysis.

Results: Concerning chemical insecticides, the highest larval mortality was observed for diazinon, with LC_{50} = 0.352 3 mg/L, followed by propoxur and cyfluthrin. The bacterial insecticide Tracer was more effective than Bactilarvae (LC_{50} = 0.008 7 mg/L and 0.011 7 mg/L, respectively) by 1.37 folds. Furthermore, *Cx. pipiens* larvae were more susceptible to insect growth regulators Baycidal (IC_{50} = 0.000 4 mg/L) if compared to Sumilarv (IC_{50} = 0.002 9 mg/L) by 7.25 folds.

Conclusions: Overall, this research added basic knowledge about the effectiveness of seven mosquito larvicides with different mechanisms of action as potential candidates for the control programs of *Cx. pipiens* mosquito populations in Saudi Arabia.

1. Introduction

Insects, ticks, and mites are dangerous vectors of deadly pathogens and parasites, which may hit as epidemics or pandemics in the increasing world population of humans and animals[1-3]. Mosquitoes are the most important group of dipterous flies in terms of public health importance, since they have the potential to transmit a wide number of pathogens and parasites to humans and animals[4]. Among Culicidae, *Culex pipiens* (*Cx. pipiens*) is distributed ubiquitously and is responsible to transmit different diseases to the human such as filariasis, Rift Valley fever and West Nile virus[5,6]. Lymphatic filariasis is caused by Filarioidea nematodes, namely, *Wuchereria bancrofti* (which is responsible for 90% of cases), *Brugia malayi* and *Brugia timori*. Nowadays, more than 1.4 billion people in 73 countries are living in areas where lymphatic filariasis is transmitted and are at risk of being infected. Globally, an estimated 25 million men suffer with genital disease and over 15 million people are afflicted with lymphoedema. Eliminating lymphatic filariasis can prevent unnecessary suffering and contribute to the reduction of poverty[7]. Although different control mechanisms were proposed, any single strategy has been

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Foundation Project: Partially funded by the Dengue Mosquito Research Station, King Abdulaziz University, Jeddah and Lab of Entomology and Toxicology, University of Tabuk (Grant No. 2/1/11/1435).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

found completely successful. In the current scenario, the selection of the insecticide type, dose and application of manures are crucial criteria for a successful control program against mosquito vectors of public health importance[8,9].

The application of synthetic insecticides has been increased dramatically to control the urban pests, agricultural pests as well as mosquitoes[10]. In the opposite side, application of these synthetic insecticides has been corresponded with the increase level of the resistance among the mosquito populations[11,12]. There are different suggested mechanisms behind the developed resistances of insects to the insecticides. One of them is the detoxification of insecticide by certain metabolism procedures in the insect's body[13]. The elevated resistance problem in the pest control management can be considered as the main reason behind the unsuccessful eradication of several insect vectors[4]. Due to the adverse effect of chemical insecticides on the human health and environment, medical entomologists focused on developing new, eco-friendly insecticides, such as bioinsecticides and insect growth regulators (IGRs)[14-16]. The bioinsecticides and IGRs have been continuously tested against different mosquito species. For example, Aziz *et al*.[9] have recently focused on the efficacy of chemical, bioinsecticides and IGRs against laboratory and field strains of *Cx. pipiens* larval populations.

In this research, we evaluated the effectiveness of seven commercial mosquito larvicides, including bioinsecticides and IGRs, against *Cx. pipiens*, the dominant mosquito species in Jeddah Governorate, Saudi Arabia. The tested classic chemical larvicides included parathyroid insecticides, organophosphate insecticides and carbamate insecticides. The tested bioinsecticides were *Bacillus thuringiensis* var. *israelensis* (*B. thuringiensis* var. *israelensis*) and Tracer 24% SC (spinosad). The tested IGRs were Baycidal (triflumuron 25%) and Sumilarv (pyriproxyfen).

2. Materials and methods

2.1. Collection sites

Following methods reported by Aziz *et al*.[9], *Cx. pipiens* larvae were collected from domestic and outside containers around homes throughout Jeddah City, Saudi Arabia, located between latitude 21°29'31" N and longitude 39°11'24" E.

2.2. Mosquito rearing

Cx. pipiens larvae were reared at the Dengue Mosquito Research Station, King Abdulaziz University (Saudi Arabia) at (27 ± 1) ℃, relative humidity (70 ± 5) %, and constant photoperiod (light: dark, 14 h:10 h). Pupae were transferred from water medium to standard mosquito rearing cages (30 cm × 30 cm × 30 cm). Adults were kept in similar cages and fed with a cotton wick soaked with 10% glucose solution. After a period of 4 days, sugar-fed females were starved for 24 h prior to blood feeding using blood-feeding machine. Blood-fed females were allowed to assimilate the blood meals for 48 h. Gravid females were given access to oviposition sites consisting of small glass containers (23 cm × 17 cm × 8 cm) lined with filter paper as egg deposition sites. Eggs were dried under laboratory conditions. Samples of eggs from filial generation 13 were hatched in cool sterilized water. Newly enclosed larvae were reared in plastic trays and fed every two days with a powdered mixture of biscuits, dried yeast, and fat-free milk (1:1:1). Late 3rd or early 4th instar larvae of generation 12 were used for larval bioassay testing. Adult bioassays were conducted using sugar-fed (10% glucose solution) 3–5-day-old adults derived from wild larvae after one generation under laboratory conditions.

2.3. Insecticides

The conventional larvicides tested in the study were the pyrethroid Block 5% (cyfluthrin 5% w/v), the organophosphate Sweeper 600EC (diazinon 60% w/v) and the carbamate Blattaney EC20 (propoxur 20% w/v). The tested bioinsecticides were Bactilarvae (*B. thuringiensis* var. *israelensis*) and Tracer 24% SC (spinosad). The tested IGRs were Baycidal (triflumuron 25%) and Sumilarv (pyriproxyfen).

2.4. Larval bioassay

Experiments were conducted following the method by Aziz *et al*.[17]. Treatments were carried out by exposing early 4th instar larvae of *Cx. pipiens* to various concentrations of the tested compounds for 24 h, in groups of glass beakers containing 100 mL of tap water. Five replicates of 20 larvae, each per concentration and control trials were carried out. The larvae were fed following the method by Aziz *et al*.[9]. Larval mortality was recorded at 24 h post-treatment for the chemical insecticides cyfluthrin 5% w/v, diazinon 60% w/v and propoxur 20% w/v, as well as for the biocides Bactilarvae and Tracer 24% SC. As regards to IGRs, triflumuron 25% and pyriproxyfen, cumulative mortalities of larvae and pupae were recorded daily. Live pupae were transferred to untreated water in new beakers for further observation, *i.e.* normal emergence, presence of morphologic abnormalities or death. Partially emerged adults or these found completely emerged but unable to leave the water surface were recorded and scored as dead. Therefore, the biological effect of triflumuron 25% and pyriproxyfen was expressed as the percentage of larvae that do not develop into successfully emerging adults, or the inhibition of adult emergence[9].

2.5. Data analysis

Mortality percentages were corrected according to Abbott[18]. The dosage-mortality data were subjected to probit analysis according to Finney[19]. The concentration that is corresponding to the

mortality of 50% and 90% of mosquito larvae at 24 h (LC_{50} and LC_{90} respectively) was applied to evaluate the efficacy of the tested insecticide. Biological activity data were analyzed using Two-way ANOVA with two factors, the treatment (*i.e.* IGR insecticides) and the dose. Means were separated using Tukey's honest significant difference test ($P < 0.05$).

3. Results

This study was carried out to investigate the toxicity of three different groups of insecticides on the laboratory strains of *Cx. pipiens* larval population. The susceptibility levels of the larvae of *Cx. pipiens* against chemical insecticides were shown in Table 1. Among the tested chemical insecticides, the organophosphate diazinon was highly effective against *Cx. pipiens* larvae, with LC_{50} and LC_{90} values of 0.352 3 and 1.323 7 mg/L, respectively when compared to the pyrethroid cyfluthrin (*i.e.* 0.078 5 and 0.119 8 mg/L) and the carbamate propoxur (*i.e.* 0.076 7 and 0.415 5 mg/L) by about 0.218 75 and 0.974 6 folds, respectively. The results of the larvicidal assay clearly indicated that the percentage of mortality was directly proportional to concentration of the insecticides. Each test included a control group with five replicates for each individual concentration. After exposure to the test concentrations, the treated larvae exhibited restlessness, sluggishness, tremors, and convulsions, followed by paralysis. As a general trend, the sensitivity of the larvae varied according to the type of the insecticide, its mode of action and the concentration of the active ingredient.

Table 2 provides the results of larval toxicity assays conducted with bioinsecticides. A single treatment with Bactilarvae (*B. thuringiensis* var. *israelensis*) and Tracer 24% (spinosad) was able to evoke high larval mortality on *Cx. pipiens*. The percentage mortality rates of *Cx. pipiens* exposed to Bactilarvae and Tracer 24% were 48%–96% and 16%–91% for laboratory strains, at concentrations ranging from 0.01 to 0.08 mg/L and 0.004–0.02 mg/L, respectively. The values of LC_{50} and LC_{90} of Bactilarvae (*B. thuringiensis* var. *israelensis*) against *Cx. pipiens* larvae were 0.011 7 and 0.068 2 mg/L and LC_{50} and LC_{90} of Tracer 24% (spinosad) were 0.008 7 and 0.020 3 mg/L by about 1.37 folds (Table 2).

The biological effects of IGR were highly effective against the laboratory strains of *Cx. pipiens* (Table 3). As a general trend, the mortality rates were associated mainly with failure to molt, the fourth larval instar of *Cx. pipiens* to pupation stage by given intermediate larvae-pupae. The post-effect of Baycidal and Sumilarv on the adult stage of *Cx. pipiens* was evaluated to study percentage of hatchability to adult stage. We used IC_{50} which is a measure

Table 1
Susceptibility of fourth instar larvae of *Cx. pipiens* to different chemical insecticides in Saudi Arabia.

Treatment	Tested doses (mg/L)	Larval mortality[a] (%)	Statistical calculations[b]			
			LC_{50} (mg/L) (95% LCL–UCL)	LC_{90} (mg/L) (95% LCL–UCL)	χ^2	Slope
Cyfluthrin	0.04–0.20	16–92	0.078 5 (0.072 6–0.085 0)	0.119 8 (0.108 8–0.135 6)	3.48	3.67
Diazinon	0.20–1.50	31–95	0.352 3 (0.293 1–0.407 8)	1.323 7 (1.105 4–1.689 0)	2.04	2.23
Propoxur	0.05–0.50	33–94	0.076 7 (0.062 4–0.090 8)	0.415 5 (0.315 9–0.619 8)	3.73	1.74

[a]: Five replicates, 20 larvae each; Control: No larval mortality; [b]: Tabulated; $\chi^2 = 7.8$; $df = 3$; $\alpha = 0.05$ level of significance indicates homogeneity of results; LCL: Lower confidence limit; UCL: Upper confidence limit.

Table 2
Susceptibility of fourth instar larvae of *Cx. pipiens* to non-conventional bioinsecticides in Saudi Arabia.

Treatment	Tested doses (mg/L)	Larval mortality[a] (%)	Statistical calculations[b]			
			LC_{50} (mg/L) (95% LCL–UCL)	LC_{90} (mg/L) (95% LCL–UCL)	χ^2	Slope
Bactilarvae	0.010–0.080	48–96	0.011 7 (0.008 5–0.014 7)	0.068 2 (0.053 2–0.098 4)	2.31	1.67
Tracer 24%	0.004–0.020	16–91	0.008 7 (0.007 9–0.009 5)	0.020 3 (0.018 0–0.239 0)	6.05	3.48

[a]: Five replicates, 20 larvae each; Control: No mortality; [b]: Tabulated; $\chi^2 = 7.8$; $df = 3$; $\alpha = 0.05$ level of significance indicates homogeneity of results.

Table 3
The biological effects of the IGR Baycidal and Sumilarv on the developmental stages of *Cx. pipiens* laboratory strains.

Compound	Concentrations (mg/L)	Larval mortality[a] (%)	Pupae produced (%)	Adult emergence (%)	Growth inhibition (%)
Baycidal	0.000 2	3.22 ± 0.14[b]	97.16 ± 0.11[b]	78.12 ± 0.12[b]	22.14 ± 0.11[b]
	0.000 5	4.18 ± 0.16[c]	96.24 ± 0.15[c]	42.30 ± 0.81[c]	58.20 ± 0.15[c]
	0.000 8	8.20 ± 0.14[d]	92.12 ± 0.13[d]	36.14 ± 0.24[d]	47.10 ± 0.18[d]
	0.001 0	11.24 ± 0.18[e]	89.14 ± 0.11[e]	9.18 ± 0.13[e]	91.70 ± 0.81[e]
	0.003 0	18.14 ± 0.12[f]	82.20 ± 0.15[f]	2.24 ± 0.15[f]	98.08 ± 0.13[f]
	Control	2.06 ± 0.08[a]	98.08 ± 0.08[a]	97.10 ± 0.10[a]	3.06 ± 0.08[a]
Sumilarv	0.001 0	7.26 ± 0.11[b]	93.18 ± 0.10[b]	74.20 ± 0.18[b]	26.22 ± 0.17[b]
	0.004 0	10.34 ± 0.11[c]	90.16 ± 0.08[c]	49.14 ± 0.21[c]	51.24 ± 0.05[c]
	0.008 0	13.20 ± 0.15[d]	87.20 ± 0.10[d]	28.26 ± 0.11[d]	72.14 ± 0.20[d]
	0.010 0	20.26 ± 0.11[e]	80.30 ± 0.43[e]	12.20 ± 0.15[e]	88.24 ± 0.11[e]
	0.040 0	30.34 ± 0.13[f]	70.16 ± 0.18[f]	6.28 ± 0.13[f]	94.16 ± 0.15[f]
	Control	2.04 ± 0.89[a]	98.12 ± 0.08[a]	96.22 ± 0.19[a]	4.10 ± 0.07[a]

[a]: Five replicates, 20 larvae each; Values followed by the same letter(s) were not significantly different (Tukey's honest significant difference, $\alpha = 0.05$).

of the dose of a given compound inhibiting the adult emergence in the 50% of tested mosquitoes. The percentage mortality rates of *Cx. pipiens* exposed to Baycidal and Sumilarv were 18%–3% and 30%–7% for laboratory strains, at concentration ranging from 0.003 0–0.000 2 mg/L and 0.040–0.001 mg/L, respectively. Table 4 shows the value of IC_{50} and IC_{95} were 0.000 4 and 0.001 3 for Baycidal and 0.002 9 and 0.024 8 for Sumilarv, by about 7.25 folds (Table 4).

Table 4
Susceptibility levels of mosquito larvae of *Cx. pipiens* to two IGRs.

Probit analysis	IGRs	
	Baycidal	Sumilarv
IC_{50} (mg/L)	0.000 4	0.002 9
LCL 95%	0.000 3–0.000 5	0.000 8–0.005 0
IC_{95} (mg/L)	0.001 3	0.024 8
UCL 95%	0.001 1–0.001 6	0.020 8–0.225 3
Slope	2.591 0	1.379 0
Tabulated χ^2	7.810 0	7.810 0
Calculated χ^2	4.710 0	6.310 0

Values were means of five replicates, 20 larvae each; control: No mortality.

4. Discussion

Mosquitoes are the carriers of important pathogens and parasites, such as malaria, arboviral encephalitis, dengue fever, chikungunya fever, West Nile virus, yellow fever and Zika virus. The present investigation was performed to determine the susceptibility of the West Nile vector *Cx. pipiens* to some chemical, bioinsecticides and IGRs in Jeddah Province of Saudi Arabia. The assessment of insecticide susceptibility status is important for vector control interventions and enables the prevention or management of resistance[20]. The results from bioassays with diazinon, cyfluthrin and propoxur showed significant mortality against the laboratory strains of *Cx. pipiens* larvae with LC_{50} of 0.352, 0.078 and 0.076 mg/L. A dose-dependent effect was found, in agreement with previous evidences of Aziz *et al.*[9] who reported that the variation in the larval mortality increased correspondingly with the increase in the insecticide concentration. Further, Ataie *et al.*[21] noted the resistance of *Cx. pipiens* to dichlorodiphenyl trichloroethane, deltamethrin, lambda-cyhalothrin, malathion and propoxur with LT_{50} values of 134.750, 10.430, 24.370, 8.025 and 36.105 min. Also Chavshin *et al.*[22] reported that susceptibility of *Anopheles maculipennis* to six insecticides (permethrin, deltamethrin, propoxur, bendiocarb, malathion and dieldrin) belonging to four different classes in West Azarbaijan Province, Northwestern Iran.

The bioinsecticides Bactilarvae (*B. thuringiensis* var. *israelensis*) and Tracer 24% (spinosad) evoked high mortality rates against the laboratory strains of *Cx. pipiens* larvae at very low doses; LC_{50} were 0.011 7 and 0.008 7 mg/L (Table 2). The bacterial insecticides have been proved to be effective against the vector of bancroftian filariasis *Culex quinquefasciatus*[23]. For example, Al-Solami *et al.*[24] showed that the bioinsecticide spinosad proved to be more effective than VectoBac against *Aedes aegypti* larvae with LC_{50} values 0.009 mg/L (spinosad) and 0.1 mg/L (VectoBac) by about 11.1 times. Further, Aziz *et al.*[9] reported the toxicity of different commercial brands of *B. thuringiensis* var. *israelensis* (*i.e.* tested concentrations 0.05–0.50 mg/L) and reported that the values of LC_{50} and LC_{90} were 0.104 and 0.435 mg/L, respectively. Furthermore, Panneerselvam *et al.*[14] showed that *B. thuringiensis* var. *israelensis* were highly effective against the laboratory strains of larvae of *Anopheles stephensi* with LC_{50} ranging from 1.72 g/L (I instar) to 2.42 g/L (IV instar). In addition, Kovendan *et al.*[25] explored that bacterial insecticide spinosad was toxic against larvae of *Aedes aegypti* with LC_{50} ranging from 51.76 mg/L (I instar) to 93.44 mg/L (pupa). We hypothesized that the toxicity of bioinsecticides against filarial vectors was due to the variation in the commercial brands and production criteria.

IGRs, also known as the third generation insecticides, are effective tools to control a variety of arthropod vectors[26]. IGRs and microbial insecticides are intrinsically non-toxic, biologically specific, and environmentally safe compared to conventional chemical larvicides. In the present study, the activity of Baycidal and Sumilarv was evaluated against *Cx. pipiens*. The results showed that Baycidal formulations were more effective (IC_{50} = 0.000 4 mg/L) if compared to Sumilarv (IC_{50} = 0.002 9 mg/L), by about 7.25 folds. Aziz *et al.*[9] reported that IGR of Baycidal at concentration 0.000 2–0.002 0 mg/L was applied to study the susceptibility of the *Cx. pipiens* larvae (laboratory and field strains). The emergence rate of the adult in this research ranged from 2% to 78% and the IC_{50} was higher (0.000 4 mg/L) compared to the study by Aziz *et al.*[9]. It has been reported that application of IGR showed deformed abnormalities in developmental stages of *Cx. pipiens* after treatment and other intermediate stages including larval siphon, pupal trumpets, unmelanized pupa and failure of adults to emerge from the pupal skins. These observed abnormalities on developmental stages could be due to morphological aberrations leading to the failure of successful emergence from exuviae of pupal stages[27]. Overall, this research added basic knowledge about the effectiveness of seven mosquito larvicides with different mechanism(s) of action as potential candidates for the control programs of *Cx. pipiens* in Saudi Arabia. Further research on nanoencapsulation of *Cx. pipiens* larvicides in field conditions is urgently required[28].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We are grateful to team of the Dengue Mosquito Research Station, King Abdulaziz Univeristy, Jeddah, Saudi Arabia, as well as to the Entomology and Toxicology Unit, University of Tabuk, Department

of Biology. This research was partially funded by the Dengue Mosquito Research Station, King Abdulaziz University, Jeddah and Lab of Entomology and Toxicology, University of Tabuk (Grant No. 2/1/11/1435).

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