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ASSESSMENT OF SOME CHEMICAL AND BIORATIONAL INSECTICIDES AGAINST *CULEX PIPIENS* (DIPTERA: CULICIDAE) IN ABHA CITY, SAUDI ARABIA

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Abstract

The present study was tested certain chemical insecticides (Propetamphos and Cypermethrin) and a Bioinsecticide (Baciloid 5000: *Bacillus thuringiensis*) against *Culex pippins*, the dominant mosquito species in Abha City. LC_{50} value of Propetamphos was 0.0162 ppm against the 3rd instar larvae of laboratory strain compared with field strain which was 0.0442 ppm, and LC_{90} of Propetamphos was 0.8109 ppm against the 3rd instar larvae of laboratory strain, comparing to field strain (3.31ppm). Cypermethrin was also very effective where LC50 was 0.0132 ppm against the adult females of laboratory strain, comparing to (0.1192 ppm) of laboratory strain. On the other hand, residual activity of *Bacillus thuringiensis* var. *israelensis* reached from 4 to 20 days of concentrations ranged between 0.001 to 100 ppm (lethality was 11.7 to 96.8%) in laboratory strain in the 1st week, whereas ranged between 0.0 to 70.8% in the 2nd week with same concentrations finally ranged between 0.0 to 12.7% in the 3rd week. While reached 6 -23 days at concentrations 0.001 to 100ppm in field strain in the 1st week, whereas ranged between 0.0 to 70.8% in the 2nd week with the same concentrations and ranged between 0.0 to 12.7% in the 3rd week. So, the field collected larvae of *Cx. pipens* were more susceptible and have prolonged residual effect as compared to laboratory reared larvae.

Key words: Saudi Arabia, conventional and non-conventional insecticides, *Culex pipiens*.

Introduction

Mosquitoes (Diptera: Culicidae) are annoying, serious problem in man's domain. Mosquito-borne diseases take a tremendous toll on human populations, especially in developing nations In the last decade, scientists developed mosquitoes, which have been genetically modified to prevent transmission of mosquito-borne diseases, and field trials were conducted (Resnik, 2014). Some mosquitoes have been rendered infertile, some have been equipped with a vaccine they transmit to humans, and some have been designed to resist diseases. Econometric model for malaria, which is responsible for more than 1 million deaths every year, suggests that countries with intensive malaria have income levels only 33% of that of those without malaria (Al-Agroudi *et al*, 2017).

Apart from malaria, *Aedes aegypti* transmits viral hemorrhagic fevers as Yellow Fever, Dengue Fever, and chikungunya (Heikal *et al*, 2011) and Zika Fever, which would remain a significant challenge to public health in the Gulf States (Binsaeed *et al*, 2016). Also, mosquitoes transmit encephalitis, Rift Valley Fever, dog heartworm, West Nile Fever, Eastern Equine Encephalitis …etc. (El-Bahnasawy *et al*, 2013), besides, *Cx. pipiens* is the main vector of bancroftian filariasis in Saudi Arabia (Sebai *et al*, 1974). In Saudi Arabia, RVF epidemic occurred and mortality reached 13.9% (Madani *et al*, 2011). They are vector of Japanese encephalitis, meningitis, and urticaria (Nassar, 2000) Moreover, skin irritation by an allergic reaction to its saliva caused red bump and itching or Skeeter Syndrome (Abdel-Motagaly *et al*, 2017).

This work aimed to study the effect of some conventional and non-conventional chemicals on the field and laboratory strain *Culex pipiens* in Abha City, Saudi Arabia

Materials and Methods

Adult mosquito was collected using the black hole mosquito trap "Gangnam-gu, Seoul, 135-744, KORE (Abu Ras, 2007) and identified according to standard key (Lorenz *et al*, 2015). *Culex pipiens* were reared using standard conditions to generate similar-sized individuals. Others observed this time period to be sufficient for *Cx. pipiens* to complete the gonotrophic cycle prior to the oviposition (Derek Charlwood *et al*, 12016) who stated that mosquito survival, oviposition interval and gonotrophic concordance are important determinants of vectorial capacity. These vary among species or within a single species due to the environment. They may be estimated by examination of the ovaries of host-seeking mosquitoes. Five days post-blood feeding, all adult mosquitoes were aspirated from cages and transferred according to treatment group to new cages containing an empty 100ml plastic cup affixed to bottom. Plastic cup would be used later to contain an oviposition substrate. GPS device determined the coordinates of breeding spots and drop it on the maps to show areas with a high density of larvae in Abha City.

Insecticides: Commercial formulations of insecticides were used in this study applied on mosquito control which includes organophosphorous (Propetamphos), synthetic pyrethroide (Cypermethrin) and *Bacillus thuringiensis* var. *israelensis*, Baciloid 5000.

Bioassays: a- Larval instars, dipping method for treatment of insect larvae with different insecticide concentrations was applied as four replicates to 3rd instar mosquito-larvae (El-Hela *et al*, 2013) with some modifications. All the tests were run at 14:10 (light: dark) and maintained at 28±2°C & 70-80% humidity, whereas those of the controls were dipped in tap water. After being dipped for exactly 30 sec, larvae were transferred to the rearing jars containing food for account the number of emerging flies, and b- Adult stages CDC (complement mediated cytotoxicity) bottle bioassay (Sukontason *et al*, 2004). For adult stage of *Cx. pipiens*, assays were adapted (Shin *et al*, 2011). Twenty-five females were exposed to the treated surface of test tube. Adults transferred for mortality count after one and 24 hours.

Statistical analysis: Mortality was evaluated after 24 hours. Data were subjected to Probit Analysis. Mortality was corrected after Abbott's (1925). Levels of resistance in field colony strains of the two insects were calculated as follows:

$$\text{Resistant ratio (R.R)} = \frac{LC_{50} \text{ or } LC_{90} \text{ of the field colony strains}}{LC_{50} \text{ of } LC_{90} \text{ the laboratory strain}}$$

Data were analyzed of variance (ANOVA) and means were compared by LSD test at 0.05 levels, using SAS program (SAS Institute, 1988). Data showed relationship between mosquito densities and climatic factors by using correlation and multiple regression techniques. Also, relationship between climatic factors (temperature, relative humidity and rainfall) and mosquito density were analyzed (Fitzmaurice *et al*, 2004).

Results

The results are shown in tables (1, 2 & 3) and figures (1 & 2).

Table1: Susceptibility of *Cx. pipiens* larvae (lab & field strains) to Propetamphos 20% by dipping technique then exposure for 24 hr.

Insecticide	*Cx. pipiens* strain	Effective conc. (ppm)	Larval mortality (%)[a]	Statistical parameters								
				LC_{50}	LC_{90}	Slope	X^2 (Chi)^2 C	X^2 (Chi)^2 T	P	R C	R T	RR*
Propetamphos 20%	Lab St.	0.5-0.001	97-19	0.1062	0.8109	0.7537	15.74	9.5	0.0004	0.927	0.811	2.7
	Field St.	0.5-0.001	90-15	0.0442	3.31	0.6838	12.15	9.5	0.0011	0.933	0.878	

a: Five replicates, 20 larvae each; control mortalities ranged from 0.0%-3.0%. b:. When tabulated (Chi)2 larger than calculated at 0.05 level of significance indicates the homogeneity of results C = Calculated, T= Tabulated, RR = Resistant ratio

Table 2: Susceptibility females *Cx pipiens* (lab. & field strains) to Cypermethrin10 for 24h using CDC bioassay.

Insecticide	Strain	Effective conc. (ppm)	Mortality (%)[a]	Statistical parameters								
				LC_{50}	LC_{90}	Slope	X^2 $(Chi)^2$		P	R		RR
							C	T		C	T	
Cyperme-thrin 10%	Lab.	0.5-0.001	98-20	0.0132	0.6303	0.7631	20.69	9.5	0.0004	0.91	0.81	9.03
	Field	0.5-0.001	89-19	0.1192	1.8522	1.076	19.70	9.5	0.0006	0.94	0.81	

Table 3: Residual activity (maximum /day) of *Bacillus th. var. israelensis* and mortality of *Cx. pipiens* larvae/week

Conc. (ppm)	Max. days of residual activity	Mortality% /week		
		1st week	2nd week	3rd week
Laboratory strain				
0.001	4	11.7	0.0	0.0
0.01	8	19.4	0.0	0.0
0.1	10	61.9	7.4	0.0
1	14	75.8	27.9	0.0
10	17	93.7	51.5	2.7
100	20	96.8	70.8	12.7
Field strain				
0.001	6	9.2	0.0	0.0
0.01	9	15.5	0.0	0.0
0.1	11	58.4	0.0	0.0
1	16	71.4	21.9	0.0
10	19	90.8	42.7	0.0
100	23	94.1	67.5	10.1

Discussion

The present study revealed that LC_{50} of Propetamphos was 0.0162 ppm against the 3rd instar larvae of *C. pipiens* of laboratory strain as compared with field strain which was 0.0442 ppm. But, LC_{90} of propetamphos was recorded 0.8109ppm and 3.31 ppm of laboratory and field strains respectively. The slope function of laboratory strain was 0.7537 in case of Propetamphos and in filed strain was 0.6838. The X^2 $(Chi)^2$ was 9.5, while calculated X^2 $(Chi)^2$ was 15.74 of laboratory stains of larval stages of *Cx. pipiens* when treated with Propetamphos. On the other hand, the tabulated $(Chi)^2$ was 9.5 and calculated $(Chi)^2$ was 12.15 against the field strains. The average of resistance of ratio was 2.73 fold indicated that the field strain of Propetamphos was sensitive. The results agreed Abd El-Samie and Abd El-Baset (2012) who tested the efficacy of the most used insecticides belonging to organophosphate, carbamate, synthetic and pyrethroid against *Cx. pipiens* four different field populations.

The results showed that Cypermethrin was the effective insecticide (LC_{50} = 0.0ppm) against the laboratory strain 3rd instar larvae, and against field strain Cypermethrin gave (LC_{50} = 0.1192 ppm). The mortality of Cypermethrin ranged between 20-98% against laboratory strain females and ranged between 19-89% with field strain.

In the present study, the slope of laboratory and field stains due to cypermethrin on larval stages was 0.7631 & 1.076 respectively. Also X^2 $(Chi)^2$ was 9.5 while calculated X^2 $(Chi)^2$ of laboratory and field stains was 20.69 & 19.70, respectively. The levels of resistance in *Cx. pipiens* of field colony illustrate monitoring resistance ratio in the 3rd instar larvae of *Cx. pipiens* field colony strain collected from Abha City. Concerning the population of Abha City, the resistant ratios in the 3rd instar larvae to the toxicity of cypermethrin that mentioned at LC_{50} levels were 0.0132 & 0.1192 against laboratory and field strains, respectively. Pyrethroid compounds prevent sodium gates from closing in nerves of insects and potent neuropoisons, their mechanism of action on the nervous system. The nerve excitation occurred as a result of changes in nerve membrane permeability to sodium and potassium ions, and therefore any effect of pyrethroids could be interpreted in terms of such permeabilities. This agreed with Al-Sarar (2010) who studied the developing resistance against mosquitoes in Riyadh. Two populations from Wadi Namar (WN1 & WN2) were highly resistant to deltamethrin (187.1- and 161.4-folds respectively). The field population from Al-Wadi district (Al-W) showed low resistance to lambda-cyhalothrin (3.8-folds) and moderate resistance to beta-cyfluthrin and bifenthrin (14- & 38.4-folds respectively). No resis-tance to fenitrothion was observed in WN1 population. Fenitrothion concentrations required to inhibit 50% of Acetylcholinesterase (AChE) activity in both WN1 population and the laboratory susceptible strain (S-LAB) were 786 and 0898ppm respectively. Piperonyl butoxide suppressed resistance to pyrethroid insecticides (> 90%) in field populations indicating that oxidases and/or esterases played an important role in the reduction of pyrethroids toxicity. These results should be considered in the current mosquitoes control programs in Riyadh (Al-Sarar *et al*, 2005). The following results in agreement with the present results. Also high resistance levels of *Cx. pipiens pipiens* (233 and 453-fold) to deltamethrin which reported in Tunisia (Daaboub *et al,* 2008).

In the present study, the residual activity of *Bacillus thuringiensis* var. *israelensis* reached from 4 to 20 days of concentrations ranged between 0.001 to 100 ppm in case laboratory strain, while reached between 6-23 days of concentrations ranged between 0.001 to 100 ppm in case field strain. Also, the mortality percentage ranged between 11.7 to 96.8% of concentrations and ranged between 0.001 to 100ppm against laboratory strain in the 1st week, whereas it ranged between 0.0 to 70.8 in the 2nd week against the same concentrations and ranged 0.0 to 12.7 in the 3rd week. On the other hand, the mortality percentage ranged between 9.2 to 94.1% of concentrations and ranged between 0.001 to 100ppm against field strain in the 1st week, whereas ranged 0.0 to 67.5 in the 2nd week against the same concentrations and ranged 0.0 to 10.1 in the 3rd week. The *Bti* showed the highest level of biological activity with different levels of toxicity to mosquito species. The *Culex* and *Aedes* were highly susceptible but, *Anopheles* was less susceptible (Charles *et al*, 1996). The results agreed with Jahan *et al*. (2013), they found that field collected *Cx. quinquefasciatus* larvae were more susceptible and gave prolonged residual effect as compared to laboratory reared *A. stephensi* against *Bsph* while *Bti* gave a vice versa effect. Also, El-Zahran *et al*. (2013) found the most effective tools for *Cx. pipiens* larvae eradication was by using some binary mixtures of these tested control measures, which gave better control, being safe and less expensive. Residual effect for field evaluation of *Bti* WDG using low dose (0.2mg/litre) against *Cx. quinquefasciatus* lasted 14 days showing more efficacy for field bioassays compared to the laboratory one. Charles and Nielsen (2000) found that biological control with *Bti* and *Bsph* larvicides proved highly effective, less expensive and environmentally safe to non-target organisms as well as for human exposure. Hampel *et al*. (2010) stated that the pharmaceuticals are emerging pollutants widely used in everyday urban activities which can be detected in surface, ground, and drinking waters. Their presence is derived from consumption of medicines, disposal of expired medications, release of treated and untreated urban effluents, and from the pharmaceutical industry. Their growing use has become an alarming environmental problem which potentially will become dangerous in the future. However, there is still a lack of knowledge about long-term effects in non-target organisms as well as for human health. The toxicity testing indicated a relatively very low action on the fish species, but no information is available on possible sub lethal effects.

Conclusion

The outcome results showed that *Bacillus* products were most effective, more or less environmental friendly to fauna and flora and can be produced locally.

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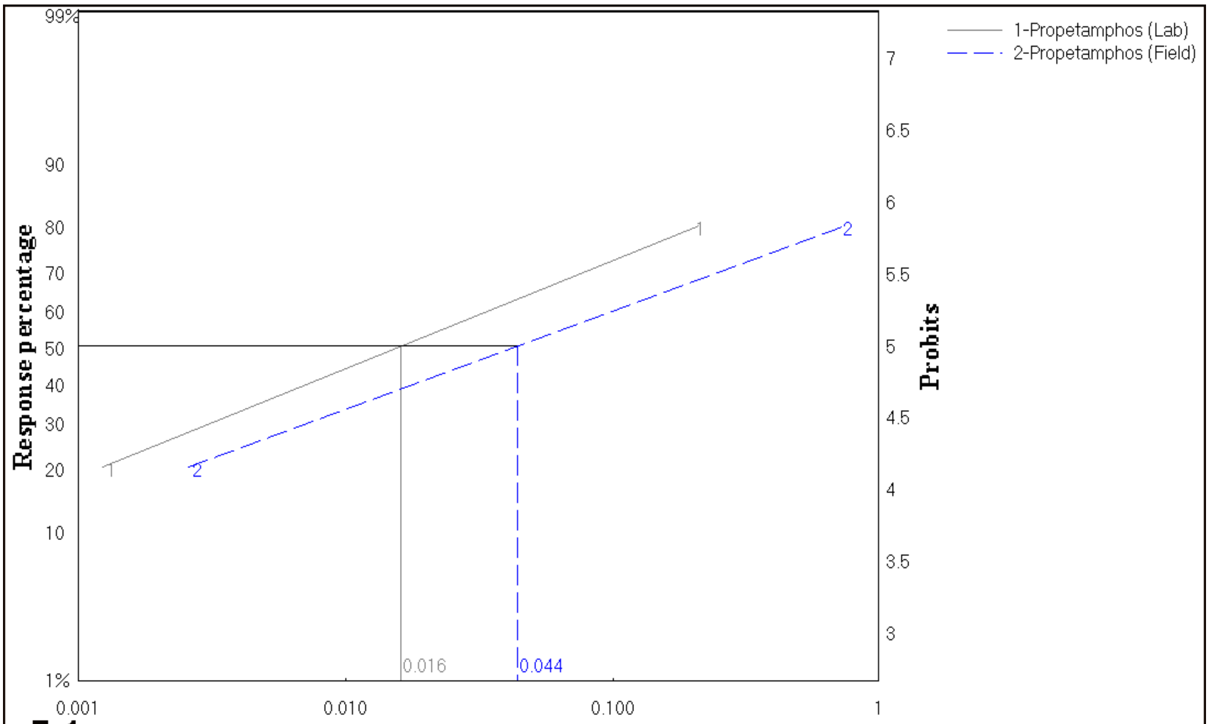
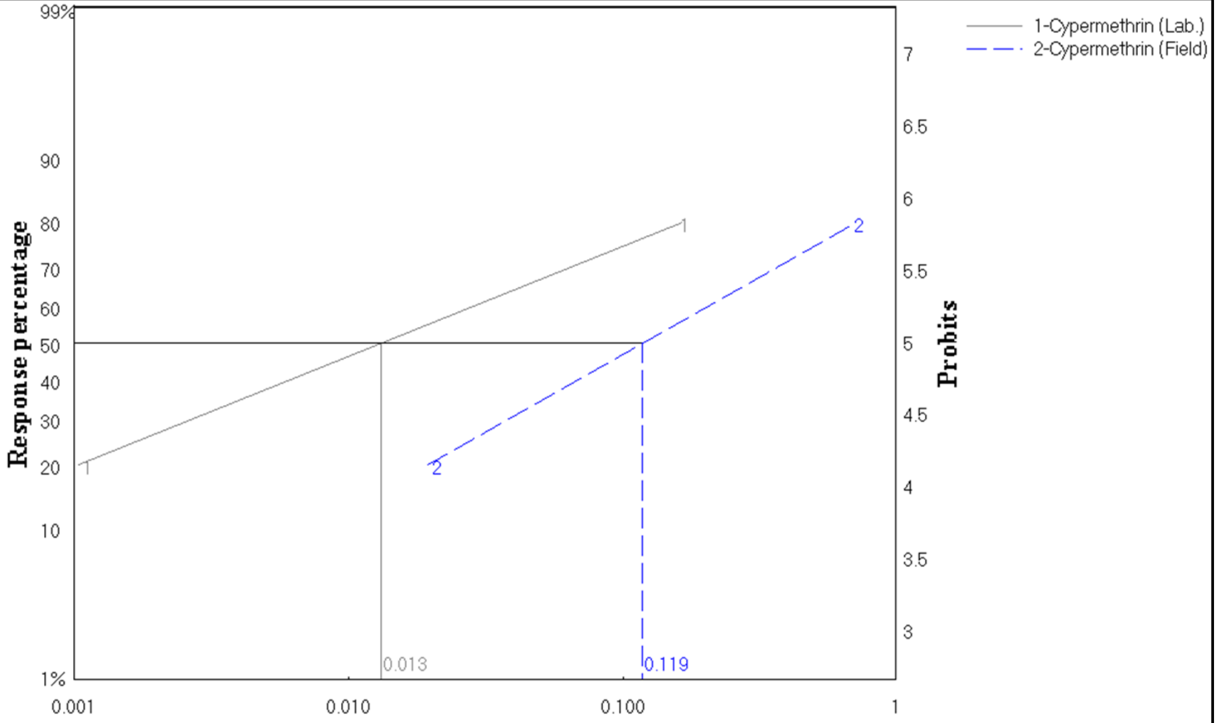
Explanation of figures

Fig. 1: Regression lines for Propetamphos 20% bioassay of larvae (laboratory & field strains) *Cx. pipiens* using dipping technique.
Fig. 2: Regression lines for Cypermethrin 10% bioassay of *Cx. pipiens* females (laboratory & field strains) using CDC bioassay technique.



F. 1



F. 2