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# Insecticide susceptibility in larval populations of the West Nile vector *Culex pipiens* L. (Diptera: Culicidae) in Saudi Arabia

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ABSTRACT

**Objective:** To investigate the susceptibility to some conventional and non-conventional insecticides in laboratory and field larval populations of the West Nile vector *Culex pipiens* L. (*Cx. pipiens*), the dominant species in Jeddah Province, Saudi Arabia.
**Methods:** The tested conventional insecticides were Actikil and Pesgard, while the non-conventional ones were Bacilod, Dudim and Baycidal. Probit analysis and photo-microscopical observations were carried out to shed light on acute toxicity in laboratory and field *Cx. pipiens* strains.
**Results:** *Cx. pipiens* were more susceptible to Pesgard ($LC_{50}$: 0.045 and 0.032 mg/L) than Actikil (0.052 and 0.038 mg/L) and Bacilod (0.129 and 0.104 mg/L), for the field and laboratory strains, respectively. Results showed that treatments with the chitin synthesis inhibitor Dudim and Baycidal evoked morphological effects similar to those induced by other insect growth regulators. According to $IC_{50}$ values obtained (concentration which to inhibit the emergence of 50% of mosquito adults), the compound Dudim (0.000 3 and 0.000 1 mg/L) was more effective against *Cx. pipiens* L. mosquitoes than Baycidal (0.000 4 and 0.000 3 mg/L) for both the field and laboratory strains, respectively.
**Conclusions:** Our results provide baseline data to enhance control programs and orient public health decisions on the selection of pesticides against mosquito vectors in Saudi Arabia.

## 1. Introduction

The development and use of insecticides have produced immeasurable benefits for humankind as they kill unwanted insect pests by disruption of their vital processes through chemical action. Therefore, they have been a major contributor to the upsurge in agricultural productivity over the past three decades. Their use has not only resulted in foodstuffs of the highest quality but also has saved millions of lives through eradication of disease-carrying insects [1].

Mosquitoes are considered as the most important group of insect which have ability to spread the disease in the world [2–4]. Chemical control is one of the main ways to combat mosquito vectors of medical and veterinary importance. In the beginning of the 19th century, the discovery of a group of chlorinated hydrocarbons such as DDT and its derivatives boosted up pest control. Later on, many synthetic chemical insecticides [*i.e.*, organophosphates, carbamates and insect growth regulators (IGRs)] were successfully used to control mosquitoes [5,6]. Over the last century, natural and synthetic insecticides usage

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has increased in the agricultural and public health sectors [7,8]. This has saved millions of tons of agricultural food resources and human lives. However, the introduction of synthetic insecticides has arisen the problem of resistance in vector-borne diseases [9]. Resistance is the developed ability in a strain of insects to tolerate the doses of insecticides, which may be lethal to the majority of individuals in a normal population of the same species. This is reflected in repeated failure of an insecticide to achieve the expected level of control of insects when used according to the product label recommendations and where problems of product storage, application and unusual climatic or environmental conditions can be eliminated as causes of the failure [10]. To overcome these problems, scientists discovered other types of chemicals known as IGRs. IGR is a chemical group of pesticides, which may be used as mosquito larvicides and divided into two families; the juvenoid family, which affects the growth process of the insects, and the chitin synthesis inhibitors, which disrupt the transformation processes. In general, IGRs are safer to fishes, amphibians, mammals and birds, if compared with other pesticides [11]. Pyriproxyfen, belonging to this family, is an IGR that affects the physiology of morphogenesis, reproduction and embryogenesis of insects. It exhibits a high level of activity against mosquito larvae inhibiting adult emergence at low dose [12,13]. Furthermore, Vythilingam *et al.* [14] showed the long-term effectiveness of pyriproxyfen against dengue vectors in Asia. Similarly, Sihunincha *et al.* [15] showed that, pyriproxyfen prevented adult emergence at extremely low concentrations ($LC_{50}$ = 0.012 mg/L) when applied to late mosquito instars. In this research, we determine the susceptibility levels on the West Nile vector *Culex pipiens* L. (*Cx. pipiens*) testing conventional and non-conventional insecticides in Jeddah province of Saudi Arabia. Results provide baseline data to enhance control programs and orient public health decisions on the selection of pesticides in Saudi Arabia.

## 2. Materials and methods

### 2.1. Collection sites

Mosquito 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.

All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

### 2.2. Mosquito collection and colonization

*Cx. pipiens* larvae were maintained at the laboratory of University of Tabuk under conditions of controlled temperature [(27 ± 1) °C] and relative humidity [(70 ± 5)%] with a 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). Subsequently, adults were kept in cages and provided with a cotton wick soaked with 10% glucose solution for post-emergence. After a period of 4 days, sugar-fed females were starved for 24 h prior to feed on artificial blood suppliers. 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 bioassays. Adult experiments 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

Conventional insecticides: the Actikil 50% (active ingredient: pirimiphos-methyl 5%), and Pesguard Fg161 (active ingredient: D-tetramethrin 4%; cyphenothrin 6%).

Non-conventional insecticides: Bacilod 5000 ITU (active ingredient: *Bacillus thuringiensis* var. *israelensis*), Dudim 4G (active ingredient: diflubenzuron 4%), and Baycidal (active ingredient: triflumuron 25%).

### 2.4. Larval bioassays

Experiments were conducted following the method by Aziz *et al.* [16]. Batches of 20 larvae were added to glass beakers filled with 200 mL of water containing different concentrations of the five insecticides: *i.e.*, Actikil, Pesguard, Bacilod, Dudim and Baycidal. When larvae were introduced into the beakers, 0.02 g of the powdered mixture was added to avoid death by starvation.

The concentrations applied for conventional insecticides Actikil and Pesguard were 0.02–0.15 and 0.02–0.20. The concentrations applied for non-conventional insecticides, Bacilod, Dudim and Baycidal were 0.05–0.5, 0.000 1–0.00 4 0, and 0.000 2–0.002 0, respectively. These concentrations of each insecticide were tested on fourth instar larvae in five replications, for both laboratory and field-strains. In each case, the same number of glass beakers with the same treatment but without insecticide served as controls. Beakers were inspected 24 h after introduction of larvae and the numbers of dead larvae were recorded.

### 2.5. Data analysis

In the Actikil, Pesguard and Bacilod insecticides bioassay experiment, the numbers of dead larvae were determined after 24 h. Following [17], larvae incapable of reaching the water surface for oxygen and those showing no diving reaction characteristics when the water was disturbed were considered dead. For IGR insecticides Dudim and Baycidal, daily inspections were carried out until adult emergence, dead larvae were recorded. The distortion was calculated. Results were excluded from analysis if mortality rate was above 20%. In addition, if the percentage ranged between 5% and 20%, the mortality was corrected using the Abbott's formula [18]. Data from larval bioassays were subjected to probit analysis [19]. The concentrations of agents that killed 50% and 90% of mosquito larvae in 24 h ($LC_{50}$ and $LC_{90}$, respectively) were used to judge the larvicidal activity of the tested products [20]. The resistance status was determined according to World Health Organization [21].

The following formula was used to evaluate resistance according to Aziz *et al.* and Pungasem *et al.* [16,22].

$$\text{Resistant ratio (RR)} = \frac{LC_{50}\ \text{(Field strains) or}\ IC_{50}\ \text{(Field strains)}}{LC_{50}\ \text{(Laboratory strains) or}\ IC_{50}\ \text{(Laboratory strains)}}$$

where, RR < 2 is susceptible strain, 10 > RR > 2 is tolerant strain, RR > 10 is resistant strain.

## 3. Results

### 3.1. Susceptibility of *Cx. pipiens* larvae

The different patterns of larval susceptibility were reported in Table 1. The lowest percentage mortality (12%) was recorded among wild larvae exposed to Actikil, whereas the highest mortality rate (98%) was observed for laboratory strain larvae exposed to Actikil and Pesguard. The percentage mortality rates of *Cx. pipiens* exposed to larvicides were lower in the field strain than in the laboratory strain. The $LC_{50}$ of Actikil for the laboratory strain was 0.038 mg/L, which was 1.37 fold lower than that of the field strain. The $LC_{50}$ of Pesguard for the laboratory population was 0.032 mg/L, which was 1.41 fold lower than that of the field strain. The $LC_{50}$ of Bacilod for the laboratory strain was 0.104 mg/L, which was 1.24 fold lower than that of the field strain. Pesguard was the most effective larvicide in both field and laboratory strains, followed by Actikil (Figure 1A–C). The slopes were greater for the laboratory strain than the wild strain for Actikil (3.11 *vs.* 2.97, respectively), Pesguard (2.449 *vs.* 2.098, respectively), and Bacilod (2.435 *vs.* 1.827, respectively), indicating homogeneity of response to the tested larvicides. Overall, field larvae were less susceptible to the larvicides than the laboratory-adapted larvae.

### 3.2. Impact of IGR Dudim and Baycidal on young instars

Treated 4th instar larvae of *Cx. pipiens* field and laboratory strain with Dudim delayed effects insecticides at 0.000 1 and 0.004 0 concentrations showed percentage mortalities from 2%

**Table 1**
Susceptibility of the 4th larval stage of *Cx. pipiens* L. to different insecticides.

| Insecticide | *Cx. pipiens* strain | Dose (mg/L) | Larval mortality (%)[a] | Statistical parameters[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $LC_{50}$ (mg/L) | $LC_{90}$ (mg/L) | Slope | $\chi^2$ | | Resistant ratio |
| | | | | | | | C | T | |
| Actikil | Lab strain | 0.02–0.15 | 20–98 | 0.038 | 0.097 | 3.11 | 0.702 | 7.8 | 1.37 |
| | Field strain | | 12–93 | 0.052 | 0.141 | 2.97 | 0.849 | 7.8 | |
| Pesguard | Lab strain | 0.02–0.20 | 32–98 | 0.032 | 0.108 | 2.449 | 0.224 | 7.8 | 1.41 |
| | Field strain | | 23–92 | 0.045 | 0.184 | 2.098 | 1.240 | 7.8 | |
| Bacilod | Lab strain | 0.05–0.50 | 24–96 | 0.104 | 0.435 | 2.435 | 0.753 | 7.8 | 1.24 |
| | Field strain | | 14–92 | 0.129 | 0.574 | 1.827 | 0.876 | 7.8 | |

[a]: Five replicates, 20 larvae each; [b]: Litchfield and Wilcoxon (1949) [20].
C: Calculated; T: Tabulated; $\chi^2$: T larger than C at 0.05 level of significance indicates homogeneity of results.

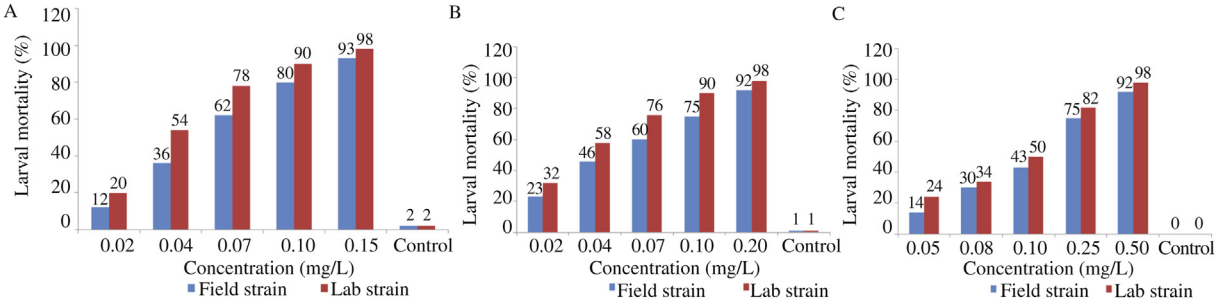


**Figure 1.** The effect of larval treatments with Actikil (A), Pesguard (B) and Bacilod (C) on the 4th larval stage of *Cx. pipiens*.

**Table 2**
Impact of the IGR Dudim on the developmental stages of laboratory and field strains of *Cx. pipiens*.

| Dose (mg/L) | *Cx. pipiens* strain | Larval mortality (%)[a] | Pupal mortality (%) | Adult mortality (%) | Survival rate (%) | Adult emergence inhibition | |
|---|---|---|---|---|---|---|---|
| | | | | | | Observed | Corrected[b] |
| 0.000 1 | Lab strain | 5 | 3 | 32 | 60 | 40 | 35.4 |
| | Field strain | 2 | 0 | 26 | 72 | 28 | 22.6 |
| 0.000 4 | Lab strain | 7 | 0 | 54 | 39 | 61 | 58.1 |
| | Field strain | 10 | 0 | 50 | 40 | 60 | 57.0 |
| 0.000 7 | Lab strain | 12 | 1 | 68 | 19 | 81 | 79.6 |
| | Field strain | 9 | 0 | 64 | 27 | 73 | 71.0 |
| 0.001 0 | Lab strain | 21 | 3 | 64 | 12 | 88 | 87.1 |
| | Field strain | 3 | 0 | 79 | 18 | 82 | 82.0 |
| 0.004 0 | Lab strain | 21 | 0 | 77 | 2 | 98 | 97.8 |
| | Field strain | 30 | 0 | 65 | 5 | 95 | 92.6 |
| Control | 4 | 0 | 3 | 93 | 7 | 0 | |

[a]: Five replicates, 20 larvae each; [b]: Corrected with Abbott's formula [18].

to 30% and 5% to 21%, respectively (Table 2). Whereas the percentage mortality was 3%–31% for field strain and 4%–23% for laboratory-reared larvae exposed to Baycidal at 0.0002–0.0020 concentrations (Table 3). The percentage mortality of larvae exposed to cuticle synthesis inhibitors was lower in the field strain than in the laboratory strain.

Generally, the mortality rates were associated mainly with failure to pupate. The post-effect of Dudim and Baycidal on the adult stage of *Cx. pipiens* was evaluated studying percentage of adult emergence. Some *Cx. pipiens* individuals that have succeeded to reach to the adult stage have folded wings (Figure 2). We used $IC_{50}$ which is a measure of 50% inhibition of adult

**Table 3**

Impact of the IGR Baycidal on the developmental stages of laboratory and field strains of *Cx. pipiens*.

| Dose (mg/L) | *Cx. pipiens* strain | Larval mortality (%)[a] | Pupal mortality (%) | Adult mortality (%) | Survival rate (%) | Adult emergence inhibition | |
|---|---|---|---|---|---|---|---|
| | | | | | | Observed | Corrected[b] |
| 0.0002 | Lab strain | 4 | 2 | 26 | 68 | 32 | 0.0 |
| | Field strain | 3 | 1 | 21 | 75 | 25 | 0.0 |
| 0.0004 | Lab strain | 6 | 2 | 46 | 47 | 53 | 0.0 |
| | Field strain | 7 | 3 | 37 | 57 | 43 | 0.0 |
| 0.0006 | Lab strain | 10 | 2 | 58 | 30 | 70 | 0.0 |
| | Field strain | 7 | 1 | 60 | 32 | 68 | 0.0 |
| 0.0008 | Lab strain | 22 | 2 | 61 | 15 | 85 | 0.0 |
| | Field strain | 5 | 10 | 64 | 21 | 79 | 0.0 |
| 0.0020 | Lab strain | 23 | 3 | 79 | 5 | 95 | 0.0 |
| | Field strain | 31 | 5 | 54 | 10 | 90 | 0.0 |
| Control | 2 | 2 | 0 | 96 | 4 | 0 | |

[a]: Five replicates, 20 larvae each; [b]: Corrected with Abbott's formula [18].


**Figure 2.** Morphological abnormalities in the developmental stages of *Cx. pipiens* after treatment with Baycidal or Dudim.
A: Larval–pupal intermediate showing larval siphon (a) and pupal trumpets (b); B: Unmelaninized pupa (albino pupa), pupal, adult intermediate; C: Early adults which failed to emerge from the pupa.

emergence following insecticide exposure. The percentage mortality rates of *Cx. pipiens* exposed to Dudim were 22.6%–92.6% and 35.48%–97.88% for field strain and laboratory strains respectively, at concentrations ranging from 0.000 1 to 0.004 0 mg/L, respectively. The value of $IC_{50}$ was 0.000 3 and 0.000 1 mg/L for field and laboratory strains, respectively (Figure 3).

The effective concentration of Baycidal was between 0.000 2 and 0.002 mg/L, and the given adult emergence inhibition was 25%–90% and 32%–95% for field population and laboratory population respectively. Whereas, the $IC_{50}$ was 0.000 4 and 0.000 3 for field strain and laboratory strains (Table 4). According to the resistance ratio obtained the 4th larvae stage of *Cx. pipiens*, the field strain was susceptible to Baycidal compound (Resistant ratio = 1.33), as compared from the tolerant strain for Dudim (Resistant ratio = 3) (Table 4).

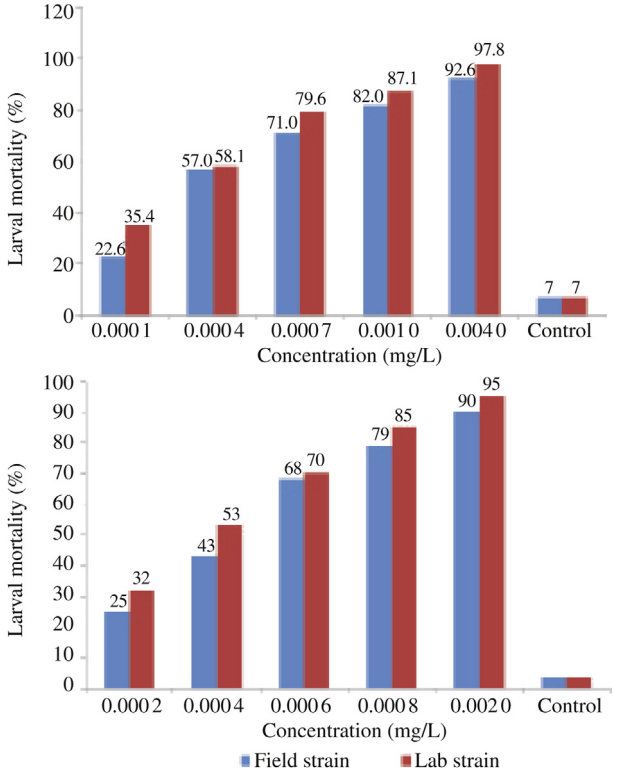


**Figure 3.** Effect of larval treatment with the IGR insecticides on survived adults of *Cx. pipiens*.

## 4. Discussion

The present study was performed to determine the susceptibility of the West Nile vector *Cx. pipiens* to some conventional and non-conventional insecticides in Saudi Arabia. Larvae of *Cx. pipiens* were more susceptible to Pesgard (0.045 and 0.032 mg/L) over Actikil (0.052 and 0.038 mg/L) and Bacilod (0.129 and 0.104 mg/L), both for field and laboratory strains. On the other hand, the response of 4th instar of *Cx. pipiens* to tested insecticides (Actikil 50%, Pesguard and Bacilod) depends on the mode of action and the concentration of the active ingredient. According to $IC_{50}$ values obtained, the compound Dudim was more effective over Baycidal both for lab and field strains. In agreement with our findings, Saleh *et al.* [23,24] reported that the differences in the percentages of larval mortality increased proportionally with the increase in the use of concentration. Furthermore, mortality in the pupal stage and damage to adult emergence was also observed. Results revealed that larvae exposed to Dudim and Baycidal showed abnormalities in developmental stages such as larval siphon, pupal trumpets, unmelanized pupae (albino pupae), and/or adults failure to emerge from the pupae [25,26]. The resistance ratio of *Cx. pipiens* treated with Actikil, Pesguard, Bacilod and Baycidal showed that this mosquito species was susceptible, whereas it was tolerant against Dudim, even if it did not reach the resistance level. This may be linked to the fact that the authority of Jeddah Province recently used a successful vector control programs against *Cx. pipiens* larvae, with proper rotation between the insecticidal groups, to avoid resistance development. The slightly difference in the effective concentration range among these studies could be linked to the differences between strains, biological response of the tested larvae, formulation of compounds and experimental conditions. Long-term follow-up studies are needed to determine how the environmental conditions affect the larvicidal effectiveness of these pesticides in field control programs. The reason may be due to the difference susceptibility of *Cx. pipiens* to different active ingredients [27]. This result showed that the larval mortality increased with concentration raised. In addition, the findings of this study were in agreement with several studies conducted in different parts of the world about the effect of insecticide against the mosquitoes [28–30]. Our findings revealed that the larvae treated with IGRs 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

**Table 4**
Susceptibility of the 4th larval stage of *Cx. pipiens* to delayed effects of different insecticides.

| Insecticide | *Cx. pipiens* strain | Dose (mg/L) | Adult emergence inhibition (%)[a] | Statistical parameters[b] | | | | | Resistant ratio |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $IC_{50}$ (mg/L) | $IC_{90}$ (mg/L) | Slope | $\chi^2$ | | |
| | | | | | | | C | T | |
| Dudim | Lab strain | 0.000 1–0.004 0 | 35.4–97.8 | 0.000 1 | 0.003 9 | 1.474 3 | 9.406 | 7.8 | 3 |
| | Field strain | | 22.6–92.6 | 0.000 3 | 0.004 0 | 1.145 7 | 16.922 | 7.8 | |
| Baycidal | Lab strain | 0.000 2–0.002 0 | 32.0–95.0 | 0.000 3 | 0.001 2 | 2.248 5 | 2.445 | 7.8 | 1.333 |
| | Field strain | | 25.0–90.0 | 0.000 4 | 0.001 6 | 2.133 8 | 5.253 | 7.8 | |

[a]: Five replicates, 20 larvae each; [b]: Litchfield and Wilcoxon (1949) [20].
C: Calculated; T: Tabulated; $\chi^2$: T larger than C at 0.05 level of significance indicates homogeneity of results.

morphological aberrations leading to the failure of successful emergence from exuviae of pupal stages [31].

## Conflict of interest statement

We declare that we have no conflict of interest.

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