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Al Thabiani Aziz
Biology Department, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

Correspondence
Al Thabiani Aziz
Biology Department, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

Insecticidal activity of three insect growth regulators towards the dengue and Zika virus vector *Aedes aegypti* in Saudi Arabia

Al Thabiani Aziz

Abstract
Objectives: To explore the insecticidal activity of insect growth regulators (IGRs) against the dengue vector *Aedes aegypti* (*Ae. aegypti*) (Diptera: Culicidae) in Saudi Arabia.
Methods: LC_{50} and LC_{90} values were calculated in laboratory conditions using Probit analysis.
Results: I tested three selected insect growth regulators (IGR) Diflox flowable, Sumilarv and Baycidal. The results explored that, among three IGRs, the highest larval and pupal mortality was observed for diflox flowable, with 0.178 ppm (larva) and 0.178 ppm (pupae) followed by sumilarv i.e. 0.146 (larvae)0.146 (pupae) and baycidal i.e. 0.142 (larva) and 0.115 (pupae) ppm respectively. Concerning adult emergence inhibition, Sumilarv 0.5G was more effective (IC_{50} = 0.0004ppm) than Diflox Flowable (IC_{50} = 0.002ppm) and Baycidal 25 wp (IC_{50} = 0.047ppm) by about 5.75 and 117.5 folds, respectively.
Conclusions: Overall, this study highlighted that the insect growth regulators (IGRs) could be used as a poential larvicidal compounds against the dengue vector *Ae. aegypti*.

Keywords: *Aedes aegypti*; dengue; insect growth regulators; diflox flowable; Sumilarv

1. Introduction
Mosquitoes (Diptera: Culicidae) pose a major threat to millions of people worldwide, as they vector important parasites and pathogens, including malaria, dengue, filariasis, and arboviruses such as dengue, West Nile and Zika virus [1-3]. Mosquito-borne diseases are endemic in over 100 countries, causing mortality of nearly two million people every year, and at least one million children die of such diseases each year, leaving as many as 2100 million people at risk around the world [4, 5]. Dengue is a mosquito-borne viral infection found in tropical and subtropical regions around the world [6]. Recently, dengue transmission has strongly increased in urban and semi-urban areas, becoming a major international public health concern. In recent years, there has been increasing incidence of dengue has grown dramatically around the world in recent decades. A recent estimate indicates 390 million dengue infections per year (95 % credible interval 284–528 million), of which 96 million (67–136 million) manifest clinically (with any severity of disease) [7]. Another study, of the prevalence of dengue, estimates that 3900 million people, in 128 countries, are at risk of infection with dengue viruses [8, 1]. There is no specific treatment for dengue. Thus, dengue prevention and control depend solely on effective vector management measures [9, 10, 11].
Mosquito vectors may be controlled in their aquatic larval stage or as aerial adults [12]. The use of synthetic pesticides (i.e., insect growth regulators, organophosphates and carbamates, was successful to control Culicidae [13, 14]. However, the widespread and frequent usage of synthetic organic insecticideshave led to disrupted natural biological control systems heading to reappearance and resistance in target species and resulting in a human health concern [15].
Among various methods of vector control, insect growth regulating (IGR) compounds are emerging as safer alternative to conventional insecticides as they inhibit development of immature stages and reduce adult emergence instead of being toxic to its target [16]. They are comparatively safer to non-target organisms [17] and recommended for mosquito control [18]. The use of insect growth regulator (IGRs) is a novel approach in insect pest management where normal activity of the endocrine system is disrupted influencing development, metamorphosis and reproduction of the target insects [19] leading to various abnormalities that impair the survival of the insects.

Nowadays, more attention has been paid to the use of non-conventional insecticides for mosquito control worldwide [20, 21], since IGRs have mechanisms of action and extended effect on the mosquito if compared to those of conventional insecticides. IGRs included pyriproxyfen, diflubenzuron, triflumuron, novaluron and s-methoprene. Pyriproxyfen had high potency on *Ae. aegypti* [22] and *Culex pipiens* larvae in Saudi Arabia [23]. The IGR triflumuron, a chitin synthesis inhibitor was highly effective against *Ae. aegypti* [24]. Also, Sihunincha *et al*. [25] showed that, pyriproxyfen prevented adult emergence at extremely low concentrations (LC_{50}=0.012 mg/L) when applied to late mosquito instars. Further, Almadiy *et al*. [26] evaluated some IGR's Baycidal, Sumilarv and Dudim against *Ae. aegypti*. In addition, the effects of 4 insect growth regulators, namely triflumuron, diflubenzuron, fenoxycarb and buprofez in rice bloodworm, *Chironomus tepperi* [27]. Therefore, in the current study, the effectiveness of three selected insect growth regulators (IGRs) Baycidal, diflubenzuron and pyriproxyfen was investigated against immature stages and adults of *Aedes aegypti*.

2. Materials and Methods

2.1 Collection sites

Following the methods reported by Aziz *et al*. [14], *A. aegypti* 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 *A. aegypti* rearing

A. aegypti larvae were reared at the Dengue Mosquito Research Station, King Abdulaziz University (Saudi Arabia) at 27 ± 1°C,70 ± 5 % RH, and photoperiod 14:10 h (L:D). 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 with10% glucose solution. After a period of 4 days, sugar-fed females were starved for 24 h prior to blood feeding on a membrane feeding apparatus. 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 generation13 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). Early IV instar larvae of generation 12 were used for larval bioassay testing.

2.3 Insecticides

The insect growth regulators (IGRs) tested in the study were:

1. Baycidal 25 wp (triflumuron), Benzamide- 2- chloro- N- [4- (trifluoromethoxy) phenyl] amino] carbonyl], supplied by Bayer Ltd., Germany.
2. Diflox Flowable (diflubenzuron), 1- (4- chlorophenyl) -3- (2,6- difluorobenzoyl)- urea, Provided by, Chemtura Europe Limited, UK.
3. Sumilarv 0.5G (pyriproxyfen), 2- [1- methyl- 2- (4- phenoxyphenoxy) ethoxy]pyridine, supplied by Sumitomo Chem&Co., Japan.

2.4 Larval bioassays

Experiments were conducted following the method [28]. Treatments were carried out by exposing early 4thinstar larvae of *A. aegypti* to various concentrations of the tested compounds for 24 h, in groups of glass beakers containing 100 ml of tap water. Three replicates of 20 larvae each per concentration and control trials were carried out. The larvae were fed following the method [14. Larval mortality was recorded at 24 h post-treatment for the IGRs Baycidal 25%, Diflox Flowable and Sumilarv 0.5G, 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 Baycidal 25%, Diflox Flowable and Sumilarv 0.5G was expressed as the percentage of larvae that do not develop into successfully emerging adults, or the inhibition of adult emergence [14].

2.5 Data analysis

Mortality percentages were corrected according [29]. The dosage mortality data were subjected to Probit analysis according [30]. The concentration that is correspondent to the mortality of 50% and 90% of mosquito larvae in 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 HSD test ($P<0.05$).

3. Results

This study was carried out to investigate the toxicity of three different groups of IGRs on the field strains of *Ae. aegypti* larval and pupal population. The susceptibility levels of the larvae and pupae of *Ae. aegypti* against insect growth regulators are shown in Tables 1, 2 and 3. Among the tested IGRs, the difloxflowable was highly effective against *Ae. aegypti* larvae and pupae, with LC_{50} were 0.178 ppm (larva) and 0.178 ppm (pupae) when compared to the sumilarv i.e. 0.146 (larvae)0.146 (pupae) and baycidal i.e. 0.142 (larva) and 0.115 (pupae) ppm respectively. The results of the larvicidal assay clearly indicate that the percentage of mortality was directly proportional to concentration of the insecticides. Each test included a control group with three 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 varies according to the type of the IGR, its mode of action and the concentration of the active ingredient.

The biological effects of IGR were highly effective against the field strains of *Ae. aegypti* (Table 4). As a general trend, the mortality rates were associated mainly with failure to molt, the fourth larval instar of *Ae. aegypti* to pupation stage by given intermediate larvae-pupae. The post effect of Diflox flowable, Sumilarv and Baycidal on the adult stage of *Ae. aegypti* was evaluated studying percentage of adult emergence. Some *Ae. aegypti* individuals that have succeeded to reach to the adult stage have folded wings (Figure 1). Mortality rates of *Ae. aegypti* exposed to three different IGR were 10.3% to 71.6% (Diflox flowable), 3% to 68% (Sumilarv) and 6% to 85% (Baycidal) for field strain (Table 4). IC_{50} (concentration which to inhibit the emergence of 50%

of adults) was 0.002 ppm (Diflox flowable), 0.0004 ppm (Sumilarv) and 0.047 (Baycidal) for field strains, respectively (Table 5). According to IC_{50} values, Sumilarv 0.5G proved to be more effective against mosquito larvae of *A. aegypti* than Diflox flowable and Baycidal 25 wp by about 5.75 and 117.5 folds, respectively.

Table 1: Susceptibility levels of mosquito larvae and pupae of *Ae. aegypti* to the Diflox flowable (IGR) tested against the field strains in Saudi Arabia

Target	Concentration (ppm)	Mortality (%)± SD[a]	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	χ^2 (*df*=3)
IV instar	Control	0±0	0.178 (0.139-0.298)	0.301 (0.221-0.551)	0.400 *n.s*
	0.02	4±2			
	0.04	8±2			
	0.06	12±3			
	0.08	15±5			
	0.1	20±10			
Pupa	Control	0±0	0.178 (0.139-0.297)	0.056 (0.034-0.070)	0.285*n.s*
	0.02	96±2			
	0.04	92±4			
	0.06	88.6±4.0			
	0.08	85±5			
	0.1	80±10			

[a]Values are mean ± SD of three replicates
No mortality was observed in the control
SD = standard deviation
LC_{50}= lethal concentration that kills 50% of the exposed organisms
LC_{90}= lethal concentration that kills 90% of the exposed organisms
UCL= 95% upper confidence limit
LCL= 95% lower confidence limit
χ^2= chi square
d.f.= degrees of freedom
n.s. = not significant (α=0.05)

Table 2: Susceptibility levels of mosquito larvae and pupae of *Ae. aegypti* to the Sumilarv (IGR) tested against the field strains in Saudi Arabia

Treatment	Concentration (ppm)	Mortality (%)± SD[a]	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	χ^2 (*df*=3)
IV instar	Control	0.0±0.0	0.146 (0.120-0.208)	0.261 (0.202-0.409)	0.086*n.s*
	0.02	8±2			
	0.04	12±2			
	0.06	17±2			
	0.08	22±2			
	0.1	31±1			
Pupa	Control	0.0±0.0	0.146 (0.120-0.208)	0.031 (0.001-0.046)	0.086*n.s*
	0.02	92±2			
	0.05	88±2			
	0.08	83±2			
	0.2	78±2			
	0.4	69±1			

[a]Values are mean ± SD of three replicates
No mortality was observed in the control
SD = standard deviation
LC_{50}= lethal concentration that kills 50% of the exposed organisms

Table 3: Susceptibility levels of mosquito larvae and pupae of *Ae. aegypti* to the Baycidal (IGR) tested against the field strains in Saudi Arabia

Treatment	Concentration (ppm)	Mortality (%)± SD[a]	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	χ^2 (*df*=3)
IV instar	Control	0.0±0.0	0.142 (0.117-0.195)	0.263 (0.206-0.390)	1.243*n.s*
	0.01	8±2			
	0.03	10±2			
	0.05	20±2			
	0.08	24.6±4.5			
	0.10	32.6±2.5			
Pupa	Control	0.0±0.0	0.115 (0.101-0.139)	0.024 (0.006-0.036)	3.380*n.s*
	0.01	92±2			
	0.03	90±2			
	0.05	80±2			
	0.08	75.3±4			
	0.10	54±23.5			

[a]Values are mean ± SD of three replicates
No mortality was observed in the control
SD = standard deviation
LC_{50}= lethal concentration that kills 50% of the exposed organisms
LC_{90}= lethal concentration that kills 90% of the exposed organisms
UCL= 95% upper confidence limit
LCL= 95% lower confidence limit

χ^2= chi square
d.f.= degrees of freedom
n.s. = not significant (α=0.05)

Table 4: The biological effects of the IGR Diflox flowable, Baycidal and Sumilarv on the developmental stages of *Ae. aegypti* field strains in Saudi Arabia

Compound	Concentration (ppm)	Adult emergence (%)	Adult inhibition (%)
Baycidal	0.02	85±5^f	15±5^f
	0.04	60±5^e	40±5^e
	0.06	39.3±4.5^d	60.6±10.5^d
	0.08	22±2^c	78±8^c
	0.1	6±2^b	94±4^b
	Control	4.67±5.03^a	95.33±5.03^a
Sumilarv	0.02	68±8^f	32±8^f
	0.04	40±10^e	60±10^e
	0.06	26±6^d	74±6^d
	0.08	13±3^c	87.3±3.0^c
	0.1	4±1^b	96±1^b
	Control	3±1^a	97±1^a
DifloxFlowable	0.01	71.6±7.6^f	28.3±7.6^f
	0.03	50±5^e	50±5^e
	0.05	22±2.6^d	78±2.6^d
	0.08	15±5^c	85±5^c
	0.10	10.3±4.5^b	89.6±4.5^b
	Control	3.33±0.57^a	96.67±0.57^a

Within each treatment, values followed by the same letter(s) were not significantly different (ANOVA, Tukey'sHSD, α = 0.05).

Table 5: Susceptibility of the 4th larval stage of *A. aegypti* to delayed effects of different insecticides

Compound Tested	IC*	Concentration (ppm)	95% Upper Confidence limit (ppm)	95% Upper confidence limit (ppm)	Slope	χ^2**
Baycidal	50	0.047	0.0433	0.0513	3.64	5.73n.s.
	90	0.107	0.0948	0.1256		
DifloxFlowable	50	0.002	0.0013	0.0033	2.03	8.28n.s.
	90	0.01	0.0076	0.024		
Sumilarv	50	0.0004	0.0003	0.0005	1.77	0.87n.s.
	90	0.0021	0.0016	0.0028		

*Concentration inhibiting the emergence of 50 or 90% of adults
**Not significantχ^2 indicates homogeneity of results (P>0.05)

4. Discussion

Dengue is currently considered the most important arbovirus for Public Health and has been reported in several countries as large epidemics with high rates of morbidity and mortality [31]. Hence, the present study was performed to determine the susceptibility of the dengue vector *Ae. aegypti* to IGRs in Saudi Arabia. IGRs are a special new class of insecticides which influence insect mortality and growth inhibition in an environment friendly way [32, 33]. In this research, IGRs Difloxflowable, Sumilarv and Baycidal showed varying levels of toxicity against 4th instar larvae and pupae of dengue vector, *Ae. aegypti*. The results from bioassays, the IGR diflox flowable more effective, with LC_{50} = 0.142 ppm, over sumilarv and baycidal. Results revealed that the variation in the larval mortality increased correspondingly with the increase in the insecticide concentration [28]. Further, the highest larval mortality was observed for diazinon, with LC_{50} = 0.352 3 mg/L, followed by propoxur and cyfluthrin [32]. In addition, Alsobhi *et al*. [34] have reported that the slow release formulation of Natular DT was more effective against *Culexpipiens* over Tap 60 and VectoBac granule of about 1.3 and 5.8 times. Indeed, mortality in the pupal stage and damage to adult emergence or lost their antennae, mouthparts and legs attached in the pupal exuvia was also observed (Figure 1). In agreement with our findings, Mahyoub [35] have reported that the IGRs alsystin and pyriproxyfen highly effective against 4thinstar larvae and puape of *Ae. aegypti*. Further, various sythetic IGRs such as diflubenzuron, lufenuron, triflumuron, novaluron, and methoprene have been used against mosquito and different insect pest [36, 37]. Also, Suman *et al*. [38] have reported that the ovicidal properties of IGRs such as azadirachtin, diflubenzuron and pyriproxyfen against *Aedes albopictus*, *Ae. aegypti*, *Ae. atropalpus* and *Culex pipiens*.

Also, this study demonstrated an effective adult emergence inhibition of *A. aegypti*. The results showed that Sumilarv formulations were more effective (IC_{50} = 0.0004ppm) if compared to Diflox Flowable (IC_{50} = 0.002ppm), and Baycidal (IC_{50} = 0.047 ppm) by about 5.75 and 117.5 folds. For instance, Khan *et al*. [33] have reported that the IGRs, pyriproxyfen 1.0 WDG was found best over pyriproxyfen 0.5 WDG and methoprene that exhibited significantly high emergence inhibition against *Culex* and *Aedes* spp. Another study by Belinato *et al*. [39] highlighted that the triflumuron was effective in emergence inhibition (EI) of *Cx. quinquefasciatus* ($EI_{50=}$ 5.28 µg/L) and *Ae. albopictus* ($EI_{50=}$ 1.59 µg/L). Also, Batra *et al*. [40] studied that the inhibition of adult mosquitoes was 80% in one week post-application at 0.25 ppm, but 100% inhibition of emergence remained up to five weeks at 1 ppm. In addition, the adult emergence was inhibited completely in *Spodoptera litura* (F.) with diflubenzuron at 50 ppm and in *Culex quinquefasciatus* with 23 diphenylureas chitin inhibitors at 1 ppm [41]. Adult emergence inhibition suggested a general toxic effect of the IGRs, which was found to be dose dependent. The metamorphic abnormalities like larval inability to moult to

next stage and larval pupal intermediates noticed were higher when compared to control group. Inability of adults to shed completely its exuvia, which remained attached to its appendages, was also noticed. The treated adult could not fly above normal level and rested for longer period on the water surface when compared to untreated adult mosquitoes. In this context of observation, exposure of larvae (all three vector mosquito species) to aqueous extract resulted in death at larval-pupal moult and pupal-adult eclosion suggesting inhibition of molting process [42].

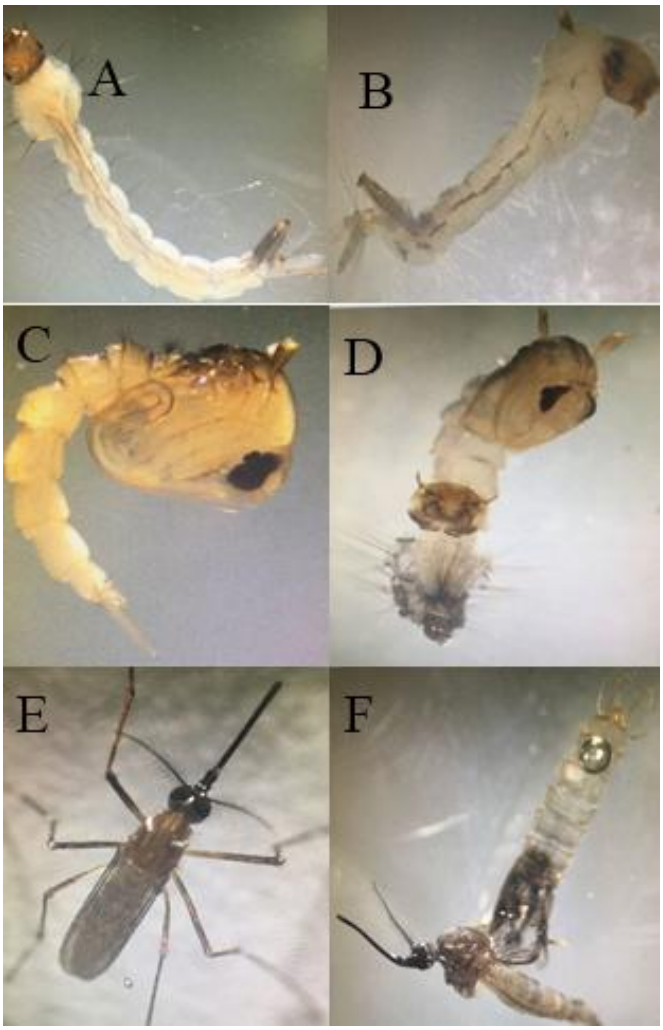

Fig 1: Morphological abnormalities in the developmental stages of *Ae. aegypti* after treatment with Baycidal or Sumilarv or diflox flowable **a**: normal larvae (control), **b**: Larval–pupal intermediate showing larval siphon; **c**: normal pupae **d:** Pupal-adult intermediate with poorly development adult characters**; e:** normal adult; **f:** Incompletely emerged adult with legs attached to the exuviae Curved tarsi in the dead adult.

5. Conclusions

In conclusion, the IGRs (Diflox flowable, Sumilarv and Baycidal) were highly effective in inducing several morphogenetic abnormalities and developmental arrest in the field strain of dengue vector *Ae. aegypti.* Therefore, the selective IGRs may provide excellent potential for control *Ae. aegypti* population, including resistant populations.

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