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Molting inhibitory and lethal effect of two Juvenile Hormone Analogues on *Culex pipiens* L

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Abstract
Culex pipiens is a dominant mosquito species in Saudi Arabia and it is responsible for the transmission of several diseases to human. One way to control *Cx. pipiens* is to use conventional insecticides which have an effective role in reducing mosquito population. However, this control has been accompanied with some public concern and mosquito resistance of insecticides. Therefore, new insecticides have been developed such as Insect Growth Regulators (IGRs) which are safer on human and environment. The present study aims to evaluate two IGRs compound against the *Cx. pipiens*. Larvae were exposed to several concentrations of Difox flowable and Moskill 4G. The lethal concentration required to kill 50% of larvae was 0.0022 in Difox flowable and 0.1182 ppm in Moskill. As only 0.01 ppm of Difox flowable caused 95% of adult inhibition compared to 0.5 ppm of Moskill which caused 91%. Moreover, morphological deformations were observed such as contraction of body segments.

Keywords: *Culex pipiens*, filariasis, insect growth regulators, Moskill 4G, Difox Flowable, Adult inhibition, Deformations.

1. Introduction
Culex pipiens L. is one of the most common mosquitoes and has a worldwide distribution, which cover all the temperate regions of the world [1]. *Cx. pipiens* is also a vector of several diseases that are transmitted to hosts through biting behavior [2]. For example, it is considered the primary vector of filariasis that causes extreme swelling in the arms or legs, known as" Elephantiasis" and might lead to permanent disability [3]. Kilpatric *et al*. [4] mentioned that the *Cx. pipiens* and *Cx. restuans* are responsible for 80% of human West Nile Virus (WNV) infections in the north-eastern region of the United States. In Saudi Arabia *Culex spp*. was found to be more abundant compared to other medically important genera such as *Aedes spp*. and *Anopheles spp.* [5, 6, 7]. It was reported that some cases of filariasis were recorded in the south-eastern region of Saudi Arabia and *Culex. pipiens* mosquitoes might act as a potential vector of this disease [8, 9]. During 2000-2001 Ministry of Health in Saudi Arabia reported that 516 cases with 87 deaths of Rift Valley fever occurred in the south-western region of the kingdom [10], suggesting these three species; *Cx. pipiens complex*, *Cx. tritaeniorhynchus* and *Ae. vexans arabiensis* are epidemic vectors of RVF in Saudi Arabia [11]. Furthermore, surveillance and control of the targeted insects including mosquitoes are the substantial way to protect human and reduce risk of mosquito population in environment. In Saudi Arabia chemicals insecticides have been intensively applied for controlling malaria and dengue fever and other Vector-borne diseases, as they are toxic to their target. For example, Pyrethroids and Organophosphate groups were used and proved to be effective in reducing mosquitoes populations [12]. However, this success of mosquito control has been accompanied with some public concerns regarding the human health and environment. Also some mosquito species exhibited a resistance against these compound as a result of regular and frequent use [13]. Therefore, new and eco- friendly insecticides have been developed such as InsectGrowth Regulators (IGRs) which are safer on human, animals and environment. These are known as non-conventional insecticides or the three-generation insecticides that are supposed to take place widely. These types of insecticides cause adverse effect on insect's growth and act as an inhibitor for chitin synthesis [14]. Other type of IGR is called Juvenile Hormone Analogues (JHAs) which interfere and disrupt the endocrine system of insects [15] leading to an abnormal development and early mortality [14]. For example, a recent study showed that treating the 4th instar larvae of *Cx. pipiens* with IGR Dudim (0.0040 ppm) caused percentage mortalities from 5% to 21% [16]. Alsolami *et al.* [17] conducted a study on *Ae. aegypti* and found that the IGRs

Sumilarv (0.02 ppm) and Dudim (0.004 ppm) led to 89.2% and 93% inhibition of adult emergence respectively and the value of IC50 was 0.004 in sumilarv while only 0.00038 in dudim. Moreover, the 4th instar larvae of Ae. *aegypti* was exposed to IGRs Alsytin and Pyriproxyfen, ranged from 0.0002-0.004 ppm and 0.002-.02 ppm, and caused mortality from 3-25% and 8-17% respectively. These results also showed that some adult mosquitoes did not emerge completely or emerged without antennae or deformed mouthparts or legs were adhered in the pupal exuviae [18]. In this study, we aim to evaluate two IGRs insecticides in terms of causing mortality or an abnormal growth development in the 4th instar larvae of *Cx. Pipien* instead of using conventional chemical insecticides, which have harmful effects on human and environment.

2. Materials and Methods

2.1 Samples location and experiment period: A field adult strain of *Cx. pipiens* (L.) was collected from Baljurashi province (south of Saudi Arabia) and then maintained under laboratory conditions of 27 ± 1 °C and 70 ± 5% R.H., with 14: 10 (L : D), in Experimental Station of Dengue fever Mosquitoes, King Abdulaziz University during winter 2016.

2.2 Mosquito rearing: These adults were fed on animal blood (pigeon) for three days with average of two hours a day. The following generations were also reared to get abundant larvae for experimental purpose.

2.3 The compound test and experiment procedure: Two non-conventional insecticides (IGRs group) were used which are Difox flowable (a.i: Diflubenzuron 10%) and Moskill 4G (a.i: Diflubenzuron 4%). Larval susceptibility tests were conducted according to the method of WHO. Treatments started by exposing the 4th instar larvae of *Cx. pipiens* to several concentrations of the two IGRs chemicals for 24 hr, using waxed paper cups (400 ml capacity) containing 100 ml of tap water. 20 larvae were placed into each cup with various concentration (Difox flowable: 0.001, 003, 005, 0.008 and 0.01 and Moskill 4G: 0.05, 0.08, 0.1, 0.3, 0.5) as well as control trials were set up. Besides that, all larvae were provided with usual food to avoid starvation cases. The experiment was replicated five times and larval mortalities were recorded after 24 hr post treatment. In addition, the adult emergence and abnormal developments were recorded on a daily basis.

2.4 Data analysis: Data was analysed to calculate the percentage of larval mortalities and inhibition of adult emergence was compared with control mortalities by using Abbott's formula [19], also the IC_{50} (Concentration in which inhibit the emergence of 50% of adults) was determined for treatment trials.

3. Results and Discussion

Initially, the two IGRs Difox flowable and Moskill 4G showed varied biological effects against the 4th instar larvae of *Cx. pipiens* as shown in Table 1. In IGR Difox Flowable the effective concentration ranged from 0.001 - 0.01 ppm that caused larval mortality from 10 – 30% and the corresponding of adult inhibition was 29- 95% respectively. Whereas, higher concentrations of Moskill 4G (0.05 – 0.5 ppm) were required to exhibit 5 – 19% mortality with corresponding adult inhibition of 20 – 91% respectively. Similar results were found that other IGRs such as Alsystin and pyriproxyfen did not show high mortilatiy at larval which ranged from 3-25% and 8-17% respectively [19]. In addition, these IGRs showed low percentage of pupae mortality, whereas Bridges *et al.* [21] mentioned that most pupae died before the adult emerged and that might have occurred due to using high concentration of IGRs compound or because of the variation of active ingredient. These results also showed that only 0.01 ppm of Difox Flowable has given high percentage of adult inhibition (95%) which correspond with other study that found 0.02 ppm of IGR Sumilarv led to 89.2% adult inhibition [17].

Furthermore, results also showed the value of IC_{50} was 0.0022 in Difox Flowable and 0.1182 in Moskill 4G which indicate that The Difox Flowable is more effective against *Cx. pipiens* than Moskill 4G by about 53 fold (Table 2) and both samples of the treated larvae exhibited homogeneity in responding to different concentrations of those IGRs compound as shown in Fig.1. Other study showed that less concentrations of IGRs such as Dudim and Baycidal killed 50% of larvae when the concentrations were only 0.0001 and 0.0003 pmm consequently [16]. However, the present strain of mosquito might require less concentration of dudim and baycidal, as pest control in Baljurashi is limited compared to Jeddah province in which lots of insecticides have been used frequently and this provides more tolerance and resistance against them. In addition, the results showed some morphological deformations on the treated larvae; for instance, emergence of intermediate stage between larvae and pupa or between pupa and adult, body segments contraction, body pigmentation (dark or black spots), deformed mouthparts and elongation of larvae neck and albino larvae, Fig.2. These observations are correspondent to other laboratory studies that used the IGRs group [18, 16] which confirm the effectiveness of IGRs in controlling insects.

Table 1: The biological effect of IGRs Difox Flowable and Moskill 4G on the development of the 4th instar larvae of *Cx. pipiens*.

IGRs compound	Effective concentrations (ppm)	Larval Mortality* (%)	Pupation (%)	Adult emergence (%)	Adult Inhibition (%)
Difox Flowable	0.001 -0.01	10 – 30	90 - 70	71 - 5	29 - 95
Moskill 4G	0.05 - 0.50	5 – 19	95 - 81	80 – 9	20 - 91
control	0	2	98	96	4

** Five replicates, 20 larvae each*

Table 2: The lethal concentration required to kill 50% of larvae (IC_{50}) in IGRs Difox Flowable and Moskill 4G.

IGRs Compound	Con. IC_{50} ppm	Lower limit ppm	Upper limit ppm	Slope	Chi
Diflox Flowable	0.0022	0.0018	0.0026	1.99	7.377
Moskill 4G	0.1182	0.0695	0.1897	1.9865	10.3678

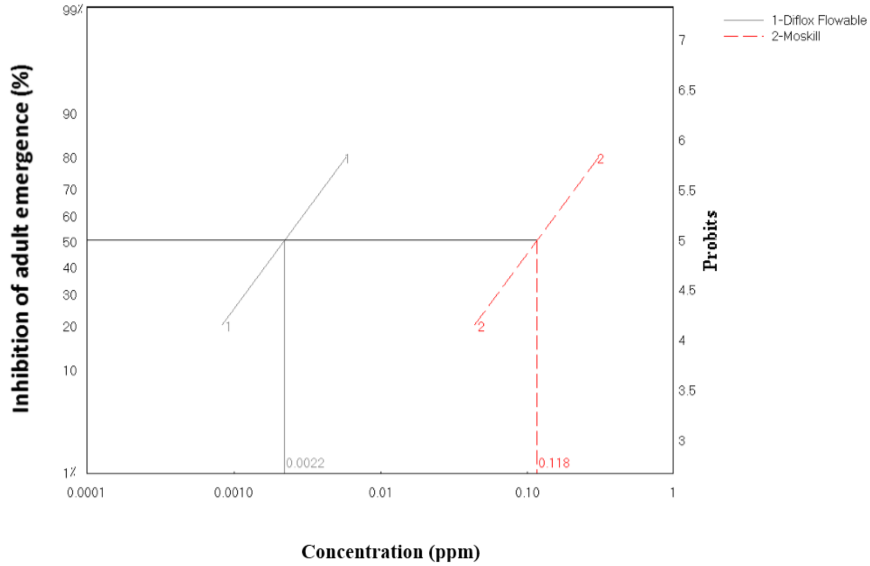

Fig 1: Laboratory toxicity line of the two IGRs Difox Flowable and Moskill 4G.

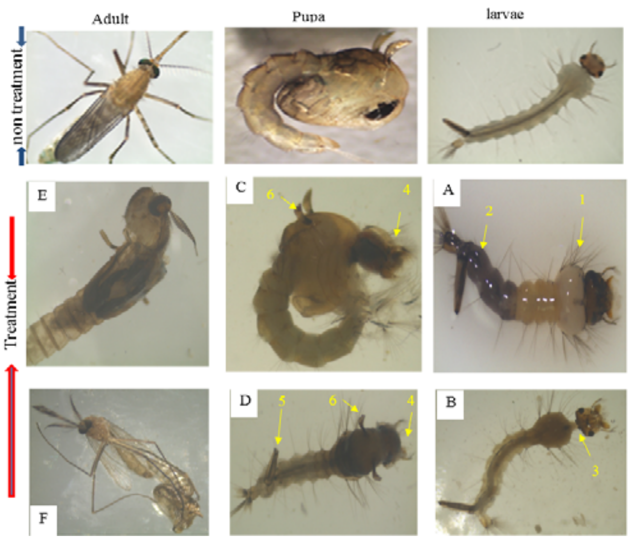

Fig 2: Some biological effects of the compounds tested against *C. pipiens*

A) misshapen larvae, deformed thorax (1) and abdomen pigmentation (2)
B) neck elongation (3)
C) & D) Intermediate stage between larvae and pupae:
4) Larval head capsule
5) Terminal segments of larval siphon.
6) Cephalothorax of a pupae with respiratory trumpets.
E) Incomplete adult emerged with antenna, mouth parts and legs attached in the pupal exuvia
F) Adult completely emerged but it is unable to fly, legs attached in the pupal exuvia.

4. Conclusion

A bioassay test was conducted for IGRs Difox Flowable and Moskill 4G to evaluate their effectiveness against the 4th instar larvae of *Cx. pipiens*. The IC50%was 0.0022 in Difox Flowable and 0.1182 ppm in Moskill4G which indicate that the Difox Flowable is more effective by about 53 fold. Only 0.01 ppm of Difox Flowable caused 95% of adult inhibition compared to 0.5 ppm of Moskill4G, which caused 91%. Moreover, morphological deformations were observed such as contraction of body segments. This study illustrated a safer approach to control vector-borne diseases using low concentration of IGRs that leads to harmful effect on mosquitoes and proved to be more eco-friendly method in comparison to conventional insecticides.

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