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EFFECT OF INSECT GROWTH REGULATING COMPOUNDS 'METHOPRENE' ON OVIPOSITED AND SHORT TIME EXPOSURE AGAINST A SELECTED THREE VECTOR MOSQUITOES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author PR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MM managed the analyses of the study. Author MS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The mosquitoes are the principal vector for many of the vector in borne diseases affecting human beings and other animals. The aim of the present study is to evaluate the Oviposited activity of Insect Growth Regulator (IGR) Methoprene and Short time exposure of three species of mosquitoes, viz., *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston).

Keywords: Oviposited; short time exposure; methoprene.

1. INTRODUCTION

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. They act as vectors for several human diseases like malaria, yellow fever, dengue fever, chikungunya fever and filariasis in different parts of the world [1].

Mosquito control is critical for managing the spread of disease agents and is based primarily on the use of chemical insecticides. Drawbacks associated with widespread use of these conventional insecticides for mosquito control have not only resulted in attaining physiological resistance in mosquito strains but also caused long-term harmful effects on non-target organisms and other environmental components [2,3,4]. Therefore, more attention has been recently paid to the use of non-conventional insecticides such as bioinsecticides, insect growth regulators (IGRs) for controlling mosquito vectors around the world [5,6,7].

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Most IGRs provided good larvicidal effect for the control or targeted mosquitoes, depending on the active ingredients, formulations, dosages and the habitats treated Pyriproxyfen a new generation of IGR. It is a juvenile hormone analogue and a relatively stable aromatic compound. It functions as an insecticide by overloading the hormonal system of the target insect, ultimately affecting its egg production, brood care and other social interactions, and inhibiting its growth [8]. Pyriproxyfen works well against public health insects like houseflies and mosquitoes [9]. Pyriproxyfen is reported to exhibit 95% inhibition of the emergence of mosquito larvae and its effects on mosquito larvae having lasted for two months after application [10]. Although the treated mosquito larvae continue to pupate, however, their emergence is inhibited by the action of pyriproxyfen [11]. The influence of short time exposure to an insect growth regulator, novaluron on mortality and adult emergence of *Cx.quiquefasciatus*, *Ae.aegypti* and *An.stephensi*was also reported [12] (Rajasekar and Jebanesan, 2011).

An insect poisoned with a CSI cannot make chitin and so cannot moult. Because moulting must take place for the insect to reach the adult stage, a CSI poisoned insect also cannot reproduce. Eventually, the insect dies. Because humans do not make chitin, CSIs are not considered toxic to humans. However, CSIs are very toxic to any organism that has an exoskeleton, such as crustaceans (shellfish), and should be used with great care, if at all, in areas where they could contaminate the environment [13]. The inhibition of *Aedes aegypti* on simulated domestic unter-storage containers by using a controlled release formulation of Pyriproxyfen reported by [14].

Anopheles mosquitoes breed in clean water collections. Therefore, breeding increases dramatically in the rainy season because many artificial water collections occur. *Anopheles* species are the most important species as they are capable vector for malaria parasites. Malaria parasite alone can kill more than a million people every year [15]. The estimated five hundred fifteen million cases of human malaria each year are generally caused by four species, including *Plasmodium falciparum, P. ovale, P. vivax*and *P. malariae*, are transmitted by the bites of female *Anopheles* mosquitoes [16] Globally, malaria remains a leading cause of ill health, causing an estimated two hundred forty three million cases of clinical malaria and eight hundred sixty three thousand deaths [17]. More than 85% of malaria cases and 90% of malaria deaths occur in Africa, south of Sahara. In Africa, the vast majority of cases and deaths occur in young children. Approximately half of the world's population is at risk of malaria, particularly those living in lower-income countries. It infects more than five hundred million people per year and kills more than one million [18].

Culex mosquitoes are painful and persistent biters and are responsible for causing filariasis to man. These mosquitoes are very common in Indian sub-continent. Lymphatic Filariasis, commonly known as elephantiasis, is a painful and profoundly disfiguring disease. The disease is caused by three species of nematode thread-like worms known as *Wuchereria Bancrofti*, *Brugiamalayi*and *Brugiatimori*. An estimated one hundred twenty million people in tropical and subtropical areas of the world are infected with lymphatic filariasis; of these, almost twenty five million men have genital disease (most commonly hydrocele) and almost fifteen million, mostly women, have lymphoedema or elephantiasis of the leg. Approximately 66% of those at risk of infection live in the WHO South-East Asia Region and 33% in the African Region [19].

Aedes mosquitoes on the other hand are also persistent biters. *Aedes aegypti*is responsible for spreading Dengue and Chikungunya. Dengue is prevalent throughout the tropics and subtropics. The World Health Organization estimates that around 2.5 billion people are at risk of dengue. Infections have dramatically increased in recent decades due to increased urbanization, trade and travel. No effective drug or vaccine is available so far. Only solution is to prevent the disease-carrying mosquito from breeding and biting humans. Dengue is the most important mosquito spread viral disease and a major international public health concern. It is a self limiting disease found in tropical and sub- tropical regions around the world, predominantly in urban and semi-urban areas. DF/DHF is caused by dengue virus which belongs to genus Flavivirus, family Flaviviridae and includes serotypes 1, 2, 3 and 4 (Den-1, Den-2, Den-3 and Den-4) [19].

Therefore, the present study was undertaken to investigate the Effect of Insect Growth Regulating Compounds ‘Methoprene’on Oviposited and short time exposure against a selected three vector mosquitoes*i*. This study may help to predict and guide the development of IGRs with more advantages and higher potency within the legal confers as other insecticides in the coming years.

2. MATERIALS AND METHODS

2.1 Test Insects

Cx. quinquefaseiatus, *Ae. Aegypti* and *An. Stephensi* mosquitoes were obtained from a stock colony being

maintained in the insectary at 27±1&C and 75±5% relative humidity at laboratory, Department of Zoology, Jamal Mohamed College.10%sucrose was provided to females. Female mosquitoes were fed on rabbit blood for 4-5 days. Five days after blood feeding, the gravid female mosquitoes were used for bio assay experiments.

2.2 Test Chemicals

IGR compounds namely Methoprene chemically known as isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate and an isopropyl ester urea was received as gratis (10% EC formulation Makhteshim Agan of North S America).

2.3 Contact Effect on Gravid and Oviposited Female Mosquitoes to Methoprene Treated Water

In this experiment, one bowl with 200 ml of the test solution at the desired concentration and another with tap water and ethanol (control) were kept separately in two mosquito cages. Observations on the mortality of gravid and oviposited females were made after 16-20 h. Since the egg laid and the dead gravid females in the test solutions were found sunk the bottom of the bowl, the possible role of surface tension of water with varying concentration of Methoprene in comparison with the technical material of Methoprene was determined by using the method described [20]. Analysis of variance (ANOVA) was performed on the percentage mortality to determine significant ($P<0.05$) treatment differences.

2.4 Effect of Minimum time Exposure on Adult Emergence

Twenty-five fourth instar of three species of mosquitoes were exposed to varying concentrations of Methoprene for 10, 20, 30, 50 and 60 minutes. The larvae were exposed to 250 ml of departure with desired concentration of Methoprene (0.0002–0.2 mg/l) in 500 ml beaker. Six replicates along with a control were maintained. The larvae were washed in running water after the required exposure time and then transferred to beakers with untreated tap water. Larval food was provided till pupation. Observations on the responses of treated larvae after limited exposure (such as larval mortality, larval-pupal intermediate, pupal mortality, pupal-adult intermediate or incompletely emerged dead adults and normal adult emergence) were recorded and expressed in percentage. Percentage inhibition in adult emergence at different exposure times and at various dosages were analysed by Analysis of variance (ANOVA) after transforming the percentages to arcsine values to normalize the variance [21].

3. RESULTS

3.1 Methoprene

3.1.1 Contact effect on gravid and oviposited females

The mortality rate of the gravid and oviposited females of the selected three species of mosquitoes in response to contact with treated water of Methoprene are given in Table 1. The results showed that the mortality of both the gravid and ovipositor female mosquitoes display significant ($P<0.05$) variation among three species. The death of the gravid females and oviposited females on the water surface was found to be significantly ($P<0.05$) more in higher dosages.

In *Cx. quinquefasciatus*, the mortality of the gravid females at the dose of Methoprene 0.01mg/l was not found to vary significantly ($P>0.05$) from that in control. The number of dead gravid females was significantly ($P<0.05$) more than two times at dosages 0.1 and 1.0 mg/l whereas the oviposited females showed a significantly ($P<0.05$) higher mortality at 1.0 mg/l.

In *Ae. aegypti*, the mortality of the gravid females at the dose of 0.01mg/l was not found to vary significantly ($P>0.05$) from that in control. The number of dead gravid females was significantly ($P<0.05$) more than three times at dosages 0.1 and 1.0 mg/l whereas the oviposited females showed a significantly ($P<0.05$) higher mortality at 1.0 mg/l.

In *An. stephensi*, the mortality of the gravid females at the dose of Methoprene 0.01mg/l was not found to vary significantly ($P>0.05$) from that in control. The number of dead gravid females was significantly ($P<0.05$) more than four times at dosages 0.1 and 1.0 mg/l whereas the oviposited females showed a significantly ($P<0.05$) higher mortality at 1.0 mg/l.

By analysing one sample t-test the effect of Methoprene on contact effect of gravid and oviposited females of three mosquitoes *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* showed a significant ($P<0.05$) reduction.

3.1.2 Short time exposure of mosquitoes of Methoprene

The influence of short term exposure (10-60 min) on percentage adult emergence of *Cx. quinquefasciatus*,

Ae. aegypti and *An. stephensi* was evident when fourth instar were treated with Methoprene at varying dosages (Tables 2 to 4). The percentage emergence inhibition obtained in *Cx. quinquefasciatus* was found to be significant (F=186.924, P=0.000) when larvae were exposed for short time (10-60 min) at varying dosages. While complete emergence inhibition was evident when larvae were exposed for 10-60 min at 0.002-0.2 mg/l, only about 75% inhibition in adult emergence was obtained at 0.0002 mg/l for the same exposure time. Significant reduction in adult emergence was evident in *Ae. aegypti* when fourth instar were exposed for 10-60 min at various concentrations (F=239.279, P=0.000). The exposure time (10-60 min) considerably influenced (F=275.568, P=0.000) the adult emergence of *An. stephensi* larvae when treated with Methoprene at varying concentrations.

From the overall analysis the short time exposure (10-60 min) values it is evident that all stages of *Cx. quinquefasciatus* are comparatively more susceptible than *Ae. aegypti* and *An. stephensi* respectively. On comparison of short time (10-60 min) exposure of both IGR compounds against adult mosquitoes species, the Methoprene compound showed more emergence inhibition effect.

4. DISCUSSION

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis etc., causing millions of deaths every year [22]. Human beings are compelled to fight against them using available technical ornaments. There was initial success in controlling vectors by using synthetic insecticides. Since 1900, the World Health Organization has warned about the possible emergence and reemergence of arthropod-borne disease due to combined human, biological, environmental and climatic factors [19].

The most effective way to combat with this mosquito infestation is the prevention of mosquito breeding through the use of larvicides, synthetic insecticides such as organophosphates have been used as larvicide in several countries for the last 30 years [23]. However, one major drawback with the use of these chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment [24].

Moreover, increasing awareness of the environmental hazards of these chitin synthetic insecticides and development of resistance has forced the scientists to look for other alternatives of vector control. Hence, biologically active IGR compounds with complex chemical substances of different composition found with selective anti-larval and anti-adult properties are being paid attention to replace the synthetic ones.

The results of present study are interesting. The findings indicate the importance of traditional knowledge in science. The selected two IGR compounds Methoprene were tested against the mosquito in laboratory condition for ovicidal activity and short time exposure.

4.1 Contact Effect on Gravid and Oviposited Females to Methoprene Treated Water

The mortality rate of gravid females and oviposited females of *An. stephensi* against Methoprene were significant at concentration of 1.0 mg/l. The high degrees of mortality observed in gravid females and ovipositing females soon after coming in contact with Methoprene treated water surface (at concentration higher than 0.1 mg/l) implied the possible contact toxicity of the compound received through the specific sensitive parts of the mosquitoes that enable than the in oviposition. The drowning of the gravid females of all three species of the test mosquitoes and the sinking of the oviposited eggs of *Cx. quiquefasciatus* were also observed. Such disrupted or scattered eggs and sunken eggs did not hatch at all.

The drowning of gravid females or the sinking of the eggs at higher dosages might be due to lowering of surface tension by the treatment of these IGRs. The lowering of surface tension could be due to the present of considerable amount of emulsifiers in the EC formulation of Methoprene [25].

This study inferred the importance of IGRs with deterrent activity in the transmission of mosquito-borne disease. In addition to inhibition of adult emergence achieved by employing IGRs in control operations, mosquitoes can also be deterred from the treated breeding and ovipositing sides. Considerable contact effect observed for Methoprene can affect the completion of the oviposition cycle by killing the oviposited females and disrupt the transmission of the disease pathogen.

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Table 1. Mortality rate of gravid and oviposited females of *Cx. quinquifasciatus, Ae. aegypti* and *An. stephensi* in response to Methoprene treated water

Species	Percentage mortality (Mean ± SE)								t value
	Concentration(mg/l)								
	0.01		0.1		1.0		Control		
	Gravid females	Oviposited females	Gravid females	Oviposited females	Gravid females	Oviposited females	Gravid females	Oviposited females	
Cx. quinquifasciatus	3.55 (1.56)	11.35 (3.52)	32.54 (9.32)	24.32 (10.53)	64.46 (11.25)	32.00 (1.00)	3.26 (1.36)	7.65 (2.65)	2.389
Ae. aegypti	5.24 (3.21)	8.35 (4.31)	28.46 (7.83)	52.56 (11.31)	72.62 (11.65)	60.62 (6.45)	2.34 (1.42)	15.30 (4.32)	2.904
An. stephensi	8.35 (2.65)	15.23 (5.24)	20.36 (4.23)	30.42 (3.2)	86.32 (11.25)	41.00 (0.10)	2.42 (1.00)	15.65 (2.35)	2.144

Values are expressed as Mean ± SE (Parenthesis) on six replicates

Table 2. Effect of short time exposure of Methoprene on the adult emergence of *Culex quinquefasciatus*

Exposure time (min)	Mortality and adult emergence in percentage[a] (mean ± SE) at different dosages									
	0.0002mg/l					0.002mg/l				
	I	**II**	**III**	**IV**	**AE**	**I**	**II**	**III**	**IV**	**AE**
10	12.1	0.0	2.5	0.0	85.0	19.0	44.1	35.0	1.5	0.0
	0.4	0.0	0.1	0.0	0.4	0.2	0.9	0.8	0.1	0.0
20	13.2	0.0	3.8	0.0	82.7	20.4	45.2	32.1	2.0	0.0
	0.3	0.0	0.1	0.0	0.3	0.4	0.3	0.4	0.1	0.0
30	17.1	0.0	6.0	0.0	76.5	43.9	45.6	9.7	0.5	0.0
	0.2	0.0	0.1	0.0	1.0	0.8	0.6	0.2	0.1	0.0
40	24.1	0.0	2.5	0.0	73.2	50.7	42.6	6.0	0.0	0.0
	0.2	0.0	0.1	0.0	0.2	0.5	0.3	0.1	0.0	0.0
50	25.2	0.0	4.3	0.0	70.3	58.5	40.2	1.0	0.0	0.0
	0.1	0.0	0.1	0.0	1.3	0.8	0.8	0.1	0.0	0.0
60	26.8	0.0	5.0	0.0	68.0	83.9	15.0	0.8	0.0	0.0
	0.5	0.0	0.2	0.0	0.4	0.9	0.5	0.1	0.0	0.0
Control	7.5	0.0	2.1	0.0	90.2	4.8	0.0	1.0	0.0	94.0
	0.3	0.0	0.2	0.0	0.4	0.1	0.0	0.0	0.0	0.1
	0.02mg/l					**0.2mg/l**				
	I	II	III	IV	AE	I	II	III	IV	AE
10	21.0	13.2	48.2	16.2	1.1	25.3	56.1	16.2	2.0	0.0
	0.4	0.4	0.9	0.6	0.0	0.5	1.1	0.8	0.0	0.0
20	29.4	21.3	40.2	8.6	0.0	27.9	60.8	10.9	0.0	0.0
	0.5	0.2	0.5	0.4	0.0	0.5	0.7	0.5	0.0	0.0
30	47.8	22.5	21.9	7.5	0.0	43.1	52.4	4.2	0.0	0.0
	0.6	0.7	0.6	0.5	0.0	1.6	1.2	0.1	0.0	0.0
40	54.2	22.3	23.1	0.0	0.0	64.8	32.0	3.0	0.0	0.0
	0.7	0.8	0.6	0.0	0.0	0.9	0.5	0.1	0.0	0.0
50	58.3	29.2	12.2	0.0	0.0	74.8	23.5	1.4	0.0	0.0
	1.0	0.8	0.3	0.0	0.0	0.8	1.1	0.1	0.0	0.0
60	68.5	22.6	8.5	0.0	0.0	87.6	12.0	0.0	0.0	0.0
	0.9	0.6	0.1	0.0	0.0	1.0	0.4	0.0	0.0	0.0
Control	5.9	0.0	1.8	0.0	92.1	5.2	0.0	2.5	0.0	92.0
	0.2	0.0	0.0	0.0	0.1	0.2	0.0	0.2	0.0	0.1

a: n = 400; AE: emerged adults, Values are expressed as Mean ± SE on six replicates

Mortality at: I-Larval Stage: II – Larval-Pupal intermediate stage: III –Pupal stage; IV-Pupal-adult intermediate stage.

0.0002-0.2, Culex quinquefasciatus, F=Value (F=186.924, P=0.000)

Table 3. Effect of short time exposure of Methoprene on the adult emergence of *Aedes aegypti*

Exposure time (min)	Mortality and adult emergence in percentage[a] (mean ± SE) at different dosages									
	0.0002 mg/l					0.002 mg/l				
	I	**II**	**III**	**IV**	**AE**	**I**	**II**	**III**	**IV**	**AE**
10	12.1	57.5	0.0	0.0	0.0	13.5	1.3	2.9	0.0	82.5
	0.3	0.2	0.0	0.0	0.0	0.3	0.2	0.2	0.0	0.6
20	22.8	76.9	0.0	0.0	0.0	16.3	1.1	5.8	6.5	70.0
	0.6	0.9	0.0	0.0	0.0	0.4	0.1	0.1	1.4	1.3
30	56.2	53.5	0.0	0.0	0.0	22.0	0.4	6.5	2.2	68.5
	0.8	0.5	0.0	0.0	0.0	0.4	0.1	0.1	0.1	0.6
40	77.6	22.1	0.0	0.0	0.0	24.2	0.0	8.0	0.6	66.9
	0.7	0.8	0.0	0.0	0.0	0.5	0.0	0.3	0.1	0.8
50	99.3	0.3	0.0	0.0	0.0	30.2	2.1	3.0	1.9	62.5
	0.9	0.1	0.0	0.0	0.0	0.5	0.2	0.1	0.2	0.7
60	100.0	0.0	0.0	0.0	0.0	29.6	1.4	9.0	2.2	57.4
	0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.3	0.1	1.0
Control	1.9	0.0	1.2	0.0	96.0	4.2	1.1	4.0	0.0	90.5
	0.1	0.0	0.1	0.0	0.1	0.3	0.2	0.2	0.0	0.4
	0.02mg/l					**0.2mg/l**				
	I	II	III	IV	AE	I	II	III	IV	AE
10	15.8	71.1	10.2	2.5	0.0	17.1	54.5	13.9	9.2	4.9
	0.2	0.5	0.3	0.3	0.0	0.2	0.5	0.1	0.4	0.4
20	33.6	44.9	16.1	5.0	0.0	20.4	50.1	17.9	8.4	3.0
	0.4	1.1	0.7	0.3	0.0	0.3	0.4	0.2	0.4	0.2
30	34.1	45.7	16.2	3.9	0.0	31.7	48.9	11.9	7.3	0.0
	0.5	0.7	0.5	0.2	0.0	0.5	0.5	0.3	0.2	0.0
40	37.8	48.9	9.9	3.1	0.0	39.3	38.1	13.2	9.1	0.0
	0.6	0.5	0.3	0.2	0.0	0.8	0.6	0.5	0.3	0.0
50	38.7	49.6	5.9	5.8	0.0	50.9	25.5	16.2	7.0	0.0
	0.5	0.7	0.5	0.5	0.0	0.5	0.7	0.6	0.4	0.0
60	55.2	38.2	6.1	0.5	0.0	66.2	17.2	13.2	3.2	0.0
	0.2	0.3	0.3	0.1	0.0	0.7	0.5	0.4	0.2	0.0
Control	3.0	0.0	0.6	0.0	96.2	2.5	0.0	0.0	0.0	97.2
	0.1	0.0	0.1	0.0	0.6	0.3	0.0	0.0	0.0	0.3

a: n = 400 AE: emerged adults, Values are expressed as Mean ± SE on six replicates
Mortality at: I-Larval Stage: II – Larval-Pupal intermediate stage: III –Pupal stage IV-Pupal-adult intermediate stage.
Concentration 0.0002-0.2, Aedes aegypti, F=Value (F=239.279, P=0.000)

Table 4. Effect of short time exposure of Methoprene on the adult emergence of *Anopheles stephensi*

Exposure time (min)	Mortality and adult emergence in percentage[a] (mean±SE) at different dosages									
	0.0002mg/l					0.002mg/l				
	I	II	III	IV	AE	I	II	III	IV	AE
10	14.0	0.0	5.3	1.0	79.6	20.6	0.0	19.0	44.5	15.5
	0.2	0.0	0.3	0.1	0.6	0.4	0.0	0.2	0.8	0.6
20	16.3	0.0	12.0	0.0	71.6	22.4	0.0	34.9	27.5	8.9
	0.5	0.0	0.5	0.0	0.7	0.8	0.0	0.9	1.1	1.0
30	20.4	0.0	14.2	0.0	64.3	55.7	1.0	21.2	21.5	0.2
	0.5	0.0	0.6	0.0	0.5	0.5	0.1	0.7	0.8	0.1
40	25.2	0.0	16.3	0.0	58.4	84.8	1.5	12.9	0.5	0.0
	0.4	0.0	0.4	0.0	0.6	0.3	0.1	0.3	0.1	0.0
50	28.4	0.0	14.7	0.0	56.8	89.8	1.9	4.9	0.0	0.0
	0.5	0.0	0.5	0.0	0.7	0.4	0.2	0.2	0.0	0.0
60	32.3	0.0	12.3	0.0	54.3	98.3	0.2	1.1	0.0	0.0
	0.5	0.0	0.3	0.0	0.6	0.2	0.1	0.2	0.0	0.0
Control	1.3	0.0	1.5	0.0	97.0	3.0	0.0	1.0	0.0	95.7
	0.1	0.0	0.2	0.0	0.3	0.2	0.0	0.1	0.0	0.3
	0.02mg/l					0.2mg/l				
	I	II	III	IV	AE	I	II	III	IV	AE
10	25.5	0.0	20.5	25.7	27.9	27.4	0.1	26.5	20.0	24.7
	0.6	0.0	0.4	0.5	0.7	0.8	0.1	1.0	0.5	0.3
20	26.7	5.5	20.1	28.2	19.2	18.5	0.0	24.6	19.8	36.9
	0.5	1.2	0.5	1.0	0.6	1.0	0.0	1.3	1.0	2.0
30	29.4	0.0	27.5	28.0	15.0	41.2	0.0	20.1	23.2	15.1
	1.2	0.0	1.2	0.6	0.3	1.4	0.0	0.5	0.9	0.6
40	34.2	0.0	25.0	27.2	13.2	50.2	0.0	13.2	28.1	8.3
	0.9	0.0	1.2	1.0	0.4	1.5	0.0	0.6	1.2	0.3
50	44.3	3.0	19.5	24.0	8.9	65.2	0.0	12.2	8.1	14.2
	0.4	0.3	0.7	0.8	0.2	1.2	0.0	0.4	0.5	1.0
60	47.1	26.2	17.0	4.3	5.2	89.1	0.9	3.2	5.0	1.6
	1.1	1.5	0.5	0.3	0.2	1.0	0.3	0.5	0.7	0.5
Control	1.0	0.0	0.9	0.0	97.7	1.5	0.0	5.1	0.0	93.3
	0.1	0.0	0.1	0.0	0.2	0.1	0.0	0.5	0.0	0.2

a: n = 400 AE: emerged adults, Values are expressed as Mean ± SE on six replicates
Mortality at: I-Larval Stage: II – Larval-Pupal intermediate Stage: III –Pupal stage IV-Pupal-adult intermediate stage.
Concentration 0.0002-0.2, Anopheles stephensi , F=Value (F=275.568, P=0.000)

Variations in the susceptibility levels of *A. aegypti*against the test IGRs may be attributed to the differential mode of action of the present IGRs and its effective concentrations. Laboratory and field studies in this respect were carried out by several investigators using different formulations of IGRs against various mosquito species such as the IGR triflumuron against *C. quinquefasciatus* in polluted water [26]. The IGRs Diflubenzuron and Methoprone against *A. aegypti* [27]. The IGRs pyriproxyfen and Methoprene against *A. albopictus* and *C. Quinquefasciatus* [28]. The IGR pyripoxyfen against *C. quinqueasciatus* in catch basins [29]. The IGR Halofenozide towards *C. pipiens* [30].

4.2 Effect of Minimum Time Exposure on Adult Emergence

This IGR induced notable abnormalities in larvae, pupae and adults of the three species of *Cx. quiquefasciatus, Ae. aegypti*and *An. Stephensi*, similar to those often induced by other IGRs [31,32,33].

From the observation the effect of short time 10-60 minutes exposure periods of concentrations of Methoprene 0.0002 to 0.2 against first instar, second instar, third instar and fourth instar and adult emergence of three vector species of *Cx. quiquefasciatus,Ae. aegypti* and *An. stephensi* were tested. The maximum mean percentage of mortality and emergence inhibition were observed in the first instar stage of *An. stephensi* by Methoprene compounds and maximum emergence inhibition in the *Cx. quiquefasciatus* was observed at the time exposure of 10 minutes.

The minimum mean concentrations of against three mosquito species were observed for the Methoprene compound on the first instar stages of *Cx. quiquefasciatus* at the duration of ten minutes. The minimum mean concentration of Buprofezin against on the adult emergence in *Cx. quiquefasciatus* were observed at the duration of 10 minutes. Previous reports indicated that the combination of temphos with the pheromone could result in the implementation of the attracted and kill strategy [34].

From the observation and analysis it was established the effectiveness of IGR is dependent on exposure time as well as on the concentration. There was a significant time concentration interaction observed in the adult emergence of the three vector species. At the application rates of 0.0002,0.002,0.02 and 0.2 mg/l this IGR could completely prevent development of immature and emergence of *Cx. quiquefasciatus and Ae. aegypti* adults, even at a minimum exposure time of 10 min.

This IGR induced notable abnormalities in larvae, pupae and adults of the three species of mosquitoes similar to those often indused by other IGR's [31 and 33]. Most of the larvae showed extended body and characteristic dorsal splitting of thoracic cuticle after treatment. Many larvae were found attached to the previous moult and were observed moving rapidly from the water surface to the bottom of the beakers in attempts the larvae died at prepupal stage [35] with thoracic busbous projection as larval-pupal intermediate with both larval and pupal characteristic such larval-pupal intermediate or prepupal stage larvae were immobile and dead shortly thereafter presumably from suffocation as reported earlier by [33] in *Cx. quiquefasciatus* when treated with cyromazine.

Morphogenetic aberrations other kinds were also noticed in pupae. Many pupae succeeded in splitting their exuviate and liberated themselves in part from the larval cuticle. Others retained their larval head capsule. In few others the larval head capsule remained attached to the anterior portion of their cephalothorax while their entire body was free of the larval culture. Most of the pupae that were able to shed larval cuticle were soft body but without characteristic sclerotization of the pupae cuticle. Such forms were earlier referred to as "albino" by [31] most of the treated dead pupae were longer than normal pupae with distended body. Pupal – adult intermediates were found with poorly developed adult characters along with pupal structures. Many of the incompletely enclosed adults were attached to the exuviate by their legs or wings or both many dead adults were also found with curve tarsi and crippled wings.

5. CONCLUSION

The present study suggests that Methoprene was effective in all the mortality rate of gravid females and oviposited females of *An. stephensi* against Methoprene were significant at concentration of 1.0 mg/l. The drowning of gravid females or the sinking of the eggs at higher dosages might be due to lowering of surface tension by the treatment of these IGRs. The lowering of surface tension could be due to the present of considerable amount of emulsifiers in the EC formulation of Methoprene [25]. The maximum mean percentage of mortality and emergence inhibition were observed in the first instar stage of *An. stephensi* by Buprofezin compounds and maximum emergence inhibition in the *Cx. quiquefasciatus* was observed at the time exposure of 10 minutes. This IGR induced notable abnormalities in larvae, pupae and adults of the three species of *Cx. quiquefasciatus, Ae. aegypti* and *An. Stephensi*,

similar to those often induced by other IGRs [33].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction, 2nd edn. Cambridge: Cambridge University Press. 2010;80.
2. A1- Ghamdi KH, Saleh MS, Mahyoub JA, A1Fifi ZI. La Laboratory evaluation of the insect growth regulator dudim and the plant extract neem oil against Culex pipiens mosquitoes with reference to its side effects on aquatic non target organisms. Biosciences, Biotechnology Research Asia. 2009;6(2):509-512.
3. El-Heneidy A. Laboratory evaluation of the effect of insecticides on non-target organisms: 2-the egg parasitoid, *Trichogramma evanescens* West (Hymenoptera: Trichogammatidae). Egyptian Academic Journal of Biological Sciences. 2018;11(3): 35-44.
4. Suman DS, Wang Y, Bilgrami AL, Gaugler R. Ovicidal activity of three insect growth regulators against *Aedes* and *Culex* mosquitoes. Acta Tropica. 2013;128(1):103-109.
5. Rathy MC, Sajith U, Harilal CC. Plant diversity for mosquito control: A preliminary study. Int J Mosq Res. 2015;2(1):29-33.
6. Amira K, Touahria Chouaib NE, Boudjelida H. Laboratory study of larvicidal efficacy of a local plant *Hertiacheirifolia* against the most abundant mosquito species, in Algeria. Journal of Entomology and Zoology Studies. 2018; 6(1):258-262.
7. Lau KW, Chen CD, Lee HL, Low VL and Sofian-Azirun M. Bioeficacy of insect growth regulators against Aedes albopictusand *Culex quinqueasciatus* (Diptera: Culicidae) from Sarawak, Malaysia: A statewide Survey. Journal of Economic Entomology. 2018; 111(3):1388-1394.
8. Glancey BM, Reimer N, Banksk WA. Effects of IGR Fenoxycarb and Sumitomo S-31183 on the queens of two myrmicine ant species. In: Applied Myrmecology: A World Perspective. Eds. Robert K. Vander Meer, Klaus Jaffe, and Aragua Cedeno. Boulder: Westview Press. 1990;604-613.
9. Worthing CR, Hence R.J. The British crop protection council. The pesticide manual: A world compendium London: Unwin. Brothers Limited. 1991;9:12-16.
10. Miyamoto J, Hirano M, Takimoto Y, Hatakoshi M. Insect growth regulators for pest control with emphasis on juvenile hormone analogs: present status and future prospects. Pest Control with Enhanced Environmental Safety. 1993;524:144-68.
11. Sihuincha M, Zamora-Perea E, Orellana-Rios WJ, Stancil D, Lozpez-Sifuentes V, Vidal-Ore C, Devine GJ. Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru.J. Med. Entomol. 2005;42:620–630.
12. Rajasekar P, Jebanesan A. Emergence inhibition effect of insect growth regulator Novaluron and Buprofezin against mosquitose (Diptera: Culicidae). J. Pest. 2011;35:34-36.
13. Valles SM, Koehler PG. Antagonism of fipronil toxicity by piperonylbutoxide and S,S,S-tributylphosphorotrithioate in the german cockroach (Dictyoptera: Blattellidae). Household Struc. Insects. 2005;90:1254-1258.
14. MohSeng C, Setha T, Chanta N, Socheat D, Guillet P, Nathan MB. Inhibition of adult emergence of Aedes aegyptiin simulated domestic water-storage containers by using a controlled release formulation of pyriproxyfen. J. Am. Mosq. Cont. Assoc. 2006;22:152-154.
15. Daily BF, Bishop J, Steiner R. The mediating role of EMS teamwork as it pertains to HR factors and perceived environmental performance. Journal of Applied Business Research. 2007;23(1):95-109.
16. Snow AL, Shepson PB. Snowpack production of formaldehyde and its effect on the Arctic troposphere. Nature. 2008;398:230–233.
17. WHO. Dengue and Dengue haemorrhagic fever. Fact. Sheet No. 117. WHO, Geneva. Global prevalence of vitamin A deficiency in populations at risk 1995–2005: WHO global database on vitamin A deficiency. Geneva, World Health Organization; 2009.

18. WHO. Centralized Information System Infectious Diseases (CISID) database; 2010.
19. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction, 2nd edn. Cambridge: Cambridge University Press. 2010;80.
20. Murugaiyan T, Jayaraman T, Dharmalingam M. Popular practical physics. Asian Book Co., Madras. 1984;46-48.
21. Sokal RR, Rohlf FJ. Biometry, Freeman, New York 2nd edition. 1981;78.
22. Ranson H, Rossiter L, Ortelli F, Jensen B, Wang X, Roth CW, Collins FH, Hemingway J. Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*. J. Biochem. 2001;359: 295–304.
23. Chavasse DC, Yap HH. Chemical methods for the control of vector and Pests of public health importance. WHO/CTD/ WHOPES/97.2. WHO, Geneva, Switzerland; 1997.
24. Omena MC de, Navarro DMAF, Paula de. JE, Luna JS, Ferreira de, Lima MR, Sant' Ana AEG. Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. Bioresour. Technol. 2007;98:2549-2556.
25. Becker JL. Fusions in vitro de cellules somatiques en culture de Drosophila melano-gaster, induitespar la Concanavaline-A. C. R. Acad. Sci. 1972;275:2969-2972.
26. Batra CP, Mittal PK, Adak T and Ansari MA. Efficacy of IGR compound Starycide 480 SC (Triflumuron) against mosquito larvae in clear and polluted water. Journal of Vector Borne Diseases. 2005;42(3):109-116.
27. Silva JJ, Mendes J, Lomônaco C. Effects of sublethal concentrations of diflubenzuron and methopreneon *Aedesaegypti* (Diptera: Culicidae) fitness. International Journal of Tropical Insect Science. 2009;29(1):17- 23.
28. Khan GZ, Khan I, Khan IA, Salman M, Ullah K. Evaluation of different formulations of IGRs against *Aedes albopictus* and *Culex quinquefasciatus* (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine. 2016; 6(6):485-491.
29. Mian LS, Dhillon MS, Dodson L. Field evaluation of pyriproxyfen against mosquitoes in catch basins in Southern California. Journal of the American Mosquito Control Association. 2017;33(2):145-147.
30. Bouaziz A, Amira K, Djeghader N, Aissaoui L and Boudjelida H. Impact of an insect growth regulator on the development and the reproduction potency of mosquito. J. Ent. And Zoo. Studies. 2017;5(3):1662-1667.
31. Arias JR, Mulla MS. Morphogenetic aberrations induced by a juvenile hormone and analogue in the mosquito *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 1975; 12:309-316.
32. Kelada LN, Gaaboub I, Rawash AA. Comparison of the juvenilizing effect of six juvenile hormone like activity compounds on Egyptian Culex pipiens. J. Agri. Sci. 1980;95: 203-12.
33. Awad TI, Mulla MS. Morphogenetic and histopathological effects of the insect growth regulator Cyromazine in larvae of *Culex quinquefasciatus* (Diptera: Culicidae). J. Med. Ent. 1984;21:427-431.
34. Michalakis Y, Briese DT, Sheppard AW. The taxonomic status of Larinuscynarae F. and Larinuslatus Herbst (Coleoptera, Curculionidae), and its implications for the biological control of Onopordum (Asteraceae: Cardueae) in Australia Biocon. Sci. Tech. 2007;2:275-280.
35. Sacher RM. Mosquito larvicides with favourable environmental properties, Mosq. News. 1971;31:513-516.