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## Use Of Methoprene And Diflubenzuron For Long-Term Control Of Aedes Aegypti, The Vector Of Dengue Fever In Jeddah Governorate.

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### ABSTRACT

During this investigation, the insect growth regulator and the insect chitin synthesis inhibitor diflubenzuron tablets and the insect juvenile hormone analogue methoprene briquets were used as slow release formulations against the dengue fever mosquito transmitter (vector) *Aedes aegypti* in Jeddah governorate. The study was conducted in a simulated field study area inside experimental tabs with dimensions 50 x 50 x 50 centimeters by using two types of water (pond and tap water). Based on the duration of effective control, per day where our results showed that the use of diflubenzuron, the insect chitin sensitive inhibitor gave long term continued effective control for 62-86 days against the mosquito larvae whereas the juvenile hormone methoprene against the same mosquito larvae has shown 90% - 100% inhibition (the period of emergence of adult mosquitoes for a period of 37 and 67 days occurred from tap and pond water respectively). All preparations gave more effective emergence from the tap water when compared with pond water with the range of 74 and with the double effect of 1.8 for the diflubenzuron tablets and methoprene briquets respectively. From this current study, we recommend the urgency of using these slow release preparations with the goals of long term suppression of mosquito larvae within the premises and localities of Jeddah governance since both formulations prove to be considered excellent mechanisms which will reduce the repetitive insecticidal spray, reduce the cost of control and reduction of environmental pollution.

**Keywords:** Slow- release formulations, Insect growth regulators, dengue fever, long-term control, *Aedes aegypti*.

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## INTRODUCTION

Dengue fever is caused by a flavivirus transmitted by a female mosquito (*Aedes aegypti*). It has been estimated that 50 million human being is infected by dengue fever by *A. aegypti* every year. The spread of the virus through *A. aegypti* is very fast and different strategies are explored to control the infection into a human (Heather and Sott, 2018). Due to the topographical distribution of these mosquito vectors, the population of over 2 billion is at risk, causing dengue hemorrhage fever (DHF). The disease is now epidemic to over 100 countries and 4 different serotypes have been reported from the insects and human. Recently a 5th serotype is also described (Mustafa et al.,2015). So far the containment and vaccine strategies are not effective to control the mosquito and spread of the virus.

Previous studies have shown that the use of conventional chemical pesticides against mosquitoes continuously for more than 60 years has not achieved its purpose in the eradication and/or reduction in their numbers in the environment. However, the usage has resulted in many problems, amongst which the emergence of strains of mosquitoes that are highly resistant to these chemical pesticides. The pesticides have caused environmental pollution, which has led scientific research centers to limit the use of these chemicals in the environment, with growing interest in the development of non- conventional insecticides and safe-to-use alternative control methods (Al-Ghamdi et al, 2008; Qusti et al, 2010).

During the last four decades of the 20<sup>th</sup> century, scientific research has been directed towards insect growth regulators (IGRs) that affect the biology and behavior of insects during their growth and therefore fall within non-conventional suppression methods for reducing insect communities which were widely used against many types of mosquitoes worldwide (Saleh and Wright, 1989; Mulla et al, 2003; Silva et al,2009).

Studied have been conducted to prepare a variety of insect growth formula on industrial scales of slow-release formulations to tackle mosquito larvae. The idea of these preparations is to incorporate the active ingredient of the IGR compound, which regulates the growth of insects. Addition of the materials, allowing for slow flow and release of the effective material in water while, at the same time, confirms fortification of the vigorous material from the factors of hydrolysis and disintegration, which increases the effectiveness of the compound for long periods. These additions decrease the pollution of the environment while ensuring a continuous reduction in the community numbers and population factions of mosquito larvae (Alsobhi et al.,2016; Alkenani,2017).

The aim of the present study was to evaluate the effectiveness of two of the slow-release formulations for insect growth regulators: Methoprene and Diflubenzuron tablets against *A. aegypti* larvae grown in drinking water and cultivated in pond water as the vector of dengue fever in the province of Jeddah.

## MATERIALS AND METHODS

The study was conducted in a field strain of the *A. aegypti* larvae, collected from a pond in Al Muntazahat district in Umm al-Salam locality in Jeddah municipality. The larvae were kept in water basins and reared on media, containing yeast powder, dry bread powder and skimmed milk powder in equal proportions. The culture was maintained under laboratory conditions of  $37 \pm 1^\circ\text{C}$ , humidity  $70 \pm 5\%$ , and light periods of 14 hours light and 10-hour darkness. The larvae were reared until pupation and adult emergence and were transferred inside breeding cubicles and fed on a 10% sugar solution. In order to obtain eggs for continuous breeding in consecutive generations, the adult mosquito females were fed on a blood alive pigeon with legs tied and wing feathers removed from the chest area for 1-2 hours in the breeding chamber under semi-dark conditions. After 4-5 days, the female mosquitoes laid eggs that were hatched after about 1-2 days. The same larvae were incubated to preserve the mosquito breed and to perform the necessary tests.

### Compounds tested

Chemicals e.g., methoprene (Zoecon Corp, U.S.A) and diflubenzuron tablets were used (Chemtura Europe Limited, UK) in the experimental tests. The tests were conducted in plastic basins (50 × 50 × 30 cm), each containing 20 L of drinking water and others containing pond water obtained from one of the permanent foci to breed mosquito larvae. In each basin, 1.3 g of methoprene and/or 0.133 g of the diflubenzuron, were added per 20 L of drinking water.

In each basin, 25 larvae of the fourth instar mosquito *A. aegypti* were kept with larval feed for experiment as well as for control. The rate of mortality of the larvae as well as the number of virgins evolved from these larvae were recorded. This was considered to be the first test. The experiment was repeated by adding new *A. aegypti* mosquito larvae to the same basins treated, with water added to compensate for evaporation. The rate of mortality was recorded until completion of the process of pupation and the exiting of whole insects. That was considered to be the second test. The tests were continued until they were stopped when the efficiency of the preparations against the mosquito larvae dropped to less than 90% inhibition in the hatching of the total insects resulting from these treatments. The experiment was consisted of tree replication and experiment was repeated four times and the same.

The duration of effectiveness of each compound under the test was rescored, being the number of continuous days in which the efficiency of the preparations used in the water is capable of causing 90-100% inhibition of adult emergence (Saleh et al, 1981).

### RESULTS AND DISCUSSION

Tables 1-4 showed the results of the slow-release formulations tests for two of the insect growth regulators: methoprene and diflubenzuron tablets, against the larval and the pupae until adult emergence of *A. aegypti*. Effective control of these formulations was determined against the mosquito. Effect was monitored in the life cycle of *A. aegypti*. The effectiveness of these two compounds, in the term of days (time duration) that the formulations was kept continue to be effective in treated water, causing 90-100% inhibition of complete insect hatching, resulting from larval interactions (Saleh et al, 2003). The results showed that treatment with the tested compounds provided an active control fraction against the *A. aegypti* larvae that continued for several consecutive weeks, replicated in the death and deformity in mosquito stages (Figure 1). It was noted that the effectiveness of these preparations varied when used upon mosquitoes that were raised in tap water than those raised in pond water. The results of this study established that treatment of methoprene and diflubenzuron tablets against *A. aegypti* resulted in an effective and continuous inhibition ratio from 90-100% (when not reaching full development) within 62 and 84 days consecutively (Tables 1,2). These results exhibited that the slow-release formulations of the diflubenzuron tablets against *Ae. aegypti* in tap water was more effective than the methoprene compound by about 1.36 times.

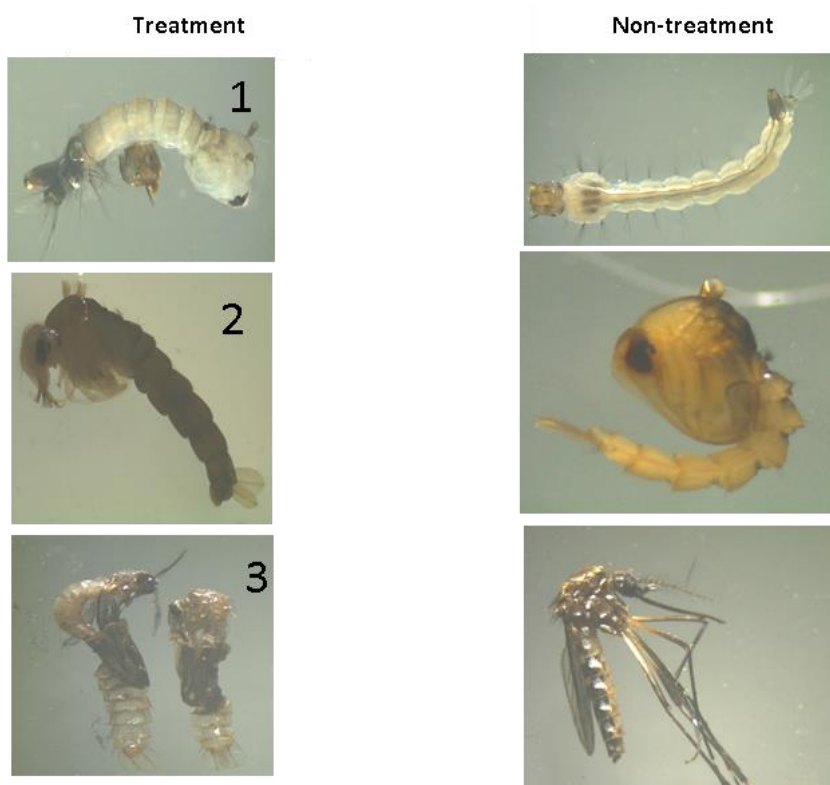


Fig 1: Some malformation of the compounds tested against *Ae. aegypti*

- 1) An intermediate stage between larvae and pupae
- 2) An intermediate stage between pupae and Adult
- 3) Failure of ecdysis of the adult stage

**Table 1: Efficacy of slow-release formulations of methoprene against larvae of *Aedes aegypti* raised in tap water**

No. Of Tests	Dead larvae* (%)	Pupae produced (%)	Adults Emerged (%)	% I.E	Duration of effectiveness (days)
1	4 (12) <sup>b</sup>	96	0	100	62
2	5 (11) <sup>b</sup>	95	0	100	
3	7 (12) <sup>b</sup>	93	2	98	
4	9 (13) <sup>b</sup>	91	6	94(93.3) <sup>c</sup>	
5	9 (14) <sup>b</sup>	91	7	93 (92.4) <sup>c</sup>	
6	6 (12) <sup>b</sup>	94	20	80	
7	11 (10) <sup>b</sup>	89	34	66	

a: Four replicates, 25 larvae each; control mortalities ranged from 4–9 % I.E.

b: The number of days after treatment until larval death or adults emerged.

c: Corrected for control mortalities (Abbott 1925).

**Table 2: Efficacy of slow-release formulations of diflubenzuron against larvae of *Aedes aegypti* raised in tap water**

No. of Tests	Dead larvae* (%)	Pupae produced (%)	Adults Emerged (%)	% I.E	Duration of effectiveness (days)
1	5 (11) <sup>b</sup>	95	0	100	84
2	5 (11) <sup>b</sup>	95	0	100	
3	6 (10) <sup>b</sup>	94	7	93(92.6) <sup>c</sup>	
4	8 (10) <sup>b</sup>	92	9	91(90.3) <sup>c</sup>	
5	7 (11) <sup>b</sup>	93	0	100	
6	6 (11) <sup>b</sup>	94	0	100	
7	6 (10) <sup>b</sup>	94	9	91(90.3) <sup>c</sup>	
8	5 (10) <sup>b</sup>	95	7	93(91.4) <sup>c</sup>	
9	5 (11) <sup>b</sup>	95	15	85	
10	6 (10) <sup>b</sup>	94	24	76	

a: Four replicates, 25 larvae each; control mortalities ranged from 4–9 % I.E.

b: The number of days after treatment until larval death or adults emerged.

c: Corrected for control mortalities (Abbott 1925).

**Table 3: Efficacy of slow-release formulations of methoprene against larvae of *Aedes aegypti* raised in pond water**

No. of Tests	Dead larvae* (%)	Pupae produced (%)	Adults Emerged (%)	% I.E	Duration of effectiveness (days)
1	5 (12) <sup>b</sup>	95	0	100	40
2	6 (13) <sup>b</sup>	94	2	98	
3	7 (13) <sup>b</sup>	93	2	98	
4	9 (12) <sup>b</sup>	91	8	92(91.2) <sup>c</sup>	
5	8 (11) <sup>b</sup>	92	17	83	
6	6 (11) <sup>b</sup>	94	31	69	
7	10 (10) <sup>b</sup>	90	33	67	

a: Four replicates, 25 larvae each; control mortalities ranged from 4–9 % I.E.

b: The number of days after treatment until larval death or adults emerged.

c: Corrected for control mortalities (Abbott 1925).

**Table 4: Efficacy of slow-release formulations of diflubenzuron against larvae of *Aedes aegypti* raised in pond water**

No. of Tests	Dead larvae* (%)	Pupae produced (%)	Adults Emerged (%)	% I.E	Duration of effectiveness (days)
1	5 (11) <sup>b</sup>	95	0	100	61
2	6 (11) <sup>b</sup>	94	0	100	
3	6 (10) <sup>b</sup>	94	9	91	
4	7 (9) <sup>b</sup>	93	9	91(90.1) <sup>c</sup>	
5	7 (10) <sup>b</sup>	93	10	90	
6	6 (10) <sup>b</sup>	94	9	91	
7	6 (10) <sup>b</sup>	94	25	75(74.4) <sup>c</sup>	
8	5 (10) <sup>b</sup>	95	18	82	
9	5 (11) <sup>b</sup>	95	35	65	

a: Four replicates, 25 larvae each; control mortalities ranged from 4–9 % I.E.

b: The number of days after treatment until larval death or adults emerged.

c: Corrected for control mortalities (Abbott 1925).

On the other hand, test formulations in pond water began to lose its efficacy against *Ae. aegypti* larvae after 40 days of treatment of methoprene tablets (Table 3) and over 61 days of treatment when using the diflubenzuron compound tablets (Table 4). The results confirmed that the biological activity of two compounds against mosquito populations in pond water led to a 35.48% and 27.38% decreases respectively as compared to tap water treatments. The low decrease in effective control periods of these formulations tested against *Ae. aegypti* mosquito larvae in pond water may be attributed to the adhesion of the active of these compounds to the organic matter and soil components suspended in the water. The adhesion property might led to a reduction in its deadly activity against the *Ae. aegypti* (Ramoska et al,1982; Saleh,1989).

Lastly, the results of the present study are consistent with the results of much earlier research using release formulations (SRFs) for insect growth regulators against various types of mosquito larvae (Al-Ghamdi et al, 2010). Use of slow-release formulations of bacterial insecticide and insect growth regulators (IGRs), against mosquito larvae. These are better tactics to integrated pest management and insecticide resistance management ( Alkenani, 2018). Saleh et al. (2103) used a chitin synthesis inhibitor Dudim® against *A. aegypti*. The results showed that the test formulations provided long-term residual control against the larvae. Effective control giving 90–100 % inhibition of adult emergence was achieved for 10 weeks post-treatment for Dudim®. It was found that the use of Altosid in the form of pellets controlled in its flow had, caused more than 95% delay growth in insect life cycle such as *Ae. taeniorhunchus* (Flooe et al, 1990), *A. nigromaculis* (Cornel et al,2000) and *Ae. dorsalis* (Lawler et al,2000). It was mentioned that the use of diflubenzuron inhibition in the form of slow-acting tablets has been shown to provide effective three-month long-term effective control of *Ae. aegypti* and *Ae. albopictus* mosquitoes and communities, whilst the effect of the similar compound have been effective for several weeks against *Ae. albopictus* and *Ae. aegypti* (Thavara et al,2007).

results of the present study confirmed that the use of insect growth regulators in slow-release formulation (SRFs) against mosquito larvae provies effective control for a long period of time. The effects may be spread for several successive weeks when used once in the breeding environment of the mosquito communities and populations of this mosquito without the need to repeat spraying of conventional chemical pesticides as it leads to the rationalization of the use of pesticides and reduces the pollution of the environment (Vythilingam et al,2005).

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