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Efficacy of Spinosad and Flubex against Dengue Fever Vector *Aedes aegypti* in Jeddah Governorate, Saudi Arabia

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Abstract
The biological effects of both spinosad (4.7%) and flubex (diflubenzuron DT 2%) against *Aedes aegypti (Ae. aegypti* (L.) mosquito larvae were assessed under laboratory conditions. The LC_{50} values of the spinosad and diflubenzuron were 0.22 ppm and 0.0019 ppm respectively, against *Ae. aegypti* larvae. The mortality rate of mosquito larvae ranged from 35- 96 % and 2-20 % for those spinosad and diflubenzuron separately. The results revealed that the spinosad formulation was highly effective against larvae comparing with flubex. Larval treatment with the IGR diflubenzuron reduced the reproductive potential of adult mosquito that emerged from these treatments by 16-84%. These results revealed that although flubex is an IGR, its larvicidal activity is better than spinosad. Further assessments and field investigation on IGRs products as insecticides alternatives should be carried out for managing *Ae. aegypti* mosquitoes.
Keywords: Spinosad, flubex, *Aedes*, biological, larvicides.

INTRODUCTION

Mosquitoes are the most medically important insect species due to their capacity in carrying and transmitting both animal and human diseases (Ikhlak *et al.,* 2016; Snow *et al.,* 1999; Roth *et al.,* 2010; Weaver *et al.,* 2010). *Aedes aegypti* (L.) is widely spread throughout the world, including tropical and subtropical areas and it is recognized as the most important vector for transmitting serious diseases such as dengue, chikungunya and Zika viruses (Benelli, 2016 a,b)

In Saudi Arabia, the first dengue outbreak in over 50 years, was in Jeddah city in 1994 (Gubler, 2002). Since that time, dengue fever has emerged as a major public health problem in Jeddah city (WHO 2010) and dengue virus surveillance was established after that time (Fakeeh and Zaki, 2001). In 2006, dengue fever reported cases had risen drastically compared to the earlier recorded numbers (Aburas, 2007).

Due to extensive use of chemical insecticides for several decades to manage mosquitoes populations (Hemingway and Ranson, 2000), mosquitoes have developed resistance to these insecticides. Furthermore, health concerns have promoted research to find alternative insecticides for effective control of vector mosquitoes (Uragayala *et al.,* 2015). The key criteria for an effective mosquito larvicide is low mammalian toxicity, low impact on the environment, the broad spectrum of activity against all target species of mosquito and a long duration of effect that reducing application frequency. The researchers started to look for new insecticides having new modes of action to either prevent or reduce the impact of insecticide resistance to the previous generation of insecticides (Darriet and Corbel, 2006; Perez *et al*., 2007 and WHO, 2012). Insect growth regulators (IGRs) appeared as alternatives to such chemical larvicides due to their low mammalian toxicity, biologically specific and environmentally safe and have been recommended for *Ae. aegypti* control (Thavara *et al*., 2004;Silva *et al*., 2009).

Spinosad DT is a naturally derived insecticide composed of a mixture of two metabolites (spinosins A and D) obtained by fermentation process employing the soil bacterium *Saccharopolyspora spinosad* (*Actinomycetales*). It is highly virulent by both contact and ingestion to several dipterous insect pests (Bacci L *et al*., 2016; Prabhu *et al*., 2011). Due to its lower mammalian toxicity and its environmental impact, lower persistence and lower toxicity to a number of predaceous insects, it has been approved to control mosquito larvae in drinking water (Tomlin, 2000; Williams *et al*., 2003; WHO, 2010). Three formulations (granules, aqueous suspension concentrate and tablets) of spinosad have been evaluated by WHO (2007) for mosquito larvae control. It has been used as a larvicide at 0.25–0.5 mg/l active ingredient for controlling *Ae*. *aegypti* in drinking-water containers. Technical and formulated spinosad has been evaluated against larvae of several important mosquito species under laboratory conditions. Both LC_{50} and LC_{90} technical material of the Spinosad against *Ae. aegypti* larvae ranged from 0.155 to 0.35 mg/L AI and 0.185 to 0.92 mg/L AI respectively (WHO ,2007). Another study was conducted against *Ae. aegypti, Anopheles gambiae,* and *Cx. quinquefasciatus* larvae with technical material dissolved in ethanol (Darriet *et al.,* 2005). Spinosad was found to be more active against larvae of *An. gambiae*, followed by *Cx. quinquefasciatus* and *Ae. aegypti*. The LC_{50} and LC_{90} values were: 0.01–0.032 mg/L AI (*An. gambiae*), 0.093–0.49 mg/L AI (*Cx. quinquefasciatus*) and 0.35– 0.92 mg/L AI (*Ae. aegypti*) respectively. A couple of formulations of spinosad, direct application tablet (DT) and 0.5% granules (GR), at 3 dosages (0.25, 0.5 and 1.0 mg/l) were evaluated against *Ae*. *aegypti* larvae (Thavara *et al*., 2009). A percentage of 79-100 IE for 34 days was produced by The DT formulation at the highest concentration (1.0 mg/l) whilst after 62 days 90-100% IE was obtained. These results indicate a longer residual period of such formulation.

Flubex (Diflubenzuron 2%) is another GR mainly works through ingestion leading to inhibition of both synthesis and deposition of the chitin in the body wall of the treated immature stages of the insects that finally causing death (Sihuincha *et al*., 2005). The Cuticle of treated larvae is unable to withstand increased pressure during the ecdysis process and fail to provide adequate muscular support during molting. Such larvae are unable to throw their exuviae and finally die due to either starvation or rupture of the new, delicate, malformed cuticle. According to WHOPES recommendation (WHO, 2006), Diflubenzuron wettable powder has been used in mosquito larvae control since the mid-1970's.

Several studies have evaluated insect growth regulators (IGRs) for mosquito control in different regions of the world (Mulla *et al*., 2003; Cetin *et al*., 2006 ;Silva *et al*., 2009 Jacups *et al*., 2014; Anjum, *et al*., 2017). In the present investigation, two nonconventional insecticides, diflubenzuron 2% DT and spinosad 7.48% DT, were used to control *Ae. aegypti* larvae based on recommendations made by WHO (2007,2009) for controlling the mosquito larvae.

MATERIALS AND METHODS

Mosquito Rearing

The mosquito *Ae.aegypti* (L.) was chosen an experimental insect for the present study, because it is considered as one of the most important biting and nuisance mosquitoes and the major vector for dengue fever in the study area. Larvae were obtained from the Municipality of Jeddah and were reared to produce a colony under laboratory conditions. The colony was maintained in insectary at room temperature (27± l °C),

relative humidity of 70±5% and 14:10 (L: D) controlled photoperiod. Larvae were fed on fish food and males were fed on 10% glucose sugars whilst females get their blood meals from domestic pigeons.

Insecticides

Two commercial formulations of IGR_S, flubex and spinosad, were used in larval bioassays. Flubex (diflubenzuron 2% DT) was obtained from Agricultural office-Jeddah, Saudi-Arabia and spinosad (7.48% DT.) was obtained from the Municipality of Jeddah. These insecticides were selected for larval bioassay due to their usage as larvicides to control the container - breeding *Ae. aegypti* in vector control programs in Jeddah city, Saudi Arabia.

Larval bioassays

Bioassays were undertaken according to instructions of WHO (1981). A stock solution of both spinosad and diflubenzuron were obtained by grinding and dissolving the tablets in a suitable solvent. A stock solution of spinosad and diflubenzuron were prepared by dilution with distilled water and homogenized by shaking until completely dissolved, required concentrations were diluted immediately prior to use in bioassays.

The late third or early emerged fourth instar larvae of *Ae.aegypti* were selected for use in bioassays. Larvae were subjected to series of concentrations from Spinosad 7.48% DT (0.125-2 ppm) and flubex 2% (0.0004-0.008 ppm). Twenty five larvae were placed in 249 ml of de-chlorinated tap-water plus 1ml from the concentration of the insecticide in pyrex beakers. Each concentration has four replicates and control. Mortality was monitored at 24 hours intervals after initial exposure. Larvae that showed lack of movement in response to continued probing were considered dead. Larval and pupal mortalities were recorded daily whilst alive pupae were transferred to untreated water in new beakers and left until the emergence of the last mosquito. Both partially emerged mosquitoes and those found completely emerged but unable to leave the water surface were recorded and considered as dead adults.

Statistical analysis

Results of bioassays were corrected by using Abbott's formula (Abbott, 1925) when control mortality exceeded 10% which never happened then subjected to probit analysis (Finney, 1952). Probit regression analysis programme was used to analyze mortality data to obtain LC_{50} and LC_{95} of tested compounds. Statistical differences between LC_{50} values were determined based on overlapping of 95% confidence intervals. The chi-square test was used to calculate the respective slope lines.

RESULTS AND DISCUSSION

Two non-conventional insecticides (IGR) flubex 2% DT and spinosad 7.48% DT were evaluated against *Ae.aegypti* larvae. The cumulative mortalities of the development stage have been considered as a criterion for the evaluation of the IGRS due to their delayed action against such stages (WHO, 2005a). Larval and pupal mortality percentages as well as inhibition of adult emergence were shown in tables (1, 2) and figure (1).

As shown in the table (2) the mortalities of larvae treated with effective concentrations of diflubenzuron compound were very low and ranged from 2 to 20%. These results could be due to either the delayed or the cumulative effects of this compound on the developmental stages of mosquitoes. These results are in agreement with Georghiou and Lin (1974) who mentioned that we should not use larval mortality as indicator when we evaluating effects of these compounds against mosquito larvae due to their delayed or cumulative effects on mosquitoes developmental stages. Therefore IC_{50} (Concentration that inhibits the emergence of 50% of mosquito) was used as the criterion rather than LC_{50} (Concentration that kills 50% of mosquito larvae) in the present work. In contrary, spinosad was highly effective against the *Ae.aegypti* larvae and showed 35- 96 % mortality (Table 1), whereas the corresponding percentages of adult emergence inhibition were 16-84% for diflubenzuron. The IC_{50} and IC_{90} that prevented adults emergence from larvae treated with diflubenzuron were 0.0019 and 0.0022 ppm respectively.

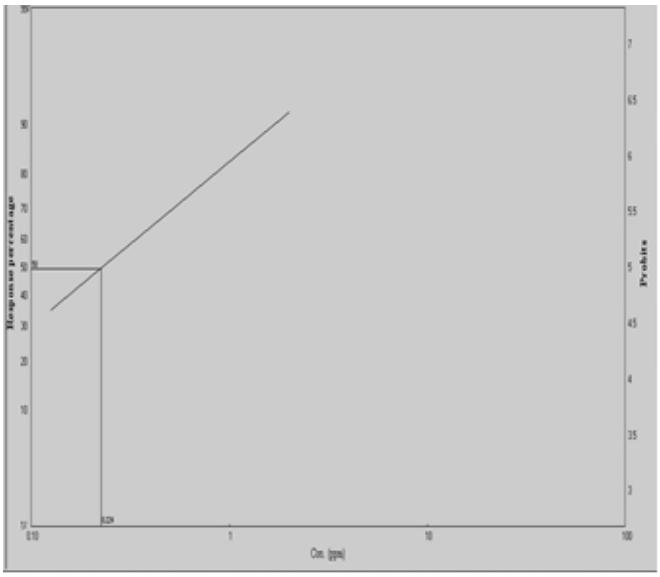
Results revealed that the spinosad formulation was highly effective against larvae (Table 1) with LC_{50} = 0.22 ppm. However, diflubenzuron was more effective in inhabiting adult emergence. The present study indicated that diflubenzuron showed a significant effect in inhibiting adult emergence with higher mortality in the pupal stage (84%) and lower mortality in the larval stage (20%). Contrarily, Spinosad revealed high mortality percentage of larvae (96%) compared with diflubenzuron. Furthermore, pupal mortality and incomplete adult emergence were recorded.

Table 1. Larvicidal effects and statistical parameters of spinosad on *Ae. aegypti*.

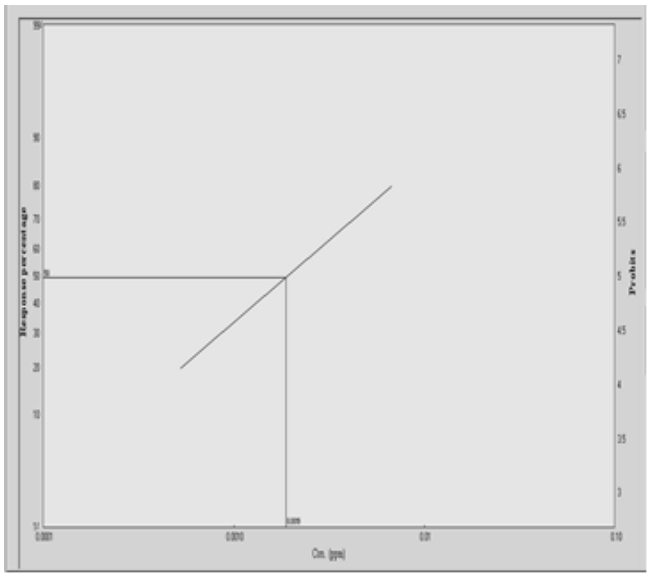
Conc.(ppm)	Larval mortality %	Statistical parameters	Larval stage
0.250	52	95%(*F.L)	0.172-0.276
0.500	70	LC 95 (ppm)	2
1.000	86	95%(*F.L)	1.961-6.361
2.000	96	Slope	1.47
		Tabulated $(Chi)^2$	7.8
Control	0	Calculated $(Chi)^2$	1.24
		R-Squared	80.1%

Table 2. Biological effects and statistical parameters of IGR diflubenzuron 2% on developmental stages of *Ae. aegypti*.

Con.(ppm)	Larval Mortality (%)[A]	Pupa Produced	Adult%		Statistical parameters	Adult Stage
			Emergence	Inhibition (%)[B]		
0.0004	2	94	84	16	LC_{50} (ppm)	0.0019
0.0008	11	83	72	28	95%(*F.L)	0.0016-0.0023
0.002	17	80	48	52	LC_{95} (ppm)	0.022
0.005	15	85	27	73	95%(*F.L)	0.0152-0.0397
0.008	20	88	16	84	Slope	1.51
Control	2	95	93	7	Tabulated $(Chi)^2$	7.8
					Calculated $(Chi)^2$	0.18
					R-Squared	84.6%



(A)



(B)

Fig.1. The relation between concentrations of spinosad 4.7% (A), diflubenzeroun 2% (B) and mortality percentage of *Ae.aegypti* larvae.

Figure (2) showed morphological abnormalities (intermediate stages such as larval siphon, pupal trumpets) in developmental stages of *Ae. aegypti* resulted from treatment with diflubenzuron. Several previous studies (Bridges *et al*., 1977 ; Kelada *et al.,* 2006 ;Thangaraj *et al*., 1987 and Baruah and Dus,1996; Bond *et al.,* 2004; Khan *et al.,* 2016) are inconsistent with these findings. Mulla (1995) stated that these abnormalities affect the developmental stages leading to failure in successful adult emergence from pupal exuviae. Additionally, findings of the present study are in agreement with findings of several studies conducted in different regions of the world (Romi *et al*. 2006;Thavara *et al*., 2007 and 2009, Seccacini *et al.*, 2008; Jiang , Mulla, 2009; Kamal, H., Khater, E. 2010, and Suman *et al*., 2010; Saleh *et al*., 2013). The slight difference in the efficacy range of the compounds among these studies could be due to differences in mosquito strain, biological response of the tested larvae, compounds formulation and experimental conditions.

(A)

(B)

Fig. 2. Abnormalities in the developmental stages of *Ae. aegypti* after treatment with Flubex 2%, (A) a larval-pupal intermediates. (B) Untreated larvae.

CONCLUSION

The study showed that both spinosad and flubex have high efficacy against the larval stage and adult emergence of *Ae.aegypti* mosquitoes.

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CONFLICT OF INTEREST

The authors declare that no there is no conflict of interest for this study.

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