Semi-Field Evaluation of some Slow-Release Insecticide Formulations against the Dengue Fever Mosquito *Aedes aegypti* (L)

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ABSTRACT

Semi-field trials were conducted to evaluate the efficacy of slow-release formulations (SRFs) of Bactimos briquets, Spinosad tablets, Altosid briquets and Dudim tablets against mosquito larvae of *Aedes aegypti*. The records showed that SRF treatments provided long-term effective control against mosquito larvae using a single application of the test formulations. Effective control with 90-100% inhibition of adult emergence was obtained for 4, 9, 10 and 12 weeks post-treatment by using SRFs of Bactimos, Spinosad, Altosid and Dudim, respectively. On the other hand, the results showed that larval treatments with the test SRFs affected the blood feeding activity and reproductive capacity of mosquito adult survivors.

Key words: *Aedes aegypti*, slow-release formulations, semi-field trials, blood feeding activity, reproductive capacity.

INTRODUCTION

Currently, dengue fever is the most important arthropod-borne viral disease in different parts of the world. *Aedes aegypti* is generally recognized as the primary vector of dengue viruses causing 50 million cases of infection and 300,000 deaths each year in tropical and subtropical areas (Darriet *et al.*, 2010).

With the current trends in dengue incidence worldwide and without an effective vaccine, it is expected that the widespread use of insecticides will continue. Many insecticide formulations have been developed and tested for their efficacy against a wide spectrum of mosquito vectors. In this concern, a great impetus has been given to the use of slow-release insecticide formulations against mosquito larvae (WHO, 2005). Such formulations are likely to enhance residual larvicidal activity *via* greater stability and maximized contact with the target mosquito larvae (Mulla *et al.*, 1988; Cornell *et al.*, 2000; Bond *et al.*, 2004, Seng *et al.*, 2008; Jacups *et al.*, 2014).

The present research work was planned to evaluate the larvicidal efficacy of four slow-release formulations of bacterial insecticides and insect growth regulators (IGRs) against mosquito larvae of *A. aegypti*, the primary vector of dengue fever in Jeddah governorate, Saudi Arabia. The blood feeding activity and reproductive capacity of mosquito adult survived from larval treatments with the tested formulations were also studied.

MATERIALS AND METHODS

Mosquito strain

Tests were performed on a field strain of *A. aegypti* (L.) raised from wild larvae, collected from Al-Jamaeh District, Jeddah governorate, Saudi Arabia, and had been maintained under laboratory conditions of 27±1°C and 70±5% R.H. with 14: 10 (L:D). The larvae were reared until pupation and adult emergence took place for maintaining the stock culture.

SRFs tested

The following SRFs were used:

1- Two SRFs of bacterial insecticides: Bactiomos briquets (*Bacillus thuringiensis israelensis*, 7000 ITU; 10% a.i., briquet weight 12.5gm, supplied by Summit Chem. Co., Baltimore, M.D., USA) and Spionsad DT tablets (*Saccharopolyspora spinosa*, 7.48% a.i., tablet weight 1.37 mg, provided by Clark Company, Roselle, IL, USA).

2- Two SRFs of IGRs: Altosid XR-briquets (Methoprene 2.1% a.i., briquet weight 48 gm, proided by Zoecon, USA) and Dudim DT tablets (Diflubenzuron 2% a.i., tablet weight 2 gm, supplied by DGM Italia SrL).

Experiments

Semi-field trials were conducted at dengue mosquito research station, Dept. of Biological Science, Fac. of Science, King Abdul-Aziz Univ., Jeddah, Saudi Arabia. Experiments were carried out in white plastic pools (50×50×30 cm) containing 30 L of tap water. The pools were placed in the shade under a roof and were kept covered with muslin cloth sheets to prevent debris and ovipositon by wild mosquitoes. Each pool received a batch of 25 third instar larvae of *A. aegypti* and the test formulation. The dosage of each formulation required for larval treatments (Aquarter pieces, ~ 3.2 gm of Bactioms briquet; 0.2gm of Spinosad tablet; 7 gm of Altosid briquet and 0.3 gm of Dudim tablet) was determined according to the recommended dosages for

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Received May 6, 2015, Accepted june 2, 2015

field control. Pools without formulations were used as controls. The larvae were given the usual larval food during the tests. Water was slowly added to the pools every other day to compensate evaporation. New live batches of 3rd larval instars of *A. aegypti* were added weekly to the test pools. All treatments and controls were replicated four times. Any pupae produced were transferred to small plastic cups containing water and placed in adult cages for emergence. The efficacy of the test formulations was calculated as the number of emerging adults compared to the initial number of larvae added or the inhibition of emergence (%IE). The assessment of effectiveness was made at weekly intervals until the level of efficacy decrease to <50% IE.

Subsequent trials were also carried out to study the delayed effects of larval treatments with the test formulations on the blood feeding activity and reproductive potential of mosquito adults that emerged from these treatments. Adult survivors were isolated in clean cages. Four days later, emerged females were subject to a pigeon as a source of the blood meal. The percentage of biting was estimated after one hour and was expressed as the number of blood-fed females / total number of test females ×100. Afterwards, each engorged female was kept with a male in small plastic cups, half-filled with water and covered with Nylon cloth held in place with a rubber band. They were fed on a 10% sugar solution through the Nylon cloth using cotton pads. The reproductive potential of mosquitoes (based on egg production and egg hatchability) was also considered for the 1st gonotrophic cycle. Differences between treatments and controls were compared and analysed using the t-test.

RESULTS AND DISCUSSION

The efficacy of two bacterial insecticides Bactioms and Spionsad as SRFs against 3rd instar mosquito larvae of *A. aegypti* is shown in Table 1 and illustrated by Fig. 1. The effective control was defined as 90–100% inhibition of adult emergence (%IE).

The results showed that larval treatments with Bactioms briquets produced ineffective control (81% IE) for one week post-treatment and then the formulation began to give continuous effective control with 90–100% IE for about 4 weeks (Fig. 1). On the other hand, larval treatments with Spionsad tablets provided excellent effective control with 90–100% IE for 9 weeks post-treatments, after which its efficacy declined to 58% IE at week 12 post-treatment and 35% IE by the end of week 17 (Fig. 1). Taking the durations of effective control into consideration, the records showed that Spinosad tablets (9 weeks) proved to be highly effective against mosquito larvae of *A. aegypti* than Bactimos briquets (4 weeks) by about 2.25 times.

Table 2 shows the larvicidal effectiveness of two IGRs Altosid and Dudim as SRFs against *A. aegypti*. The results showed that treatments with Altosid XR-briquets produced high levels of residual efficacy against the present mosquito larvae as indicated by > 90–100% IE for 10 weeks post-treatment (Fig. 2). However, satisfactory control > 85% IE was observed for 12 weeks post-treatment. On the other hand, effective control (90-100% IE) was obtained with Dudim tablet treatments for a period of 12 weeks post-treatment, after which its effectiveness decreased to 70.2% IE at week 15, and then fluctuated between 38.7 and 26.6% IE during the last five weeks of trials (Fig. 2). In general, these records indicate that treatment of *A. aegypti* larvae with Dudim tablets is 1.2 times more effective than Altosid briquets. This was highly pronounced on the basis of durations of effective control (90–100% IE) of SRFs of Altosid (10 weeks) and Dudim (12 weeks). However, variations in the durations of efficacy among the test SRFs may be attributed to the differential mode of action of the active ingredients and the dosages tested (Saleh *et al.*, 2013). Similar studies in this respect were conducted by several investigators using different SRFs of bacterial insecticides (Nasci *et al.*, 1994; Saleh *et al.*, 2003; Thavara *et al.*, 2009; Tripathi *et al.*, 2013) and IGRs (Knepper *et al.*, 1992; Nayer *et al.*, 2002; Seng *et al.*, 2008; Jacups *et al.*, 2014) against many species of mosquito vectors. They pointed out that larval treatments with SRFs provided continuous good to excellent control against different mosquito larvae for several weeks. Such SRFs may be particularly useful for application in any location near the household where water collects and remains for long period such as pond, irrigated pastures and artificial containers.

Table 3 shows the blood feeding activity *A. aegypti* mosquito females that survived from larval SRF treatments. The records indicated that the blood feeding activity of females emerged from larval treatments with Bactimos briquets (84%) and Spinosad tablets (80%) was reduced by about 8.7 and 16.7%, respectively, when compared with their control which were in respect 92 and 96%. A similar depression in the blood feeding response was obtained with mosquito females of *A. aegypti* that survival from larval treatments with Altosid briquets (56%) and Dudim tablets (64%) by about 36.4 and 30.4%, respectively, as compared with control ones. Depression in the blood feeding activity may be due to larval SRF treatments affected the structure and function of muscles responsible for the movements of maxillae in mouth parts of female survivors (Vasuki, 1992). Similar reduced feeding responses have be reported by Saleh and Wright (1990) using Cyromazine against *A. epacticus* and Sithiprasasna *et al.* (1996) using Methoprene against *Anopheles dirus*. However, the reduction in blood feeding activity of mosquito

females may led to a decrease in the number of fully engorged females and accordingly affect the reproductive capacity of mosquitoes.

The results showed that treatment of *A. aegypti* larvae with Bactioms briquets did not significantly affect the mean number of eggs produced by female survivors (36.2 eggs) during 1st gonotrophic cycle as compared with the control which was 40.6 eggs (Table 4).

Table 1. Effectiveness of SRFs of Bactimos briquets and Spinosad tablets against mosquito larvae of *A. aegypti*

Weeks post-treatment	Dead larvae*		Pupae produced (%)		Adult emerged (%)		IE (%)	
	B	S	B	S	B	S	B	S
1	76	100	24	0.0	19	0.0	81	100
2	98	100	2	0.0	1.0	0.0	98.9	100
3	100	100	0.0	0.0	0.0	0.0	100	100
4	96	100	4	0.0	3	0.0	96.8	100
5	89	100	11	0.0	7	0.0	93	100
6	71	93	29	7	21	5	79	95
7	56	97	44	3	36	3	61.7	96.8
8	39	90	61	10	58	4	42	96
9	22	91	78	9	70	3	24.7	96.7
10	24	83	76	17	73	13	20.6	85.9
11	20	78	80	22	71	16	29	84
12	18	53	82	47	77	42	18.1	58
13		40		60		51		45.7
14		24		76		71		29
15		31		69		66		28.3
16		26		74		68		32
17		31		69		65		35

* Four replicates, 25 larvae each; control mortalities ranged from 4–9% IE.
B = Bactimos briquets; S = Spinosad tablets
IE = Inhibition of emergence, corrected for control mortalities (Abbott, 1925)

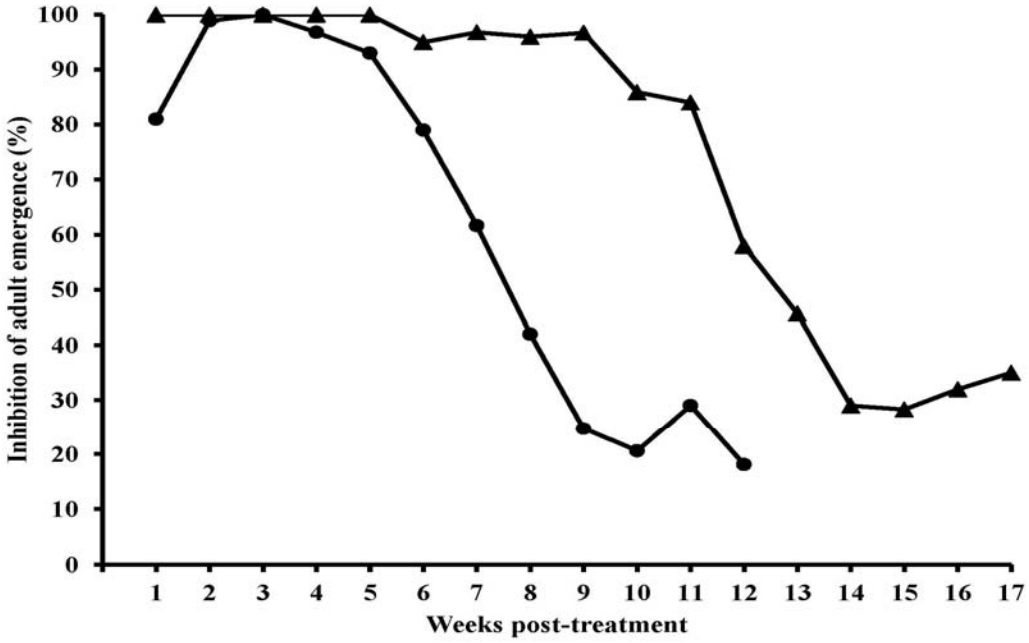

Fig. 1. Percentage emergence inhibition of *A. aegypti* after the treatment of 3rd instar larvae with SRFs of Bactimos briquets (●) and Spinosad tablets (▲)

Table 2. Effectiveness of SRFs of Altosid briquets and Dudim tablets against mosquito larvae of *A. aegypti*

Weeks post-treatment	Dead larvae*		Pupae produced (%)		Adult emerged (%)		IE (%)	
	A	D	A	D	A	D	A	D
1	32	29	68	71	6	8	93.5	91.4
2	41	38	59	62	0.0	6	100	93.5
3	36	43	64	57	2	8	97.8	91.5
4	39	46	61	54	0.0	9	100	90.3
5	44	36	56	64	4	0.0	95.6	100
6	32	54	68	46	4	8	95.5	91.1
7	31	44	69	56	5	0.0	94.6	100
8	28	49	72	51	3	0.0	96.7	100
9	26	40	74	60	5	7	94.6	92.5
10	30	32	70	68	6	5	93.5	94.6
11	32	37	68	63	10	2	88.9	97.8
12	34	39	66	61	13	9	85.5	90.0
13	29	31	71	69	22	18	75.8	80.0
14	26	28	74	72	51	21	45.7	77.6
15	19	35	41	65	66	28	29.9	70.2
16	27	27	73	73	60	57	35.5	38.7
17	22	26	78	74	63	64	31.5	30.4
18	19	18	81	82	74	66	17.8	28.3
19		20		80		74		23.6
20		15		85		69		26.6

* Four replicates, 25 larvae each; control mortalities ranged from 6–10% IE.

A = Altosid briquets; D = Dudim tablets

IE = Inhibition of emergence, corrected for control mortalities (Abbott, 1925)

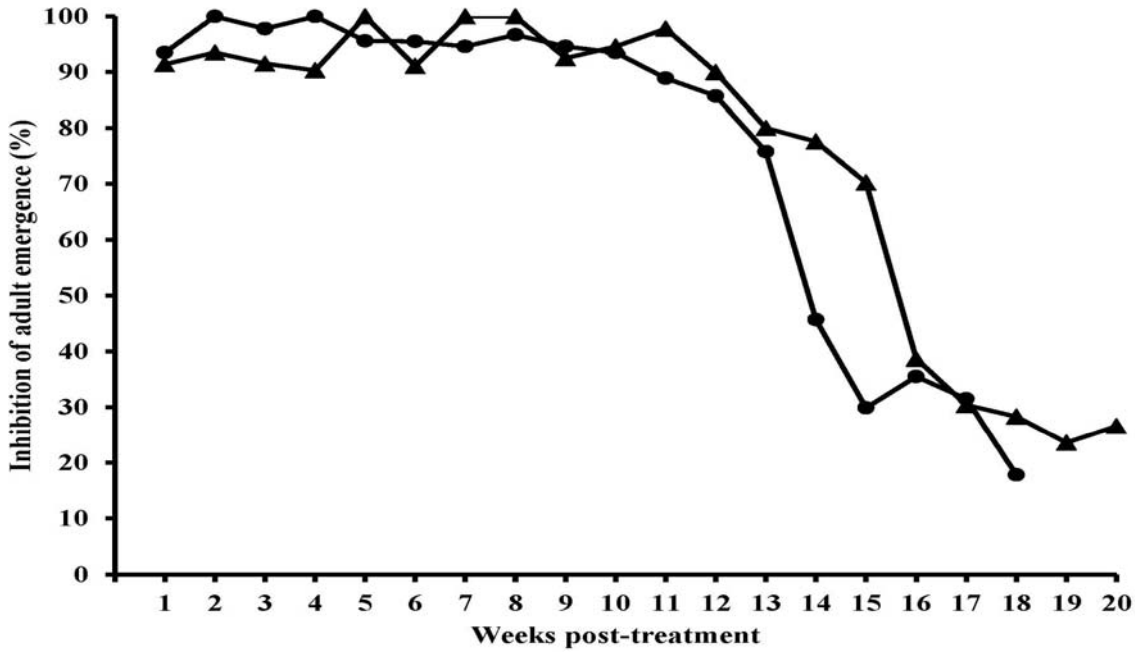

Fig. 2. Percentage emergence inhibition of *A. aegypti* after the treatment of 3rd instar larvae with SRFs of Altosid briquets (●) and Dudim tablets (▲)

Table 3. The effect of larval treatments with SRFs on the blood feeding activity in mosquito females of *A. aegypti* that survived from these treatment

SRFs	No. of engorged females*		Feeding activity (%)		Reduction (%)
	Treatment	Control	Treatment	Control	
Bactimos briquets	21	23	84	92	8.7
Spinosad tablets	20	24	80	96	16.7
Altosid briquets	14	22	56	88	36.4
Dudim tablets	16	23	64	92	30.4

* 25 unfed mosquito females were used.

Table 4. The effect of larval treatments with SRFs on the reproductive potential of *A. aegypti* adults that emerged from survived larvae

SRFs	Egg production		Total No. of larvae hatched	Hatchability (%)	Reduction (%)
	Total	Mean*±S.E.			
Bactimos briquets					
Treatment	724	$36.2^{a} \pm 9.3$	636	87.8	5.1
Control	812	$40.6^{a} \pm 10.1$	753	92.5	
Spinosad tablets					
Treatment	533	$26.7^{a} \pm 10.8$	447	83.9	7.4
Control	756	$37.8^{b} \pm 9.5$	685	90.6	
Altosid briquets					
Treatment	564	$28.2^{a} \pm 10.8$	435	77.1	15.5
Control	827	$41.3^{b} \pm 11.1$	764	91.2	
Dudim tablets					
Treatment	508	$25.4^{a} \pm 9.7$	367	72.2	22.9
Control	792	$39.6^{b} \pm 10.3$	742	93.7	

* Mean of 20 engorged mosquito females; means of each formulation followed by the same letter are not significantly different (P = 0.05).

The reduction in this mean per female was about 10.8%. However, larval treatments with SRFs of Spinosad, Altosid and Dudim caused a marked decrease in the egg-laying capacity of *A. aegypti* female survivors. The mean number of eggs/ female in the above SRF treatments was in respect 26.7, 28.2 and 25.4 eggs as compared with their controls 37.8, 41.3 and 39.6 eggs, respectively. The records thus indicate that larval treatments with these SRFs caused about 29.5, 31.8 and 35.9% reduction in egg production of female survivors. Statistically, the records showed that the differences in the mean number of eggs between treatment trials and control ones were significant. Moreover, the results presented in Table 4 indicated that the hatchability of eggs produced by *A. aegypti* females that emerged from larval treatments with Bactimos briquets (87.8%), Spinosad tablets (83.9%), Altosid briquets (77.1%) and Dudim tablets (72.2%) was reduced as compared with their controls (92.5, 90.6, 91.2 and 93.7%) by about 5.1, 7.4, 15.5 and 22.9%, respectively. A possible explanation is that the exposure of mosquito larvae to residues of SRFs for long periods may be affect its gonads and accordingly the reproductive capacity of adult survivors (Robert and Olsom, 1989; Vasuki, 1999; Saleh *et al.*, 2013). However, long term follow-up studies are needed to determine how physico-chemical factors (i.e.; water pH, high organic matter contents, water temperature and sunlight) affect the larvicidal effectiveness of such SRFs when used for field control measures.

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الملخص العربي

تقييم شبه حقلي لبعض تجهيزات المبيدات الحشرية بطيئة الانسياب ضد بعوضة حمى الضنك *Aedes aegypti*

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أجريت تجارب شبه حقلية لتقيـيم فاعليـة تجهيـزات المبيدات الحشرية بطيئة الانـسياب لأقـراص بكتيمـوس، أقراص أسبينوساد، أقراص ألتوسيد، أقراص دوديم وذلـك ضد يرقات بعوض *Aedes aegypti*. أظهـرت النتـائج أن المعاملة بالتجهيزات بطيئة الانسياب مرة واحدة قد أعطـت مكافحة فعالة طويلة الأمد ضـد يرقـات البعـوض. تـم الحصول على مكافحة فعالة بنسبة ٩٠-١٠٠% تثبيطاً في فقس الحشرات الكاملة لمدة ٤، ٩، ١٠، ١٢ أسبوعاً عقـب المعاملات وذلك باستخدام التجهيزات بطيئة الانسياب مـن بكتيموس، أسبينوساد، ألتوسيد، دوديم على التوالي.

من ناحية أخرى، أظهرت النتائج أن المعاملات اليرقية بالتجهيزات بطيئة الانسياب المختبرة قد أثرت على نـشاط التغذية على الدم والقدرة التناسلية في البعوض البالغ الـذي عاش من تلك المعاملات.