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Larvicidal efficacy of three sustained insecticides against *Culex pipiens* larvae

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Abstract
Three sustained tablet formulas of the bacterial insecticides Spinosad and VectoBac as well as the insect growth regulator Dudim were evaluated for larvicidal activity against larval stages and pupae until adult emergence of *Culex pipiens*. Effective control with 90-100% inhibition of adult emergence (% IE) was used as a criterion to evaluate the effectiveness of the test formulations. A high level of residual efficacy with 90-100% IE was achieved for 8 and 11 weeks post-treatment by using tablet formulations of Spinosad and Dudim, respectively. On the other hand, larval treatments with VectoBac tablet formulations showed ineffective control with 63% IE for the 1st week post-treatment and after that it began to give continuous excellent effective control (90-100% IE) for 5 successive weeks. These estimated times indicate that Dudim formulation proved to be more effective against *C. pipiens* larvae than formulations of Spinosad and VectoBac by about 1.4 and 2.2 times, respectively. Apart from lethal action, larval treatments with the test slow-release formulations led to a reduction in the mean number of eggs laid by mosquito female survivors during the 1st gonotrophic cycle but did not affect the hatchability of eggs.

Keywords: Slow-release formulations, *Culex pipiens*, oviposition, larvicidal

Introduction
Worldwide mosquito related disease gain attention in the recent years. Amount them malaria, filariasis, chikungunya and dengue etc are major diseases present worldwide. Vector control programmes are undertaken by many countries in order to control these diseases [1]. Some of spore producing bacterial strains has Larvicidal activity against different insects such as flies, mosquitoes, black flies and bettles [2]. The bacterial produce spore and that spore enter gut of the mosquito. It disrupt the midgut endothelium layer causing larvae death. Bacterial toxin can easily effect the mosquitoes during the aquatic feeding stages. Different formulas of these bacterial toxins are used for the control of mosquitoes.
Bacterial strain *Saccharopolyspora spinosa* produce a substance Spinosad that has the ability to kill different insects. There are two form of this chemical produces by this strain. Spinosad is used in order to control mosquitoes, mites, ants and some flies [3]. IGR Dudim is one of chitin synthesis inhibitors, responsible for disruption of different insects including mosquitoes. It is safe for animals and other living organisms compare to synthetic pesticides [4]. World Health Organization Pesticide Evaluation Scheme used Spinosad and IGR Dudim recommend it as larvicid for mosquitoes [5].
The concept of slow-release formulations (SRFs) as mosquito larvicides was explored by the U.S. Army in 1966 and tested periodically by the agency thereafter [1]. The idea of SRFs was to incorporate the insecticide into a substance (a matrix) which would permit slow-release of the toxicant into breeding sites of mosquito larvae while protecting the remaining material from hydrolysis, degradation and other types of decomposition [2; 3]. The World Health Organization Expt Committee on Insecticides [4] recommended that further developmental research on slow-release insecticide formulations should be encouraged. Recently, more attention has been paid to the use of some non-conventional insecticides such as insect growth regulators (IGRs) and bacterial agents as slow-release formulations for the control of a wide spectrum of mosquito vectors [5, 6, 7].
The current was carried out to determine the effectiveness of one insect growth regulator (IGR Dudim) and two bionsecticides Spinosad and VectoBac as sustained formulations against

Culex pipiens larvae. Furthermore effect of these formulation against the reproductive potential was also evaluatued.

Materials and Methods

Mosquito sampling and rearing

Culex pipiens, was collected from Jeddah, kingdom of Saudi Arabia.then these mosquitoes were maintained under controlled conditions of 27±1°C and 70±5% R.H. with 14 : 10 (L: D) photoperiod.

Chemicals used

Three slow-release formulations (SRFs) were used: Spinosad DT tablets (*Saccharopolyspora spinose*, 7.48% a.i., tablet weight 1.37 gm) provided by Clark company, Roselle, IL, USA.; VectoBac tablet (*Bacillus thuringiensis israelensis*, *Bti*, 5% a.i., 2700 ITU/mg, tablet weight 0.37 gm) supplied by Valent Bio Sciences Corp., IL, USA and Dudim DT tablets (Diflubenzuron, 2% a.i., tablet weight 2 gm) provided by DGM Italia Srl. The active ingredients of test formulations have different mechanisms of action. Spinosad is a natural product derived from the bacterium *S. spinose*. It affects the insect nervous system at unique sites on the nicotinic acetyl choline and GABA receptors [8]. VectoBac (*Bti*) is a gut poison and its primary action is to lyse midgut epithelial cells of affected larvae and forming pores [9] Dudim (diflubenzuron) is an insect growth regulator that inhibits the synthesis of chitin and hence interferes with moulting [10].

Ovipositional behavior of mosquitoes

(1) Experiment was carried out in suitable white plastic pools (50 × 50 × 30 cm) containing 40 L of tap water. The pools were kept covered with muslin cloth sheets to prevent debris and oviposition by wild mosquitoes. Each pool received a batch of 25 third larval instars of *C. pipiens* and the test formulation. The dosages of each formulation required for larval treatments were as follows: 0.3 gm of Spinosad, yielding 0.56 ppm; one tablet (0.37 gm) of VectoBac, yielding 0.46 ppm and half tablet (1gm) of Dudim, yielding 0.5 ppm. The test dosages were determined according the recommended dosages for field control. Pools without test formulations were used as controls. The larvae were given the usual larval food during these tests. Water lost to evaporation was replenished every other day. New live batches of *C. pipiens* larvae were added weekly to the test pools. Any pupae produced were transferred to plastic cups containing water and placed in adult cages for emergence. The efficacy of test formulations was calculated as the percentage of larvae that did not develop into successfully emerging adults or the inhibition of emergency (%IE).

Effect on reproduction of mosquitoes

Additional trials were also conducted to study the possible delayed effects of the test SRFs on the reproductive potential of mosquito adults that survived from larval treatments. Tests were carried out when the tested formulations began to give <50% IE. Surviving adults for both treatment and control groups were isolated in clean adult cages and provided with 10% sucrose solution. Four days later, mosquito females fed on a living pigeon for a blood meal. The engorged females were allowed to lay egg rafts in cups containing tap water and covered with muslin cloth. Number of eggs per female during the 1st gonotrophic cycle and hatchability of eggs were recorded.

Analysis of data

Abbotts formula was used to compare the percentage of mortality between control and treated one [11]. *t*- test was used to determine the difference between control and treated.

Results and Discussion

Results of the three slow-release tablet formulations of Spinosad, VectoBac and Dudim on the larval stages and pupae until adult emergence of *C. pipiens* are recorded in tables 1-3 and illustrated by Fig. 1. Effective control with 90-100% inhibition of adult emergence (% IE) was used as a standard to evaluate the effectiveness of these formulations.

As shown in Table 1, larval treatments with Spinosad tablets produced high levels of residual efficacy against *C. pipiens* larvae as indicated by 92-100% IE for a period of 8 weeks post-treatment, after which its efficacy decreased to 85% IE at week 9 and then fluctuated between 19.6-64.5% IE during the last four weeks of trials (Fig. 1). On the other hand, larval treatments with VectoBac tablets showed ineffective control with 63% IE against *C. pipiens* for the 1st week post-treatment (Table 2) and then it began to give continuous excellent control with 90-100% IE for 5 successive weeks (Fig. 1). However, the results presented in Table 3 showed that larval treatments with tablets of Dudim against *C. pipiens* produced effective control with 90.4-100% IE for 11 weeks post-treatment and 27% IE by the end of week 15 (Fig. 1).

Generally, taking the durations of effective control (in days) into consideration, the results showed that Dudim tablets (77 days) proved to be highly effective against mosquito larvae of *C. pipiens* followed by Spinosad tablets (56 days), while tablets of VectoBac (35 days) were the less effective ones. The results thus indicate that larval treatment with Dudim formulation against *C. pipiens* is 1.4 and 2.2 times more effective than formulations of Spionsad and VectoBac, respectively. Variations in the durations of efficacy of the test SRFs may reflect differences in their active ingredients and mode of action. However, similar laboratory and field trials in this respect were carried out by serveral investigators using different SRFs of Spinosad [12, 13], VectoBac [6, 14] and Dudim [15, 16] against many species of mosquito vectors.

Table 4 shows the delayed effects of larval treatments with the test SRFs on the reproductive potential of adult survivors. The results showed that tablet formulations of Spinosad and Dudim caused a marked decrease in the egg-laying capacity of *C. pipiens* female survivors. The mean number of egg / female during the 1st gonotrophic cycle was in respect 98.8 and 90.1 eggs as compared with their controls 129.7 and 141.6 eggs, respectively. These records indicate that larval treatments with SRFs of Spinosad and Dudim induced about 23.8% and 36.4% reduction in eggs produced by *C. pipiens* female survivors. Statistically, the results showed that differences in the mean number of eggs between treatment trials and control ones were significant. On the other hand, larval treatments with tablet formulation of Vectobat did not significantly affect the fecundity of mosquito females survived from these treatments. The mean number of eggs produced by female survivors was 134.7 eggs/female when compared with the control which was 147.9 eggs/female. The reduction in this mean per female was about 8.9% (Table 4). Morever, the hatchability of eggs produced by mosquito

females that survived from larval treatments with tablet formulations of Spinosad (84.1%), VectoBac (81.1%) and Dudim (85.2%) was decreased when compared with their control ones (88.7%, 91.7%, and 93.1%) by about 5.2%, 11.6% and 8.5%, respectively (Table 4).

However, such a reducing effect of insecticide formulations was previously recorded by several authors using different formulations of IGRs [17, 18] and bacterial insecticides [19, 20] against different mosquito species. It has been suggested that there are two factors may be caused such a reduction in the reproductive capacity of mosquitoes. First, the exposure of larvae continuously to residues of SRFs may be affect the gonads and accordingly the fecundity of adult survivors, and second, some engorged mosquito females that survived from larval treatments ultimately oviposite very few eggs, most of which fail to hatch successfully [21, 22].

Generally, in the present work, it can be suggested that long-term effective control of *C. pipiens* can be achieved economically with a single application of the test formulations in mosquito breeding sites such as ditches, pond and artificial containers. However, long term follow-up trails are needed to elucidate the possible delayed effects of such formulations on some biological and behavioural aspects of mosquito adult survivors.

Table 1: Efficacy of Spinosad as sustained formulation against *C. pipiens* larvae

Weeks post-treatments	Dead larvae* (%)	Pupae produced (%)	Adult emerged (%)	% IE**
1	100	0.0	0.0	100
2	100	0.0	0.0	100
3	100	0.0	0.0	100
4	92	8	7	93
5	95	5	3	96.7
6	93	7	5	94.7
7	91	9	8	92
8	92	8	5	95
9	81	19	15	85
10	66	34	33	64.5
11	41	59	58	42
12	22	78	74	19.6
13	20	80	75	25

* Control mortalities ranged from 3-8% IE, Four replicates, twenty five third instar larvae each
** control mortalities correlation [11].

Table 2: Efficacy of VectoBac as sustained formulation against *C. pipiens* larvae

Weeks post-treatments	Dead larvae* (%)	Pupae produced (%)	Adult emerged (%)	% IE**
1	61	39	37	63
2	100	0.0	0.0	100
3	92	8	8	90
4	98	2	2	98
5	93	7	6	94
6	88	12	9	91
7	82	18	16	84
8	69	31	27	70.6
9	51	49	46	54
10	38	62	59	41
11	30	70	66	28.3
12	24	76	74	26
13	19	81	77	18.1

* Control mortalities ranged from 3-8% IE, Four replicates, twenty five third instar larvae each
** control mortalities correlation [11].

Table 3: Efficacy of Dudim as sustained formulation against *C. pipiens* larvae

Weeks post-treatments	Dead larvae* (%)	Pupae produced (%)	Adult emerged (%)	% IE**
1	28	72	9	91
2	35	65	0.0	100
3	33	67	7	93
4	41	59	3	96.8
5	30	70	0.0	100
6	29	71	2	97.9
7	37	63	8	92
8	36	64	6	94
9	22	78	9	90.4
10	26	74	8	92
11	32	68	9	91
12	19	81	48	52
13	26	74	69	31

14	18	82	67	27.9
15	21	79	73	27

* Control mortalities ranged from 3-8% IE, Four replicates, twenty five third instar larvae each
** control mortalities correlation [11].

Table 4: Oviposition and egg-hatchability of *C. pipiens* mosquito adults survived from larval treatments with sustain formulation of Spinosad, VectoBac and Dudim

Formulation	Egg production			Total No. of larvae hatched	Hatchability (%)	Reduction (%)
	Total*	Mean**±SE	Reduction			
Spinodad	1976	98.8b±2.8	23.8	1662	84.1	5.2
Control	2594	129.7a±4.1		2300	88.7	
VectoBac	2693	134.7a±3.1	8.9	2184	81.1	11.6
Control	2958	147.9a±1.7		2712	91.7	
Dudim	1802	90.1b±1.9	36.4	1535	85.2	8.5
Control	2832	141.6a±3.3		2637	93.1	

* 20 engorged mosquito females were used.
** Mean of each formulation (P=0.05).

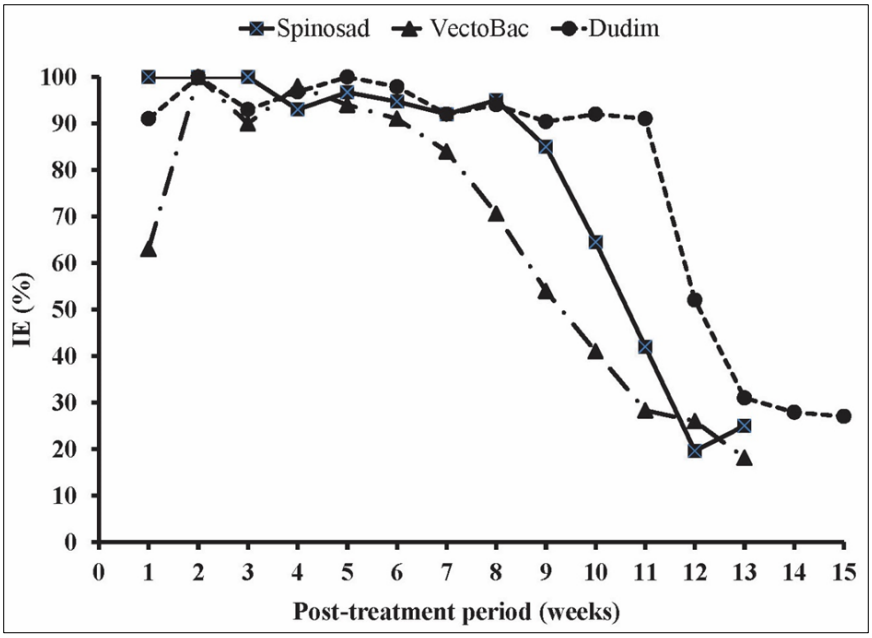

Fig 1: Percentage emergence inhibition of *C. pipiens* after treatment with sustain formulations of Spionsad, VectoBac and Dudim

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