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Slow release formulations of *Bacillus thuringiensis israelensis* (AM 65-52) and spinosyns: effectiveness against the West Nile vector *Culex pipiens* in Saudi Arabia

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ABSTRACT

Objective: To investigate the effectiveness of slow release formulations of *Bacillus thuringiensis israelensis* (AM 65-52) (*B. thuringiensis israelensis*) and spinosyns against the West Nile vector *Culex pipiens* (*Cx. pipiens*) in Saudi Arabia.
Methods: We tested slow release insecticide formulations of Natular DT, Tap 60 and VectoBac granule against II instars of *Cx. pipiens* larvae in 50 L laboratory arenas.
Results: Slow release formulations of *B. thuringiensis israelensis* and spinosyns gave continuous control against *Cx. pipiens* for several weeks. Natular DT was more effective over Tap 60 and VectoBac granule of about 1.3 and 5.8 times, respectively. Variations in the durations of effective control among the tested slow release formulations may reflect differences in their active ingredients and the mode of action.
Conclusions: Our results highlighted the effectiveness of *B. thuringiensis israelensis* and spinosyns against an important West Nile vector, providing baseline data to develop eco-friendly mosquito control programs in Saudi Arabia.

1. Introduction

Mosquitoes (Diptera: Culicidae) pose a major threat to millions of people worldwide, as they vector important parasites and pathogens, including malaria, dengue, chikungunya, Japanese encephalitis, lymphatic filariasis and Zika virus[1-3]. *Culex pipiens* L. (*Cx. pipiens*) is an important vector of West Nile virus, Rift Valley fever and bancroftian filariasis. Filariasis is caused by Filariodidea nematodes, namely, *Wuchereria bancrofti*, which is responsible for 90% of cases, *Brugia malayi*, and *Brugia timori*[4]. Nowadays, more than 1.4 billion people in 73 countries are living in areas where lymphatic filariasis is transmitted and are at risk of being infected. Globally, an estimated 25 million men suffer with genital disease and over 15 million people are afflicted with lymphoedema. Eliminating lymphatic filariasis can prevent unnecessary suffering and contribute to the reduction of poverty[4].

The current strategy of integrated pest management comprises the general approach of eco-friendly control measures may involve several complements[5]. Until a few years ago, only the adults were sprayed, but now, a more efficient way of reducing mosquito populations is to target the egg and larval instars[6-8]. The global use of insecticides for mosquito vector control in recent decades have negative effects on the human health and the environment, and lead to development of insecticide resistance[7]. To deal with

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these crucial challenges, in recent years biological insecticides have been developed[7,8].

Many biological control agents have been evaluated against larval stages of mosquitoes, of which the most successful ones comprise bacteria *Bacillus thuringiensis* (*B. thuringiensis*) and *Bacillus sphaericus*[9]. VectoBac granule (VectoBac G) is granular formulations of *Bacillus thuringiensis israelensis* (AM 65-52) (*B. thuringiensis israelensis*) for the control of mosquito larvae. *Bacillus* sp. produces large, spreading, gray-white colonies with irregular margins. A unique characteristic of this bacterium is its ability to produce endospores when environmental conditions are stressful. *B. thuringiensis* is currently marketed worldwide as control agents of many important plant pests, mainly Lepidoptera, mosquito and black flies larvae[10]. The toxic action of *B. thuringiensis* starts when the larvae ingested the insecticidal crystalline protein spore complex. In the midgut, the insecticidal crystalline protein is dissociated to protoxins and activated by gut proteases, inducing the arrest of feeding and leading to larval death[11]. The National Dengue Control Program in Brazil employed VectoBac wettable granule (VectoBac WG) for routine treatment of reservoirs of drinking water, controlling temephos-resistant *Aedes aegypti* (*Ae. aegypti*) larvae. VectoBac WG was the most suitable Bti formulation[12,13].

Natular DT (spinosad), a mixture of spinosyns A and D, known as fermentation products of a soil actinomycete *Saccharopolyspora spinosa*[14], is a biological neurotoxic insecticide that was approved and registered by the US Environmental Protection Agency as a larvicide for mosquito control in October 2007[15]. Spinosad is highly active by both contact and ingestion to numerous pests in the orders Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera and Hymenoptera[16,17]. Spinosad has little toxicity to vertebrates and has recently been approved for use as a mosquito larvicide in human drinking water[18]. The spinosad degrades rapidly, minimizing potential exposure[19,20]. Spinosad also establishes a new standard for low environmental and human risks and offers new approaches to integrated pest and insecticide resistance management. Hence, the use of microbial insecticides provides alternatives to chemical insecticides. Slow release larvicides have been recognized as efficacious and cheap mosquitocides. Such formulations can reduce the frequency and cost of insecticide application, especially in situations where large or inaccessible bodies of water require repetitive treatments.

In this research, we investigated the effectiveness of slow release formulations of *B. thuringiensis israelensis* and spinosyns against the West Nile vector *Cx. pipiens* in Saudi Arabia. Following the World Health Organization method, we tested slow release insecticide formulations of Natular DT, Tap 60 and VectoBac G against II instars of *Cx. pipiens* larvae.

2. Materials and methods

2.1. Collection sites

A field strain of *Cx. pipiens* L. was used in this study. The parental strain was raised from wild larvae collected from Jeddah City, Saudi Arabia, and maintained under laboratory conditions of (27 ± 1) °C and (70 ± 5)% relative humidity, with natural photoperiod.

2.2. Slow release formulations

Three slow release formulations were tested, *i.e.* Natular DT (direct application tablets), Tap 60 and VectoBac G (Figure 1). Spinosad is a natural product derived from the bacterium *Saccharopolyspora spinosa*. It effects as a GABA neurotransmitter agonist and kills insects by hyperexcitation of the insect nervous system.

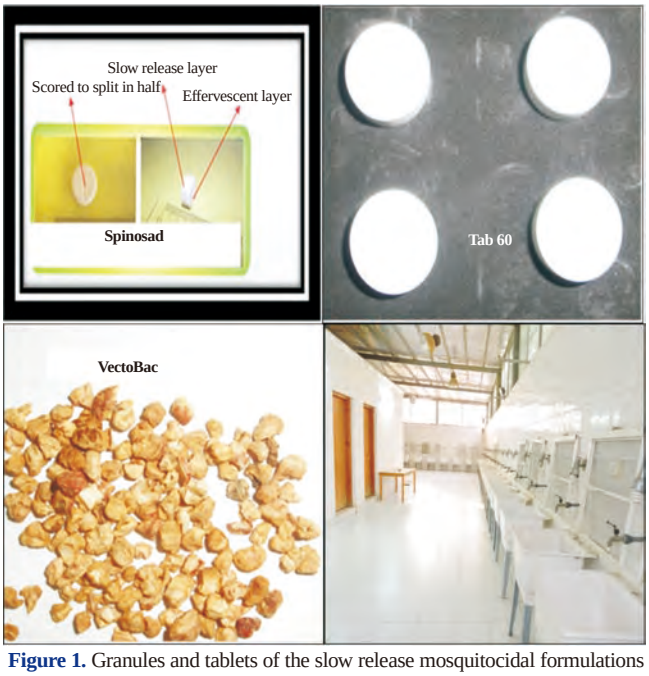

Figure 1. Granules and tablets of the slow release mosquitocidal formulations were tested in this study. The fourth image represented the experimental arenas where *Cx. pipiens* larvae were tested.

2.3. Semi-field experiments

Experiments were carried out in glass pools (54 cm × 52 cm × 30 cm) containing 50 L of tap water. Each pool received a batch of 25 larvae (II instar) of *Cx. pipiens* plus the tested formulations[21]. Untreated pools were used as controls. The dosage of each formulation required for larval treatments were 0.35 g for Natular DT, 0.34 g for Tap 60 and 0.40 g for VectoBac G. They were determined by calculating the total surface of water in the pool as well as accordingly to the recommended dosages for field trials. The larvae were fed during the tests. All tests and controls were replicated four times. Water lost to evaporation was

replenished every day. Larval mortalities were recorded daily until all larvae either died or pupated. The live pupae were transferred to untreated water in clean glass beakers for emergence. When complete larval mortality occurred, new live larvae were added to the test pools. This procedure was continued consecutively until the efficacy of each formulation reached a low level (*i.e.* less than 50% inhibition of adult emergence).

2.4. Data analysis

Mortality data were corrected using the Abbott's formula[22], then analyzed by ANOVA followed by Tukey's honestly significant difference (HSD) test. Statistical parameters were calculated according the method[23].

3. Results

3.1. Efficacy of slow release formulation of Natular DT

The effectiveness of a slow release formulation of Natular DT on the larval and pupal stages of *Cx. pipiens* was showed in Table 1 and Figures 2 and 3. Effective control was defined as 90%–100% inhibition of adult emergence. In our experiemnts, the treatments with slow release formulation were gave continuous effective control against *Cx. pipiens* for several weeks. Table 1 shows the lethal toxicity of the product Natular DT on the larval stage as well as the inhibition of adult emergence in *Cx. pipiens*. During one to five weeks, exposure to Natular DT produced 100% larval mortality and 100% inhibition of adult emergence when compared to 10 weeks where the larval mortality reached to 60% and 40% of pupation. The records showed that larval treatments with Natular DT provided tremendous effectiveness against *Cx. pipiens* with 90%–100% inhibition of adult emergence for 70 days post-treatment (Table 1 and Figure 2).

Table 1
Efficacy of slow release formulation of Natular DT on larvae of the West Nile vector *Cx. pipiens*.

Number	Larval mortality (%)[a]	Pupation (%)	Adult emergence (%)	Inhibition (%)	DEC (days)[d]
1	$100.0 \pm 0.0^{*(6)b}$	$0.0 \pm 0.0^{*}$	$0.0 \pm 0.0^{*}$	$100.0 \pm 0.0^{*}$	70
2	$100.0 \pm 0.0^{*(6)b}$	$0.0 \pm 0.0^{*}$	$0.0 \pm 0.0^{*}$	$100.0 \pm 0.0^{*}$	
3	$100.0 \pm 0.0^{*(6)b}$	$0.0 \pm 0.0^{*}$	$0.0 \pm 0.0^{*}$	$100.0 \pm 0.0^{*}$	
4	$100.0 \pm 0.0^{*(6)b}$	$0.0 \pm 0.0^{*}$	$0.0 \pm 0.0^{*}$	$100.0 \pm 0.0^{*}$	
5	$100.0 \pm 0.0^{*(6)b}$	$0.0 \pm 0.0^{*}$	$0.0 \pm 0.0^{*}$	$100.0 \pm 0.0^{*}$	
6	$95.0 \pm 2.0^{*(20)b}$	$5.0 \pm 5.0^{*}$	2.0 ± 2.0	$98.0 \pm 1.0^{*}$	
7	$94.0 \pm 2.0^{(20)b}$	6.0 ± 2.0	6.0 ± 1.0	$94.0^{(93.62)c} \pm 1.0$	
8	$86.0 \pm 2.0^{(3)b}$	14.0 ± 2.0	14.0 ± 2.0	$86.0^{(85.11)c} \pm 2.0$	
9	$71.0 \pm 2.0^{(3)b}$	29.0 ± 1.0	29.0 ± 1.0	71.0 ± 2.0	
10	$60.0 \pm 3.0^{(1)b}$	40.0 ± 4.0	40.0 ± 2.0	60.0 ± 3.0	

DEC: Duration of effective control; [a]: Four replicates, 25 larvae each; [b]: Number of treatments were carried out before recording larval mortality, pupation or adult emergence; [c]: Corrected with Abbott's formula to correct the percentage of inhibition of metamorphosis (*i.e.* the transition from larva stage to pupa stage), control mortality ranged from 4% to 6%; [d]: Number of days of effective control, *i.e.* from 90% to 100% inhibition of adult emergence after treatment; [*]: Within a column, means ± SD followed by the asterisks were not significantly different (Tukey's HSD test, $P < 0.01$).

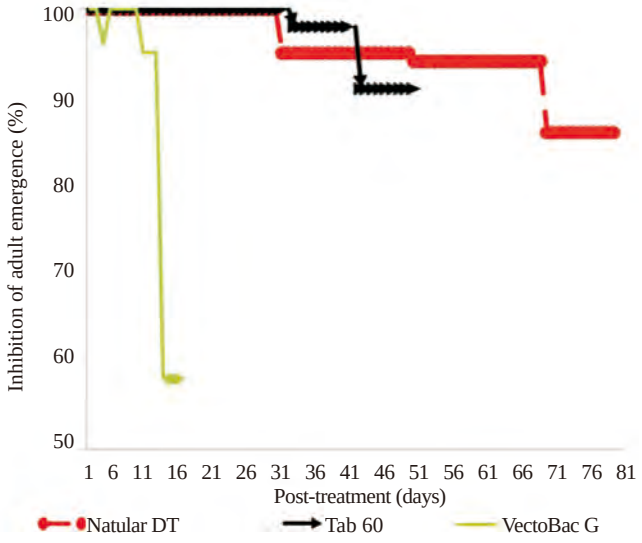

Figure 2. Mortality (%) of *Cx. pipiens* larvae (II instar) post-treatment with slow release formulations of Natular DT, Tab 60 and VectoBac G.

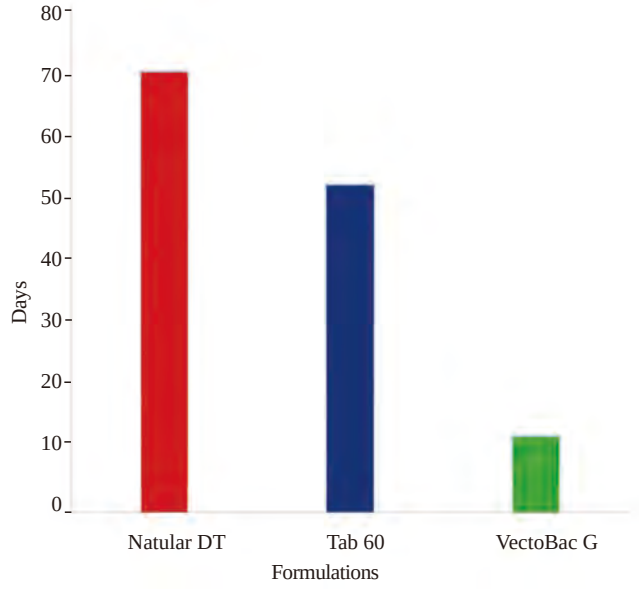

Figure 3. Total duration (days) of the effectiveness of slow release formulations of Natular DT, Tab 60 and VectoBac G against *Cx. pipiens* larvae.

3.2. Efficacy of slow release formulations of Tap 60 and VectoBac G

The efficacy of Tap 60 and VectoBac G on the larval stage and pupal until adult emergence of *Cx. pipiens* was showed in Tables 2 and 3. During the first five weeks Tap 60 produced 6%–16% of larval mortality and 90%–100% inhibition of adult emergence, while it began to lose its effectiveness after 52 days (Figure 2). Concerning VectoBac G, effective control with 90%–100% inhibition of adult emergence was achieved after six/seven days post-treatment (Figure 4). The percentage inhibition of adult emergence after seven days of post treatment ranges from 95%–64%. Overall, recommended dosage information and number of days of treatment were provided in Table 4.

Table 2
Evaluation of the efficacy of slow release formulation of Tab 60 on the West Nile vector *Cx. pipiens*.

Number	Larval mortality (%)[a]	Pupation (%)	Adult emergence (%)	Inhibition (%)	DEC (days)[d]
1	10.0 ± 1.0$^{(10)b}$	90.0 ± 1.0	0.0 ± 0.0*	100.0 ± 0.0*	52
2	16.0 ± 2.0$^{*(11)b}$	84.0 ± 2.0*	0.0 ± 0.0*	100.0 ± 0.0*	
3	12.0 ± 2.0$^{*(11)b}$	88.0 ± 2.0*	0.0 ± 0.0*	100.0 ± 0.0*	
4	6.0 ± 1.0$^{*(10)b}$	94.0 ± 2.0*	2.0 ± 1.1	98.0 ± 1.0$^{(97.8)c}$	
5	11.0 ± 1.0$^{*(10)b}$	91.0 ± 2.0*	9.0 ± 1.0	91.0 ± 1.0$^{(90.22)c}$	
6	4.0 ± 1.0$^{(10)b}$	96.0 ± 1.0	18.0 ± 2.0	82.0 ± 2.0	
7	7.0 ± 1.0$^{*(11)b}$	93.0 ± 2.0*	23.0 ± 3.0	77.0 ± 3.0	
8	4.0 ± 1.0$^{*(10)b}$	96.0 ± 2.0*	38.0 ± 2.0	62.0 ± 4.0	

DEC: Duration of effective control; [a]: Four replicates, 25 larvae each; [b]: Number of treatments were carried out before recording larval mortality, pupation or adult emergence; [c]: Corrected with Abbott's formula to correct the percentage of inhibition of metamorphosis (*i.e.* the transition from larva stage to pupa stage), control mortality ranged from 4% to 6%; [d]: Number of days of effective control, *i.e.* from 90% to 100% inhibition of adult emergence after treatment; [*]: Within a column, means ± SD followed by the asterisks were not significantly different (Tukey's HSD test, $P < 0.01$).

Table 3
Evaluation of the efficacy of slow release formulation of VectoBac G on the West Nile vector *Cx. pipiens*.

Number	Larval mortality (%)[a]	Pupation (%)	Adult emergence (%)	Inhibition (%)	DEC (days)[d]
1	100.0 ± 0.0$^{*(1)b}$	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	12
2	100.0 ± 0.0$^{*(1)b}$	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
3	100.0 ± 0.0$^{*(1)b}$	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
4	100.0 ± 0.0$^{*(1)b}$	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
5	100.0 ± 0.0$^{*(1)b}$	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
6	100.0 ± 0.0$^{*(1)b}$	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
7	100.0 ± 0.0$^{*(1)b}$	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
8	95.0 ± 2.0$^{*(3)b}$	5.0 ± 2.0	5.0 ± 2.0	95.0 ± 2.0	
9	84.0 ± 2.0$^{*(2)b}$	29.0 ± 1.0	29.0 ± 1.0	71.0 ± 2.0	
10	64.0 ± 2.0$^{*(3)b}$	36.0 ± 3.0	36.0 ± 2.0	64.0 ± 2.0	

DEC: Duration of effective control; [a]: Four replicates, 25 larvae each; [b]: Number of treatments were carried out before recording larval mortality, pupation or adult emergence; [c]: Corrected with Abbott's formula to correct the percentage of inhibition of metamorphosis (*i.e.* the transition from larva stage to pupa stage), control mortality ranged from 2% to 4%; [d]: Number of days of effective control, *i.e.* from 90% to 100% inhibition of adult emergence after treatment; [*]: Within a column, means ± SD followed by the asterisks were not significantly different (Tukey's HSD test, $P < 0.01$).

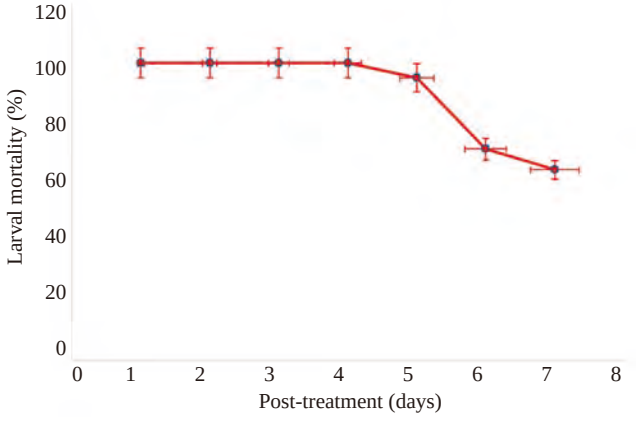

Figure 4. Mortality (%) of the *Cx. pipiens* larvae (II instar) post-treatment with a slow release formulation of VectoBac G.

Table 4
Effectiveness of slow release insecticide formulations against the larval instars of *Cx. pipiens*.

Treatment	Recommended dose	Ration use	Days
Natular DT	1.4 g/200 L	0.070 g/10 L	70
Tap 60	2.0 g/300 L	0.067 g/10 L	52
VectoBac G	8.0 g/1 000 L	0.080 g/10 L	12

4. Discussion

Nowadays, mosquito vector control is challenging due to the emergence of resistance to conventional synthetic insecticides, warranting either counter measures or development of newer insecticides[24]. *B. thuringiensis israelensis* is a biocontrol agent ideal for the control of *Anopheles* and *Culex* mosquitoes, due to its prolonged killing action[25,8]. It has been proved to be effective against *Culex quinquefasciatus* (*Cx. quinquefasciatus*), a vector of bancroftian filariasis, breeding in urban and peri-urban areas[26]. In this study, Natular DT was more efficient against *Cx. pipiens* by about 1.3 folds than Tap 60 and 5.8 folds than VectoBac G, respectively. Interestingly, Natular DT has little toxicity to vertebrates and has recently been approved for use as a mosquito larvicide in human drinking water[18]. It has been shown to be effective in preventing or reducing the development of immature aquatic stages of important vector species, particularly *Ae. aegypti*, *Aedes albopictus*, *Anopheles gambiae*, *Anopheles pseudopunctipennis*, *Anopheles albimanus*, *Cx. pipiens* and *Cx. quinquefasciatus*[15]. In addition, Kovendan *et al.*[27] pointed out that the bacterial insecticide spinosad is highly effective on larvae of the chikungunya vector *Ae. aegypti* with LC_{50} values ranging from 51.76 mg/L (I instar larvae) to 93.44 mg/L (pupae). Kumar *et al.*[28] highlighted that spinosad is highly effective against the larvae and pupae of *Anopheles stephensi* (*An. stephensi*) and *Ae. aegypti*. After 24 h of exposure, LC_{50} values against *An. stephensi* were 384.19 (I instar larvae) and 572.63 mg/L (pupae). LC_{50} values against *Ae. aegypti* were 210.68 mg/L (I instar larvae) and 305.85 mg/L (pupae). Furthermore, Madhiyazhagan *et al.*[29] reported that azadirachtin and spinosad may be successfully employed as larvicides against *Chironomus kiiensis*. Recently, Duchet *et al.*[30] reported that *B. thuringiensis israelensis* and spinosad were effective in reducing adult emergence of the non-biting midges *Polypedilum nubifer* and *Tanytarsus curticornis*. In this research, the deviation in the durations of efficacy among the tested formulations may be attributed to the differential mode of action of the active ingredients and the concentration tested[31]. We hypothesized that the toxicity of spinosad against *Cx. pipiens* may be the excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and paralysis[32].

On the other hand, the larvicidal performance of VectoBac G was relatively poor with one week of complete control of *Cx. pipiens* larvae per each season. The low persistence of *B. thuringiensis*-based product, also reported in previous trials, is particularly evident when exposed to direct sunlight[33]. In this study VectoBac G effective control with 90%–100% inhibition of adult emergence was achieved after six-seven days of post-treatment. Percentage inhibition of adult emergence after seven days of post treatment ranges from 95%–58%. For instance, Karch *et al.*[34] highlighted that polluted gutter water, the breeding site of *Cx. quinquefasciatus*,

was treated with 2, 4, and 6 L/ha of VectoBac 12 aqueous suspension. At all doses larval mortality was higher than 95% on post-treatment day 1, while larval mortality was less than 40% on post-treatment day 2 and the larval population began to recover 7 days after treatment. Similarly, in this study the larval mortality was 90%–100% on post treatment from day 1 to day 7 as compared from day 8 to day 12. Also, Lee *et al*.[35] reported that wettable granule formulation of *B. thuringiensis israelensis*, VectoBac WG against dengue vectors, *Ae. aegypti* and *Aedes albopictus* in the state of Selangor, Malaysia. Further, the aqueous suspension of *B. thuringiensis israelensis* (VectoBac 12 aqueous suspension) are highly toxic against *An. culicifacies* and *An. stephensi* in laboratory and field conditions[36]. Furthermore, Aldemir *et al*.[37] showed the commercial formulation of VectoBac 12 aqueous suspension and VectoBac G was highly effective against *Anopheles sacharovi*, *Anopheles maculipennis*, *Cx. pipiens*, and *Culex thelleri*. Recently, Djenontin *et al*.[38] reported that the VectoBac granules (potency 200 International Toxin Units per milligram), a new formulation of bacterial larvicide *B. thuringiensis israelensis* was highly effective in field trials against *Anopheles gambiae* and *Cx. quinquefasciatus*. In addition, Panneerselvam *et al*.[39] highlighted that *B. thuringiensis* are highly toxic against *An. stephensi*, LC_{50} values ranging from 1.72 g/L (I instar larvae) to 2.42 g/L (pupae). The renewal of interest in the integrated methods of vector control during the early 1980s was renewed with the use of environmental friendly approaches in vector control[40], and naturally occurred insecticides may play a more prominent role in mosquito control programs in the future[41].

In this research, the effectiveness of Natular DT was higher on *Cx. pipiens* larvae, if compared to Tap 60 and VectoBac G. Spinosad outperformed Tap 60 and VectoBac G, which provided brief or intermediate periods of Culicidae control. Due to the very low mammalian toxicity[42] and rapid breakdown in the environment[43], there can be little doubt that spinosyns represent an important improvement over conventional mosquitocides in terms of safety. Overall, our results highlighted the effectiveness of spinosyns against an important West Nile vector, providing baseline data to develop eco-friendly mosquito control programs in Saudi Arabia.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Mehlhorn H, Al-Rasheid KA, Al-Quraishy S, Abdel-Ghaffar F. Research and increase of expertise in arachno-entomology are urgently needed. *Parasitol Res* 2012; **110**: 259-65.

[2] Benelli G, Mehlhorn H. Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. *Parasitol Res* 2016; **115**(5): 1747-54.

[3] Benelli G, Canale A, Higuchi A, Murugan K, Pavela R, Nicoletti M. The recent outbreaks of Zika virus: Mosquito control faces a further challenge. *Asian Pac J Trop Dis* 2016; **6**(4): 253-8.

[4] World Health Organization. Lymphatic filariasis. Geneva: World Health Organization; 2016. [Online] Available from: http://www.who.int/mediacentre/factsheets/fs102/en/ [Accessed on 25th May, 2016]

[5] Ruiu L. Insect pathogenic bacteria in Integrated Pest Management. *Insects* 2015; **6**: 352-67.

[6] Benelli G. Research in mosquito control: current challenges for a brighter future. *Parasitol Res* 2015; **114**: 2801-5.

[7] Benelli G. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. *Parasitol Res* 2015; **114**: 3201-12.

[8] Panneerselvam C, Murugan K, Kovendan K, Kumar PM, Subramaniam J. Mosquito larvicidal and pupicidal activity of *Euphorbia hirta* Linn. (Family: Euphorbiaceae) and bacterial insecticide, *Bacillus sphaericus* against *Anopheles stephensi* Liston. (Diptera: Culicidae). *Asian Pac J Trop Med* 2013; **6**(2): 102-9.

[9] Suryadi BF, Yanuwiadi B, Ardyati T, Suharjono S. Evaluation of entomopathogenic *Bacillus sphaericus* isolated from lombok beach area against mosquito larvae. *Asian Pac J Trop Biomed* 2016; **6**(2): 148-54.

[10] Ramanujam B, Rangeshwaran R, Sivakmar G, Mohan M, Yandigeri MS. Management of insect pests by microorganisms. *Proc Indian Nat Sci Acad* 2014; **80**(2): 455-71.

[11] Ruiu L, Satta A, Floris I. Emerging entomopathogenic bacteria for insect pest management. *Bull Insectol* 2013; **66**(2): 181-6.

[12] Vilarinhos PTR, Monnerat R. Larvicidal persistence of formulations of *Bacillus thuringiensis* var. *israelensis* to control larval *Aedes aegypti*. *J Am Mosq Control Assoc* 2004; **20**: 311-4.

[13] Brazilian Ministry of Health. Rules for the employ of *Bacillus thuringiensis israelensis* insecticides in *Aedes aegypti* control programs. Technical Note N. 06/05/CGPNCD/DIGES/SVS/ MS, 2005.

[14] Crouse GD, Sparks TC, Schoonover J, Gifford J, Dripps J, Bruce T, et al. Recent advances in the chemistry of spinosyns. *Pest Manag Sci* 2001; **57**: 177-85.

[15] Hertlein MB, Mavrotas C, Jousseaume C, Lysandrou M, Thompson GD, Jany W, et al. A review of spinosad as a natural product for larval mosquito control. *J Am Mosq Control Assoc* 2010; **26**: 67-87.

[16] Bacci L, Lupi D, Savoldelli S, Rossaro B. A review of spinosyns, a derivative of biological acting substances as a class of insecticides with a broad range of action against many insect pests. *J Entomol Acarol Res* 2016; **48**: 40-52.

[17] Prabhu K, Murugan K, Nareshkumar A, Bragadeeswaran S. Larvicidal and pupicidal activity of spinosad against the malarial vector *Anopheles stephensi*. *Asian Pac J Trop Med* 2011; **4**(8): 610-3.

[18] World Health Organization. Spinosad DT in drinking water: use for vector control in drinking-water sources and containers. Geneva: World Health Organization; 2010. [Online] Available from: http://www.who.int/water_sanitation_health/dwq/chemicals/spinosadbg.pdf [Accessed on 25th May, 2016]

[19] United States Environmental Protection Agency. Pesticide Fact Sheet: Spinosad. Washington: United States Environmental Protection Agency, Office of Pesticides and Toxic Substances; 1999. [Online] Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-110003_19-Jul-99.pdf [Accessed on 25th May, 2016]

[20] Medina P, Morales J, Smagghe G, Vinuela E. Toxicity and kinetics of spinosad in different developmental stages of the endoparasitoid *Hyposoter didymator* (Hymenoptera: Ichneumonidae) and its host *Spodoptera littoralis* larvae (Lepidoptera: Noctuidae). *Biocontrol* 2008; **53**: 569-78.

[21] World Health Organization. Guidelines for laboratory and field-testing of mosquito larvicides. Geneva: World Health Organization; 2005. [Online] Available from: http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf [Accessed on 25th May, 2016]

[22] Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; **18**(2): 265-7.

[23] Litchfield JT Jr, Wilcoxon F. A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* 1949; **96**: 99-113.

[24] Karunamoorthi K, Sabesan S. Insecticide resistance in insect vectors of disease with special reference to mosquitoes: a potential threat to global public health. *Health Scope* 2013; **2**(1): 4-18.

[25] Kovendan K, Murugan K, Vincent S, Barnard DR. Studies on larvicidal and pupicidal activity of *Leucas aspera* Willd. (Lamiaceae) and bacterial insecticide, *Bacillus sphaericus*, against malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). *Parasitol Res* 2011; **110**: 195-203.

[26] Mwangangi JM, Kahindi SC, Kibe LW, Nzovu JG, Luethy P, Githure JI, et al. Wide-scale application of Bti/Bs biolarvicide in different aquatic habitat types in urban and peri-urban Malindi, Kenya. *Parasitol Res* 2011; **108**: 1355-63.

[27] Kovendan K, Murugan K, Naresh Kumar A, Vincent S, Hwang JS. Bioefficacy of larvicdial and pupicidal properties of *Carica papaya* (Caricaceae) leaf extract and bacterial insecticide, spinosad, against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). *Parasitol Res* 2012; **110**: 669-78.

[28] Kumar PM, Kovendan K, Murugan K. Integration of botanical and bacterial insecticide against *Aedes aegypti* and *Anopheles stephensi*. *Parasitol Res* 2013; **112**: 761-71.

[29] Madhiyazhagan P, Murugan K, Nareshkumar A, Nataraj T, Amerasan D, Kalimuthu K, et al. Larvicidal effect of spinosad and azadirachtin against dengue vector, *Aedes aegypti* (Insecta: Diptera: Culicidae) and *Chironomus kiiensis* (Chironomidae). In: Verma AK, Singh GD, Gupta VK, editors. *Perspectives in animal ecology and reproduction*. New Delhi: Astral International (P) Ltd; 2013, p. 159-88.

[30] Duchet C, Franquet E, Lagadic L, Lagneau C. Effects of *Bacillus thuringiensis israelensis* and spinosad on adult emergence of the non-biting midges *Polypedilum nubifer* (Skuse) and *Tanytarsus curticornis* Kieffer (Diptera: Chironomidae) in coastal wetlands. *Ecotoxicol Environ Saf* 2015; **115**: 272-8.

[31] Thavara U, Tawatsin A, Asavadachanukorn P, Mulla MS. Field evaluation in Thailand of spinosad, a larvicide derived from *Saccharopolyspora spinosa* (Actinomycetales) against *Aedes aegypti* (L.) larvae. *Southeast Asian J Trop Med Public Health* 2009; **40**: 235-42.

[32] Kumar AN, Murugan K, Madhiyazhagan P, Prabhu K. Spinosad and neem seed kernel extract as bio-controlling agents for malarial vector, *Anopheles stephensi* and non-biting midge, *Chironomus circumdatus*. *Asian Pac J Trop Med* 2011; **4**(8): 614-8.

[33] Batra CP, Mittal PK, Adak T. Control of *Aedes aegypti* breeding in desert coolers and tires by use of *Bacillus thuringiensis* var. *israelensis* formulation. *J Am Mosq Control Assoc* 2000; **16**: 321-3.

[34] Karch S, Manzambi ZA, Salaun JJ. Field trials with Vectolex (*Bacillus sphaericus*) and Vectobac (*Bacillus thuringiensis* (H-14)) against *Anopheles gambiae* and *Culex quinquefasciatus* breeding in Zaire. *J Am Mosq Control Assoc* 1991; **7**: 176-9.

[35] Lee HL, Chen CD, Masri SM, Chiang YF, Chooi KH, Benjamin S. Impact of larviciding with a *Bacillus thuringiensis israelensis* formulation, vectobac WG, on dengue mosquito vectors in a dengue endemic site in Selangor State, Malaysia. *Southeast Asian J Trop Med Public Health* 2008; **39**(4): 601-9.

[36] Mittal PK. Biolarvicides in vector control: challenges and prospects. *J Vector Borne Dis* 2003; **40**: 20-32.

[37] Aldemir A. The efficacy and longevity of VectoBac 12 AS and VectoBac G (both based on *Bacillus thuringiensis* subsp. *israelensis*) for the control of mosquitoes in Turkey. *Turk J Zool* 2007; **31**: 317-23.

[38] Djenontin A, Pennetier C, Zogo B, Soukou KB, Ole-Sangba M, Akogbéto M, et al. Field efficacy of Vectobac GR as a mosquito larvicide for the control of anopheline and culicine mosquitoes in natural habitats in Benin, West Africa. *PLoS One* 2014; **9**(2): e87934.

[39] Panneerselvam C, Murugan K, Kovendan K, Kumar PM, Ponarulselvam S, Amerasan D, et al. Larvicidal efficacy of *Catharanthus roseus* Linn. Family: Apocynaceae) leaf extract and bacterial insecticide *Bacillus thuringiensis* against *Anopheles stephensi* Liston. *Asian Pac J Trop Med* 2013; **6**(11): 847-53.

[40] Saleh MS. Effects of six insect growth regulators on mosquito larvae of *Aedes aegypti*. *Int J Trop Insect Sci* 1985; **6**: 609-11.

[41] Wandscheer CB, Duque JE, da Silva MA, Fukuyama Y, Wohlke JL, Adelmann J, et al. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. *Toxicon* 2004; **44**: 829-35.

[42] Breslin WJ, Marty MS, Vedula UV, Liberacki AB, Yano BL. Developmental toxicity of Spinosad administered by gavage to CD rats and New Zealand white rabbits. *Food Chem Toxicol* 2000; **38**(12): 1103-12.

[43] Cleveland CB, Mayes MA, Cryer SA. An ecological risk assessment for spinosad use on cotton. *Pest Manag Sci* 2002; **58**: 70-84.