BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2018. Vol. **15**(4), p. 841-849

Evaluation of Effectiveness and Persistence of three Slow Release Formulations against *Aedes Aegypti* (L) (Diptera: Culicidae) under Laboratory Conditions

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http://dx.doi.org/10.13005/bbra/2693

(Received: 09 October 2018; accepted: 14 December 2018)

A laboratory evaluation of three slow-release formulations (SRFs) of spinosad tablets, sumilarv granules and du-dim tablets was carried out in 20 liters plastic containers. The tested formulations were applied according to recommended doses to evaluate their efficacy and longevity against *Ae. aegypti*. The results revealed that the tested SRFs achieved complete inhibition of adult stages emergence of *Ae. aegypti* in the first four weeks and gave continuous effective with 50-100% for 98, 86 and 78 days post -treatment by using spinosad tablets, sumilarv granules and du-dim tablets respectively. The highest larval mortality was observed for spinosad followed by sumilarv and du-dim, it gave 1.3 folds than sumilarv and 1.1 folds with du-dium, whereas sumilarv and du-dium were more effective on pupal mortality than spinosad. On the other hand, morphological abnormalities were observed in larval and pupal stages of *Ae. aegypti* as delayed effects by tested formulations. This study highlighted that these SRFs could be used as potential larvicidal compounds in mosquito control programs as a single treatment and provide satisfactory results and continuous control against the dengue vector *Ae. aegypti* for several weeks.

Keywords: *Aedes aegypti*, Slow-Release Formulations, persistence, conditions, evaluation.

Mosquitoes are an annoying insects transmitting several diseases to human causing millions of deaths worldwide annually or lead to interfere with their work performance and spoil leisure time (WHO.2015). Dengue Fever is a viral disease which mainly transmitted by *Ae*. *aegypti* that became more widely dispersed than at any time in the past (Halstead, 2008). Outbreaks of dengue fever were reported in several tropical and sub-tropical countries and have increased 10 folds in the last 30 years (Simmons and Farrar, 2009 and WHO, 2015). Around 128 countries are at risk, with more than 3 billion infected people with dengue virus , and more than 20 thousand deaths annually(Bhatt, *et al*. 2013,WHO, 2015, Suresh , *et al* .,2015 and Rajaganesh *et al* .,2016).

In Saudi Arabia, the first dengue outbreak occurred in over 50 years. (Gubler, 2002) Since 1994, when the dengue fever infection was first officially documented in Jeddah, Saudi Arabia, it became a major public health problem in Jeddah city(Fakeeh and Zaki 2001). In 2006, dengue fever reported cases had risen drastically compared to the earlier recorded numbers[9].Ghaznawi reported that the water storage containers, which found inside or outside construction sites, play an important role

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as breeding sites for *Ae. aegypti* and distribution of dengue virus infection in Jeddah (Aburas, 2007). Mosquito control strategy is the only effective method to control the disease, becuse there is no a specific drug to treat the dengue virus and no vaccine is available (Suresh *et al.,* 2015 and Rajaganesh *et al.,* 2016). The widespread and frequent usage of the chemical insecticides has led to resistant mosquito populations resulting in a human health concern (Benelli, 2016).

Basically, the effective control depends upon prevention and elimination of aquatic habitats that are necessary for the development of the immature stages of mosquitoes. Such habitats sometimes cannot be drained or removed to eliminate, therefore the application of IGRs formulation to these potential larval development sites consider the primary or the sole measure and sustainable way which will drastically reduce the population of *Ae. aegypti* and prevent disease outbreaks (WHO, 2004). Thus, the searching for alternatives, including bioregulators-synthetic analogs of insect hormones and biological-entomopathogenic bacteria products is very necessary and considerably important. Among these recommended products that could be used as alternatives to synthetic insecticides might be Spinosad, Sumilarv and Du-dium, due to their slow toxicity for humans and non-targets organisms and a highly effective against pests such as mosquitoes (Attaran *et al.,* 2000, Suman *et al.,* 2010 and WHO, 2010).

Spinosad contains 2 insecticidal factors, spinosyn A and spinosyn D, present in a 85:15 ratio within the final product (Kirst *et al.,* 1992). It has very low acute mammalian toxicity. It had established a new standard for low environmental and other non-target fauna, human risks and degraded rapidly in the environment (Miles and Dutton, 2000, Cisneros *et al*., 2002 and Williams *et al.,* 2003) . It's mode of action is unique against larvae involving the postsynaptic nicotinic acetylcholine and gamma-amino butyric acid receptors (GABA), acted as a stomach , contact poison and neurotoxin and affecting on these receptors function(Watson, 2001 and Cisneros *et al.,* 2002).Mosquito larvicidal activity of Spinosad was first reported in laboratory bioassays screening extracts from the fermentation broth of *Saccharopolyspora Spinosa*.

Bond *et al.* (2004) reported the Tracer®, a commercial suspension concentrate formulation (Tracer® Naturalyte® Insect Control) of spinosad, had high larvicidal toxicity against *Ae. aegypti* L. and *Anopheles albimanus* in the laboratory (Bond *et al.* 2004). The 24-h LC_{50} against 3rd and 4th instars of *Ae. aegypti* and *An. Albimanus* was estimated at 0.025 mg (AI) /liter and 0.024 mg (AI) /liter, respectively. Several researchers evaluated the naturally - derived insecticide spinosad against larvae of mosquitoes species (Romi *et al.,*2006 ; Bahgat *et al.,* 2007 ; Jiang and Mulla , 2009 ; Thavara *et a1*.,2009 and Hertlein *et al.,* 2010). Semi-field and field studies have confirmed that different formulations of SRFs products could produce satisfactory effective control against *Ae. aegypti* and *Culex* spp (WHO, 2008 , and Hertlein *et al.,* 2010).

Diflubenzuron was the first bioinsecticides benzoylurea compound. Different studies on its mode of action against insects indicated that metabolism played a major role in the determination of the toxicological efficiencies of these compounds (Neumann and Guyer, 1987). Diflubenzuron has been recommended for controlling mosquitoes in drinking water. Its insecticidal effect came from inhibition of chitin synthesis in the cuticle of treated larvae (WHO, 2008).Numerous of studies showed that the diflubenzuron compounds have good efficacy for controlling the larvae of mosquitoes (Mulla *et al*. 2003 ; Thavara *et al*., 2007 ; Seccacini *et al*., 2008 ; Romeo *et al*., 2009 ; Silva *et al.*, 2009 and Suman *et al.,* 2010).

Pyriproxyfen (a growth regulator) is a juvenile hormone analogue which is mainly active against the developmental pupal stages of mosquitoes. Its mode of action to disrupt the regulation insects hormones leading to the inhibition of development, disturbed behaviour, decrease in adult fertility, inhibitor of embryogenesis, metamorphosis, and adult formation (Ishaaya and Horowitz ,1992). It has low toxicity for mammals with an LD_{50} above 5000 mg/kg for rats[36]. Pyriproxyfen had been evaluated for its action against mosquitoes and recommended to use for the control of some mosquito species (WHO, 2001). Concentrations of less than or equal to one part per billion of pyriproxyfen cause inhibition of adult *Ae. aegypti* emergence and

remains effective up to ûve months, longer than *Bacillus thuringiensis israeliensis*, methoprene, or temephos. Adult mosquitoes emerged from larvae exposed to pyriproxyfen have decreased fecundity and such contaminated adults can disseminate lethal doses from treated to untreated sites (Sihunincha *et al*., 2005).. Field and laboratory studies have revealed that pyriproxyfen have good residual activity against the pupal stage of *Ae. aegypti* (Nayar *et al.,* 2002 ; Seccacini *et al.,* 2008 and Aziz , 2017).

The present work was planned to evaluate the efficacy and longevity of three SRFs, spinosad, du-dium and Sumilarv, against the developmental stages of *Ae. aegypti*, the primary vector of dengue fever.

MATERIALS AND METHODS

Mosquito strain

This investigation involved evaluation and determining the efficacy and longevity of three selected slow release formulations under laboratory conditions. Larval stages of *Ae. aegypti* were collected from breeding habitats in Al-Balad-Jeddah city and had maintained in the laboratory under controlled conditions of 27±1°C and 70±5% R.H., with 14:10 (L:D) photoperiod.

Insecticides

Three slow release formulations (SRFs) were used: Spinosad (7.4% tablet) and Du-Dim (diflubenzuron 2 % tablet). They were supplied by Mosquito Research laboratory of the Municipality of Jeddah and Sumilarv (pyriproxyfen 0.5 % WDG) manufactured by Summit Chemical Company Japan, was obtained from Agricultural office-Jeddah, Saudi Arabia.

Test experiments

Test procedures were conducted according to standard WHO guidelines (2005).Experiments were carried out in transparent plastic containers (40× 31× 21 cm) containing 20 liters of tap water. Lids for covering the plastic containers were cut from the middle and muslin were glued in all lids which were in place all the time to prevented either debris or adult mosquitoes from entering the containers. The lids just opened during addition of larvae and inspecting the efficacy of tested formulation. Each containers received 25 second instar larvae of *A. aegypti* from lab rearing colonies and the tested formulations. The amount of each formulation (0.14gm Spinosad; 1 gm Du-dim tablet; and 0.2gm Sumilarv) required for treating mosquito larvae was determined according to the recommended dosages for field trials as well as by calculating the total volume of water in the container. The larvae were given the larval food during these tests. Three untreated containers were kept as control. The treatments were made in four replicates. The plastic containers were inspected daily and larval mortalities were recorded until all larvae either pupated or died. Alive pupae were counted and transferred to the lab rearing untreated water in glass beakers to observe adult emergence or mortality. New sets of alive larvae were added to the test containers when complete larval mortality occurred. Every two weeks one third of water volume in all containers was removed and refilled, to simulate the field conditions. Temperature (25-27 C) and PH (7.6-8.2) of water in all treatments were measured every day during experiments. Fifty percentage inhibition of adult emergence was calculated and taken as criteria for low level of larvicidal effectiveness activity and persistence of tested formulations mortality percentage of larvae in all containers as well as the reduction percentage and inhibition of emergence (IE%) of adult.

Data analysis

Percentages of mortality were corrected using Abbot's formula. The data were statistically analyzed to obtain ANOVA and least significant different using SAS (V 9.0).

RESULTS AND DISCUSSION

Different artificial containers such as water-storage that used for either holding or storing water consider suitable habitats and play an important role for the development of *Ae. aegypti* inside or outside buildings (Thavara *et al*., 2004). Evaluation of efficacy and longevity of three slow release formulations against the field strain of *Ae. aegypti* was conducted. The results demonstrated that the mortality occurred either in the immature stages (larvae and pupae) or at inhibition adult emergence.

Efficacy and longevity of slow release formulation of spinosad

As shown in table(1) and Figs (1and2) the efficacy of slow release formulations Spinosad,

Nature DT against the immature and adult stages during the period of experiment (98 days) achieved 95-100% mortality of larval stages and 90-100 % inhibition of adult stages emergence in the first eight weeks. Spinosad showed higher larval mortality compared with sumilarv and du-dium. It produced the highest larval mortality and gave 1.3 fold than sumilarv and almost the same effectiveness with du-dium but its effectiveness started to decrease gradually after 77 days post treatment.

Efficacy and longevity of slow release formulation of Sumilarv

The results in table (2) and Figures (1and 2) revealed the efficacy of the juvenoid action of sumilarv against the immature and adult stages

Table 1. Efficacy and longevity of Spinosad 7.4% on developmental stages of dengue fever vector *Ae. aegypti* from 1-98 days

No. of tests	larva mortality(%)[a] Means ±SD	Pupation %	Adult emergence %	Inhibition %	Duration effectiveness (days)
1	100.0 ± 0.0* (7)*b*	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	98
2	100.0 ± 0.0* (6)*b*	0.0 ± 0.0*	0.0 ± 0.0*	100.0± 0.0*	
3	100.0 ± 0.0* (6)*b*	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
4	100.0 ± 0.0* (7)*b*	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
5	98.0 ± 0.8(7)*b*	2.0 ± 0.8	1.0 ± 1.0	99.0 ± 1.0	
6	100.0 ± 0.0* (8)*b*	0.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
7	100.0 ± 0.0* (7)*b*	0.0 ± 0.0*	2.0 ± 1.7	98.0 ± 1.7	
8	100.0 ± 0.0* (6)*b*	0.0 ± 0.0*	1.0 ± 0.8	99.0 ± 0.8	
9	98.0 ± 1.4(8)*b*	2.0 ± 1.4	2.0 ± 0.8	98.0 ± 0.8	
10	95.0 ± 0.8 (7)*b*	5.0 ± 0.8	10.0 ± 1.6	90.0 ± 1.6	
11	86.0 ± 2.1(8)*b*	14.0 ± 2.1	20.0 ± 8.1	80.0 ± 8.1	
12	82.0 ± 8.1(7)*b*	18.0 ± 8.1	22.0 ± 1.8	78.0 ± 1.8	
13	80.0 ± 3.1(7)*b*	20.0 ± 3.1	25.7 ± 3.0	74.2 ± 3.0	
14	65.0 ± 2. 4(7)*b*	35.0 ± 2.4	48.0 ±3 .6	52.0 ± 3.6	

[a]: 25 larvae for each replicate and four replicates for each test.
[b]: Days post-treatment until complete mortality or pupation.
*: Not significantly different within the same column ($P < 0.0001$)

Table 2. Efficacy and longevity of Sumilarv 5% WDG on larvae of dengue fever vector Ae. aegypti from 1-86 days

No. of tests	larva mortality(%)[a] Means ±SD	Pupation %	Adult emergence %	Inhibition %	Duration effectiveness (days)
1	0.0 ± 0.0* (9)b	100.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
2	0.0 ± 0.0* (10)b	100.0.± 0.0*	0.0 ± 0.0*	100 .0 ± 0.0*	86
3	0.0 ± 0.0* (9)b	100.0 ± 0.0*	0.0 ± 0.0*	100.0 ± 0.0*	
4	6.0 ± 0.8 (9)b	94.0 ± 0.8	0.0 ± 0.0*	100.0 ± 0.0*	
5	4.0 ± 0.8 (9)b	96.0 ± 0.8	16.0 ± 0.8	84.0 ± 0.8	
6	2.0 ± 1.6 (10)b	98..0 ± 1.6	20..0 ± 0.8	80.0 ± 0.8	
7	5.0 ± 0.8 (11)b	95.0 ± 0.8	26.0 ± 2.1	74.0 ± 2.1	
8	6.0 ± 0.8 (9)b	94.0 ± 08	40.0 ± 1.6	60.0 ± 1.6	
9	3.0 ± 0.8 (10)b	97.0 ± 0.8	50.0 ± 1.4	50.0 ± 1.4	

a: 25 larvae for each replicate and four replicates for each test.
b: Days post-treatment until complete mortality or pupation.
*: Not significantly different within the same column ($P < 0.0001$)

during the period of experiment (86 days). The mortality recorded of larval stages was 2-6%, whereas the inhibition of adult stages emergence was 80-100% in the first six weeks. Sumilarv showed higher pupal mortality compared with spinosad and its effectiveness started to decrease gradually after 56 days post treatment.

Efficacy and longevity slow release formulation of du-dium

The results in Table (3) and Figures (1and 2) represented the efficacy of slow release formulation of du-dium against the immature and adult stages during the period of experiment (78 days). The larval mortality yielded 14-32% whereas the inhibition of adult stages emergence was 08-100% in the first eight weeks, the IE fluctuated between 54-100% during the period of study. Du-dium showed higher pupal mortality compared with spinosad and its effectiveness started to decrease gradually after 62 days post treatment.

Results of the present study revealed that the three SRFs achieved complete inhibition of adult stages emergence of *Ae. aegypti* in the first four weeks and gave continuous effective with 50-100% for 98, 86 and 78 days post treatment by using spinosad tablets ,sumilarv granules and du-dim tablets, respectively. The results demonstrated that the spinosad, sumilarv and du-dium achieved 100% inhibition of adult emergence up to 28 days post treatment (Fig.1). The complete adult

Table 3. Efficacy and longevity of Du-dium 2% on larvae of dengue fever vector *Ae. aegypti* from 1-78 days during

No. of tests	larva mortality(%)[a] Means ±SD	Pupation %	Adult emergence %	Inhibition %	Duration effectiveness (days)
1	20.0 ± 0.8$^{(9)b}$	80.0 ± 0.8	0.0 ± 0.0*	100.0 ± 0.0*	
2	32.0 ± 3.5$^{(8)b}$	68.0 ± 3.6	0.0 ± 0.0*	100.0 ± 0.0*	78
3	27.0 ± 2.5$^{(8)b}$	73.0 ± 2.6	0.0 ±0.0*	100.0 ± 0.0*	
4	20.0 ± 1.6$^{(7)b}$	80.0 ± 1.6	0.0 ± 0.0*	100.0 ± 0.0*	
5	17.0 ± 1.4$^{(8)b}$	83.0 ± 1.4	2.0 ± 1.4	98.0 ± 1.4	
6	15.0 ± 1.8$^{(8)b}$	85.0 ± 1.8	4..0 ± 0.8	96.0 ± 0.8	
7	16 .0 ± 1.3$^{(7)b}$	84.0 ± 1.2	5.0 ± 0.8	95.0 ± 0.8	
8	14.0 ± 1.5$^{(7)b}$	86.0 ± 1.5	19.0 ± 1.8	81.0 ± 1.8	
9	11.0 ± 0.8$^{(8)b}$	89.0 ± 0.8	25.0 ± 1.8	75.0 ± 1.8	
10	7.0 ±.1.5$^{(8)b}$	93.0 ± 1.5	46.0 ± 2.9	54.0 ± 2.9	

a: 25 larvae for each replicate and four replicates for each test.
b: Days post-treatment until complete mortality or pupation.
*: Not significantly different within the same column (P < 0.0001)

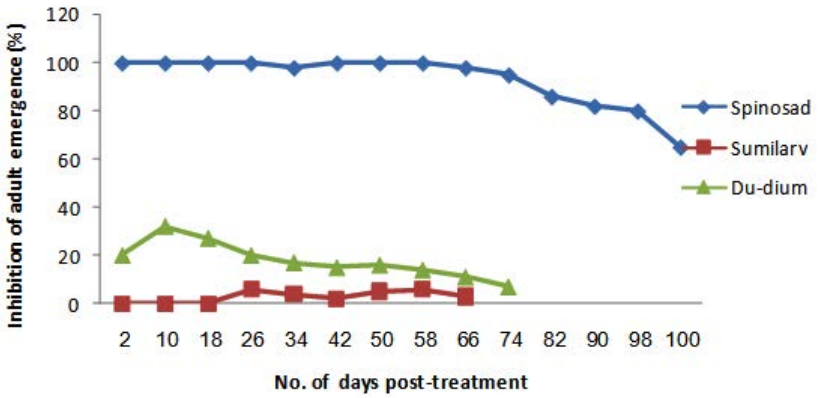

Fig. 1. Larval mortality percentage of *Ae. aegypti* after treatment with slow release formulations of spinosad, sumilarv and du-dium(P < 0.0001)

emergence observed in the present study could be due to morphological aberration that lead to failure in adult emergence (Mulla, 1995. They began to gradually decreasing their effectiveness after 77, 62 and 56 days post treatment for spinosad, sumilarv and du-dium respectively. The reason for this fluctuating of decreasing might be to different mode of actions and the type of formulations

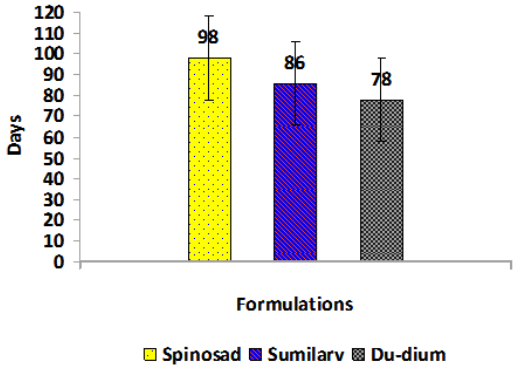

Fig. 2. Effectiveness of slow release formulations of spinosad, sumilarv and du-diumin days (P < 0.0001)

(Thavara, 2009). Even with replacement of third of water in the containers in all treatments, the three tested formulations in this study provided high efficacy and long-term adult inhibition. This agrees with findings of Chang *et al*. (2006) and Seng *et al*. (2008) who reported that the removing and replacing two-thirds of the volume of the water didn't reduce the duration efficacy of a controlled-release formulation of pyriproxyfen against *Ae. aegypti* mosquito in simulated domestic water storage containers.

As shown in tables 2 and 3, both sumilarv and du-dium have poor larvicidal effects against *Ae. aegypti* compared with spinosad , in contrast the spinosad (Tab. 3) revealed the highest larval mortality. It gave 1.3 folds than sumilarv and 1.1 folds effectiveness than du-dium. Both sumilarv and du-dium were more effective on pupal mortality than spinosad because they have complementary mode of action (Darriet and Cobel, 2006 and Lee *et al.,* 2005) . Sumilarv inhibits adults and prevent their emergence, conversely spinosad showed highly larval mortality, despite that there were some survivorship of larvae. On the other

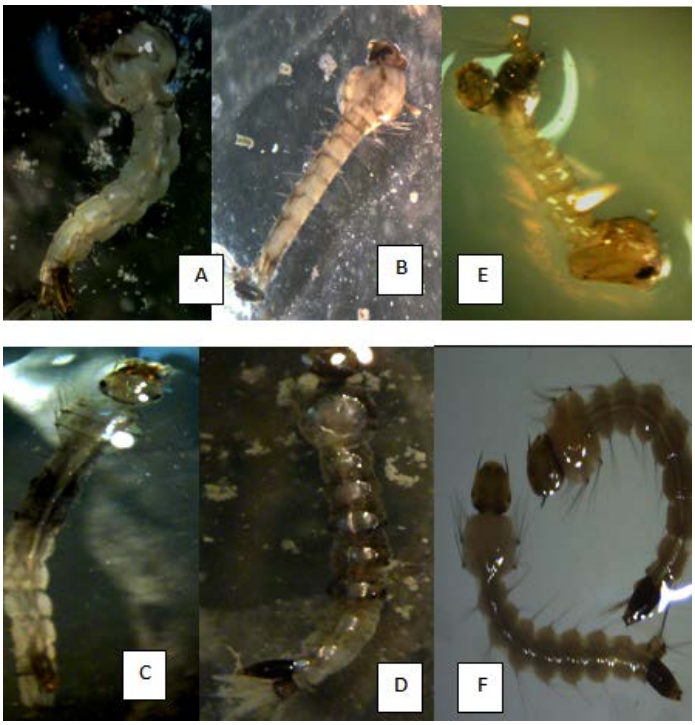

Fig. 3. Morphological abnormalities in the larval and pupal stages of *Ae. aegypti* post-treatment with spinosad. (A) Sumilarv, (B) (intermediate stage) and spinosad, (C) Sumilarv, (D) Black area, (E) Incompletely emerged pupa, (F) Normal untreated larvae (Control)

hand, morphological abnormalities were observed in larval and pupal stages of *Ae. aegypti* as delayed effects by tested formulations (Fig.3) which agrees with Vythilingam *et al.* (2005) who reported that the IGRs induce abnormalities or delayed effects on mosquitoes larvae lead to decline in reproduction or fecundity.

Overall, in this study the three SRFs showed high efficacy and longevity against *Ae. aegypti* for several weeks in water containers and have low risk to human, environment and non-target organisms(Williams *et al.,* 2003 and WHO, 2010).Nemours similar studies were carried out by several researchers using different SRFs of insecticide such as pyriproxyfen, diflubenzuron and spinosad against different species of mosquito vectors (El-Shazly and Refaie, 2002 Thavara *et al*., 2007, Vythilingam *et al.,* 2005 ; Seng *et al*., 2008; Martins*, et al*., 2008; Kamal and Khater, 2010 and Aziz, 2017 , Al-Azab and Shaalan, 2018).

CONCLUSION

It could be concluded that, three SRFs showed high efficacy and longevity against *Ae. aegypti* mosquito for several weeks in water storage under lab conditions. Thus we suggest that the slow-release formulations used in this study, could be used as a suitable bioinsecticides alternative for chemical insecticides in vector control program by a single application. Furthermore, mixture of spinosad with either sumilarv or du-dium formulations could be applied in mosquito breeding sites especially artificial water containers or tanks to achieve larval and pupal mortality and prevent their survivorship and consequently

ACKNOWLEDGEMENT

The authors are grateful to all work colleagues and friends for their co-operation and encouragement during this work. The authors also thankful to editor and reviewers of this Journal for their valuable comments and suggestions to the improvement of this paper.

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