Semi-field assessment of Spinosad in combination with Altosid briquet and Dudim DT tablets against *Aedes aegypti* mosquito larvae reared in pond water

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**Abstract**

This study aims at evaluating the larvicidal efficacy of slow-release formulations of bacterial insecticide Spinosad blended with two insect growth regulators (IGRs), Altosid XR–briquets and Dudim DT tablets against mosquito larvae of *Aedes aegypti*. This insect is the cause of dengue arthropod-borne viral disease. Treatment based on vector control using chemicals poses risks to human and environment, while the strategy based on the use of natural products poses lower risks. The results of the present study indicated that the mixture of Spinosad plus Dudim or Altosid tablets continued their fatal activity against the 3rd instar larvae for a longer period than the treatment with Spinosad, Altosid or Dudim tablets alone. The treatment of Spinosad plus either Altosid or Dudim has given effect to female mosquitoes in laying eggs in egg cups containing the treated pond water compared to the control egg cups. The results also showed that the treatment of Spinosad plus Altosid did not affect the egg hatching rate in the treated pond water, while the treatment of Spinosad combination with Dudim showed a significant decrease in the percentage of egg hatching. Use of natural products with larvicidal activities offers better approaches to integrated pest management and insecticide resistance management.

**1. Introduction**

Dengue is a serious arthropod-borne viral disease that occurs world-wide. This virus is transmitted by mosquitoes (*Aedes aegypti*) (Capeding et al., 2013). This disease causes an acute infection which can kill human faster than the immune disease AIDS. The prevention chemical method is the classical method of vector control although (Pavela, 2009). But, this method has environmental and human risks (Pavela, 2008). Therefore, high attention has been recently given to the use of natural products in vector control (Pirali-Kheirabadi and da Silva, 2010).

It is a major task to detect efficient mosquito control with no consequence of developing resistant insects to the larvicide. Therefore, the World Health Organization (WHO) promotes the potential use of several insecticides with varying modes of action. This action can be followed either using mixture of insecticides, making rotations across different growing seasons, or combination of both (WHO, 2003). The natural product-based insecticide Temephos is used in combination with *Bacillus thuringiensis israelensis* (Bti) against *Culex quinquefasciatus* and gave good results under different growing conditions (Andrade, 1989). Chung et al. (2001) used the insecticide mixture of Pirimephos methyl and Bti and found that the combination very effective in terms of mortality against different growth phases of *A. aegypti*. It was obvious that the latter combination has a long-term efficacy in the control. Wirth et al. (2000) indicated a high insecticidal activity of *Bacillus sphaericus* against *A. aegypti* when used in combination with Bti. The synergism exhibited between the two components is the major cause of high mortality. A recent study indicated adult emergence inhibition against *A. Aegypti* due to the use of controlled release formulations of pyriproxyfen (a juvenile hormone mimic) (Nayar et al., 2002). Spinosad was used in the present study as it is known as a friendly compound with low environmental and human risks (Williams et al., 2003). In addition, Spinosad provides schemes of integrated pest management (IPM) and insecticide resistance management (Salgado, 1997; Darriet et al., 2005).

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Peer review under responsibility of King Saud University.
without an effective vaccine. 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; Cornel et al., 2000; Bond et al., 2004; Seng et al., 2008; Jacups et al., 2014).

The aim of this study was to evaluate larvicidal efficacy of slow-release formulations of bacterial insecticide Spinosad against mosquito larvae of A. aegypti in the presence/absence of two insect growth regulators namely Altosid XR-briquets and Dudim DT tablets. A. aegypti is known as the primary vector of dengue fever in Saudi Arabia. The joint toxicity action resulting from mixing the bacterial insecticide with insect growth regulators will also be monitored.

2. Material and methods

2.1. Tests of formulation mixtures

The evaluation of the active larval fatality for some mixtures that are made from two of slow-release formulations and at the same level of concentration that in special for each preparation for the tested mixtures include tablets of the bacterial insecticide Spinosad plus tablets of the insects growth regulator Altosid in one hand. One the other hand, they include tablets of the bacterial insecticide Spinosad plus tablets of the insects growth regulator Dudim.

2.2. Mosquito material

The strain of A. aegypti used in this study was collected from Al-Ajwad district, Jeddah, Saudi Arabia (N213531.22, E39168.10). The mosquitoes were maintained in the insectary under laboratory conditions of 27 ± 1 °C, 70 ± 5% relative humidity (RH) and a period of 14 h light and 10 h darkness.

The larvae were reared in Dengue Mosquito Research Station (DMRS) at King Abdulaziz University until pupation and adult emergence took place for maintaining the stock culture.

2.3. Insecticide (compounds tested)

The bacterial insecticide Spinosad DT tablets (Saccharopolyspora spinosa at 7.4% a.i., weight of 1.37 g) provided by Clark company (Roselle, IL, USA). Two slow-release formulation of insect growth regulator, e.g., Altosid XR-briquets (methoprene at 2.1% a.i., briquet weight of 48 g) provided by Zocon (USA), and Dudim DT tablets (Difubenuron at 2% a.i., and tablet weight of 2 g) supplied by DGM Italia Srl.

2.4. Experiments

Semi-field trials of this study were done at DMRS, Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Experiments were carried out in white plastic pools (50 × 50 × 30 cm) containing 30 L pond water. Pools were placed in shade under a roof and kept covered with muslin cloth sheets to prevent debris and oviposition 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 (0.2 g Spinosad DT tablet; 7 g Altosid briquet; and 0.3 g Dudim tablet) was determined according to the recommended dosage for field. Control pool without formulations was used as a control. The larvae were given the usual larval food during the tests. Pond water was slowly added to the pools every other day to compensate evaporation. New live batches of 3rd larval instar of A. aegypti were added weekly to the test pools. All the mixtures of the above mentioned preparations have been tested against the 3rd instar larvae of the mosquito A. aegypti in the experimental troughs, which contains 30 L pond water with 4 replicates and the control. Any pupae produced were transferred to small plastic cups containing water and placed in adult cages for emergence. Calculations of the efficacy of the test formulations were done in terms of the number of emerging adults compared to the initial number of added larvae or the inhibition of emergence (IE%). The assessment of effectiveness was made on a weekly basis towards the decrease of efficacy in order to reach 50% IE or more. Calculations of inhibition percentage of emergence of adult as well as cycle of the effective centers for each mixture was done on a weekly basis.

Percentage of larval mortalities and inhibition of adult emergence were corrected for control mortalities using Abbott’s formula (Abbott, 1925). Additional trials were also conducted during the effective control periods of the slow release formulations, which resulted in 90–100% IE against mosquito larvae grown in pond water as the following.

A. aegypti for laboratory strain was fed on a blood meal from a live dove for about an hour. Groups of engorged females were collected from manual cages by manual suction. Each group of 10 females is fed into a fresh, clean cage with a 10% sugar solution and inside it are two small cups of plastic, one of which has 100 ml water taken from the treated ponds, and the other cup has 100 ml pond water. The test consisted of three replicates of 10 females fed on blood/duplicate. The number of eggs that were placed in both the treated water and its control was recorded to determine the behavior of the female mosquitoes in favor of oviposition site preference by calculating the oviposition activity index (OAI) according to the following equation (Kramer and Mulla, 1979):

\[
\text{OAI} = \frac{\text{NT} - \text{NC}}{\text{NT} + \text{NC}}
\]

NT = No. of eggs in treated larvae
NC = No. of eggs in the control larvae

On the other hand, the number of larvae that were deposited from the eggs that were placed in treated and untreated water was recorded to calculate hatching levels.

3. Results

Table 1 showed that the treatment on 3rd instar larvae of A. aegypti reared in pond water with a mixture of the bacterial insecticide Spinosad plus the insect’s growth regulator Altosid. The results proved that the larval fatalities of the mixture continued for a number of weeks with 90–100% inhibition of the emerging adult stage (Fig. 1). The obtained results in Table 2 and Fig. 2 showed that the efficacy of the mixture made of the bactericide Spinosad plus the insect growth regulator Dudim tablets against 3rd instar larvae of A. aegypti in the pond water started to lose its efficacy giving less than 90% inhibition of adult emergence 11 weeks post-treatment.

The present results indicate that the mixture of Spinosad plus Dudim tablets continued its fatal activity against the 3rd instar larvae for a long period of effective control and gave an increase of 2.2 or 1.5 folds than the treatment with Spinosad or Dudim tablets alone, respectively. The results have proved the possibility of limiting the problem of the efficacy reduction of the slow-release formulations when treating the pond water larvae of the mosquito
Table 1
The efficacy of Spinosad plus Altosid briquet tablet mixture as slow-release formulations against 3rd instar larvae A. aegypti mosquito reared in pond water.

| Weeks post-treatment | Dead larvae (%) | Pupae produced (%) | Adult emerged (%) | IE (%) | Duration (wks) |
|----------------------|-----------------|--------------------|-------------------|--------|----------------|
| 1                    | 100             | 0.0                | 0.0               | 100    |                |
| 2                    | 100             | 0.0                | 0.0               | 100    |                |
| 3                    | 100             | 0.0                | 0.0               | 100    |                |
| 4                    | 100             | 0.0                | 0.0               | 100    |                |
| 5                    | 97              | 3                  | 0.0               | 100    | 9              |
| 6                    | 98              | 2                  | 0.0               | 100    |                |
| 7                    | 91              | 9                  | 0.0               | 100    |                |
| 8                    | 84              | 16                 | 4                 | 95.5   |                |
| 9                    | 58              | 42                 | 7                 | 93     |                |
| 10                   | 53              | 47                 | 16                | 82.4   |                |
| 11                   | 37              | 63                 | 23                | 73.3   |                |
| 12                   | 33              | 67                 | 29                | 68.8   |                |
| 13                   | 28              | 72                 | 51                | 43.3   |                |

a Four replicates, 25 larvae each; control mortalities ranged from 4 to 11% IE.
b IE = Inhibition of adult emergence, corrected for control mortalities (Abbott, 1925).
c Duration of effective control with 90–100% IE.

Table 2
The efficacy of Spinosad plus Dudim DT tablet mixture as slow-release formulations against 3rd instar larvae A. aegypti reared in pond water.

| Weeks post-treatment | Dead larvae (%) | Pupae produced (%) | Adult emerged (%) | IE (%) | Duration (wks) |
|----------------------|-----------------|--------------------|-------------------|--------|----------------|
| 1                    | 100             | 0.0                | 0.0               | 100    |                |
| 2                    | 100             | 0.0                | 0.0               | 100    |                |
| 3                    | 100             | 0.0                | 0.0               | 100    |                |
| 4                    | 100             | 0.0                | 0.0               | 100    |                |
| 5                    | 100             | 0.0                | 0.0               | 100    |                |
| 6                    | 97              | 3                  | 0.0               | 100    | 11             |
| 7                    | 89              | 11                 | 2                 | 98     |                |
| 8                    | 84              | 16                 | 2                 | 97.7   |                |
| 9                    | 71              | 29                 | 9                 | 91     |                |
| 10                   | 64              | 35                 | 8                 | 91.2   |                |
| 11                   | 49              | 51                 | 6                 | 93.3   |                |
| 12                   | 32              | 68                 | 21                | 76.9   |                |
| 13                   | 27              | 73                 | 39                | 58.5   |                |
| 14                   | 22              | 78                 | 52                | 47.8   |                |

a Four replicates, 25 larvae each; control mortalities ranged from 4 to 11% IE.
b IE = Inhibition of adult emergence, corrected for control mortalities (Abbott, 1925).
c Duration of effective control with 90–100% IE.
The efficacy of Spinosad tablets plus Altosid – briquets and Dudim Tablet mixture as slow-release formulations against *A. aegypti* mosquito reared in pond water on ovipositional behavior of mosquito females with reference to egg hatchability.

| Pond water          | %IE (Wk. No.) | Total No. of eggs laid | OAI | Total no. of larvae hatched | Hatchability (%) |
|---------------------|----------------|------------------------|-----|-----------------------------|------------------|
| Spinosad + Altosid  | 100 (wk 6)     | 860                    | 0.46| 640                         | 74.4             |
| Control             |                |                        |     |                             |                  |
| Spinosad + Dudim    | 98 (wk 3)      | 453                    | -0.28| 230                         | 52.9             |
| Treatment           |                |                        |     |                             |                  |
| Control             |                | 773                    |     | 690                         | 89.3             |

a Three replicates, 10 engorged mosquito females.

b Oviposition activity index (Kramer and Mulla, 1979).

c All newly hatched larvae were died within 1–2 days.

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**Fig. 2.** The inhibition percentage in the hatchability of the mosquito *A. aegypti* adults emerging from larvae reared in pond water after treatment with a mixture of Spinosad tablet plus Dudim DT tablets as slow-release formulations.

**Fig. 3.** The oviposition activity index of the mixture made of the bactericide Spinosad plus the insect growth regulator Altosid XR briquets and Dudim tablets against 3 instar larvae of *A. aegypti* in the pond water.
A. aegypti. However, the water might be accumulated from rains, nursery, garden irrigation or stagnant water with house precincts especially those under construction by mixing 2 slow-release formulations from the non-conventional insecticides, which differ in its effect like the biological bactericides or the insect growth regulators. Hence, the mixing of 2 compounds led to the increase of their residual effect of the active material within their breeding sites, which might increase the period of effective control of the mixture better than using each compound alone.

Table 3 indicates the effects of treatment of slow-release formulations of Spinosad plus Altosid and Spinosad plus Dudim against A. aegypti larvae reared in pond water (week 6 and week 3 respectively), on the behavior of eggs laid by female mosquitoes in the treated pond water. The results showed that the treatment of Spinosad tablets plus Altosid has given an attractive effect to female mosquitoes to lay eggs in egg cups containing the treated pond water (860 eggs) compared to the control egg cups (320 eggs). Then, the ovipositional behavior of mosquito females with reference to egg hatchability for female mosquitoes in pond water (860 eggs) compared to the control egg cups (320 eggs). The ovipositional behavior of mosquito females with reference to egg hatchability for female mosquitoes in pond water was –0.28 (Fig. 3). The results also showed that the treatment of Spinosad plus Altosid did not affect the egg hatching rate in the treated pond water (74.4%). On the other hand, the results of the treatment of Spinosad combination with Dudim showed a significant decrease in the percentage of egg hatching in the treated water. The percentage of egg hatching in pond water cups was 52.9%, while 89.3% in the control. Overall, all newly hatched larvae died within 1–2 days.

4. Discussion

The recovered result proved the effectiveness of the mixture in the inhibition of adult emergence. From the larval treatment of 100% extending for 6 weeks continuously, and the effective control has extended to record 93.3% at the end of the 11th week post larval treatment. These results assured that mixture of Spinosad and Altosid briquets was more effective against the pond water 3rd instar larvae of A. aegypti as compared with the treatment of the larvae with Spinosad tablets (5 weeks) or Altosid tablets alone (6 weeks) with 1.8 and 1.5 folds difference, respectively (Alkenani et al., 2015). The results proved that the treatment of the pond water on 3rd instar larvae with a mixture has yielded an actively effective fatality of larvae with an effective control for longer periods of time from 2.2 or 1.6 time as compared with the application of Spinosad or Dudim tablets alone, in which larvae has lost activity after 5 or 7 weeks after treatment, respectively. The present results have proved the possibility of limiting the problem of the efficacy reduction of the slow-release formulations when treating the pond water larvae of the mosquito A. aegypti.

Additionally, the present results are in agreement with Darriet et al. (2010) who assured that the mixture made of the bacterial insecticides Spinosad and the insect growth regulator pyriproxyfen gave more efficacy against larvae of A. aegypti under field conditions for 8 consecutive months giving effective control for periods of 3 or 5 months when used by Spinosad or Pyriproxyfen tablets singularly, respectively.

From another perspective, the mixture of two unconventional insecticides with different mode of action is considered one of the effective methods in the programs of mosquito abatement especially against species that has resistance to conventional insecticides because it is yet known that the use of an insecticide alone (especially the conventional ones) for long intervals of time in the control program could lead to the increase of the dosages of that particular insecticide, which evidently leads to the building of resistance in the mosquito strains for that insecticides and for any other insecticide from the same chemical group.

Generally, the present investigation had proven that the slow-release unconventional insecticides like the bacterial insecticides or the insect growth regulators against the larvae of the mosquito A. aegypti had produced effective control at 90–100% in the inhibition of the emerging adults. This is continued for a number of weeks when larvae treated with the tested preparation for one treatment in the breeding site without the need for the repetition of the frequency of control when compared with that through the use of the conventional chemical insecticides. However, this might lead to the rational use of the insecticides, which automatically reduces the cost of control and reduces environment pollution (Darriet et al., 2010).

Acknowledgements

The author would like to acknowledge and appreciate Prof. Dr. Khalid M. Alghmied and Prof. Dr. Mustafa S. Saleh, Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University for their advice, encouragement and significant support.

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