FIELD EVALUATION IN THAILAND OF SPINOSAD, A LARVICIDE DERIVED FROM *SACCHAROPOLYSPORA SPINOSA* (ACTINOMYCETALES) AGAINST *AEDES AEGYPTI* (L.) LARVAE

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Abstract. Two formulations of spinosad, direct application tablet (DT) and 0.5% granules (GR), at 3 dosages (0.25, 0.5 and 1.0 mg/l) in 200-liter earthen jars were evaluated against the larvae of *Aedes aegypti.* Two water regimens were used in the jars: jar full all the time and a full jar in which half the volume of the water was removed and replaced at each assessment interval. All treatments and controls were replicated 4 times and challenged with cohorts of 25 third-instar larvae of *Ae. aegypti* at weekly intervals during the study. The number of pupal skins (indicating successful emergence of adults) in the treated and control regimens were counted 7 days post-addition and they were used to calculate inhibition of emergence (% IE) based on the original number of larvae used. The DT formulation at the highest concentration (1.0 mg/l) yielded 79-100% IE for 34 days in the full jars, efficacy declining beyond this period. However, the longevity of this dosage was much longer with 90-100% IE for 62 days post-treatment in the water exchange regimen. The target and manufacturer-recommended concentration of 0.5 mg/l of DT gave good control (92-100% IE) for 20 days, declining below 92% IE thereafter in full jars. This dose also yielded good control with IE of 97-100% for 27 days in the water exchange regimen. The 0.5% GR formulation at all 3 dosages showed higher efficacy and greater longevity in the jars than the DT. In the full jars, all 3 dosages produced IE of 76-100% for 55 days post-treatment. In the water exchange regimen, the efficacy and longevity were increased by about one week, up to 62 days post-treatment. It is clear that the DT formulation can be used effectively against *Ae. aegypti* larvae at a target dose of 0.5 mg/l in 200-liter jars. This dose can be increased to 1.0 mg/l if slightly longer residual activity is desired. In containers where water is consumed and more water added, the longevity of efficacy will be longer for the DT than in jars which remain full all the time. GR (0.5%) gave longer control than DT. GR (0.5%) floated on the surface and produced scum and an oily film, features not desirable in stored water.

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INTRODUCTION

Spinosad is a natural product derived from the bacterium *Saccharopolyspora spinosa.* This bacterium is normally responsible for the decomposition of organic material in soil. This organism was first isolated in certain

soil samples in 1988 (Thompson *et al*, 1997). The most active metabolites from spinosad fermentation were identified as Spinosyn A and D (Sparks *et al*, 1997). Spinosad exhibits stomach and contact poisoning properties and affects specifically the function of γ-aminobutyric acid (GABA) receptors and nicotinic acetylcholine receptors of the target insects (Salgado 1997, 1998). This product has been widely tested against injurious insects in a variety of crops, such as cotton (Banerjee *et al*, 2000), wheat (Fang *et al*, 2002) and tobacco (Blanc *et al*, 2004). In Thailand, spinosad has also been evaluated against tomato pests, such as thrips, *Ceratothripoides claratris* (Shumsher) (Premachandra *et al*, 2005) and the sweetpotato whitefly, *Bemisia tabaci* (Genadius) (Kumar and Poehling, 2007). This bioactive agent has also been shown to have a high level of activity against larvae of various mosquito species, such as *Aedes aegypti* (L.), *Ae. albopictus* (Skuse), *Culex quinquefasciatus* Say, *Cx. pipiens* L., *Anopheles albimanus* Weidemann, *An. stephensi* Liston and *An. quadrimaculatus* Say in recent studies (Bond *et al*, 2004; Cetin *et al*, 2005; Dariet *et al*, 2005; Dariet and Corbel, 2006; Paul *et al*, 2006; Romi *et al*, 2006; Pridgeon *et al*, 2008).

The objective of this study was to evaluate a novel formulation (DT) of spinosad and compare it with GR (0.5%) in earthen jars (200 liter water) according to WHO guidelines (WHO, 2005). Multiple dosages of each formulation were employed in two water regiments of either full jars or full jars with half the volume of water removed and replaced weekly. This regimen simulated water-use patterns in rural Thailand and elsewhere.

MATERIALS AND METHODS

Field study site

During the course of this research, the experimental field facilities in Bang Bua Thong District, Nonthaburi Province, Thailand, were employed. We have used these facilities successfully in the past evaluating larvicides (Mulla *et al*, 2004; Fansiri *et al*, 2006; Tawatsin *et al*, 2007; Thavara *et al*, 2007). Earthen water-storage jars in the tests were placed on a concrete slab covered with a roof, but open on the sides. The containers were in shade at all times. The facility is located in a semi-rural area about 50 km from Bangkok. The jars were fitted with aluminum fabricated lids covering the jars at all times except during assessment for about 3-4 hours per week.

Test units

Earthen water-storage jars, the most commonly used containers were placed in rows on the concrete slab, on each side of a gutter. The jars have a capacity of 200 liters, when filled have a depth of 62 cm, when half full (100 liter water), the depth was 32 cm. Fitted aluminum lids covered the mouths of the jars at all times except during filling, emptying, adding larvae and counting larvae and pupal skins. The covers precluded light entry and prevented deposit of debris and oviposition by wild mosquitoes, as well as invasion by predacious macro-invertebrates. The jars were filled with tap water, 0.5 g of ground up larval food was added initially and then 25 third-instar larvae from a laboratory colony of *Ae. aegypti* were added at weekly intervals. Water loss due to evaporation was replenished on a monthly basis and larval food (0.25 g) was added weekly. One month after the start of the experiment, a food suspension (25 g ground up mouse food/ 100 ml water) was prepared, then 1 ml of this food suspension (equal to 0.25 g dry food/container) was added weekly.

Formulations and treatments

The spinosad product in this study was a mixture of Spinosyn A and D with ratio of 85% to 15%, respectively. Two formula-

tions of spinosad: a direct application tablet (DT: GF-1855, Lot NB 115-44-29) and granules (0.5% GR: GF-1578, Lot NB104-35-32) were employed in this study and were provided by Dow Agro Sciences, Indianapolis, USA. Both the DT (7.5% active ingredient) and 0.5% GR (0.5% active ingredient) formulations were administered at 3 dosages: 0.25, 0.5 and 1.0 mg/l. Both formulations were applied directly to water in the jars without stirring. Controls were also run. All treatments and controls were replicated 4 times and challenged with cohorts of 25 third-instar larvae (lab reared) of *Ae. aegypti* at weekly intervals after treatment. Two water regimens were used in the jars: water jars full all the time and full jars from which half the water volume (100 liters) was removed and refilled weekly at each assessment interval. For water removal, a submersible water pump was lowered to the mid-depth of the jar and the water was then pumped out. Water exchange began one week after the granules sank or 13 days after treatment.

Assessment of efficacy

After treatment, 25 third-instar larvae/jar were added weekly to challenge the efficacy of the treatments. By the 7th day post-addition, all surviving larvae had pupated and emerged as adults. The exact number of pupal skins (indicating successful emergence of adults) was counted 7 days post-addition by removing pupal skins which float on the water surface along the margins of the water using a syringe. The pupal skins in each container were removed and placed in a white pan containing water. The efficacy was reported as the level of inhibition of emergence (IE%) calculated on the basis of successful surviving larvae, papae or emergence (based on pupal skins) in the treated and control regimens divided by the original number of larvae used (25 larvae/jar). The assessment of efficacy was made at weekly intervals until the level of control dropped below 80%.

RESULTS

Data regarding DT efficacy is found in Fig 1 (without water exchange) and Fig 2 (water exchanged weekly). This formulation at all 3 dosages (1.0, 0.5 and 0.25 mg/l) resulted in 100% inhibition of emergence (IE) in the full jars for 13 days (Fig 1). The DT formulation at the highest concentration (1.0 mg/l) yielded 79-100% IE for 48 days in the full jars, with the efficacy declining beyond this period. However, in the water exchange regimen, the longevity of efficacy was much longer with 90-100% IE for 62 days post-treatment (Fig 2). There was one reading on Day 27 when the IE was 69%, an obvious anomaly since the 5 subsequent readings gave 90-100% IE (Fig 2). The manufacturer-recommended dose of 0.5 mg/l of DT gave good control (IE 92-100%) for 20 days, declining below 92% IE thereafter reaching 79% IE 27 days post-treatment in the full jars (Fig 1). This dose also yielded good control with an IE of 97-100% for 27 days in the water exchange regimen and thereafter the efficacy fluctuated between 74% and 96% IE from 34 to 55 days post-treatment (Fig 2). The DT formulation at the lowest dosage of 0.25 mg/l provided satisfactory control (about 92-100% IE) in the jars with water exchange for 27 days post-treatment, one week longer than those in the jars without water exchange (20 days post-treatment). Beyond these periods, this dosage showed a continuing decline in efficacy in both water regimens.

Regarding the 0.5% GR formulation, the dosages of 1.0, 0.5 and 0.25 mg/l produced almost complete efficacy (IE 98-100%) in both the full and exchanged water jars for 34 days post-treatment (Figs 3 and 4). Beyond this period, the highest dosage (1.0 mg/l) still showed more than 80% IE for up to 62 days

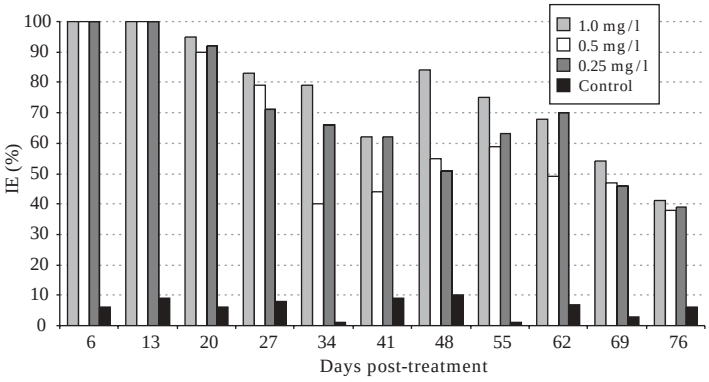

Fig 1–Field evaluation of spinosad (DT tablets) against larvae of *Ae. aegypti* in 200 liter water in earthen jars (water full jars) at Bang Bua Thong, Nonthaburi, Thailand (treated on April 18, 2007).

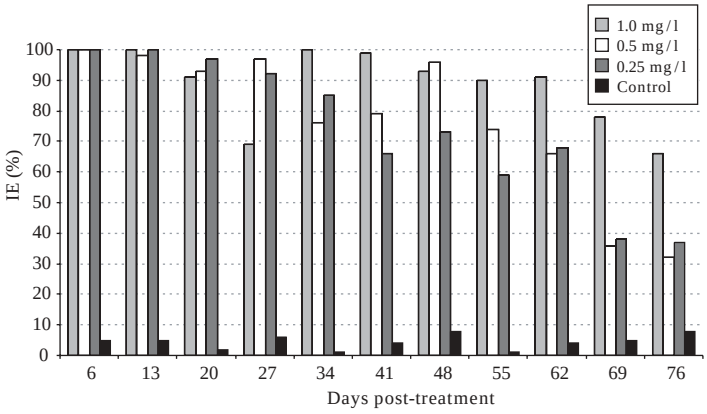

Fig 2–Field evaluation of spinosad (DT tablets) against larvae of *Ae. aegypti* in 200 liter water in earthen jars (water full jars, 1/2 emptied - refilled weekly) at Bang Bua Thong, Nonthaburi, Thailand (treated on April 18, 2007).

post-treatment in both water regimens. At 69 and 76 days post-treatment, this dosage (1.0 mg/l) exhibited a lower efficiency with an IE of 70% or lower in both water regimens. The GR formulation at 0.5 mg/l provided 76-81% IE in full jars 62 days post-treatment (Fig 3) and yielded at least 82% IE for up to 62 days post-treatment in the water-exchanged jars (Fig 4). In contrast, the 0.5% GR formulation at the lowest dosage (0.25 mg/l) in the jars without water exchange demonstrated somewhat better efficacy of least 82% IE (from 41 to 55 days post-treatment) better than the jars with water exchange regimen (Figs 3 and 4).

DISCUSSION

The efficacy and longevity of both formulations of spinosad in our study were dose dependent. Overall, the DT and 0.5% GR formulations at dosages of 0.25, 0.5 and 1.0 mg/l provided at least 90% IE for 20-62 and 41-62 days, respectively. However, the duration of the residual efficacy of the 0.5% GR formulation in this study was shorter than that obtained in our previous study in 2006 (83-111 days) under the same dosages and simulated field conditions (WHO, 2007). In comparison, a similar study conducted in Malaysia with the same testing protocol showed that the DT formulation of spinosad yielded excellent control (97-100% mortality) against *Ae. aegypti* larvae in earthen jars at all three concentrations tested (0.25, 0.5 and 1.0 mg/l) for up to 16 weeks in both water regimens while the granular formulation (0.5% GR) also gave the same results for up to 16 weeks in the jars without water exchange, but shorter longevity (12 weeks) in the jars in which water was removed and refilled weekly (Jaal, 2007). These differences in efficacy and longevity could be due to various factors, such as the test material, biological response of the test larvae, differences in earthen jars used or in the environment. According to the results obtained from WHOPES supervised trials, the WHO (2008) has recommended the use of the spinosad DT formulation at dos-

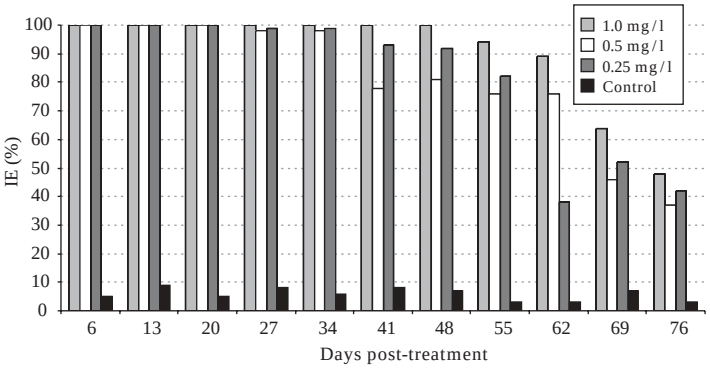

Fig 3–Field evaluation of spinosad (GR 0.5) against larvae of *Ae. aegypti* in 200 liter water in earthen jars (water full jars) at Bang Bua Thong, Nonthaburi, Thailand (treated on April 18, 2007).

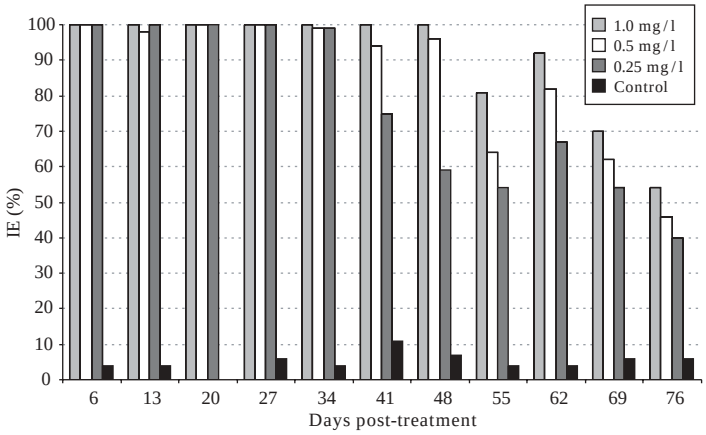

Fig 4–Field evaluation of spinosad (GR 0.5) against larvae of *Ae. aegypti* in 200 liter water in earthen jars (water full jars, 1/2 emptied - refilled weekly) at Bang Bua Thong, Nonthaburi, Thailand (treated on April 18, 2007).

ages of 0.25-0.5 mg/l to control *Aedes* larvae in breeding containers with an expected duration of residual efficacy for 4-6 weeks under field conditions. Prior to this study, spinosad 12% SC and 0.5% GR have been also recommended by the WHO for the control of container-bred mosquitoes at dosages of 0.1-0.5 mg/l, with an expected duration of efficacy of 10-12 weeks (WHO, 2007).

The status and behavior of the two formulations after application were also observed in this study. These observations are important in terms of the physical characteristics, ease of application and acceptability of the formulations in water storage containers. The granules prepared on corn grit were of uniform size (15/20 mesh estimated size) with good flow ability. After application a considerable amount of the granule formulation sank to the bottom of the earthen jars, but a substantial amount (about 25%) remained floating on the water surface. At the two higher dosages of 1.0 and 0.5 mg/l, the amount of the granules formulation floating in the containers was quite large which will be objectionable for use in water-storage containers. The floating granules made counting the larvae, pupae and pupal skins difficult. Six days post-treatment the granules were mixed with the surface water using a spoon whereupon all the granules sank to the bottom. The granules at the two higher dosages produced a film of scum on the water surface. A film, scum or floating granules are objectionable in water storage containers. The film disappeared after one month and was not visible untill the date of the termination of the experiment. No such scum or film was seen with the DT treatments.

The speed of action of spinosad was also observed in order to provide a critical insight into the activity and efficacy of the two formulations. During the first month after treatment, mosquito larvae with the granular treatment died within one to two hours of

being placed in the treated water. The larvae were noted to undulate and curve attempting to bite their siphons. A quick effect was not noted with the DT treatment. With the DT treatment mortality occurred more slowly and later. This phenomenon is reflected by the activity and efficacy of DT, which were shorter compared to the granules (Figs 1, 2, 3 and 4). On termination of the experiment (83 days post-treatment), none of the larvae in any of the treatments (DT and 0.5% GR) succumbed quickly. They were noted to be active and alive, even at high dosages of DT and 0.5% GR.

The activity and longevity of many mosquito larvicides depends on the characteristics of the formulation. This also applies to spinosad. Spinosad has been formulated as SC (11.6%), GR (0.5%) and recently as controlled release tailor-made direct treatment tablets (DT), weighing 1.35 g, containing 7.5% spinosad. DT is a unique formulation, consisting of two homogeneous layers. Each layer has a different concentration of spinosad and a different release profile. The first layer is effervescent, facilitating quick release of spinosad for initial concentration and action. The second layer containing a different concentration of spinosad will ensure slow release of the active ingredient for long-lasting control of mosquito larvae. The tablets were easy to apply. They sank to the bottom instantly, started to fizz and kept moving on the bottom surface and resettled soon. After fizzing, the tablets remained stationary. They remained mostly intact and visible at the bottom of the containers until the termination of the experiment 83 days post-treatment. There was no scum or visible film with the DT treatments. DT, therefore, should have greater acceptability in treating artificial water-storage containers. The DT application at all dosages provided greater and longer efficacy in jars with the water exchange regimen as compared to the full jars. Water removal and replenishment either increased the release of spinosad from DT or facilitated dissolution of spinosad from the active ingredients absorbed and adsorbed on surfaces. Our prior studies with other chemicals also showed this trend in jars (Mulla *et al*, 2004; Thavara *et al*, 2007). In general, the IE (%) was higher in water exchange regimens than in full jars, except in one case, the dosage of 1.0 mg/l at 27 days post-treatment. This anomaly could be due to the interruption releasing of spinosad from the second layer of the DT formulation. In all assessments up to 62 days, the IE (%) was consistently higher in the water-exchange jars than in the full ones.

In conclusion, it is clear that the DT formulation can be used effectively against *Ae. aegypti* larvae at the dose of 0.5 mg/l in 200 liter jars. This dose can be increased to 1.0 mg/l if slightly longer residual activity is desired. In containers where water is consumed and added, the longevity of efficacy is longer for DT than in the jars which remained full all the time. 0.5% GR gave longer control than the DT formulation. With the GR formulation, there was not much difference between full and water exchanged jars. The spinosad DT formulation is a satisfactory choice for controlling *Ae. aegypti* larvae in water-storage containers.

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