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# EFFECTIVENESS OF SPINOSAD AND TEMEPHOS FOR THE CONTROL OF MOSQUITO LARVAE AT A TIRE DUMP IN ALLENDE, NUEVO LEON, MEXICO

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ABSTRACT. The effectiveness of spinosad and temephos for the control of mosquito larvae was evaluated in a tire dump in Allende, Nuevo Leon, Mexico. Three groups of 12 to 17 tires located in tree shade were utilized for this study. After the larvicides were applied, samples were collected weekly from 7 randomly chosen tires. The data showed a significant difference between the larvicides and control. Under the conditions of the present study, the effectiveness of spinosad against mosquito larvae was similar to that of temephos, both being effective for up to 91 days postapplication. In addition, spinosad allowed the establishment of the mosquito predator *Toxorhynchites* sp.

**KEY WORDS** Spinosad, temephos, *Aedes aegypti*, biological control, larvicides

## INTRODUCTION

Spinosad is a natural metabolite produced by the soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao, which shows a very low toxicity to mammals. Spinosad consists of a mixture of spinosyns A and D, which have been shown to be toxic against various species belonging to the orders Lepidoptera, Thysanoptera, and Diptera (Bret et al. 1997, DeAmicis et al. 1997, Bond et al. 2004, Jiang and Mulla 2009). Spinosad acts both as a contact and stomach poison. After ingestion, it kills insects within 24 h. Spinosad is a neurotoxin that binds to nicotinic acetylcholine and gamma-aminobutyric acid receptors (Salgado 1997, 1998; Watson 2001; Cisneros et al. 2002; Bahgat et al. 2007).

Extracts and formulations based on spinosad have been evaluated against larvae of various species belonging to the genera *Aedes*, *Anopheles*, *Culex*, and *Psorophora*, in different conditions with very promising results. Spinosad has good potential for controlling mosquito larvae (Bond et al. 2004, Cetin et al. 2005, Darriet et al. 2005, Darriet and Corbel 2006, Romi et al. 2006, Pridgeon et al. 2008, Antonio et al. 2009, Jiang and Mulla 2009, Allen et al. 2010, Hertlein et al. 2010).

Unlike spinosad, temephos is an organophosphorus insecticide that has been used as a larvicide for many years in various countries around the world in campaigns for vector control, especially against species in the genus *Aedes*, some of which can be vectors of such diseases as dengue and yellow fever (Macoris et al. 2003, Biber et al. 2006, Seccacini et al. 2006, Floore et al. 2009). The aim of this study was to evaluate and compare the effectiveness of spinosad and temephos against mosquito larvae in a tire dump in the municipality of Allende, Nuevo Leon.

## MATERIALS AND METHODS

The study was conducted at a tire dump known as ''Cementerio o Panteon de Llantas'' in Allende, Nuevo Leon; located at coordinates 25°17′10.77″N, 100°00′24.88″W, at an elevation of 431 m. The study began in early September 2009 after a period of heavy rains and continued until December 2009. The tire dump contained numerous tires piled in various groups, which were removed daily for trituration and disposal. We used a site that contained groups of 12 to 17 tires with a diam of 60 cm located under the shade of some trees. In one group of tires, the recommended dose of spinosad was applied in the form of a tablet with an effervescent component and another for slow release designed to last for a month (1 tablet of Natural® DT with 0.105% AI per 200 liters; Clarke Mosquito Control, Roselle, IL). In a 2nd group of tires, temephos at a dose utilized by the Ministry of Health was applied in a granular formulation (field rate of 20 g per 200 liters water, Abate® 1 SG; BASF, The Chemical Company, Florham, NJ). A 3rd group of untreated tires served as control.

A white plastic dipper of 350 ml was used to obtain 7 samples of each group of tires. Sampling was carried out before and after the treatments at weekly intervals. Due to the difficulty of separating *Aedes aegypti* (L.) from *Ae. albopictus* (Skuse)

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Table 1. Mean number (± standard deviation) of total *Aedes* larvae[1] observed in control, spinosad-, and temephos-treated waste tires for 98 days postapplication at a tire dump facility in Allende, Nuevo Leon, Mexico.

| Days post-application | Control | Spinosad | Temephos |
|---|---|---|---|
| 0 | 29.28 ± 8.96 | 16.14 ± 4.09 | 18.85 ± 5.69 |
| 7 | 15.86 ± 6.19 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 14 | 15.14 ± 12.69 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 21 | 10.28 ± 11.56 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 28 | 4.71 ± 3.50 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 35 | 8.71 ± 4.96 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 42 | 6.12 ± 2.62 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 49 | 6.29 ± 3.30 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 56 | 10.72 ± 5.22 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 63 | 7.29 ± 3.90 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 70 | 5.57 ± 3.87 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 77 | 2.86 ± 2.21 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 84 | 2.86 ± 2.19 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 91 | 1.86 ± 1.35 | 1.28 ± 0.95 | 2.84 ± 1.30 |
| 98 | 2.15 ± 1.68 | 2.14 ± 1.77 | 1.70 ± 0.75 |

[1] *Aedes aegypti* and *Ae. albopictus* combined.

collected, we pooled these species and tabulated them as total larval density and density of early and late instars of *Aedes* spp. We also collected *Culex quinquefasciatus* Say in small numbers and tabulated as total. Finally, we determined the density of pupae of mosquitoes without specifying the genus or species.

The densities of total, early-stage, and late-stage larvae of *Aedes* spp. (mostly *Ae. aegypti*), total larvae of *Cx. quinquefasciatus*, and mosquito pupae were statistically analyzed using a completely randomized design to determine possible difference between the treatments; differences among the means were evaluated using ANOVA and Tukey's test. All data were analyzed utilizing the statistics package Olivares-Saenz (1994) and SPSS (2006).

Table 2. Mean number (± standard deviation) of early-stage *Aedes* larvae[1] observed in control, spinosad-, and temephos-treated waste tires for 98 days postapplication at a tire dump facility in Allende, Nuevo Leon, Mexico.

| Days post-application | Control | Spinosad | Temephos |
|---|---|---|---|
| 0 | 18.14 ± 4.59 | 12.80 ± 2.19 | 13.25 ± 1.60 |
| 7 | 7.57 ± 2.70 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 14 | 12.00 ± 10.34 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 21 | 9.57 ± 11.09 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 28 | 1.14 ± 0.69 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 35 | 5.71 ± 3.50 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 42 | 2.85 ± 1.21 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 49 | 3.00 ± 2.45 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 56 | 6.29 ± 4.68 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 63 | 4.29 ± 2.56 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 70 | 3.00 ± 1.73 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 77 | 1.29 ± 0.75 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 84 | 1.29 ± 1.11 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 91 | 1.29 ± 1.11 | 1.28 ± 0.95 | 1.57 ± 1.30 |
| 98 | 1.29 ± 1.11 | 2.14 ± 1.77 | 1.42 ± 0.75 |

[1] *Aedes aegypti* and *Ae. albopictus* combined.

Table 3. Mean number (± standard deviation) of late-stage *Aedes* larvae[1] observed in control, spinosad-, and temephos-treated waste tires for 98 days postapplication at a tire dump facility in Allende, Nuevo Leon, Mexico.

| Days post-application | Control | Spinosad | Temephos |
|---|---|---|---|
| 0 | 11.14 ± 3.02 | 3.34 ± 1.88 | 5.57 ± 1.13 |
| 7 | 8.29 ± 4.75 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 14 | 3.14 ± 1.34 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 21 | 0.71 ± 0.48 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 28 | 3.57 ± 3.55 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 35 | 3.00 ± 2.58 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 42 | 3.27 ± 1.88 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 49 | 3.29 ± 2.29 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 56 | 4.43 ± 2.30 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 63 | 3.00 ± 2.58 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 70 | 2.57 ± 2.15 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 77 | 1.57 ± 0.97 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 84 | 1.57 ± 1.40 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 91 | 0.57 ± 0.53 | 0.00 ± 0.00 | 1.57 ± 0.97 |
| 98 | 0.86 ± 0.69 | 0.00 ± 0.00 | 0.28 ± 0.48 |

[1] *Aedes aegypti* and *Ae. albopictus* combined.

## RESULTS

In our study, we found spinosad as effective as temephos in controlling the larvae of several mosquito species. During the study, we found larvae of both *Ae. aegypti* and *Ae. albopictus*, with the former occurring predominantly in waste tires. Unlike *Aedes*, *Cx. quinquefasciatus* larvae were found at low densities. Moreover, both control and spinosad-treated tires had larvae of *Toxorhynchites* sp., suggesting that spinosad did not impact this predacious mosquito. Before the treatment, the larval density of *Aedes* spp. was 16.14, 16.82, and 29.28 larvae/dip in spinosad-, temephos-treated, and control tires, respectively (Table 1). Unlike control tires which consistently showed *Aedes* spp. larvae, all treated tires had no mosquito larvae until 91 days postapplication. Statistical analysis of the data on total numbers of *Aedes* larvae revealed significant differences between the treatments and control, but no differences between the 2 larvicides used ($P > 0.05$). Pretreatment density of the early-stage *Aedes* spp. were 12.8, 13. 25, and 18.14 in spinosad-, temephos-treated, and control waste tires, respectively (Table 2). Similarly, pretreatment density of late-stage *Aedes* larvae was 3.34, 5.57, and 11.14 larvae/dip for spinosad-, temephos-treated, and control tires, respectively (Table 3). As with the total larvae, data on both early- and late-stage *Aedes* larvae did not show significant differences between the larvicidal treatments. However, larval density levels in control tires were significantly higher than in

Table 4. Mean number (± standard deviation) of *Culex quinquefasciatus* larvae observed in control, spinosad-, and temephos-treated waste tires for 98 days postapplication at a tire dump facility in Allende, Nuevo Leon, Mexico.

| Days post-application | Control | Spinosad | Temephos |
|---|---|---|---|
| 0 | 2.29 ± 2.21 | 2.14 ± 1.06 | 2.28 ± 1.38 |
| 7 | 0.29 ± 0.76 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 14 | 1.43 ± 1.13 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 21 | 3.71 ± 2.56 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 28 | 1.86 ± 1.07 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 35 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 42 | 1.85 ± 1.57 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 49 | 2.71 ± 2.21 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 56 | 7.57 ± 1.72 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 63 | 3.28 ± 1.11 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 70 | 3.29 ± 2.56 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 77 | 2.57 ± 2.07 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 84 | 1.14 ± 1.07 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 91 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 98 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Table 5. Mean number (± standard deviation) of mosquito pupae observed in control, spinosad-, and temephos-treated waste tires for 98 days postapplication at a tire dump facility in Allende, Nuevo Leon, Mexico.

| Days post-application | Control | Spinosad | Temephos |
|---|---|---|---|
| 0 | 2.70 ± 1.97 | 1.20 ± 0.75 | 1.68 ± 0.95 |
| 7 | 1.00 ± 0.57 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 14 | 1.85 ± 1.57 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 21 | 0.28 ± 0.48 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 28 | 0.85 ± 0.69 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 35 | 1.00 ± 1.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 42 | 0.42 ± 0.53 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 49 | 1.28 ± 1.11 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 56 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 63 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 70 | 0.42 ± 0.43 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 77 | 0.14 ± 0.37 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 84 | 0.14 ± 0.37 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 91 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 98 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

tires treated with larvicides. Late-stage *Aedes* larvae were not found in temephos- and spinosad-treated tires until 91 and 98 days postapplication, respectively.

Due to the small numbers of larvae of *Cx. quinquefasciatus* collected in this trial, only total larvae were considered for statistical analysis that indicated the same pattern as for *Aedes* larvae (Table 4). The larvicide treatments differed significantly from the control, with no significant differences observed between the 2 larvicides tested.

Mosquito pupae were observed in the control treatment and only at the beginning of the evaluation in tires treated with the larvicides (Table 5). Once again, the data showed significant differences between the larvicidal treatments and control, but no significant differences between the larvicides.

It should be mentioned that larvae of the mosquito predator *Toxorhynchites* sp. were collected during the present evaluation, where they were found on 7 of 15 sampling dates in the control and 8 of 15 sampling dates in the spinosad-treated group; this suggests that the predator established itself in places where spinosad had been applied.

## DISCUSSION

The results of the present study clearly show that spinosad controlled larvae of both *Aedes* spp. and *Cx. quinquefasciatus*, up to 91 days postapplication. These findings are in agreement with the results obtained by Bond et al. (2004), Jiang and Mulla (2009), and Thavara et al. (2009). Perez et al. (2007) also found no difference between spinosad and temephos, as seen in our study. The manufacturer claims a slow release of spinosad to last for 60 days. In our study, spinosad-treated waste tires did not show mosquito larvae for up to 91 days postapplication.

One of the advantages of spinosad is its minimal risk to human health. However, the effect on nontarget organisms needs to be thoroughly studied. As reported in earlier studies by Miles and Dutton (2000) and Williams et al. (2003), we also observed larvae of the mosquito predator *Toxorhynchites* sp. in the tires treated with spinosad. Spinosad appears to be an effective natural product to be used in the integrated management of mosquito larvae. Spinosad may be valuable in situations where local strains are highly resistant to other insecticides.

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