Comparison of novaluron, pyriproxyfen, spinosad and temephos as larvicides against *Aedes aegypti* in Chiapas, Mexico

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Abstract

Objective. To compare the efficacy of three modern larvicides with the organophosphate temephos for control of *Aedes aegypti* in water tanks in Chiapas. **Materials and methods.** Trials were performed to compare the efficacy of pyriproxyfen, novaluron, two formulations of spinosad (granules and tablets) and temephos in oviposition traps and domestic water tanks. **Results.** Pyriproxyfen and temephos provided 2-3 weeks of complete control of larvae in oviposition traps, whereas spinosad granules and novaluron provided 7-12 weeks of control. Treatment of water tanks resulted in a significant reduction in oviposition by *Ae. aegypti* in houses ($p<0.001$). Higher numbers of larvae were present in temephos and pyriproxyfen-treated water tanks compared to novaluron and spinosad tablet treatments during most of the study. **Conclusion.** Spinosad formulations and novaluron were effective larvicides in this region. The poor performance of temephos may be indicative of reduced susceptibility in *Ae. aegypti* populations in Chiapas.

Keywords: larvicides; spinosad; growth regulators, insect; oviposition

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Resumen

Objetivo. Comparar la eficacia de tres larvicidas modernos para el control de *Aedes aegypti* en tanques de agua doméstica en Chiapas. **Material y métodos.** Se comparó la eficacia de piriproxifeno, novalurón, dos formulaciones de spinosad (gránulos y tabletas) y temefos en ovitrampas y tanques domésticos de agua. **Resultados.** El piriproxifeno y el temefos proporcionaron de 2 a 3 semanas de control de larvas en ovitrampas, mientras que los gránulos de spinosad y novaluron proporcionaron de 7 a12 semanas. Los tanques de agua tratados produjeron una reducción significativa en la oviposición por *Ae. aegypti* en las casas ($p<0.001$). Se encontró gran cantidad de larvas en los tanques tratados con temefos y piriproxifeno en comparación con los tratados con novaluron y tabletas de spinosad durante la mayor parte del estudio. **Conclusión.** Las formulaciones de spinosad en tabletas y novaluron fueron larvicidas efectivos en esta región. El bajo desempeño de temefos puede indicar una susceptibilidad reducida en poblaciones de *Ae. aegypti* en Chiapas.

Palabras clave: larvicidas; spinosad; reguladores del crecimiento de insectos; oviposición

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Given its role in the transmission of dengue, yellow fever, chikungunya and Zika viruses, *Aedes aegypti* is now the most important vector of human disease in tropical urban habitats.[1] In southern Mexico, vector control teams apply a granular formulation of the organophosphate insecticide temephos to control the immature stages of *Ae. aegypti* in domestic water tanks that can harbor an important fraction of the immature mosquito population.[2] However, there are frequent reports of the development of resistance to this compound in Latin America and elsewhere.[3,4] To date, resistance to temephos has not been reported in Mexico, although high levels of detoxifying esterases were reported in *Ae. aegypti* populations from Quintana Roo,[5] and artificial selection resulted in a rapid increase in resistance to temephos in laboratory-reared insects from Mexico.[6] To address this issue, alternative larvicides are currently approved for control of *Ae. aegypti* in Mexico; these include spinosad and the insect growth regulators (IGRs) novaluron and pyriproxyfen.[7,8] The World Health Organization (WHO) has classified these compounds as safe for use in potable water.

Spinosad is a naturally-derived product with a favorable ecotoxicological profile, which is toxic to mosquito larvae.[9,10] Spinosad-based larvicides include sustained-release granular and tablet commercial formulations. Novaluron is a benzoyl-phenyl urea chitin-synthesis inhibitor that affects larval mortality, development and sex ratio.[11] Pyriproxyfen is an insect juvenile hormone mimic that inhibits metamorphosis and prevents the emergence of adults.[12]

Here, we compared the efficacy of these larvicides in oviposition traps and domestic water tanks in an urban environment in Chiapas, southern Mexico. Following established procedures, the efficacy of larvicidal compounds was evaluated using oviposition traps placed inside houses.[13] Granule and tablet commercial formulations of spinosad were tested alongside liquid formulations of pyriproxyfen and novaluron, and compared with 1% temephos mineral granules.

Materials and methods

Insecticides

Novaluron was obtained as a commercial emulsifiable concentrate containing 100 g active ingredient (a.i.)/l. Pyriproxyfen was obtained as a commercial emulsifiable concentrate containing 103 g a.i./l. Spinosad used in ovitraps was obtained as a 2.5% a.i. granular formulation of spinosad applied to oviposition traps, whereas an extended-release tablet formulation containing 7.48% a.i. spinosad was used in water tanks. Temephos was obtained as a generic mineral granular formulation comprising 1% a.i. provided by the Ministry of Health (*Secretaría de Salud*), Mexico City.

Study area

Experiments were performed in an area of ~12 hectares in the town of Huixtla (population ~51 000), Chiapas, Mexico (15° 08′ N; 92° 28′ W, altitude 40 m), with an average annual temperature of 27 °C and annual precipitation of 2 500 mm that occurs mainly from May to October. The study area contained ~550 houses with ~2 500 inhabitants. Verbal consent was obtained from the heads of 75 households distributed in the area. All the activities described in this study were authorized by the Ethics Committee of the National Institute of Public Health (Instituto Nacional de Salud Pública [INSP]).

Persistence of larvicides in oviposition traps

The experiment to evaluate the persistence of larvicides ran for 13 weeks from May 27 to August 19, 2015, during the rainy season. Houses were assigned to treatments following a balanced blocks design with five treatments and 15 repetitions. A circular black plastic oviposition trap, 10 cm in diameter by 20 cm in height, containing 1 l water, was placed in each house. Each oviposition trap was assigned to one of five treatments: 1) 0.5 mg a.i./l pyriproxyfen, 2) 0.5 mg a.i./l novaluron, 3) three granules of spinosad (mean ± SE: 5.5 ± 0.1 mg); 4) 0.1 g temephos granules, 5) untreated water as control. These treatments corresponded to the concentrations recommended by the National Center for Preventative Programs and Disease Control for mosquito control in Mexico.[7] To avoid loss of temephos granules, these were placed in a 1.5 ml perforated plastic tube that was removed prior to each sample. Weekly counts were performed to determine the number of living larvae and pupae present in each container. All insects (living and dead) were removed and discarded at each sampling, and the water that had evaporated was replaced in all treatments. Oviposition was monitored by placing a strip of filter paper in contact with the water, attached to the top of the container. Filter papers were replaced during weekly sampling and taken to the laboratory where eggs were counted. The prevalence of eggs on each filter paper that had hatched in the week since the previous sample, was recorded as proportion of egg hatch. Unhatched eggs were placed in water and larvae that emerged were reared to adulthood using a powdered laboratory rodent diet[14] and identified to species. A single oviposition trap was lost during the study in the control and pyriproxyfen treatments, compared with two traps lost in the spinosad and novaluron treatments, and three traps, in the temephos treatment.

Efficacy of larvicides in domestic water tanks

This experiment was performed in 40 houses distributed in the same area of Huixtla as the previous experiment. The houses were assigned following a balanced blocks design with four treatments, each with 10 replicates (houses). The selected houses had an internal brick-built water tank with an average capacity of 1 668 liters.

The experiment began with three weeks of pretreatment sampling from August 25 until September 15, 2015**.** The same day (September 15), one of the following treatments was assigned to each tank, based on its estimated capacity: 1) 0.5 mg a.i./l pyriproxyfen (mean ± SE volume: 1 677±158 liters; range: 714-2 445 liters), 2) 0.5 mg a.i./l novaluron (mean: 1 762±162 liters; range: 825-2 379 liters), 3) spinosad - one tablet for every 200 liters water (mean: 1 606±175 liters; range: 590-2 420 liters), and 4) 10 g temephos granules for every 100 liters water (mean: 1 655±232 liters; range: 140-2 342 liters). To monitor the efficacy of larvicides in water tanks over time, two ovitraps with untreated water were placed in each house. Untreated water tanks were not used as a control because cases of chikungunya and dengue fever were present in the study zone.

In order to improve the persistence of liquid larvicides, pyriproxyfen and novaluron treatments were adsorbed onto 70 g of crushed pumice for a 24 h period prior to application. This resulted in fine granules, similar to the granular formulation of the temephos treatment. Each treatment (spinosad tablets, temephos granules and pyriproxyfen-treated or novaluron-treated pumice) was placed in a 6x22 cm perforated nylon bag, tied to an empty 600 mL PET soda bottle that floated on the water, with the gauze bag suspended ~15 cm below the surface.

Water tanks and untreated oviposition traps were monitored weekly to evaluate the persistence of larvicides during 12 weeks post-treatment from September 22 to December 8, 2015. Tanks were sampled using cone shaped nets, with an orifice diameter of 25 cm and a pore size of 0.7×0.17 mm. Two samples were taken from each tank; a perimeter sample was taken by dragging the net around the edge and another X-shaped sample was taken from corner-to-corner. Immature mosquitoes were counted, identified, registered according to genus and a sub-sample (~10 larvae) was placed in a plastic bag with water, transported to the laboratory and reared to adulthood on powdered rodent diet for species identification. For oviposition traps, larvae + pupae were counted, paper filters were changed and evaporated water replaced, as described in the previous section. The number of eggs on each of the paper filters and the proportion of hatched eggs were determined by direct observation in the laboratory.

Statistical analyses

Mixed effects models were fitted to each of the variables measured using R v. 3.4.0. Egg counts from oviposition traps in both experiments were analyzed by specifying a negative binomial error distribution. The prevalence of egg hatching was analyzed by specifying a binomial error distribution for treated oviposition traps (first experiment) and a normal error distribution for untreated oviposition traps (second experiment). The numbers of pupae were generally low, so that, for analysis, counts of larvae and pupae were pooled within replicates for each sample time and were analyzed by specifying a Poisson error distribution. Sample times were considered as a categorical variable.

Results

Persistence of larvicides in oviposition traps

The mean (±SE) air temperature during this study was 32.6±0.3°C (range 26.0-41.1°C), while relative humidity averaged 57.6±1.0% (range 33-74%). Average container water temperature at the moment of sampling was 28.5±0.08°C (range 20-39°C).

A total of 52 563 eggs of *Aedes* spp. were registered in the oviposition traps in houses (figure 1A). Average weekly egg counts ranged from 48.9±4.5 eggs/trap in the control to 83.8±7.2 eggs/trap in novaluron-treated traps, but did not differ significantly among treatments (χ^2=2.93, df=4, *p*=0.57); however, egg numbers/trap fluctuated significantly over time (treatment*time: χ^2=63.5, df=44, *p*=0.029).

Overall, a proportion of 0.405 eggs (N=21 296) from oviposition traps were found to have hatched in the preceding week since the previous sample was taken (figure 1B). The mean proportion of egg hatch ranged from 0.318±0.023 in the control to 0.495±0.024 in pyriproxyfen-treated traps and differed significantly between treatments (χ^2=17.1, df=4, *p*=0.002) and between sampling dates (treatment*time: χ^2=1 610, df=44, *p*<0.001). The prevalence of egg hatching in pyriproxyfen-treated ovitraps was significantly higher than that observed in the control in eight sampling points and was higher than all other larvicide treatments in six out of 12 sampling points (figure 1B). None of the larvicides exhibited marked ovicidal effects.

In total, 3 473 *Aedes* spp. larvae + pupae were observed developing in experimental containers. The number of larvae + pupae differed significantly between treatments (χ^2=1 413, df=4, p<0.001) and between sampling points (χ^2=1 715, df=11, p<0.001). The average number of immature *Aedes* spp. in the control was 8.9±1.4 insects/container over the 12-week period (figure 1C). Among the larvicide treatments, the average numbers of larvae + pupae were highest in the pyriproxyfen treatment followed by the temephos treatment, with average densities of 6.3±0.5 and 4.2±1.0 insects/container, respectively. These larvicides provided 3-4 weeks of complete inhibition of immature development during the study. In contrast, the presence of larvae + pupae in spinosad- and novaluron-treated containers was lowest, with an average density <1.5 insects/container, although densities in these treatments increased at 9-12 weeks post-treatment. The spinosad treatment provided seven weeks of complete inhibition of *Aedes* spp. in oviposition traps, while the presence of immature mosquitoes was sporadic, but always low, in the novaluron treatment (figure 1C).

Laboratory rearing revealed that 95% of insects (N=354) in control oviposition traps were *Ae. aegypti*, compared with 94-100% (N=30-254, depending on treatment) of this species in larvicide-treated traps. All other individuals were identified as *Ae. albopictus*. As IGRs can affect the production of pupae, we calculated the percentage of emergence inhibition (IE) during laboratory rearing (table I).[15] Small numbers of pupae first appeared in pyriproxyfen-treated traps at 4 weeks post-treatment and were first reared to adulthood at seven weeks post-treatment, resulting in an IE value of 94%. In the novaluron treatment, pupae were first observed at 10 weeks post-treatment, and none of the insects collected developed to adulthood (IE=100%). Pupae were observed in the temephos and spinosad tablet treatments at four and eight weeks, respectively, and laboratory rearing resulted in IE values of 51% in both treatments (table I).

Efficacy of larvicides in water tanks

During the sampling period, the average air temperature was 31.5±0.1 °C (range 27-34 °C) and the mean of relative humidity was 61.2±0.8% (range 42-92%). The average tank water temperature was 26.8±0.05 °C (range 24-31 °C).

The average number of eggs in oviposition traps was significantly higher in the pre-treatment samples (range: 36.7-75.0 eggs/trap/week depending on

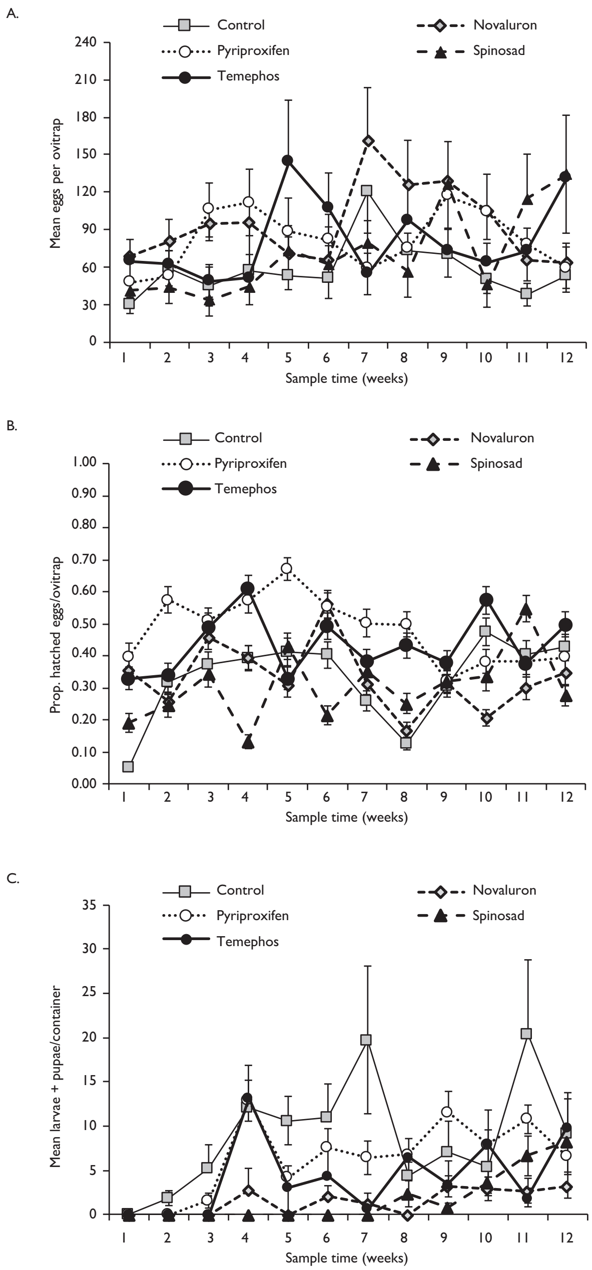

Figure 1. Efficacy of larvicides in oviposition traps in terms of (A) mean weekly counts of eggs, (B) proportion of hatched eggs and (C) mean number of larvae + pupae in larvicide-treated traps placed in houses in Huixtla, Chiapas, Mexico, during a 12 week study (May-August, 2015). Vertical bars indicate SE; for clarity, only half the bar is shown at some points

treatment) compared with samples taken post-treatment (range: 28.9-44.3 eggs/trap/week) ($z = -4.114$, $p < 0.001$). Following treatment of water tanks, the average number of *Aedes* eggs in untreated oviposition traps fluctuated significantly over time (χ^2=8 800, df =1, $p < 0.001$) (figure 2A), but was significantly lower in houses that received the novaluron and spinosad treatments compared with those treated with temephos or pyriproxyfen (χ^2=14.4, df=3, p=0.002). Overall, novaluron was significantly more effective than spinosad tablets in reducing *Aedes* spp. oviposition in traps (z=2.379, p=0.017).

The proportion of hatched eggs observed at each sample time (table II, figure 2B) varied between 0.268±0.020 in oviposition traps located in pyriproxyfen treatment houses and 0.363 ± 0.024 in traps in temephos treatment houses, and was intermediate in the spinosad and novaluron treatment houses (χ^2=8.31, df=3, p=0.04).

Mean numbers of larvae + pupae in untreated oviposition traps, taken as an indicator of *Aedes* spp. population density during the experiment, fluctuated between 3.4 and 26.7 insects/trap during the post-treatment period (figure 2C). Larvicide treatment resulted in a significant reduction in numbers of larvae + pupae in water tanks (χ^2=2 614, df=4, $p<0.001$). Temephos treatment provided just one week of absolute inhibition of immature mosquitoes (figure 2C), compared with seven weeks for spinosad and eight weeks for novaluron, although the numbers of larvae + pupae remained extremely low in both these treatments until the end of the study. Pyriproxyfen did not eliminate mosquito development at any time point, except at week 12. The mean number of *Aedes* larvae + pupae sampled in treated water tanks was highest in the pyriproxyfen treatment (3.11±0.7 larvae + pupae/tank), lowest in the novaluron and spinosad treatments (0.09 - 0.68 larvae + pupae/tank) and intermediate in the temephos treatment (1.84±0.4) (table II). Overall, the efficacy of spinosad tablets did not differ significantly from that of novaluron (z=0.788, p=0.43).

Of the insects reared from water tanks, a proportion of 0.965 (N=726) and 0.958 (N=1 350), were *Ae. aegypti* in pre-treatment and post-treatment samples, respectively. The remainder were *Ae. albopictus* and very small numbers of *Culex* spp. (N=15-42 insects) (table II). Pupae were first observed in the pyriproxyfen-treated water tanks at three weeks post-treatment and were first reared to adulthood at seven weeks post-treatment (table I). Inhibition of adult emergence (IE) in laboratory reared insects was 89% in this treatment. In contrast, no pupae were observed in the novaluron or spinosad tablet treatments. IE values were 71% and 37% in the spinosad tablet and temephos treatments respectively, based on laboratory rearing of small numbers of larvae (table I).

Table I

Presence of *Aedes aegypti* pupae in sampled oviposition traps (experiment 1) and water tanks (experiment 2) and development to adulthood during laboratory rearing of samples collected in Huixtla, Chiapas, Mexico in 2015

Treatment	*Sample in which pupae were first observed (weeks post-treatment)*	*Sample in which pupae first reared to adulthood (weeks post-treatment)*	*Total number of pupae observed*	*Percentage of inhibition of adult emergence (IE*) from larvae + pupae reared in laboratory (sample size)*
Oviposition traps				
Control	5	5	22	-
Novaluron	10	None	38	100 (132)
Pyriproxyfen	4	7	9	94 (294)
Spinosad (granules)	8	8	9	51 (66)
Temephos	4	4	16	51 (75)
Water tanks				
Untreated traps‡	1	1	20	-
Novaluron	None	None	None	100 (0)
Pyriproxyfen	3	7	8	89 (82)
Spinosad (tablet)	None	None	None	71 (8)
Temephos	4	4	6	37 (22)

* IE was calculated as IE = 100 - (T/C 100), where T and C are percentages of emergence in the treatment and control, respectively.[15]

‡ Untreated oviposition traps were used to monitor oviposition by *Aedes* spp. in houses with larvicide-treated water tanks.

Adult emergence in control samples was 96% (n=124) in oviposition trap samples and 87% (n=196) in water tank samples.

Table II

PRE-TREATMENT AND POST-TREATMENT MEAN NUMBERS OF EGGS AND PROPORTION EGG HATCHING IN UNTREATED OVIPOSITION TRAPS PLACED IN HOUSES, MEAN NUMBERS OF LARVAE + PUPAE IN LARVICIDE-TREATED WATER TANKS AND PREVALENCE OF *AEDES AEGYPTI* IN LABORATORY-REARED SAMPLES IN HUIXTLA, CHIAPAS, MEXICO, IN 2015

Treatment	*Mean (± SE) eggs in untreated oviposition traps per week (sample size)*	*Mean (±SE) proportion hatched eggs in untreated oviposition traps*	*Mean (± SE) number of larvae + pupae in water tanks per week*	*Proportion of* Ae. aegypti *in samples from water tanks determined by laboratory rearing (sample size)*
I. Pre-treatment				
Novaluron	36.7 ± 6.8 (1 433)	0.216 ± 0.042	6.3 ± 2.2	0.87 (23)
Pyriproxyfen	39.2 ± 6.1 (1 997)	0.324 ± 0.040	6.9 ± 1.9	0.95 (19)
Spinosad (tablet)	75.0 ± 13.4 (3 225)	0.291 ± 0.046	5.7 ± 1.5	0.86 (22)
Temephos	61.1 ± 8.8 (3 115)	0.226 ± 0.038	5.5 ± 1.6	0.89 (18)
II. Post-treatment				
Novaluron	28.9 ± 3.1 (5 729)	0.269 ± 0.023	0.09 ± 0.1	- (0)
Pyriproxyfen	38.2 ± 3.7 (7 378)	0.268 ± 0.020	3.11 ± 0.7	1.00 (8)
Spinosad (tablet)	34.2 ± 2.7 (7 718)	0.313 ± 0.023	0.68 ± 0.1	1.00 (2)
Temephos	44.3 ± 3.5 (8 680)	0.362 ± 0.024	1.84 ± 0.4	0.92 (12)

SE: standard error of the mean.

Discussion

The efficacy of larvicides was tested in houses in Huixtla, Chiapas, over a 12 week period, which is the usual duration for which temephos granule treatments remain effective in this region. Studies on treated oviposition traps and water tanks gave broadly similar results in that granular and tablet formulations of spinosad and novaluron (adsorbed onto pumice) provided significantly better control of *Ae. aegypti* than temephos or pyriproxyfen.

Water tanks are difficult habitats to treat with larvicides because they are readily accessible to mosquitoes, but due to the frequent use, washing and refilling of these tanks, the persistence of larvicides in these settings can be a major challenge. To address this issue, we compared granular slow-release products (spinosad and temephos) and two liquid larvicides (pyriproxyfen and novaluron) adsorbed onto pumice, which is an inert mineral that is cheap and readily available in Mexico.

Studies on treated ovitraps are valuable because these can be used as safe ovitraps in vector surveillance programs, being prevented from becoming productive containers by the presence of the larvicide. In this study, we obtained no evidence for ovicidal activity (egg hatching) of the compounds at the concentrations tested. Spinosad, pyriproxyfen and temephos have low ovicidal activity.[16-18] Novaluron has ovicidal activity in Lepidoptera,[19] but its effects on mosquito eggs have not been studied.

Both novaluron and spinosad granules and tablets showed similar efficacy in oviposition traps and water tanks, with 7-8 weeks of absolute or near absolute control in both types of habitat. For spinosad, these findings are in broad agreement with previous studies in which a granular formulation provided six weeks of absolute control in an urban cemetery in Chiapas,[20] with similar findings elsewhere in Mexico,[21] and other countries.[22,23] Liquid formulations of spinosad can also be highly effective larvicides.[24-26]

The use of novaluron as a larvicide in Mexico was approved following the study on its efficacy for control of medically-important mosquitoes in natural and urban habitats.[27] Others have reported up to 20 weeks of control of *Ae. aegypti* in novaluron-treated habitats.[28,29] Inhibition of adult emergence was observed in larval and pupal samples from pyriproxyfen (IE=89-94%) and novaluron (IE=100%) treatments in both experiments. The effectiveness of novaluron and pyriproxyfen was not affected by being adsorbed onto pumice as other studies that have reported similar IE values (>90%) in pyriproxyfen-treated water containers,[20,30,31] although numbers of larvae may remain high.[32-34] As such, monitoring of larvae may not be a reliable indicator of pyriproxyfen efficacy, whereas for novaluron, development of larvae, pupae and adults was effectively inhibited.

The poor performance of temephos in this region may be due to the continuous dilution of this compound in water,[35] or possible resistance in the mosquito population. This is a cause for concern as this product is used to

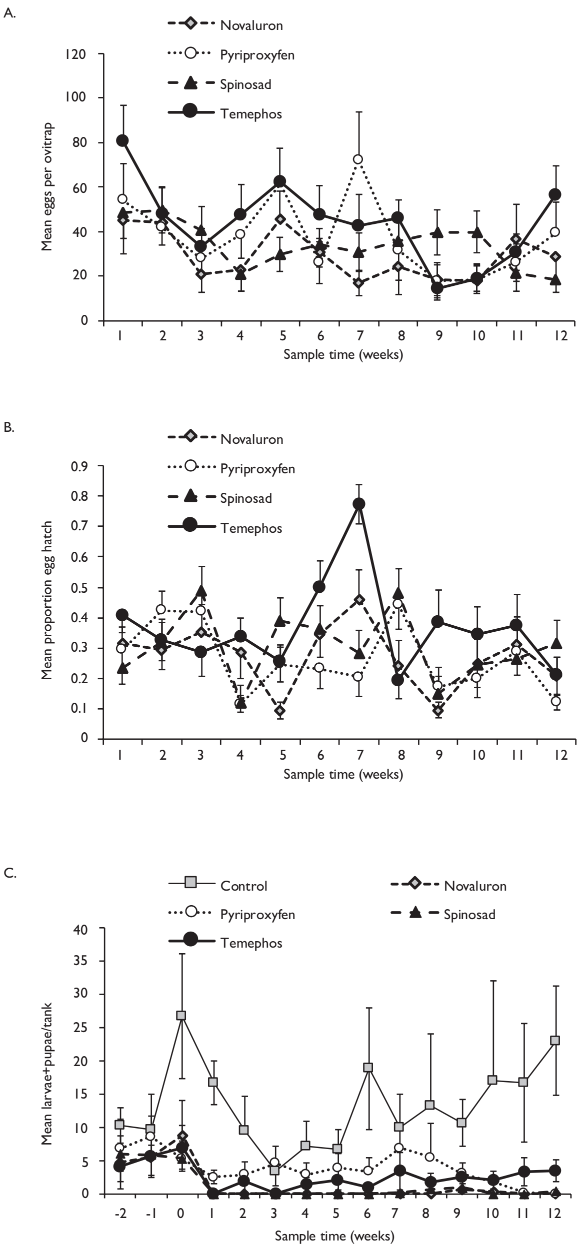

Figure 2. Efficacy of larvicides in water tanks in terms of (A) mean weekly counts of eggs and (B) proportion of hatched eggs in untreated oviposition traps placed in houses with larvicide-treated water tanks. (C) Mean number of larvae + pupae sampled in treated water tanks in houses in Huixtla, Chiapas, Mexico, during a 12 week study (August-December, 2015). Untreated oviposition traps acted as controls in (C). Vertical bars indicate SE; for clarity, only half the bar is shown at some points

treat water tanks and containers that cannot be emptied or eliminated.[35] Clearly, there is an urgent need to assess the susceptibility of *Aedes* spp. populations to temephos in southern Mexico. We conclude that the sustained-release formulations of spinosad granules and tablets and novaluron adsorbed onto pumice may contribute to the control of *Aedes* spp. in southern Mexico.

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Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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