

INSECTICIDE SUSCEPTIBILITY IN TWO POPULATIONS OF *Aedes aegypti* L. (Diptera: Culicidae) FROM GUERRERO, MEXICO, AND RECOMMENDATIONS FOR THEIR MANAGEMENT

Nelson Erik López-Zerón¹, J. Concepción Rodríguez-Maciél^{2*}, Claudia Yanet Wilson-García³, Ángel Lagunes-Tejeda¹, Gonzalo Silva-Aguayo⁴

¹Centro de Bachillerato Tecnológico Agropecuario No. 178. Carretera San Luis Horcasitas km 0.5, San Luis Acatlán, Guerrero, Mexico. C. P. 41600.

²Colegio de Postgraduados Campus Montecillo. Posgrado en Fitosanidad-Entomología y Acarología. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.

³Universidad Autónoma Chapingo. Sede San Luis Acatlán. Carretera San Luis Acatlán-Tlapa de Comonfort km 5, San Luis Acatlán, Guerrero, Mexico. C. P. 41600.

⁴Universidad de Concepción. Facultad de Agronomía, Departamento de Producción Vegetal. Casilla 537, Chillán, Ñuble, Chile.

* Author for correspondence: concho@colpos.mx

ABSTRACT

The *Aedes aegypti* L. mosquito is a vector of diseases such as classic and hemorrhagic dengue (DENV), chikungunya (CHIKV), Zika (ZIKV), yellow fever (YFV), and Mayaro (MAYV). In 2019 alone, in Latin America, there were 2.7 million cases and 1206 deaths. In Mexico, government programs for managing this pest include the elimination of larval habitats and chemical control of larvae and adults. The objective of this study was to evaluate the response of two larvae and adult populations of *A. aegypti* from the state of Guerrero, Mexico, under laboratory conditions, to insecticides with different modes of action and to make considerations for their management. In the bioassays, larvae showed susceptibility to permethrin, malathion, deltamethrin, and *Bacillus thuringiensis* var. *israelensis*; low resistance to Temephos and propoxur; as well as moderate resistance to Spinosad. Both adult populations showed resistance to permethrin and susceptibility to deltamethrin, while the Acapulco population showed resistance to alpha-cypermethrin. For larvae, Spinosad is suggested to be used in times of high incidence of dengue. During drought, it is advisable to include rotations of *B. thuringiensis* var. *israelensis* with organophosphates. For adults, the use of imidacloprid in combination with botanical insecticides is considered a rational alternative to rotations with organophosphates and pyrethroids.

Keywords: vector control, yellow fever mosquito, dengue mosquito, plague, resistance management.

Citation: López-Zerón NE, Rodríguez-Maciél JC, Wilson-García CY, Lagunes-Tejeda Á, Silva-Aguayo G. 2024. Insecticide susceptibility in two populations of *Aedes aegypti* L. (Diptera: Culicidae) from Guerrero, Mexico, and recommendations for their management. *Agrociencia*. <https://doi.org/10.47163/agrociencia.v58i7.3041>

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: July 03, 2023.
Approved: August 29, 2024.
Published in Agrociencia:
October 03, 2024.

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INTRODUCTION

The yellow fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae), is one of the most important insect species in public health, as it is a vector of viral diseases such as classical and hemorrhagic dengue (DENV), chikungunya (CHIKV), Zika (ZIKV), Mayaro fever (MAYV), and yellow fever (YFV) (WHO, 2019). In Mexico, during the 33rd epidemiological week of 2022, 3134 cases of dengue were confirmed. The highest number of cases coincides with the rainy season (CENAPRECE, 2022). Regarding confirmed cases of Zika, a total of 12 996 have been documented between 2015 and 2022. In February 2017, the first case of microcephaly due to Zika virus infection was also detected (CENAPRECE, 2022).

The species *A. aegypti* was considered to colonize only regions with an elevation below 1700 m; however, at the end of the last decade, its presence was documented in Mexico City at 2200 m (Kuri-Morales *et al.*, 2017). Currently, management strategies for this species are based on awareness campaigns to promote the elimination of breeding sites and the use of insecticides against larvae and adults (WHO, 2019). In Mexico, for more than 30 years, Temephos (organophosphate) insecticides have been applied uninterruptedly against larvae, and for the last 10 years, permethrin (pyrethroid) has been used against adults (Flores *et al.*, 2013). Consequently, in both biological states, numerous cases of resistance have been documented in the states of Veracruz (Flores *et al.*, 2013), Coahuila (Flores *et al.*, 2009), Quintana Roo (Flores *et al.*, 2006), Guerrero (Chino-Cantor *et al.*, 2014), and Baja California (Flores *et al.*, 2005).

The use of Spinosad, *Bacillus thuringiensis* var. *israelensis*, and Temephos to control *A. aegypti* larvae is authorized in Mexico, but Temephos is the most widely used larvicide. The active ingredients permethrin, deltamethrin, chlorpyrifos, malathion, and propoxur are authorized against adults of this vector (CENAPRECE, 2017). Currently, the number of insecticides used to reduce the density of the dengue mosquito has diversified, so it is expected that the resistance levels that were present when chemical control was based on a few options have decreased. Therefore, the present study aimed to evaluate the response to insecticides in two populations of *A. aegypti* from the state of Guerrero, Mexico, and to make recommendations for their management.

MATERIALS AND METHODS

Populations

During April and May 2017, 148 ovitraps for *A. aegypti* mosquitoes were placed in Acapulco, Guerrero, Mexico (16° 51' 46" N, 99° 53' 13" W), and 165 in San Luis Acatlán (16° 48' 23" N, 98° 43' 58" W). The ovitraps were kept at the peridomestic sites and checked weekly. Subsequently, they were sent to the Toxicology Laboratory of the Colegio de Postgraduados Montecillo Campus to obtain enough F_1 individuals for the bioassays. The New Orleans race of *A. aegypti* was used as the susceptible reference population. Field and reference populations were bred using the method proposed by the World Health Organization (WHO, 2005).

Insecticides used

For the larvae bioassays, the commercial formulations used were Spinosad (NATULAR EC CLARKE[®], 20.6 %, emulsifiable concentrate, 230 g active ingredient (a. i.) L⁻¹; Public Health Supply and Equipment de México S. A. de C. V., Mexico); *Bacillus thuringiensis* var. *israelensis* (VECTOBAC WDG[®], 34.7 % water-soluble granules; Bayer de México S. A. de C. V., Mexico); Temephos (TEMEPHOS 500 CE, 500 g a.i. L⁻¹, emulsifiable concentrate; Química Lucava S.A. de C.V., Mexico); chlorpyrifos ethyl (CLORPIRIFÓS, 122.8 g a. i. L⁻¹, liquid in mineral oil; Public Health Supply and Equipment de México S. A. de C. V., Mexico); permethrin (AQUA RESLIN SUPER[®], 108.7 g a. i. L⁻¹, aqueous solution; Bayer de México S. A. de C. V., Mexico); deltamethrin (AQUA K-OTHRINE[®], aqueous emulsion 20 g a.i. L⁻¹; Bayer de México S. A. de C. V., Mexico); deltamethrin (AQUA K-OTHRINE[®], aqueous emulsion 20 g a. i. L⁻¹; Bayer de México S. A. de C. V., Mexico); malathion (VERTHION[®], 410 g i. a. L⁻¹ concentrated solution; Agricultura Nacional S.A. de C.V., Mexico), and technical grade propoxur (99.5 % purity; Chem service, West Chester, PA, USA).

For the adult bioassays, permethrin (technical grade, mixture of isomers, 99.4 % purity; Chem service, West Chester, PA, USA), deltamethrin (technical grade, 99.1 % purity; Chem service, West Chester, PA, USA), and alpha-cypermethrin (technical grade, 99.5 % purity; Chem service, West Chester, PA, USA) were used. For the formulated insecticides, distilled water was used to prepare the required concentrations, and for the active ingredients, analytical-grade acetone (Meyer[®]; Tláhuac, Mexico City, Mexico) was used.

Bioassays with larvae

Bioassays with larvae were performed according to the standardized procedure of the World Health Organization (WHO, 2012). In 120 mL polystyrene containers, 100 mL of distilled water and 20 early fourth instar larvae were placed. Subsequently, 1 mL of the required concentration of the insecticide treatment to be evaluated was added. Initially, the biological response window was determined with the range of concentrations that caused between 0 and 100 % mortality. Subsequently, for each insecticide, nine intermediate concentrations were evaluated to cover this range. After 24 h of exposure to the toxicant, larvae that were not able to perform vertical movements or that did not show the characteristic diving reaction when the water was disturbed were considered dead (Flores, 2014).

Five replicates of each insecticide concentration were made, and each replicate included an untreated control. The maximum natural mortality accepted for the control was 10 %, and this was adjusted with the formula by Abbott (1925). Resistance ratio values at the level of the lethal concentrations LC₅₀ (RR₅₀) and LC₉₅ (RR₉₅) were obtained by dividing LC₅₀₍₉₅₎ of the field population by LC₅₀₍₉₅₎ of the susceptible population. The Mazzarri and Georgiou (1995) criterion was considered to define the resistance level. It was established that a resistance factor < 5 indicates a low resistance level, between 5 and 10 it is considered moderate resistance, and > 10 is high resistance.

Bioassays with adults

For bioassays with *A. aegypti* adults, the recommended diagnostic doses of alpha-cypermethrin, deltamethrin (10 µg per bottle), and permethrin (15 µg per bottle) were evaluated (CDC, 2010). The required concentrations were prepared using a micropipette (Thermo Fisher Scientific, Waltham, MA, USA) of 1 mL capacity in amber glass bottles, and analytical grade acetone was used as diluent (Meyer®, Mexico City, Mexico). Individually, 1 mL of the insecticide concentration was added to the 250 mL Wheaton glass bottles, while 1 mL of acetone was applied to the control. To form a uniform layer of the insecticide inside the bottles, these were capped and placed in an inverted position for 30 s. Subsequently, they were laid horizontally. The lid was removed and placed on a rotating roller machine (Star MFG International Inc., Maplewood, MO, USA) until the acetone evaporated, which occurred in approximately 10 min.

With a manual aspirator, 20 females (1–2 days old) previously in blood quarantine were introduced and provided with 10 % sugar water *ad libitum*. The treated insects were checked every 5 min for half an hour, and this period was considered the diagnostic time. The exposure time was approximately 30 min. Adult mosquitoes that could not remain in normal position or fly in a coordinated manner were categorized as knocked down (Flores, 2014). The maximum number of downed mosquitoes accepted in the control was 10 %. Five replicates of each insecticide were conducted on different days, and a control was included in each replicate. Mortality was adjusted using the Abbott (1925) formula.

The World Health Organization (WHO, 2016) criteria were used to categorize resistance as follows: with 98–100 % knockdown, the population was considered susceptible; with 90–97 %, it was considered as possible resistance, in which case the bioassay was repeated to confirm; and with a knockdown value of less than 90 %, the population was considered resistant to the insecticide evaluated (WHO, 2016).

Data analysis

Data from the larval bioassays were analyzed using the PROC PROBIT SAS procedure ver. 9.4 (SAS Institute, 2016). The slope values and their standard error, the mean, 95 % lethal concentrations (LC_{50} and LC_{95}), and their respective confidence intervals (95 %) were estimated. The significant difference in response between the two populations at the LC_{50} or LC_{95} level was determined by the absence of overlap between their confidence intervals (Mazzarri and Georghiou, 1995). The mortality result with the adult diagnostic dose was plotted with the Excel 2013 software package (Microsoft Corporation, USA).

RESULTS AND DISCUSSION

The two mosquito larvae populations showed susceptibility to *Bacillus thuringiensis* var. *israelensis*, as their resistance factor (RR_{50}) was $1.14 \times$ (Acapulco) and $0.95 \times$ (San

Luis Acatlán), and there was overlap of their confidence intervals (LC_{50} and LC_{95}) with the susceptible New Orleans control population (Table 1). These results agree with reports of *A. aegypti* from the field that have shown susceptibility to *B. thuringiensis* var. *israelensis* (Araújo *et al.*, 2013; IRAC, 2017). For more than 10 years, populations under selection pressure have been studied under laboratory conditions and showed no changes in susceptibility to this insecticide. This can be attributed to the composition of the commercial product containing a mixture of four proteins from this bacterium (Cry4Aa, Cry4Ba, Cry11Aa, and Cyt1Aa), each with different receptors or active sites, with activity in the midgut of mosquito larvae (Tetreau *et al.*, 2013).

Table 1. Toxicity of microbial insecticides on the larval population of *Aedes aegypti* L. from the state of Guerrero, Mexico.

Insecticide	Population	[†] n	[‡] b ± EE	[§] LC ₅₀ (LC 95 %)	[¶] Pr > χ^2	[‡] RR ₅₀	LC ₉₅ (LC 95 %)	RR ₉₅
Spinosad	New Orleans	500	3.78 ± 0.36	0.11 (0.10–0.12)	0.23		0.31 (0.25–0.41)	
	San Luis Acatlán	500	3.13 ± 0.23	0.18 (0.16–0.20)	0.63	1.63	0.62 (0.51–0.79)	2.00
	Acapulco	600	3.37 ± 0.29	0.15 (0.13–0.17)	0.75	1.36	0.47 (0.38–0.62)	1.51
<i>Bacillus thuringiensis</i> var. <i>israelensis</i>	New Orleans	500	2.11 ± 0.16	0.021 (0.018–0.025)	0.40		0.12 (0.099–0.180)	
	San Luis Acatlán	500	2.67 ± 0.27	0.02 (0.017–0.023)	0.20	0.95	0.083 (0.062–0.120)	0.69
	Acapulco	500	3.26 ± 0.31	0.024 (0.021–0.028)	0.30	1.14	0.079 (0.062–0.110)	0.65

[†]n: total number of treated insects; [‡]b ± EE: slope and standard error; [§]LC: lethal concentration (mg L⁻¹); [¶]Pr > χ^2 : Chi-square probability of fit to a straight line; [‡]RR: resistance factor.

In the case of Spinosad, the San Luis Acatlán population showed low levels of resistance (RR < 5 ×), both at the LC_{50} and LC_{95} levels, while the Acapulco population was only at the LC_{50} level. Therefore, it is necessary to implement rational management of this insecticide to avoid higher levels of resistance, as well as to monitor the biological effectiveness in the field that contributes to making the right decisions, as suggested by Marina *et al.* (2012).

Both larvae populations showed susceptibility to deltamethrin (Table 2). This insecticide is a type II pyrethroid, with a metabolic mechanism of resistance based on the action of Multiple Function Oxidases (MFO), unlike type I pyrethroids such as permethrin, which produce MFO and are also metabolized by esterases. (Atencia *et al.*, 2016). Both larval populations reported low LC_{50} levels to malathion (FR < 5 ×). Chino-Cantor *et al.* (2014) found similar results when evaluating three field populations with this insecticide and determined resistance levels < 5 ×.

Table 2. Toxicity of synthetic insecticides on the population of *Aedes aegypti* L. larvae from the state of Guerrero, Mexico.

Insecticide	Population	[†] n	[‡] b ± EE	[§] LC ₅₀ (LC ₉₅ %)	[¶] Pr > χ ²	[‡] RR ₅₀	LC ₉₅ (LC ₉₅ %)	RR ₉₅
Permethrin	New Orleans	800	1.65 ± 0.19	0.0098 (0.0069–0.0130)	0.052		0.096 (0.054–0.250)	
	San Luis Acatlán	500	2.24 ± 0.19	0.045 (0.040–0.052)	0.67	4.50	0.24 (0.18–0.37)	2.50
	Acapulco	600	2.19 ± 0.25	0.014 (0.011–0.017)	0.52	1.42	0.08 (0.055–0.140)	0.83
Deltamethrin	New Orleans	900	1.97 ± 0.13	0.0083 (0.0070–0.0097)	0.98		0.056 (0.042–0.079)	
	San Luis Acatlán	600	2.07 ± 0.25	0.01 (0.008–0.012)	0.63	1.20	0.062 (0.043–0.110)	1.10
	Acapulco	600	2.53 ± 0.22	0.0028 (0.0024–0.0032)	0.69	0.33	0.012 (0.0098–0.0170)	0.21
Temephos	New Orleans	600	2.48 ± 0.22	0.017 (0.014–0.019)	0.73		0.077 (0.058–0.110)	
	San Luis Acatlán	500	2.31 ± 0.20	0.076 (0.067–0.087)	0.34	4.47	0.39 (0.29–0.58)	5.06
	Acapulco	600	3.10 ± 0.25	0.032 (0.028–0.036)	0.88	1.88	0.10 (0.08–0.18)	1.29
Propoxur	New Orleans	500	3.67 ± 0.37	0.014 (0.012–0.016)	0.73		0.039 (0.031–0.054)	
	San Luis Acatlán	600	2.36 ± 0.21	0.024 (0.021–0.028)	0.10	1.71	0.12 (0.09–0.18)	3.07
	Acapulco	700	2.39 ± 0.30	0.028 (0.021–0.040)	0.039	2.00	0.13 (0.082–0.360)	3.33
Malathion	New Orleans	600	1.56 ± 0.18	0.017 (0.014–0.022)	0.22		0.19 (0.11–0.45)	
	San Luis Acatlán	600	2.31 ± 0.21	0.031 (0.026–0.036)	0.74	1.82	0.16 (0.11–0.24)	0.84
	Acapulco	600	1.88 ± 0.18	0.03 (0.025–0.037)	0.87	1.76	0.22 (0.15–0.4)	1.15
Chlorpyrifos	New Orleans	900	1.48 ± 0.15	0.096 (0.07–0.13)	0.074		1.23 (0.68–3.00)	
	San Luis Acatlán	800	1.65 ± 0.14	0.29 (0.24–0.36)	0.18	3.02	2.92 (1.90–5.26)	2.37
	Acapulco	800	1.12 ± 0.19	0.33 (0.19–0.77)	0.0083	3.43	9.50 (2.61–212.10)	7.72

[†]n: total number of treated insects; [‡]b ± EE: slope and standard error; [§]LC: lethal concentration (mg L⁻¹); [¶]Pr > χ²: Chi-square probability; [‡]RR: resistance factor.

In the case of Temephos, at the LC₅₀ level, the Acapulco and San Luis Acatlán populations showed low resistance, whereas the latter showed moderate resistance at the LC₉₅ level. Both populations also had low resistance to propoxur at the LC₅₀

level. These results agree with Chino-Cantor *et al.* (2014), who documented moderate resistance to Temephos in larvae populations of this species from Guerrero. According to Marina *et al.* (2011), this can be attributed to the fact that Temephos has been used for more than 35 years to combat *A. aegypti* larvae in that place; in addition, this insecticide and other organophosphates are also used in agriculture and may come into contact with breeding sites located in bodies of water, contributing to resistance development. This could be the case of chlorpyrifos, which registered a RR_{95} of $7.72 \times$ in the Acapulco population, indicating moderate resistance.

Adults of both populations showed susceptibility to deltamethrin (Figure 1). At the diagnostic dose evaluated ($10 \mu\text{g}$ per bottle) and 30 min of exposure, a value $> 98 \%$ mortality was recorded. Because this insecticide was used extensively and intensively in the past (Flores *et al.*, 2013), this population is likely to contain resistance alleles not detectable by the methodology used, as suggested by Atencia *et al.* (2016).

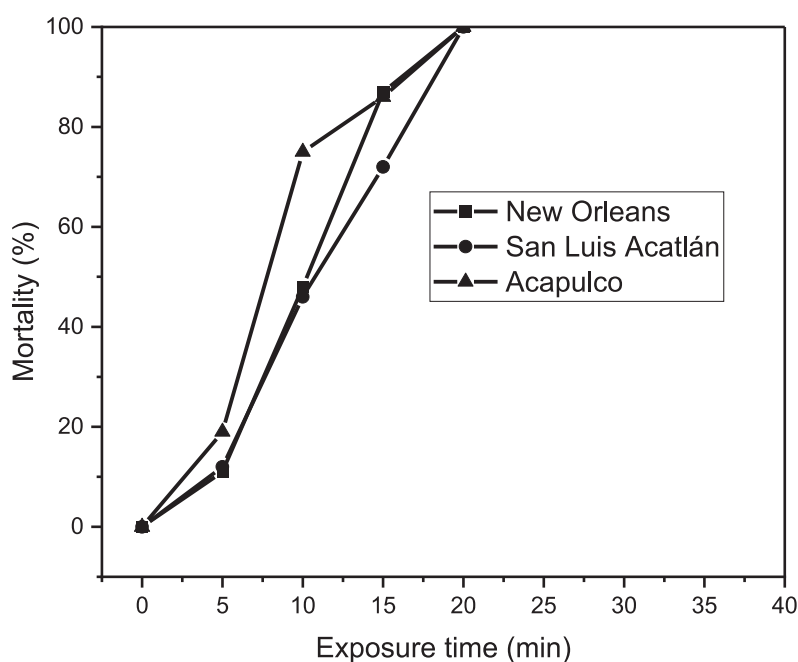


Figure 1. Toxicity of deltamethrin on *Aedes aegypti* L. populations from the state of Guerrero, Mexico, at a dose of $10 \mu\text{g}$ per bottle.

The San Luis Acatlán population was susceptible to alpha-cypermethrin since 100 % mortality was reached before the diagnostic time; however, the Acapulco population showed possible resistance to this insecticide since only 97 % mortality was reached at the diagnostic time and dose (30 min) (Figure 2).

The Acapulco population showed resistance to permethrin (Figure 3), which could be due to the use of this insecticide in Mexico for more than a decade (Flores *et al.*, 2014).

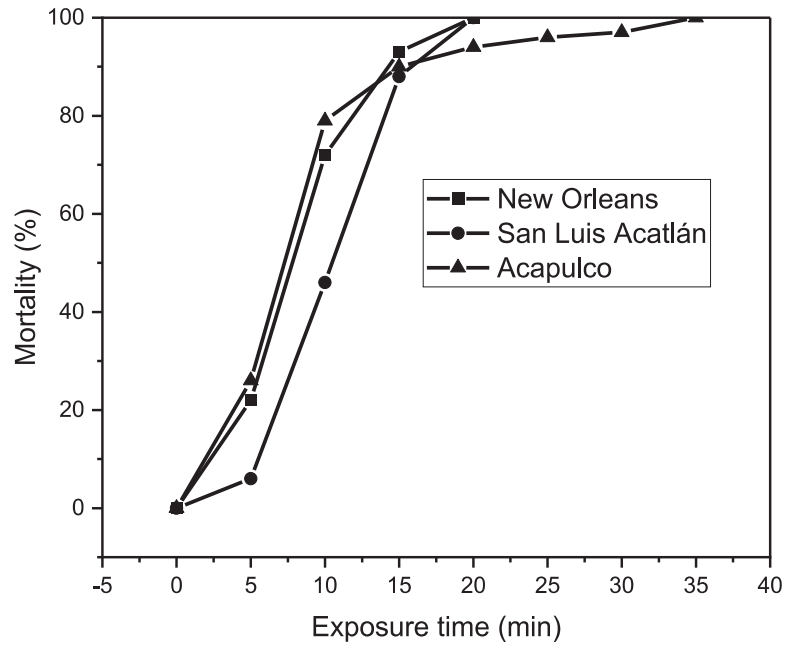


Figure 2. Toxicity of alpha-cypermethrin on *Aedes aegypti* L. populations from the state of Guerrero, Mexico, at a dose of 10 µg per bottle.

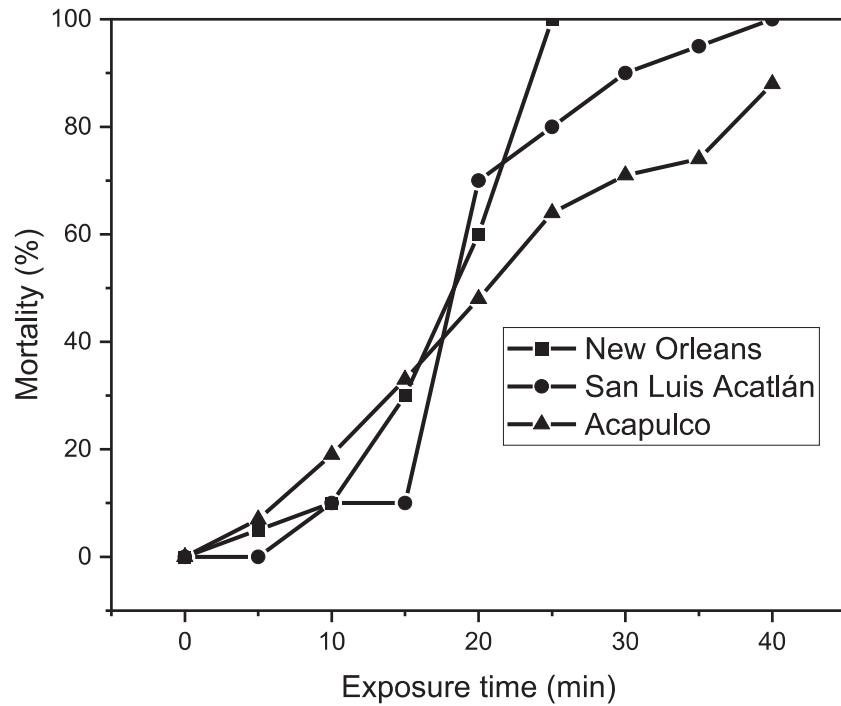


Figure 3. Toxicity of permethrin in *Aedes aegypti* L. populations from the state of Guerrero, Mexico, at a dose of 10 µg per bottle.

The results above agree with Deming *et al.* (2016), who evaluated field populations from municipalities near Mérida, Yucatán, and found that the Mérida population of adults presented higher resistance levels than nearby municipalities. This was because the frequency of application is higher in larger cities than in small towns. However, although the adults of the Acapulco population showed resistance to permethrin, the larvae were susceptible to this insecticide. Similarly, Chaverra-Rodríguez *et al.* (2012) detected a population of *A. aegypti* adults with field resistance to this insecticide and susceptibility in larval stages.

The use of Spinosad and *B. thuringiensis* var. *israelensis* as mosquito larvicides started in Mexico in 2012 (Marina *et al.*, 2012). Their use is not yet widespread since, in most cases, Temephos is used, which could explain the low resistance values in the populations evaluated.

In the case of larvae, it is advisable to use microbial insecticides such as Spinosad; however, it has a high cost, so it is recommended to apply in rainy seasons. However, *B. thuringiensis* var. *israelensis* can be used at any time of the year in rotation with organophosphates since they have a low propensity to resistance (Tetreau *et al.*, 2013). In the case of adults, although resistance occurs locally, it is suggested to restrict the use of pyrethroid insecticides because they are highly prone to resistance. Where pyrethroids are used, rotation with other groups, such as organophosphates, neonicotinoids, carbamates, or botanicals, is recommended (Rodríguez *et al.*, 2014). For adults, the use of permethrin in public health programs should be avoided until resistance levels to this adulticide decrease and it can be used in rotation with other insecticides.

In Mexico, the highest incidence of dengue cases and mosquito density coincides with the rainy season, that is, from July to September (CENAPRECE, 2022). On the other hand, in the first months of the year (January to June), cases decrease, so it is suggested to apply pyrethroids in rotation with organophosphates, mainly chlorpyrifos, at the beginning of the year in the dry months (Marina *et al.*, 2012). At the start of the rainy season, it is suggested to apply mixtures of imidacloprid with botanicals since adult density increases, and these products maintain their biological efficacy (Ahmed and Matsumura, 2012). However, they are highly prone to resistance and are also more expensive than carbamates and pyrethroids.

In Mexico, mixtures of botanical insecticides such as neem (*Azadirachta indica* A. Juss) and cinnamon (*Cinnamomum zeylanicum* Blume) are used to combat adult populations of *A. aegypti*, in addition to including them in mixtures with imidacloprid and pyrethrins. This option is considered adequate to diversify the tools to combat this vector, in addition to the use of biopesticides with less adverse effects on the environment and human health (CENAPRECE, 2017).

CONCLUSIONS

A. aegypti larvae showed susceptibility to permethrin, malathion, deltamethrin, and *Bacillus thuringiensis* var. *israelensis*, low resistance to Temephos and propoxur,

and moderate resistance to Spinosad. Adults of the San Luis Acatlán population were susceptible to alpha-cypermethrin; however, the Acapulco population was resistant. Adults from Acapulco were resistant to permethrin, but their larvae showed susceptibility to this insecticide. Adults are resistant to permethrin because it has been used to control this insect for over 30 years.

Studies revealed that Spinosad can be helpful in times of high incidence of dengue; likewise, in times of drought, it is advisable to include rotations with *Bacillus thuringiensis* var. *israelensis* and organophosphates. For adults, using imidacloprid in mixture with botanical insecticides is considered an alternative to carrying out rotations with organophosphates and pyrethroids.

ACKNOWLEDGEMENTS

Our thanks to the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) and Colegio de Postgraduados for funding and supporting the project.

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