AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Insecticide resistance level of *Aedes aegypti* L. (Diptera: Culicidae) in northern Mexico

López-Zerón, Nelson E.[1]; **Wilson-García, Claudia Y.**[2*]; **Alvarez-Vazquez, Perpetuo**[3], **Rojas-García, Rafael A.**[4]

[1] Centro de Bachillerato Tecnológico Agropecuario No. 178, San Luis Acatlán, Guerrero, México, C. P. 41600.
[2] Universidad Autónoma Chapingo, Unidad Académica San Luis Acatlán, San Luis Acatlán, Guerrero, México, C. P. 41600.
[3] Universidad Autónoma Agraria Antonio Narro, Departamento de Recursos Naturales Renovables, Buenavista, Saltillo Coahuila, México, C.P. 25315.
[4] Universidad Autónoma de Guerrero, Facultad de Medicina Veterinaria y Zootecnia No. 2, Carretera Acapulco-Pinotepa Nacional, km. 197, Cuajinicuilapa, Guerrero, México, C. P. 41940.
* Correspondence: cwilsong@chapingo.mx

ABSTRACT

Objective: To determine the response of the larvae of three *Ae. aegypti* populations from the Mexican North Pacific region to insecticides with different mode of action.

Design/Methodology/Approach: Three colonies were obtained placing ovitramps in peridomestic sites in Guadalajara (Jalisco), Culiacan (Sinaloa), and La Paz (Baja California Sur). Based on the methodology proposed by WHO, the bioassays were carried out with F_1 larvae in the early fourth instar.

Results: The larvae from the three field colonies had high resistance to permethrin and low resistance to deltamethrin; however, they were susceptible to Spinosad and *Bacillus thuringiensis* var. *Israelensis*. The Culiacan strain showed a high resistance to the malathion and propoxur insecticides.

Study limitations/Implications: The results provided valuable information about the response of these populations to insecticides, which are useful to establish resistance in the lab. Consequently, further studies should be carried out to complement the information obtained in these field tests.

Findings/Conclusions: The data indicated resistance levels to pyrethroid insecticides (mainly permethrin), as well as to organophosphates and carbamates.

Keywords: resistance, *Aedes aegypti*, biorational, pyrethroids, carbamates.

Citation: López-Zerón, N. E., Wilson-García, C. Y., Alvarez-Vazquez, P., & Rojas-García, R. A. (2023). Insecticide resistance level of *Aedes aegypti* L. (Diptera: Culicidae) in northern Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i12.2779

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: September 27, 2023.
Accepted: November 16, 2023.
Published on-line: January 24, 2024.

Agro Productividad, 16(12) suplemento. December. 2023. pp: 129-135.

INTRODUCTION

The *Aedes aegypti* L. mosquito is one of the most important insects for the public health sector, because it spreads several diseases, including classic dengue, dengue hemorrhagic fever, chikungunya virus, Zika virus, Mayaro virus, and yellow fever. This mosquito lives in urban habitats and it mainly reproduces in artificial containers. Unlike other mosquitoes, it feeds during the day; however, its biting activity intensifies in the early morning and at dusk (Souza *et al.*, 2019).

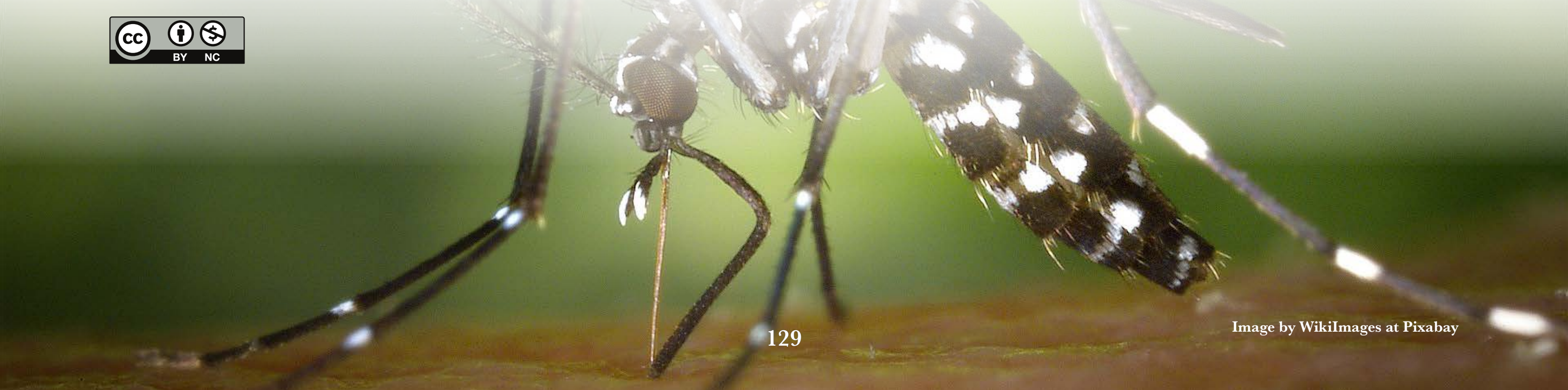

Image by WikiImages at Pixabay

Given its adaptability, *Aedes aegypti* can currently be found in climates and altitudes where it had not been previously reported. Some studies point out that dengue prevails in 128 countries, impacting 3.900 billion people (Brady *et al.*, 2012).

In Mexico, management has mainly been focused on the use of insecticides such as pyrethroids and organophosphates to control adults and larvae, respectively. Nevertheless, this practice has resulted in resistance problems in several states, including Guerrero (Chino-Cantor *et al.*, 2014), Veracruz (Flores *et al.*, 2013), and Quintana Roo (Flores *et al.*, 2006).

As a consequence of the increased range of insecticides authorized for the control of the *Aedes aegypti* larvae, the susceptibility to the said authorized insecticides must be reviewed (Zettel and Kaufman, 2008). Therefore, the objective of this study was to evaluate the current state of the response of three *Ae. aegypti* populations from the Mexican states of Jalisco, Sinaloa, and Baja California Sur to insecticides.

MATERIALS AND METHODS

Individuals from three *Ae. aegypti* populations were collected in Guadalajara, Jalisco (20° 39' 58" N and 103° 21' 07" W), Culiacan, Sinaloa (24° 48' 00" N and 107° 23' 00" W), and La Paz, Baja California Sur (24° 08' 32" N and 110° 18' 39" W). The New Orleans strain —which has been certified as susceptible to insecticides and was provided by the Universidad Autónoma de Nuevo León— was used as a point of comparison. This strain had been kept three years in the lab where the bioassays were carried out.

The field material was gathered during April and May 2017 (La Paz), June 2017 (Culiacan), and May and June 2017 (Guadalajara). The samples were collected with ovitramps. These tramps were made up of 1-L black plastic containers. The insides were covered with a 12 cm wide × 27 cm long white Pellon interlining fabric (model F-1600); the fabric had a hole in the ½ liter measurement capacity. The ovitramps were left in the peridomestic sites for a month. During that period, they were checked on a weekly basis.

The cloth with the eggs was extracted, dried, and sent to the lab. A total of 119 fabric pieces (23,746 parental eggs), 66 fabric pieces (15,353 parental eggs), and 227 fabric pieces (40,779 parental eggs) were obtained from the colonies found in Guadalajara, La Paz, and Culiacán, respectively. However, the egg feasibility percentage was $<1\%$ in all the colonies, because most of them had hatched or were dehydrated.

The larvae were fed every third day, with dust from pet food (Rodent Lab Chow®5001). The containers were kept in a TFFU2065FWA bioclimatic chamber (Thermo Fisher Scientific, Waltham, MA, USA), at 27 °C and with a 12:12 (L:O) photoperiod. The pupae were extracted and placed in polystyrene glasses. The said glasses were introduced into 30×30×45 cm entomological cages, wrapped in organza fabric, at 27 °C ± 2, with a 75% ± 5 relative humidity, and the same photoperiod than the larvae, in order to help them to reach adulthood.

Adults were fed with a 10% sugary solution. The females were given *Sus scrofa domesticus* L. pig blood, with 4 mL of heparin sodium 1,000 un/mL per liter of blood as anticoagulant. The blood was warmed in a bath Marie until it reached 37 °C. Afterwards, 5 mL of blood were poured into polystyrene glasses, covered with heat-resistant Parafilm-M® sealing film which had been soaked with human sweat on the outside. The glasses were placed upside-down at the top of the cage (Carvalho *et al.*, 2014).

Eight insecticides from different toxicological groups were used: organophosphates, carbamates, pyrethroids, spinosyns, and Bacillus (Table 1).

In the case of the insecticide formulations, distilled water was used to prepare the required concentrations, while Meyer® reagent grade acetone was used for the active ingredients.

The bioassays followed the standardized procedures of the WHO (2012). Twenty larvae in the early four instar were placed in two 120-mL polystyrene containers filled with 100 mL of distilled water. During the biological response window, nine concentrations of each insecticide were established, starting from 0.01% and logarithmically decreasing until 0 and 100% mortality results were obtained, after a 24 h exposition. Subsequently, nine intermediate concentrations (five repetitions per insecticide and an untreated control) were evaluated. In order to obtain the 0-100% mortality ranges, the dead larvae were counted. In addition, the larvae that were unable to move vertically or that did not perform their characteristic movements when touched with the stimulus-responsive brush were considered to be dead (Flores, 2014). The maximum mortality accepted for the control was 10%; this result was adjusted using Abbott's formula (Abbott, 1925).

The 50% and 95% mortality (RF_{50} and RF_{95}) factors were obtained dividing the LC_{50} or LC_{95} lethal concentration of the field population by the LC_{50} o LC_{95} of the susceptible population. The Mazzarri and Georghiou (1995) criteria were used to determine the resistance degree of the field populations: <5 resistance factor (RF) indicates a low resistance level; 5-10, a moderate resistance level; and >10, a high resistance level.

The data of the bioassays were analyzed using the PROC PROBIT procedure of the SAS software, version 9.0 (SAS Institute, 2002). The LC_{50} and LC_{95} values were calculated, as well as their confidence intervals (95%). The lack of overlap between confidence intervals was taken into account to determine if there was a significatively different response between one and two populations subjected to insecticides of the same toxicological group, regarding the LC_{50} and LC_{95} values.

Table 1. Insecticides used in the bioassays.

Active Ingredient & Trade Name	Formulation	Percentage or purity	Formulator
Spinosad, Natular EC	Concentrated emulsionable	20.6%, 230 g i. per litre	Public Health Supply and Equipment de México S. A. de C. V.
Bacillus thuringiensis var. israelensis VectoBac®WDG	Water-soluble granules	34.7%	Bayer de México S. A. de C. V.
Temefós, Temephos	Concentrated emulsionable	500 de i. a. per litre	Química Lucava S.A. de C.V.
Clorpirifós etílico, Clorpirifós	Liquid in mineral oil	122.8 g de i. a. per litre	Public Health Supply and Equipment de México S. A. de C. V.
Permetrina, Aqua Reslin Super	Aqueous solution	108.7 g de i. a. per litre	Bayer de México S. A. de C. V.
Deltametrina, Aqua K-Othrine®	Aqueous emulsion	20 g de i. a. per litre	Bayer de México S. A. de C. V.
Malatión, Verthion	Concentrated Solution	410 g de i. a. per litre	Agricultura Nacional
Propoxur	Technical Grade	Puresa 99.5%	Chem service, West Chester, PA

RESULTS AND DISCUSSION

The colonies of Guadalajara, Culiacan, and La Paz showed high resistance levels (>10 x) to permethrin, recording 40.81, 42.85, and 69.38x resistance factor (RF_{50}), respectively (Table 2). Meanwhile, the three colonies showed even higher resistance levels (RF_{95}). However, the La Paz colony recorded the highest resistance factor (217x) and RF_{50} among the three colonies.

Meanwhile, the three colonies recorded low resistance levels to the deltamethrin insecticide: 3.73, 1.56, and 2.77x (RF_{50}) for the Guadalajara, Culiacan, and La Paz colonies, respectively. For its part, RF_{95} reported a similar trend towards low resistance levels. In all cases, the slope response of the populations to this insecticide was >2. This phenomenon indicates that the populations have a drastic mortality response to a dose increase. Nevertheless, this was not the case with permethrin, whose slope results were ≤ 1.61 in all the cases. These results indicate a high resistance trend: when the dose increases, mortality slowly increases.

None of the colonies showed resistance to Spinosad. The LC_{50} and LC_{95} of the field colonies recorded lower results than the New Orleans strain control (Table 3). This insecticide was first applied in Mexico for the management of the *Aedes aegypti* larvae in 2014. However, it was not used in all the states were the health campaign against *Aedes aegypti* has been implemented and this may explain the results obtained.

The slope of all the colonies where Spinosad was applied was ≥ 2.4, which indicates that these colonies have a similar mortality response to a dose increase.

All the field colonies were susceptible to *Bacillus thuringiensis* var. *Israelensis*: their LC_{50} and LC_{95} fiducial limits overlapped with the susceptible New Orleans strain control. Likewise, the slope values of the bioassays were ≥ 3.24 in all the cases, which indicates that this insecticide has a low resistance trend.

Although the temephos insecticide is widely used to manage mosquito larvae in Mexico, the three colonies (LC_{50}) showed low resistance levels, reporting 1.42, 1.47, and 3.23x values for the colonies of Guadalajara, Culiacan, and La Paz, respectively (Table 4). LC_{95} showed a similar behavior, recording <5x resistance factors.

Table 2. Pyrethroid toxicity (mg L^{-1}) in *Aedes aegypti* larvae of the Mexican North Pacific.

Insecticide	Strain	[1]N	[2]b±SE	[3]LC_{50} LC%95	[4]$Pr>\chi^2$	[5]RF_{50}	LC_{95} LC95%	RF_{95}
Permetrina	New Orleans	800	1.65±0.19	0.0098 (0.0069-0.013)	0.052		0.096 (0.054-0.25)	
	Guadalajara	480	1.61±0.16	0.4 (0.32-0.49)	0.96	40.8	4.2 (2.98-6.86)	43.7
	Culiacán	540	1.27±0.13	0.42 (0.33-0.52)	0.98	42.8	8.42 (5.05-18.08)	87.7
	La Paz	720	1.1±0.091	0.68 (0.55-0.85)	0.4	69.3	20.89 (12.08-44.09)	217
Deltametrina	New Orleans	900	1.97±0.13	0.0083 (0.007-0.0097)	0.98		0.056 (0.042-0.079)	
	Guadalajara	480	2.29±0.16	0.031 (0.027-0.036)	0.82	3.73	0.16 (0.12-0.22)	2.8
	Culiacán	320	2.42±0.27	0.013 (0.011-0.016)	0.92	1.56	0.066 (0.051-0.097)	1.1
	La Paz	420	2.03±0.17	0.023 (0.02-0.028)	0.9	2.77	0.15 (0.11-0.23)	2.6

[1]N: number of events; [2]b±SE (SE): slope and standard error; [3]LC (LC): lethal concentration; [4]$Pr>\chi^2$: chi-square probability; [5]RF (RF): resistance factor.

Table 3. Toxicity (mg L^{-1}) of microbials in *Aedes aegypti* larvae of the Mexican North Pacific.

Insecticide	Strain	^{1}N	^{2}b±SE	$^3LC_{50}$ LC%95	$^4Pr>\chi^2$	$^5RF_{50}$	LC_{95} LC95%	RF_{95}
Spinosad	New Orleans	500	3.78±0.3	0.11 (0.1-0.12)	0.23		0.31 (0.25-41)	
	Guadalajara	420	3±0.2	0.035 (0.031-0.039)	0.82	0.31	0.12 (0.1-.16)	0.38
	Culiacán	420	2.41±0.19	0.037 (0.032-0.043)	0.51	0.33	0.17 (0.14-o.24)	0.54
	La Paz	700	3.39±0.3	0.027 (0.023-0.031)	0.11	0.24	0.082 (0.065-0.11)	0.26
Bacillus thuringiensis var. *israelensis*	New Orleans	540	2.43±0.1	0.021 (0.019-0.024)	0.55		0.1 (0.083-0.13)	
	Guadalajara	360	3.87±0.39	0.011 (0.01-0.012)	0.29	0.52	0.03 (0.024-0.04)	0.3
	Culiacán	540	3.25±0.2	0.027 (0.024-0.029)	0.72	1.28	0.086 (0.073-0.1)	0.86
	La Paz	420	3.24±0.2	0.018 (0.016-0.021)	0.8	0.85	0.06 (0.049-0.077)	0.6

^{1}N: number of events; ^{2}b±SE (SE): slope and standard error; ^{3}LC (LC): lethal concentration; $^4Pr>\chi^2$: xhi-square probability; ^{5}RF (RF): resistance factor.

Table 4. Organophosphate and carbamate toxicity (mg L^{-1}) in *Aedes aegypti* larvae in the Mexican North Pacific.

Insecticide	Strain	^{1}N	^{2}b±SE	$^3LC_{50}$ LC%95	$^4Pr>\chi^2$	$^5RF_{50}$	LC_{95} LC95%	RF_{95}
Temefós	New Orleans	540	2.15±0.15	0.021 (0.018-0.024)	0.27		0.12 (0.094-0.16)	
	Guadalajara	480	2.25±0.17	0.03 (0.026-0.035)	0.24	1.42	0.16 (0.12-0.22)	1.33
	Culiacán	540	2±0.15	0.031 (0.027-0.036)	0.14	1.47	0.2 (0.16-0.29)	1.66
	La Paz	700	4.55±0.43	0.068 (0.06-0.079)	0.12	3.23	0.18 (0.14-0.25)	1.5
Malation	New Orleans	600	1.56±0.18	0.017 (0.014-0.022)	0.22		0.19 (0.11-0.45)	
	Guadalajara	420	2.93±0.28	0.1 (0.095-0.11)	0.18	5.88	0.38 (0.29-0.55)	2
	Culiacán	480	2.6±0.3	0.29 (0.23-0.36)	0.022	17.05	1.24 (0.84-2.39)	6.52
	La Paz	420	2.81±0.38	0.1 (0.08-0.13)	0.07	5.8	0.4 (0.26-0.93)	2.1
Clorpirifós	New Orleans	600	3.58±0.33	0.013 (0.012-0.015)	0.18		0.039 (0.031-0.052)	
	Guadalajara	480	2.05±0.17	0.045 (0.038-0.053)	0.94	3.46	0.28 (0.2-0.45)	7.17
	Culiacán	420	2.34±0.58	0.096 (0.066-0.28)	0.0017	7.38	0.48 (0.2-22.12)	12.3
	La Paz	420	2.89±0.22	0.022 (0.02-0.026)	0.51	1.69	0.084 (0.067-0.11)	2.15
Propoxur	New Orleans	900	1.48±0.15	0.096 (0.07-0.13)	0.074		1.23 (0.68-3)	
	Guadalajara	420	4.03±0.57	1.21 (1-1.49)	0.01	12.6	3.11 (2.29-5.7)	2.52
	Culiacán	420	2.93±0.54	1.79 (1.15-2.75)	0.03	18.6	6.52 (3.87-23.18)	5.3
	La Paz	420	3.36±0.45	2.64 (2.04-3.58)	0.006	27.5	8.15 (5.45-17.34)	6.62

^{1}N: number of events; ^{2}b±SE (SE): slope and standard error; ^{3}LC (LC): lethal concentration; $^4Pr>\chi^2$: chi-square probability; ^{5}RF (RF): resistance factor.

The field colonies of Guadalajara and La Paz showed a moderate resistance to Malathion in their LC_{50}, both recording 5.88x resistance factor values. However, the Culiacan strain recorded a high resistance to this insecticide and its RF_{50} reached 17.05x.

The Guadalajara and La Paz strains showed low resistance levels to clorphyrifos in their LC_{50}: 3.46 and 1.69x, respectively. However, the Culiacan strain recorded a moderate resistance (7.38x RF_{50}). Comparing the LC_{95}, the La Paz strain recorded a low resistance (>5x), while the Guadalajara and the Culiacan strains showed moderate (7.17x) and high resistance (>10x), respectively. Meanwhile, the response of the three colonies to the three organophosphate insecticides recorded slopes values between 2 and 3. Meanwhile, only the La Paz strain recorded a 4.55 slope against the temephos insecticide. Among the three colonies this strain recorded the highest RF_{50} against this insecticide.

All the field strains had a high resistance to propoxur (carbamate): their RF_{50} were 12.6 (Guadalajara), 18.64 (Culiacan), and 27.5x (La Paz). Nevertheless, regarding the resistance factors of the LC_{95}, the Guadalajara strain had a low resistance (2.52x), while the Culiacan and La Paz strains recorded a moderate resistance (5.3 and 6.62x, respectively). The highly variable slope values ranged from 2.93 to 4.03, indicating that the response to this insecticide is not even (Table 4).

The three colonies showed low resistance levels (>5x) to deltamethrin, a type II insecticide; however, they recorded high resistance levels to permethrin, a type I pyrethroid. The only metabolic resistance mechanism of the first type of insecticide is made up of mixed function oxidase (MFO). Meanwhile, permethrin has a metabolic resistance mechanism made up of esterase and MFO. In addition, permethrin has been used for a longer period in the campaign against adult mosquitoes and is widely applied in the agricultural sector. For its part, the use of deltamethrin is recent and is mainly recommended for the impregnation of pavilions; consequently, the pressure to select a single insecticide is lower than in the apple cultivation sector, where permethrin is the chosen product.

Although neither deltamethrin pyrethroids, nor permethrin were applied to the larvae, Zettel and Kaufman (2008) pointed out that the use of adulticides can have a marginal selection effect on the larvae, because spraying can spread on water bodies where the larvae develop. Therefore, the larvae frequently become resistant to adulticides.

Meanwhile, although several other species (mainly agricultural pests) have shown resistance to Spinosad, only one resistance case (*Culex quinquefasciatus*) has been recorded among urban pests; this case was induced by selection pressure in the lab (Su and Cheng, 2014). Consequently, field strains frequently keep their susceptibility status. In addition, this insecticide was introduced for urban use in Mexico in 2014.

There is no record about the mosquitoes' resistance to *Bacillus thuringiensis* var. *Israelensis*, although it has been used in some places for more than 10 continuous years, as the only option for the control of larvae (Tetreau *et al.*, 2013).

The abuse in the use of temephos in Mexico —which has been employed since 1980— as a control method against Ae. aegypti larvae has caused numerous resistance cases. Although temephos recorded low resistance levels (>5x), the selection pressure of this insecticide causes a crossed resistance with other insecticides of the same toxicological group (such as chlorpyrifos) and other toxicological groups (such as propoxur) (Rodríguez *et al.*, 2002; Rodríguez *et al.*, 2005).

CONCLUSIONS

The data obtained indicate certain resistance level against pyrethroid insecticides (mainly permethrin). Organophosphates and carbamates also registered resistance levels. Consequently, this work should encourage other researchers to carry out further field tests to confirm that the application of these insecticides is not impacting the conditions. If this situation is confirmed, insecticides should be used with a rotary arrangement. Another proposal is to stop using some insecticides, at least until the resistance levels decrease. In order to achieve this reduction, bioassays should be carried out to monitor mosquito larvae.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267.

Brady, O.J., Gething, P.W., Bhatt, S., Messina, J.P., Brownstein, J.S., Hoen, A.G., Moyes, C.L., Farlow, A.W., Scott, T.W. y Hay, S.I. 2012. Refining the Global Spatial Limits of Dengue Virus Transmission by Evidence-Based Consensus. *PLoS Neglected Tropical Diseases 6*(8): 760. DOI: 10.1371/journal.pntd.0001760

Carvalho, D.O.; Nimmo, D.; Naish, N.; Mckemey, A.R.; Gray, P.; Wilke, A.B.B.; Marrelli, M.T.; Virginio, J.F.; Alphey, L.; Capurro, M.L. 2014. Mass Production of Genetically Modified *Aedes aegypti* for Field Releases in Brazil. *Journal of Visualized Experiments* 83. DOI: 10.3791/3579

Chino-Cantor, A.; Sánchez-Arroyo, H.; Ortega-Arenas, L.D; Castro-Hernández, E. 2014. Insecticide susceptibility of *Aedes aegypti* L. (Diptera: Culicidae) in Guerrero, Mexico. *Southwestern Entomologist 39* (3): 601-612. https://doi.org/10.3958/059.039.0319

Flores, A. E. 2014. Detección de resistencia a insecticidas en mosquitos con énfasis en *Aedes aegypti*. https://www.researchgate.net/profile/Adriana_Flores4/publication/272156554

Flores, A.E.; Grajales, J.S.; Salas, I.F.; Garcia, G.P.; Becerra, M.H.L.; Lozano, S.; Brogdon, W.G.; Black, W.C.; Beaty, B. 2006. Mechanisms of insecticide resistance in field populations of *Aedes aegypti* (L.) from Quintana Roo, Southern Mexico. *Journal of the American Mosquito Control Association 22*(4): 672-677. DOI:10.2987/8756-971X(2006)22[672:MOIRIF]2.0.CO;2

Flores, A.E.; Ponce, G.; Silva, B.G.; Gutierrez, S.M.; Bobadilla, C.; Lopez, B.; Mercado, R.; Black, W.C. 2013. Wide Spread Cross Resistance to Pyrethroids in *Aedes aegypti* (Diptera: Culicidae) From Veracruz State Mexico. *Journal of Economic Entomology 106*(2): 959-969. DOI: 10.1603/ec12284

Mazzarri, M.B.; Georghiou, G.P. 1995. Characterization of resistance to organophosphate, carbamate, and pyrethroid insecticides in field populations of *Aedes aegypti* from Venezuela. *Journal of the American Mosquito Control Association-Mosquito News 11*(3): 315–322. https://pubmed.ncbi.nlm.nih.gov/8551300/

Rodríguez, M.M.; Bisset, J.A.; De Armas; Ramos, F. 2005. Pyrethroid insecticide-resistant strain of *Aedes aegypti* from cuba induced by deltamethrin selection. *Journal of the American Mosquito Control Association 21*(4): 437-445. DOI: 10.2987/8756-971X(2006)21[437:PISOAA]2.0.CO;2

Rodríguez, M.M.; Bisset, J.; Ruiz, M.; Soca, A. 2002. Cross-resistance to pyrethroid and organophosphorus insecticides induced by selection with temephos in *Aedes aegypti* (Diptera: Culicidae) from Cuba. *Journal of medical entomology 39*(6): 882–888. DOI: 10.1603/0022-2585-39.6.882

SAS Institute. 2016. SAS/Stat® 9.1User's Guide. SAS Institute, Inc., Cary, NC.

Souza-Neto, JA., Powell, JR., Bonizzoni, M. 2019. *Aedes aegypti* vector competence studies: A review. *Infection, Genetics and Evolution 67*(2019) 191–209. DOI: 10.1016/j.meegid.2018.11.009

Su, T.; Cheng, M. L. 2014. Cross Resistances in Spinosad-Resistant *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Medical Entomology 51*(2): 428-435. DOI: 10.1603/me13207

Tetreau, G.; Stalinski, R.; David, J. P.; Despres, L. 2013. Monitoring resistance to *Bacillus thuringiensis* subsp. *israelensis* in the field by performing bioassays with each Cry toxin separately. *Memórias do Instituto Oswaldo Cruz 108*(7): 894-900. DOI: 10.1590/0074-0276130155

World Health Organization. 2012. Global strategy for dengue prevention and control, 2012-2020. Geneva, Switzerland: World Health Organization. https://www.who.int/publications/i/item/9789241504034

Zettel, C.P; Kaufman. 2008. Yellow fever mosquito, *Aedes aegypti* (Linnaeus) (Insecta: Diptera: Culicidae). University of Florida. EENY-434: 1-8. DOI:10.32473/edis-in792-2009