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Wide spread cross resistance to pyrethroids in *Aedes aegypti* (L.) from Veracruz State Mexico

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Abstract

Seven F_1 strains of *Aedes aegypti* (L.) were evaluated by bottle bioassay for resistance to the pyrethroids *d*-phenothrin, permethrin, deltamethrin, λ-cyalothrin, bifenthrin, cypermethrin, α-cypermethrin and z-cypermethrin. The New Orleans strain was used as a susceptible control. Mortality rates after a 1h exposure and following a 24h recovery period were determined. The resistance ratio between the 50% knockdown values (RR_{KC50}) of the F_1 and New Orleans strains indicated high levels of knockdown resistance (kdr). The RR_{KC50} with α-cypermethrin varied from 10–100 among strains indicating high levels of kdr. Most of the strains had moderate resistance to *d*-phenothrin. Significant but much lower levels of resistance were detected for λ–cyalothrin, permethrin and cypermethrin. For *z*-cypermethrin and bifenthrin, only one strain exhibited resistance with RR_{KC50} values of 10- and 21-fold, respectively. None of the strains showed RR_{KC50} >10 with deltamethrin, and moderate resistance was seen in three strains, while the rest were susceptible. Mosquitoes from all strains exhibited some recovery from all pyrethroids except *d*-phenothrin. Regression analysis was used to analyze the relationship between RR_{LC50} and RR_{KC50}. Both were highly correlated ($R^2 = 0.84 – 0.97$) so that the slope could be used to determine how much additional pyrethroid was needed to insure lethality. Slopes ranged from 0.875 for *d*-phenothrin ($RR_{LC50} \simeq RR_{KC50}$) to 8.67 for λ–cyalothrin (~8.5 fold more insecticide needed to kill). Both RR_{LC50} and RR_{KC50} values were highly correlated for all pyrethroids except bifenthrin indicating strong cross resistance. Bifenthrin appears to be an alternative pyrethroid without strong cross resistance that could be used as an alternative to the current widespread use of permethrin in Mexico.

Keywords

Aedes aegypti; pyrethroids; insecticide resistance; cross resistance

Aedes aegypti (L.) is the primary vector in Mexico of the four dengue virus serotypes (DENV1-4), the causative agents of dengue fever (DF) and dengue hemorrhagic fever (DHF). Although *Ae. albopictus* (Skuse) is present in many regions of the country; it still has not been incriminated as an important vector. Mexico is severely affected by DF and

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DHF because DENV1-4 viruses co-occur with *Ae. aegypti* in most states of Mexico. In 2007, 28 of 32 states registered cases of DF and DHF, in 2008, 26 states and in 2009, 25 states registered confirmed cases.

The coastal regions of Mexico are most affected by DF and DHF and the state of Veracruz, with an area of 72,815 km^2 and a coastline of 684 km, has had the greatest numbers of DF and DHF cases. In 2007 alone, there were 15,253 DF cases of which 2,645 progressed to DHF. In 2008 there were 4,117 DF cases of which 2,051 progressed to DHF and in 2009 there were 6,390 DF cases of which 2, 978 eventually progressed to DHF (Boletin de Epidemiologia, Mexico 2007, 2008, 2009).

The official Mexican policy for the surveillance and control of vectors, NOM-032-SSA2-2002, recommended the use of the organophosphate larvicide temephos (Abate® granules) at a concentration of 1 ppm for the control of larval *Ae. aegypti* and a formulation of permethrin as an adulticide has now been used for more than 10 consecutive years (1999–2010). On June 1, 2011, a new policy was published (NOM-032-SSA2-2010) that established the characteristics that insecticides should have for the control of disease vectors, including *Ae. aegypti,* in Mexico without specifying which agents should be used, stating instead that it would depend on proven effectiveness, resistance and safety characteristics related to exposure.

In Mexican populations of *Ae. aegypti*, resistance to permethrin is conditioned in part by esterases (Flores et al. 2005, 2006). The presence of a new mutation (Ile1,016) in the voltage-gated sodium channel gene is also associated with kdr to pyrethroids. This mutation was originally found in a permethrin resistant strain from Isla Mujeres, off the coast of Cancun (Saavedra-Rodriguez et al. 2007). High frequencies of this resistance allele were subsequently found in collections of *Ae. aegypti* from 78 sites in Mexico with some of the highest frequencies detected in collections from Veracruz state (Ponce et al. 2009, Siller et al. 2011).

The practice of utilizing a single insecticide until the appearance of resistance has become a standard practice that quickly reduces the number of insecticides available for vector control. Rotations, mosaics and mixtures have instead been proposed as strategies for insecticide resistance management (Curtis 1985, Curtis et al. 1993, Roush 1989). Mathematical models have been applied for estimating how these tools could be used in an optimal manner (Tabashnik 1989). However, these models have been rarely tested under field conditions, especially for insect vectors, due to the difficulties in determining changes in frequencies of resistance genes in large samples of insects from resistant populations (Hemingway et al. 1997).

In Mexico, there was a large-scale field trial with *Anopheles albimanus* that used rotations or mosaics of insecticides substituting the simple use of DDT or of a specific pyrethroid (Hemingway et al. 1997; Penilla et al. 1998). Changes in the frequency of resistance genes were monitored for four years (Penilla et al. 1998). The results were promising and predicted that rotations or mosaics of insecticides are viable long-term strategies for the sustainable use of insecticides in disease control programs. With that goal in mind, the aim of the present study was to determine the levels of resistance to eight pyrethroids in seven collections of *Ae. aegypti* from the state of Veracruz. This knowledge will facilitate the selection of viable alternative pyrethroids for use in a rotation program for sustained control of *Ae. aegypti* at the local, regional and possibly state-wide levels.

Materials and Methods

Study area

Seven field collections of *Ae. aegypti* were collected in 2009 from the state of Veracruz (Fig. 1). The localities sampled were: Panuco (22°03′00.00″N, 98°10′59.92”W), Tantoyuca (21°20′54.44″N, 98°13′45.90″W), Poza Rica (20°32′00.00″N, 97°26′59.84″W), Martinez de la Torre (20°03′42.55″N, 97°03′06.97″W), Veracruz (19°10′21.48″N, 96°07′59.93″W), Coatzacoalcos (18°08′16.00″N, 94°26′07.49″W) and Cosoleacaque (18°00′03.16″N, 94°37′46.90″W). The New Orleans strain was used as a susceptible reference strain.

Mosquitoes

Laboratory colonies were established from larvae collected from natural breeding sites and maintained at 25 ± 4°C and 12:12 (L:D). Upon pupation, they were placed in 250-ml flasks in cages (30×30 cm) until the adults emerged (parental generation). Adults were allowed to intermate. The male mosquitoes were fed a 10% sugar solution and the females were fed on rats (*Rattus norvegicus)* for the production of eggs, for which flasks with water, lined inside with filter paper, were provided. These eggs corresponded to the F_1 generation, which were use for all bioassays.

Bioassays

F_1 females 1–3 days of age were held without blood feeding for use in bioassays. The Brogdon and McAllister (1988) bottle bioassay was used in which a 250 mL Wheaton® bottle received a one mL solution of acetone containing technical grade insecticide (ChemService, West Chester, PA). The bottle was capped and shaken to insure uniform coverage and dried for an hour at room temperature. The insecticides tested were: *d*-(cis-trans)-phenothrin (6.3% cis, 91.7% trans), trans-permethrin (92% trans-6% cis), deltamethrin (99%), λ–cyhalothrin (99.1%), bifenthrin (99%), cypermethrin (99%), α-cypermethrin (99.5%), and *z*-cypermethrin (98%). Doses (μg/bottle) were predetermined that gave a range of knockdown rates and a 24h mortality rate from 0 – 99%. The number of different doses tested varied from 5 – 10, with three repetitions per dose and 20 females per repetition.

The numbers of knocked down mosquitoes were recorded every 10 min up to 1h. After 1h of exposure, all mosquitoes were gently transferred to a recovery container without insecticide and were offered a cotton ball soaked in a sugar solution. Mortality was recorded at 24 h. Both the bottles and recovery containers were kept at 24 ± 2 ° C and 70% RH.

Data analysis

Rates of kdr and 24 h recovery were analyzed by log-probit software (Raymond et al. 1985), according to Finney (1971). The Abbott (1925) correction formula for control mortality was applied. We determined the KC_{50} (concentration causing 50% knockdown) after one hour of exposure. The LC_{50} (concentration causing 50% mortality) was estimated from mortality data 24 h after recovery. The resistance ratio at 1 h of exposure (RR_{KC50}) was calculated by dividing the KC_{50} of a F_1 test population by the KC_{50} of the New Orleans reference susceptible strain. The RR_{LC50} was similarly calculated by dividing the LC_{50} of the test population by the LC_{50} of the New Orleans strain. The criteria of Mazzarri and Georghiou (1995) were used to classify RRs as high (> 10-fold), moderate (5- 10 fold), or low (< 5-fold). Linear regression was carried out between the RR_{LC50} vs RR_{KC50} for each insecticide in the seven strains. A R^2 was estimated to test the strength of the correlation. The slope is the ratio of the change in LC_{50} relative to the change in KC_{50} and indicates how much pesticide concentration should be increased to cause lethality vs. knockdown. A slope $\simeq 1$

suggests $RR_{LC50} \simeq RR_{KC50}$ or that most knocked down mosquitoes are dead 24 hours later. A slope > 1 suggests $RR_{LC50} > RR_{KC50}$ or that recovery occurs and more insecticide is required to kill knocked down mosquitoes.

Cluster and principal component analyses are multivariate statistical techniques for summarizing relationships among collections in which many quantitative variables (e.g. LC_{50} for eight pyrethroids) have been measured. All 56 LC_{50} values were entered into a 7 (strains) × 8 (insecticides) matrix. The Pearson's correlation coefficient (ρ) among LC_{50} values for each pesticide was calculated between each pair of collections to generate a symmetrical 8 × 8 correlation matrix. For cluster analysis, a second matrix of (1- ρ) values was calculated and subject to a UPGMA (Unweighted Pair Group Method with Arithmetic Mean - Sneath and Sokal, (1963)) cluster analysis using NEIGHBOR in PHYLIP3.6.7 (Felsenstein, 2005) to generate dendrograms indicating similarity among the eight pesticides.

Principal components were calculated as a linear combination of eight LC_{50} values multiplied by the eigenvector. There were 7 different principal components. The sum of the products of the eigenvectors and the LC_{50} values for each principal component is the eigenvalue. The eigenvalue is proportional to the magnitude of the general correlation in LC_{50} values among the 7 collections. If LC_{50} values for the eight pesticides covary among collections then the eigenvalue will be large but if LC_{50} values vary independently among collections then the eigenvalue will be small. The principal component with the largest eigenvector is defined as the first principal component while the principal component with the next largest eigenvector is defined as the second principal component. The first and second principal components should explain most of the covariance among collections in order to be useful. Bivariate plots of the first two principal components are useful in summarizing relationships among collections especially when they account for >70% of the covariance among LC_{50} values. Biplot analysis is a facet of principal components (Gabriel, 1971) that requires little additional computation and identifies the magnitude of variances and covariances among individual pesticides. Principal component and biplot analyses were performed on R version 2.11.1 (http://www.r-project.org/) using the prcomp and biplot functions.

There are three useful properties of biplots for analysis of insecticide resistance. First, the length of a biplot vector is proportional to the variance in LC_{50} values among and indicates how much a LC_{50} value varies among collections relative to other insecticides. Second, the cosine of the angle (in radians) between two biplot vectors equals the correlation in allele frequencies among collections at the two loci. If both vectors point in the same direction, then the angle between them approaches 0. The cosine of 0 radians is 1 which is also the correlation in LC_{50} values between those two insecticides. If both vectors form a right angle, cosine 90° ($\pi/2$ radians) = 0 indicating that LC_{50} values for two insecticides vary independently among collections. If vectors point in opposite directions, cosine 180° (π radians) = −1 indicating that LC_{50} values for two insecticides vary in opposite directions in collections. A third useful property of biplots is that they can be graphed alongside population principal components to identify which pesticides are causing specific patterns of differentiation among collections. All multivariate techniques were repeated for $KC_{50,}$ RR_{LC50}, and RR_{KC50} values.

Results

Knockdown resistance, RR_{KC50}

KC_{50} and RR_{KC50} values appear in Table 1. All strains showed >30 fold kdr to α-cypermethrin. Five strains showed from 7–62 fold resistance to *d*-phenothrin.

Coatzacoalcos, Poza Rica and Veracruz showed kdr with λ–cyalothrin. Moderate resistance was found in Cosoleacaque. Mosquitoes from Panuco, Tantoyuca and M. de la Torre were susceptible to λ–cyalothrin. Poza Rica and M. de la Torre exhibited 12–18 fold resistance to permethrin, while Coatzacoalcos, Veracruz and Panuco exhibited moderate resistance. Only Cosoloeacaque and Tantoyuca were susceptible to permethrin. Similar findings were obtained for cypermethrin, in which Poza Rica and M. de la Torre exhibited high resistance, and moderate resistance was noted in Coatzacoalcos and Cosoleacaque. Only Coatzacoalcos exhibited 10 fold resistance to z-cypermethrin, while moderate resistance was exhibited by Poza Rica and Tantoyuca. Panuco, M. de la Torre, Veracruz and Cosoleacaque were susceptible to *z*-cypermethrin. For bifenthrin, Veracruz mosquitoes exhibited 21 fold resistance, while moderate resistance was seen in Cosoleacaque. Panuco, Tantoyuca, Poza Rica and Coatzacoalcos, were susceptible with RR_{KC50} values ≤ 2. No strains exhibited resistance to deltamethrin, and only Coatzacoalcos, Veracruz and Tantoyuca displayed moderate resistance. Panuco, Tantoyuca, M. de la Torre and Cosoleacaque were susceptible to deltamethrin.

Surprisingly, high levels of resistance to α-cypermethrin were detected, with most strains exhibiting > 100 fold resistance. Moderate resistance (14–62 fold) was seen for *d*-phenothrin; for which two-thirds of the strains displayed resistance. Significant but much lower levels (17–24 fold) of resistance were detected for λ–cyalothrin in half of the strains. Resistance was even lower for permethrin in which a third of the strains showed kdr. Similar results were obtained for cypermethrin with two of the strains showing 11–15 fold greater values. For the pyrethroids z-cypermethrin and bifenthrin, only one strain demonstrated resistance (RR_{KC50} 10 – 21 fold, respectively). For deltamethrin, none of the strains had RR_{KC50} values >10, moderate resistance was found in three strains, while the rest were susceptible. RR_{KC50} is a useful statistic to describe the amount of kdr in a population. It would be interesting to relate the magnitude of the kdr with the frequency of Ile1,016 mutations. This could be very helpful when the necessary molecular tools are lacking, as the case in many countries in Latin America.

Resistance to 1 h exposure and 24 h post-recovery, RR_{LC50}

Mortality 24 h following an initial 1 hour insecticide exposure provides a means of implicating other, possibly metabolic, forms or resistance. Table 2 lists LC_{50} and resistance ratio (RR_{LC50}) beginning with the insecticides with the highest resistance values. Six strains exhibited resistance to α-cypermethrin, with RR_{LC50} values greater than 100 fold for five of the strains. Cosoleacaque was relatively susceptible to α-cypermethrin (4.5 fold). For λ–cyalothrin, Coatzacoalcos, Poza Rica, Veracruz and Cosoleacaque had RR_{LC50} values >10. Martinez de la Torre showed moderate resistance and Panuco and Tantoyuca and were susceptible. Poza Rica, Veracruz, Coatzacoalcos and M. de la Torre exhibited resistance to *d*-phenothrin, while Tantoyuca and Cosoleacaque were moderately resistant and Panuco and Tantoyuca were susceptible. Veracruz, Cosoleacaque and Poza Rica exhibited resistance to bifenthrin. Coatzacoalcos displayed moderate resistance and Panuco, Tantoyuca and M. de la Torre were susceptible. Coatzacoalcos was the most resistant to *z*-cypermethrin, followed by Poza Rica and Tantoyuca; Veracruz and M. de la Torre were moderately resistant and Panuco and Cosoleacaque were susceptible. In the case of deltamethrin, even though no strain showed kdr, recovery was observed at 24 h post-exposure in Coatzacoalcos, Veracruz and Poza Rica. Panuco, Tantoyuca and Cosoleacaque were susceptible. Poza Rica exhibited the greatest resistance to permethrin, followed by M. de la Torre and Veracruz. Coatzacoalcos and Tantoyuca were moderately resistant and Panuco and Cosoleacaque were susceptible to permethrin. Poza Rica and M. de la Torre were resistant to cypermethrin, while only Coatzacoalcos displayed moderate resistance and Panuco, Tantoyuca, Veracruz and Cosoleacaque were susceptible with RR_{LC50} <5.

The results of mortality at 24 h demonstrated that the majority of the pyrethroid insecticides showed recovery of mosquitoes with the exception of *d*-phenothrin. For α-cypermethrin and cypermethrin, the results for both resistance after 24 h and kdr were similar. For permethrin, 3 strains exhibited resistance at 24 h, and two exhibited kdr. Surprisingly, three strains showed resistance to deltamethrin at 24 h, the same strains that had moderate kdr of $RR_{KC50} \leq 5$. For z-cypermethrin, three strains showed resistance at 24 h, where one had already shown kdr and where two were moderately resistant. There was a large recovery rate in mosquitoes exposed to λ–cyalothrin.

Cluster analysis

Cluster analysis was performed on the LC_{50}, KC_{50}, RR_{LC50}, RR_{KC50} for each of the 8 compounds (Fig. 2). The resulting dendrograms for LC_{50} and $RRLC_{50}$ were almost identical so that only a single dendrogram is presented. The same was true for KC_{50} and $RR_{KC50.}$ The resistance profiles were highly correlated for deltamethrin and λ-cyhalothrin ($\rho = 0.924$), followed by permethrin with cypermethrin ($\rho = 0.911$) and *d*-phenothrin with α-cypermethrin ($\rho = 0.796$). The resistance statistics for bifenthrin were not correlated with any of the other 7 compounds.

Principal component and biplot analyses were performed on the LC_{50}, KC_{50}, RR_{LC50}, and RR_{KC50} values. All four plots were extremely similar and so only the results for RR_{KC50} are shown (Fig. 3). The first and second principal components accounted for 58.9 and 23.4% (cumulatively 82.3%) of the covariance among LC_{50} values thus representing a good summary of resistance profiles among collections. The distribution of population principal components suggests that the first principal component represents overall resistance. Tantoyuca, Cosoleacaque, Panuco and M. de la Torre were more susceptible in general to all pyrethroids (except bifenthrin) compared with Veracruz, Coatzacoalcos and Poza Rica. The second principal component represents resistance to bifenthrin. Veracruz displayed 21 fold resistance. The remainder exhibited moderate resistance or were susceptible. The biplot vectors for all pyrethroids, except bifenthrin, point in a similar direction. As noted in the cluster analysis, note that biplots vector are very similar for deltamethrin and λ-cyhalothrin, permethrin and cypermethrin and for *d*-phenothrin and α-cypermethrin.

Regression analysis

Recovery 24 h after exposure provides evidence for additive, possibly metabolic resistance mechanisms. Regression analysis between RR_{LC50} and RR_{KC50} are shown in Fig. 4. For *d*-phenothrin $R^2 = 0.93$ with a slope of 0.875. This indicates that the majority of mosquitoes knocked down with *d*-phenothrin did not recover. In contrast for permethrin $R^2 = 0.96$ with a slope of 1.91. This indicates that approximately twice the concentration of permethrin sufficient to knockdown mosquitoes is required to eventually kill those mosquitoes. A similar pattern was seen for the other pyrethoids except for λ–cyalothrin where the slope was 8.67, indicating that approximately 9 fold higher concentration of λ–cyalothrin is necessary to eventually kill knocked down mosquitoes. However, this interpretation should be validated in laboratory bioassays with increased concentrations of permethrin and λ–cyalothrin.

Discussion

In Mexico, as in the majority of Latin American countries, various insecticides have been used for vector control. For example DDT, was used extensively in 1960s for the control of malaria, and was eventually followed by the use of organophosphate insecticides such as malathion (adulticide) and temephos (larvicide). For *Ae. aegypti* control, malathion was used until 1989 in spatial applications, and temephos granules were applied to water containers as potential breeding sites for *Ae. aegypti* larvae (Fernandez 1999). The use of temephos has been uninterrupted up to the present. Numerous reports have indicated that DDT has conferred cross resistance to pyrethroids (Hemingway et al 1989). In areas such as the Caribbean and South America, there are reports of resistance to pyrethroids in populations of *Ae. aegypti* in Venezuela (Field et al. 1984, Mazarri and Georgiou 1995), Puerto Rico (Hemingway et al. 1989) and Dominican Republic (Mekuria et al. 1991) associated with cross-resistance to DDT. Reports have shown that resistance associated with pyrethroids is due not only to mutations in the voltage-gated sodium channel gene, but also to enzymatic mechanisms (Brengues et al. 2003, Rodriguez et al. 2005). Similarly, there are numerous reports indicating that the prolonged use of temephos for more than 30 years has produced resistance in *Ae. aegypti* populations (Paeporn et al. 2003, Bisett et al. 2004, Braga et al. 2004, Rodriguez et al. 2004, Saelim et al. 2005, Montella et al. 2007). In Mexico cross-resistance to permethrin is mediated in part by esterases (Flores et al. 2005, 2006).

An unusual aspect of the present study was the comparison of RR_{LC50} and RR_{KC50} values. This aspect was studied following the observation that many of the mosquitoes that were knocked down following permethrin exposure in the bottle bioassay recovered when removed to the insectary away from the pesticide. With the exception of *d*-phenothrin, from 2–9 fold greater amounts of pyrethroids were required to eventually kill exposed mosquitoes. However, we are uncertain about the relevancy of these results to the field. Assuming a knocked down mosquito falls into an insecticide free environment, it seems to us very likely that the mosquito will die due to desiccation, predation or a greater likelihood of being crushed or damaged. However our results suggest that at least a proportion of knocked down mosquitoes may recover from their initial exposure. This may be especially important with the Ile1,016 resistance allele in which Saavedra-Rodriguez et al. (2007) observed that 90.6% (221/244) of Ile1,016/Val1,016 heterozygotes were knocked down after a 1 hour exposure to permethrin but that 57.4% (127/221) of knocked down heterozygotes eventually recovered. Thus the rate of recovery following knockdown is inherited as an additive genetic trait with heterozygotes exhibiting a intermediate phenotype. The rate of response to selection is predicted to be much more rapid for a trait with an additive genetic inheritance than for a trait with a strictly recessive inheritance (e.g. kdr). This may explain why Ile1,016 has spread so rapidly in Mexico (Ponce et al. 2009; Barbosa et al 2011).

Mosquitoes are capable of developing resistance to the majority of insecticides used for their control, such that the established effective dose suddenly fails. Generally, the immediate response to control failure is to increase the frequency of treatment or the concentration of the insecticide, thus aggravating the problem further. Instead a rational strategy for managing resistance requires understanding of the actual physiological, biochemical and genetic mechanisms involved. Early detection as a preventive method should be complemented with a strategy of rotation of active insecticides which permits the utilization of an alternative compound preferably with a different mode of action so that resistance does not continue to increase. The development of new formulations that reduce resistance or potentiate the action of the insecticide (Curtis et al. 1993, Roush 1989) would also be applicable. These strategies require constant monitoring of populations exposed to these compounds to predict increases in the resistance. Pyrethroid resistance is clearly increasing despite the initial optimism over their rapid action and novelty (Malcom, 1988). Evidence of resistance to permethrin in *Ae. aegypti* populations in Mexico due to enzymatic mechanisms (Flores et al, 2005, 2006, 2009) and target-site insensitivity (kdr) (Saavedra et al. 2007, 2008; Ponce et al. 2009) suggest that the widespread use of permethrin has conferred cross resistance to many other pyrethroids including those not commonly used in mosquito control in Mexico.

The analyses presented here show that the field strains from the state of Veracruz are resistant to most of the pyrethroids analyzed. Even though RR_{KC50} were < 10 fold (no knockdown resistance present) for deltamethrin, treated mosquitoes in three strains recovered after 24h. The results suggest that populations in the state of Veracruz have been exposed to strong selection pressure, resulting from the continuous application permethrin for more than 9 years, together with the effect of insecticides applied for the control of agricultural pests and other urban pests. In light of the prevalence of cross resistance to pyrethroid in Veracruz state, it is pertinent to ask if pyrethroids should continue to be insecticides of first choice in Mexico for controlling *Ae. aegypti* populations.

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References cited

Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925; 8:265–267.

Barbosa S, Black WC, Hastings A. Challenges in Estimating Insecticide Selection Pressures from Mosquito Field Data. PLoS Neglected Tropical Diseases. 2011; 5:e1387. [PubMed: 22069506]

Bisset JA, Rodríguez MM, Fernandez D, Perez O. Estado de la resistencia a insecticidas y mecanismos de resistencia en larvas del municipio Playa, colectadas durante la etapa intensiva contra el *Aedes aegypti* en Ciudad de La Habana, 2001–2002. Rev Cub Med Trop. 2004; 56:61–66.

Boletin de Epidemiologia. Sistema Nacional de Vigilancia Epidemiologica. Vol. 24. Secretaria de Salud; Mexico: 2007.

Boletin de Epidemiologia. Sistema Nacional de Vigilancia Epidemiologica. Vol. 25. Secretaria de Salud; Mexico: 2008.

Boletin de Epidemiologia. Sistema Nacional de Vigilancia Epidemiologica. Vol. 26. Secretaria de Salud; Mexico: 2009.

Braga IA, Lima JBP, Soares SS, Valle D. *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the States of Rio de Janeiro, Sergipe, and Alagoas, Brazil. Memórias do Instituto Oswaldo Cruz. 2004; 99:199–203. [PubMed: 15250476]

Brengues C, Hawkes NJ, Chandre F, McCarroll L, Duchon S, Guillet P, Manguin S, Morgan JC, Hemingway J. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. Med Vet Entomol. 2003; 17:87–94. [PubMed: 12680930]

Brogdon WG, McAllister JC. Simplification of adult mosquito bioassays through use of time-mortality determinations in bottles. J Am Mosq Control Assoc. 1998; 14:159–164. [PubMed: 9673916]

Curtis CF. Theoretical models of the use of insecticide mixtures for the management of resistance. Bull Entomol Res. 1985; 75:259–265.

Curtis CF, Hill N, Kasim SH. Are there effective resistance management strategies for vectors of human disease? Biol J Linn Soc. 1993; 48:3–18.

Felsenstein, J. PHYLIP (Phylogeny Inference Package) version 3.6. Department of Genome Sciences, University of Washington; Seattle: 2005. Distributed by the author

Fernandez, SI. Manual de operaciones. Monterrey, Mexico: 1999. Biología y control de *Aedes aegypti*. Editorial UANL

Field WN, Hitchen JM, Rees AT. Esterase activity in strains of *Aedes aegypti* (Diptera: Culicidae) tolerant and susceptible to the organophosphate insecticide malathion. J Med Entomol. 1984; 21:412–418. [PubMed: 6492084]

Finney, DJ. Probit Analysis. 3. Cambridge University; 1971. p. 50-80.

Flores AE V, Albeldaño W, Fernandez SI, Badii MH, Loaiza H, Ponce GG, Lozano FS, Brogdon WG, Black WC, Beaty BJ. Elevated α-esterase levels associated with permethrin tolerance in *Aedes aegypti* (L.) from Baja California, Mexico. Pesticide Biochemistry and Physiology. 2005; 82:66–78.

Flores AE, Grajales JS, Fernandez SI, Ponce GG, Loaiza H, Badii MH, Lozano FS, Brogdon WG, Black WC, Beaty BJ. Mechanisms of insecticide resistance in field populations of *Aedes aegypti* (L.) from Quintana Roo, Southern Mexico. J Am Mosq Control Assoc. 2006; 22:672–677. [PubMed: 17304936]

Flores AE, Reyes G, Fernandez SI, Sanchez RFJ, Ponce GG. Resistance to permethrin in *Aedes aegypti* (L.) in northern Mexico. Southwestern Entomologist. 2009; 34:167–177.

Gabriel KR. The biplot graphic display of matrices with application to principal component analysis. Biometrika. 1971; 58:453–467.

Hemingway J, Boddington RG, Harris J. Mechanisms of insecticide resistance in *Aedes aegypti* (L.) (Díptera: Culicidae) from Puerto Rico. Bull Ent Res. 1989; 79:123–30.

Hemingway J, Penilla RP, Rodriguez AD, James BM, Edge W. Resistance management strategies in malaria vector mosquito control. A large scale trial in Southern Mexico Pest Sci. 1997; 51:375–382.

Malcom CA. Current status of pyrethroid resistance in anophelines. Parasitology Today. 1988; 4:S13–S6. [PubMed: 15463083]

Mazzarri MB, Georghiou GP. Characterization of resistance to organophosphate, carbamate, and pyrethroid insecticides in field population of *Aedes aegypti* from Venezuela. J Am Mosq Control Assoc. 1995; 11:315–322. [PubMed: 8551300]

Mekurian Y, Gwinn TA, Williams DC, Tidwell MA. Insecticide susceptibility of *Aedes aegypti* from Santo Domingo. Dominical Republic J Am Mosq Control Assoc. 1991; 7:69–72.

Montella I, Martins A, Fernandez P, Pereira J, Braga I, Valle D. Insecticide Resistance Mechanisms of Brazilian Aedes aegypti populations from 2001 to 2004. Am J Trop Med Hyg. 2007; 77:467–477. [PubMed: 17827362]

Norma Oficial Mexicana NOM-032-SSA-2-2002 para la vigilancia epidemiologica, prevencion y control de enfermedades transmitidas por vector (Diario Oficial de la Federacion, julio 2003) Mexico.

Norma Oficial Mexicana NOM-EM-003-SSA2-2010 para la vigilancia epidemiologica, prevención y control de enfermedades transmitidas por vector (Diario Oficial de la Federacion, junio 2011) Mexico.

Paeporn P, Komalamisra N, Deesin V, Rongsriyam Y, Eshita Y, Thongrungkiat S. Temephos resistance in two forms of *Aedes aegypti* and its significance for the resistance mechanism. Southeast Asian J Trop Med Public Health. 2003; 34:786–92. [PubMed: 15115088]

Penilla RP, Rodríguez AD, Hemingway J, Torres JL, Arredondo JI, Rodríguez MH. Resistance management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against Anopheles albimanus in Mexico. Med Vet Entomol. 1998; 12:217–233. [PubMed: 9737593]

Ponce G, Flores A, Fernández I, Saavedra K, Reyes G, Lozano S, Bond J, Casas M, Ramsey J, García J, Domínguez M, Ranson H, Hemingway J, Eisen L, Black LWC IV. Recent rapid rise of a permethrin knock down resistance allele in *Aedes aegypti* in México. PLOS Neglected Tropical Diseases. 2009; 3:531–560.

Raymond M. Presentation d' un programme d'analyse log-probit pour microordinateur cahiers Orstrom Ser Entomo. Med Parasitol. 1985; 23:117–121.

Rodriguez MM, Bisset JA, Fernandez D, Perez O. Resistencia a insecticidas en larvas y adultos de *Aedes aegypti* : prevalencia de la esterasa A4 asociada con la resistencia a temefos. Rev Cubana Med Trop. 2004; 56:54–60. [PubMed: 15849910]

Rodriguez MM, Bisset JA, De Armas Y, Ramos F. Pyrethroid insecticide-resistant strain of *Aedes aegypti* from Cuba induced by deltamethrin selection. J Am Mosq Control Assoc. 2005; 21:437–445. [PubMed: 16506569]

Roush RT. Designing resistance management programmes: how can you choose? Pestic Sci. 1989; 26:423–42.

Saavedra K, Urdaneta L, Rajatileka S, Moulton M, Flores AE, Fernandez I, Bisset J, Rodriguez M, Mccall PJ, Donnelly MJ, Ranson H, Hemingway J, Black WC IV. Mutations in the voltage gated sodium channel gene associated with pyrethroid resistance in Latin American Aedes aegypti. Insect Molecular Biology. 2007; 16:785–798. [PubMed: 18093007]

Saavedra K, Strode C, Flores A, Fernandez I, Ranson H, Hemingway J, Black WC IV. QTL mapping of genome regions controlling permethrin resistance in the mosquito Aedes aegypti. Genetics. 2008; 180:1137–1152. [PubMed: 18723882]

Saelim V, Brogdon WG, Rojanapremsuk J, Suvannadaba S, Pandii W, Jones JW, Sithiprasasna R. Bottle and biochemical assays on temephos resistance in *Aedes aegypti* in Thailand. Southeast Asian J Trop Med Public Health. 2005; 36:417–425. [PubMed: 15916049]

Siller Q, Ponce G, Lozano S, Flores AE. Update on the frequency of Ilde1016 mutation in voltage-gated channel gene of *Aedes aegypti* in Mexico. J Am Mosq Control Assoc. 2011; 27:357–362. [PubMed: 22329266]

Sneath, PHE.; Sokal, RR. Principles of Numerical Taxonomy. Freeman & Co; San Francisco: 1963.

Tabashnik BE. Managing resistance with multiple pesticide tactics: theory, evidence and recommendation. J Econ Entomol. 1989; 82:1263–1269. [PubMed: 2689487]

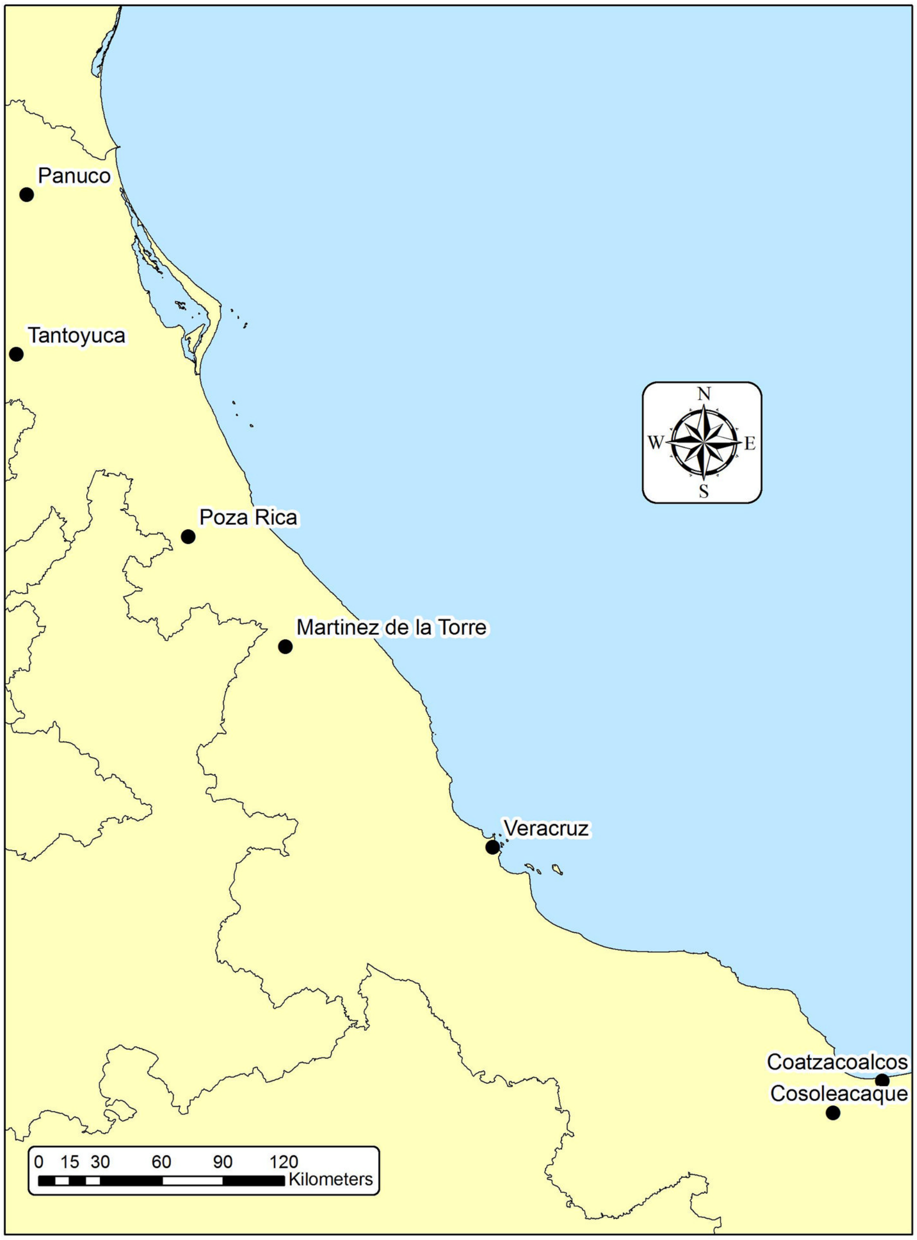

Figure 1.

Map of the seven field populations of *Ae. aegypti* collected from state of Veracruz, Mexico.

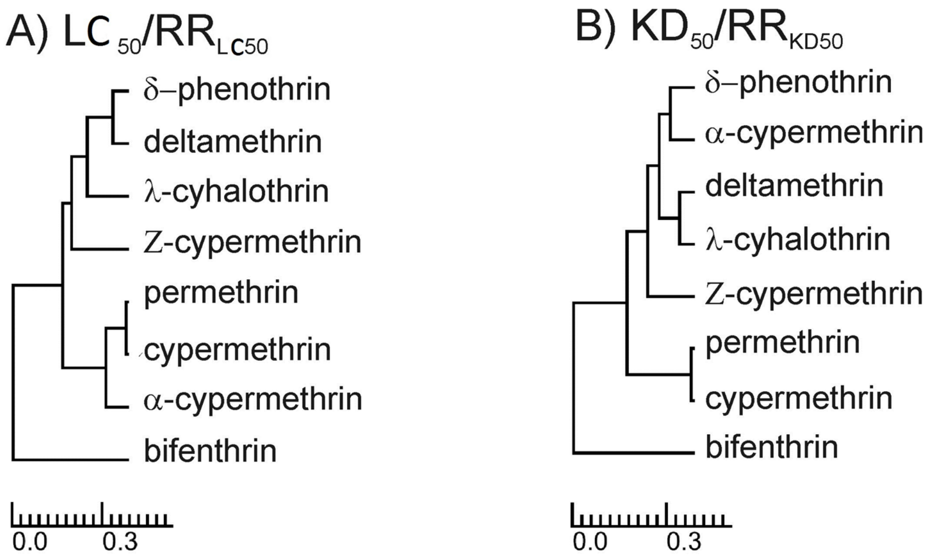

Figure 2.

UPGMA dendrograms arising cluster analysis of one minus Pearson correlation coefficient for LC_{50}, $KD_{50,}$ RR_{LC50}, $RR_{KD50,}$for each of the eight pyrethroids.

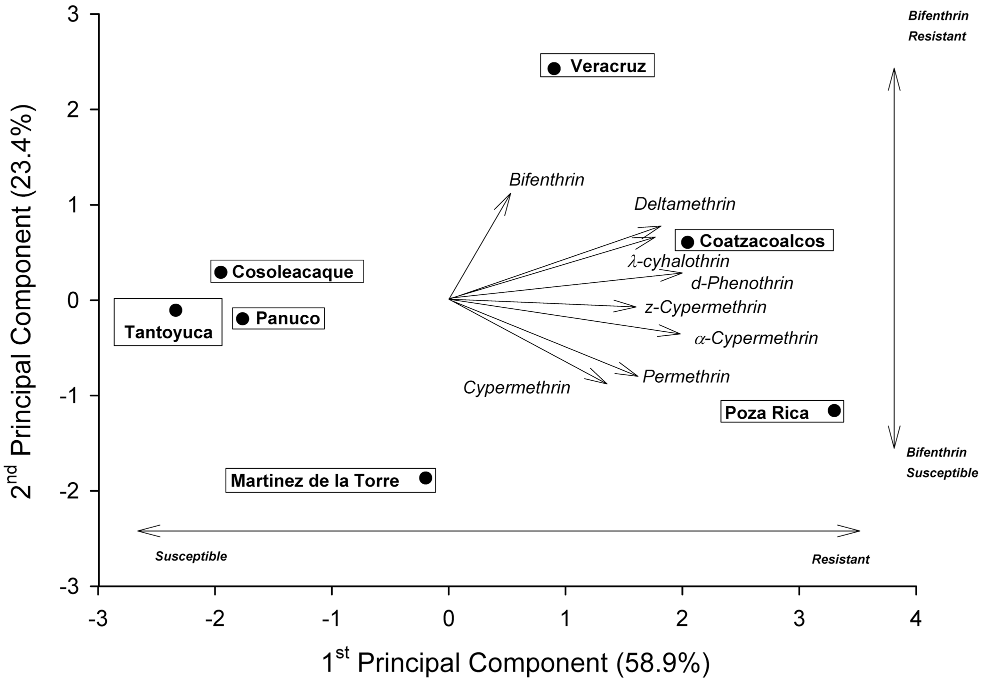

Figure 3.

Biplot analyses of RR_{KD50} values. The first and second principal components are represented as solid dots for each of the seven strains. Biplots are represent as arrows for each of the eight pyrethroids.

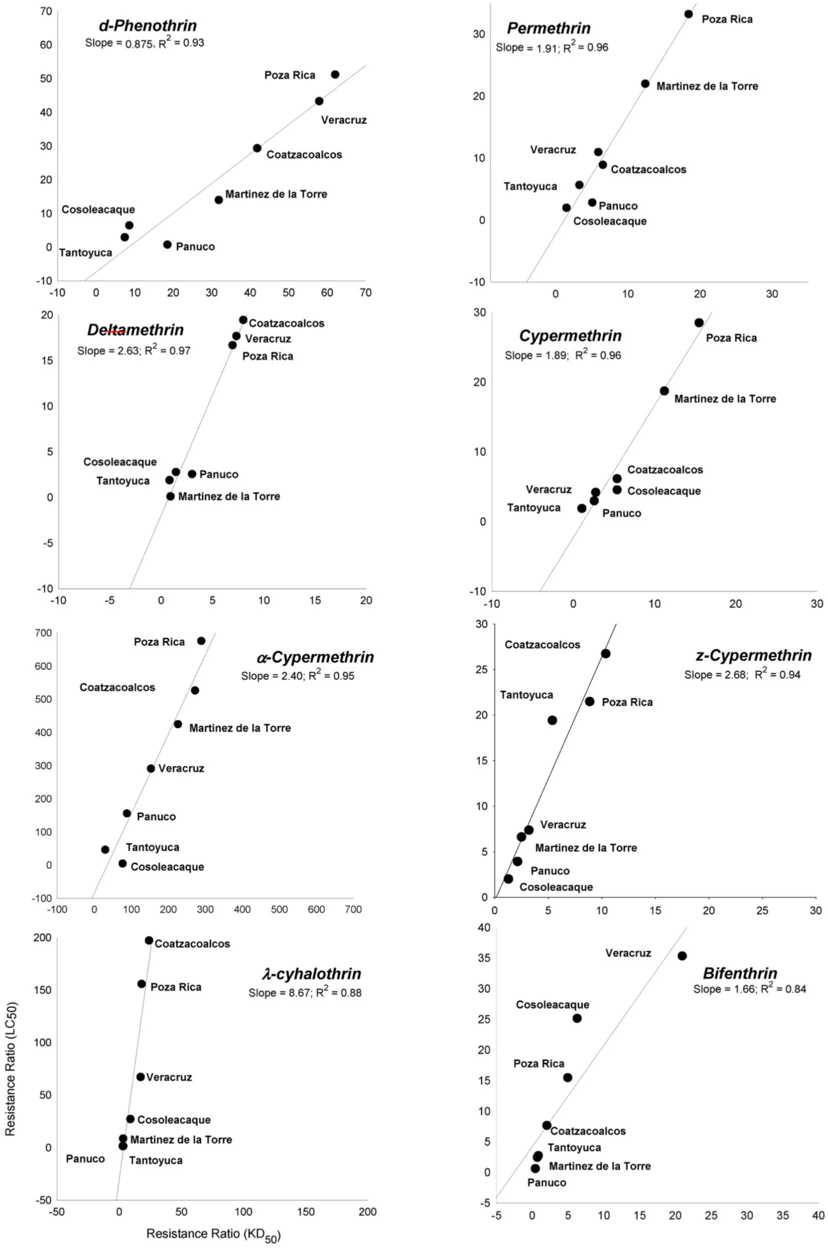

Figure 4.

Regression analysis between RR_{LC50} and RR_{KD50}. The proportion of the total variance accounted for by the linear model is $R^{2.}$ The estimated slope is also provided.

Table 1

Knockdown concentration (KC_{50}) and resistance ratio (RR_{KC50}) of 8 pyrethroids against *Aedes aegypti* females from Veracruz, Mexico.

STRAIN	***d*-PHENOTHRIN**	**PERMETHRIN**	**DELTAMETHRIN**	**CYPERMETHRIN**	**α-CYPERMETHRIN**	**Z-CYPERMETHRIN**	**λ–CYHALOTHRIN**	**BIFENTHRIN**
Panuco	421[1]	413	311	342	359	294	480	480
KC_{50} [2]	8.13 (4.67–14.68)	2.28(1.71–2.96)	0.063(0.046–0.089)	0.263(0.190–0.368)	0.141(0.113–0.177)	0.223(0.180–0.282)	0.014(0.010–0.019)	0.09(0.060–0.140)
RR_{KC50} [3]	18.48	5.07	3.00	2.58	88.12	2.14	2.76	0.43
b (± SE)[4]	0.533 (0.068)	1.179 (0.121)	1.139 (0.123)	1.063 (0.111)	1.534 (0.158)	1.792 (0.177)	0.905 (0.077)	0.629 (0.062)
x^2 (df)[5]	10.64 (4)	12.32 (3)	0.68 (3)	8.08 (4)	25.35 (4)	6.05 (3)	7.21 (6)	8.78 (6)
Tantoyuca	720	660	483	343	404	345	270	450
KC_{50}	3.26(2.53–4.16)	1.48(1.27–1.70)	0.017(0.015–0.020)	0.108(0.076–0.148)	0.047(0.032–0.070)	0.561(0.432–0.727)	0.016(0.009–0.029)	0.18(0.140–0.230)
RR_{KC50}	7.41	3.29	0.81	1.06	29.37	5.39	3.24	0.86
b (± SE)	0.998 (0.066)	1.889 (0.143)	1.992 (0.164)	1.045 (0.120)	0.807 (0.087)	1.338 (0.140)	0.484 (0.068)	1.540 (0.191)
x^2 (df)	8.32 (10)	15.17 (9)	7.07 (6)	1.36 (4)	18.03 (5)	14.15 (4)	5.72 (5)	8.35 (4)
Poza Rica	664	600	299	325	339	318	480	405
KC_{50}	27.29(24.54–29.96)	8.29(7.15–9.57)	0.146(0.130–0.160)	1.580(1.410–1.762)	0.462(0.385–0.560)	0.923(0.790–1.062)	0.090(0.070–0.120)	1.04(0.900–1.200)
RR_{KC50}	62.02	18.42	6.95	15.49	288.75	8.87	18.00	4.95
b (± SE)	2.678 (0.226)	1.801 (0.144)	3.487 (0.325)	3.247 (0.350)	2.009 (0.184)	2.640 (0.268)	1.332 (0.129)	2.132 (0.180)
x^2 (df)	67.46 (9)	8.27 (8)	0.021 (3)	22.52 (4)	1.94 (4)	9.42 (4)	11.62 (7)	9.22 (8)
M.de la Torre	300	300	379	418	287	341	369	517
KC_{50}	14.01(10.60–18.30)	5.59(4.61–6.58)	0.019 (0.014–0.026)	1.145(1.00–1.294)	0.361(0.307–0.428)	0.260(0.199–0.339)	0.016(0.014–0.019)	0.153(0.127–0.187)
RR_{KC50}	31.84	12.42	0.90	11.22	225.62	2.5	3.20	0.73
b (± SE)	1.461 (0.142)	2.258 (0.270)	1.112 (0.112)	2.824 (0.245)	2.319 (0.270)	1.405 (0.128)	1.749 (0.144)	1.810 (0.175)
x^2 (df)	22.62 (3)	12.68 (3)	19.03 (4)	17.88 (6)	16.27 (3)	6.09 (4)	6.29 (6)	15.81 (4)
Veracruz	353	430	278	285	337	286	390	315
KC_{50}	25.47(21.70–29.80)	2.66(2.23–3.14)	0.154(0.132–0.177)	0.281(0.231–0.339)	0.245(0.204–0.297)	0.333(0.259–0.426)	0.086(0.060–0.120)	4.40 (3.870–4.970)
RR_{KC50}	57.89	5.91	7.33	2.75	153.12	3.20	17.20	20.95
b (± SE)	2.293 (0.220)	1.913 (0.164)	2.767 (0.291)	2.108 (0.214)	1.904 (0.186)	1.557 (0.167)	1.159 (0.118)	2.814 (0.266)
x^2 (df)	15.10 (4)	4.18 (5)	5.35 (3)	5.10 (3)	9.80 (4)	8.38 (3)	7.38 (5)	20.10 (6)
Coatzacoalcos	380	361	302	370	334	283	225	270

STRAIN	**_d_-PHENOTHRIN**	**PERMETHRIN**	**DELTAMETHRIN**	**CYPERMETHRIN**	**α-CYPERMETHRIN**	**Z-CYPERMETHRIN**	**λ–CYHALOTHRIN**	**BIFENTHRIN**
KC_{50}	18.40(15.84–21.19)	2.94(2.48–3.45)	0.168(0.149–0.189)	0.550(0.447–0.662)	0.435(0.344–0.562)	1.078(0.937–1.232)	0.120(0.080–0.180)	0.43(0.310–0.600)
RR_{KC50}	41.82	6.53	8.00	5.39	271.87	10.36	24.00	2.05
b (± SE)	2.395 (0.229)	2.192 (0.198)	3.236 (0.318)	2.105 (0.179)	1.524 (0.147)	3.108 (0.387)	1.005 (0.117)	1.306 (0.168)
x^2 (df)	6.62 (4)	11.22 (4)	7.51 (3)	16.09 (5)	10.82 (4)	33.15 (3)	6.40 (4)	15.96 (3)
Cosoleacaque	737	481	293	447	364	246	360	360
KC_{50}	3.78(3.05–4.46)	0.68(0.57–0.80)	0.030(0.023–0.038)	0.256(0.198–0.319)	0.122(0.089–0.167)	0.203.(0.157–0.254)	0.045(0.030–0.060)	1.32(1.060–1.660)
RR_{KC50}	8.59	1.51	1.43	5.39	76.25	1.29	9.00	6.29
b (± SE)	1.224 (0.086)	1.693 (0.154)	1.582 (0.181)	1.537 (0.130)	1.102 (0.102)	1.806 (0.207)	0.896 (0.087)	1.498 (0.174)
x^2 (df)	9.10 (10)	5.77 (6)	15.62 (3)	14.78 (6)	8.00 (4)	3.43 (2)	21.63 (4)	19.51 (4)
New Orleans	287	300	340	375	426	370	360	360
KC_{50}	0.44(0.38–0.49)	0.45(0.37–0.51)	0.021(0.017–0.024)	0.102(0.084–0.122)	0.0016(0.0013–0.0018)	0.104(0.081–0.135)	0.005(0.0002–0.001)	0.21(0.170–0.280)
b (± SE)	3.018 (0.327)	2.787 (0.330)	2.798 (0.298)	1.860 (0.174)	1.994 (0.167)	1.342 (0.125)	0.998 (0.103)	1.446 (0.140)
x^2 (df)	10.97 (3)	13.51 (3)	10.14 (4)	19.55 (4)	15.18 (5)	1.18 (4)	15.00 (4)	45.17 (4)

[1] Number of females tested,

[2] KC_{50}, 50% knockdown concentration in μg/bottle, 95% confidence interval in parentheses,

[3] RR_{KC50}, resistance ratio: KC_{50} resistant strain/KC_{50} New Orleans strain,

[4] Slope of regression line Probit-log, standard error (± SE) are in parentheses,

[5] Chi-square (degree of freedom).

Table 2

Toxicity (LC$_{50}$) and resistance ratio (RR$_{LC50}$) of 8 pyrethroids against *Aedes aegypti* females from Veracruz, Mexico

STRAIN	*d*-PHENOTHRIN	PERMETHRIN	DELTAMETHRIN	CYPERMETHRIN	α-CYPERMETHRIN	Z-CYPERMETHRIN	λ–CYHALOTHRIN	BIFENTHRIN
Panuco	421[1]	413	311	342	359	294	480	480
LC$_{50}$[2]	0.24(0.13–0.41)	0.62(0.43–0.83)	0.023(0.016–0.031)	0.196(0.129–0.299)	0.171(0.135–0.222)	0.200(0.158–0.258)	0.006(0.003–0.114)	0.033(0.019–0.056)
RR$_{LC50}$[3]	0.71	2.82	2.56	2.97	155.45	3.92	1.43	0.61
b (± SE) [4]	0.797 (0.070)	1.289 (0.115)	1.100 (0.125)	0.801 (0.099)	1.407 (0.157)	1.568 (0.167)	0.469 (0.065)	0.526 (0.059)
x^2 (df)[5]	6.70 (4)	4.62 (3)	7.45 (3)	9.98 (4)	11.92 (4)	8.29 (3)	26.43 (6)	5.68 (6)
Tantoyuca	720	660	483	343	404	345	270	450
LC$_{50}$	0.98(0.68–1.34)	1.24(1.05–1.44)	0.017(0.014–0.018)	0.124(0.085–0.177)	0.051(0.032–0.0825)	0.990(0.784–1.284)	0.001(0.0004–0.002)	0.147(0.113–0.188)
RR$_{LC50}$	2.88	5.64	1.89	1.88	46.36	19.41	1.43	2.72
b (± SE)	0.814 (0.061)	1.727 (0.131)	2.141 (0.171)	0.921 (0.116)	0.651 (0.083)	1.519 (0.167)	0.344 (0.064)	1.540 (0.192)
x^2 (df)	11.96 (10)	28.79 (9)	7.13 (6)	6.99 (4)	12.55 (5)	24.65 (4)	3.60 (5)	6.91 (4)
Poza Rica	664	600	299	325	339	318	480	405
LC$_{50}$	17.40(15.16–19.64)	7.31(4.78–10.71)	0.150(0.130–0.170)	1.881(1.502–2.514)	0.743(0.595–0.972)	1.096(0.882–1.366)	0.109(0.081–0.143)	0.835(0.737–0.947)
RR$_{LC50}$	51.18	33.23	16.67	28.50	675.45	21.49	155.71	15.46
b (± SE)	2.602 (0.206)	2.105 (0.157)	2.594 (0.268)	1.436 (0.282)	1.674 (0.179)	1.598 (0.218)	1.129 (0.120)	2.521 (0.193)
x^2 (df)	23.07 (9)	47.30 (8)	9.61 (3)	29.98 (4)	9.61 (4)	6.30 (4)	15.32 (7)	37.92 (8)
M.de la Torre	300	300	379	418	287	341	369	517
LC$_{50}$	4.74(3.39–6.37)	4.84(3.94–5.74)	0.001(0.0004–0.002)	1.234(1.055–1.444)	0.467(0.373–0.624)	0.337(0.262–0.434)	0.006(0.0042–0.0074)	0.131(0.110–0.158)
RR$_{LC50}$	13.94	22.00	0.11	18.70	424.54	6.61	8.57	2.43
b (± SE)	1.378 (0.130)	2.223 (0.257)	0.521 (0.085)	2.073 (0.193)	1.604 (0.240)	1.528 (0.137)	1.104 (0.116)	1.903 (0.187)
x^2 (df)	16.54 (3)	10.58 (3)	23.09 (4)	36.98 (6)	10.80 (3)	35.25 (4)	18.82 (6)	18.01 (4)
Veracruz	353	430	278	285	337	286	390	315
LC$_{50}$	14.72(12.22–17.39)	2.41(2.03–2.83)	0.159(0.126–0.197)	0.276(0.227–0.333)	0.320(0.259–0.408)	0.376(0.267–0.538)	0.047(0.029–0.073)	1.908(1.508–2027)
RR$_{LC50}$	43.29	10.95	17.67	4.18	290.91	7.37	67.14	35.33
b (± SE)	2.270 (0.211)	2.003 (0.166)	1.703 (0.242)	2.112 (0.214)	1.612 (0.178)	1.056 (0.151)	0.776 (0.104)	2.521 (0.279)
x^2 (df)	6.26 (4)	2.98 (5)	16.09 (3)	7.73 (3)	7.63 (4)	9.38 (3)	28.51 (5)	37.09 (6)
Coatzacoalcos	380	361	302	370	334	283	225	270

STRAIN	***d*-PHENOTHRIN**	**PERMETHRIN**	**DELTAMETHRIN**	**CYPERMETHRIN**	**α-CYPERMETHRIN**	**Z-CYPERMETHRIN**	**λ–CYHALOTHRIN**	**BIFENTHRIN**
LC_{50}	9.97(8.16–11.82)	1.96(1.64–2.30)	0.175(0.156–0.195)	0.405(0.315–0.509)	0.579(0.448–0.776)	1.364(1.216–1.545)	0.138(0.084–0.243)	0.413(0.281–0.608)
RR_{LC50}	29.32	8.91	19.44	6.14	526.36	26.74	197.14	7.65
b (± SE)	2.110 (0.201)	2.137 (0.191)	3.528 (0.331)	1.538 (0.135)	1.419 (0.148)	3.734 (0.502)	0.758 (0.108)	1.100 (0.160)
x^2 (df)	12.03 (4)	26.54 (4)	4.11 (3)	21.74 (5)	10.78 (4)	6.34 (3)	9.35 (4)	20.89 (3)
Cosoleacaque	737	481	293	447	364	246	360	360
LC_{50}	2.18(1.68–2.77)	0.43(0.35–0.51)	0.025(0.019–0.033)	0.155(0.108–0.207)	0.005(0.0008–0.014)	0.102(0.066–0.140)	0.019(0.010–0.033)	1.358(1.082–1.722)
RR_{LC50}	6.41	1.95	2.78	2.35	4.54	2.00	27.14	25.15
b (± SE)	1.063 (0.078)	1.749 (0.162)	1.380 (0.175)	1.243 (0.121)	0.447 (0.088)	1.422 (0.194)	0.588 (0.081)	1.424 (0.168)
x^2 (df)	10.96 (10)	7.14 (6)	20.68 (3)	41.69 (6)	20.05 (4)	0.189 (2)	44.37 (4)	22.66 (4)
New Orleans	287	300	340	375	426	370	360	360
LC_{50}	0.34(0.28–0.39)	0.22(0.15–0.27)	0.009(0.003–0.015)	0.066(0.054–0.081)	0.0011(0.0009–0.0013)	0.051(0.041–0.064)	0.0007(0.0002–0.001)	0.054(0.040–0.071)
b (± SE)	2.521 (0.277)	1.737 (0.226)	1.616 (0.173)	1.771 (0.169)	2.403 (0.194)	1.612 (0.150)	0.494 (0.091)	1.378 (0.134)
x^2 (df)[5]	12.87 (3)	17.62 (3)	9.87 (4)	30.55 (4)	43.11 (5)	44.65 (4)	31.02 (4)	21.83 (4)

[1] Number of females tested,

[2] LC_{50}, 50% lethal dose in μg/bottle, 95% confidence interval in parentheses,

[3] RR_{LC50}, resistance ratio: LC_{50} resistant strain/LC_{50} New Orleans strain,

[4] Slope of regression line Probit-log, standard error (± SE) are in parentheses,

[5] Chi-square (degree of freedom).