Susceptibility Status to Temephos in Larval Aedes aegypti and Aedes albopictus (Diptera: Culicidae) Populations from Quintana Roo, Southeastern Mexico

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Source: Florida Entomologist, 105(3) : 255-257

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.105.0312

Susceptibility status to temephos in larval *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) populations from Quintana Roo, southeastern Mexico

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Aedes aegypti (L.) (Diptera: Culicidae) has a wide distribution in tropical and subtropical regions of Mexico, covering most of the states. In addition, *Aedes albopictus* (Skuse) (Diptera: Culicidae) is an invasive species initially discovered in northern Mexico (Francy et al. 1990), with a distribution that now includes 17 states (Moo-Llanes et al. 2021). Both species are vectors of the dengue virus and have adapted to urban areas, feeding almost exclusively on humans. The lack of effective medication and vaccines to combat this disease has caused control efforts to rely heavily on chemical applications to reduce vector abundance (Maciel-de-Freitas et al. 2014; Deming et al. 2016). However, continual exposure of a species to insecticides can favor genetic mutations that confer resistance to the chemicals used for its control. This factor is a significant concern for controlling dengue vectors because resistance has arisen already to carbamates, pyrethroids, organochlorides, and organophosphates in other areas of the world (Ranson et al. 2010).

For several yr, the organophosphate insecticide temephos has been used widely in Mexico for vector control. However, prolonged exposure to this compound has caused resistance in *Ae. aegypti* and *Ae. albopictus* larvae targeted by such applications (Ponce-García et al. 2009; Ranson et al. 2010; López-Solís et al. 2020; Villegas-Ramírez et al. 2020). In the Sovereign State of Quintana Roo, temephos is applied monthly for larval control of *Aedes* mosquitoes in areas where dengue transmission is at risk, especially in urban cities such as Cancún, Playa del Carmen, and Chetumal. Likewise, this compound is applied constantly in southern rural locales for the control of *Anopheles* mosquitoes. Therefore, we evaluated the status of temephos susceptibility in *Ae. aegypti* and *Ae. albopictus* larval populations obtained from various locations in Quintana Roo, Mexico.

Initially, ovitraps were placed and monitored weekly in Cancún, Chetumal, Playa del Carmen, and José María Morelos, Quintana Roo, Mexico (Fig. 1) by trained staff from the Ministry of Health as part of "Entomological Surveillance with Ovitraps" (a national program for the prevention of dengue) from Oct through Dec 2020. Ovitraps consisted of 1 L black plastic containers (Cresar, Puebla, Puebla, Mexico) half filled with tap water and Pellon mesh fabric (F1600) (Industrias Notesa, Tlalnepantla, Mexico) added as an oviposition substrate. Eggs were removed from ovitraps, hatched in plastic containers (Sterilite, Townsend, Massachusetts, USA), and then reared to adults in a climate-controlled room (29 °C ± 2 °C, RH 70% ± 10%, 12:12 h [L:D] photoperiod) at the Entomological Research and Bioassays Unit of Quintana Roo. Larvae were reared in 30 × 23 × 3 cm plastic trays (Industrias Boris, León, Guanajuato, Mexico) and fed with a mixture (2:8) of dehydrated yeast (AB Calsa, Mexico City, Mexico) and fish flakes (Grupo Acuario Lomas, Mexico City, Mexico). Emerged mosquitoes were identified to species using the taxonomic keys of Darsie and Ward (2005), and each species was placed in separate screened insect rearing cages (Bioquip, Rancho Dominguez, California, USA) (with 10% sugar solution soaked in cotton pads and bovine blood for egg production). Individuals of the F_1 generation then were used for the determination of larvicide susceptibility (Brogdon & Chan 2010).

Bioassays were performed using 1× the discriminant concentration (0.012 mg per L) for *Aedes* species (WHO 1992) from a 1 mg per L stock solution of temephos (50% active ingredient) (Quimix, Mexico City, Mexico). Although technical grade insecticides provide more precise results than formulated, it is possible to use formulated insecticides as a basis to give an approximation of the status of insecticide susceptibility in mosquitoes (Zamora-Perea et al. 2009; Brogdon & Chan 2010). If our bioassays detected resistant phenotypes in a population, the species was exposed to 5× the discriminant concentration (0.06 mg per L) (WHO 2016). Twenty-five larvae, second to third instar (F_1 generation), were placed into each of 4 waxed cups (Graphic Packaging International, Atlanta, Georgia, USA) containing 222.3 mL distilled water and 2.7 mL of the stock solution. The 5× discriminant dose consisted of 5.4 mL of stock solution and 211.5 mL of distilled water. Four repetitions from each location per species and concentration were completed.

We found that *Ae. aegypti* larval mortality from all Quintana Roo locations was less than 98% at the discriminant concentration (Table 1). According to the World Health Organization (WHO 2016), when the mortality of individuals exposed to a discriminate concentration is less than 98%, it confirms phenotypic resistance in

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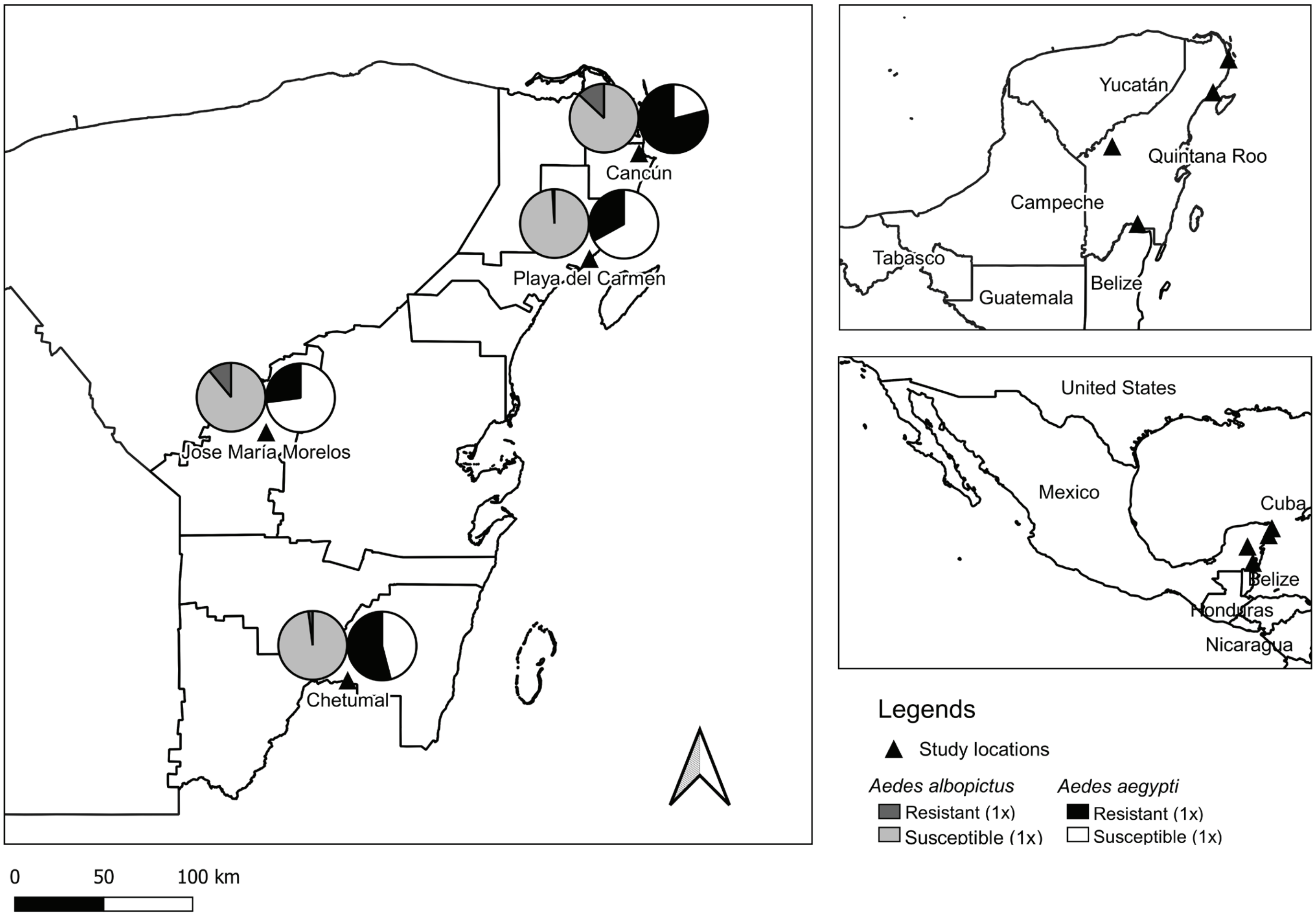

Fig. 1. Susceptibility status to temephos (1× the discriminant dose) of *Aedes albopictus* (grey pie charts) and *Aedes aegypti* (black and white pie charts) larvae from 4 communities of Quintana Roo, Mexico.

that population. Larvae from Cancún had the lowest percentage of mortality (21%), and José María Morelos exhibited the greatest at 73%. All *Ae. aegypti* populations exposed to 5× the diagnostic dose reached 100% mortality. This data indicates that the temephos phenotypic resistance in *Ae. aegypti* larvae in Quintana Roo was considered low-intensity based on the criteria of WHO (2016) because there were no survivors at 5×.

Mean *Ae. albopictus* larval mortality from Playa del Carmen (99%) and Chetumal (98%) exhibited the greatest mortality to the 1× concentration of temephos (Table 1). Furthermore, mortality from larvae obtained at the remaining locations was slightly lower. Although *Ae. albopictus* from Playa Del Carmen and Chetumal were considered susceptible to temephos, Cancún and José María Morelos exhibited slight tolerance to this larvicide.

Table 1. Mean mortality of *Aedes aegypti* and *Aedes albopictus* from Quintana Roo at 1× the discriminant dose of temephos.

		Aedes aegypti	*Ae.albopictus*
Location	*n*	Mean ± SE	Mean ± SE
Cancún	4	21.00 ± 5.51	87.00 ± 6.61
Chetumal	4	46.00 ± 6.83	98.00 ± 1.15
Playa del Carmen	4	66.67 ± 1.91	99.00 ± 1.91
José M. Morelos	4	73.00 ± 1.15	89.00 ± 1.00

Our study revealed that susceptibility of temephos to *Ae. albopictus* larvae was greater than that of *Ae. aegypti* (Table 1). As mentioned earlier, *Ae. albopictus* was first reported in Quintana Roo in 2011 (Salomón-Grajales et al. 2012) suggesting that this species had not been exposed to temephos at the same intensity as *Ae. aegypti*. Also, the preference of *Ae. albopictus* to develop in natural bodies of water rather than artificial containers also may have influenced the frequency of exposure to larvicides applied by vector control programs that focus on *Aedes* container-inhabiting mosquitoes (Dom et al. 2013). The greatest amount of Temephos-resistance in *Ae. aegypti* and tolerance in *Ae. albopictus* was in Cancún, the most populated city in Quintana Roo. In this city, urbanization has been unplanned primarily with insecticides routinely applied outdoors by private pest control companies as well as households; these activities may play an important role in the development of resistance, especially in peridomestic mosquito species (Dusfour et al. 2019). Future research to evaluate the susceptibility of temephos and other larvicides against *Aedes* container-inhabiting mosquitoes in Mexico is warranted at the operational level. Such effort is needed to measure the effects of alternate strategies (such as the use of bacterial larvicides or growth inhibitors) to prevent as well as delay the onset of potential resistance to larvicides and improve vector control of mosquito vectors.

We greatly appreciate the assistance of the entomology technicians from the Health Services of Quintana Roo in this project, especially those in charge of ovitrap surveillance.

Summary

In Mexico, the larvicide temephos (an organophosphate) has been used widely for vector control for a number of yr. Repeated exposure to this larvicide has caused resistance in *Aedes* container-inhabiting larvae. In this study, the susceptibility status to temephos was evaluated in *Ae. albopictus* and *Ae. aegypti* larvae from 4 locales within Quintana Roo, Mexico. *Aedes aegypti* exhibited low-intensity phenotypic resistance in Cancún, Playa del Carmen, José María Morelos, and Chetumal. *Aedes albopictus* was found to be susceptible to temephos in Chetumal and Playa del Carmen, but low-intensity phenotypic resistance (tolerance) was observed in Cancún and José María Morelos. The changes in susceptibility status to temephos in both species could be caused by different degrees of exposure to this larvicide. However, more in-depth studies are required to confirm this.

Key Words: organophosphate; dengue; arbovirus; vector; larvicide

Sumario

En México, el larvicida temefos (organofosforado) se ha utilizado ampliamente para el control de vectores durante varios años. La exposición prolongada a este compuesto ha provocado resistencia en las larvas del género *Aedes*. En este estudio, el estado de la susceptibilidad a temefos fue evaluado en larvas de *Ae. albopictus* y *Ae. aegypti* de cuatro comunidades de Quintana Roo, México. *Aedes aegypti* mostró una resistencia fenotípica de baja intensidad en Cancún, Playa del Carmen, José María Morelos, y Chetumal. Por otro lado, las poblaciones de *Ae. albopictus* de Chetumal y Playa del Carmen fueron susceptibles al larvicida, sin embargo, se observó resistencia fenotípica de baja intensidad en Cancún y José María Morelos. Los cambios en el estado de susceptibilidad a temefos de ambas especies puede deberse a los diferentes niveles de exposición al larvicida, sin embargo, se requiere de estudios más profundos para confirmarlo.

Palabras Clave: organofosforados; dengue; arbovirus; vector; larvicida

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