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SCIENTIFIC NOTE

TEMEPHOS RESISTANCE STATUS OF *AEDES AEGYPTI* POPULATIONS FROM HAVANA, CUBA

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ABSTRACT. *Aedes aegypti* chemical control remains an indispensable alternative to prevent dengue, Zika, and chikungunya outbreaks in Havana, Cuba. The city of Havana requires constant surveillance because of its bioecological characteristics that favor the proliferation of mosquito vectors of these viruses, which constitutes a high risk to the health of its inhabitants. The goal of this study was to determine the impact of the stopping of temephos applications during the 2 years of the COVID-19 pandemic on the level of susceptibility of *Ae. aegypti* in 5 municipalities of Havana, Cuba. Larval susceptibility was evaluated by bioassays as described by the World Health Organization. All *Ae. aegypti* populations tested showed high resistance to temephos. The National Control Program of *Ae. aegypti* in Cuba will need to promote insecticide rotation policies to prevent the evolution of temephos resistance in Havana.

KEY WORDS Insecticide, mosquito, resistance, temephos, vector

The approach for the control of *Aedes aegypti* (L.) since the last century has mainly been based on environmental sanitation, through community participation and the application of chemical insecticides due to the presence of larval stages in a variety of natural and artificial containers (Rodriguez et al. 2012, Bisset et al. 2017). Temephos, an organophosphate insecticide, is the most widely used larvicide to control *Ae. aegypti*. (Rawlins 1998, Flores et al. 2006, Tikar et al. 2008). Its intensive use has generated insecticide resistance in different *Ae. aegypti* populations associated with the metabolic action of enzymes in locations such as such as Brazil (Melo-Santos et al. 2010), the Martinique Islands (Marcombe et al. 2009), and India (Kumawat et al. 2021).

The first report of temephos resistance in Cuba was in 1997, with the subsequent occurrence of a dengue outbreak in Santiago de Cuba (Rodriguez et al. 1999). In a study carried out in Havana, not only was it shown that esterase enzymes play an important role in temephos resistance (Bisset et al. 2011) but later it was shown that the reversal of temephos resistance was possible due to the mechanism involved in the development of mosquito resistance (Bisset el al. 2019). Temephos resistance is a phenomenon that has been evolving rapidly, posing a threat to vector control. It is an issue that needs greater attention in Cuba because of the continued use of this larvicide as part of the interventions carried out by the National Vector Control Program of *Ae. aegypti* and *Ae. albopictus* (Skuse) established in Cuba since 1981. However, temephos application was stopped by the National Vector Control Program during 2020–2022 because of the COVID-19 pandemic. The objective of this study was to determine the impact of the stopping of temephos applications during the 2 years of the COVID-19 pandemic on the susceptibility of *Ae. aegypti* in 5 municipalities of Havana, Cuba.

The study area selected to evaluate *Ae. aegypti* resistance to temephos was 5 municipalities: Playa, Diez de Octubre, Arroyo Naranjo, Marianao, and La Lisa in Havana province (Fig. 1). Playa is located north and west of the capital, Havana, 23°05′39″N, 82°26′56″W, and covers a total area of 35 km^2. Diez de Octubre is located west of the capital at 23°05′17″N, 82°21′35″W and covers a total area of 12.27 km^2. Arroyo Naranjo is located at 23°02′37″N, 82°19′58″W and covers a total area of 83 km^2. Marianao is located at 23°05′00″N, 82°26′00″W and covers a total area of 22 km^2. La Lisa is located on the western outskirts of the capital at 23°01′29″N, 82°27′47″W and covers a total area of 37.5 km^2.

Entomological sample collections in the field were carried out using ovitraps from January to May 2022, following the sampling protocols of the National Vector Control Program of Cuba (MINSAP 2012). An *Ae. aegypti*–susceptible strain named Bora Bora, collected in French Polynesia, provided by the Institute Pasteur de Guadeloupe was used in this study. This susceptible reference strain has been colonized in the laboratory since 2018. The rearing of late third and/or early fourth larval instars of F1 generation of field-collected *Ae. aegypti* populations from Havana province were maintained following the methodology by Perez et al. (2004). Artificial blood meal was provided to female mosquitoes using a Hemotek system

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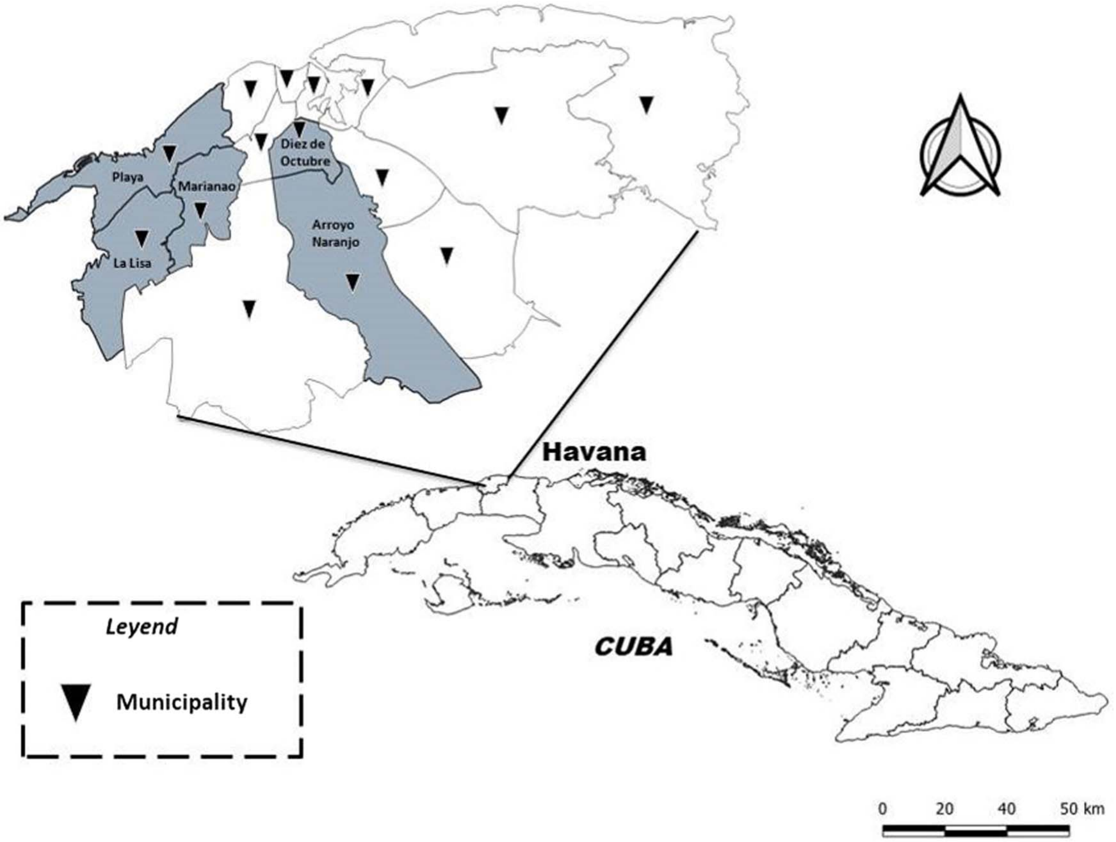

Fig. 1. Municipalities from Havana province where *Aedes aegypti* larvae collection was carried out for this study.

(Hemotek Ltd., Blackburn, UK) for the maintenance of the colonies used in this study. The morphological characterization of the larvae was carried out in the Vector Control Department, Institute of Tropical Medicine Pedro Kouri, using the taxonomic keys by Gonzalez (2006).

Temephos susceptibility level was evaluated in larval bioassays (WHO 1981). Nine concentrations (from 0.03 to 3.0 ppm) of temephos were tested with 3 or 4 replicates each and a control. Twenty late third instar and/or early fourth instars of uniform size were tested in plastic cups containing 99 ml of tap water and 1 ml of prepared insecticide solution. Mortality was determined 24 h after the insecticide application.

The results of the larval bioassays were analyzed using the Probit test implemented in the statistical program IBM SPSS Statistics version 21.0 (IBM Corporation, USA). The resistance ratio (RR_{50}) was calculated comparing the value of lethal concentrations (LC_{50} and LC_{90}: lethal concentrations that cause 50% and 90% mortality, respectively) of field colonies with the Bora Bora strain. Populations were classified as resistant or susceptible using the following criteria: $RR_{50} \leq 5$: susceptible, $5 < RR_{50} \leq 10$: moderate resistance, and $RR_{50} > 10$: high resistance (Mazzarri and Georghiou 1995).

The resistance ratios (RR_{50} and RR_{90}) and the slope of the probit regression lines for temephos resistance in *Ae. aegypti* populations are shown in Table 1. The results showed all populations presented a high resistance ($RR_{50} > 10$) to temephos, with 3 municipalities (Arroyo Naranjo, Diez de Octubre, and Playa), showing values of $26 < RR_{50} < 43$ (Table 1). The Marianao population was a notable exception and was considered the most resistant ($RR_{50} > 80$) of all colonies. Mosquito populations of La Lisa ($RR_{50} = 42.5$, $RR_{90} = 29.44$) and Arroyo Naranjo ($RR_{50} = 34.75$, $RR_{90} = 28.88$) municipalities showed similar resistance ratios (RR), although they were higher than those shown by Diez de Octubre ($RR_{50} = 30$, $RR_{90} = 16.94$) and Playa ($RR_{50} = 26.87$, $RR_{90} = 16.66$) (Table 1). Resistance ratios in both *Ae. aegypti* populations (Diez de Octubre and Playa) were also similar to each other, whereas resistance ratio of the Marianao population ($RR_{50} = 82.5$, $RR_{90} = 42.5$) was the highest of all of the field-collected mosquito populations (Table 1). The Marianao and Diez de Octubre populations showed the highest slope values (3.5 and 3.1) of the probit regression line (Table 1). These results confirm that temephos resistance was most homogeneous in *Ae. aegypti* populations from both municipalities.

Table 1. Temephos resistance level expressed as resistance ratio (RR_{50} and RR_{90}), calculated from the insecticide concentration that caused 50% (LC_{50}) and 90% (LC_{90}) of mortality in *Aedes aegypti* populations from 5 municipalities in Havana province (first five rows), from January to June 2022. Five hundred larvae evaluated in each colony.

Ae. aegypti colonies	LC_{50} (ppm)[1]	RR_{50}[2]	LC_{90} (ppm)[1]	RR_{90}[2]	Slope (±SD)[3]	Chi-square[4]
Marianao	0.66 (0.4–1.2)	82.5	1.53 (0.94–12.4)	42.5	3.5 (±0.35)	36.82
Arroyo Naranjo	0.278 (0.169–0.419)	34.75	1.04 (0.648–2.786)	28.88	2.23 (±0.16)	26.32
Playa	0.215 (0.115–0.351)	26.87	0.6 (0.365–1.983)	16.66	2.87 (±0.2)	30.09
Diez de Octubre	0.24 (0.199-0.284)	30	0.61 (0.5-0.81)	16.94	3.1 (±0.18)	12.44
La Lisa	0.34 (0.177–0.618)	42.5	1.06 (0.593–6.473)	29.44	2.6 (±0.22)	17.72
Bora Bora	0.008 (0.006–0.01)	—	0.036 (0.024–0.069)	—	1.90 (±0.15)	12.38

[1] Lethal concentration (LC_{50} and CL_{90}) in mg/liter, 95% confidence limits (CL) in parentheses.

[2] Resistance ratio (RR_{50} and RR_{90}): LC_{50} or LC_{90} strain to be evaluated/LC_{50} or LC_{90} Bora Bora strain.

[3] Slope of the Probit-log line, standard deviation (±SD) in parentheses.

[4] Chi-square of the Probit test to determine goodness of fit.

The indiscriminate use of chemical insecticides results in the development of resistance in mosquitoes of medical importance, and it is one of the factors that reduces the chances of vector control programs being successful (Cui et al. 2006). The emergence of resistance to insecticides is a complicated phenomenon involving physiological, genetic, ecological, and behavioral factors combined with insecticide application (Karunaratne et al. 2018).

The first incidence of temephos resistance in an *Ae. aegypti* population from Cuba was reported in 1997 and later coincided with an outbreak of dengue in Santiago de Cuba, located in the eastern part of the country (Rodriguez et al. 1999). Temephos resistance was also reported in *Ae. aegypti* populations from Guanabacoa and Playa municipalities during the dengue epidemic in Havana in 2001–2002 (Bisset et al. 2004, Rodriguez et al. 2004). Subsequently, resistance was confirmed in Boyeros municipality in 2006 (Rodriguez et al. 2009). A study carried out in 2008 reported a high resistance to temephos in *Ae. aegypti* populations from 15 municipalities of Havana induced by its intensive use (Bisset et al. 2011). The results of this study showed high resistance ($RR_{50} > 10$) to temephos in all populations evaluated. Marianao, Playa, and La Lisa showed an increase in their resistance ratios ($RR_{50} = 82.5$, $RR_{50} = 26.87$, and $RR_{50} = 42.5$) compared to those reported from more than 10 years ago (Bisset et al. 2011). However, Arroyo Naranjo and Diez de Octubre showed a slight reduction in resistance ratio values ($RR_{50} = 34.75$ and $RR_{50} = 30$) with respect to those reported from more than 10 years ago (Bisset et al. 2011). These results warrant further analyses of the populations studied to determine the mechanisms involved in temephos resistance. Reversal of temephos resistance has been reported in a resistant laboratory strain in the absence of selection pressure for 6 generations (Bisset et al. 2019), which could have influenced the slight decrease in the resistance ratio values in Arroyo Naranjo and Diez de Octubre because of the stopping of larvicide application during the COVID-19 pandemic. The slope values obtained by the regression lines (Table 1) reflected that temephos resistance was more homogeneous in the Marianao and Diez de Octubre populations. It would be appropriate to carry out a study of population genetics and resistance mechanisms to elucidate the genetic differences of both colonies that allow them to express a more homogeneous resistance to temephos compared to the other populations studied.

High levels of temephos resistance in *Ae. aegypti* are of great concern for the control actions carried out by the National *Ae. aegypti* Control Program, which should be conducted more frequently because some authors have reported a significant decrease in the residual effect of temephos in highly resistant mosquito populations ($RR > 10$) being effective for a period of 13 days and susceptible populations ($RR \leq 5$) for 18 days (Bisset et al. 2011).

The results of this study showed an urgent need to implement new integrated vector control strategies, such as those using alternative insecticides based on *Bacillus thuringiensis var israelensis* (*Bti*) de Barjac or pyriproxyfene (insect growth inhibitor) to avoid the continued increase of temephos resistance in these municipalities. A successful strategy carried out in the capital municipality of Boyeros showed how the resistance levels decreased in *Ae. aegypti* populations when temephos application was discontinued and replaced by *Bti* (Rodriguez et al. 2012). Similar results were obtained in Brazil using this microbial control agent and using growth inhibitors (Andrighetti 2008; Lima et al. 2011; Rahman et al. 2021). It has also been shown that temephos susceptibility can be recovered because its metabolic resistance mechanisms are reversed when its use is discontinued (Melo-Santos et al. 2010; Bisset et al. 2019). Preferably, these strategies could be carried out by promoting insecticide rotation policies to preserve the effectiveness of insecticides.

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