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Susceptibility of *Aedes albopictus* (Skuse, 1894) against the organophosphorus insecticide temephos, in Chidambaram, Tamil Nadu, India

Soliang Manyu, C. Elanchezhiyan*, K. Sivasankaran and P. Basker#

Department of Zoology, Annamalai University, Annamalai Nagar, Chidambaram 608002, Tamil Nadu, India.
#National Centre for Disease Control, Ministry of Health and Family Welfare, Government of India, New Delhi110 054, India.
Email: chezhiyan6@gmail.com

ABSTRACT: Investigation showed that *Aedes albopictus* (Skuse, 1894) of Chidambaram-Town, Annamalai-Nagar and Muthiah-Nagar of Tamil Nadu are still susceptible to the insecticide organophosphorus temephos with 98–100 mortality percentages. The resistance ratios of all the three sentinel sites are negligible. LC_{50} value was 0.002 - 0.003 ppm with high significance. It was the first temephos bioassay case study conducted on DENV vector *Ae. albopictus* in the selected sentinel sites and estimated lethal concentrations.

KEY WORDS: Dengue, vector, mortality, resistance ratio, sentinel site

INTRODUCTION

World Health Organization dengue reported, increase of 8-fold cases over the last twenty years (Park *et al.,* 2022). The primary vectors for dengue virus are mosquito species belonging to the genus *Aedes* and *Ae. albopictus* (Skuse, 1894) plays a crucial role in the transmission of dengue virus–DENV (Rebecca, 1987; Muthusamy *et al.,* 2015; Amorin and Birbrair, 2022; Dalpadado *et al*., 2022; WHO, 2022). Dengue track record in India is engrossing. It first debuted in 1780 (Chaturvedi and Nagar, 2008) and then reappeared in 1963–64 in East-Coast India (Pavri *et al*., 1964; Chatterjee *et al*., 1965; Carry *et al*., 1966). Thereafter, frequent cases are reported from different parts of India against all four dengue virus (DENV) serotypes (Dash *et al*., 2004; Dar *et al*., 2006). Currently, dengue is prevalent throughout the country and in Tamil Nadu in all the districts since 2000 (Samuel *et al*., 2021). Chemical control measures have been employed heavily to keep the vector population in check (Horstick *et al*., 2010). In such scenario, application of temephos has gained momentum for elimination of immature *Aedes* mosquitoes in many countries (Ponlawat *et al*., 2005; Jacquet *et al*., 2015) as well as in India (Mukhopadhyay *et al*., 2006). It is a non-systemic organophosphorus insecticide, used to control mosquito larvae and other insect pests. It was initially registered by US EPA in 1965 (by American Cyanamid Co, now BASF) and re-registered in 1991, and in India temephos is registered as 50 per cent EC for dengue mosquito larvae control (WHO, 2011). However, prolonged application of such measure has led to detection of insecticide

* Author for correspondence

resistance in a vector (Ocampo *et al*., 2011; Bonizzoni *et al*., 2013). However, resistance status of *A. albopictus* to temephos in the selected sentinel sites is still unknown, despite frequent application of temephos in the region over dengue outbreaks. Therefore, the present investigation was undertaken to assess the susceptibility/ resistance status of *Ae. albopictus* against organophosphorus temephos, in Chidambaram, Tamil Nadu, so as to provide a precise application rate of temephos against the targeted vector in sampled areas.

MATERIALS AND METHODS

The study was carried out in Chidambaram, Tamil Nadu, India (11° 23' 53.4984'' N; 79°41'43.2888'' E). Based on recent vicious dengue outbreaks in the area (Basker and Kolandaswasmy, 2015), *Ae. albopictus* larvae were collected from three different sentinel sites from Chidambaram-Town, Annamalai-Nagar, and Muthiah-Nagar. Sampling of the specimen was done two ways; a) Larvae were gently collected from their natural breeding habitats using a plastic dropper and dipper cup with a handy magnifying glass and transferred into a plastic cup as per the guidelines given in (WHO, 2016); and b) Ovitrap surveillance was conducted in the month of October 2021 as per (IAEA, 2017).

Specimen from each station was colonized until 1st generation (F1) and late 3rd instar larvae were used for the bioassay and susceptibility tests. The specimen was identified morphologically following the illustrated keys (Reuda, 2004) and then molecular identification was conducted at TRI-BIOTECH, Trichy Research Institute of Biotechnology Pvt. Ltd., Thillai-Nagar, Tamil Nadu, India (Soliang *et al*., 2022). Samples of *Ae. albopictus* larvae and eggs (post-hatching) were maintained in allocated mosquito insectary at Department of Zoology, Faculty of Science, Annamalai University. Temperature and humidity of the colony were maintained following the methods (Govindarajan and Sivakumar, 2011) with temperature ranging between 27±3°C and relative humidity was kept at 70 - 80 per cent.

The larval specimens from each site were pooled and transferred into a larval tray of 40 x 30 x 8 cm in dimension. Larvae were fed on with larval diet, which consisted of pup-start (Puppy feed) and yeast in 60:40 ratios totalling 3g in 100 ml of water for a 500-1000 larvae population. Newly emerged adult was kept in a mosquito cage of 30 x 30 x 30 cm dimension and fed on sugar feed for 2-3 days post-emergence. Feeding was met with 10 per cent sucrose solution and overnight soaked raisins for better nourishment. Following sugar feed, before blood feed, one-day sugar feed abstinence was observed for a quality blood feed. The live mouse was exposed for a period of one hour per day for the next 2-3 days. Thereafter, whatman filter paper in a black cup with water occupying 1/2 of the cup was put in for oviposition. The eggs obtained are then hatched to produce F1 progeny. Third to fourth instar larvae were used for larval bioassay and susceptibility tests.

Temephos of organophosphate was selected for the present study due to its availability and as it is primary insecticide used for vector control. Technical grade temephos 50 per cent EC was sponsored by the Deputy Director of Health Service, Cuddalore, Tamil Nadu.

Baseline bioassay was conducted according to WHO standardized procedure (WHO, 2005; WHO, 2016) in the laboratory on late 3rd and early 4th instar stages. Technical grade temephos used had a 50 per cent efficacy concentration. Therefore, 2 ml of temephos was dissolved in one litre of double distilled water to yield a 1ppm stock solution. Following six discrete concentrations were chosen for the narrow range bioassay; 0.002 ppm, 0.003 ppm, 0.004 ppm, 0.005 ppm, 0.006 ppm and 0.007 ppm yielding between 30 to 100 per cent larval mortality in 24 h. Four replicates for each concentration were set up for treated and two replicates for control assays. Batches of 25 larvae were transferred with the help of a dropper into the disposable cups of 120 ml capacity. The test containers are held at 27±3°C and preferably a photoperiod of 12 h light followed by 12 h dark (12 L: 12 D). After 24 hours of exposure time, the larval mortality was recorded in standard test form made available by World Health Organization (WHO, 2005). Mortality of the larvae was detected by lightly stirring them with a clean plastic pipette. Moribund

larvae were counted as dead. The bioassay results were subjected to Probit Analysis (Finney, 1971), for lethal concentrations by using SPSS software V22 with significance value of 0.05. The resistance ratio (RR) was calculated based on the computed LC_{50}, LC_{90} and LC_{99} values, using the following formula:

$$\text{Resistance ratio (RR)} = \frac{LC_{50} / LC_{90} / LC_{99.9} \text{ of field strain}}{LC_{50} / LC_{90} / LC_{99.9} \text{ of laboratory strain}}$$

Guidelines of (Mazzarri and Georghiou, 1995) were used to classify the RRs as high (≥10 fold), medium (between 5 and 10 fold) or low (≤5 fold). Mortality correction through (Abbott, 1987) was not accounted as the pupated percentage and larval mortality in the test were negligible.

Susceptibility bioassay was conducted according to WHO (2005; 2016) to determine phenotypic resistance using discriminating or diagnostic concentrations drawn from the aforementioned baseline bioassay result. It is taken as double the concentration corresponding to 99.9% mortality (the $LC_{99.9}$ value), at which all the individuals in a susceptible population will be killed. This is conventionally known as the discriminating (or diagnostic) concentration (i.e., 1x). For each station, four replicates were taken for both treated and control samples with equal batches of larvae, i.e., 25 larvae of early 3rd and 4th instar stages. Unlike baseline bioassay, susceptibility assay is run for one hour. The discriminating concentrations used for Chidambaram-Town, Annamalai-Nagar and Muthiah-Nagar were estimated as 0.022 ppm, 0.024 ppm and 0.018ppm respectively. The data were interpreted following the guidelines of (WHO, 2016), which categorizes the result into three parts based on the susceptibility assay mortality percentage; i) Susceptible-larval mortality > 98 per cent; ii) Possible resistance- larval mortality 90-98 per cent; iii) Resistant-larval mortality < 90 per cent.

RESULTS AND DISCUSSION

Baseline bioassay: The larval bioassay result LC_{50} LC_{90} and $LC_{99.9}$ estimated for Chidambaram-Town were 0.003 ppm, 0.006 ppm and 0.011 ppm respectively. LC_{50} LC_{90} and $LC_{99.9}$ estimated for Annamalai-Nagar were 0.003 ppm, 0.007 ppm and 0.012 ppm respectively and LC_{50} LC_{90} and $LC_{99.9}$ estimated for Muthiah-Nagar were 0.002 ppm, 0.005 ppm and 0.009 ppm respectively. The resistance ratio (RR) in all the case was negligibly low with value much lower than 5 fold resistance ratio categorisation. Moreover outcome of the study was observed highly significant with statistical significant value of 0.000 ($P<0.05$ (Table 1).

Susceptibility bioassay: The susceptibility test serves as a tool to detect the existence of resistant vectors against any insecticide available in the public domain. Primary database required for the assay is discriminating concentration, which can be evaluated through a baseline bioassay. The result of susceptibility bioassay is illustrated in table 2, which indicates that vector population from the selected sites are still susceptible to on-going temephos, with mortality percentage of 98 for Chidambaram-Town and Annamalai-Nagar, and 100 for Muthiah-Nagar. According to insecticide resistance classification of WHO, *Aedes albopictus* larvae from Muthiah-Nagar were observed highly susceptible to temephos, while the specimen from Chidambaram-Town and Annamalai-Nagar are prompt to build resistance early.

Despite intense application of control measures, dengue vector population continued to dominate the public health (Mirresmailli and Isman, 2014). The main cause is interruption of vector control efficacy by insecticide resistance development (Meenambigai *et al*., 2022) and lack of efficient drugs (Porretha *et al*., 2022). Measures like application of insecticides in rotation manner and resistance management have been adopted to overcome incidence of resistance development (Araújo *et al*., 2013; Morgan *et al*., 2022). Moreover early detection of resistance ensures primary success of vector control measures. This is achieved by performing susceptibility bioassay (Reyes-Solis *et al*., 2014) which can detect existence of resistant vector population and help in duly resistance management (Kraemer *et al*., 2015).

Table 1. Bioassay of *Aedes albopictus* larvae against temephos from Chidambaram-Town; Annamalai Nagar and Muthiah Nagar, Tamil Nadu

SentinelSite	Conc. (ppm)	T	M%	`95% Confidential Interval (CI)			P Value
				LC_{50} (ppm) RR_{50}	LC_{90} (ppm) RR_{90}	$LC_{99.9}$ (ppm) $RR_{99.9}$	
Chidambaram Town	0.002	100	40	0.003 [0.001-0.004]	0.006 [0.004-0.018]	0.0011 [0.007-0.118]	
	0.003	100	47				
	0.004	100	59				
	0.005	100	78	1.33	1.5	1.18	0.00
	0.006	100	96				
	0.007	100	100				
	Control	50	1				
Annamalai Nagar	0.002	100	30	0.003 [0.002-0.004]	0.007 [0.005-0.013]	0.012 [0.008-0.48]	
	0.003	100	42				
	0.004	100	59				
	0.005	100	70	1.67	1.14	1.17	0.00
	0.006	100	89				
	0.007	100	100				
	Control	50	2				
Muthiah Nagar	0.002	100	48	0.002 [0.001-0.003]	0.005 [0.004-0.008]	0.009 [0.006-0.028]	
	0.003	100	60				
	0.004	100	74				
	0.005	100	89	1.5	1.2	1.11	0.00
	0.006	100	99				
	0.007	100	100				
	Control	50	2				

Conc. (Concentration); T (Total number of exposed larvae to temephos for 24 hours); M% (Mortality percentage: ratio of total death divided to total number of larvae exposed multiplied by 100); RR(Resistance ratio: ratio of lethal concentration of field population to lab population); LC_{50} (Lethal concentration that kills 50% of the exposed larvae); LC_{90} (Concentration that kills 90% of the exposed larvae); $LC_{99.9}$ (Concentration that kills 99.9% of the exposed larvae); P (Statistical significance, which was found to be highly significant with $p<0.05$)

Diagnostic concentration or discriminating concentration is prerequisite data required for resistance surveillance and it differs widely from one station to another. In the present study also, though the sentinel sites are under same taluk, their discriminating concentration varied widely (Table 2), where the discriminating concentration for Chidambaram-Town, Annamalai-Nagar and Muthiah-Nagar were 0.022 ppm, 0.024 ppm, and 0.018 ppm respectively. Diagnostic concentrations

are formulated from lethal concentration, which are obtained through baseline bioassay (WHO, 2016; 2005). It is estimated as double of $LC_{99.9}$ (WHO, 2016). The $LC_{99.9}$ obtained in the current study for Chidambaram-Town, Annamalai-Nagar and Muthiah-Nagar was 0.011 ppm, 0.012 ppm and 0.009 ppm respectively. Findings of the study revealed that *Ae. albopictus* larvae are still susceptible to temephos in Chidambaram-Town, Annamalai-Nagar and Muthiath-Nagar with 98 mortality percentage.

Ae. albopictus is cosmopolitan (Romiti *et al., 2022;* Sivasankaran *et al., 2022)* and most invasive vector (Vanlandingham *et al*., 2016), alarming public health concern with its ability to cause 32 proven pathogen diseases, subsuming dengue, chikungunya, and zika (Goubert *et al.,* 2016; Liu *et al.,* 2022; Morgan *et al.,* 2022a,b). Incidence of *Ae. albopictus* was observed at Arupathi (Mayiladuthurai district, Tamil Nadu, India) and Sityan-Gam (Lohit district, Arunachal Pradesh, India) in addition to the selected sentinel sites for the study. Like overseas countries (Bharati and Saha, 2021), in India also, temephos is specifically subjected to control of dengue vector larvae (Ocampo *et al*, 2011; Romiti *et al*., 2022) and it has led to development of resistance (Singh *et al*., 2014; Yadav *et al*., 2015; Wu *et al*., 2022). Tamil Nadu state lies in tropical climate zone and is endemic to DENV and to other vector borne disease as well (Shimono *et al*., 2021; Lesmana *et al*., 2022). Resistant dengue vector population to temephos are reported from the state (Fatima and Syed, 2018). The vaccines for dengue are made available but failed to gain public attention due to their low efficacy and hope for a reliable vaccine is still a long wait (Rai *et al*., 2020; Hassan *et al*., 2021). Vector control with chemical measures continues with timely resistance surveillance. Thus the present study provides effective vector control in the present scenario with precise kill using new formulated lethal concentrations (Table 1) and it sets primary database for monitoring *Ae. albopictus* resistance status in Chidambaram-town, Annamalai-Nagar and Muthiah-Nagar. With $LC_{99.9}$ value of 0.012 ppm, Annamalai-Nagar showed to have highest lethal concentration amongst the three selected stations and has potential to develop resistance early. LC_{50} and mortality percentage value of Annamalai-Nagar and Chidambaram-Town were found to be same, this could be due to close proximity of the stations sharing similar environment. Station Muthiah-Nagar showed to have the least lethal concentrations and cent per cent mortality indicating highly susceptible. Similar studies are conducted in different parts of the country where temephos is used as primary chemical control measure and the results are reported resistant (Sivan *et al*., 2015). The current study yielded LC_{50} and RR_{50} of all the selected stations much lesser than that of (Sivan *et al*., 2015) findings with RR_{50} of 15.3 and LC_{50} of 1.177ppm. In addition to source reduction vector control measures, susceptibility and resistance status surveillances have become the key point in today's vector control planning. Present investigation on insecticide resistance proved, *A. albopictus* larvae from the selected sentinel sites are susceptible to temephos. However, it is important to limelight, the specimen from Chidambaram-Town and

Table 2. Analysis of phenotypic resistant via susceptibility bioassay with the application of discriminating concentration (1x) against *Aedes albopictus* larvae (n=100)

Population Strain	1x (ppm)	Mortality%	Status
Chidambaram-Town	0.022	98	Susceptible
Annamalai-Nagar	0.024	98	Susceptible
Muthiah-Nagar	0.018	100	Susceptible

Mortality percentage with exposure period of one hour; Discriminating concentration (1x) is double the concentration of $LC_{99.9.}$

Annamalai-Nagar are likely to build resistance speedily. This study is the first insecticide resistance case study on *Ae. albopictus* resistance status against temephos in the selected sentinel sites. Findings of present investigation revealed that the vector species is still susceptible to on-going application of temephos. However, due and periodic resistance surveillance in the future is highly advised with the present results as baseline database.

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