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Susceptibility of Dengue/ Chikungunya vector, *Aedes aegypti* against carbamate, organochlorine, organophosphate and pyrethroid insecticides

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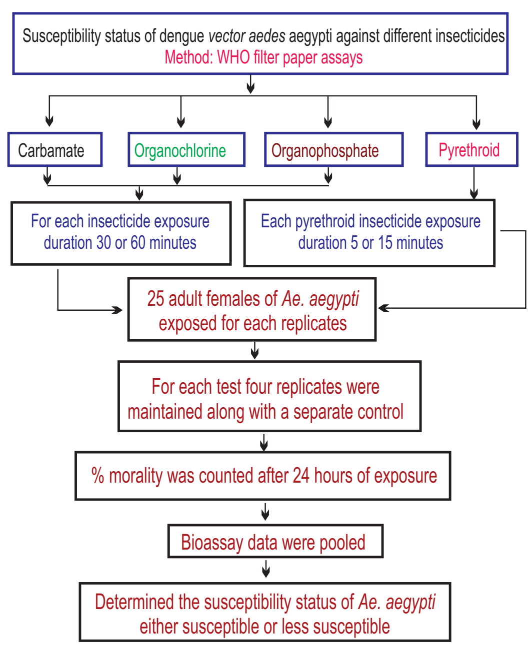
Abstract

Aim : Controlling vectors through source reduction measures and larviciding and fogging operation with insecticides are being carried out by the state health department during outbreak period. The present study was conducted to understand the susceptibility status of dengue vector *Aedes aegypti* (Linnaeus, 1762) field strain towards various insecticides as a base line data.

Methodology : Twenty five adult females of *Ae. aegypti* were exposed to organochlorine and organophosphate insecticide for 30 and 60 min and exposure duration of pyrethroid compounds for 5 and 15 min with four replicates for respective insecticides as experiment and a separate control was also maintained. The percentage mortality count was made 24 hrs after the exposure and data were pooled to determine the level of resistance / susceptibility status.

Results : *Ae. aegypti* females were exposed to 0.1% carbamate propoxur for 30 and 60 min and the observed mortality after 24hrs was 96% and 100%. Similarly, exposure to 0.4% Dieldrin resulted in 48% and 100% mortality whereas 4% Dieldrin for the same duration recorded 88% and 100% morality. Exposure to 0.1% Fenitrothion for the same period led to 40.0% and 100% mortality, whereas 5.0 %Malathion, exposure recorded 28% and 100% mortality respectively. A total of five different pyrethroid compounds include Cyfluthrin (0.15% and 0.5%), Deltamethrin (0.05%), Etofenprox (0.5%) Lambda Cyhalothrin (0.05%) and Permethrin (0.75%) were tested for exposure duration of 5 and 15 min against *Ae. aegypti*. Based on the mortality after 24 hrs the efficacy of insecticides for 5 min exposure was in the following order Lambdacyhalothrin> Cyfluthrin > Deltamethrin > Permethrin and > Etofenprox, whereas exposure for 15 minutes the order was Cyfluthrin > Permethrin > Deltamethrin > Lambdacyhalothrin and >Etofenprox, respectively.

Interpretation : *Ae.aegypti* was found to be relatively less susceptible to organophosphorous compounds, however, they were found invariably susceptible to all tested pyrethroid compounds. The data suggests that utilization of various insecticides in this region has been minimal for past several decades and fogging operations of insecticides are intensified during the outbreak period of dengue or chikungunya. The present study will form a baseline for initiating in depth studies on molecular and biochemical enzyme activities.



Introduction

Vector control, which includes both anti-larval and anti-adult measures, constitutes an important feature of any mosquito control programmes in different parts of the world. Controlling vector mosquitoes is becoming difficult due to the development of resistance in vectors to conventional insecticides. The insecticide resistance has been documented in insect vectors of human diseases (Hemingway and Ranson, 2000).Though people are affected by various kinds of vector borne diseases (VBDs), emerging and re-emerging diseases like dengue/chikungunya is wide spread in India with more number of recorded cases in south India (Mariappan 2013; Mariappan *et al.,* 2013). Dengue fever which is transmitted by *Aedes aegypti*, is a well known public health problem not only in India but also the entire globe. Although, different types of organophosphorus compounds such as Malathion, Fenitrothion and Pirimiphosmethyl have been used worldwide in the past to control various vectors, currently, these insecticides were replaced by pyrethroids (WHO, 2001; 2009). Deltamethrin and cypermethrin the known synthetic pyrethroids are used to control adult *Aedes* mosquitoes during through spray operation apart from using technical Malathion in all dengue affected regions in many parts of the world (WHO, 2009). In India, under the national vector-borne disease control programme (NVBDCP), various insecticides are being used to control different vectors of malaria, lymphatic filariasis and other VBDs either in the form of indoor / outdoor residual spray or space spray in addition to utilization of insecticide impregnated mosquito nets by the community and larviciding with organophosphorus and pyrethroid compounds (NVBDCP, 2015a). The first record of DDT resistance was reported in the adult *Ae. aegypti* from Jharia, Bihar, India in 1967 (Azeez, 1967) followed by other documented scientific reports on the resistance from different parts of the country (Madhukar and Pillai, 1968; Kaul, 1976; Mahadev *et al.,* 1993). Temephos, an organophosphorus compound was used for several decades as larvicide for controlling *Ae. aegypti* (NVBDCP, 2015b), apart from the insecticidal space spray with either organophosphorus or pyrethroid compounds. In a recent study on susceptibility status of *Ae. aegypti* to Temephos from 3 districts of Tamil Nadu (Salem, Namakkal and Dharmapuri) reported that field populations have shown resistance through continuous exposure to Temephos (Muthusamy and Shivakumar, 2015). The development of resistance to organophosphate and pyrethroids in *Ae.aegypti* population was due to increased activity and metabolism of both insecticides (Muthusamy *et al.,* 2014). It was reported that the profound use of insecticides led to the development of resistance in insect vectors including *Ae.albopictus* (Momidas and Prafulla Dutta 2014). It has also been reported that resistant and susceptible strains of insects frequently differ in fitness components, including longevity, fecundity and fertility in addition to their susceptibility to insecticides (Arnaud and Haubruge 2002; Arnaud *et.al*., 2002). This study was conducted as part of the requirement of operational research activities to understand the susceptibility status of *Ae. aegypti* mosquitoes against different conventional insecticides used in public health. In the present study, the adult susceptibility of *Ae.aegypti* was determined as per standard procedure recommended by World Health Organization (WHO, 1985; 1991) against organochlorine, organophosphate and pyrethroid insecticides.

Materials and Methods

Samples of *Aedes* larvae and pupae were collected from breeding habitats of various containers including cement cisterns and tanks in Thathaneri of Madurai. Pupae obtained from the field samples were kept for emergence of species identification at the Laboratory of Centre for Research in Medical Entomology (CRME) using standard mosquito identification key (Barraud, 1934). *Ae. aegypti* adults obtained from F1 generation were fed with 10% sucrose and used for insecticide susceptibility tests. Insecticide impregnated papers (Boxes) were obtained from Vector Control Research Unit, School of Biological Sciences, University Sains Malaysia,11800 Minden, Penang, Malaysia. The following insecticides with WHO recommended diagnostic dosages, given in parentheses of the respective insecticides, were used: Carbamate (Propoxur 0.1%), Organochlorine (Dieldrin 0.4% and Dieldrin 4%), Organophosphate (Fenitrothion 1% and Malathion 5%,) and Pyrethroid (Cyfluthrin 0.15% & 0.5%, Deltamethrin 0.05%, Etophenoprox 0.5%, Lambdacyhalothrin 0.05% and Permethrin 0.75%). Non-blood fed adult females of *Ae. aegypti* aged 3-5 days old were exposed to the diagnostic dosages of standard WHO insecticide paper (WHO, 1981). Propoxur, Dieldrin, Fenitrothion and Malathion were tested for exposure time of 30 and 60 min, while other pyrethroid compounds were tested for exposure time of 5 and 15 min, respectively. In each exposure tube, 25 females were exposed to the insecticide paper, while mosquitoes used as controls were exposed to paper without insecticide at 25±2 ºC. Mosquitoes were transferred to clean holding tubes and provided with cotton pads soaked in 10% sucrose solution. The percent mortality was counted after 24 hours of exposure. Interpretation of results of the bioassay data were pooled and determined the level of resistance / susceptibility status. Mortality was corrected with observed control mortality by Abbott's formula (Abbott, 1925).

Mortality rates were calculated as number of dead/alive for each test replicate, where no mortality in control replicate was seen. In case of (5% - 20%) mortality in control, mortality rate of the test group was adjusted using Abbott's formula as: Mortality (%) = (Y-X) / (100-Y)*100 (where, X is the percentage mortality in the insecticide treated sample and Y is the percentage mortality in control (WHO, 1975; 1981). Mortality higher than 20% was not observed in any of the control replicates. The mean mortality rate was determined across all cohorts of mosquitoes tested for a particular insecticide in the laboratory. Percentage mortality in

Table 1 : Mortality data and susceptibility status of *Aedes aegypti* against different insecticides

Insecticide with diagnostic dosages used in percentage	Exposure period in minutes	No. of mosquitoes exposed	No. of dead mosquitoes	% of corrected mortality	Susceptibility status
Carbamate					
Propoxur (0.1%)	30.0	100	96.00	96.00	Susceptible
	60.0	100	100.00	100.00	Susceptible
Organochlorine					
Dieldrin (0.4%)	30.0	100	48.00	48.00	Less susceptible
	60.0	100	100.00	100.00	Susceptible
Dieldrin (4.0%)	30.0	100	88.00	88.00	Susceptible
	60.0	100	100.00	100.00	Susceptible
Organophosphate					
Fenitrothion (1.0%)	30.0	100	40.00	40.00	Less susceptible
	60.0	100	100.00	100.00	Susceptible
Malathion (5.0%)	30.0	100	28.00	28.00	Less susceptible
	60.0	100	100.00	100.00	Susceptible
Pyrethroid					
Cyfluthrin (0.15%)	5.0	100	80.00	80.00	Susceptible
	15.0	100	84.00	84.00	Susceptible
Cyfluthrin (0.5%)	5.0	100	84.00	84.00	Susceptible
	15.0	100	96.00	96.00	Susceptible
Deltamethrin (0.05%)	5.0	100	79.00	79.00	Susceptible
	15.0	100	88.00	88.00	Susceptible
Etofenprox (0.5%)	5.0	100	54.00	54.00	Susceptible
	15.0	100	60.00	60.00	Susceptible
Lambdacyhalothrin (0.05%)	5.0	100	90.00	90.00	Susceptible
	15.0	100	100.00	100.00	Susceptible
Permethrin (0.75%)	5.0	100	56.00	56.00	Susceptible
	15.0	100	90.00	90.00	Susceptible

Note: Percentage mortality in different exposure time considered as either susceptible or less susceptible

different exposure time was considered either as susceptible or resistant to various insecticides.

Results and Discussion

Susceptibility status of *Ae.aegypti* females against different insecticides is presented in Table 1. *Ae. aegypti* females were exposed to 0.1% Propoxur for 30 and 60 min and mortality after 24hrs was 96% and 100%. Similarly, 0.4% Dieldrin exposure resulted in 48% and 100% mortality, while 4% Dieldrin for same exposure period showed 88% and 100% mortality. The heterogeneity population of *Ae. aegypti* was found susceptible to Propoxur. However, 0.4% Dieldrin was found less susceptible to 30 min exposure, else the population was found relatively to be more susceptible towards Dieldrin. The results showed that one of the oldest generations of organochlorines was not highly exposed to *Ae.aegypti* during past several decades. Exposure of *Ae. aegypti* to 1.0% Fenitrothion for 30 and 60 min resulted in 40.0% and 100% mortality, respectively. In the case of 5.0% malathion for same duration, mortality was 28% and 100%. However, exposure of malathion to *Ae. aegypti was* found to be less susceptible at 30 min than 60 min. As such, the results indicated that Propoxur, Dieldrin, Fenitrothion and Malathion could be used in operational programme to control *Ae. aegypti* on rotational basis in order to delay the resistance mechanisms. A total of five different pyrethroid compounds were tested against *Ae. aegypti* females with exposure period of 5 and 15 min. The results indicated that exposure to 0.15% and 0.5% of cyfluthrin for less than 5 min, resulted in 80% and 84% mortality. The observed mortality was 79% for 0.05% for deltamethrin, 54% for 0.5% Etofenprox, 90% for 0.05% Lambdacyhalothrin and 56% for 0.75% Permethrin, respectively. Based on the mortality, the descending order of pyrethroids were Lambdacyhalothrin > Cyfluthrin > Deltamethrin> Permethrin and > Etofenprox. Exposure of 15 min, the observed mortality for Cyfluthrin (0.15%) and (0.5%) was 84% and 96%, respectively. In the case of other pyrethroids, mortality for 0.05% Deltamethrin was 88%, 0.5% Etofenprox was 60%, 0.05% Lambdacyhalothrin was 84% and 0.75% Permethrin was 90%. Based on the results, the efficacy in descending order of pyrethroids was Cyfluthrin > Permethrin > Deltamethrin > Lambdacyhalothrin and > Etofenprox. The results indicated that all the pyrethroid compounds were found to be more susceptible for both the exposure duration. This might be due to the usage of pyrethroids in public health operational programme during the past few decades. During the last five decades, insecticides use in agriculture and public Health has led to the development of resistance in mosquito vectors in many endemic countries (Kamgang *et al*., 2011; Dusfour *et.al.,* 2011; Singh *et al.,* 2011 and Dhiman *et al.,* 2013). However, *Ae. aegypti* was found to be more susceptible to various insecticides due to less exposure. It was noted that the selection pressure of these compounds on dengue vector was minimal in this study. Studies which were undertaken at Delhi and Koderma (Jharkhand) regions reported that the susceptibility status of *Ae. aegypti* population against malathion, permethrin, deltamethrin, lambdacyhalothrin and cyfluthrin was also found to be more susceptible (Katyal, 2001; Singh *et al.,* 2011). The present study indicated that all types of compounds could be used against *Ae. aegypti* population due to discontinuous exposure of different insecticides over a period of time. The insecticides usage is very important during dengue/chikungunya outbreak period only. Similar views were also expressed based on the studies on insecticidal activity of some synthetic pyrethroids against vector mosquitoes in arid zone (Bansal and Singh, 2006; Bansal *et al.,* 2012). Studies also highlighted the development of resistance of *Ae.aegypti* against small number of pyrethroids like Deltamethrin and Permethrin in north east of Thailand (Ponlawat *et al.,* 2005; Pimsamarna *et al.,* 2009). However, observed mortality of *Ae.aegypti* adults to 30 or 60 min exposure revealed that population was susceptible to carbamate, organochlorine and organo phosphates and less susceptible to 30 min exposure. The reason is that the insecticidal exposure in Thathaneri of Madurai was low and thermal fogging operations with insecticides were intensified during the outbreak period of dengue / chikungunya. Use of pyrethroid compounds in the National Control Programmes has also been low during the last two to three decades, whereas other organochlorine and organophosphorous compounds are continued under the operation for more than seven decades (NVBDCP, 2015b). Thus, *Ae.aegypti* population was found susceptible for various types of compounds in the present study. At present, the use of pyrethroids against *Ae. aegypti* in field has certainly led to the development of resistance, however to delay the rotational use of various pyrethroids is essential. In a study carried out on relative susceptibility of some common mosquito vector larvae to synthetic insecticidal compounds in north-western Rajasthan reported that both larval and adult population has developed resistance against different disease vectors (Bansal and Singh, 2007). This study is immensely important to develop future resistance management strategies and will also lead to the selection of suitable insecticides for mosquito control to apply in particular area. The study of the susceptibility status of Madurai strain *Ae. aegypti* to different insecticides has projected as an overview. The observations made in the present study will be a baseline to initiate in depth studies and would help to guide insecticide-based strategies. Also, broader monitoring of insecticide resistance on this species is required as a continuous process to determine the molecular and biochemical mechanisms involved in insecticide resistance. Since dengue cases are rising annually, it is recommended that regular resistance surveillance should be focused in different endemic areas by using large numbers of vectors in order to target the specific interventions.

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