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Biochemical mechanisms of insecticide resistance in field population of Dengue vector *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT
Insecticide resistance has been known to be prevalent in several insect species including mosquito. It has become a major problem in vector control programme due to pesticide resistance through detoxification enzymes. The present study investigated the toxicity of *Ae. aegypti* to organophosphates and pyrethroid insecticide and biochemical mechanisms involved in insecticide resistance in larval population. Larval bioassay revealed an LC_{50} value of 0.734 ppm for dichlorvos and 1.140 ppm for λ-cyhalothrin exposure. Biochemical assay revealed increased activity of AChE (0.3 µmole/mg protein) and GST in dichlorvos (1-1.5 µmole/mg protein) treatment and esterase activity in λ-cyhalothrin treated compared to control activity. These studies suggest that AChE and GST is associated with organophosphate and esterase associated with pyrethroid resistance in *Ae. aegypti*.

Keywords: Dengue vector, Glutathione S-transferase, resistance, pyrethroid, organophosphate.

1. Introduction
Vector borne diseases are among the major causes of illness and death in tropical and subtropical countries and worldwide more than one million people die due to vector borne diseases every year [1]. Mosquitoes are one of the important vectors transmitting a variety of diseases in humans and domesticated animals, among which *Aedes aegypti* (*Stegomyia aegypti*) is the primary vector for dengue and Chikungunya in India [2]. The mosquito control, therefore, continues to be an important strategy in controlling and preventing the mosquito-borne diseases [3, 4]. Chemical insecticides have been the most favourite vector control method throughout the world; among insecticides organophosphates and pyrethroids have been used for the control of larval and adult mosquito population [5]. The widespread use of chemical insecticides has led to development of insecticide resistance in several insects including mosquitoes [6].

Currently insecticide resistance management is considered as a serious public health challenge throughout the world. For the control of mosquito population, continuous monitoring of insecticide resistance is important. Mechanisms of insecticide resistance in mosquito vectors are being studied worldwide, as they elucidate the pathways of resistance development and help in newer strategies of preventing and delaying insecticide resistance. Biochemical estimations have been method of choice for understanding the mechanism of insecticide resistance among insects. With sophisticated and sensitive biochemical assays, it is now possible to analyze the mechanisms of insecticide resistance with a fair degree of accuracy. Among the metabolic based insecticide resistance mechanism, non-specific esterase, Glutathione S-transferase (GSTs) and, P450 mediated monooxygenase (MFOs) are known to be involved in the detoxification of organophosphate, pyrethroid, and carbamate insecticides [5]. In insects, AChE has mainly been studied in relation to insecticide resistance because the enzyme is the target of organophosphate and carbamates insecticides and its insensitivity to insecticides is one of the main factors accounting for resistance [7]; thereby these enzymes are used as reliable marker to assess the impact of toxic compounds on a range of test organisms [8].

Many studies have been conducted throughout the world to understand the mechanisms of pyrethroid resistance in insects, especially in mosquitoes. Using biochemical methods, the increase of enzyme activities of esterase, GST and P450- mediated monooxygenase has been

reported to play a role in the metabolism of pyrethroids, which has led to failures in vector borne disease control, especially in dengue control [9]. Hence the present study was investigated to understand the mechanism of insecticide resistance in *Aedes aegypti* field larval population.

2. Materials and Methods

2.1 Mosquito culturing and Maintenance

Aedes aegypti egg mass were collected from Salem city by placing ovitraps in fresh water household containers and immature of *Ae. aegypti* collected from tyres and tanks around Salem city in Tamil Nadu, India. The colonies were reared in the laboratory and early 4th instar F1 larval population were used as test sample, the cultures were maintained under controlled conditions of 26±1 °C, 12 h light and 12 h dark photoperiod. The control larvae used in the present study was obtained from the laboratory stock culture F5.

2.2 Insecticides

Dichlorvos, 76% EC was obtained from Syngenta India Ltd., India and λ-cyhalothrin, 5% EC insecticides was purchased from commercial market Valsad, Gujarat, India.

2.3 WHOPES Larval bioassay

Bioassay method was followed as per WHOPES protocol [10]. The different concentrations of insecticide solution were prepared in water ranging from 0.001, 0.01, 0.1, 0.2, 0.5, 1, 2, 5, 10 ppm. Larval released in water alone served as Control (without treatment). Three replicates were kept for each concentration and in each replicate 25 early 4th instar larvae were released. Larval mortality was recorded after 24 hours observation and mortality was calculated using Abbott's formula [11]. The mortality data were further subjected to probit analysis for estimating LC_{50} and LC_{90} values using Finney method [12].

2.4 Enzyme preparation

Batches of 50 early 4th instar larvae from control and live larvae in treatments were homogenized in 1ml of ice-cold 0.01M phosphate buffer (pH-7) using pre-chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 30 minutes at 4˚C. Solid debris and cellular matrix was discarded. The supernatant was collected and stored at -20 ºC and immediately used as enzyme source. Protein content of supernatant was determined as described by Lowry *et al.* (1951) [13] and bovine serum albumin was used as the standard protein.

2.4.1 Acetylcholinesterase assay

Acetylcholinesterase (AChE) was determined using acetylthiocholine-iodide (ATChI) as substrate according to the method of Ellman *et al.* (1961) [14]. A total of 3.6 - ml reaction mixture (0.1 ml of supernatant, 2.4 ml of 100 mM phosphate buffer [pH 7.4], and 0.1 ml mixture [0.075 M acetylthiocholine-iodide]: 0.01M 5,5˙-dithio-bis [2-nitrobenzoicacid] was first incubated for 15 min at 27 ºC, and then 0.001 M eserine was added and mixed. Absorbance at 412 nm was recorded with spectrophotometer.

2.4.2 Esterase assay

Esterase activity was determined using the Kranthi method described earlier by Kranthi, (2005) [15]. A total of 6 ml reaction mixture (0.2 ml of enzymatic source, and 0.1 ml of 0.3 mM α -napthylacetate) was first incubated for 20 minutes at 30 ºC in dark, then 1.0 ml mixture of fast blue BB salt and sodium dodecyl sulfate (SDS) solution at 2:5 ratio) was added and mixed. Absorbance at 412 nm was recorded using spectrophotometer. The activity of α-naphthyl acetate in different treatment was calculated from a standard curve.

2.4.3 Glutathione S-transferase assay

GST activity was carried out using the method of Habig *et al.* (1974) [16]. 3-ml reaction mixture contain (0.05 ml of 50 mM CDNB [1-chloro-2, 4-dinitrobenzene], 0.15 ml of reduced glutathione (GSH) were added to 2.79 ml of 40 mM buffer saline (pH 6.8) and 0.01 ml enzyme source) was added. The mixture was incubated for 2-3 min at 20 ºC. The increasing in absorbance value (OD) was recorded for 5 min, and the GST activity (μM mg $protein^{-1}$ min^{-1}) was then calculated. All enzyme activities are subjected to One Way ANOVA analysis.

3. Results and Discussion

The results of larval bioassay to dichlorvos presented an LC_{50} value of 0.734 ppm, whereas λ-cyhalothrin presented a LC_{50} of 1.140 ppm as compared to laboratory population LC_{50} value 0.0032 and 0.034 ppm respectively, which suggest the presence of organophosphate and pyrethroid resistance in field populations. Similar results were found in *Aedes aegypti* population from Brazil which showed resistance to both organophosphate and pyrethroid insecticide in field population [17]. Pimsaman *et al.* (2009) reported high level of resistance to permethrin and cypermethrin in the north-east of Thailand [18].

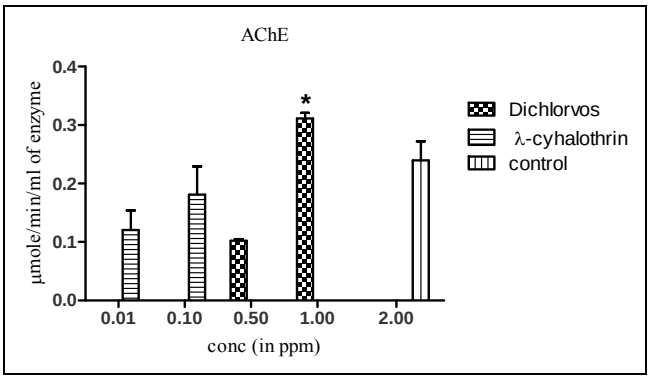
AChE insensitivity is a common resistance mechanism observed in several species of arthropods, which is due to the selection of a modified AChE less sensitive to these insecticides [19]. Numerous studies have demonstrated that the increased metabolic detoxifications were involved in organophosphate resistance in many insect pests [20]. In the present study, increased activity of AChE was observed in dichlorvos treatment $p<0.05$, (0.3 μmol/min/mg of protein) as compare to control activity (Figure 1), suggest that organophosphate resistance is rather increased activity of detoxification enzymes occurred in the field population.

Detoxification mechanism mediated through non-specific esterases is another major mechanism of resistance in insects. These esterases detoxify organophosphate (OP), carbamates (CB) and synthetic pyrethroid (SPs) pesticides by two main ways, hydrolysis of the ester bond and binding of the pesticide (OP) to the active site of esterase [21]. Most of the insecticide groups contain ester linkages which are susceptible to hydrolysis by esterase. Resistant insects usually show a very high activity of estrases [22, 23]. In this study esterase activity increased in λ-cyhalothrin treatment, which suggests that esterase based pyrethriod detoxification, is important mechanism in *Ae. aegypti* larvae (Figure 2). On the contrary there was a very high activity of GST in dichlorvos treatment $p<0.05$, (1.5 μmol/min/mg of protein) than control activity (Figure 3), which support that GST may also be involved in organophosphate detoxification in *Ae. aegypti* mosquito population [24]. The present data suggest that development of resistance to organophosphate and pyrethroids in *Aedes aegypti* larvae is due to increased activity and metabolism of both insecticides by AChE, GST and esterase metabolic enzymes.

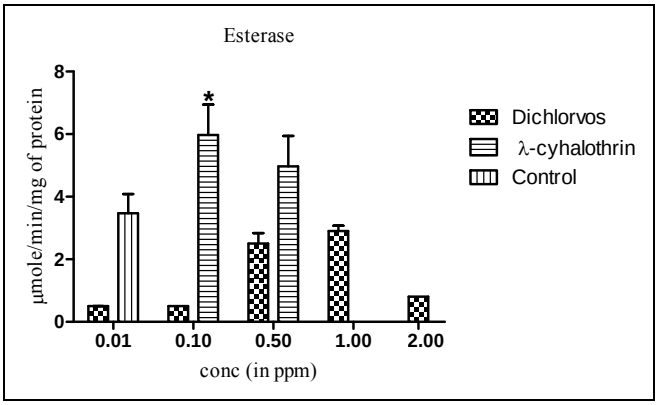
Table 1: Toxicity of *Ae. aegypti* against dichlorvos and λ-cyhalothrin after 24 hours observation compared with laboratory colony.

Pesticides	population	LC_{50} (95% Fl)[a]	LC_{90} (95% Fl) [a]	df	χ^2
Dichlorvos	field	0.734 (0.087-1.271)	2.711 (1.813-3.271)	2	1.321
λ-cyhalothrin	field	1.140 (0.891-2.301)	3.735 (2.410-4.110)	2	0.422
Dichlorvos	lab	0.0032 (0.003-0.0035)	0.005 (0.005-0.006)	1	0.023
λ-cyhalothrin	lab	0.034 (0.032-0.040)	0.071 (0.065-0.082)	1	0.045

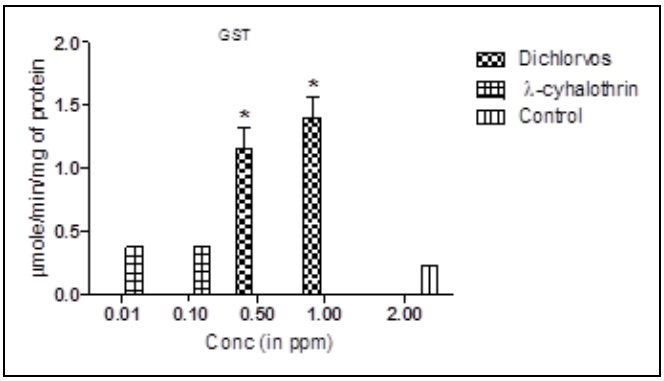
[a] Lethal concentrations (ppm) for 50 % and 90% of the larvae after 24h exposure.



(Fig. 1)



(Fig. 2)



(Fig. 3)

Fig: 1-3. Detoxification enzyme profile of (AChE, Esterase, GST and control) on 4th instar *Ae. aegypti* larval exposure to dichlorvos and λ-cyhalothrin insecticide.
Indicates significant difference between treatments at $p<0.05$ (One Way ANOVA- Bonferronii multiple comparison tests).

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