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BIOCHEMICAL STUDIES OF INSECTICIDE RESISTANCE IN *AEDES AEGYPTI* BY USING AN ORGANOPHOSPHATE (TEMEPHOS) INSECTICIDE

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ABSTRACT

Mosquito borne diseases are dramatically affect public health and represent a major burden in terms of economy and development worldwide. Vector borne diseases are global problem it is trend that may only increases if global temperature rises and demographic trends continue and their economic and social impact are enormous. The mosquitoes control largely relies on insecticide applied to control the larval habitats, indoors against adult mosquito population worldwide and there is evidence that it has compromised the success of control interventions. Insecticide play a vital role in the fight against the diseases by controlling the vectors in order to improve the public health and however resistance to commonly used insecticides such as temephos. The present study was carried out to determine the metabolic resistance of detoxifying enzyme level in the resistant strain of five generation of Aedes aegypti. Biochemical analysis was done on Aedes (stegomyia) aegypti mosquitoes to determine the activities of enzymes such as α and β esterases, MFO, GST and AchE. These tests were performed in five generation of resistant strain of Aedes aegypti. The resistant generation shows the increased mean value compared with the control and susceptible strain. This result indicates the detoxifying enzyme level was progressively increased from R1 to R2 and shows a level of significant was 0.001. The result of present observation was indicating the resistance would develop among the population of Aedes aegypti.

KEY WORDS

Vector borne diseases, insecticide, resistance, mosquito, temephos, detoxifying enzymes.

INTRODUCTION

Aedes aegypti is the major vectors of arboviral diseases such as dengue fever, yellow fever and chickungunya (Weaver and Reisen., 2010). Dengue fever is a major public health concern in India. The major focus in dengue diseases control program of the island is vector control through elimination of breeding sites and application of insecticides. Spraying of insecticides has been widely used for several years in India to controlling of dengue vectors especially during diseases outbreaks. Four major groups of synthetic insecticides are organophosphate, organochlorides, carbamates and pyrethroids are commonly used in pest control programmes. The majority of cases of insecticide resistance are either based on increased metabolic detoxification or reduction in the sensitivity of the insecticide's target site to inhibition. The major metabolic enzymes involved in resistance are esterases, oxidases and glutathione-s- transferases (GST) (Brown & Brogdon, 1987). *Aedes aegypti* is the main vector of dengue and yellow fever; it has a significant of public health importance in the tropics. The global incidence of dengue has increased dramatically in the past decade and now there are approximately 2.5 billion people at risk with an estimated 50–100 million cases of dengue fever and 250,000–500,000 cases of dengue

hemorrhagic fever in worldwide (WHO, 2008). At present, there is no treatment or vaccine available for dengue, and therefore vector control is the only available means of prevention. However, this method is threatened by increasing reports of *Ae. aegypti* resistance to common classes of insecticides including organochlorines, organophosphates, carbamates and pyrethroids (Georghiou and Lagunes-Tejeda, 1991). Dichloro diphenyl trichloroethane (DDT) was the main insecticide used to control Caribbean populations of *Ae. aegypti* during the first half of the last century but was later replaced by organophosphates due to the problem of insecticide resistance. This replacement was short-lived because resistance quickly developed to this group of insecticides (Georghiou *et al*., 1987; Rawlins and Ou Hing Wan, 1995; Rawlins, 1998) prompting the introduction of pyrethroids. However, pyrethroid resistance was reported in *Ae. aegypti* from Puerto Rico (Hemingway *et al.,* 1989), the Dominican Republic (Mekiuria *et al.,* 1991) and Cuba (Rodríguez *et al*., 2005). In mosquitoes esterases are the primary mechanisms involved in organophosphate, carbomate, pyrithroid resistance. Resistant in insects may be detoxify or destroy the toxin faster than susceptible insects or quickly rid their bodies of the toxic molecules. Metabolic resistance is the most common mechanism and often presents the greatest challenge. Insects used their internal enzyme systems to break down insecticides. Resistant strains may possess higher levels or more efficient forms of these enzymes. In addition to being more efficient, these enzyme systems also may have a broad spectrum of activity (i.e., they can degrade many different insecticides). Metabolic resistance is caused by alterations in levels or activities of detoxification enzymes; elevated activities of cytochrome P450 monooxygenase, glutathione-s-transferase (GST) and carboxylesterases. These enzymes act to metabolize insecticide to non-toxic materials with a very fast rate, or reverse binding of the insecticide (hijacking process) causing it to no longer become effective (Hemingway et al., 1998, Nazni *et al.*, 2004). The synthetic pesticides are more effective and fast acting, repeated and indiscriminate application often lead to the development of resistance, resulting in rebound of the vector population and its disease potential. Quantitative metabolic Enzymes assay have been commonly used in the detection of insecticide resistance because it is very simple, sensitive and gives results rapidly even at low frequencies (Brogdon, 1989 and Lee, 1990). Metabolic resistance is a dynamic process involving potent regulation of the mosquito detoxification system in order to counteract the chemical aggregation caused by insecticides. Metabolic resistance consists of elevated levels of enhanced activities of insecticides detoxifying enzymes in resistant insects. As a resulting in a sufficient proportion of insecticides molecules being metabolized before reaching their target in mosquitos' nervous system (Brooke and Koekemoer, 2010). Insecticide detoxification can be the consequence of the over production or structural modification of a single enzyme but different enzymes from the same or different families can also act together simultaneously or sequentially to confer resistance. To date most studies were focused on the over production of detoxification enzymes while the selection of particular detoxification enzymes alleles conferring enhanced insecticide degradation has been rarely studied in mosquitoes (Hardstone *et al*., 2010).

MATERIALS AND METHOD

Collection of mosquitoes

Aedes aegypti was collected as larvae from the household region in and around the annamalai nagar, chidhambaram. Larvae were collected and reared in to adult under laboratory conditions for developing a resistance strain of *Aedes aegypti*. The resistance developing larvae was used to analyse the biochemical studies of insecticide by using temephos. The concentration was chosen the present study were 0.002ppm, 0.004ppm, 0.006ppm, 0.009ppm, 0.012ppm, 0.015ppm, 0.020ppm, 0.025. Immature stages was transported in 500ml flasks to the insectorium where the F1 to F5 generations were obtained under controlled conditions of temperatures is 28°C ± 2 °C, relative humidity (60% ±10%) and a photo period of 12h light and 12h dark.

Biochemical Assay

Five different detoxifying enzymes were quantified for each mosquitos' larvae for fourth instars. α, β-esterases, MFO, GST and AchE (acetylcholine esterase) followed by biochemical assay protocol (Brogdon, 1989; Brogdon *et al*., 1990; Brogdon and McAllister, 1997; Valle *et al.*, 2006). These assays were carried out for 50 fourth instars larvae of *Aedes aegypti*. Each larvae was individually homogenized in 100µl of 0.01M of potassium phosphate solution, pH is 7.2 and suspended

in 2ml of the same buffer, Aliquots of 100 µl were transferred to microtier plates, each individual sample was analyzed in triplicates on each. To measure the acivity of α, β- esterases 100 µl of α, and β naphthyl acetate was added to each well for 10min of incubation at room temperature. Then 100 µl of dianisidine was added followed by 2min of incubation. Absorbance was read at wavelength of 540nm. For the MFO assay 200µl tetramethyl benzidine (TMBZ) previously dissolved in methanol and 0.25 M sodium acetate buffer were added to each well. Subsequently 25 µl of 3% of hydrogen peroxide was added. After 5 min of incubation at room temperature the microplate was read at a wave length of 620nm. For GST 100 µl of reduced glutathione and 100 µl 1-choloro2, 4'- dinitrobenzene (previously diluted in acetone and KPO_4 buffer) were added to each well. Absorbance readings were taken immediately (T_0) at a wave length of 340nm and a second reading was done after 10min (T_{10}). The absorbance values obtained at T_0 were subtracted from the values obtained at T_{10}. For the AchE assay which determines if altered acetylcholine site is present. 100 µl of acetylcholine iodide (ATCH) with propoxur and 10 µl of dithiobis-2-nitrobenzoic acid (DTNB) were added to each well. The plate was read immediately (T_0) at a wavelength of 414nm and after 10 min (T_{10}) at the same wavelength. We subtracted the T_0 reading from the T_{10}. Positive and negative control was included for MFOs and esterases. The same volume of homogenate used in the respective assays was used in the controls. For α and β esterases, α-and β-napthyl acetate solutions were used respectively. Cytochrome-C solution was the positive control for the MFO assays. KPO4 buffer was used as a negative control.

Statistical analysis

All the enzyme were calculated and the mean of enzyme activities in each *Aedes aegypti* mosquito sample for the five generation were compared with the control by analysis of variance (ANOVA) using SPSS statistical program (SPSS Inc., 2001). Fisher's least significant difference (LSD) test was used to separate mean at a =0.001.

RESULT

The detoxifying enzyme avititvites were crboxylesterases (α and β), GST, MFO, AchE for *Aedes aegypti* are shown in table: 1. the organo phosphorous insecticide temephose treated mosquitoes shows the biochemical assay of the mean value located in the table. The resistance strain was developed in five generation. The α and β estreases, mixed function oxidases, glutathione -s -transferase and acetyl choline esterase shows highest detoxification of enzyme level in every resistance stain (R1 –R5). The lowest mean value was observed in the control and susceptible strain.

Table: 1 Enzyme activities of *Aedes aegypti* treated with temephos

Resistance stain of *Aedes aegypti*	α-esterases (nmole/α-naphthol/min/mg/protein)	β- esterases (nmole/β naphthol/min/mg/protein)	MFO (nmole product/min/mg protein)	GST (nmole CDNB/min/mg protein)	AchE
Control	265.19 ± 27.83	43.51± 3.07	29.75± 1.75	23.51±3.09	11.39±0.83
R1	366.91±20.98	49.39±1.53	48.93±3.96	28.79±1.77	15.20±2.10
R2	390.45±9.59	55.85±1.67	55.16±2.02	45.12±5.28	18.55±0.54
R3	414.88±69.68	72.046±5.62	71.10±5.89	62.97±5.58	22.21±1.05
R4	1102.60±59.27	97.46±13.71	118.14±1.20	71.55±4.68	28.74±1.52
R5	1239.84±29.31	186.92±16.41	137.63±16.12	103.92±15.40	33.86±0.38

Mean ± S.E. Significant increase in compared to the control (p<0.001, fisher's least significant difference test).

DISCUSSION

The present study was demonstrated that toxicological research confirmed the high level of resistance was observed in the resistance stain of *Aedes aegypti* to the organophosphate insecticide of temephose at the larval stage. The carboxyl esterases based resistance mechanism is a major mechanism of organophosphate resistance in insects (Hemingway and Karunaratne 1998). *Ae.aegypti* resistance to organophasphate in the caribean linked to elevated carboxyl esterases activities was described by Rodriguez *et al.*, 2001. The significant increased carboxylesterses was observed in fenitrothion organophasphate resistance in *Ae. aegypti* in Nakhon Sawan Jirakan- janakit., 2007. The esterase-based mechanisms was reported by Ranasinghe & Georghiou, Rodriguez et al., 2002 and are responsible for

temephose organophosphate resistance *Culex quinequefaciatus* and *Ae. Aegypti*. The MFO was a prominenet enzyme responsible for pyrithroid resistance in *Ae. aegypti* in Thailand (Pethuan *et al.*, 2006). This present study proved that the resistance level was increased in every resistance stain of *Ae. aegypti*. The increased level of MFO indicates the importance of metabolic resistance mechanisms in Martinique. High mean values of esterases activity resulting in fenitrothion resistance in Nakhon Sawan could be explained by its history of insecticide uses of pyrithroids temephos and malathion. GST activity and DDT-resistance was first detected in houseflies by Clarke & Shamaan (1984) and a similar relationship has since been demonstrated in the mosquitoes *Aedes aegypti, Anopheles gumbiae. Atz.culicifacies, An.subpictus* and *Culex quinquefasciatus* (Grant & Matsumura, 1989; Hemingway *et al.,* 1985; Herath *et al.,* 1988; Amin & Hemingway, 1989). Acetylcholinesterases (AchE) is critical for hydrolysis of acetylcholine at cholinergic nerve synapses and is a target for organophoshphate and carbomate insecticides (Anthony *et al*., 1995). Altered AchE is an important resistance mechanism to organophosphates in many insects. The existent of enzyme production in mosquito through the prior insecticide or chemical pressure in the area could constitute resistence against alternate insecticides. The present study was demonstrated that the increased mean value of the detoxifying enzyme activities was proved to develop the resistance of *Aedes aegypti.*

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