



CARBOXYL ESTERASE AND GST LEVELS OF *CULEX QUINQUEFASCIATUS* SAY AND *AEDES AEGYPTI* L. COLLECTED FROM THE SELECTED SITES OF MALAPPURAM DISTRICT

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ABSTRACT

Mosquitoes are the dreadful insects which transmits bacterial, viral and parasitic diseases including Malaria, Filariasis, Yellow fever, Chikunguniya, Dengue fever, Japanese encephalitis, Zika, West Nile fever etc. A careful and prolonged control of the vector can help to eliminate these diseases, but it is not an easy task due to its natural tolerance and development of resistance to available insecticides. The early detection of resistance in vector mosquitoes will help the local government to plan and select appropriate alternative control measures or insecticides for effective control. Quantitative metabolic enzymes assay have been commonly used in the detection of insecticide resistance because it is very sensitive and gives results rapidly even at low frequencies. In the present study, Carboxylesterase and Glutathione S-transferase (GST) activity in *Culex quinquefasciatus* Say and *Aedes aegypti* L. of the selected sites from Malappuram District were studied. Enzyme activities were analysed to estimate the detoxification status of these mosquito species. There is a significant difference in carboxylesterase and GST activities in laboratory and field populations. *Cx. quinquefasciatus* and *Ae. aegypti* of Ponnani area shows higher enzyme activity when compared to Tirur and Calicut University. Regular spraying of insecticides occurs in Ponnani and Tirur, but there is no insecticide application in Calicut University. The results reinforce the need for constant surveillance of mosquito populations susceptibility against the insecticides used in control programs as well as their effectiveness in the field.

KEY WORDS: *Culex quinquefasciatus*, *Aedes aegypti*, Carboxylesterase, Glutathione S-transferase (GST), Detoxification.

INTRODUCTION:

Synthetic insecticides are the main strategy in the management of medical and veterinary important insect vector populations (Zain and Jambulingam 2007). During the past two decades, considerable progress has been made in the development of natural and synthetic compounds that are capable of interfering with the growth and development process of the target insects. Man's effort to control insect pests and vectors, especially by application of synthetic chemical pesticides such as DDT, will not provide any considerable success after the regular and improper usage of insecticides and it also lead to the selection of insecticide resistant populations in the field (Beard *et al.*, 1998)

The mechanism contributing to this undesirable trait of insecticide resistance is characterized into four factors mainly: increased metabolism for non-toxic compounds, decreased site sensitivity, decreased rates of insecticide penetration and the increased rates of insecticide excretion. Among these factors, the metabolic resistance is the most important one as it enhances the activities of specific enzymes, thus aiding in the detoxification and degradation of insecticides. There are three major groups of such enzymes; Glutathione-S-transferase (GST), monooxygenases and carboxylesterases (Hemingway *et al.*, 2004).

The present study aims to find out the detoxification enzyme levels mainly glutathione s transferase and carboxyl esterase of *Cx. quinquefasciatus* and *Ae. aegypti* collected from Ponnani, Tirur and Calicut University Campus of Malappuram District, Kerala.

MATERIALS AND METHODS:

Cx. quinquefasciatus Say. and *Ae. aegypti* (L) larvae were collected from three different localities of Malppuram District in 2015 and were fed with yeast and dog biscuit in the ratio 3:1. Emerged adults were identified and the F1 generation were used for the study, mainly fourth instar larvae were used for all the assays. Susceptible laboratory population were collected from CRME (ICMR), Madurai and reared in our Laboratory without exposure to any chemical insecticides.

Areas of Collection: Malappuram is situated in the midland area of the Kerala state. Tirur is a municipal town in Malappuram district. It spreads over an area of 16.55 square kilometres. It is one of the business centres of Malappuram district and is situated 26 kilometres west of Malappuram and 41 kilometres south of Kozhikode. Tirur is also a major regional centre for fish and betel leaf and has an average elevation of two metres. Ponnani is a municipality in Ponnani taluk and Malappuram district. It spreads over 19.23 kilometres. It is situated at the mouth of Bharathappuzha and is bounded by the Arabian Sea on the west. The tidal port is an important fishing centre in the region. Ponnani is one of the oldest ports on Malabar Coast and has contributed to the trade and economy of Malabar from very early times. Calicut University is located at Thenhipalam Panchayath in Malappuram. It is established in 1968. The campus locales spread over around 500 acres (2.0 km²) in rural outskirts of Malappuram, had the feel of a hill station.

Biochemical assays:

Biochemical assays were used to quantify levels of non-specific esterases, in 4th instar larvae of the F1 generation. 30 fourth instar larvae were taken from each of

the samples for the assays. The assays were done according to the protocol provided in techniques to detect insecticide resistance mechanism; Field and Laboratory Manual, [Techniques to detect insecticide resistance mechanisms, WHO/CDS/CPC/MAI: /98; 6]. Total protein was measured using the protocol of Bradford, 1976.

Statistical Analysis:

Statistical analysis (ANOVA) was performed using Statistical package SPSS 20.0.

RESULTS AND DISCUSSION:

Metabolic detoxification is the most common resistance mechanism that occurs in almost all insects after the continuous exposure to the insecticides. The best method of choice for understanding the mechanism of insecticide resistance among insects is the biochemical estimations, which are sophisticated and highly sensitive (Hemingway and Ranson, 2000).

Esterase based resistance to organophosphorous and carbamate insecticides are common in almost all insects. The esterase either produce broad spectrum insecticide resistance through rapid binding and slow turnover of insecticide or narrow spectrum resistance through metabolism of a very restricted range of insecticides containing a common ester bond. Resistance to organophosphate insecticides has been associated with the carboxylesterase activity changes in many insects and the nature of changes varies widely according to the sensitivity and differences in strains. Elevated esterase activity accounts for resistance to organophosphates, carbamate and pyrethroid insecticides (Hemingway and Karunaratne, 1998).

Figure 1 & 2 shows the data regarding α & β esterase levels in the mosquitoes collected from Calicut University Campus, Tirur, Ponnani and laboratory population. From the graph it was clear that the carboxylesterase levels were high in the mosquito populations which were collected from regularly insecticide spraying area. In Calicut University campus scarcely the spraying occurs, but in Ponnani and Tirur regular spraying of insecticides occurs. There was a significant increase in α & β esterase activity ($p < 0.05$; ANOVA) and the alteration in the activity of α & β esterase levels indicating the detoxification levels were higher in Ponnani population where insecticide application occurs regularly. In these places malathion was used for fogging and temephos and *Bti* used as larvicide.

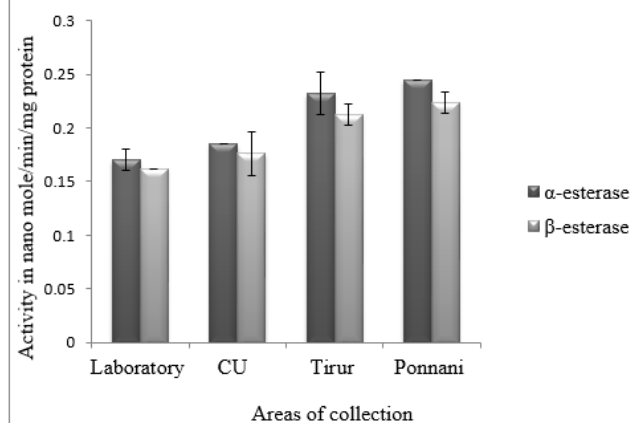


Fig 1: Carboxylesterase activity of *Cx. quinquefasciatus*

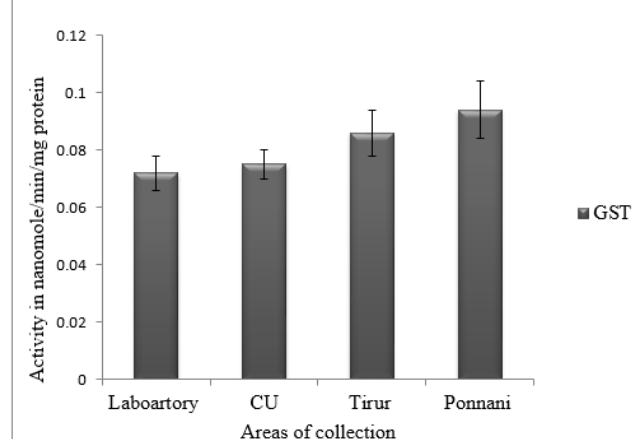


Fig 4: GST activity of *Ae. aegypti*

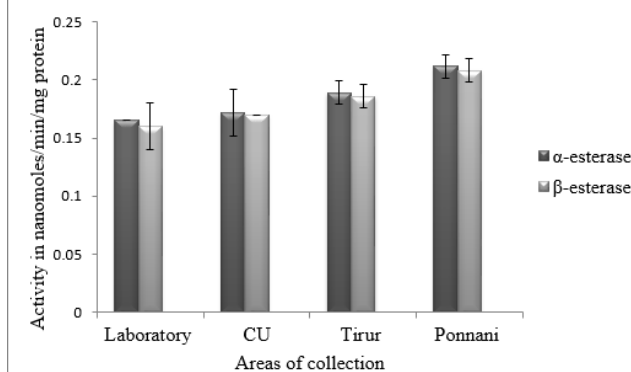


Fig 2: Carboxylesterase activity of *Ae. aegypti*

Several studies have shown that insecticide-resistant insects have elevated levels of Glutathione S-Transferase (GST) activity in crude homogenates, which suggests the role of GSTs in insecticide resistance towards DDT (Hemingway and Ranson, 2000), Organophosphates (Fournier *et al.*, 1992), Organochlorines (Grant *et al.*, 1992) and pyrethroid insecticides (Kostaropoulos *et al.*, 2001). The GSTs often act as a secondary resistance mechanism in conjunction with a P 450 - or esterase-based resistance mechanism (Hemingway *et al.*, 1991).

In the present study there is significant difference in the activity of GST in *Ae. aegypti* and *Cx. quinquefasciatus* collected from the different areas. In both cases the maximum activity of GST was shown by Ponnani population followed by Tirur and Calicut University population.

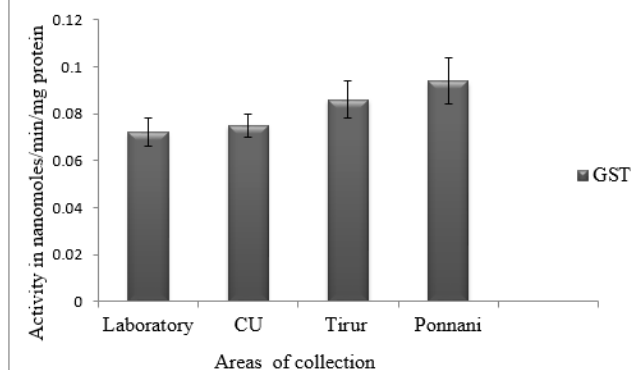


Fig 3: GST activity of *Cx. quinquefasciatus*

Table 1 shows the data regarding the ratio of detoxification enzyme levels of field and laboratory population. Detoxification ratio or resistance ratio (RR) was calculated using dividing the value of Mean enzyme level of field strain with Mean enzyme level of laboratory strain (Dhang *et al.*, 2009). For all the three enzymes the detoxification ratio was higher in *Cx. quinquefasciatus* collected from Ponnani. In the case of *Ae. aegypti* also Ponnani population shows higher value as compared with Tirur and Calicut University mosquito population. It indicates the relation of detoxification and exposure to insecticides.

Table 1: Detoxification ratio of enzymes of field populations with that of laboratory population

Mosquito population	α-esterase detoxification ratio	β-esterase detoxification ratio	GST detoxification ratio
<i>Cx. quinquefasciatus</i>			
CU	1.09	1.09	1.06
Tirur	1.36	1.31	1.2
Ponnani	1.44	1.38	1.4
<i>Ae. aegypti</i>			
CU	1.04	1.06	1.04
Tirur	1.15	1.16	1.19
Ponnani	1.28	1.3	1.31

Specific activities of α esterase, β esterase and GST in *Cx. quinquefasciatus* and *Ae. aegypti* were studied and it reveals that there was significant difference in the activity of carboxylesterase and GST enzyme levels between the laboratory and field populations. Based on the results it was found that *Cx. quinquefasciatus* and *Ae. aegypti* of Ponnani area shows higher α esterase, β esterase and GST activity as compared with mosquitoes collected from Tirur and Calicut University area. All the three enzymes activity were less in Calicut University sample as compared with the other two study sites and it points out the role of exposure to insecticides in the detoxification enzyme elevations.

The present study observed that there is significant difference ($p < 0.05$) in the detoxifying enzyme levels in the field and laboratory population. The development of detoxification mechanism is also influenced by the mode of insecticide application in the field. From the results it was clear that the repeated exposure to insecticides, will results in development of detoxification mechanisms in faster rates.

Biochemical analysis showed significant increase of esterase, glutathione-S-transferase activities in the field populations of both *Cx. quinquefasciatus* and *Ae. aegypti* collected from Ponnani and Tirur as compared with Calicut University sample and Laboratory population. Actually the resistance development to insecticides is a multifactorial trait that may be affected by the nature of breeding sites, the amount, frequency and time of exposure of insecticide application. The elevated enzyme levels in *Cx. quinquefasciatus* and *Ae. aegypti* populations reinforces the need for constant surveillance of mosquito populations susceptibility against the insecticides used in control programs. This must begin before the use of insecticides in the field, such that initial levels of resistance were determined and it will facilitate the resistance management.

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REFERENCES:

1. Zain, M and Jambulingam (2007) Global insecticide use of vector-borne disease control, 3rd edn. WHO, Geneva.
2. Beard, C. B., Durvasula, R. V. & Richards, F. F. (1998). Bacterial symbiosis in arthropods and the control of disease transmission. *Emerging Infectious Diseases*, 4(4), 581–591.
3. Hemingway, J., Hawkes, N. J., McCarroll, L., & Ranson, H. (2004). The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology*, 34(7), 653–665.
4. World Health Organization/Department of Communicable Disease Prevention Control and Eradication. (1998). *Techniques to detect insecticide resistance mechanisms. Field and laboratory manual*. (Dr M. Zaim/WHOPES, Ed.). Geneva: World Health Organization.
5. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254.
6. Hemingway, J., & Ranson, H. (2000). Insecticide Resistance in Insect Vectors of Human Disease. *Annual Review of Entomology*, 45(1), 371–391.
7. Hemingway, J., & Karunaratne, S. H. (1998). Mosquito carboxylesterases: a review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Medical and Veterinary Entomology*, 12(1), 1–12.
8. Fournier, D., Karch, F., Bride, J. M., Hall, L. M. C., Bergé, J. B., & Spierer, P. (1989). *Drosophila melanogaster* acetylcholinesterase gene. Structure, evolution and mutations. *Journal of Molecular Biology*, 210(1), 15–22.
9. Grant, D., Bender, D. M. & Hammock, B. (1992). Genetic and molecular evidence for a trans-acting regulatory locus controlling glutathione S-transferase-2 expression in *Aedes aegypti*. *MGG Molecular & General Genetics*, 234(2), 169–176.
10. Kostaropoulos, I., Papadopoulos, A. I., Metaxakis, A., Boukouvala, E., & Papadopoulou-Mourkidou, E. (2001). Glutathione S-transferase in the defence against pyrethroids in insects. *Insect Biochemistry and Molecular Biology*, 31(4–5), 313–319.
11. Hemingway, J., Miyamoto, J., & Herath, P. R. J. (1991). A possible novel link between organophosphorus and DDT insecticide resistance genes in *Anopheles*: Supporting evidence from fenitrothion metabolism studies. *Pesticide Biochemistry and Physiology*, 39(1), 49–56.