ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 09, pp.2449-2454, September, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

DETOXIFICATION ENZYME ACTIVITIES IN LABORATORY COLONIZED CARBOFURAN RESISTANT *Culex quinquefasciatus* SAY

[*1]Aneesh, E. M. and [2]Vijayan, V. A.

[1]Communicable Disease Research Laboratory, Department of Zoology, St. Josephs College, Irinjalakuda, Thrissur
[2]Vector Biology Research Laboratory, Department of studies in Zoology, University of Mysore, Manasagangothri, Mysore –06, Karnataka, India

ARTICLE INFO

Article History:
Received 25th June, 2013
Received in revised form
18th July, 2013
Accepted 27th August, 2013
Published online 14th September, 2013

Key words:
Culex quinquefasciatus,
glucose-6 phosphate,
dehydrogenase (G6PD).

ABSTRACT

Carbofuran is a systemic insecticide/nematicide used extensively in modern agriculture to combat various major insect pests and vectors. Selection experiments for analyzing carbofuran resistance development were carried out for ten generations at Mysore with *Culex quinquefasciatus*, a widely dispersed domestic mosquito and the vector of lymphatic filariasis. The mosquito populations were continuously exposed to different carbofuran concentrations following the WHO method. Simultaneously detoxifying enzymes such as alpha esterase and glucose-6 phosphate dehydrogenase (G6PD) have been assayed qualitatively and quantitatively to relate the biochemical mechanisms involved in tolerance development. The selection experiments and enzyme assay have revealed a steady increase in tolerance build up and the target enzyme level. The results of larval selection test up to F10 revealed a significant increase in tolerance development in every generation compared to the susceptible F1. The results revealed up to 16.22 times tolerance after 10 generations.

INTRODUCTION

Carbofuran (2,3- dihydro-2, 2- dimethyl- 7- benzofuranyl methyl carbamate) is a broad-spectrum carbamate insecticide. It is applied in granular form or aerially, and the former is currently banned in the U.S. However it is used on a variety of crops, the most common being corn, grapes, banana and wheat in India. Further carbofuran is used to control soil dwelling or foliar-feeding insects and vectors such as corn rootworm, mosquitoes, weevil, and aphids (trotter *et al*., 1991) Working on contact or ingestion, it is a cholinesterase inhibitor, but the short-term effects on nervous system are reversible. Carbamate insecticides are widely used in commercial agriculture and home gardening (EXTOXNET., 2001). Insecticides play a central role in controlling major vectors of diseases such as mosquitoes, sandflies, fleas, lice, tsetse flies and triatomid bugs. In 1955 the World Health Organization (WHO) assembly proposed the global eradication of the most prevalent vector-borne human disease, malaria by the use of residual house spraying of DDT. However, the insecticide euphoria soon ended and in 1976 WHO officially reverted from malaria eradication to malaria control. This marked shift from malaria eradication to primary health care was an emotive issue, eliciting a rapid and complete change of rhetoric from WHO (Bradley, 1998). Several issues had prompted this switch, but a major cause of the change in policy was the appearance of DDT resistance in a broad range of the mosquito vectors. In 1975 WHO reported that 256 million people were living in areas where DDT and/or BHC resistance was undermining malaria control efforts. This did not include the African region, where 90% of malaria occurs and where DDT resistance had already been noted in *Anopheles gambiae,* the major malaria vector. The resistance problem continued with the switch to newer insecticides such as the organophosphates, carbamates and pyrethroids. Operationally, many control programms have switched from blanket spraying of house interiors to focal use of insecticides on bed nets. Focal spraying limits the insecticides of choice largely to pyrethroids due to the speed of kill required to protect the occupant of the bed net and the safety margin needed for insecticides used in such close contact with people. Today the major emphasis in resistance research is on the molecular mechanisms of resistance and rational management, with a view to control the development and spread of resistant vector populations. In Africa, WHO and the World Bank have instigated major new initiatives with other major donors and the scientific community internationally to ''roll back'' malaria.

Resistance development and prolific breeding of vectors could lead to public disillusionment and subsequent non co-operation during control programmes. Carbofuran and the majority of pyrethroids recommended for treatment of mosquito nets are classified by WHO as class 2, i.e. moderately hazardous (Yang, *et al.,* 2002). Experimental hut studies have shown that the performance of the carbamate, carbofuran on nets against pyrethroid resistant *Anopheles* and carbamate resistant *Culex* mosquitoes is equivalent or better than that of pyrethroids against susceptible mosquitoes. However carbofuran resistance was reported in *Culex pipiens fatigans* in the year 1970 at USA (Shrivasthava, *et al.,* 1970). Keeping all these reports in view, in order to evaluate the potential of resistance development to carbofuran in *Culex quinquefasciatus* larval stage at

Corresponding author: Aneesh, E. M., Communicable Disease Research Laboratory, Department of Zoology, St. Josephs College, Irinjalakuda, Thrissur

Mysore, the present study was undertaken by the authors in the Vector Biology Research Lab, Department of Studies in Zoology, University of Mysore, India. Further, the quantitative estimation of two well known detoxifying enzymes such as α-esterase (A-est), and glucose-6-phosphate dehydrogenase (G6PD) were undertaken. In addition all of the above enzymes were subjected to qualitative (electrophoretic) analysis with a hope that the out come could be useful in vector management programmes against lymphatic filariasis.

MATERIALS AND METHODS

Insecticide and other chemicals

Technical grade carbofuran [purity 100%(HPLC)] procured from the Accustandard; Inc (125 market street, New Haven,CT 06513,USA.). Fast blue BB salt [4-Benzoylamino-2,5-diethoxybenzenediazonium Chloride Hemi (Zinc Chloride) Salt] was produced from M/s. Loba-Chemie Pvt. Ltd, India. All the other chemicals employed for biochemical assay and gel electrophoresis were of analytical grade (AR), procured from M/s. Hi-Media, Mumbai, India, M/s. SRL Chemicals, Mumbai, India and M/s SD Fine Chemicals, Mumbai, India.

Mosquito colony

Culex quinquefasciatus larvae were collected in and around Mysore city (12º 30`N, 76º 65` E) and maintained in an insectary at the Vector Biology Research Laboratory, Department of Studies in Zoology, University of Mysore. The adults were reared in cages (30 x 30 x 30 cm) fitted with mosquito netting and fed first on freshly water-soaked raisin. Female mosquitoes were allowed to feed on a mouse placed in resting cage overnight on the third day post-emergence. Intuitional animal ethics committee (IAEC) clearance was taken before commencement of rearing. Small bowls filled with dechlorinated water were used to collect the egg rafts for hatching. The larvae were reared in large enamel or plastic trays (30 x 24 x 5 cm), containing dechlorinated water and fed with finely powdered dog biscuits and dry yeast in the ratio of 2:1. The food was sprinkled on the surface of the water twice daily. The rearing water was changed daily until pupation. Pupae were collected daily and transferred to small bowls containing dechlorinated water, by using a Pasteur pipette. The bowls were placed in cages for adult emergence. The test population was maintained in the laboratory under environmentally controlled conditions (26 $\pm$ 2°C and 75 $\pm$ 5% relative humidity) with a photoperiod of 14 hours light and 10 hours dark. Some physico- chemical parameters of the water employed for larval rearing such as pH, total hardness and dissolved oxygen were determined as per standard method of Trivedy and Goel (1984).

Selection experiment

Forth instar larvae of a susceptible laboratory colonized *Culex quinquefasciatus* were placed under selection pressure with carbofuran for 10 generations. Earlier the susceptible parental line colonized for 15 generations free of any insecticidal exposure, was designated as F_1. The larvae of this line were subjected to bioassay and selection with different concentrations to get LC_{50} at each generation. Larval selection was carried out employing WHO standard method (WHO 1981). Mortality encountered in controls between 5 and 20%, was subjected to a correction factor employing the Abbott's formula. (Abbott 1925). The corrected mortality data was subjected to regression analysis of probit-mortality on log dosage (Finney 1971). LC_{50} values were considered to be significantly different if the 95% fiducial limits of two values did not overlap each other (Yang 2002).

Qualitative enzyme assay – Polyacrylamide gel electrophoresis (PAGE)

Qualitative enzyme analysis and comparison was made on susceptible and insecticide selected lines of *Culex quinquefasciatus.* Larvae were used for enzyme assay so to assess the stage specific activity of the enzymes. For the present study, two enzymes such as α-esterase (Est-A) and glucose 6-phospho dehydrogenase (G6PD), were analysed to establish the differential isozyme profiles in the two lines of *Culex quinquefasciatus.*Early 4th instar mosquito larvae were individually homogenized with 25µl of 40% (w/v) sucrose solution in an eppendrof tube using a Knot`s pestle. The samples were homogenised by keeping the setup on ice and later centrifuged at 2,400rpm for 5 minutes at 4ºC. For G6PD, larvae were homogenised in a mixture of 0.1M Tris-HCl (P^H 8.5), 20% sucrose and NADP (2mg/ml). Samples were centrifuged at 14,000rpm for 5 minutes. An equal (12-20µl) volume of supernatant was carefully loaded to each well. The gels were initially run at 40V for 20-30 minutes at room temperature and increased to 60V at 4ºC employing a refrigerator until the marker dye front touched the sealing gel (nearly 5 hours).

Quantitative assay (Microplate assay)

Quantitative enzyme assay was carried out according to the procedures outlined by Hemingway (1998) by using microplate assay method. Batches of thirty early 4th instar larvae were individually homogenised in 200µl of distilled water using a teflon or plastic pestle taken in 1.5ml capacity eppendrof tube which was kept on ice. The homogenate was spun at 5000 rpm for 10 minutes at 4˚C. Replicate of the 20µl supernatant from each sample was transferred to a fresh eppendrof tube and the final volume was made to 100µl by adding 0.1M (P^H7.2) potassium phosphate (KPO_4) buffer. This procedure was repeated for the sample preparation of all enzymes such as α/β esterase and G6PD in both susceptible and selected lines. Protein assay of the early fourth instar larvae were carried out as per the standard procedures of Lowry (Lowry-Rosebrough *et al.,* 1951).

Statistical Analysis:

Allelic frequencies of different enzyme loci between the two populations were compared. The frequency of the common alleles for each enzyme locus in the two groups of larvae was compared to see the level of significant difference, if any. The corresponding *P* value for the Z statistics was obtained from the standard normal distribution tables. For a two-sided test, if Z was greater than (+) 1.96 or smaller than (-) 1.96, then, *P* value is considered as less than 0.05 (P<0.05), then null hypothesis of equality of allele frequency at two different tested lines was rejected (Daly and Bourke, 2000).

RESULTS

Culex quinquefasciatus was placed under carbofuran selection pressure and each generation was tested for its susceptibility to the chemical using dose mortality relationships. Table-1 provides the results of the selection experiments in *Culex quinquefasciatus.* The LC_{50} and LC_{90} values with Fiducial limits and the regression equation with slope andheterogeneity (λ^2) along with the resistance ratio (RR) for 10 generations are also provided in the Table-2. The increase in resistance was based on the comparative susceptibility of parent generation (F_1) (LC_{50}= 0.08ppm and LC_{90}= 0.122ppm). Both the LC_{50} and LC_{90} values increased gradually under continuous carbofuran selection. The LC_{50} showed a gradual increase from 0.08ppm in the F_1 to 1.298ppm in F_{10} (16.225 fold). Here too the variation in LC_{90} did not show any definite pattern though gradual increase was recorded from F_1 to F_{10} i.e., from 0.122ppm to 1.784ppm (14.59) fold. The ratio of increase in tolerance based on the LC_{50} was found to be significant ($p<0.05$) in all the generations compared to F_1. The slope of regression lines for test data from each generation were computed and the values were found to vary from 6.946 to 15.294ppm. The results of qualitative analysis of enzymes such as α-esterase (A-est), and glucose-6-phosphate dehydrogenase (G6PD) by employing polyacrylamide gel electrophoresis (PAGE) are provided in Table- 3. The electromorphs of these enzymes are depicted in Figs-1 and 2. In Zymogram, each band represents the product of one allele.

Table 1. Data on bioassay results of carbofuran selection on *Culex quinquefasciatus*

F_1		F_2		F_3		F_4		F_5	
Concentration (ppm)	Mortality (%)	Concentration (ppm)	Mortality (%)	Concentration (ppm))	Mortality (%)	Concentration (ppm))	Mortality (%)	Concentration (ppm)	Mortality (%)
0.04	8	0.068	8	0.1125	16	0.15	30	0.175	10
0.05	14	0.085	18	0.123	30	0.16	36	0.187	24
0.06	20	0.097	42	0.134	36	0.173	52	0.2	30
0.074	28	0.11	52	0.145	60	0.186	56	0.216	64
0.09	58	0.125	80	0.16	72	0.2	82	0.232	66
0.11	75			0.175	90			0.25	84
0.14	98								
Control	0		0		0		0		0

Table 1. Contd. Data on bioassay results of carbofuran selection on *Culex quinquefasciatus*
*** Mortality in controls corrected with the Abbott's formula (Abbott 1925).**

F_6		F_7			F_8		F_9			F_{10}	
Concentration (ppm)	Mortality (%)	Concentration (ppm)	Mortality (%)	Corrected* Mortality (%)	Concentration (ppm))	Mortality (%)	Concentration (ppm)	Mortality (%)	Corrected* Mortality (%)	Concentration (ppm)	Mortality (%)
0.02	12	0.4	4	2.04	0.437	22	0.5	12	9.2	0.866	20
0.325	36	0.42	14	12.2	0.516	32	0.6	26	23.7	0.966	24
0.346	48	0.44	32	30.6	0.608	48	0.721	32	29.8	0.998	36
0.37	60	0.46	52	51.02	0.72	64	0.866	48	46.3	1.2	44
0.395	78	0.48	64	63.26	0.847	80	1.04	72	71.1	1.344	50
0.421	82	0.5	84	83.67	1	94	1.25	92	91.7	1.5	84
0.45	92										
Control	0		2			0		3			0

Table 2. Bioassay results of carbofuran selection experiments on 4th instar larvae of *Culex quinquefasciatus*

RR: resistance ratio (compared to LC_{50} of F_1).

Generations	LC 50 (mg/lit)	Fiducial limits	LC 90 (mg/lit)	Fiducial limits	Slope ±SE	Heterogeneity (χ^2) (df)	Regression equation	RR
1	0.08	0.07092	0.123	0.10518	6.9465±0.976	24.80 (4)	6.9465X+12.6075	_
		0.09094		0.16706				
2	0.104	0.10181	0.147	0.13904	8.6434±0.66	5.41 (3)	8.6434X+13.4747	1.3*
		0.10770		0.15856				
3	0.139	0.13621	0.184	0.17703	10.5071±0.767	4.18 (4)	10.5071X+14.0041	1.74*
		0.14182		0.19361				
4	0.172	0.16313	0.216	0.19843	13.1828±2.074	9.95 (3)	13.1828X+15.0544	2.15*
		0.18279		0.27316				
5	0.212	0.20969	0.261	0.25433	14.2372±0.940	5.35 (4)	14.2372X+14.5682	2.65*
		0.25433		0.27151				
6	0.375	0.37049	0.456	0.44450	15.2942±1.028	4.33 (4)	15.2942X+11.5025	4.68*
		0.38083		0.47010				
7	0.451	0.44679	0.524	0.51300	13.7425±1.498	1.12 (4)	19.7425X+11.8165	5.62*
		0.45644		0.53967				
8	0.608	0.58136	0.992	0.92244	6.0321±0.5018	7.51 (4)	6.0321X+6.3024	7.57*
		0.63455		1.09266				
9	0.826	0.72681	1.297	1.09718	6.5499±1.015	14.54 (4)	6.5499X+5.5408	10.32*
		0.93992		1.88144				
10	1.298	1.20361	1.784	1.55067	9.2932±1.5104	21.48 (4)	9.2932X+3.9445	16.225*
		1.46340		2.52148				

* The difference in LC50 is significant based on the non- overlapping of 95% fiducial limits.

Table 3. Allelic frequency of three detoxifying enzymes in susceptible (F_1) and carbofuran selected (F_{10}) lines of *Culex quinquefasciatus*.

	Electromorph	Frequency		Z- value
		F1	F10	
α - esterase	0.53	-	0.65	-
	0.6	-	0.28	-
	0.8	0.97	0.61	3.124*
	1	0.94	0.9	0.289
G6PD	1	1	1	___
	1.24	0.94	0.9	0.854

* Values significant ($p \leq 0.05$)

Table 4. Differential activity of two detoxifying enzymes in susceptible and carbofuran selected lines of *Culex quinquefasciatus.*

Enzyme	Susceptible (Mean ± SD)	Selected (F_{10}) (Mean ± SD)	Fold Increase
α-esterase (A-est), nMoles of α-naphthol produced/min/mg protein	0.1702± 0.0273	0.2211±0.0224	1.29**
G6PD nMoles NADP produced/min/mg protein	0.463+0.04	0.892+0.04	1.93**

** $p \leq 0.01$

Different alleles of an enzyme are designated by using a number as superscript for the abbreviated form of the enzyme in an ascending order. For example, for α-esterase (A-est), A-est$^{0.8}$ and A-est$^{1.0}$ are the designated bands. These numericals are provided in the order of decreasing mobility of each enzyme band. Thus A-est$^{1.0}$ is the fastest moving allele and A-est$^{0.8}$ is the slowest. In other words A-est$^{0.8}$ is more cathodal and A-est$^{1.0}$ is more anodal. The elecropherogram of the α-esterase and enzymes in the susceptible and carbofuran selected lines show variation in the mobility, intensity and number of bands (Fig- 1). However, elecropherogram of G6PD shows variation in the intensity only (Fig-2).

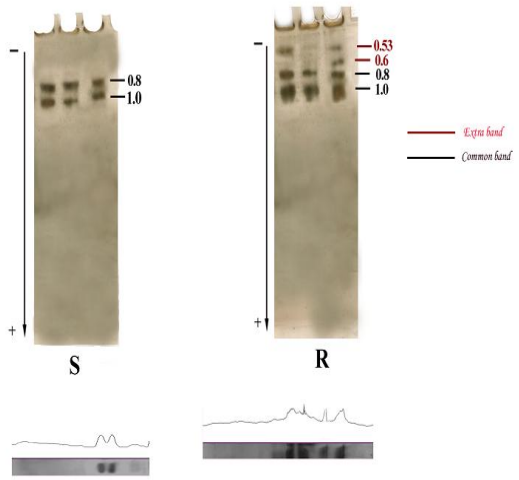

Fig. 1. Isozyme profile of α- esterase in susceptible (S) and carbofuran selected (R) lines of Culex *quinquefasciatus*

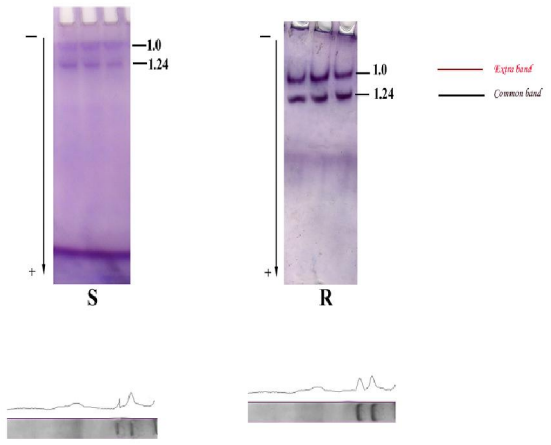

Fig. 2. Isozyme profile of G6PD in susceptible (S) and carbofuran selected (R) lines of *Culex quinquefasciatus*

The results of quantitative analysis of a few enzyme such as α-esterase (A-est) and glucose-6-phosphate dehydrogenase (G6PD) for *Culex quinquefasciatus* from susceptible and selected lines are given in Table-4. The enzyme assays were performed on the susceptible (F_1) and carbofuran selected (F_{10}) generations of *Culex quinquefasciatus with* sample size of 30 individuals per generation and each assay. Enzyme activities were found to increase in the carbofuran selected line. The mean value of α-esterase activity in susceptible line of *Culex quinquefasciatus* was 0.1702 nMoles of α-naphthol produced/min/mg protein while; it is 0.2211 nMoles of α-naphthol produced/min/mg protein in carbofuran selected line. The increase in α-esterase activity in the selected line was 1.29 times more than that of susceptible one and the mean difference was statistically significant at 0.01 level ($p \leq 0.01$. The G6PD activity difference between the larvae of *Culex quinquefasciatus* from susceptible (0.463 nMoles NADP/min/mg protein) and selected line (0.892 nMoles NADP/min/mg protein) too was found to be significant at 0.01 level ($p \leq 0.01$). The activity in selected population was found to be 1.93 times (mean values) more than that of susceptible line.

DISCUSSION

As mosquito populations are being exposed to different classes of organic insecticides in their respective localities, the chances of resistance and cross resistance will be high as the available pesticides have almost similar mode of action (Hemingway and Ranson, 2000; Sarkar *et al*, 2009). Extensive exposure of insect vectors to insecticides eventually selects for insecticide resistance. Widespread occurrence of resistance to insecticides has been a serious threat to the public health, especially for mosquito control as they are the most important vectors of communicable diseases worldwide. So monitoring the susceptibility status of the mosquito fauna to insecticides and probing alternate strategies is one basic and essential components of integrated vector control. Isozymes have been shown to be the ideal tool for investigating the pattern of gene products during resistance development because any change in the protein pattern directly depicts the alteration in the gene function. With this in view, isozyme pattern of two enzymes such as α-esterase and G6PD in *Culex quinquefasciatus*, a filariasis vector was studied during the present investigation. Experiment carried out with 4th instar larvae of *Culex quinquefasciatus* showed a gradual but steady increase in LC_{50} and LC_{90} values against carbofuran with every generation compared to the parental susceptible colony (Table 1 & 2). The present results indicate that development of resistance to carbofuran is quite fast in *Culex quinquefasciatus* under selection pressure in the laboratory during ten generation of exposure. The data indicate 16.26 and 14.59 fold increase in LC_{50} and LC_{90} values (LC_{50} = 0.08ppm LC_{90} =1.298ppm) respectively after ten generation of exposure, compared to the parental susceptible colony. The current studies shows that the laboratory colony of *Culex quinquefasciatus has* high tendency to develop carbofuran resistance within a short span of time. The present data confirms the fear that continuous insecticide pressure may lead to development of resistance even against the most effective pesticides in Mysore population as well. The observation is in strong agreement with the studies of Shrivasthava and georghiou, (1970) at USA. According to them selection with carbofuran for 5 consecutive generation shows 10 fold increase in tolerance for *Culex Culex pipiens fatigans.*

Previous studies from the same laboratory have reported the isozyme profiles of the above said enzymes under insecticide pressure in different species of mosquitoes. For example, studies made by Urmila and Vijayan (2009) at Mysore demonstrated significant changes in the allelic frequency of α-esterase, β-esterase and G6PD in a pyrethroid resistant *Ae. aegypti.* Similarly increased isozyme patterns of esterases were observed in cypermethrin selected lines of *An. stephensi* at Mysore recently (Aivazi, 2009). The present investigation too shows marked variation in the isozyme profiles between control and carbofuran treated larvae. Similarly, Chakraborti *et al.,* (1993) from Pune have studied the esterases in 5 different populations of *Ae. albopictus* from the field and compared it with the laboratory strain. They have found more bands in the field populations as compared to the laboratory strain. Perusal of literature also reveals that the qualitative changes of detoxifying enzymes due to insecticide resistance. Carbofuran resistance was reported in *Culex quinquefasciatus* in the year 1970 in USA (Shrivastava, *et al.,* 1970) and at France in the year 1996 (Fabrice *et al.,* 1997). Selection of the susceptible *Plutella xylostella* (diamondback moth) with carbofuran for seven generations resulted in about 170-fold resistance at USA. Further in the field population of Colorado potato beetle, *Leptinotarsa decemlineata* progression of resistance was found to be inherited via a single, autosomal, incompletely dominant gene, resulting in decreased acetyl cholinesterase sensitivity (Philippos *et al.,* 1992). Georghiou

et al., (2000) have investigated the rate of development and other characteristics of carbamate resistance in *Culex quinquefasciatus* at California. Selective pressure against the larvae for 35 generations resulted in 25.4 fold resistance in Baygon (a carbamate insecticide) selected larvae, but only 3 to 5 fold resistance in adults. Broad-spectrum resistance to organophosphates and carbofuran caused by insensitive acetyl cholinesterase mechanisms have been identified in *Anopheles gambiae* from Cote d`lvoire (N`Guessan, 2003). However such reports are scanty in Indian scenario.

Hence, *Culex quinquefasciatus* being the only filariasis vector in India and the likelihood of carbofuran resistance in this species compared to other are the reasons being choosing it by the author to study the underlying resistance mechanisms. Additionally, all insecticide groups including carbofuran are being increasingly used for protection against agricultural pests in India. Agricultural land use is often closely associated with an increase in the prevalence of vector- born disease. This is because the vectors such as *Culex quinquefasciatus* can breed in the agricultural field also. There are many examples indicating the relationship between vector resistance with agricultural activities (Brown, 1986; Lacey and Lacey, 1990). Besides, more than 90% of all insecticides produced have been used for agricultural purposes and that has created serious problems in mosquito control programmes (Roberts and Andre, 1994). So insecticide resistance in natural populations of *Culex quinquefasciatus* is likely to have arisen from the indiscriminative and widespread use of insecticides in public health and agricultural fields. Mosquito larvae could be collected from pools, stream margins, stream bed, palm irrigation canal and rivers where insecticide reach in small amount leading to the development or enhancement of resistance in the larvae (Davari *et al.,* 2007). Esterases are complex enzymes acting on a variety of substrates and are capable of hydrolyzing ester bonds. Association of pyrethroid resistance with increased band number of α-esterase and β-esterase in *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* has been reported in earlier studies by (Kumar *et al.,* 1991; Vulule *et al.,* 1999; Ganesh *et al.,* 2002; Ganesh *et al.,* 2003; Corbel *et al.,* 2007; Urmila and Vijayan, 2009). The present study too revealed variations in number and intensity of bands, i.e two extra α-esterase bands are associated with carbofuran selected *Culex quinquefasciatus* line (F10) compared to the susceptible line (F1). The selected line of *Culex quinquefasciatus* registered 4 alleles for α-esterase compared to the 2 in susceptible line. Allelic frequency of α-esterase between two lines is statistically different ($P \leq 0.05$). A variation in esterase profiles was noticed in a deltamethrin selected line at Mysore (Ganesh *et al.,* 2002). Likewise four extra bands of both α-esterase and β-esterase have been reported in *Aedes aegypti* after 20 generations of deltamethrin selection (Urmila and Vijayan, 2009). Accordingly, increased band number and intensity has been observed in different carbamates resistant strains of mosquitoes (Georghiou, *et al.,* 1966). In West Africa, *Culex quinquefasciatus* displayed large variation in resistance to carbamates and clear differences were observed among populations within and between the two countries (Chadre, *et al.,* 1997).

The allelic frequency of dehydrogenase (glucose-6-phosphate dehydrogenase) is provided in table 24. Though G6PD registered two bands in both susceptible and selected lines of *Culex quinquefasciatus* indicating the bands were more intense in the selected line. Such an observation was made in a previous study on the susceptible line of *Culex quinquefasciatus* at Mysore. (Madhu and Vijayan, 2009). In G6PD (dehydrogenase), the reduced NADH or NADPH formed during the reaction oases electrons through an intermediate electron carrier, usually phenozine methosulphate to a tetrazolium compound, resulting in the information of an insoluble purple diformazen dye at the site of the enzyme activity. The NADPH generated by G6PD in redox reaction is utilized by monoxygenases. Thus increase in monooxygenase activity must be reflected in G6PD activity also as suggested by kumar *et al.,* (1991).

G6PD registered significant increase of activity ($P \leq 0.01$) in carbofuran selected line of *Culex quinquefasciatus as* depicted in Fig-19. The differences in the G6PD activity in normal and treated lines were also noticed by Kumar *et al.,* (1991) and Ganesh *et al.,* (2002). Further investigations on pyretroid resistance in *Anopheles stephensi* (Enayati *et al.,* 2006; Davari *et al.,* 2007, Aivazi *et al.,* 2009), *Aedes aegypti* (Urmila and Vijayan, 2009) and *Culex quinquefasciatus* (Hardstone *et al.,* 2009)have been attributed to the involvement of G6PD which supports the present results. Developments of quantitative technique such as spectrophotometric and microtire plate assay are being used nowadays to detect resistance levels considering some important detoxifying enzymes as targets. On the other hand, as different isozymes are inherited co dominantly at individual loci, it is possible to detect the genotype of an individual from the electrophoretic profile analysis of resistance is clearly the method of choice for understanding long and short-term effects of insecticide use on population structure. Quantitative enzyme assay technique will provide a reasonably complete evaluation of an individual insects ability to metabolise a given class of insecticide. In general, quantitative increase in these enzymes, associated with gene amplification of overproduction under selection pressure, confers insecticide resistance (Mouches *et al.,* 1990). Therefore, it has become apparent that such techniques hold considerable promise in relating specific target enzyme to resistant genotypes rather than phenotypes in a single insect. It is in this regard the present quantitative analysis gains importance in *Culex quinquefasciatus* at Mysore after exposing to carbofuran for 10 consecutive generations. Esterase enzymes cleave carboxylester and phosphodiester bonds. They are extremely important in resistance organophosphate and carbamate resistance (Marquadrt and Kondratieff, 2005). The author too has found difference in esterase level between susceptible and carbofuran lines of *Culex quinquefasciatus.* There was a significant ($P \leq 0.01$) increase to the tune of 1.29 fold (0.15 to 0.50 nMoles of α-naphthol produced/min/mg protein) in α-esterase and 1.32 times (0.05 to 0.15 nMoles of β -naphthol produced/min/mg protein) in β-esterase in *Culex quinquefasciatus*. These findings are in strong agreement with the studies of Enayati and Ladonni, (2006) which showed an increase of esterases in field populations of *Anopheles stephensi* compared to susceptible lab population. To sum up, the present data are supported by all above mentioned works, clearly show the important role of enzymatic mechanism of carbofuran tolerance by *Culex quinquefasciatus at* Mysore. The perusal of literature on carbamate tolerance and the present study reveal that the resistance mechanism may be similar in *Culex quinquefasciatus* with different classes of chemicals. Among 2 enzymes analysed, all seems to play an important role in the detoxification of carbofuran in *Culex quinquefasciatus.* Further results of the present work on 2 enzymes of *Culex quinquefasciatus* reiterate the idea that biochemical approach for the detection of insecticide resistance/ tolerance will help us in integrated vector management in endemic areas, where vector species might have an earlier exposure to insecticides.

Acknowledgement

The authors are thankful to UGC for financial assistance and the Chairman, Department of Studies in Zoology, University of Mysore and the Principal, St. Josephs College, Irinjalakuda, Thrissur

REFERENCES

Abbott, W. S. 1925. A method of computing of the effectiveness of an insecticide. *J Econ Entomol,* 8: 265-267.

Aivazi, A. and Vijayan, V. A. 2009. Larvicidal activity of oak *Quercus infectoria* Oliv (Fagaceae) gall extracts against *Anopheles stephensi* Liston. *Parasitol. Res*. 104:1289-1293.

Bradley, D. J. 1998. The particular and general issues of specificity and verti- cality in the history of malaria control. *Parassitologia.* 40: 5–10.

Brown, A. W. A. 1986. Insecticide resistance in mosquitoes: a pragmatic review. *J. Am. Mosq. Control Assoc.* 2: 123–40.

Chakraborthi, S., Mourya, D. T., Gokhale, M. D. and Banerjee, K. 1993. Insecticide susceptality status and enzyme profile of *Aedes albopictus* from different localities of Maharashtra State. *Indian. J. Res.,* 97: 37-43.

Enayathi, A. A. and Motevalli-Haghi, F. 2007. Biochemistry of pyrethroid resistance in German Cockroach (Dictyoptera: Blatellidae) from hospitals of Sari, Iran. *Iran biomed journal*. 11(4): 251-258.

EXTONET. 2001. Carbofuran-available online: http:/ace.ace.orst.edu/into/extoxnet/pips/carbotur.html.

Georghiou, G. P., Taylor, C. E. 1986. Factors influencing the evolution of resistance. In: Pesticide Resistance: Strategies and Tactics for Management, Committee on Strategies for the Management of Pesticide Resistant Pest Populations. *National Academy Press*, Washington, DC. 157–169.

Hardstone, M. C., Leichter, C., Harrington, L. C., Kasai, S., Tomita, T. and Scott, J. G. 2009. Cytochrome P450 monoxygenase-mediated permethrin resistance confers limited and larval specific cross-resistance in the southern house mosquito, *Culex pipiens quinquefasciatus*. *Pestic. Biochem. Physiol*., 89: 175- 184.

Hemingway, J and Ranson, H. 2000. Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol*., 45: 371-391.

Kumar, S., Thomas, A. and Pillai, M. K. 1991. Invovlment of monoxygenases as a major mechanism of deltamethrin resistance in larvae of three species of mosquitoes. *Indian J Exp Biol.,* 29: 379-84.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Proteinmeasurement with folin phenol reagent. *J Biol Chem.,* 193: 265-275. Macedo, M.E., Consoli, R. A. G. B., Grandi, T. S. M., dos Anjos., A. M. G., de

Madhu, S. K. and Vijayan, V. A. 2010. Evaluation of the larvicidal efficacy of extracts from three plants and their synergistic action with propoxur against larvae of the filarial vector *Culex quinquefasciatus* (Say). *Toxicol Environ Chem.* 92: 115-126.

Matsumura, F., Brown, A. W. A. 1961. Biochemical study of malathion tolerant *Aedes aegypti*. *Mosq. News.* 21:192–94.

Philippos, M. L., Edward, J. G., Joel, M. W., Mark, E. W. and Robert, M. H. 1992. Selection inheritance and characterization of carbofuran resistance in the Colorado potato beetle. *Am J potato Res.* 83: 485-494.

Sarkar,S. D., Latif, Z. and Greay, A. I. 2005. Natural products Isolation. 2nd .ed. *Humana press inc*; Totowa., pp.515.

Shrivastava, S. P., Georghiou, G. P. 1970. Carbomate resistance in mosquitoes. *Bull. Wld Hlth Org.* 42: 931-942.

Tottor, D. 1991. Aquatic fate and effects of Carbofuran. *Crit. Rev. in Environ. Cont.* 21(2): 137-176.

Trivedy, R. K. and Goel, P. K. 1986. Chemical and biological method of water pollution studies. *Environmental publications*, Karad.

Urmila, J. and Vijayan, V. A. 2009. Biochemical characterization of deltamerthrin resistance in a laboratory selected strain of *Aedes aegypti. Parasitol. Res.,* 104:1431-1436.

Vulule, J. M., Beach, R. F., Atieli, F. K., Roberts, J. M., Mount, D. L., Mwangi, R. W. 1994. Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya. *Med. Vet. Entomol.* 8:71–75.

Yang, X., Buschman, L. L., Zhu, K. Y. and Margolies, D .C. 2002. Susceptibility and detoxifying enzyme activity in two spider mite spoecies (Acari: Tetranychidae) after selection with three insecticide. *J Econ Entomol*. 95(2): 399-406.

****