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ESTERASE BANDING PATTERN IN DIFFERENT DEVELOPMENTAL STAGES OF *Culex quinquefasciatus* SAY 1823 (DIPTERA: CULICIDAE)

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ABSTRACT
Polyacrylamide gel electrophoresis (PAGE) was used to analyze esterase patterns in different age group of the developmental stages of *Culex quinquefasciatus* Say using α- and β- naphthyl acetate as substrates. Altogether seven esterase bands (Est-1, Est-2, Est-3, Est-4, Est-5, Est-6 and Est-7) could be revealed. Egg and 1-6 days old larvae showed no bands. The number of esterase bands increased with the increases larval age of 7 days and onward. Four (Est-2, Est-5, Est-6 and Est-7) bands were found in the 7-9 day old (3rd instar) larvae, six (Est-1, Est-2, Est-3, Est-5, Est-6 and Est-7) bands were found in 10-11 days old larvae (4th instar). Number of esterases decreases with onset of pupation three bands were (Est-1, Est-2 and Est-4) found and only two bands (Est-2, Est-3) were found in adult (female). The esterase bands preferred α-naphthyl acetate to β-naphthyl acetate as substrates. Among these seven esterases, six (Est-1, Est-3, Est-4, Est-5, Est-6 and Est-7) appeared to be carboxylesterase or cholinesterase, as they were inhibited by malathion.

KEYWORDS: *Culex quienquefasciatus* Say, esterase, malathion, insecticide resistance.

INTRODUCTION

Organophosphate compounds (OP) are commonly used to control the mosquitoes in many places of the world. In many insect populations, development of resistance to certain OP insecticides has been found to be associated with their repeated treatment (IRAC, 2008). OP resistance in *Culex quinquefasciatus* Say is a major concern for the mosquito control authorities as well as for the common peoples, since this mosquito species spreads bancroftian filariasis and causes biting irritation (Curtis and Feachem, 1981, Subra, 1981). No reports are available on Dhaka city *Culex* population regarding insecticide resistance mechanism. Three broad enzyme classes involved in insecticide detoxication are the mixed function oxidases (MFO), esterases and glutathione S-transferases (WHO, 2008). The major mechanism of resistance to organophosphate insecticides in *Cx. quinquefasciatus* Say is through highly active non-specific esterase isoenzymes, which appear to metabolize the insecticide to harmless products (Villani *et al*., 1983). In insects they take part in biological processes such as regulation of juvenile hormone, digestion, reproduction and insecticide resistance. Esterases have been found to vary among populations and species and are an important tool for analysis of genetic differentiation and evolutionary relationships (Lima-Catelani *et al*., 2004). Stage-specific and tissue-specific expression patterns have also been observed for esterase in mosquito (Lima-Catelani *et al*., 2004). It is the most significant enzymes for insecticide detoxication in insects. Organophosphate, carbamate and pyrethroids contain carboxylester and phosphotriester bonds that are subject to attack by esterase enzymes (Brattsten, 1992). Insect esterases are very diverse and can include monomers, dimmers and multimers, which mean that their relative molecular mass can cover a wide range. Polymorphism is a notable characteristic of insect esterases. Multiple forms of esterases are present in the soluble, cytosolic fraction of insect (Brattsten, 1992, Dauterman, 1985). Of the multiple forms of esterase isozymes that exist in insects, few participate in insecticide metabolism (Maa and Terrier, 1983). Each isozymes probably has a certain range of substrates. Different types of esterases (A1, B1, A2 and B2) have been recognized in OP resistant populations of *Cx. pipiens* complex throughout the world (Poirie *et al.*, 1992) due overproduction of two nonspecific carboxylesterases A and B. This overproduction is caused either by the coamplification of both A and B esterase structural genes (Rooker *et al*., 1996 and Guillemaud *et al.*, 1997), or by the amplification of the B esterase gene alone (Guillemaud *et al.*, 1997 and Mouches *et al*., 1986), or else by the up regulation of the A esterase gene expression with no amplification (Rooker *et al.*, 1996). Increased esterase detoxification is a common mechanism of resistance to OPs in insects. In *Culex*, it is due to the overproduction of esterases that have a high binding affinity with insecticides but a very low rate of hydrolysis and thus mainly act by sequestering insecticides before they reach their acetylcholinesterase target (Vaughan *et al*., 1995). In *Anopheles stephensi*, the rate of development of deltramethrin resistance differed significantly depending on whether larval or adult stages were subjected to selection pressure (Gayathri *et al*., 2006). The development of resistance has led to serious problems in the *Cx. quinquefasciatus* Say mosquito control programme of Dhaka city (Kabir, 1987). This programme invariably failed for exclusive reliance on pesticides. To avoid the insecticide resistance problem, investigations of resistant

populations of mosquitoes are necessary. Identification of resistance mechanism helps determine the cross-resistance spectrum, facilitates the choice of alternative insecticides and allows detailed mapping of areas with resistant populations. Thus, determining the trend and nature of resistance in *Cx. quinquefasciatus* Say population of Dhaka city would be of great use in devising sustainable control strategy in mosquito. Esterase banding pattern in different stages of *Cx. quinquefasciatus* Say were studied and compared in the present study.

MATERIALS & METHODS

Cx. quinquefasciatus Say mosquitoes were frequently collected from different areas of Dhaka and these were reared in the Zoological Garden, University of Dhaka following Ameen *et al*. (1984). The study of esterase polymorphism was carried out by using egg, larvae (1day to 12 day old larvae), pupa and adults (female). All the larval stages, pupa and adult female mosquito samples were prepared for PAGE. Each of the samples was separately squashed in 10 µl of 1 X Tris –Borate-EDTA buffer in an eppendorf tube 10 µl 2 X Bromophenol blue dyes followed by the addition of 5 minutes centrifugation at 12000rpm. The supernatant obtained there from were subjected to electrophoresis using a standard slab gel system (Vaughan *et al*., 1995). 10 µl of each individual mosquito sample was put on to the slot the gel. Electrophoresis was carried out at 120V for 1 to 1.15 hour until the tracking dye (Bromophenol blue) reachedto the gel bottom. The gel was recovered and put onto a staining tray containing 30ml of substrate mixture (0.2 M Monobasic sodium phosphate, 0.2 M Dibasic sodium phosphate, 0.04% α-napthyl acetate and 0.04% β-napthyl acetate dissolved in acetone and distilled water). After 15 minutes, substrate mixture was out poured and 30 ml of fast blue solution (0.02g Fast blue RR salt and 120 ml distilled water) was added to the gel and incubated 25 minutes at 37ºC. Photograph of the gel was taken by a zoom lens camera putting it on a white background. Finally the gel stored in a buffer (TBE) solution at room temperature for later observation. Esterases were identified in the gels following the technique described by Johnson *et al.* (1966) and Steiner and Johnson (1973), relative mobility (Rs) values are calculated using the ratio of the distance from the center of a band to the origin and the distance of the marker to the origin. To calculate the relative mobility of different bands the value of the most frequent band are consider as unit 1.

Inhibition tests for biochemical characterization of esterases involved the use of organophosphorus insecticide malathion (0.4mM) included in the preincubation and stain solutions. The overall procedure of PAGE was same as above. The only exception was that 0.4mM of malathion was added in substrate mixture (α-napthyl acetate and β-napthyl acetate with staining buffer) for 15 minutes before incubation. Then added 0.4mM of malathion in the stain solution (Fast Blue) for 30 minutes at 40ºC. Esterase bands from 4th instar larvae were used in the inhibition experiments.

RESULTS & OBSERVATIONS

To find out the substrate specificity or preference of the esterases, α and β-naphthyl acetates were tested separately. Substrates α and β-naphthyl acetates gave black and red bands respectively on the gel. Total seven esterase bands, Est-1, Est-2, Est-3, Est-4, Est-5, Est-6 and Est-7 were observed in the mosquito *Cx. quinquefasciatus*. Number of specific bands in different life stages was expressed as shown in the Fig. 1. In case of egg and 1-6 day old larvae, no esterase band appeared in the gel. To be certain whether this observation is due to very little body mass of the samples, some egg rafts were squashed together and subjected to PAGE. But the result was same indicating that no esterase was expressed in the eggs. Same approach in the early larvae (up to 6days old) revealed no esterase. Esterases were observed in the 7 day old larvae onwards. Frequencies of the particular esterase bands in larva, pupa and adult have been presented in the Table 1. Esterase pattern varied with the life stages. Only three esterases of different relative mobility have been observed in 7 day old larvae whereas six in 11-12 day old larvae. Est-2 appeared in all larvae of 7-12 days age, 50% of pupae and 20% of adult female. This is an indication that Est-2 has a pivotal role in the development of the mosquito (Lima-catelani *et al*., 2004). All the 12 days old larvae possessed Est-1, 2, 5, 6 and 7 and this pattern of expression differs from that of other stages and age groups (Table 1).

TABLE 1: % occurrence of various esterase bands in the different age groups (egg, larvae, pupae, adult) in 7.5% PAGE stained with α and β Napthylacetate

Bands No.	Relative Mobilities	Egg	1-6 days old larvae	7 days old larvae	8 days old larvae	9 days old larvae	10 days old larvae	11 days old larvae	12 days old larvae	Pupae	Adult (Female)
Est – 1	1.18 ± 2	-	-	-	-	-	-	+	+	+	-
Est – 2	1	-	-	+	+	+++	+++	+++	+++	+	+
Est- 3	0.78 ± 2	-	-	-	-	-	++	++	++	-	++
Est – 4	0.68 ± 2	-	-	-	-	-	-	-	-	++	-
Est – 5	0.53 ± 2	-	-	+	+	+++	+++	+++	+++	-	-
Est – 6	0.36 ± 4	-	-	+	+	++	++	+++	+++	-	-
Est – 7	0.24 ± 5	-	-	-	+	++	++	+	+	-	-

*scored from stained gel (Here, - = absent, + = intensive band, ++ = moderately intensive band and +++ = highly intensive band)

All the pupae had Est-4 which was absent in all other stages. Thus, this esterase appeared to have specific functions in the pupal stage of the mosquito. Adult female had only two esterases, Est-2 and 3. All the adult females possessed Est-3. Different levels of esterase band activity, denoted by different thickness and degrees of staining, were another kind of variation observed among the developmental stages (Plate I). It shows that even esterases that are necessary in different stages may be under control, increasing or decreasing their expression level while ageing (Lima-catelani *et.al*., 2004).

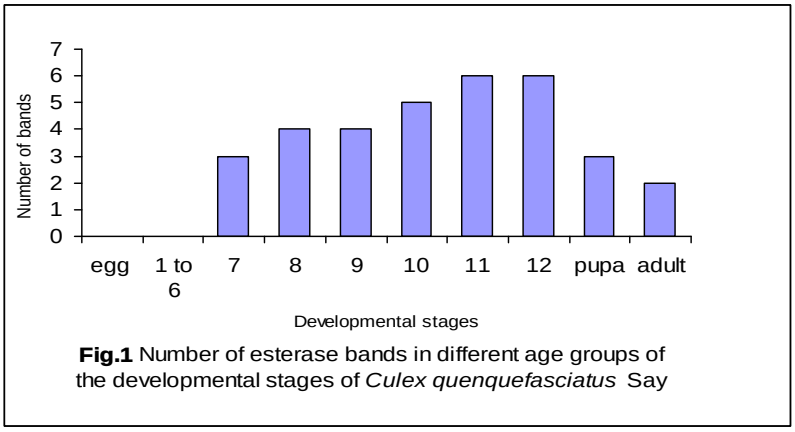

Fig.1 Number of esterase bands in different age groups of the developmental stages of *Culex quenquefasciatus* Say

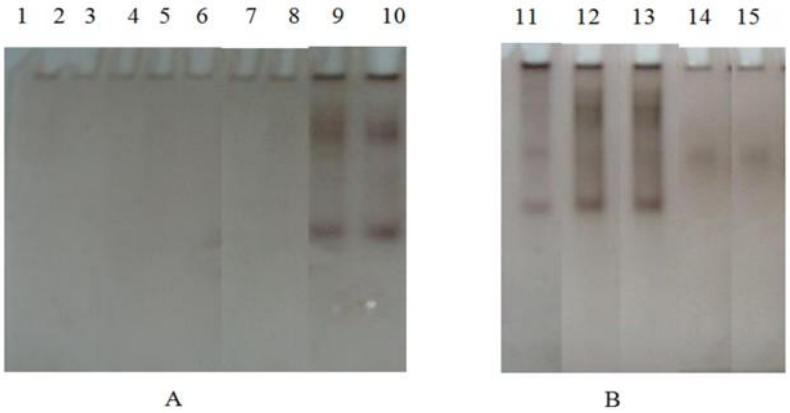

PLATE I. Acrylamide gel electrophoresis of different developmental stages of *Cx. quinquefasciatus* Say stained for esterase activity. (Lane 1 – egg, 2-13 represents 1-12 day old larvae respectively, 14 pupae, 15 adult female)

Overall, it can be concluded that there is variation in esterase pattern along the life stages of the mosquito. And this variation could be attributed to specific physiological functions involved with developmental stages. Malathion as organophosphorus insecticide was used as inhibitor. This malathion inhibit Est-1, Est-3 to Est-7 (Plate II). Faint Est-2 band was found in the malathion-inculation gel. The esterase banding pattern of the 4th instar larvae scored from stained control and malathion-inculation gels are presented in the Table II.

TABLE II: Esterase activity of early 4th larvae of *Cx. quinquefasciatus* Say on 7.5% PAGE the control gel and malathion incubated gel.

Gel	Sample	Est–1	Est–2	Est–3	Est–4	Est–5	Est–6	Est-7
Control	1-10	-	+	+	-	+	+	+
Malathion - incubation gel	11-20	-	+	-	-	-	-	-

*scored from stained (Here + = Present and - = Absent)

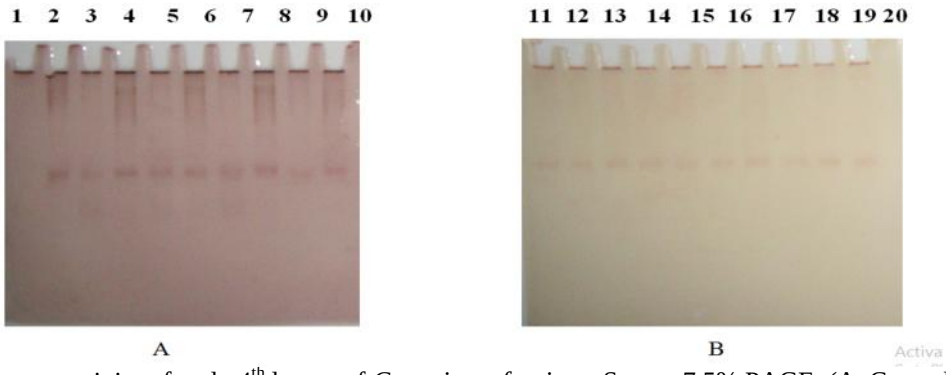

PLATE II. Esterase activity of early 4th larvae of *Cx. quinquefasciatus* Say on 7.5% PAGE. (A. Control gel and B.Gel incubated Malathion).

DISCUSSION

Differences in esterase patterns among stages reflect gene regulation of protein production during development, coordinating the synthesis as required (Hames, 1986). In the present study, one of the seven loci proposes were active throughout development of *Cx. quinquefasciatus* Say: Est-2 (Estα2/ Estβ2). The most active stage in synthesis of esterases was the larval one. Among the

larvae it seems that esterase activity is increasing with an increase in age**.** In 1–6 day old larvae (2nd Instar), no esterase bands was observed. From 7 to 12 day old larvae, the number of bands increased as the age of larvae increase. Considering very low body mass of individual 1-6 day old larvae, 10-15 individuals of them were squashed together to find out esterase activity. In this case also, no band was found. Est-1(Estα1/Estβ1) bands were the fastest but faintest ones. These were observed in the 11 and 12 day old larvae only. The presence of highly active esterase Est-2 (Estα2/Estβ2), in all larvae tested in the present study. While Est-3 (Estα3/Estβ3) bands were present in all 12 day old larvae, and in some 11 and 10 day old larvae. Est-5 (Estα5/Estβ5) and Est-6 (Estα6/Estβ6) found in 7-12 day old larvae. But and Est-7 found in 8-12 day old larvae. Est- 4 band not appeared in larval stages in the present study. It is reasonable to think that esterase requirements in larval stages are greater than in other stages due to high level of food ingestion, juvenile hormone metabolism and specific development processes. The esterase bands of pupae significantly different from larvae and adults. Estα4/Estβ4 found in all pupae of *Cx. quinquefasciatus* Say quit different from larvae. This Est-4 did not appear in other developmental stages. Est-5 (Estα5/ Estβ5), Est-6 (Estα6/ Estβ6) and Est-7 (Estα7/ Estβ7) were not expressed in pupae. Est-1 (Estα1/ Estβ1) and Est-2 (Estα2/ Estβ2) also found in pupae. In the pupal stage, the food intake ceases, so may be that reason requirements of esterase became less.

The esterase bands of female differ from larvae and pupae. Only Est-2 and Est-3 found in female mosquito. Highly active esterase Est-3(Estα3/Estβ3), found in all female mosquitoes tested in the present study. Est-2(Estα2/Estβ) found in some female mosquitoes, but not all females in the present study. In adult, development is completed so, the esterase requirements lower than that of larvae. The esterase enzymes in this last stage are probably involved mainly with reproduction, digestion and nervous system physiology. There are many reports of variations in electrophoretic esterase patterns at different stages of insect development (Simon, 1969; Georghiou and Pasteur, 1978; Maruyama, 1969). The results presented here indicated that this is also the case with the present population of *Cx. quinquefasciatus* Say.

Different levels of esterase band activity, denoted by different thicknesses and degrees of staining, were another kind of variation observed among developmental stages in the present study. It shows that even esterases that are necessary in different stages may be under control, increasing or decreasing their expression level during development.

According to Oakeshott *et al*. (1993), esterases inhibited by organophosphate (malathion) and carbamate (eserine sulphate) are classified as cholinesterases, esterases inhibited only by organophosphate (malathion) are classified as carboxylesterases and esterases that are inhibited only by sulphydryl reagents (PMSF) are arylesterases. In the present study, malathion inhibited completely Est-1, Est-3 to Est-7 bands. So, it can be said that these esterases are either carboxylesterases or cholinesterases. Est-2 not inhibited by malathion, so it may be arylesterase.

CONCLUSION

"Differences in esterase synthesis among stages are probably due to regulatory mechanisms acting in agreement with the requirements of a variable number of processes in which esterases are involved" (Lima-Catelani *et al.,* 2004). The larval stages are more active during development and intake more diverse food compared to other stages. These features may be related to increase esterase production these stages.

REFERENCES

Brattsten, L.B. (1992) Potential role of plant allelochemicals in development of insecticide resistance. In: Molecular mechanism of insecricide resistance, diversity among insects. American Chemical Society Symposium Series. Mullin, C. A., & Scott, J. G. [eds]. pp. 313-348, Washington DC.

Curtis, C.F. and Feachem, R.G. (1981) Sanitation and *Culex pipiens* mosquitoes: A brief review. *J Trop Med Hyg*. 84, 17–25.

Dauterman, W.C. (1985) Insect metabolism: Extramicrosomal. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology. Kerkut. G. A. & Gilbert, L. I. [eds]. pp. 713-730, Pergamon Press, Oxford.

Gayathri, V., Balakrishna, P. and Murthy (2006) Reduced susceptibility to deltamethrin and kdr mutation in *Anopheles stephensi* Liston, a malaria vector in India. J Am Mosq Control Assoc. 22, 678-88.

Georghiou, G.P. and Pasteur, N. (1978) Electrophoretic esterase patterns in Insecticide–resistant and susceptible mosquitoes. J. Econ. Entomol. 71, 201- 205.

Guillemaud, T., Makate, N., Raymond, M., Hirst, B. and Callaghan, A. (1997) Esterase gene amplification in *Culex pipiens.* Insect Mol. Biol. 6, 319-327.

Hames, B.D. (1986) An introduction to polyacrylamide gel electrophoresis in gel electrophoresis of proteins. pp. 1- 45. RIL Press Limited.Oxford. Washington, D.C.

Johnson, F.M., Kanapi, C.G., Richardson, R.H., Wheeler, M.R. and Stone, W.S. (1966) An operational classification of *Drosophila* esterases for species comparison. Univ. Texas Public. 6615, 517 – 532.

Kabir, S.M.H. (1987) Role of insecticides in mosquito control and resistance problem. Bangladesh J. Zool. 2, 43 – 49.

Lima-Catelani, A.R.D.A., Ceron, C.R. and Bicudo, H.E. M.D.C.B. (2004) Variation of genetics expression during, development, revealed by esterase patterns in *Aedes aegypti* (Diptera:Culicidae). Biochem Genet. 42, 69 – 84.

Maa, W.C. & Terrier, L.C. (1983) Age dependent variation in enzymic and electrophoretic properties of house fly (*Musca domestica*) carboxylesterases. Comp. Biochem. Physiol. 74, 461-467.

Maruyama, Y., Yastsutomi, K. and Ogita, Z. (1969) Electrophoretic analysis of esterase isozymes in organophosphate resistant mosquitoes (*Culex pipiens*). Insect Biochem. 14, 181 – 188.

Mouches, A. C., Pasteur, N., Berge, A, J. B., Hyrien, O., Raymond, M., Robert De Saint Vi.ncent, B. (1986) Amplification of an esterase gene is responsible for insecticide resistance in a California *Culex* mosquito. Sci. 233, 778-780.

Oakeshott, J.G., Papenrecht, V., Boyee, E.A., Healy, T.M. and Russel, R.J. (1993) Evolutionary genetics of *Drosophila* esterases. Genetica. 90, 239 – 268.

Poirie, M., Raymond, M. & Pasteur, N. (1992) Identification of two distinct amplification of the esterases B locus in *Culex pipiens* (L.) mosquitoes from Mediterranean countries. Bioch Gen. 30, 13-26.

Rooker, S., Guillemaud, T., Berge Â, J., Pasteur, N. and Raymond, M. (1996) Co amplification of A and B esterase genes as a single unit in *Culex pipiens* mosquitoes. Heredity, 77, 555-561.

Simon, J. P. (1969) Esterase isoenzymes in the mosquito. *Culex pipiens fatigans:*. Develop-mental and genetic variations. Ann. Entomol. Soc. Am. 62**,** 1307.

Steiner, W.W.M. and Johnson, W.F. (1973) Techniques for electrophoresis of Hawaian *Drosophila* US-IBP. Islan Ecosyst. Tech. Rep. 30, 1 – 21.

Subra, R. (1981) Biology and control of *Culex pipiens quinquefasciatus* Say 1823 (Diptera, Culicidae) with special reference to Arica. Insect Sci. Application. 1, 319 – 338.

Villani, F., White, G.B., Curtis, C.F. and Miles, S.J. (1983) Interitance and activity of some esterases associated with organophosphate resistance in mosquitoes of the complex of *Culex pipiens* L. (Diptera: Culicidae). Bull. Ent. Res. 73, 153 – 170.

Vaughan, A., Rodriguez, M. and Hemingway, J. (1995) The Independent gene amplification of electrophoretically indistinguishable B esterases from the insecticide-resistant mosquito *Culex quinquefasciatus.* Biochem. J. 305, 651-658.