Comparison between the Activity of Alpha, Beta and both Alpha & Beta Substrate in Esterase Enzyme of Mosquito Fish, *Gambusia affinis*

Md. Monjurul Ahasan[1*], Md. Anisur Rahman[2], Md. Sabir Hossain[3] , Sayeda Zannatul Sakina Mazid[2] and Md. Akbar Hossain[1]

[1]Department of Pharmacy, Dhaka International University, Banani, Dhaka-1213, Bangladesh
[2]Genetics & Molecular Biology Laboratory, Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh
[3]Department of Biochemistry & Molecular Biology, Jahangirnagar University Savar, Dhaka-1342, Banngladesh

Abstract

For this experiment the samples just after birth, 7, 14 days old fry and 21, 28, 35, 42, 49 days old male and female were used and stained with α, β and both α & β napthyl acetate as substrate by using 7.5% polyacrylamide gel electrophoresis during the month of December 2008 to May 2009 in Genetics and Molecular Biology Laboratory, Department of Zoology, University of Dhaka. Altogether three esterase bands named as Est-1, 2 and 3 were observed based on decreasing mobility from the origin. Some bands were absent with only β napthyl acetate and maximum bands were α specific. But all bands are present, when stained with both α and β napthyl acetate.

Keywords: Esterase, isozyme, *Gambusia affinis*, α & β napthyl acetate substrate.

Introduction

The fast-acting residual insecticides are used widely for mosquito control throughout the world which are shorter-lasting, costly and affect the environment adversely. Owing to the evolution of resistant mosquito strains, these insecticides are less effective (Bay, 1976a; WHO, 1986). So, the development of an effective alternative method is desirable. The biological control of mosquito by *G. affinis* can be one of such approaches. The mosquito fish, *G. affinis* (Poeciliidae: Cyprinodonti-formes) is one of the exotic species in Bangladesh which is native to the watershed of the Gulf of Mexico (Gebrich and Laird, 1968) and is well known as a biological agent in controlling the larval population of mosquito (Bay, 1976b; Rees, 1934; Rees, 1945; Nelson *et al.,* 1992; Hubbs *et al.,* 1991;Boklund, 1997). All sizes and ages of *Gambusia* readily feed on mosquito larvae and fry only a few hours old will attack young instars. *G. affinis* was introduced in our country in 1930s / 1940s but successful management system is not developed yet. Synthetic insecticides are used widely for mosquito control throughout the world, and some of this has long residual activities which affect the environment adversely. Many mosquito species has developed resistance to a variety of insecticides (WHO, 1986). It is necessary to know the genetic makeup of a species for any successful management. Moreover, knowledge about the genetic variability in different age group of the fish is a fundamental necessity for biological control of mosquitoes. So far a few genetic or biochemical researches have been done on fisheries in Bangladesh and the present study is the first. There is no measure to determine the extent of genetic abnormality due to the application of different insecticides in a view to control mosquitoes. Esterase isozymes are one of the lipid-hydrolyzing enzymes, possess high significance in genetics and toxicology (Market and Moller, 1959; Callaghan *et al.,* 1994). This esterase enzyme is a very interesting enzyme because on one hand it is implicated in synaptogenesis while on the other hand it is known to be involved in neuro-degeneration in adult tissue (Ahasan *et al.,* 2009). Esterases are also used as bioindicators to measure the toxic potency of pesticide residues usually applied in agriculture. The residual effect of pesticide in aquaculture specifically in fish is high which, in turn, cause death of fish particularly, after the rainy season (Debnath, 1978; Sahib and Rao, 1980). Esterase isozyme appears to be playing a critical role in offering resistance

Correspondence author: Md. Monjurul Ahasan, Department of Pharmacy, Dhaka International University, Banani, Dhaka-1213, Bangladesh.
E-mail: labu63@gmail.com

to insecticides (Karunaratne *et al.*, 1999). Organophosphate acts as an inhibitor of certain esterases. When organisms are treated with insecticides, continuous nerve impulse transmission due to inhibition of acetylcholine esterase causes them to be shaky and have writhing movements. This may result sudden death of an organism. The condition occurs due to low production of esterase or lack of gene that produce this isozyme. If any animal tissue bears sufficient esterase, the organism will be more resistant against poisonous agent. As guppy are use for mosquito control in different aquatic habitat where insecticides are applied for same purpose, it is a requirement to investigate how much resistant the guppy is and its various stages of fry to insecticide. An organism may develop resistance to insecticides by producing large amount of specific esterase enzymes which either break down the insecticide molecules or bind to it so tightly that it can not function. Considering the above facts, it is essential to understand the genetic status in terms of esterase variability. The paper deals in polymorphic pattern of esterase isozymes in different age group of *G. affinis* with α, β and both α & β napthyl acetate.

Materials and Methods

G. affinis were collected from the drains of Curzon Hall, University of Dhaka, Dhaka, Bangladesh. The samples just after birth, 7, 14 days old fry, 21, 28, 35, 42, 49 days old male and female were used during this experiment. Each sample of *G. affinis* was taken into an eppendrof tube and squashed in Tris-Borate EDTA buffer. After squashing, 2X TBE-Bromophenol blue solution was added to each sample. Samples were then centrifuged at 12,000 rpm for 5 minutes at 4 ºC. Supernatants were used for electrophoresis. About 7.5 ml acrylamide and bisacrylamide were used in the ratio of 30: 0.8; 6.0 ml of TBE (5X) buffer, which contain Tris base, Boric acid, 0.5M EDTA (Ethylendiamine Tetra acetic Acid); TEMED (Tetramethylethylene diamine); 10% AMPS (Amonium persulfate) and distilled water were used for gel preparation. Gels were pre run at 120 volts for about 30 minutes. Samples (10 µl) were then carefully loaded into the wells using a micropipette, one sample to one well. Electrophoresis was done at 120 volt (constant voltage). In this condition, the electrophoresis apparatus was kept undisturbed for at least 1 hour and 15 minutes, until the tracking dye (Bromophenol blue) comes out from the sandwich. Staining solution for esterase contained with of 0.2 M Monobasic sodium phosphate and 0.2 M Dibasic sodium phosphate and the substrate was α, β and both α & β napthyl acetates used separately. Running gel was kept in this mixture for 15 minutes at 25 ºC. After 15 minutes, substrate mixtures were poured out and the gel was immersed in staining mixture. Staining mixture was prepared by dissolving Fast Blue RR salt in water every day just before use. Then gel was stained at 37ºC incubation for 25 minutes. After staining, the Fast Blue staining solution was drained out and the gel was washed with distilled water. Stained gel was photographed after decolorizing for 30 minutes. Esterase bands were scored from the stained gel (Ahasan *et al.*, 2009).

Results and Discussion

Esterase isozyme studies were made to generate genetic data on the basis of the banding pattern, number of esterase loci and their variability. It is necessary to know the genetic make up of a species for any successful management. Concerns about the biological control of mosquitoes as well as coordinated actions for successful management have increased the interests to researchers to develop genetic knowledge of the fish. Esterases were identified by using α and β napthyl acetate as substrate following basically the technique described by Johnson and Denniston (1964) and Steiner and Joslyn (1979). When only α napthyl acetate was hydrolyzed, the bands in the gel turned black and named as α esterases, when only β napthyl acetate, the bands were red and named as β esterases (Figure 1).

Relative mobility: Isozyme analysis is a powerful technique with numerous applications including the amount of genetic variability. The esterase enzyme loci were assigned to an increasing numbers based on the decreasing mobility from the origins following Webb (1964). Relative mobility of each band was determined by comparing with the most frequent band 2 selected as a standard and it was present in all age groups. The value of most mobile band was considered as one when the relative mobility values of different isozyme bands were measured (Shahjahan *et al.,* 2001). The relative mobilities of the bands were 1.44 ± 0.02, 1.00 ± 0.02 and 0.27 ± 0.02 respectively, showed in (Table 1). The highest relative

mobility value of esterase band was 1.44 ± 0.02 and was possessed by Est-1, located near the anode (+) pole, slowest relative mobility value was 0.27 ± 0.02 and was possessed by Est-3, found in the cathode (-) pole.

Banding pattern of esterase isozyme: Polyacrylamide gel electrophoresis (9.8 cm × 12 cm gel, running time = 1 hour and 15 minutes) was conducted to examine the esterase pattern. Different types of bands were revealed in PAGE when stained for esterase activity. The banding patterns of esterases are shown in (Figures 1, 2). Altogether three esterase bands namely Est-1, Est-2 and Est-3 were found in *G. affinis* (Figures 1, 2 and Tables 1, 2). Five esterase bands were found in Nile tilapia (*Orechromis niloticus*) (Shahjahan *et al.,* 2008). Five esterase bands were found in *Pangasius hypothalamus* (Begum *et al.,* 2008). Seven esterase bands were found in Sword tail fish (*Xiphophorus helleri*) (Ahuja *et al.,* 1977). According to previous Mendelian inheritance studies on these esterase loci, each of the bands corresponds to one allele (Stordeur, 1976). These esterase bands had tissue and substrate specific expression. All the bands were expressed when stained with both α and β napthyl acetate as substrate, fewer bands were observed with only one substrate and most of the bands were α specific (Figure 1). The bands also showed an intensity variation among different age groups (Figure 1 and Table 2).

Esterase-1: Esterase-1 was observed in all the samples when stained with both α and β napthyl acetate as substrate but absent in slot 1, 2, 6, 8, 9, 10 and 12 when stained with β napthyl acetate (Figure 1). In the liver tissue of Nile tilapia (*O. niloticus*), four esterase bands were expressed in α napthyl acetate but Esterase 1 was absent when stained with β napthyl acetate as substrate (Shahjahan *et al.,* 2008).

Esterase-2: Esterase-2 was observed in all the samples when stained with both α and β napthyl acetate as substrate but absent in slot 1, 2, 6, 8, 9, 10 and 12 when stained with β napthyl acetate (Figure 1). Three esterase bands Est-2, 3 and 4 were expressed when stained with both α and β napthyl acetate in fore, mid and hind gut of Nile tilapia (*O. niloticus*) but exception was observed in hind gut when stained with β napthyl acetate as substrate where Est-2 was absent (Shahjahan *et al.,* 2008).

Esterase-3: Esterase-3 was observed in all the samples when stained with both α and β napthyl acetate but absent in slot 1, 2, 6, 7, 8, 9, 10 and 12 when stained with β napthyl acetate (Figure 1). Four esterase bands namely Est-2, 3 4 and 5 were observed in α napthyl acetate but in β napthyl acetate Est-5 was absent and three esterase bands were observed (Shahjahan *et al.,* 2008).

In the present study, in 28 days female, Est-1, 2 and 3 were deeply stained in both α and β napthyl acetate but Est- 1 and 2 was medium stained and Est-3 was faint when stained with β napthyl acetate. In 49 days female, Est-1, 2 and 3 were deeply stained in both α and β napthyl acetate but Est-1 and 2 were deeply stained and Est-3 was medium when stained with β napthyl acetate (Figure 1). Banding difference was also observed in the tissues of *Carassius* sp. (Shengming *et al.,* 1988). The observed variability in the banding intensity showing a variation of its activity based on the samples. These bands were used to analyze genetic variation. Different levels of esterase band activity, denoted by different thickness and degree of staining were another kind of variation that has been observed among the different samples. This observation was important because high degree of staining and thickness are indicative of great enzymatic activity. Lima-Catelani *et al.* (2004) observed differences in esterase synthesis among *Musca domestica* from different regions are probably due to regulatory mechanisms acting in agreement with the requirements of a variable number of processes in which esterase are involved. Among thirteen observed samples, in the present study, the esterase activity was most abundant in females compared to that of males (Figure 1). Above results indicate that tissue specific differences was observed in the banding pattern of esterases in *G. affinis*. Significant banding difference in esterase also noticed in the brain, kidney, spleen and heart tissues of *Carassius* sp. revealed a similar trend in esterase diversity among different fish (Shengming *et al.,* 1988). Sharma and Al-Daham (1979) noted in a study from Iraq that the male *Gambusia* was a poorer consumer of mosquito larvae than the female. But the genetic basis of their findings is not understood. The variation of consumption rate was due to reduced metabolism of male fish as was suggested by Rees (1945). In the present study as stated earlier reveals that females possess much esterases than males. Esterase activities are associated with digestion, metabolism and physiological function (Jones and Brancoft, 1986; Sastry, 1974). The present

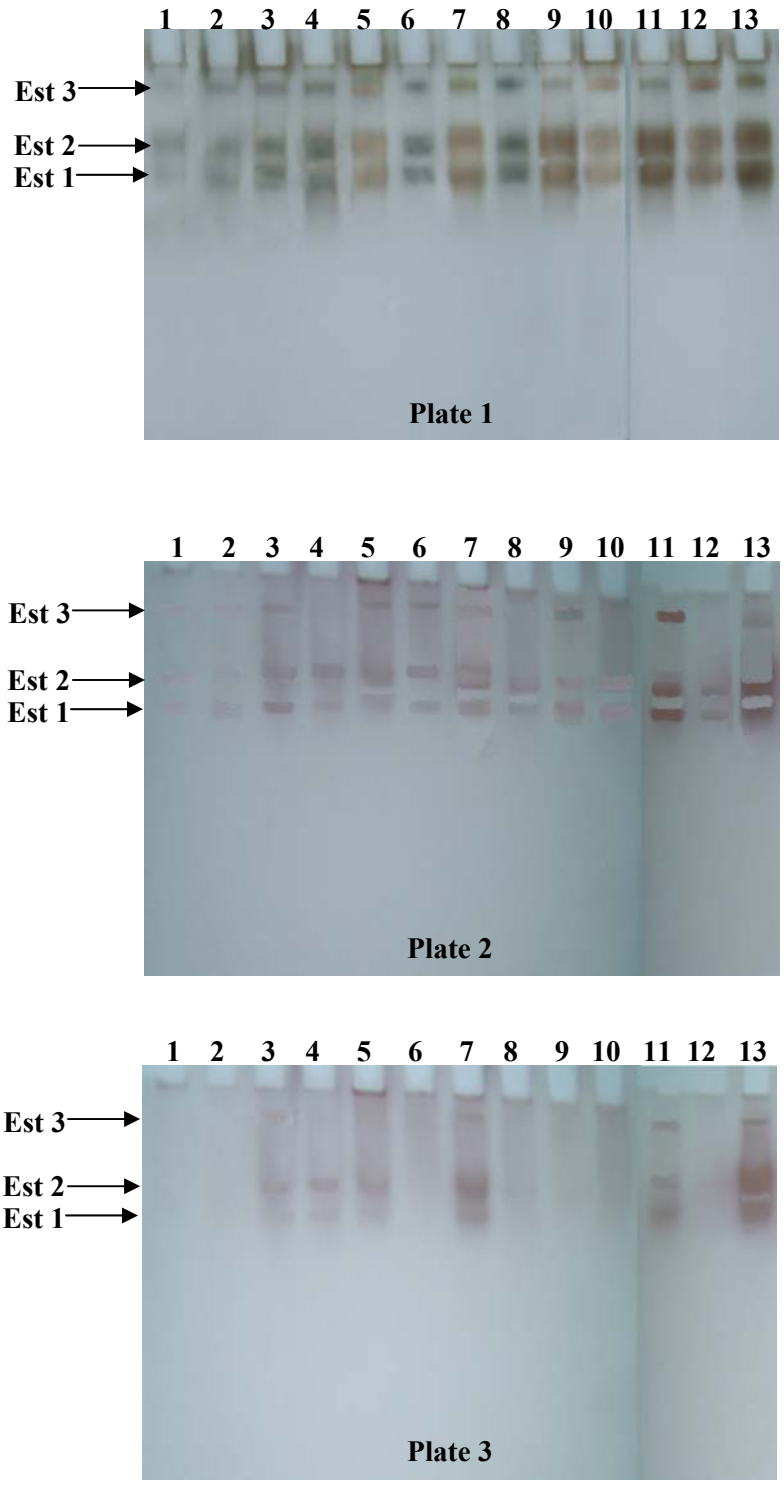

Figure 1. Esterase isozyme pattern in different age groups of *G. affinis* stained in both α and β napthyl acetate as substrate (Plate 1), α napthyl acetate as substrate (Plate 2) and β napthyl acetate (Plate 3). Where lane (1) denotes samples from at birth; (2) 7 ; (3) 14 ; (4) 21 M; (5) 21 F; (6) 28 M; (7) 28 F; (8) 35 M; (9) 35 F; (10) 42 M; (11) 42 F; (12) 49 M; (13) 49 F. Arrow indicates position of esterase bands.(Note: M = Male, F = Female.)

Table 1. Electrophoretic banding pattern showing the relative mobilities (Rm) of the esterase bands in different age groups of *G. affinis* scored from α and β napthyl acetate as substrate.

Slot no.	Age group (days)	Esterase								
		Est-1			Est-2			Est-3		
		M	Rm	Mean±SD	M	RM	Mean±SD	M	Rm	Mean±SD
1	At birth	3.3	1.43		2.3	1.00		0.7	0.30	
2	7	3.3	1.43		2.4	1.04		0.6	0.26	
3	14	3.3	1.43		2.3	1.00		0.6	0.26	
4	21 M	3.4	1.47		2.4	1.04		0.7	0.30	
5	21 F	3.3	1.43		2.3	1.00		0.7	0.30	
6	28 M	3.3	1.43		2.3	1.00		0.7	0.30	
7	28 F	3.3	1.43	1.44±0.02	2.3	1.00	1.00±0.02	0.6	0.26	0.27±0.02
8	35 M	3.3	1.43		2.3	1.00		0.6	0.26	
9	35 F	3.4	1.47		2.3	1.00		0.6	0.26	
10	42 M	3.3	1.43		2.3	1.00		0.6	0.26	
11	42 F	3.4	1.47		2.3	1.00		0.6	0.26	
12	49 M	3.3	1.43		2.3	1.00		0.7	0.30	
13	49 F	3.4	1.47		2.3	1.00		0.6	0.26	

Note: M = Mobility, Rm = Relative mobility, M = Male, F = Female

Table 2. Electrophoretic banding pattern showing the intensity variation of esterase isozymes in different age groups of *G. affinis* scored from both α and β napthyl acetate.

Slot no.	Sample age (days)	Est-1 (1.44±0.02)	Est-2 (1.00±0.02)	Est-3 (0.27±0.02)
1	At birth	+	+	+
2	7	+	+	+
3	14	++	++	++
4	21 M	++	++	++
5	21 F	++	++	++
6	28 M	++	++	++
7	28 F	+++	+++	+++
8	35 M	+++	+++	+++
9	35 F	+++	+++	+++
10	42 M	++	++	++
11	42 F	+++	+++	+++
12	49 M	+++	+++	+++
13	49 F	+++	+++	+++

Note: +++ = Deep stained ++ = Medium stained + = Faintly stained M = Male, F= Female

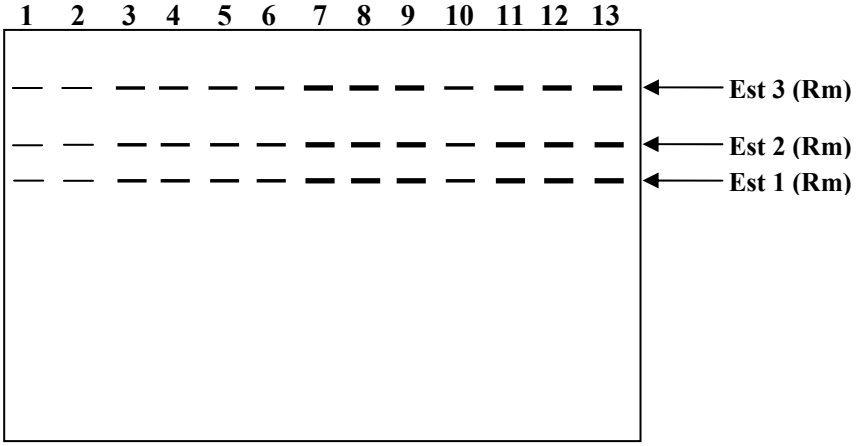

Figure 2. Zymogram for esterase banding pattern in *G. affinis* scored from both α and β napthyl acetate.Where lane (1) denotes samples from at birth; (2) 7 ; (3) 14 ; (4) 21 M; (5) 21 F; (6) 28 M; (7) 28 F; (8) 35 M; (9) 35 F; (10) 42 M; (11) 42 F; (12) 49 M; (13) 49 F. Arrow indicates position of esterase bands. (Note: M = Male, F = Female)

study revealed that Est-2 was most abundant and most of the samples contained it (Figure 1). Est-3 was found in all the tissues of *Pangasius hypothalamus* (Begum *et al.,* 2008). Champion et al. (1975) reported that high level of esterase activity in earliest embryonic stages declined during subsequent development toward hatching in green sunfish (*Lepomis cyanellus*). But the present study revealed that high level of esterase activity proceeds during subsequent development toward being sexually mature. Banding pattern of esterases of different tissues has a good potential used in the identification of species. Al-Amin *et al.* (2005) reported that isozyme banding pattern of the intestine could be used for identification of two species of Pangasius *(P. sutchi* and *P. pangasius*). Between the two species the intestine of *P. sutchi* and *P. pangasius* possesses 4 and 6 bands, respectively. Furthermore, two species of *Anabus* (A. *testudineus and A. oligolepis*) was identified using esterase bands of liver, kidney, skeletal muscle, heart and egg (Ramaseshaiah and Dutt, 1984).

Conclusion

Est-1, Est-2 & Est-3 were observed in all the samples when stained with both α and β napthyl acetate as substrate but absent in some slot when stained separately with α napthyl acetate & β napthyl acetate, so the enzymes activity depends on the substrate. The findings in the present study may be extended to use as genetic marker in various fields of taxonomy, physiology and toxicology in *G. affinis.*

References

Ahasan, M.M., Shahjahan, R.M., Begum, R.A., Sakina S.Z. and Jamal Uddin, A.F.M. 2009. Esterase in different age group of farm chicken (*Gallus gallus*) with reference to the nervous tissue. *Int. J. Sustain. Agril. Tech*. **5**, 98-101.

Ahuja, M.R., Schawb, M. and Anders, F. 1977. Tissue-specific esterases in the Xiphorine fish *Platypoecillus maculates, Xiphophorus helleri* and their hybrid. *Biochem. Genet.* **15**, 601-610.

Al-Amin, M., Sufi, G.B. and Shahjahan, R.M. 2005. Esterase isozyme pattern in *Pangasius pangasius* and *P. sutchi. J. Biol. Sci.* **14**, 193-196.

Bay, E.C. 1976a. Mosquito control by fish: a present day-appraisal. *WHO Chronicle* **21**, 415-423.

Bay, E.C. 1976b. Potential for naturalistic control of mosquitoes. *Proc. Calif. Mosq. Contr. Assoc.* **35**, 34-37.

Boklund, R.J. 1997. Mosquito fish in control programs. *J. Am. Mosq. Contr. Assoc.* **13**, 99-100.

Begum, R.A., Bhadra, S.C., Shahjahan, R.M., Alam, M.S. and Begum, A. 2008. Esterase banding pattern in different tissues of *Pangasius hypothalamus* (Sauvage, 1878). *Bangladesh. J. Zool.* **36**, 287-294.

Callaghan, A., Boiroux, V., Raymofld, M and Pasteur, N. 1994. Prevention of changes in electrophoretic mobility of overproduced esterase from organophosphate-resistant mosquitoes of the *Culex pipiens* complex. *Med. veterin. Entomol.* **8**, 391-394.

Champion, M.J., Shaklee, J.B. and Whitt, G.S. 1975. Developmental genetics of the telost isozymes. *Isozymes.* 3: Acad. Press, N. Y. pp. 417-437.

Debnath, J.C. 1978. Electrophoretic and biochemical studies of proteins and isozymes of non-specific esterase, Lactate and Malate dehydrogenases in the three species of freshwater fishes of Bosnia and Hecegovina. Ph.D. thesis, University Medical Centre, Sarajevo.

Gebrich, J.B. and Laird. M. 1968. Bibliography of papers relating to the control of mosquitoes by the use of fish. *F.A.O. Fisheries Technical Paper* **75** (FRs/T75): 70.

Hubbs, C., Edwards, R.J. and Grrett, G.P. 1991. An annotated checklist for the freshwater fishes of Texas, with keys to identification of species. *The Texas J. Sci. Supp.* **43**, 1-56.

Johnson, F.M. and Denniston, C. 1964. Genetic variation of alcohol dehydrogenase in *Drosophila melanogaster. Nature* **204**, 906-907.

Jones, B.R. and Brancoft, H.R. 1986. Distribution and probable physiological role of esterases in reproductive, digestive and fat-body tissue of adult cotton boll weevil, *Anthonmus grandis. Biochem. Genet.,* **24**, 499-508.

Karunaratne, S.H.P.P., Small, G.J. and Hemingway, J. 1999. Characterization of the elevated esterase associated insecticide resistance mechanism in *Nilaparvata lugens* (Stal) and other planthopper species. *Int. J. Pest Manag*. **45**, 225-230.

Lima-Catelani,A.R.A., Ceron, C.R. and Buicudo, H.E.M.C. 2004. Genetic expression during development, revealed by esterase patterns in *Aedes aegypti* (Diptera: Culicidae). *Biochem. genet.* **42**, 69-84.

Market, C.L. and Moller, F. 1959. Multiple forms of enzymes tissue, ontogenetic and species specific pattern. *Proc. Nat. Acad. Sci.* **45**, 753-763.

Nelson, S.M. and Keenan, L.C. 1992. Use of indigenous fish species, *Fundulus zebrinus*, in a mosquito abatement program: A field comparison with the mosquitofish, *Gambusia affinis*. *J. Am. Mosq. Contr. Assoc.* **8**, 301-304.

Ramaseshaiah, M. and Dutt, S. 1984. Comparative electrophoretic studies of *Anabustestudineus* and *A. oligolepis* (Osteichthyes: Anabantidae) (climbing perch) from lake Kolleru, Andhra Pradesh, India. *Hydrobiologia*. **119**, 57-64.

Rees, D.M. 1934. Notes on mosquito fish in Utah, *Gambusia affinis*, (Baird and Girard). *Copeia* **4**, 157-159.

Rees, D.M. 1945. Supplemental Notes on mosquito fish in Utah, *Gambusia affinis* (Baird and Girard). *Copeia* **4**, 236.

Sahib, I.K.A. and Rao, K.V.R. 1980. Toxicity of malathion to the freshwater fish *Tilapia mosambica. Bull. Environ. Contam. Toxicol.* **24**, 870-874.

Sastry, V.K. 1974. Histochemical localization of esterase and lipase in the digestive system of two telost fishes. *Acta. Histochem.* **51**, 18-23.

Shahjahan, R.M., Begum, A., Hasanuzzaman, M. and Yesmin, F. 2001. Electrophoretic banding patterns of Alcohol dehydrogenase (ADH) isozyme in third instar larvae of melon fly, *Bactrocera cucurbitae* (Coq.). *Dhaka Univ. J. Biol. Sci.* **10**, 183-189.

Shahjahan, R.M., Karim, A., Begum, R.A., Alam, M.S. and Begum, A. 2008. Tissue specific esterase isozyme banding pattern in nile tilapia (*Oreochromis niloticus*). *Univ. J. Zool. Rajshahi Univ.* **27**, 1-5.

Sharma, P.K. and Al-Daham, N.K. 1979. Comparative studies on the efficiency of *Achanius disper* (Ruppell) and *Gambusia affinis* (Baird and Girard) in mosquito control. *Comp. Physiol. Ecol.* **4**, 106-109.

Shengming, H., Changgeng, Q. and Thukui, T. 1988. Comparative studies on the electrophoregram of esterase isozyme and lactate dehydrogenase of *Carassius aukatus* gibelio bloch and *Carassius* sp. *Zool. Res.* **9**, 69-78.

Steiner, W.W.M. and Joslyn, D.J. 1979. Electrophoretic techniques for the genetic study of mosquitoes. *Mosq. News.* **39**, 35-54.

Stordeur, D.F. 1976. Esterases in the mosquito Culex pipiens: formal genetics and polymorphism of adult esterases. *Biochem. Genet.* **14**, 481-493.

WHO. 1986. Resistance of vectors and reservoirs of disease to pesticides.Tenth report of the WHO expert committee on vector biology and control. *Technical Report Series* **737**, 87.