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ORIGINAL ARTICLE

VARIATION OF ESTERASE ISOZYME BANDING PATTERN IN *PENAEUS MONODON* WITH REFERENCE TO TISSUE SPECIFICITY, MALATHION TOXICITY AND ALLELE FREQUENCY DISTRIBUTION

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ABSTRACT: The electrophoretic banding pattern of esterase isozymes were examined in five different tissues of black tiger shrimp (*Penaeus monodon*) after staining with α and β naphthyl acetates where altogether five esterase bands (Est-1, Est-2, Est-3, Est-4 and Est-5) were observed. Single esterase band (Est-1) was observed in the dorsal (anterior) and tail (base of telson) muscle while two, four and five bands were found in ventral muscle (Est-1 and Est-4), nerve cord (Es-1, Est-2, Est-3 and Est-4) and eye (Es-1, Est-2, Est-3, Est-4 and Est-5) in order. When the nerve and eye tissue were treated with different doses of malathion, fluctuation in esterase activity in terms of intensity variation as well as switch on or off of certain allele was observed. Allele frequency distribution was counted from eye and nerve tissue of sampled population (n=50) where variation in the number of bands was observed. Most of the sampled eyes contained three or four esterase bands (40% and 46% respectively) while nerve tissues showed two or three bands (48% and 36% respectively). Each locus (band) was found to be polymorphic in nature. Polymorphism of Est-1 from both tissues was tested for Hardy-Weinberg Equilibrium (HWE) and found them within HWE.

KEYWORDS: Esterase Isozyme, Tissue Specificity, Toxicity, Allele Frequency, *Penaeus monodon*.

INTRODUCTION

Esterases are highly variable and multifunctional hydrolytic enzymes which take part in biological processes such as regulation of hormone, digestion, reproduction, insecticide resistance etc. Electrophoretic studies were done extensively on different tissues of various animals from which it was revealed that the enzyme (esterase) exists in multi molecular forms and functions (Markert and Moller, 1959). In particular, amino acid substitutions that change the electric charge of the enzyme to form its isozymes, are simple to identify by gel electrophoresis based on differential iso-electricpoints. Polyacrylamide gel electrophoresis (PAGE) made it possible to study the genetic variability involved in the expression of esterases from different tissues of *Penaeus monodon.*

Malathion, an organophosphorus insecticide and inhibitor of esterases, is usually used in pest control where shrimps become non target victims of this insecticide. It kills organism by inhibiting acetylcholinesterase (AChE) that is involved with the transmission of nerve impulses (Gordon, 2004). A substantial body of anecdotal evidences suggests that pesticide poisonings and ecological damage have become commonplace in Bangladesh (Ramaswamy, 1992; Jackson, 1991). More commonly, aquatic organisms are subjected to long-term stresses from exposure to sub lethal concentrations that have deleterious effects as do lethal concentrations, because sublethal and small effects on aquatic organisms may alter behaviors, feeding habits, school group positions, reproduction rates, quantity of fish production, etc. (Murty, 1986; Metelev *et al.,* 1983).

According to the previous Mendelian inheritance studies on these esterase loci, each of the bands corresponds to one allele (Stordeur, 1976). Populations can be scored with respect to loci scored with molecular probes. The use of allozyme variation has a long tradition in population genetics (Gitzendanner and Soltis, 2000) and nonspecific esterases are usual markers in genetic studies of animals, plants and microorganisms because they are easy to detect and appear to be highly polymorphic (Resende *et al.,* 2000). In Hardy-Weinberg Equilibrium (HWE) genotype frequencies at any locus are a simple function of allele frequencies and deviations from HWE at particular markers may suggest problems with genotyping or population structure (Wigginton *et al.,* 2005). It is now a

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common practice to check whether observed genotypes conform to HWE or not. The technique of polyacrylamide gel electrophoresis (PAGE) enables us to determine the degree to which individual genes are polymorphic (Raven and Johnson, 1986). On the other hand, the level of esterases are highly variable depending on the life stage, sex, tissue, hormones, strain, food, environmental conditions and numerous other factors (Devorshak and Roe, 1999). Hence, an attempt was taken to investigate esterase isozyme variability of *Penaeus monodon* which play an important role in the national economy of Bangladesh, in terms of tissue specificity, malathion toxicity and allele frequency distribution.

MATERIALS AND METHODS

Experimental shrimps were collected from different ghers (enclosed area characterized by an encirclement of land along the banks of tidal rivers) of Paikgacha, Khulna district of Bangladesh ($22^0 44'$N, $89^0 33'$E). The specimens were transported to the laboratory with ice cool pack and were dissected to collect measured amount (~0.016 g) of the selected tissues (dorsal, ventral and tail muscle, eye and nerve) in 'Genetics and Molecular Biology Laboratory', University of Dhaka. Each sample was separately squashed in TBE buffer (50µl), centrifuged at 12500 rpm for 15 min and 20µl aliquot from each sample was loaded on the gel slots for electrophoresis (Rashid, 2012). The entire technique for polyacrylamide gel electrophoresis (PAGE) was followed as that of Shahjahan *et al.*, (2008) and the electrophoretic bands of esterase resulting from stained gel with napthyl acetates were assigned to increasing numbers based on decreasing mobility following Richardson (1986). Malathion doses were prepared freshly considering active ingredients of molecules and tissues were treated into separate eppendroff tubes in submerged condition for 15 min before considering it to electrophoresis (Rashid, 2012). The possible epigenetic bands were prevented by the presence of EDTA in gel buffer (Callaghan *et al.*, 1994). The reproducibility of the experiments was standardized and representative gels were subjected to analysis. Allele frequency was calculated using a reiterative technique developed by *Cappellini et al.*, (1955) and slightly modified by Townson (1972). It was assumed that the F (fast) and S (slow) forms of the enzyme were controlled by two alleles Est-1^f and Est-1^s (abbreviated to f and s) and that a third allele Est-1^x (abbreviated to x), occurs at the same locus. Thus, theoretically, there could be six possible genotypic combinations: f/s and f/x producing the F_, s/s and s/x producing the S_, f/s producing the M_, and x/x producing the null_ phenotypes. Assuming equilibrium in the natural population and assuming that selection takes place at each locus, the gene frequencies were calculated from the phenotype frequencies (Falconer, 1960).

RESULTS AND DISCUSSION

Altogether, five esterase bands designated as Es-1, Est-2, Est-3, Est-4 and Est-5 were observed from different tissues of *Penaeus monodon.* Earlier study on *Macrobrachium* species showed two (*M. malcolmsonii*) and four bands (*M. rosenbergii* and *M. lamarrei*) (Rashid *et al.*, 2012a). Number of esterase bands may vary from species to species. As for example, three, four, five, six, seven and eight esterase bands were found in *Poecilia reticulata* (Ahmed *et al.*, 2011), *Heteropneustes fossilis* (Begum *et al.*, 2011), *Oreochromis niloticus* (Shahjahan *et al.*, 2008), *Ictalurus punctatus* (Knowles *et al.*, 1968), *Megalobrama amblycephala* (Sifa *et al.*, 1993) and in *Oreochromis aureus* (Hongtudo *et al.*, 1993) respectively.

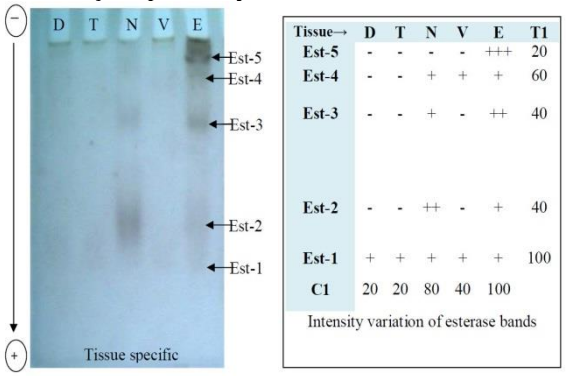
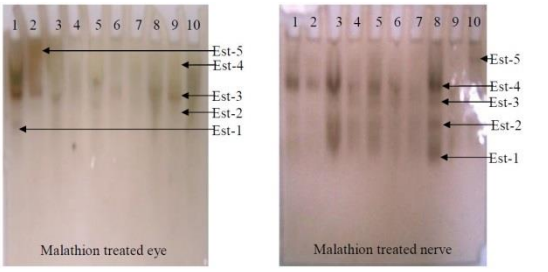


Figure 1: Tissue specific esterase isozyme variation in different tissues of Penaeus monodon stained with both α and β naphthyl acetates on 7.5% polyacrilamide gel. -, +, ++ and +++ represent absent, faint, moderate and deep stained bands. T1 personates the frequency (%) of each esterase band in all selected tissues. C1 stands for the frequency (%) of esterase bands (out of total bands) present in a certain tissue. D, T, N, V and E represent dorsal muscle, tail muscle, nerve cord, ventral muscle and eye tissue in order.

3.1. Tissue Specificity

Only one esterase band (Est-1) was observed in the dorsal (anterior) and tail (base of telson) muscle while two, four and five bands were found in ventral muscle (Est-1 and Est-4), nerve cord (Es-1, Est-2, Est-3 and Est-4) and eye (Es-1, Est-2, Est-3, Est-4 and Est-5) respectively

(Figure 1). Most of the bands were faint to medium stained except Est-5 which was deep stained. In a previous study with *Macrobrachium* species two, three and four esterase bands were found in the eye of *M. malcolmsonii, M. rosenbergii* and *M. lamarrei* respectively; while two, three and one esterase band was observed both in muscle tissue and ventral nerve cord of the above mentioned species in order (Rashid *et al.*, 2012a). Most of the esterase isozymes showed tissue specificity viz. similarity and dissimilarity in terms of staining intensity and the occurrence of the number of bands (Shahjahan *et al.*, 2008) depending on the physiological demands of each system (Witzemann and Boustead, 1981). Specific allele in specific tissue showed higher esterase activity due to biological need of that tissue specific function (Rashid, 2012). Faintly stained Est-1 was common in all the studied tissues while, Est-5 was confined to eye only which indicated that each allele might have underlying mechanisms regulating the esterase related processes (Lima-Catelani *et al.*, 2004). Certain band was also common in all the studied tissues of *H. fossilis* (Begum *et al.*, 2011), *O. niloticus* (Shahjahan *et al.*, 2008) and of *Pangasius hypophthalmus* (Begum *et al.*, 2008).



Doses (ppm)	Control (1)		5 (2)		10 (3)		20 (4)		30 (5)		40 (6)		50 (7)		60 (8)		70 (9)		80 (10)		T1
	E	N	E	N	E	N	E	N	E	N	E	N	E	N	E	N	E	N	E	N	
Est-5	-	+	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	20
Est-4	+++	+++	+	+++	+	++	++	+	+	++	+	+	+	+	+++	+	+	+	++	+	95
Est-3	++	-	++	-	++	-	+	+	+	+	+	+	-	++	+	++	+	-	+	-	70
Est-2	+	+	-	+	-	+	-	+	+	++	-	+	+	+	+	++	+	-	-	+	60
Est-1	+	+	-	-	+	+	-	+	-	+	-	-	-	-	++	-	-	-	-	-	45
C1	60	80	60	80	60	60	20	80	60	80	40	60	40	40	60	80	60	40	40	60	

Figure 2: Esterase isozyme variation in eye and nerve tissues of Penaeus monodon treated with different dose concentrations of malathion for 15 minutes. -, +, ++ and +++ represent absent, faint, moderate and deep stained bands. T1 personates the frequency (%) of each esterase band in malathion treated or non-treated nerve (N) and eye (E). C1 stands for the frequency (%) of esterase bands (out of five bands) present in malathion treated or non-treated nerve (N) and eye (E).

3.2. Malathion Effect

Fluctuation of esterases in terms of intensity variation as well as switch on or off of certain allele was observed in both nerve and eye tissue when treated with different doses of malathion (Figure 2). For example, Est-1 of eye was present in control group but switched off at the dose concentration of 5ppm of malathion. While this allele (Est-1) again switched on with lesser activity (faintly stained) in the treated eye at 10ppm of malathion. Commonly found Est-5 that was absent in control group was appeared in the treated sample of eye at 5ppm of malathion. But the allele (Est-5) was totally disappeared from the samples that were treated with higher doses of the insecticide which clearly showed its sensitivity to malathion.

Georghhiou *et al.* (1980) examined the linkage relationships between organophosphate resistance and a highly active esterase in *Culex quinquefasciatus* from California. Absence of Est-5 in control group could be explained with the differential expression and regulatory patterns of certain isozymes. This type of variation was also observed in the earlier study with *Hypophthalmicthyes molitrix* where individuals from the same locality exhibited different pattern of esterase isozyme expression and regulation (Rashid and Habib, 2012; Lizhao *et al.*, 1993). None of the nerve tissues showed more than four bands but altogether five esterase bands were observed in present study. Certain band (Est-3) that was absent in control group appeared after treated with specific doses of malathion (20 ppm) and switched off or on at higher doses. Malathion has an impact on acetylcholine esterases (Sahib *et al.*, 1980) that could promote the over production of this enzyme or could inhibit the action. Newly arisen band (Est-3) could be formed due to insecticidal action of malathion by unequal breaking down of certain isozyme. Probable cause of such kind of variation might also be due to interconversion of different forms of esterase isozymes which was shown by Jacobson *et al.*, (1970) in case of alcohol dehydrogenase. In a previous study on muscle tissue of three *Macrobrachium* species showed that total protein concentration decreased with increased dose concentrations but esterase activity fluctuated in each species (Rashid *et al.*, 2012a). Post mortal electrophoretic assay on *M. lamarrei* also showed more or less same result (Rashid *et al.*, 2012a).

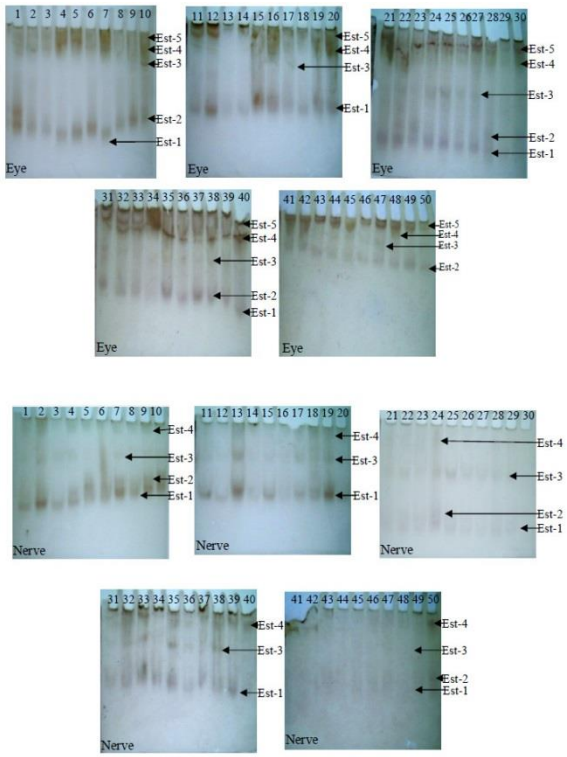


Figure 3: Esterase isozyme banding pattern in the eye and nerve tissues of Penaeus monodon (N=50) stained with both α and β naphthyl acetates on 7.5% PAGE where numerical number indicates the sample number and Est- represents the matched esterase isozyme (allele).

3.3. Allele Frequency

Most of the sampled eyes contained three or four esterase bands (40% and 46% respectively) and nerve tissues showed two or three bands (48% and 36% respectively) (Figure 3). None of the individuals showed one or five bands in eye while in nerve tissues single and four banded individuals were observed (6% and 10% in order). This variation in number of bands in the same tissue of sampled population might be due to either sub-standard sample quality or the presence of null allele at these loci (Raymond *et al.*, 1996). Variation in the number of bands was also observed in *Culex pipiens* where 0-, 1-, 2- and 3-banded individuals were found to be frequent (Raymond *et al.*, 1996). Previous study on three *Macrobrachium* species also showed differential expression of alleles in the same tissue of sampled population (Rashid *et al.*, 2013), the actual cause of which was unknown. However, these differences in the expression of esterase isozymes could be due to presence of selective forces in the natural habitat as the sampled shrimps were collected from different Ghers (enclosed water body). Moreover, variation in the number of esterase bands within population might be due to variability in the age of the individuals (Holmes and Whitt, 1970; Rashid, 2012; Begum *et al.*, 2011), as each farmer did not started shrims culture at a time. However, variation might be resulted from the biological need of specific individual that was hard to explain from the current experiment. As in the experiments with different specimens of *Hypophthalmicthyes molitrix* from the same locality exhibited different pattern of expression and regulation of these isozymes which indicated that genes encoding certain isozymes were active but exhibited different patterns of expression (Rashid and Habib, 2012; Lizhao *et al.*, 1993).

Est-5 of eye and Est-1 of nerve tissue were abundant in the population (96% and 76% respectively). It was interesting to note that a decreasing trends of abundances from higher molecular weight to lower molecular weight was observed in eye tissue while more or less opposite trends was found in nerve tissue (Figure 3). It was difficult to explain, why certain bands were profusely expressed in the sampled population, but their presence in sampled population clearly confirmed the essentiality of those particular alleles to be expressed. However, previous studies on several species suggested the same viz. Est-5 of *Macrobrachium rosenbergii* and *M. lamarrei* and Est-4 of *M. malcolmsonii* (Rashid *et al.*, 2013), Est-2 in Chironomids (Rashid and Rozy, 2013), Est-3 in *Bactrocera cucurbitae* (Rashid *et al.*, 2012b), Est-4 in *Hypophthalmicthyes molitrix* (Rashid and Habib, 2012), Est-5 in *B. dorsalis* (Rashid *et al.*, 2012c) and Est-6 in *B. tau* (Rashid *et al.*, 2012c). The finding of such a complex distribution of allozymes would seem difficult to explain on any basis other than selection (Strickberger, 1996). One of the examples of such condition was Milkman's (1973) finding i.e., in *E. coli* clones for each of five different enzymes, one particular electrophoretic band was frequent in almost all samples. Similar to earlier study on *Macrobrachium* species (Rashid *et al.*, 2013), esterase bands in some individuals were also found highly stained that indicated an esterase overproduction mechanism in this population (Raymond *et al.*, 1996).

All the esterase bands found in the sampled population of studied species were polymorphic in nature and some of the polymorphs of certain locus were abundant. Byrne and Devonshire (1993) found certain polymorphism of esterases predominated in susceptible and resistant populations of the Tobacco Whitefly from the southern United States, Middle East, Central America and Northern Europe. Similar to

current study, previous study on *Macrobrachium rosenbergii, M. malcolmsonii* and *M. lamarrei* also showed polymorphism of esterases (Rashid *et al.*, 2013). Extent of polymorphism that reflects heterozygous advantages (Riva and Robinson, 1986) might serve the advantages to be adapted with the differential environmental conditions as most allozymic variants have a minimal effect on fitness and were in population because of a combination of mutation, finite population size and migration. However, polymorphism of esterase isozymes was also studied in *Culex pipiens* (Stordeur, 1976), rainbow trout (Kingsbury and Masters, 1972), carp (Shcherbenok, 1973), *Penaeus vannameri* (Harris *et al.*, 1990) and in Russian sturgeon (Kuz'min, 2002) where remarkable polymorphism in certain locus was found. However, genetic control of these polymorphic systems was unknown, but, high enzymatic activity of these esterases, sufficient for quantitative electrophretic detection, permits utilization of these polymorphisms for phenotypic monitoring of prawn populations. Null alleles at some locus were frequent; the reason behind this was unknown. Similar results were also observed in *Macrobrachium rosenbergii, M. malcolmsonii* and *M. lamarrei* (Rashid *et al.*, 2013), the insecticide susceptible populations of *Culex pipiens* (Raymond *et al.*, 1996), Greek population of *Dacus oleae* (Krimbas and Tsakas, 1971) and *Colias eurytheme* (Burns and Johnson, 1967).

Table 1: Observed and expected frequencies of the esterase bands (Est-1)* in natural populations** of Penaeus monodon.

Tissue↓	Fob (f/f & f/x)	Fcal (p2 + 2pr)	FSob (f/s)	FScal (2pq)	Sob (s/s & s/x)	Scal (q2 + 2qr)	Nullob (x/x)	Nullcal (r2)	***X2	HWE
Nerve	40.00	45.98	18.00	10.84	12.00	18.25	30.00	24.88	4.32	Accepted
Eye	58.00	54.66	22.00	16.62	8.00	10.46	12.00	06.44	3.66	Accepted

*scored from α_ and β_naphthyl acetates stained gels
**On the basis of an equilibrium of the allele f, s and x.
***at 5% level of significance with 3 degree of freedom (Xtab=7.815).
p, q and r stands for the frequency of the allele f, s and x in order.
ob and cal represents observed and calculated frequencies respectively.

Polymorphism of Est-1 from both tissues was tested for the Hardy-Weinberg Equilibrium (HWE). Other bands were assumed to be controlled by other independent loci and were not considered for this purpose. The test results from both tissues (X^2 = 4.32 and 3.66) suggest that the sampled population followed HWE (Table 1). Similar result was also observed in an earlier study on *M. malcolmsonii* (Rashid *et al.*, 2013). However current result did not disprove selection of any kind, since it was impossible to test experimentally all the possible environmental variables (Krimbas and Tsakas, 1971). Sugama *et al.*, (2002) studied genetic variation and population structure of the giant tiger prawn in Indonesia and found six polymorphic loci in HWE at all localities. Samples were collected from different ghers (enclosed water body) of Paikgacha, Khulna district of Bangladesh where there was no direct migration channel to salt water and the population was generally consistent with the region that could be one of the causes of Hardy-Weinberg equilibrium (Sugama *et al.*, 2002). Small population size (n=50) of the sample could also be responsible for such result. However, there were evidences of selection for esterase alleles in other species, although no general pattern emerged for how selection operated (Raymond *et al.*, 1996). Allele frequency test in natural population depend critically on the accurate enumeration of alleles. Moreover, present data indicated that the assumption of one locus controlling the three alleles (f, s and x) might not be true and insufficient to be firmly established.

CONCLUSION

Allozyme study of *Penaeus monodon* is still in infancy in Bangladesh, hence this study holds enormous prospect in the development and application of molecular markers. Further studies with more molecular markers throughout their geographical ranges are highly recommended for the management of endangered inland wild populations which are commercially harvested.

REFERENCES

Ahmed MJ, Alam MS, Rashid MA, Begum RA, Shahjahan RM. Variability of esterase isozyme at some developmental stages of mosquito fish, *Poecilia reticulata.* Bangladesh J Life Sci 2011;23(1):139-142.

Begum RA, Yasmin F, Rashid MA, Alam MS, Shahjahan RM. Comparison of tissue specific esterase isozyme banding pattern in the larvae and adult of *Heteropneustes fossilis.* Indian J Soc Nat Sci 2011;1:1-7.

Begum RA, Bhadra SC, Shahjahan RM, Alam MS, Begum A. Esterase banding pattern in different tissues of *Pangasius hypophthalmus* (Sauvage, 1878). Bangladesh J Zool 2008;36:287-294.

Burns JM and Johnson FM. Esterase polymorphism in natural populations of a sulfur butterfly, *Colias eurytheme.* Science 1967;156:93-96.

Byrne FJ and Devonshire AL. Insensitive acetylcholinesterase and esterase polymorphism in susceptible and resistant populations of the tobacco whitefly, *Bemisia tabaci*. Pesti Biochem Physl 1993;45(1):34–42.

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

Cappellini R, Siniscalco M, Smith CAB. The estimation of gene frequencies in a random mating population. Ann Hum Genet 1955;20:97-115.

Devorshak C and Roe RM. The role of esterases in insecticide resistance. Rev Toxicol 1999;2:501-537.

Falconer DS. Introduction to quantitative genetics, Oliver and Boyd, London, 1960;pp:89-132.

Georghiou GP, Pasteur N, Hawley MK. Linkage relationships between organophosphate resistance and highly active esterase –B in *Culex quinquefasciatus* from California. J Econ Entomol 1980;73:301-304.

Gitzendanner MA and Soltis PS. Patterns of genetic variation in rare and wide spread plant congeners. Am J Bot 2000;87:783-792.

Gordon L, Snodgrass P, Zhu C, Chen S. Enhanced esterase gene expression and activity in a malathion-resistant strain of the tarnished plant bug *Lygus lineolaris*. Mol Biol 2004; 34:1175-1186.

Harris SEG, Dillion RT, Sandifer PA, Lester LJ. Electrophoresis of isozymes in cultured *Penaeus vannameri*. Aquaculture 1990;55:120-125.

Holmes RS and Whitt GS. Developmental genetics of the esterase isozymes of *Fundulus heteroclitus*. Biochem Genet 1970;4:471-4780.

Hongtudo F, Dequan Z, Tingting W. Isozyme of *Oreochromis aureus*. Aquaculture 1993;111:362-365.

Jackson GJ. Agrochemical Usage in Asia Region: A Reference Compendium Mimeo. 1991;58-60.

Jacobson K, Murphy J, Hartman C. Isozymes of *Drosophila melanogaster* alcohol dehydrogenase. J Bio Chem 1970;245:1075-1080.

Kingsbury N and Masters CJ. Heterogeneity, molecular weight interrelations and developmental genetics of the esterase isozymes of the rainbow trout. Bioche Biophys Acta 1972;258:455-465.

Knowles C, Aruvkar SK, Hogan JW. Electrophoretic separation of fish brain esterase. J Fish Res Bd Can 1968;25:121-129.

Krimbas CB and Tsakas S. The genetics of *Dacus oleae*. V. Changes of esterase polymorphism in a natural population following insecticide control-selection of drift. Evolution 1971;25:454-460.

Kuz'min EV. Allozyme variation of nonspecific esterases in Russian Sturgeon *Acipenser güldenstädti Brandt*. Russian J Genet 2002;38(4):408-414.

Lima-Catelani ARA, Ceron CR, Buicudo HEMC. Genetic expression during development, revealed by esterase patterns in *Aedes aegypti* (Diptera: Culicidae). Biochem Genet 2004;42:69-84.

Lizhao W, Zuxiong W, Yongchang C. Pattern of expression of isozyme genes during silver carp ontogenesis. Aquaculture 1993;111:326-327.

Markert CL and Moller F. Multiple forms of enzymes tissue, ontogenic and species specific pattern. Proc Nat Acad Sci 1959;45:753-763.

Metelev VV, Kanaev AI, Dzasokhova V. Water toxicology, Amrind Publishing Co Pvt Ltd New Delhi, 1983;120-125.

Milkman RD. Electrophoretic variation in *Echerichia coli* from natural sources. Science 1973;182:1024-1026.

Murty AS. Toxicity of pesticides to fish (Vol. II). CRC Press, Boca Raton, FL. 1986;110-125.

Ramaswamy S. Pest control and environment. Notes for discussion at a seminar on environment and agriculture. Agriculturalist Association of Bangladesh, Dhaka 1992;19-23.

Rashid MA and Habib DA. Tissue specific esterase isozymes banding pattern in Silver carp (*Hypophthalmicthyes molitrix*) at different developmental stages. Int J Res Zool 2012;2(2):18-22.

Rashid MA and Rozy F. Variability of esterase isozyme expression in larval and pupal stages of Chironomids. Int J Res Bio Sci 2013;3(1):39-42.

Rashid MA, Begum RA, Shahjahan RM. Tissue distribution of esterases and their responses

to Cypermethrin in three *Macrobrachium* species. J Asiat Soc Bangladesh Sci 2012a;38(2):227-235.

Rashid MA, Khanam M, Begum RA, Shahjahan RM. Some aspects of biology, toxicity and esterase variability of melon fly, *Bactrocera cucurbitae.* Int J Green Herb Chem 2012b;2(1):20-30.

Rashid MA, Siddika T, Begum RA, Shahjahan RM. Comparative esterase isozyme variability in two tephritid fruit flies, *Bactrocera dorsalis* and *B. tau.* J Chem Biol Phys Sci 2012c; 2(4):2467-2473.

Rashid MA, Kamruzzaman M, Ferdous Z, Alam MS, Begum RA, Shahjahan RM. Allele frequency distributions in on polymorphic esterase loci in experimental populations of three *Macrobrachium* species. J Cur Res Sci 2013;1(5):356-363.

Rashid MA. Some aspects of esterases in three *Macrobrachium* species. Lambert Academic Publishing, Germany, 2012;35-95.

Raven PH and Johnson GB. Biology. Times Mirror/Mosby College Publishing, 1986;pp:376-378.

Raymond M, Qiac CL, Callaghan A. Esterase polymorphism in insecticide susceptible populations of mosquito *Culex pipiens.*Genet Res Camb 1996;67:19-26.

Resende AG, Vidigal-Filho PS, Machado MFPS. Isozyme diversity in cassava cultivars (*Manihot esculenta* Crantz). Biochem Genet 2000;38:203-216.

Richardson BJ. Geographical distribution of electrophoretically detected protein variation in Australian commercial fishes. III. Western king prawn, *Penaeus latisulcatus* Kishinouye. Aust J Mar Fresh Res 1986;33(5):933–937.

Riva ME, Robinson AS. Induction of alcohol dehydrogenase null mutants in the Mediterranean Fruit fly *Ceratitis capitata.* Biochemical genetics 1986;24:765-774.

Sahib KA, Sailatha D, Ramana KV. Impact of malathion on acetylcholinesterase in the tissues of the fish *Tilapia mossambica* (Peters)- A time course study. *J Biosci* 1980;2:37-41.

Shahjahan RM, Karim A, Begum RA, Alam MS, Begum A. Tissue specific esterase isozyme banding pattern in Nile tilapia (*Oreochromis niloticus*). Univ J Zool Rajshahi Univ 2008;27:1-5.

Shcherbenok YI. Relation of polymorphic system of esterase and transferrins to economically valuable traits of carp. Biokhim Genet Ryb Mater Vses Soveshch 1973;1:129-137.

Sifa L, Wangi C, Biyun Z. Variation of morphology and biochemical genetic markers among population of blunt snout bream *(Megalobrama amblycephala).* Aquaculture 1993;111:117-127.

Stordeur DE. Esterase in the mosquito *Culex pipiens*: formal genetics and polymorphism of adult esterases. Biochem Genet 1976;14:481-493.

Strickberger MW. Evolution. Jones and Bartlett Publishers, Sudbury, Massachusetts 1996;pp:229-223, 528-530.

Sugama K, Haryanti P, Benzie JAH, Ballment E. Genetic variation and population structure of the giant tiger prawn, *Penaeus monodon*, in Indonesia. Aquaculture 2002;205:37–48.

Townson H. Esterase polymorphism in *Aedes aegypti*: the genetics and Km values of electrophoretically heterogeneous forms. Ann Trop Med Parastol 1972;66(2):255-266.

Wigginton JE, Cutler JD, Abecasis GR. A note on exact tests of Hardy-Weinberg Equilibrium. Am J Hum Genet 2005;76(5):887–893.

Witzemann V and Boustead C. Distribution of acetylcholinesterase molecular forms in brain, nerve and muscle tissue of *Torpedo marmorata.* Neurosci Lett 1981;26:313-318.