JEB
Journal of Environmental Biology

Journal Website : www.jeb.co.in
E-mail : editor@jeb.co.in
ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Influence of Endocel and Rogor on serum free amino acid and total protein level in *Clarias batrachus* (Linn.)

Prakriti Verma[1]*, Bipin Bihari Mishra[2] and Prabha Rani[1]

[1]Post Graduate Department of Zoology, Patna University, Patna - 800 005, India
[2]Post Graduate Department of Biochemistry, Patna University, Patna - 800 005, India
*Corresponding Author's E-mail: drprakriti@gmail.com

Publication Info

Paper received:
25 June 2013

Revised received:
29 January 2014

Re-Revised received:
12 June 2014

Accepted:
17 June 2014

Abstract

The present work was conducted to evaluate the serum free amino acids and total protein level in control and pesticide exposed fish. The fresh water catfish *Clarias batrachus* were exposed to single (1.5 µl l^{-1} Rogor and 0.008 µl l^{-1} Endocel) and combined (1µl l^{-1} Rogor + 0.004 µl l^{-1} Endocel) dose of Rogor and Endocel pesticides for 21 days. Every week blood was collected, centrifuged and serum was separated to estimate free amino acids by thin layer chromatography. Amino acids were located on chromatogram with 0.1% ninhydrin solution in acetone. Spectrophotometric reading found that each chromatogram revealed 4-5 fractions with a wide range of amino acid such as aspartic acid, glutamic acid, phenylalanine, serine, tyrosine, alanine, methionine, valine, isoleucine and threonine. The quantity and presence of each amino acid depends on the doses and exposure of pesticides. Other amino acids were present in lower concentration and quantitative estimation was not possible. Total protein content showed a decreasing trend in all the pesticide exposed group from 5.64 g dl^{-1} to 2.65 g dl^{-1} as compared to control.

Key words

Amino acid, *Clarias batrachus,* Endocel, Rogor, Thin layer chromatography, Total protein

Introduction

The gradual increasing demand for more food production has been met by adoption of better crop protection measures through pesticides (Enis *et al.,* 2012). Synthetic pesticides are deliberately sprayed on crops or agricultural land to increase food production but these agrochemicals are not very selective in producing their effects. They are toxic to many non-target species and contaminate the environment (Singh *et al.,* 2006). Increased use of chemical pesticides result in excess inflow of toxic chemicals, mainly into the aquatic ecosystem (Kalavathy *et al.*, 2001). The aquatic flora and fauna are affected by toxic substances which eventually enter into their systems or bring about external damages (Johl and Dua, 1995).

Fish represent a valuable source of protein and other nutrients in human diet of many countries. A major part of the world's food is being supplied from the fish source, so it is essential to secure the health of fish (Tripathi *et al.*, 2002). Dimethoate and Endosulfan are two such harmful pesticides that affect biochemical composition of fish (Kumble and Muley, 2000; Prasad *et al*., 2002). Free amino acids (FAA) are the major biochemical constituents and building blocks of protein (Magar *et al.*, 2012). They provide energy source for many cellular and physiological activities (Thenmozhi *et al.*, 2011). The importance of amino acid in fish has been well established from the perspective of nutrition (Cowey, 1994; Mambrini and Kaushik, 1995). Amino acid composition in tissue and blood has been well established (Hammed *et al*., 2010). But studies on the effect of pesticides on free amino acid of fish is meager (Rao *et al.*, 2010; Magar *et al.*, 2012). The present research work was an attempt to evaluate the fluctuation of free amino acids in blood serum of *Clarias batrachus*, exposed to single and combined dose of Endocel, Rogor and their combination, using thin layer chromatography.

Materials and Methods

Experimental animal : Air breathing fish, *Clarias batrachus* (commonly called Magur) were obtained from different wetlands

of North Bihar, India. The length and weight of fish were in the range of 18±2 cm and 50-60 gm, respectively. They were brought to laboratory and disinfected with 0.01% $KMnO_4$ solution and kept in large sized plexy glass aquarium. Fish were acclimated for 15 days under laboratory condition. To maintain normal water temperature, cooler and exhaust were used around the aquarium. The aerated water was changed daily. During acclimatization, fish were fed pelleted feed made in laboratory (mixture of wheat flour + egg + starch as binder) @ 5% of their body weight and water was changed daily.

For experiment pesticide Endocel (EC 35%), manufactured by "Excel Industries Ltd., and Rogor (EC 30%), manufactured by "ANU products, were used.

After acclimatization, fish were divided into four groups and were kept in four different aquaria each with 40 l water capacity each. The four groups were named as Control group, R-group, E-group and RE-group. Each group contained 18 fish. The control group was maintained in tap water without any treatment. The fish in R-group were exposed to 1.5 µl l^{-1} of Rogor (EC 30%) and in E-group were exposed to 0.008 µl l^{-1} of Endocel (EC 35%) for 21 days. The fish in RE- group were exposed to combined dose of 0.004 µl l^{-1} of Endocel + 1.00 µl l^{-1} of Rogor for 21 days, respectively.

Sublethal concentration of Endocel and Rogor were selected based on 96-hr LC_{50} value for *C.batrachus*. The LC_{50} values of Endocel and Rogor to *Clarias batrachus* for 96 hr were determined by probit analysis (Finney, 1971) and were found to be 0.02 µl l^{-1} for Endocel and 4.00 µl l^{-1} for Rogor. Therefore, the dose considered in the experimental protocol was 0.008 µl l^{-1} of Endocel for E group and 1.5 µl l^{-1} of Rogor for R group. A very low concentration of Endocel (0.004 µl l^{-1}) with Rogor (1.00 µl l^{-1}) for RE group was considered based on previous research work.

Accordingly, their stock solutions were prepared in distilled water. During exposure period, the fish were fed with feed pellets and water was changed daily. Then different dose of pesticides were added to experimental aquaria of 40 l water holding capacity. After scheduled period of exposure, blood sample of treated fish were collected at an interval of 7th, 14th and 21st day, respectively, in Eppendorf tubes through cardiac puncture with the help of sterilized syringe. Blood samples were allowed to coagulate and serum were extracted through centrifugation (5000 rpm at 4°C) and refrigerated at -20°C for amino acid and total protein estimation.

Amino acid profile in blood serum of *C. batrachus* was assayed using thin layer chromatography to arrive at Retardation factor (RF) values of standard amino acid calculated as per procedure of Wilson and Walker (2005).

Free amino acids were determined by the method of Moore and Stein (1954). Standard stock solutions of amino acids were prepared by dissolving 10 mg of each amino acid in 10 ml deionized water respectively. Each stock solution was then used to prepare the working solution.

Thin layer chromotograph sheets used in this study were silica gel coated TLC aluminium sheets No-1.05554.0007 purchased from Merck (KGa A 64271 Darmstadt, Germany). Using n - butanol : acetic acid : water (BAW) in the ratio of (4 : 1 : 5) as elutant and a 0.1% ninhydrin in acetone as spraying reagent to identify spots. The process takes approximate 3-6 hrs. Each amino acid was detected through purple colour spots by heating the ninhydrin sprayed sheets at 110°C for 15 minutes and then the Rf values of each spots were calculated by comparing it with the Rf value of standard amino acids.

To quantify the amount of amino acid in each spot, after chromatography spot located at the corresponding position in the sheet were scrapped off and then taken in a test tube adding 5 ml of acetone to it. Then 2 ml. of 1% ninhydrin solution were added. The tube were placed on a water bath for 20 minutes, full colour were developed at the end of this period. The coloured solution was transferred to measuring cylinder (10 ml) made to volume and read on Systronic UV Spectrophotometer (UV-VIS-Spectrophotometer 119) at 570 nm with the help of cuvette. Reading was compared with reading of known solution of amino acids treated in similar manner {Using lysine (1.13mg ml^{-1}) as standard for comparison}. Some amino acids were present in lower concentration and quantitative estimation was not possible.

Total protein level was estimated according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Analysis was done in BT-260 plus semi-automatic chemistry analyzer.

Statistical analysis : Six observations were taken for each parameter. Statistical analysis included mean and standard deviation. In case of Total protein, the data were analyzed for statistical significance between control and experimental groups with an analysis of variance (Two way ANOVA) and 't' test. After applying 't' test, the calculated values were referred to Fisher's table to check the level of significance at ($P<0.05$) and ($P<0.01$).

Results and Discussion

Chromatogram showed a wide range of amino acids such as glutamic acid, serine, tyrosine, alanine, methionine and glycine, but spectrophotometric readings of only few amino acids were possible. In control fish methionine (0.183 µg ml^{-1}) and serine (5.238 µg ml^{-1}) appeared quantitatively, other three amino acids i.e. glutamic acid, glycine and tyrosine were spotted but not detected quantitatively due to trace amount. In R group, methionine (0.357 µg ml^{-1}), serine (7.04 µg ml^{-1}) and tyrosine (0.062 µg ml^{-1}) were detected in fish exposed for 7 days, whereas in fish exposed for 14 days, serine was present in high concentration (6.857 µg ml^{-1}) and tyrosine in trace (0.148 µg ml^{-1}).

Glutamic acid (6.106 µg ml^{-1}), methionine (0.075 µg ml^{-1}) and tyrosine (0.525 µg ml^{-1}) were present in fish exposed for 21 days (Table 1). In E group, spots of respective amino acid were detected in chromatogram but their quantitative estimation was not possible in serum of 7 days treated fish. In 14 days treated fish, glycine (9.366 µg ml^{-1}), methionine (0.424 µg ml^{-1}) and tyrosine (0.129 µg ml^{-1}) were detected. Whereas in fish exposed for 21 days; out of five, three amino acids glutamic acid (9.366±0.003 µg ml^{-1}), methionine (6.313 µg ml^{-1}) and tyrosine (7.025 µg ml^{-1}) were detected in high concentration (Table 1). In RE group, glutamic acid (6.47 µg ml^{-1}) was detected on 7th day, glycine (13.390 µg ml^{-1}) on 14th day and serine (7.473 µg ml^{-1}) on 21st day in treated fish only. Methionine concentration in fish exposed for 7 days was 0.502 µg ml^{-1} and 2.947 µg ml^{-1} in fish treated with pesticides for 14 day but was absent in fish exposed for 21 day. Tyrosine detected on 7th day treated fish was 0.954 µg ml^{-1} but its concentration increased to 4.576 µg ml^{-1} in fish treated for 14 days, however, tyrosine was not detected in fish exposed to pesticides for 21 days (Table 1). Total protein test of blood serum showed statistically significant change in groups that were exposed to pesticides as compared to control group. The mean total serum protein level in control group was 5.64 g dl^{-1}. Whereas in experimental group (R, E and RE), mean level decreased to 5.10 g dl^{-1} ($p<0.05$) on 7th, 5.0 g dl^{-1} on 14th day and 4.45 g dl^{-1} ($p<0.05$) on 21st day as compared to control, in E group fish. In case of R group, protein level decreased to 5.0 g dl^{-1} ($p<0.01$) on 7th day, 5.1 g dl^{-1} ($p<0.01$) on 14 th day and 3.9 g dl^{-1} ($p<0.01$) on 21st day. Whereas in RE group, protein level decreased to 4.1 g dl^{-1} ($p<0.05$) on 7th day, 3.18 g dl^{-1} ($p<0.01$) on 14 th day and 2.65 g dl^{-1} ($p<0.001$) on 21st day. (Table 2)

The results of present investigation showed that 5 amino acids were present in high amount in blood serum of test fish. Toxicant induced alteration in the quantity of free amino acids has been reported by Ahmad *et al.* (2012) in *Danio rerio*. Methionine was the major affected amino acid and significant change was observed in almost all treated group of fish including control. High level of methionine in exposed fish and decline in protein content reflects possible proteolysis for metabolic purpose; low methionine level may be due to its utilization during cysteine synthesis through transulphuration (Nordrum *et al.*, 2000). Methionine also plays an important role in transport of triglyceride out of liver (Kerai *et al.*,1999) and fat digestion (Nordrum *et al.,* 2000), decreased methionine level increase accumulation of triglyceride in liver of fish (Kerai *et al.*, 1999; Espe *et al.*, 2010) leading to hepatic steatosis (Kerai *et al.*, 1999). Glutamic acid level in all group fish showed high fluctuation. The cause of fluctuation of glutamic acid may be due to proteolysis, impaired protein synthesis, which leads to increase in glutamic acid concentration. Body also increases glutamic acid level for healing purpose and acid base balance (Brosnan, 2000) while decrease in level may be due to its involvement in TCA cycle through α- keto glutarate formation (Smutna *et al.*, 2002) to meet the energy demands. Serine levels in all pesticide exposed fish were in the range of 6.857 µg ml^{-1} to 7.473 µg ml^{-1}.

Serine level gradually decreased in Rogor treated group probably organophosphate phosphorylate serine present at the active site of Acetylcholine(Ache) (Taylor, 1990) and so free serine is utilized in synthesis of new Ache (Bhattacharya, 2001). Thus, Rogor affects neural transmission. Increased concentration of serine might be attributed possibly to decreased utilization of amino acid in protein synthesis and increases protein degradation. Its degradation may be due to its utilization in TCA cycle as pyruvate for energy production (Smutna *et al.*, 2002) to meet the energy demand of cell in stress condition. Tyrosine level was detected in almost all groups ranging from 0.062 µg ml^{-1} to 7.025 µg ml^{-1} but it was in trace amount in the control group. Similar findings were reported by Rao *et al.* (2010). Tyrosine is an important aromatic amino acid and is a precursor of thyroid, adrenocortical hormones and of epinephrine and norepinephrine. It is also a precursor of dopamine which is related to control of

Table 1 : Concentration (µg ml^{-1}) of different amino acids in blood serum of *C.batrachus* treated with single and combined dose of Endocel and Rogor

Amino acid	Control	Rogor (R group)			Endocel (E group)			Rogor + Endocel (RE group)		
		7 days (R7)	14 days (R14)	21 days (R21)	7 days (E7)	14 days (E14)	21 days (E21)	7 days (RE 7)	14 days (RE14)	21 days (RE 21)
Glutamic acid	-	-	-	6.106 ±0.001	-	-	9.366 ±0.003	6.47 ±0.001	-	-
Glycine	-	-	-	-	-	9.028 ±0.002	-	-	13.390 ±0.002	-
Methionine	0.183 ±0.003	0.357 ±0.006	-	0.075 ±0.003	-	0.424 ±0.003	6.313 ±0.003	0.502 ±0.003	2.947 ±0.004	-
Serine	5.238 ±0.002	7.04 ±0.008	6.857 ±0.008	-	-	-	-	-	-	7.473 ± 0.003
Tyrosine	-	0.062 ±0.010	0.148 ±0.007	0.525 ±0.003	-	0.129 ±0.002	7.025 ±0.003	0.945 ±0.004	4.576 ±0.005	-

Values are mean of six replicates ±SD

Table 2 : Total protein level(g dl^{-1}) in blood serum of *C. batrachus* treated with single and combined dose of Endocel and Rogor

Groups	Days of exposure	Mean	t-test
Control		5.64 ± 0.280	
R group	7	5.0 ± 0.190	4.701**
	14	5.1 ± 0.196	4.715**
	21	3.9 ± 0.871	5.815**
E group	7	5.10 ± 0.196	4.715*
	14	5.0 ± 0.585	2.576NS
	21	4.45 ± 0.850	4.076*
RE group	7	4.1 ± 1.317	3.360*
	14	3.18 ± 1.213	6.210**
	21	2.65 ± 0.872	10.053***

Values are mean of six replicates ±SD; NS = Non-Significant (p> 0.05); * = Significant (p< 0.05); **= Highly significant (p < 0.01); ***= Very highly significant (p < 0.001)

stress in fish (Saavedra *et al.*, 2008) Thus, alteration in tyrosine level may be due to stress response in fish against pesticide exposure. Glycine is an important constituent of collagen and elastic tissue (Polat *et al.*, 1999).

Increase in glycine level may be due to collagen breakdown (Melendez-Hevia *et al.*, 2009) or increased protein synthesis of this particular amino acid as glycine is an important amino acid to prevent damage from oxidation and free radical stress and prevent muscle and liver degradation. Increase in concentration of FAA attributed to stepped up proteolysis or increased synthesis of free amino acid by transaminase reaction (Mohapatra and Noble, 1992). Increase in free amino acids level may also be due to breakdown of protein for energy requirements and impaired incorporation of amino acids in protein synthesis and decline in nucleic acids level (Bhavan *et al.*, 2001) The sudden rise and fall in the concentration of amino acids throughout the experiment is in agreement with Thenmozhi *et al.* (2011) who reported that initial increase in FAA in tissues and later their sudden decline was due to their utilization in the glycogenesis to compensate the energy demand under chemical stress. Decrease in amino acids may be due to their reutilization in protein turnover (Martini *et al.*, 2004).

The changes in protein level in fish treated with different doses of Rogor and Endocel during all the three durations were significant, indicating toxic status of fish. During chronic period of stress they are also a source of energy (Kumar, 2001). Parallel to our findings, decline in total protein in *Channa punctatus* (Bloch) exposed to Thiamethoxam due to protease activity has been reported by Kumar *et al.* (2010). Prasad *et al.* (2002) have also reported the effect of cypermethrin on protein metabolism of the fish *Labeo rohita* and observed that total protein level decreased in all the tissues whereas the free amino acid levels were increased.

These results agree with the observation made by Singh *et al.* (1996) who also reported decline in the protein content in different tissue of *Heteropneustes fossilis* (Bloch) exposed to sublethal concentration of aldrin and concluded that increment in FAA level is the result of protein breakdown of protein for energy requirement and impaired incorporation of amino acid in protein synthesis. The results of the present study i.e. significant increase in Amino acid and decrease in protein level due to Rogor and Endocel exposure alone and in combination were also corroborated by the reports of Narra *et al.* (2013) in different tissues of crab after chlorpyrifos exposure and Saravanan *et al.* (2000) in liver tissue of *Labeo rohita* intoxicated with Endosulfan.

The present study indicated that Rogor and Endocel intoxication significantly led to alteration in free amino acids and protein content in test fish. When these pesticides were given in combination they acted synergistically, causing more fluctuation in free amino acids and protein level. Thus, it seems that these pesticides interfere with amino acid as well as protein metabolism of the test species. Therefore, amount of these pesticides in agrifield and also in aquatic system should be monitored.

Acknowledgments

Authors are thankful to (WOS-A) scheme (NO-SR/WOS-A/LS-232/04) SERC Division, DST, New Delhi for financial assistance and Department of Zoology for providing infrastructural facilities.

References

Ahmad, M.K., D.K. Sharma, S. Ansari and B.A. Ansari: Effects of lambda-cyhalothrin and neemgold on some biochemical parameters in the gill, liver and ovary of Zebra fish (*Danio renio*). *Arch. Pol. Fish.*, **20**, 19-25 (2012).

Bhattacharya , S.: Stress response to pesticides and heavy metals in fish and other vertebrates. *Proc. Indian Nat. Sci. Acad.* (B67), **5**, 215-246 (2001).

Bhavan, P.S. and P. Geraldine: Biochemical stress responses in tissues of the prawn (*Macrobrachium malcolmsonii*) on exposure to endosulphan. *Pest. Bioch. Physiol.*, **70**, 27-41(2001).

Brosnan, J.T.: Glutamate, at the interface between amino acid and carbohydrate metabolism. *J. Nutr.*, **130,** 988–990 (2000).

Cowey, C.B.: Amino acid requirements of fish: A critical appraisal of present values. *Aquaculture* , **124**, 1- 11 (1994).

Enis Yonar, M., S.M. Yonar, M. Sener, U.S. Silici and M. Dusukcan: Protective role of propolis in chlorpyrifos-induced changes in the haemotological parameters and oxidative antioxidative status of (*Cyprinus carpio*). *Food Chem. Toxicol.,* **50**, 2703-2708 (2012).

Espe, M., R.M. Rathor, Z.Y. Du, B. Liaset and A. El-Mowafi: Methionine limitation results in increased hepatic FS activity, higher liver 18:1 to 18:0 fatty acid ratio and hepatic TAG accumulation in atlantic salmon (*Salmo salar*). *Amino Acids*, **39**, 449-460 (2010).

Finney, D.J.: Probit Analysis. 3rd Edn., Cambridge University Press, London, p. 333 (1971).

Hammed, A.M., H.A. Fashina-Bombata and O.O. Fajana: Tissue and

blood amino acids composition of an Ecotype cichlid 'wesafu', *Tilapia zilli* and *Oreochromis niloticus* using paper chromatography. *Paki. J. Nutr.*, **9,** 724 – 727 (2010).

Johl, M.S. and A. Dua: Elemental lepidological and toxicological studies in *Channa punctatus* (Bloch) upon exposure to an organochlorine pesticide, endosulfan. *Bull. Environ. Contam. Toxicol.*, **55**, 916-921 (1995).

Kalavathy, K., A.A. Siva Kumar and R. Chandran: Toxic effect of the pesticide dimethoate on fish (*Sarotherodon mossambicus)*. *J. Ecol. Res. Bioconserv.*, **2**, 27-32 (2001).

Kerai, M.D.J., C.J. Waterfield, S.H. Kenyon, D.S Asker and J.A. Timbrell: Reversal of ethanol induced hepatic steatosis and lipid peroxidation by taurine: A study in rats. *Alcohol and Alcoholism*, **34**, 529-541 (1999).

Kumar, A.V., C. Janaiah and P. Venkateshwarlu: Impact of thiamethoxam on proteases, aminases and glutamate dehydrogenase in some tissues of freshwater fish, *Channa punctatus* (bloch). *The Bioscan*, **5**, 135-137 (2010).

Kumar, S. and K. Gopal: Impact of distillery effluent on physiological consequences in fresh water teleost (*Channa punctatus). Bull. Env. Contom. Toxicol.,* **66**, 617-622 (2001).

Kumble, G.B. and D.V. Muley: Effect of acute exposure of endosulfan and chloropyrifos on the biochemical composition of the freshwater fish (*Sarotherodon mossambicus*). *Ind. J. Environ. Sci.*, **4**, 97-102 (2000).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with folin-phenol reagent. *J. Biol. Chem.*, **193**, 265 (1951).

Magar, R.S. and A. Sheikh: Biochemical changes in proteins and amino acids in *Channa punctatus* in responses to sublethal treatment with the insecticide malathion. *Trends in Life Sci.*, **1**, 19-23 (2012).

Mambrini, M. and S.J. Kaushik: Indispensable amino acid requirements of fish: correspondence between quantitative data and amino acid profiles of tissue proteins. *J. Appl. Ichthyol.*, **11**, 240-247 (1995).

Martini, W.Z., D.L. Chinkes and R.R. Wolfe: The intracellular free amino acid represents tracer precursor enrichment for calculation of protein synthesis in cultured fibroblasts and myocytes. *J. Nutr.*, **134**, 1546-1550 (2004).

Melendez-Hevia, E., P. De Paz-Lugo, A. Cornish-Bowden and M.L Cardenas: A weak link in metabolism: the metabolic capacity for glycine biosynthesis does not satisfy the need for collagen synthesis. *J. Biosci.*, **34**, 853-872 (2009).

Mohapatra, B.C. and A. Noble: RNA-DNA ratio as indicator of stress in fish. *Com. Physiol. Ecol.*, **17**, 41-47 (1992).

Moore, S. and W.H. Stein: A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem.*, **211**, 907-913 (1954).

Narra, M.R., R.R. Regatte and R. Kodimyala: Influence of chlorpyrifos stress on protein metabolism of edible crab *Barytelphusa guerini* and its recovery. *J. Stre. Physiol. Biochem.,* **9**, 219-231(2013).

Nordrum, S., A. Krogdahl, C. Rosjo, J.J. Olli and H. Holm: Effects of methionine , cysteine and medium chain triglycerides on nutrient digestibility, absorption of amino acids along the intestinal tract and nutrient retention in Atlantic salmon(*Salmo salar* L.) under pair feeding regime. *Aqua.*, **186**, 341-360 (2000).

Polat, A. and G. Beklevik: The importance of betaine and some attractive substances as fish feed additives. CIHEAM, **37**, 217-220 (1999).

Prasad, B.B., K.M. Singh and M. Rani: Dimethoate and monocil toxicity on the concentration of protein and amino acid in the serum and liver of *Channa maruius* (Ham.). *Nat. Environ. Pollut. Technol.*, **1**, 147-150 (2002).

Prasad, D. and K. Veeraiah: Effect of cypermethrin on protein metabolism of the fish, *Labeo rohita* (Hamilton). *Bull. Pure Appl. Sci.,* **21**, 27-32 (2002).

Rao, P.S., N. Bujji Babu and R. Ramesh Raju: Study the effect of chlorpyrifos on proteins in fresh water fish *Labeo rohita* by using HPLC Method. *Intern. J. Rese. Pharma. Biomed. Sci.*, **1**, 1-5 (2010).

Saavedra, M., L.E.C. Conceicao, S. Helland, P. Pousao-Ferreira and M.T. Dinis: Effect of lysine and tyrosine supplementation in the amino acid metabolism *Diplodus sargus* larvae fed rotifers. *Aquaculture*, **284**, 180-184 (2008).

Saravanan, T.S., M.A. Mohamed and R. Harikrishnan: Studies on the chronic effects of endosulfan on blood and liver of *Orechromis mossambicus. J. Ecol. Res. Biocon.*, **1**, 24-27 (2000).

Singh, N.N., V.K. Das and S. Singh: Effect of aldrin on carbohydrate, protein and ionic metabolism of a fresh water catfish *Heteropneustes fossilis. Bull. Environ. Contam. Toxicol.*, **57**, 204-210 (1996).

Singh, P.B. and V. Singh: Impact of endosulfan on the profiles of phospholipids at sublethal concentration in male *Heteropneustes fossilis* (Bloch). *J. Environ.Biol.,* **27**, 509-514 (2006).

Smutna, M., L. Vorlova and Z. Svobodova: Pathobiochemistry of ammonia in the internal environment of fish (Review). *Acta. Vet. Brno.*, **71**, 169-181 (2002).

Taylor, P.: The pharmalogical basis of therapeutics. *Macmillan Publishing Company, New York* (1990)

Thenmozhi, C., V. Vignesh, R. Thirumurugan and S. Arun: Impacts of malathion on mortality and biochemical changes of freshwater fish *(Labeo rohita). Iran. J. Environ. Hlth. Sci. Eng., 8,* 387-394 (2011).

Tripathi, G. and S. Harsh: Fenvalerate-induced macro molecular changes in catfish (*Clarias batrachus*). *J. Environ. Biol.*, **23**,143-146 (2002).

Wilson, K. and J. Walker: Principle and Techniques of Biochemistry and Molecular biology. 6th Edn., Cambridge University Press. New York, p. 546 (2005).