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Studies on biochemical changes in the tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate technical grade

T. Anita Susan[1], K. Sobha[2]*, K. Veeraiah[3] and K. S. Tilak[3]

[1]Department of Zoology, Andhra Christian College, Guntur, Andhra Pradesh, India.
[2]Department of Biotechnology, RVR and JC College of Engineering, Guntur, Andhra Pradesh, India.
[3]Department of Zoology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India.

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Fenvalerate is one among the synthetic pyrethroids, preferred for its low persistence, low toxicity to birds and mammals, broad spectrum of action on plant pests and metabolites/degradation products non toxic to man and other vertebrates. However, it is highly toxic to fish causing severe alterations in the metabolism of the organism, sometimes proving to be fatal if the concentrations are high. The present study envisages the variations in the distribution of biochemical constituents in the five major tissues viz., liver, muscle, kidney, brain and gill of the two carps, *Labeo rohita* and *Cirrhinus mrigala* exposed to sublethal and lethal concentrations of the technical grade pyrethroid, Fenvalerate. Elevation or depression in the levels of glycogen, total proteins, free amino acids and enzymes GDH, AAT and ALAT in different tissues observed were discussed in the light of metabolic stress caused due to the exposure to the toxicant.

Key words: Fenvalerate, *Labeo rohita*, *Cirrhinus mrigala*, toxicity, biochemical constituents, enzyme activity.

INTRODUCTION

Increased use of chemical pesticides results in the excess inflow of toxic chemicals, mainly into the aquatic ecosystem (Baskaran et al., 1989; Kalavathy et al., 2001). The aquatic flora and fauna are affected by the toxic substances which eventually enter into their systems or bring about external damages (Pant and Singh, 1983; Hodson, 1988; Johl and Dua, 1995). Several species of fish are susceptible to deleterious effects when exposed to heavy metals, pesticides and other environmental stressors (Khangarot et al., 1988; Arechon and Plump, 1990). Fenvalerate is highly toxic to aquatic organisms with LC_{50} values ranging from 0.008 µg/L for newly hatched mysid shrimps to 2 µg/L for stone flies (Schimmel et al., 1983). It is also highly toxic to fish, the 96-h LC_{50} values ranging from 0.3 µg/L for larval grunion to 200 µg/L for adult (EHC 95, 1990). Fenvalerate taken up by aquatic organisms is rapidly lost, when the organisms are transferred back to clean water. Therefore, in practice, the compound can be regarded as having no tendency to bioaccumulate (IPCS (International Programme on Chemical Safety), Health and Safety Guide No. 34).

However, pesticide exposure causes severe alterations in the tissue biochemistry of fishes (Kumar and Singh, 2000; Tilak et al., 2003; Mathivanan, 2004; Shrivastava and Singh, 2004). In general, the toxic effects will be more when two or more toxicants act together in a synergistic manner (Sujatha, 2006). The present study is aimed at understanding the effect of the widely used pyrethroid fenvalerate technical grade (Searle Ltd., Mumbai, India) on the biochemical composi-tion of various vital fish tissues and in turn the nutritive value of the fish harvested from ponds with pesticide contaminated water due to agricultural run off.

MATERIALS AND METHODS

Fish *Labeo rohita* and *Cirrhinus mrigala* of size 6 to 7 ± 0.5 cm and 6 to 8 ± 0.5 gm weight respectively were bought from a local fish farm and acclimatized at 28 ± 2°C in the laboratory for 96 h. The acclimatized fish were exposed for 24 h to sublethal ($1/10^{th}$ of 24 h LC_{50}) and lethal concentration (24 h LC_{50}) of fenvalerate technical

*Corresponding author. E-mail: sobhak66@gmail.com.

Table 1. The amount of total glycogen (mg/g) in tissues of *Labeo rohita* and *Cirrhinus migrala* on exposure to sub-lethal and lethal concentrations of fenvalerate technical grade.

Tissues	*Labeo rohita*			*Cirrihinus migrala*		
	Control	Sublethal	Lethal	Control	Sublethal	Lethal
Muscle	14.14 ± 1.19	@14.121 ± 5.66 (-0.134)	--	33.24 ± 2.21	**9.14 ± 1.21 (72.50)	--
Brain	18.427 ± 5.48	@19.44 ± 3.25 (5.497)	@20.23 ± 1.26 (9.784)	15.08 ± 0.86	**60.99 ± 15.29 (304.44)	*69.68 ± 21.47 (362.06)
Liver	146.99 ± 13.08	**15.22 ± 0.69 (-89.63)	**7.61 ± 1.77 (-94.82)	80.45 ± 7.34 (-52.14)	**39.09 ± 8.21 (-51.41)	**19.39 ± 2.47 (-75.89)
Gilli	8.99 ± 2.23	--	@5.43 ± 0.697 (-39.59)	21.12 ± 1.12	--	@14.46 ± 5.22 (-31.53)
Kidney	--	--	--	18.10 ± 2.33	@15.91± 0.018 (-12.09)	--

Results are the mean values of five observations and the S.D. is indicated. Figures in Parenthesis indicate per cent change over the control. Students' T Test - @ Not significant. *Significant at P<0.05. ** Highly significant at P<0.001.

grade determined by static method. The 24 h LC_{50} concentration to both the fish was found to be 0.0087 mg/L. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of glycogen, total protein, free aminoacids and for the estimation of the activity of the three enzymes viz., glutamate dehydrogenase (GDH), aspartate aminotransferase (AAT) and alanine aminotransferase (ALAT).

Glycogen, total proteins, free amino acids were estimated by the methods of Kemp et al. (1954), Lowry et al. (1951), Moore and Stein (1954) as described by Colowick and Kaplan (1957) respectively. Glutamate dehydrogenase (GDH) (L-glutamate NAD Oxidoreductase, E.C 1.4.1.3) activity was assayed by the method of Lee and Lardy (1965) with slight modification as described by Prameelamma et al. (1975). The activity of aspartate aminotransferase (AAT) (L-aspartate 2 Oxoglutarate aminotransferase: E.C.2.6.1.1) and alanine aminotransferase (ALAT) (DL – alanine 2-oxoglutarate aminotransferase: E.C. 2.6.1.2) was determined by the method of Reitman and Frankel (1957).

RESULTS AND DISCUSSION

The calculated values for various biochemical constituents and standard deviation, along with percent change over the control are given in Tables 1 to 6. Analysis of variance and student's 'T' were done on the data and levels of significance were presented in the tables. The lyotropic gradation series of various biochemical constituents is presented in Table 7.

Glycogen

Among various tissues of the control animals, higher glycogen level was observed in liver as anticipated since liver is the organ of glycogen synthesis and utilization (Table 1). Liver glycogen is largely concerned with storage and export of hexose units for maintenance of blood glucose. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself (Harper, 1985). Though brain tissue is metabolically active, lower glycogen content was observed since it lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities (Lehninger, 1983). The results indicated that the liver, a vital organ of carbohydrate metabolism was drastically affected by fenvalerate. Depletion of glycogen content was observed in the gill tissue of both the fish. While glycogen content in the muscle of *C. mrigala* under sublethal and lethal concentrations of fenvalerate showed depletion, in *L. rohita*, the glycogen content of the muscle remained similar to that of control fish. The glycogen content of the brain tissue in *L. rohita* showed increase in both sub-lethal and lethal concentrations and the increase was highly significant in the brain of *C. mrigala*. The glycogen content was not detectable in the control kidney of *L. rohita*, and also in the sublethal and lethal concentrations of the toxicant.

A highly significant decrease in glycogen content was noticed in both sublethal and lethal concentrations of technical grade fenvalerate in most of the tissues in both the experimental fish. The decrease in glycogen content was higher in sublethal concentrations than in lethal concentrations. A fall in glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in pesticide treated animals through glycolysis or Hexose monophosphate pathway (Cappon and Nicholas, 1975). The decrease in glycogen content may also be due to inhibition of the enzyme glycogen synthetase

Table 2. The amount of Total Proteins (mg/g) in tissues of fish *Labeo rohita* and *Cirrhinus migrala* on exposure to sub-lethal and lethal concentrations of fenvalerate technical grade.

Tissues	*Labeo rohita*			*Cirrihinus migrala*		
	Control	Sublethal	Lethal	Control	Sublethal	Lethal
Muscle	71.9 ± 0.01	**34.72 ± 0.052 (-51.71)	*53.7 ± 0.03 (-25.31)	156.2 ± 0.02	@119.13 ± 0.06 (-23.73)	@158.4 ± 0.03 (1.40)
Brain	99.86 ± 0.021	**35.13 ± 0.02 (-64.82)	**60.46 ± 0.05 (-39.45)	149.6 ± 0.03	**123.53 (-17.42) ±0.04	@149.9 ± 0.08 (0.2)
Liver	101.83 ± 0.03	**61.96 ± 0.015 (39.15)	**83.43 ± 0.05 (-18.06)	160.6 ± 0.03	**120.16 ± 0.04 (-25.18)	@156.2 ± 0.02 (-2.739)
Gilli	60.76 ± 0.021	**44.36 ± 0.04 (-26.99)	**53.63 ± 0.021 (-11.73)	99 ± 0.02	@87.93 ± 0.005 (-11.18)	@74 ± 0.09 (-25.52)
Kidney	77.63 ± 0.015	**28.3 ± 0.008 (-63.54)	**61.1 ± 0.04 (-21.29)	136.9 ± 0.02	@136.73 ± 0.06 (-0.124)	*125.73 ± 0.03 (-8.159)

Results are the mean values of five observations and the S.D. is indicated. Figures in Parenthesis indicate per cent change over the control. Students' T Test - @ Not significant. Significant at P<0.05. ** Highly significant at P<0.001.

or hormones which mediate glycogen synthesis (Stamp and Lesker, 1967; Edwards, 1973). The increase observed in a few tissues like brain could be due to recovery in regulatory balance between the glycogen synthesis by glycogen synthetase and breakdown by phosphorylase, thereby leading to either glucogenesis from carbohydrate precursors or gluconeogenesis from noncarbohydrate precursors (Edwards, 1973; Sreenivasa, 1983).

Koundinya (1979) reported that stepped up glycogenolysis leads to decrease in glycogen content. Similar changes in glycogen content and SDH activity have been reported in *Heteropneustes fossilis* exposed to malathion (Kabeer et al., 1983), sumithion (Koundinya and Ramamurthy, 1978), endosulfan (Vasanthi and Ramasamy, 1987), *Channa striatus* following metasystox exposure (Natarajan, 1981), tilapia mossambica following fenvalerate exposure (Radhaiah, 1988), and rat after fenvalerate exposure (Lakshmi, 1989). According to Haya (1989), fenvalerate caused a depletion of the glycogen store in the liver and muscle of juvenile Atlantic Salmon. Panigrahi and Mishra (1980) reported that accumulation of cypermethrin in brain may cause disintegration of nerve cells, clotting of blood and reduced oxygen transport to brain which may decrease lipid and glycogen contents. As reported by Smart (1978), when high energy compounds become depleted in the brain, characteristic symptoms like hyperexcitability and hyperventilation could be observed in fish. In the present study, exposure to technical grade fenvalerate caused changes in the glycogen content which may be attributed to toxic stress, resulting in the disruptions of enzymes associated with carbohydrate metabolism (Helimeyer et al., 1970). Glycogen depletion is more prevalent under hypoxic conditions (Dezwaan and Zandee, 1972) and a situation similar to hypoxia or anoxia might be occurring in the tissues of fish exposed to fenvalerate. Increased phosphorylase activity may stimulate a decrease in glycogen content resulting in altered rates in oxygen consumption.

Total sugar content in the muscle and liver tissues of fish exposed to dimethyl sulfoxide (DMSO), usually used as a carrier to toxicants, was reduced compared to theirrespective controls. Liver being the site of metabolism, the carbohydrates tend to accumulate for metabolic processes to occur (Omprakasam et al., 2008).

Total proteins

The observed variation in protein distribution (Table 2) suggests gradual difference in metabolic calibers of various tissues. The present trend in the tissues is justifiable in the wake of metabolic potential being oriented towards liver, as it is the seat for the synthesis of various proteins, and also the regulating centre of metabolism. Though muscle is rich in protein, it forms mechanical tissue intended for mobility and it does not participate in metabolism. There was a statistically significant decrease of total proteins ($P < 0.001$) in all tissues of *L. rohita* over the control. Significant change over the control was not observed in the gill, muscle and kidney of *C. mrigala*. Liver tissue of *L. rohita* evidenced a highly significant decrease in the protein content under sublethal and lethal concentrations of fenvalerate. A significant decrease was not observed in the liver of *C.*

Table 3. Free amnioacids (mg of tyrosine/g wet weight) in tissues of *Labeo rohita* and *Cirrhinus migrala* on exposure to sub-lethal and lethal concentrations of fenvalerate technical grade.

Tissues	*Labeo rohita*			*Cirrihinus migrala*		
	Control	Sublethal	Lethal	Control	Sublethal	Lethal
Muscle	5.497 ± 1.811	@7.62 ± 0.66 (38.62)	*3.97 ± 1.14 (-27.77)	4.25 ± 1.19	@6.74 ± 1.61 (58.48)	*3.34 ± 1.11 (-21.4)
Brain	3.228 ± 0.77	@2.91 ± 0.98 (-9.72)	@1.61 ± 0.85 (-50.12)	4.42 ± 1.57	@2.58 ± 0.90 (-41.62)	@4.62 ± 1.16 (4.52)
Liver	9.07 ± 2.51	**16.26 ± 2.132 (79.23)	**14.48 ± 5.32 (59.61)	8.26 ±1.55	*12.29 ± 3.36 (48.78)	*10.16 ± 0.538 (22.91)
Gilli	2.17 ± 1.68	@3.93 ± 1.566 (80.35)	@1.56 ± 0.93 (-28.4)	1.916 ± 2.16	@1.15 ± 1.26 (-39.56)	@1.23 ± 1.5 (-35.80)
Kidney	7.02 ± 2.60	@4.4 ± 3.32 (-37.32)	@6.89 ± 2.32 (-27.77)	1.55 ± 1.73	**3.87 ± 2.558 (149.6)	*2.93 ± 0.02 (89.03)

Results are the mean values of five observations and the S.D. is indicated. Figures in Parenthesis indicate per cent change over the control. Students' T Test - @ Not significant. * Significant at P<0.05. ** Highly significant at P<0.001.

mrigala under lethal concentrations of fenvalerate. There was a significant decrease in the protein content of brain, gill and kidney of *L. rohita*. The protein content in the brain of *C. mrigala*, exposed to lethal concentrations remained towards control level. Changes in brain, gill and kidney in both the fish suggests that they are relatively less affected than hepatic tissue under fenvalerate toxicity. However, an increase in protein content was noticed in the muscle of *C. mrigala* under lethal concentrations of fenvalerate. The above increase in the protein content of certain tissues in both fishes could reflect stimulated protein synthesis or detoxification enzymes at the expense of glycogen to meet additional requirement in the synthetic activity of tissue. The decreased trend of the protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or due to directing free amino acids for the synthesis of necessary proteins, or for the maintenance of osmotic and ionic regulation (Schmidt, 1975).

In the present investigation, decrease was more apparent in sublethal concentrations than in lethal concentrations. It may be due to detoxification enzymes or mechanism apparently slow. Further the insecticide probably acts continuously on the animal system for longer periods, thereby rendering detoxification mechanism less efficient and thus making recovery slow at sublethal concentrations. Koundinya (1979) reported a decrease in total protein content of *Tilapia mossambica* exposed to different pesticides. Pandi (1991) reported depletion in the protein content in muscle and liver of *Orechromis mossambicus*, *Mystus vittatus* and *Cytisusstriatus* exposed to fenvalerate. Jeba et al. (1990) recorded a decrease in protein content of L. thermalis exposed to sublethal concentrations of fenvalerate. A significant decrease was reported in the protein content of the liver and kidney in *L. rohita*, exposed to 20% active ingredient EC fenvalerate (Annamani, 1986). A similar decrease in the total and soluble protein content was observed with fenvalerate in fish (Malla and Bashamohideen, 1988; Radhaiah, 1988) and rat (Lakshmi, 1989). A decrease in protein profiles was also reported in several animals following pesticide stress (Siva et al., 1982; Pramoda and Naidu, 1987). Thus varying protein profiles in different animals probably depend on the structural configuration of various pyrethroids and also on differential tissue response.

Omprakasam et al. (2008) in their study on aquatic toxicity of DMSO to the Indian major carp, *Catla* reported that protein remains more in the muscle tissues than in the liver because of the requirement of growth factors and energy regulation needed for the swimming activity. The drastic reduction in the protein content in both the muscle and the liver tissues as compared to control suggests that the gluconeogenetic pathway has been initiated to supplement depletion of sugars by breaking down of protein to yield sugars. Exposure to sublethal doses of *Nerium indicum* latex extract in ethanol NL_{EtOH} for 24 and 96 h exposure period caused significant reduction in total protein, nucleic acids (DNA and RNA), glycogen, pyruvate level, enzyme AChE, LDH, SDH, CyO activities and also caused significant enhancement in total free aminoacids, lactate level, enzyme proteases, ALAT, AAT activity in both liver and muscle tissues of *Colisa fasciatus* (weed fish). Seven days withdrawal

Table 4. Glutamate Dehydrogenase activity (GDH) (µ moles of formazon formed/mg protein/hr) in different tissues of *Labeo rohita* and *Cirrhinus migrala* on exposure to sub-lethal and lethal concentrations of fenvalerate technical grade.

Tissues	*Labeo rohita*			*Cirrihinus migrala*		
	Control	Sublethal	Lethal	Control	Sublethal	Lethal
Muscle	0.0052 ± 0.002	**0.037 ± 0.0012 (476.92)	**0.05± 0.09 (861.53)	0.005 ± 0.0022	*0.01 ± 0.0022 (100)	*0.011 ± 0.003 (120)
Brain	0.003 ± 0.0012	**0.03 ± 0.002 (900)	**0.02 ± 0.004 (566.6)	0.0048 ± 0.005	**0.019 ± 0.0018 (295.83)	**0.015 ± 0.0011 (212.5)
Liver	0.033 ± 0.0025	**0.071 ± 0.003 (115.15)	**0.09 ± 0.001 (172.72)	0.007 ± 0.0665	**0.02 ± 0.0016 (185.7)	*0.01 ± 0.003 (42.85)
Gilli	0.007 ± 0.001	**0.037 ± 0.002 (428.57)	**0.04 ± 0.005 (471.42)	0.0066 ± 0.0037	@0.0088 ± 0.002 (33.33)	*0.01 ± 0.004 (51.51)
Kidney	0.004 ± 0.003	**0.056 ± 0.004 (1300)	**0.03 ± 0.006 (650)	0.013 ± 0.0016	@0.014 ± 0.0023 (7.692)	@0.015 ± 0.001 (15.38)

Results are the mean values of five observations and the S.D. is indicated. Figures in Parenthesis indicate per cent change over the control. Students' T Test - @ Not significant. *Significant at P<0.05. ** Highly significant at P<0.001.

experiment showed significant recovery in all the above biochemical parameters in both the tissues of the fish (Tiwari and Singh, 2009).

Free amino acids (total ninhydrin positive substances)

In the tissues of both the control fish, free amino acid content (FAA) is highest in the liver as it is the chief organ of FAA synthesis, least in the gills as they are less involved in protein metabolism (Table 3). Marked elevation in the FAA content was noticed in the liver of both the experimental fish under sublethal and lethal concentrations of fenvalerate, compared to other tissues as it is the centre for detoxification. In *L. rohita*, a slight decrease in the FAA was noticed in the brain and kidney under sublethal concentrations. A decrease was also observed in muscle, brain, gill and kidney under lethal concentrations of the toxicant. Although FAA level indicated an increase in certain tissues of *C. mrigala*, a decrease was observed in brain and gill exposed to sublethal concentrations of fenvalerate. Decrease was also apparent in the muscle and gill exposed to lethal concentrations of the toxicant.

In the present investigation, FAA content remained at higher levels from the control in sublethal concentrations than in the lethal concentrations. This rapid increase in FAA levels is probably attributed to stepped up proteolysis or increased synthesis of free aminoacids by transaminase reaction (James et al., 1979; Natarajan, 1983; Malla and Bashamohideen, 1988). The increase in FAA pool may also be useful to fish in maintenance of osmotic acid/base balance as reported in other animals (Potts, 1958; Florkin and Schoffenials, 1964). The decrease in FAA in certain tissues of *L. rohita* and *C. mrigala*, both in sublethal and lethal concentrations may be due to their utilization for the synthesis of new proteins or for the production of energy to cope with the prevailing toxic conditions due to intoxicant induced stress (Wilson and Poe, 1974; James et al., 1979). The decrease in FAA Levels provides substantial evidence for the above assumption. There are several reports on the increase in FAA content in fish and albino rat during fenvalerate intoxication (Radhaiah, 1988; Lakshmi, 1989).

However as evidenced in certain tissues of the present study, FAA levels also decreased in several animals under pyrethroid stress. Saleem and Shakoori (1986) reported that cypermethrin significantly reduced the FAA content in the sixth instar larvae of *Tribolium castaneum* and suggested that free amino acids, cholesterol and activity of the enzyme amylase were the most sensitive components of the various biochemical factors studied.

In agreement with total proteins, FAA levels have shown changes and these could be attributed to structural configuration of the insecticide in different animals.

Glutamate dehydrogenase (GDH) activity

Under sublethal and lethal exposures, the GDH activity showed significant increase in all tissues of both the fish exposed to fenvalerate (Table 4). The percent increase of enzyme activity in *L. rohita*, exposed to sublethal and lethal concentrations of fenvalerate was highly significant.

Table 5. Aspartate amino transferase activity (AAT) (µ moles of pyruvate formed/mg /hr) in different tissues of *Labeo rohita* and *Cirrhinus migrala* on exposure to sub-lethal and lethal concentrations of fenvalerate technical grade.

Tissues	*Labeo rohita*			*Cirrihinus migrala*		
	Control	Sublethal	Lethal	Control	Sublethal	Lethal
Muscle	5.39 ± 0.108	**1.66 ± 0.59 (-69.20)	@3.82 ± 0.83 (-29.12)	2.10 ± 0.06	@1.73 ± 0.64 (-17.61)	*3.32 ± 0.46 (58.09)
Brain	5.94 ± 0.052	**1.88 ± 0.39 (-68.35)	**2.74 ± 1.33 (-53.87)	4.39 ± 0.99	@1.05 ± 0.32 (-76.08)	@2.83 ± 0.61 (-35.53)
Liver	12.65 ± 0.17	**3.74 ± 0.93 (-70.43)	**7.39 ± 1.07 (-41.58)	11.92 ± 0.37	*2.73 ± 0.26 (-77.09)	@17.83 ± 0.65 (49.58)
Gilli	3.92 ± 0.04	**1.45 ± 0.04 (-63.01)	**1.87 ± 0.19 (-52.29)	1.72 ± 0.44	@1.37 ± 0.28 (-20.34)	**3.58 ± 0.58 (108.13)
Kidney	17.72 ± 1.48	**0.04 ± 0.003 (-99.77)	**5.5 ± 0.45 (-68.96)	6.8 ± 0.24	**3.11 ± 0.47 (-54.26)	**12.20 ± 0.57 (79.41)

Results are the mean values of five observations and the S.D. is indicated. Figures in Parenthesis indicate per cent change over the control. Students' T Test - @ Not significant. *Significant at P<0.05. ** Highly significant at P<0.001.

In *C. mrigala*, the percent increase of GDH activity observed under sublethal concentration of fenvalerate technical grade was highly significant in liver and brain (P < 0.001). In the present study, the activity of NAD dependent GDH was highly elevated following fenvalerate exposure indicating increased oxidation of glutamate. Since GDH catalyses the key reactions which provide substrates for either protein synthesis or carbohydrate metabolism (α – ketoglutarate) it might be regulated according to the metabolic needs of the cell. However, this increase in GDH may be accounted for coupling of transamination, leading to the formation of glutamate. Further, the forward reaction of GDH could be increased if the α – ketoglutarate formed by the oxidation of glutamate was removed by transamination (Weil – Malherbe, 1974). The increased GDH activity may lead to increased oxidation of glutamate with a consequent production of ammonia. This is augmented by the changes in transaminase activity. From the foregone account, it is evident that the operation of transamination coupled with dehydrogenation prevailed during fenvalerate toxicity, bringing into picture the role of proteins in neutralizing the energy crisis.

With the same compound fenvalerate, elevation in GDH activity presumably due to the mitochondrial permeability, lysosomal damage or induced synthesis of the enzyme was reported in fish (Radhaiah, 1988) and rat (Lakshmi, 1989). Since GDH is a mitochondrial enzyme, any alteration in the organization of mitochondria may lead to the alteration in the enzyme activity.

Aspartate (AAT) and alanine aminotransferases (ALAT) activity

Aspartate aminotransferase (AAT) activity in all the tissues of *L. rohita* showed a highly significant decrease over the control (Table 5). The decrease was more significant in sublethal concentrations than in the lethal concentrations. In *C. mrigala* exposed to sublethal concentrations, AAT enzyme activity showed a decreased trend in all the tissues but in lethal concentrations, an increased pattern was observed in different tissues except in the brain. In general, decrease in AAT activity was more conspicuous in sublethal concentrations than in the lethal concentrations. This may be due to the reason that continuous exposure to a toxicant at low concentration might, in some circumstances, be more dangerous than a brief period at a high concentration because the survivors from the latter, may be able to metabolize or excrete the toxicant over the subsequent period if no further exposure occurred (Holden, 1973).

A decreasing trend was observed in alanine aminotransferase (ALAT) activity in the muscle of both the carps on exposure to sublethal and lethal concentration of fenvalerate technical grade (Table 6). However, the percent decrease in ALAT activity in *C. mrigala* is higher under sublethal concentration except in the kidney. The possible explanation is that detoxification mechanism may not be sufficiently effective to prevent the action of the insecticide on the system under lethal concentration in *L. rohita*, while the trend was reversed in *C. mrigala*. An elevation in AAT and ALAT activity levels was reported in fish following fenvalerate intoxication (Ayyanna, 1988; Radaiah, 1988). Increase in aminotransferase activity was reported in *T. mossambica*, under pesticide exposure (Narasimha, 1983; Siva and Ramana, 1984; Girija, 1987; Radaiah, 1988). Transaminase activity is reported to increase in serum during pathological conditions (Latner, 1975; Nichol and Rosen, 1963). The same trend was also reported

Table 6. Alanine aminotransferase (ALAT) activity (µ moles of pyruvate formed/mg /hr) in different tissues of *Labeo rohita* and *Cirrhinus migrala* on exposure to sub-lethal and lethal concentrations of fenvalerate technical grade.

Tissues	*Labeo rohita*			*Cirrihinus migrala*		
	Control	Sublethal	Lethal	Control	Sublethal	Lethal
Muscle	2.28 ± 0.56	@1.15 ± 0.150 (-49.56)	@1.07 ± 0.08 (-53.07)	3.27 ± 0.36	@0.54 ± 0.029 (-83.48)	@0.653 ± 0.041 (-80.03)
Brain	3.62 ± 0.97	*1.75 ± 0.05 (-51.65)	*0.668 ± 0.047 (-81.54)	1.852 ± 0.42	**0.376 ± 0.03 (-79.69)	*0.88 ± 0.2 (-52.48)
Liver	15.25 ± 0.09	@15 ± 0.001 (-1.639)	**1.07 ± 0.08 (92.98)	19.4 ± 0.53	**2.1 ± 1.22 (-89.17)	*5.33 ± 0.32 (-72.52)
Gilli	2.62 ± 0.04	@2.09 ± 0.36 (-20.22)	*0.885 ± 0.069 (-66.22)	2.07 ± 0.02	*0.98 ± 0.01 (-52.65)	@1.88 ± 0.42 (-9.17)
Kidney	1.57 ± 0.09	*4.75 ± 0.95 (202.54)	@1.45 ± 0.03 (-7.64)	10.36 ± 2.34	@4.56 ± 1.15 (-55.98)	*1.86 ± 0.588 (-82.04)

Results are the mean values of five observations and the S.D. is indicated. Figures in Parenthesis indicate per cent change over the control. Students' T Test -@ Not significant. *Significant at P<0.05. ** Highly significant at P<0.001.

following pyrethroid intoxication in cockroach and crab (Sesha, 1989; Ayyanna (Unpublished), 1989). However, contrary to the above reports, the present study revealed a significant decrease in transaminase levels in most of the tissues in both the fish exposed to both sublethal and lethal concentrations of fenvalerate technical grade.

Relationship between the serum enzymes of fish and pesticides were investigated by several researchers. Similar decrease of glutamate oxaloacetic transaminase was reported by Lane and Scura (1970) in sailfin molly exposed to lethal concentrations of dieldrin. Significant decrease was reported in serum transaminases in all the three major carps viz., *Catla, Labeo* and *Cirrhinus* exposed to sublethal and lethal concentrations of ammonia, nitrite and nitrate (Jhansi, 1993). AAT and ALAT are located in both mitochondrial and cytosol fractions of the cell. Correlation appears to exist between the mitochondrial integrity and transaminase levels (Bonitenko, 1974) and any modification in the organization of mitochondria is bound to alter the enzyme systems associated with it. According to Schmidt and Schmidt (1973), the release of enzymes from the cell is immediately followed by a change in the catalytic activities which may either increase or decrease. Decreased release of readily and poorly extractable enzymes is due to disruptions of regulatory and organizational functions leading to cell death, passive diffusion due to concentration gradient. It may be also due to necrosis and auto-lysis as also evident from our histopathological observations.

Non recovery towards control values in the transaminase levels under sublethal and lethal concentration of fenvalerate technical grade resulting in much decreased levels suggest irreparable and irreversible action of fenvalerate on the animal system. The inhibition in the transaminase levels observed in the present study can be attributed to one or more of the above reasons.

The effects of protein synthesis inhibitors on fenvalerate-withdrawal-dependent recovery in citrate synthase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, RNA and protein of brain, liver and skeletal muscle of *Clarias batrachus* were studied to explore the possible mode of action of a pyrethroid insecticide at metabolic level. The administration of actinomycin D or cycloheximide inhibited partially or completely the withdrawal-dependent increase in enzyme activities, RNA, and protein contents substantiating *de novo* synthesis of these macromolecules during recovery. The inhibition was more pronounced in response to cycleheximide reflecting recovery in translation process. This also suggests the possibility of fenvalerate-induced impairment of metabolism through inhibition of enzyme synthesis (Tripathi and Verma, 2006). Recent study on the effects of alachlor on biochemical parameters of the fresh water fish, *Channa punctatus* (Bloch) revealed decrease in the glycogen, total proteins, DNA, RNA but the activity of the enzymes AAT, ALAT and LDH was found to increase due to toxic stress. It was also reported that percent decrease was more pronounced at lethal concentrations than at sublethal concentrations (Tilak et al., 2009).

In a nut shell, the present work revealed that the variations in biochemical parameters serve as indices in monitoring the pathological status of the pesticide treated fish. The variations were tissue and species specific and

Table 7. Variations in the Biochemical constituents in different tissues of the two carps, *Labeo rohita* and *Cirrhinus migrala* on exposure to sub-lethal and lethal concentrations of Fenvalerate Technical Grade as compared to Control.

S/No.	Biochemical constituent	*Labeo rohita*			*Cirrhinus mrigala*		
		Control	Exposed		Control	Exposed	
			Sub-Lethal	Lethal		Sub - Lethal	Lethal
1	Glycogen	Liver>Brain>Gill>Muscle and Kidney	Gill = Kidney> Muscle>Liver and Brain	Muscle = Kidney> Gill>Liver and Brain	Liver>Muscle>Gill>Kidney and Brain	Gill>Muscle>kidney, Liver and Brain	Muscle>Kidney>Gill >Liver and Brain
2	Total Proteins	Liver>Muscle>Brain >Kidney and Gill	Kidney>Muscle>Brain> Gill and Liver	Gill=Muscle>Brain>kidney and Liver	Liver>Muscle>Brain> Kidney and Gill	Gill>Muscle>Liver> Brain and Kidney	Gill>Kidney>Brain>Liver and Muscle
3	Free Amino acids	Highest in Liver and Lowest in the Gills	Brain>Gill>Muscle>Kidney and Liver	Gill>Brain>Muscle>Kidney and Liver	Highest in Liver and Lowest in the Gills	Gill>Brain>Kidney> Muscle and Liver	Gill>Kidney>Muscle >Brain and Liver
4	GDH activity	Liver>Gill>Muscle> Kidney and Brain	Significant increase in all tissues	Significant increase in all tissues	Kidney>Liver>Gill>Muscle and Brain	Significant increase in all tissues	Significant increase in all tissues
5	AAT activity	Kidney>Liver>Brain >Muscle > Gill	Kidney>Gill>Muscle>Brain and Liver	Gill>Brain>Muscle>Kidney and Liver	Liver>Kidney>Brain> Muscle>Gill	Brain>Gill>Muscle> Liver and Kidney	Brain>Muscle>Gill> Kidney and Liver
6	ALAT activity	Liver>Kidney>Muscle>Brain and Gill	Muscle>Brain>Gill>Kidney and Liver	Brain>Gill>Liver=Muscle and Kidney	Liver>Kidney>Muscle >Brain and Gill	Brain>Muscle>Gill> Liver and Kidney	Muscle>Brain>Kidney>Gill and Liver

hence can be used as meaningful indicators of pesticide pollution. Such differential behaviour with regards to tissues and fish of the above said parameters can be further examined to develop a more meaningful indicators or markers to assess or to characterize the particular pollutant and its potential for toxicity.

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REFERENCES

Annamani AK (1986). Toxicity of Fenvalerate technical grade and 20% EC to *Labeo rohita* and *Aplochelus panchax*. M. Phil. Thesis, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Arechon N, Plump JA (1990). Sublethal effects of Malathion on channel cat fish, *Ictalaurus punctatus*. Bull. Environ. Contam. Toxicol., 44: 435-442.

Ayyanna K (1988). Impact of a synthetic pyrethroid on non target animal. M.Phil. thesis, S.V. University, Tirupati, Andhra Pradesh, India.

Baskaran P, Palanichamy S, Visalakshi S, Balasubramanian MP (1989). Effects of Mineral fertilizers on survival of the fish *Oreochromis mossambicus*. Environ. Ecol., 7: 463-465.

Bonitenko YU (1974). Isoenzymes of aspartate aminotransferase in acute dichloro ethane poisoning. Gig. Tr. Prof. Zebo., 7: 46-47.

Cappon ID, Nicholas DM (1975). Factors involved in increased protein synthesis in liver microsomes after administration of DDT. Pestic. Biochem. Physiol., 5: 109-118.

Colowick SP, Kaplan NO (1957). Methods in Enzymology. Academic Press, New York. 3: 501.

Dezwaan A, Zandee DT (1972). The utilization of glycogen accumulation of some intermediates during anaerobiosis in *Mytilus edulis L*. Comp. Biochem. Physiol. B: 43: 47-54.

Environmental Health Criteria (EHC) 95 (1990). Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization.

Edwards CA (1973). Environmental Pollution by Pesticides. Plenum Press, London, New York.

Florkin M, Schoffenials E (1964). In: Studies in Comparative Biochemistry. (Manday KA ed.) Mac Milan, Pergamon press, New York.

Girija M (1987). Effect of Heptachlor and dichlorophos on structure and function of gill tissue of a fresh water teleost T. mossambica (Peters). Ph. D. Thesis, S. V. University.

Harper JW (1985). In: Harpers Review of Biochemistry (eds) Martin DW, Mayes PA, Rodwell VW. Lange Medical Publications, Maruzen Asia (Singapore). Haya K (1989). Toxicity of pyrethroid insecticides to Fish Environ. Toxicol. Chem., 8: 381-391.

Helimeyer LMG, Francois M, Haschake RM, Fisher EH (1970). Control of phosphorylase activity in muscle glycogen particle || activities of calcium. J. Biol. Chem., 2: 6649-6656.

Hodson PV (1988). The effect of metal metabolism on uptake, disposition and toxicity in fish. Aquat. Toxicol., 11: 3-18.

Holden AV (1973). Chapter 6 in Environmental Pollution by Pesticides (ed: CA Edwards) p. 542. Plenum Press.

James JH, Ziparo V, Jeppsson B, Fischer JE (1979). Hyperammonemia, plasma amino acid imbalance and blood brain aminoacid transport: A unified theory of portal systemic encephalopathy. Lancet, 2: 772-775.

Jeba Kumar SRD, Flora SDJ, Ganesan RM, Jagatheesan G, Jayaraman J (1990). Effect of short term sublethal exposure of cypermethrin on the organic constituents of the freshwater fish *Lepidocephalichthys thermalis*. J. Environ. Biol., 11(2): 203-209.

Jhansi SL (1993). Studies on the toxicity and effects of ammonia-Nitrogen, Nitrate-Nitrogen and Nitrite-Nitrogen on the three Indian major carps, Catla catla (Ham), Labeo rohita (Ham) and Cirrhinus mrigala (Ham). Ph.D. Thesis, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Johl MS, Dua A (1995). Elemental lepidological and toxicological studies in *Channa punctatus* (Bloch) upon exposure to an organochlorine pesticide, endosulfan. Bull. Environ. Contam. Toxicol., 55(6): 916-921.

Kabeer Ahamed SI, Sambasiva Rao KRS, Ramana Rao KV (1983). Dehydrogenase systems of the teleost *T. mossambica* under augmented sublethal malathion stress. J. Anim. Morphol. Physiol., 30 (1&2).

Kalavathy K, Siva Kumar AA, Chandran R (2001). Toxic effect of the pesticide Dimethoate on the fish *Sarotherodon mossambicus*. J. Ecol. Res. Bioconserv., 2 (1-2): 27-32.

Kemp AA, Kits JM, Van H (1954). A colorimetric micromethod for the determination of glycogen in tissues. Biochem. J., 56: 646-648.

Khangarot BS, Ray PK, Singh KP (1988). Influence of copper treatment on the immune response in an air breathing teleost *Saccobranchus fossilis*. Bull. Environ. Contam. Toxicol., 41: 222-226.

Koundinya PR (1979). Studies on physiological responses of teleost, *T. mossambica* to pesticide impact. Ph.D. thesis, S.V. University, Tirupati, India.

Koundinya PR, Ramamurthy R (1978). Effect of Sumithion (Fenitrothion) on some selected enzyme systems in the fish, *Tilapia mossambica* (Peters). Indian J. Exp. Biol., 18: 809-811.

Kumar S, Singh M (2000). Toxicity of dimethoate to a fresh water teleost *Catla catla*. J. Exp. Zool., India, 3(1): 83-88.

Lakshmi Rajyam Ch (1989). Evaluation of toxic effects of a synthetic pyrethroid fenvalerate in albino rat. Ph.D. thesis, S.V. University, Tirupati, India.

Lane CE, Scura ED (1970). Effects of dieldrin on glutamic oxalo acetic transaminase in *Poscilia latipinna* (sailfin molly). J. Fish Res., Bd. Can., 27: 1869.

Latner AL (1975). Clinical Biochemistry. (Ed.: Contarow and Trumper). W.B. Saunders Co., Philadelphia, pp. 182-192.

Lee YL, Lardy MA (1965). Influence of thyroid hormones on L-glycerophosphate dehydrogenases in various organs of rat. J. Biol. Chem., 240: 1427-1430.

Lehninger AL (1983). In: *Biochemistry*, Kalyani Publications and Ludhiana, New Delhi.

Lowry OM, Rosebrough NJ, Ferr AC, Randall RF (1951). Protein estimation with Folin Phenol reagent. J. Biol. Chem., 193: 265-275.

Malla Reddy P, Bashamohideen Md. (1988). Toxic impact of fenvalerate on the protein metabolism in the branchial tissue of the fish, *Cyprinus carpio*. Curr. Sci., 57: 211-212.

Mathivanan R (2004). Effects of sublethal concentration of quinalphos on selected respiratory and biochemical parameters in the freshwater fish, *Oreochromis mossambicus*. J. Ecotoxicol. Environ. Monit., 14: 57-64.

Moore S, Stein WH (1954). A modified Ninhydrin reagent for the photometric determination of aminoacids and related compounds. J. Biol. Chem., 221: 907.

Narasimha MB (1983). Studies on toxic potentials of lindane on fresh water teleost *Tilapia mossambica* (Peters) with special emphasis on nitrogen metabolism. Ph.D. thesis, S.V. University, Tirupati, Andhra Pradesh, India.

Natarajan GM (1981). Effect of Lethal LC_{50} 48 h concentrations of metasystox on selected oxidative enzymes, tissue respiration and histology of gill of fresh water air breathing fish *Channa striatus*. Curr.Sci., 50(22): 985-991.

Natarajan GM (1983). Metasystox toxicity: Effects of Lethal LC_{50} / 48 hrs concentration on free aminoacids and glutamate dehydrogenase in some tissues in the air breathing fish, *Channa striatus* (Bleeker). Comp. Physiol. Ecol., 8: 254-256.

Nichol CA, Rosen F (1963). In: Advances in Enzyme regulation. Vol. I (Ed. Weber, B) Pergamon Press, New York, p. 341.

Omprakasam M, Dayalan A, Arul Ganesan M (2008). Aquatic toxicity of dimethyl sulfoxide on the Indian major carp, *Catla catla* (Hamilton). Indian J. Sci. Technol., 1(4): 1-4.

Pandi B (1991). Use of Biochemical parameters in biomonitoring of pesticide pollution in some fresh water fishes: J. Ecotoxicol. Environ. Monitoring, 2: 101-104.

Panigrahi AK, Mishra BN (1980). Toxicological effects of sublethal concentration of inorganic mercury on freshwater fish *Tilapia mossambica* (Peters). Arch. Toxicol., 44: 269-278.

Pant JC, Singh T (1983). Inducement of metabolic dysfunction by corbomate and organophosphorus compounds in a fish, *Puntius conchonius* Pestle. Biochem. Physiol., 20: 294-298.

Potts WTH (1958). The inorganic and aminoacid composition of some lamellibranch mussels. J. Exp. Biol., 35: 749-764.

Prameelamma Y, Kesava Rao KV, Swamy KS (1975). Some aspects of regulation of succinate dehydrogenase activity in gastrocnemius muscle of frog. Indian J. Exp. Biol., 3: 177-179.

Pramoda Kumari J, Naidu BP (1987). Effect of permethrin on the organic constituents of the silk worm *Bombyx mori*. Environ. Ecol., 5: 717-719.

Radhaiah V (1988). Studies on the toxic impact of a pyrethroid insecticide fenvalerate on some metabolic aspects and histopathology of a fresh water teleost *Tilapia mossambica* (Peters). Ph.D. thesis, S.V. University, Tirupati, India.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic-pyruvic transaminases. Am. J. Clin. Pathol., 28: 56.

Saleem MA, Shakoori AR (1986). Biochemical effects of sublethal doses of cypermethrin on the sixth instar larvae of *Tribolium castaneum* (Herbst.). Arch. Insect Biochem. Physiol., 3: 447-455.

Schimmel SC, Garnas RL, Patrick JM, Moore JC (1983). Acute toxicity, bioconcentration, and persistence of AC 222,705, benthiocarb, chlorpyrifos, fenvalerate, methyl parathion, and permethrin in the estuarine environment. J. Agric. Food Chem., 31(1): 104-113.

Schmidt E, Schmidt FW (1973). Gamma glutamyl transpeptidase. Dtsch. Med. Wschr., 98: 1572-1577.

Schmidt Nielson B (1975). Osmoregulation: Effect of salinity and heavy metal. Fed. Proc., 33: 2137-2146.

Sesha Reddy (1989). Toxicity Evaluation of a synthetic pyrethroid on a target insect. M.Phil Thesis, S.V. University, Tirupati, Andhra Pradesh, India.

Shrivastava S, Singh S (2004). Changes in protein content in the muscle of *Heteropneustes fossilis* exposed to carbaryl. J. Ecotoxicol. Environ. Monit., 14: 119-122.

Siva Prasada Rao K, Ramana Rao KV (1984). Tissue specific alteration of aminotransferases and total ATPases in the fish, *Tilapia mossambica* under methyl parathion impact. Toxicol. Letts., 20: 35-57.

Siva Prasada Rao K, Sambasiva Rao KRS, Sreenivasa Babu K, Ramana Rao KV (1982). Toxic impact of phenthoate on some functional aspects of nitrogen metabolism in the tissue of the fish, *Channa punctatus*. Indian J. Fish., 29: 229-233.

Smart GR (1978). Investigation of toxic mechanisms of ammonia to fish – gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acute lethal concentrations. J. Fish Biol., 12: 93-104.

Sreenivasa MK (1983). Modulation of carbohydrate and associated metabolism in the selected tissues of fresh water mussel *Lamellidens*

marginalis during induced methyl parathion stress. Ph.D. thesis, S.V. University, Tirupati, India.
Stamp WG, Lesker PA (1967). Enzyme studies related to sex difference in mice with hereditary muscular dystrophy. Am. J. Physiol., 213: 587.
Sujatha LB (2006). Studies on the physiology, haematology and histopathology in the Indian major carp *Catla catla* (Hamilton) as influenced by individual and synergistic toxic effects of a pesticide and two metallic compounds. Ph.D. thesis, University of Madras.
Tilak KS, Satyavardan K, Thathaji PB (2003). Biochemical changes induced by Fenvalerate in the freshwater fish *Channa punctatus*. J. Ecotoxicol. Environ. Monit., 13: 261-270.
Tilak KS, Wilson Raju P, Butchiram MS (2009). Effects of alachlor on biochemical pramaters of the fresh water fish, *Channa punctatus* (Bloch). J. Environ. Biol., 30(3): 421-426.
Tiwari S, Ajay Singh (2009). Changes in some biochemical parameters in the liver and muscle of Colisa fasciatus due to toxicity of ethanolic extract of *Nerium indicum* Mill. (Lal Kaner / Latex). Natural Product Radiance, 8(1): 48-54.
Tripathi G, Verma P (2006). Mechanism of Fenvalerate-withdrawal-dependent recovery in metabolism of a catfish, *Clarias batrachus*. Pesticide Biochem. Physiol., 86 (3): 167-171.
Vasanthi M, Ramasamy M (1987). A shift in metabolic pathway of *Sarotherodon mossambicus* (Peters) exposed to thiodon (endosulfan). Proc. Indian Acad. Sci., (Anim. Sci.), 96: 56-69.
Weil-Malherbe H (1974). Ammonia formation in Brain Slices. Mol. Cell. Biochem., 4: 31-44.
Wilson RP, Poe WE (1974). Nitrogen metabolism in channel catfish *Ictalurus punctatus*: Relative pool sizes of free aminoacids and related compounds in tissues of cat fish. Comp. Biochem. Physiol., 48: 545-556.