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Impact of Cypermethrin on enzyme activities in the freshwater fish *Cirrhinus mrigala* (Hamilton)

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ABSTRACT
Freshwater fish *Cirrhinus mrigala* were exposed to lethal concentration (5 µg/L) for 1,2,3,4 days and sublethal concentration (1 µg/L) for 1,7,14,21 days of cypermethrin to examine the enzymes activity, in functionally three different tissues namely, gill, liver and muscle. Activities of Aspartate aminotransferase (AAT), Alanine aminotransferase (AlAT) and Glutamate dehydrogenase (GDH) increased in all the tissues with increase in exposure time. But in sublethal concentration at 14 and 21 day, a decreasing trend was observed in all the tissues exposed to cypermethrin. At most instances, fish in lethal medium were affected more compared to sublethal concentration.

Keywords: *Cypermethrin, Enzymes Activity, AAT, AlAT, GDH,* Cirrhinus mrigala.

INTODUCTION
Indiscriminate use of different pesticides in agriculture to prevent the crop from pest peril has been increased over the years, especially in the developing countries (Santhakumar and Balaji, 2000). These pesticides, even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like pounds and rivers and alter the physico-chemical properties of water (Bhalchandra *et al.,* 2001). These are proving to be highly toxic, not only to fishes but also to other organisms, which form food of the fishes (Madhab Prasad *et al.,* 2002). In recent years, synthetic pyrethroids have been developed for major uses in agriculture and public health purposes. The current commercial products were evolved from the natural pyrethrins, which possess high insecticidal potency low mammalian toxicity and very short persistence. These are highly toxic to fish and some aquatic invertebrates (Coats *et al.,* 1989).

The activity of aspartate and alanine amino transferases (AAT and AlAT), which serve as strategic links between protein and carbohydrate metabolisms, which is known to alter under several physiological and pathological conditions (Shivaknmar, 2005). Glutamate dehydrogenase (GDH), a mitochondrial enzyme, catalysis the oxidative deamination of glutamate, providing α-ketoglutarate to the kerbs cycle (Reddy and Venugopal, 1990). This enzyme is having several metabolic functions with great physiological significance. It is closely associated with the detoxification mechanisms of tissues. GDH in extra-hepatic tissues could be utilized for channelling of ammonia released during proteolysis for its detoxification into urea in the liver. Hence, the activities of AAT, AlAT and GDH are considered as sensitive indicators of stress (Gould *et al.,* 1976). Therefore attempt has been made to study the effect of the synthetic pyrethroid, cypermethrin on aspartate aminotransferase, alanine amino transferase and glutamate dehydrogenase activity in the economically important freshwater fish, *Cirrhinus mrigala.*

MATERIALS AND METHODS
Fish, *Cirrhinus mrigala,* weighing 5±1g and 7.5 cm in length were collected from

Karnataka State Fisheries Department fish farm Dharwad and acclimated to laboratory conditions for 15 d. They were fed with rice bran, oil cake, and soy beans in the ratio of 2:2:1 daily. Water in the tank was replaced two or three times per week. The physico-chemical characterisation of the water used for fish bioassay was carried out according to the method described in Standard Methods (APHA-AWWA-WEF, 1998): temperature 28±2^{0}C, pH 7.4-7.6, dissolved oxygen 5.7 to 6.0 mg/L, chloride 46.3 mg/L, carbon dioxide 2.08 mg/L, total hardness 110 mg/L, salinity 83.64 mg/L, total alkalinity 102 mg/L as $CaCO_3$, specific gravity 1.00374, calcium 16 mg/L and magnesium 9.3 mg/L.

Technical grade cypermethrin (95%) was obtained from United Phosphorus Ltd., Bombay.

After the normal process of acclimatization, a group of ten fish each were transferred to plastic tubs (15 L capacity) containing 10 L water and a lethal (5 µg/L) and sub-lethal concentration (1/5th of LC_{50} i.e. 1 µg/L) of cypermethrin. Predetermined exposure of lethal concentration 1,2,3 and 4 day and sub-lethal concentration 1,7,14, and 21 day (Finney, 1971). Control and exposed fishes were sacrificed at end of each day. Gill, liver and muscle tissue was isolated and immediately transferred to deep freezer prior to analysis. Aspartate aminotransferase and alanine aminotransferase were assayed by the colorimetric method of Retiman and Frankell, (1957). Alanine aminotransferease activity is expressed as µM pyruvate formed/mg protein/h and the AAT activity as µM oxaloacetate formes/mg protein/h. Glutamate dehydrogenase was assayed by the method of Lee and Hardy, (1965). GDH activity is expressed as µM formozan formed /mg protein/h. The experiments were repeated for six times to get concurrent values. The data were subjected to analysis of variance and the means were compared by Duncan's new multiple range test at 0.05% level (Duncan, 1955).

RESULTS

The present results raveled that cypermethrin induced alterations are time dependent, tissue-specific, and they point to disrupted activity GDH, AAT and AlAT enzymes has shown significant elevation in all tissues after lethal and sub lethal exposure (Tables 1-3). A progressive increase was observed in th e activities of AlAT, AAT and GDH in all the organs of the fish exposed to cypermethrin. This suggests the active transdeamination of amino acids for the incorporation of ketoacids into the TCA cycle to release necessary energy required for the synthesis of new proteins (Sreedevi *et al*., 1992; Sivaramakrishna and Radhakrishnaiah, 1998). Elevation in these enzymes indicates the utilization of amino acids. Enhancement in GDH activity in the tissues provided ketoglutarate and reduced nucleotides, which may fulfill the energy requirements during toxicity manifestations (Chandravathy and Reddy 1994). Increases in AAT and AlAT levels indicate that fish are under toxic stress. The amino acids appear to be mobilized to get transamination to 2-keto acids, for use in the production of energy rich compounds (David, 1995; Rajamannar and Manohar, 1998; Deva, 2000).

GDH is also known to play crucial role in ammonia metabolism and is known to be affected by a variety of effectors (David, 1995). After several metabolic functions with great physiological significance and known to be closely associated with the detoxifyycation mechanisms of tissues. GDH in extrahepatic tissue could be utilized for its ultimate detoxification to urea in the liver. In the present study the significant elevation in activities of these enzymes in the organs of fish exposed to the lethal concentration of cypermethrin indicates greater association of oilgomers of these enzymes in response to toxic stress. This shows that oxidative demination is contributing higher ammonia production. The high levels of ammonia produced is not eliminated but is salvaged through GDH activity which is utilized for amino acid synthesis through transaminases (David, 1995; Deva, 2000 and Prashanth 2003).

Alterations in the activities of the amino transferases would often be reflected in nitrogen metabolism and interdependent biochemical reactions. The increased levels of amino transferase might be attributed to tissue damage under toxic stress (Raju and Ramana Rao, 1985). AAT, a key enzyme of nitrogen metabolism and energy mobilezation in invertebrates, is often used as a biochemical indicator of stress (Shobha Rani *et al*., 2001; Reddy and Venugopal, 1990).

The GDH elevation in all tissue (Table, 1) also suggests the possibility of a need for α-ketoglutarate to the TCA cycle for the liberation of energy. Increased activities of AAT (Table, 2) and AlAT (Table, 3) in the study indicate that there is an active transamination of amino acids, possibly to provide keto acid in the TCA cycle.

Table 1. GDH activity (µM glutamine / mg protein / h) in the organs of fish, *Cirrhinus mrigala* on exposure to the lethal and sub lethal concentrations of cypermethrin.

Organ	Control	Exposure period in days							
		Letha				Sub lethal			
		1	2	3	4	1	7	14	21
Gill	0.1208 H	0.1370 G	0.1455 G	0.1671 F	0.1829 E	0.2804 B	0.2971 A	0.2399 C	0.2038 D
SD ±	0.0035	0.0023	0.0025	0.0008	0.0034	0.0050	0.0081	0.0046	0.0111
% Change		13.4087	20.4166	38.2673	51.3726	132.0596	145.9098	98.5653	68.6440
Muscle	0.1567 H	0.1608 G	0.1840 E	0.2217 B	0.2348 A	0.1931 D	0.2021 C	0.2187 B	0.1726 F
SD ±	0.0021	0.0018	0.0029	0.0048	0.0029	0.0073	0.0016	0.0028	0.0039
% Change		2.6056	17.4093	41.4868	49.8139	23.2160	16.7181	39.5299	10.1351
Liver	0.3696 I	0.4196 E	0.4428 C	0.5088 B	0.5307 A	0.3965 F	0.4267 D	0.3868 H	0.3908 G
SD ±	0.0016	0.0054	0.0026	0.0045	0.0021	0.0025	0.0019	0.0045	0.0051
% Change		13.5294	19.8115	37.6793	43.6051	7.2788	15.4505	4.6721	5.7545

Means are ± SD (n=6) for a tissue in a column followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncun's multiple range (DMR) test.

Table 2. The ASPARTATE AMINOTRANSFERASE (AAT) activity (µM oxalo acetate / mg protein / h) in the organs of fish, *Cirrhinus mrigala* on exposure to the lethal and sub lethal concentrations of cypermethrin.

Organ	Control	Exposure period in days							
		Lethal				Sub lethal			
		1	2	3	4	1	7	14	21
Gill	1.2423 I	1.4978 F	1.5847 E	1.7295 C	1.9297 A	1.6321 D	1.8101 B	1.4314 G	1.3316 H
SD ±	0.0079	0.0010	0.0481	0.0041	0.0040	0.0045	0.0017	0.0010	0.0084
% Change		20.5635	27.5610	39.2165	55.3260	16.1739	45.7043	15.2214	7.1854
Muscle	1.8780 I	2.3205 E	2.6862 C	2.7996 B	2.9343 A	2.2051 F	2.3859 D	2.1723 G	2.0942 H
SD ±	0.0079	0.0113	0.0068	0.0056	0.0339	0.0047	0.0016	0.0036	0.0410
% Change		23.5634	43.0382	49.0748	56.2492	17.4176	27.0476	15.6737	11.5150
Liver	2.1820 I	2.6784 E	2.9017 C	3.0658 B	3.2799 A	2.5817 F	2.7263 D	2.4153 G	2.3592 H
SD ±	0.0055	0.0095	0.0251	0.0352	0.0531	0.0035	0.0045	0.0053	0.0276
% Change		22.7488	32.9802	40.5038	50.3143	18.3172	24.9441	10.6912	8.1209

Means are ± SD (n=6) for a tissue in a column followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncun's multiple range (DMR) test.

Table 3. The ALANINE AMINOTRANSFERASE (AlAT) activity (µM pyruvate formed / mg protein/h) in the organs of fish, *Cirrhinus mrigala* on exposure to the lethal and sub lethal concentrations of cypermethrin.

Organ	Control	Exposure period in days							
		Lethal				Sub lethal			
		1	2	3	4	1	7	14	21
Gill	1.3500 H	1.7641 E	1.8759 D	2.0675 B	2.3670 A	1.6537 F	1.9735 C	1.9412 C	1.4713 G
SD ±	0.0126	0.0103	0.0061	0.0551	0.0545	0.0434	0.0052	0.0015	0.0059
% Change		30.6728	38.9580	53.1457	75.3358	22.4938	46.1827	17.8815	8.9852
Muscle	4.0975 H	4.7475 E	5.7130 C	5.9923 B	6.5145 A	4.7019 E	5.0651 D	4.4659 F	4.3119 G
SD ±	0.0344	0.0381	0.0278	0.0442	0.0711	0.0199	0.0565	0.0231	0.0041
% Change		15.8633	39.4259	46.2418	58.9861	14.7496	23.6127	8.9908	5.2328
Liver	5.7759 I	7.0193 F	7.7377 E	8.7495 B	9.8918 A	6.5058 G	8.0100 C	7.9111 D	6.1171 H
SD ±	0.0462	0.0440	0.0709	0.0357	0.0626	0.0491	0.0074	0.0023	0.0117
% Change		21.5278	33.9663	51.4843	71.2612	12.6382	38.6811	36.9688	5.9076

Means are ± SD (n=6) for a tissue in a column followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncun's multiple range (DMR) test.

The steady rise in the activities of GDH, AAT and AlAT in the organs of fish exposed to sublethal concentration of cypermethrin (Table 1-3) may be due to the synthesis of these enzymes under sub acute cypermethrin stress. The increase in these enzyme activities could be helpful to the fish for structural reorganization of proteins and incorporation of keto acids in to the TCA cycle to favor gluconeogenesis or energy production. The increase in transaminases can also link to the formation of urea (Ramana Rao, and Ramamurthi, 1983).

The steady increase in the activities of AAT AlAT and GDH lead to metabolic compensation and allow the animal to adapt to the imposed toxic stress. The elevation in GDH activity at the sub lethal concentration (Table, 1) could lead to increased production of glutamate in order to eliminate ammonia. To have an insight into the role of these enzymes in the altered metabolism of cypermethrin intoxicated fish, the activities of both AAT and AlAT were investigated in the present experiment. Elevated levels of AAT and AlAT indicate the enhanced transamination of amino acids, which may provide keto acids to serve as precursors in the synthesis of essential organic elements. It is likely that toxic stress imposed by cypermethrin might be one of the factors for the observed activities of AAT and AlAT in the present study.

GDH, a mitochondrial enzyme, catalyzes the oxidative deamination of glutamate generating α-ketoglutarate, an important intermediate of the TCA cycle. The GDH activity (Table, 1) in the present study exhibited a progressive enhancement in all tissues (gill, muscle and liver), throughout the exposure, suggesting a need for α-ketoglutarate. The regulatory roles of this enzyme as observed in mammalian models in checking the deamination process were reported earlier (Philip *et al*., 1988; Ramana Rao *et al.,* 1990; Reddy and Venugopal, 1990; Reddy and Yellama, 1991; David, 1995; Deva, 2000 and Shobha Rani *et al.,* 2001).

On the whole, it has been observed that the sublethal exposure of cypermethrin produced less change in the protein metabolism. It has also been noticed that the liver, gill and muscle were affected and the disturbances were found to be more in those tissues than that of muscle tissue. Despite the toxic effects on exposure to sublethal concentration of cypermethrin, the fish tries to withstand the toxic effects imposed by the pesticide by the modulating their physiological and metabolic response towards proper utilization of enzymes and proteins for synthetic processes.

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