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Impact of Cypermethrin on Na^+–K^+, Ca^{2+} and Mg^{2+} ATPases in Indian Major Carp, *Cirrhinus mrigala* (Hamilton)

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Abstract Activities of ATPase in different organs of *Cirrhinus mrigala* exposed to cypermethrin with lethal (5.13 μg/L) and sublethal (1.02 μg/L) concentration was investigated. Decrease of Na^+–K^+-ATPase activity was observed in gills, liver and muscle to about 60.22%, 22.13% and 48.89% respectively, by lethal concentration. Whereas in sublethal concentration activity was increased to about 7.84%, 10.70% and 5.96%. Similarly, in case of Mg^{2+}-ATPases activity as 57.61%, 48.82% and 28.59% for lethal and for sublethal it was 10.15%, 4.64% and 11.92%. Ca^{2+}-ATPase activity was observed to be 55.24%, 41.66% and 47.66% by lethal and for sublethal it was 8.02%, 24.89% and 6.47%.

Keywords Cypermethrin · *Cirrhinus mrigala* · ATPases

Discharges of industrial effluents, agricultural runoff and domestic wastewaters have posed a threat on the survival of fish and other aquatic organisms in the freshwater ecosystems. Although, pesticides do produce good results in the control of pests, their harmful effects on the non-target animals is not considered. The contaminated water poses a constant threat to all classes of living organisms including fishes (Magare and Patil 2000). Synthetic pyrethroids constitute a potent group of insecticides. Although many analogs of natural pyrethroids have been synthesized, few have succeeded commercially and only recently developed ones exhibit sufficient photostability to show promise for wide scale use in agriculture or forestry. It is known that arthropods and fishes are highly sensitive to synthetic pyrethroids (Malla Reddy and Harold Philip 1988).

Adenosine triphosphatase (ATPase) enzymes in vertebrates are vital for regulating oxidative phosphorylation, ionic transport, muscle function and several other membranes transport dependent phenomena. Na^+–K^+ ATPase has a central role in branchial transepithelial ion transport in fish (Fenwick and Lam 1988). This enzyme is present in the cell membrane of all vertebrates and particularly abundant in tissues associated with ionic and osmotic regulation. It represents a complex enzyme system, which requires Mg^{2+}, Ca^{2+}, Na^+–K^+ ions for their activity. Na^+–K^+ ATPase is a biochemical expression of active transport of Na^+–K^+ in the cells and Mg^{2+} ATPase is involved in the biosynthesis of ATP in the mitochondrial system (Chandravathy and Reddy 1995). To the best of our knowledge, there are less no existing reports on effect of pyrethroid insecticides on fish ATPases. Hence, the present investigation was carried out on cypermethrin [(R.S.)-α-Cyano-3-Phenoxybenzyl-2, 2-dimethyl-(IR, -IS)-*cis*, *trans*-3-(2,2-dichlorovinyl)cyclopropane carboxylate)], a pyrethroid insecticide, and its toxic impact on Na^+–K^+, Ca^{2+} and Mg^{2+} ATPases of economically important freshwater fish, *Cirrhinus mrigala* and the observed results are discussed in this paper.

Materials and Methods

Freshwater fish, *Cirrhinus mrigala* (length 15 ± 1 cm; weight 10 ± 1 g) was obtained from Karnataka State

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Fisheries Department Fish Farms, B.R. Project, India. The fish species were reared in large cement tank. During acclimation, the fish were fed with rice bran and oil cakes in the ratio of 2:1 on every alternate day. Water of the tank was changed daily to avoid fungal and bacterial contaminations, if any.

The physico-chemical characteristics of the water used for fish bioassay were determined according to the procedures described in Standard methods (APHA 1998). The water quality parameters were as follows: pH 8, temperature 28°C, DO 6.7–7.2 mg/L, salinity 108 mg/L, Cl 46.3 mg/L, Na 12.2 mg/L, K 30.5 mg/L, Ca 17 mg/L, Mn 1 mg/L, CO_2 9.0 mg/L, Hardness 115 mg/L, $CaCO_3$ 57 mg/L and specific gravity 1.00374.

Technical grade of cypermethrin (95%) was obtained from Rallis India Ltd, Bangalore. The pesticide stock solution was prepared by dissolving 10 mg of cypermethrin in 10 mL of analytical grade acetone. For experiment purpose, the pesticide was drawn from the stock solution. Maximum amount of acetone present in the highest concentration tested was less than 0.1 mL/L and the same quantity of acetone was added to the controls. Acetone was found to be non-toxic to fish (Pickering et al. 1962).

In order to understand the influence of time over the toxic effect of cypermethrin at lethal concentration on *Cirrhinus mrigala* at different periods of exposure. Before experimentation, healthy fishes were collected from the large cement tank with the help of big nylon net and hand net. They were acclimatized to laboratory conditions in glass troughs for fifteen days. Each trough contained 15 L of water with uniform sized (length 15 cm; weight 10 g). And were fed with commercial fish food pellets during acclimatization. After 15 days, fishes with normal behavioral activity and good health conditions were selected for further experiment purpose. The fishes were divided into two groups. Group I fishes were not exposed to pesticide and served as control. Whereas group II fishes were exposed to lethal and sublethal concentrations of cypermethrin for 1st, 2nd, 3rd and 4th day. And fishes were chosen on 1st, 7th, 14th and 21st day to observe the short-term and long-term effects respectively. Water was renewed after every 24 h to maintain the pesticide concentration. The LC_{50} value for 96 h was determined by probit analysis method (Finney 1971) and was found to be 5.13 µg/L. And sublethal concentration, one-fifth of LC_{50} value (1.026 µg/L) was considered for experimentation.

The inorganic phosphates liberated were estimated by the method of APHA (1998). All these three ATPase activities are expressed as µM Pi liberated/mg protein/h. The data were subjected to analysis of variance (ANOVA).

Results and Discussion

The toxic effect of cypermethrin at lethal and sublethal concentration on ATPase activities in *Cirrhinus mrigala* at different periods of exposure demonstrated that Na^+–K^+, Ca^{2+} and Mg^{2+} ATPases activity in all the three selected tissues (gill, muscle, and liver) was in concurrence with that of the toxic strength of cypermethrin and showed measurable decrease due to the exposure to lethal concentration, with slight fluctuation in activity due to the sublethal concentration and finally showed elevation in their activity on day 21 for sublethal concentration as recorded. The results can be depicted from Tables 1, 2 and 3.

ATPases, a membrane bound enzyme group, are responsible for the movements of different ions across the membranes. In fish various toxicants enter through gill surface by diffusion. An interaction with the membranes may disrupt the osmotic and ionic regulation of the membrane permeability, mainly due to inactivation of the ATPases in the brancheal epithelial cells (Chhaya et al. 1997). Freshwater fish take up salts from their ambient medium to compensate the water loss through excretion (David 1995). The regulation between the energy and osmoregulation in aquatic animals well is documented by Potts and Parry (1964).

In the present investigation, it was observed that the activities of Na^+–K^+, Ca^{2+} and Mg^{2+} ATPases was decreased in gill, muscle and liver in the fish on exposure to cypermethrin at lethal concentration (Tables 1, 2, 3). The decrease in their activities indicates the demolition of cellular ionic regulations in the organs of the fish as reported earlier (Renfro et al. 1974; Schmidt Nielson 1975). This disruption may be due to the toxic effect of cypermethrin on passive movement of ions (Chandravathy and Reddy 1995). Decrease in ATPases activity indicates the effects of cypermethrin on osmoion-regulations of these fishes. Further, greater imbalance caused to the gill structures, may also be probable reason for changes observed in ATPases activities. At cellular level pesticide interact with the ATPases and the interaction mainly depends on the cell surface area (David 1995).The inhibition of Na^+–K^+ ATPases is probably due to disturbance in Na^+–K^+ pump, resulting in an uncontrollable entry of Na^+ ions into the cell along the concentration gradient, with water molecule following along the osmotic gradient (Table 1). This process may cause swelling of the cell and finally membrane ruptures (Chandravathy and Reddy 1995). It was clearly observed in histological studies (Prashanth 2003).

The activity of Mg^{2+} ATPases was decreased in gill, muscle and liver of the fish on exposure to cypermethrin (Table 3). The inhibition of Mg^{2+} ATPases ion specific

Table 1 Na^+–K^+ ATPase activity (μM of Pi formed/mg protein/h) in the organs of fish, *Cirrhinus mrigala* on exposure to the lethal and sublethal concentrations of cypermethrin (5.13 and 1.02 μg/L)

Organ	Control	Exposure period in days							
		Lethal (5.13 μg/L)				Sub lethal (1.02 μg/L)			
		1	2	3	4	1	7	14	21
Gill	7.36	5.27	4.59	3.76	2.93	5.69	6.09	6.84	7.94
SD±	0.12	0.01	0.14	0.04	0.20	0.02	0.06	0.16	0.04
% Change		−28.39	−37.66	−48.99	−60.22	−22.77	−17.32	−7.09	7.85
Muscle	4.82	4.16	3.84	3.54	2.46	4.45	3.47	4.55	5.11
SD±	0.04	0.04	0.08	0.07	0.08	0.02	0.09	0.06	0.06
% Change		−13.61	−20.28	−26.50	−48.89	−7.65	−28.08	−4.05	5.96
Liver	3.81	3.63	3.43	3.11	2.97	2.76	2.52	3.53	4.22
SD±	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.05
% Change		−4.94	−10.12	−18.48	−22.13	−27.63	−33.98	−7.48	10.70

Means are ±SD (n = 6) for tissues in a column followed by the same letter are not significantly different ($p \leq 0.01$) from each other

Table 2 Mg^{2+} ATPase activity (μM of Pi formed/mg protein/h) in the organs of fish, *Cirrhinus mrigala* on exposure to the lethal and sub lethal concentrations of cypermethrin (5.13 and 1.02 μg/l)

Organ	Control	Exposure period in days							
		Lethal (5.13 μg/L)				Sub lethal (1.02 μg/L)			
		1	2	3	4	1	7	14	21
Gill	4.86	4.11	3.59	3.18	2.06	4.08	3.85	4.56	5.36
SD±	0.02	0.03	0.03	0.07	0.12	0.10	0.03	0.13	0.14
% Change		−15.41	−26.20	−34.62	−57.62	−16.10	−20.88	−6.22	10.15
Muscle	5.01	4.03	3.87	3.58	2.99	4.60	4.15	4.91	5.60
SD±	0.07	0.01	0.06	0.19	0.08	0.03	0.09	0.07	0.09
% Change		−19.55	−22.65	−28.59	−40.25	−8.20	−17.09	−1.93	11.92
Liver	7.51	5.21	5.15	4.73	3.84	6.30	5.69	6.66	7.85
SD±	0.04	0.00	0.02	0.04	0.06	0.13	0.24	0.15	0.16
% Change		−30.61	−31.45	−37.01	−48.83	−16.1	−24.18	−11.27	4.64

Means are ±SD (n = 6) for a tissue in a column followed by the same letter are not significantly different ($p \leq 0.01$) from each other

Table 3 Ca^{2+} ATPase activity (μM of Pi formed/mg protein/h) in the organs of fish, *Cirrhinus mrigala* on exposure to the lethal and sub lethal concentrations of cypermethrin (5.13 and 1.02 μg/L)

Organ	Control	Exposure period in days							
		Lethal (5.13 μg/L)				Sub lethal (1.02 μg/L)			
		1	2	3	4	1	7	14	21
Gill	8.71	6.76	6.55	5.00	3.90	7.12	7.83	8.36	9.41
SD±	0.44	0.20	0.06	0.09	0.16	0.06	0.03	0.05	0.10
% Change		−22.48	−24.88	−42.56	−55.24	−18.28	−10.15	−4.10	8.02
Muscle	5.44	4.02	3.84	3.62	2.85	4.42	3.92	4.75	5.79
SD±	0.09	0.01	0.00	0.06	0.05	0.00	0.01	0.34	0.09
% Change		−26.16	−29.40	−33.44	−47.67	−18.74	−27.95	−12.63	6.47
Liver	2.92	2.44	2.21	1.94	1.70	2.62	2.31	2.74	3.65
SD±	0.06	0.02	0.01	0.05	0.02	0.00	0.01	0.06	0.11
% Change		−16.63	−24.23	−33.73	−41.66	−10.28	−21.02	−6.38	24.89

Means are ±SD (n = 6) for a tissue in a column followed by the same letter are not significantly different ($p \leq 0.01$) from each other

ATPases could be attributed to the loss of sodium and potassium ions due to cellular leakage into the body fluids. Non-availability of substrates like ATP molecules may also result in inhibition of these ion-specific ATPases (Chandravathy and Reddy 1995). The physiological significance of Mg^{2+} ATPases activity changes is difficult to interpret, since many types of phosphates are involved, each of which having a different physiological function. Mg^{2+} ATPases is also found associated with both Na^+–K^+ and Na^+, NH_4^+ ATPases and is related to the transport of Mg^{2+} across the gill epithelium (Sargent et al. 1980). This is also essential to the integrity of the cellular membrane, intracellular elements and to the stabilization of branchial permeability (Raju 2000). The inhibition of ATPase activities in the present investigation showed greater decrease in the levels of ions observed in the gill, muscle and liver tissues of fish, exposed to lethal concentration of cypermethrin, indicating the effects of cypermethrin on osmo ion-regulations of the experimental animal. As the ion-regulatory capacity is energy dependent process, the greater decrease in the energy releasing pathways in fish subjected to lethal intoxication provides support for the more decrease in the levels of Na^+–K^+ and Ca^{2+} ions.

Due to the exposure of cypermethrin at sublethal concentration, the Na^+, K^+ and Ca^{2+} levels were significantly decreased with ion competent inhibition associated Na^+–K^+, Ca^{2+} and Mg^{2+} ATPases activity in the organs of fish at day 1 and 7 exposures (Tables 1, 2, 3). Significant elevation in the ionic levels and enzyme activities was observed on day 14 and 21 indicate that prolonged exposure to sublethal concentration of cypermethrin could not elicit inhibitory effect on the uptake of ions by activities of ATPases and instead it posed to stimulate the uptake. Possibly the inhibition of ATPases activity is dependent on the functional groups of the active pocket residue of the enzyme and the amount of cypermethrin available for the competitive replacement of the substrate.

Further, recruitment of chloride cells has been proposed as a fundamental and physiologically significant response of freshwater fish to increase the capability to uptake Na^+, K^+ and Ca^{2+} from water (Lenio et al. 1987; Fu et al. 1990). ATPases activities were found to be increased in the gills, muscle and liver of fish from 14 to 21 days. The increased ATPases level may be helpful to the animals to prevent the entry of toxic cypermethrin by maintaining cation concentration gradient. During the post-exposure recovery period the enzyme activities were found to reach normal levels on 21st day. However, during day 7 and 14 of recovery, enzymes failed to recover and this could be due to slow elimination of cypermethrin from these tissues. The metabolism and slow elimination of the cypermethrin from the tissue, is coupled with the upregulation of enzymes which might be the possible reason for the progressive recovery. Many authors have reported the recovery of ATPases in sub lethal concentration (Reichert et al. 1979; Sargent et al. 1980; Spehar et al. 1981; Holcombe et al. 1982; David 1995; Chandravathy and Reddy 1995; Raju 2000; Shivakumar 2005).

Living system needs a continuous input of energy for the buildup and maintenance of their organization. The energy derived during the cellular oxidation of organic fuels is stored in the phosphate bond primarily with adenosine in the form of ATP (adenosine triphosphate) and sometime in the form of CP (creatine phosphate). Adenosine triphosphatases has a central role in physiological functions of cells as energy transducers by coupling the chemical reactions of ATP hydrolysis. Membrane bound Na^+–K^+ ATPase is the enzymatic machinery for the active transport of sodium and potassium across the cell membrane.

In the present investigation, it was clear that the exposure to cypermethrin demonstrated decreased ATPases activity. This is attributed to the lipophilic nature of the pyrethriod and its direct action on the enzymes in general. The synthesis of ATP by phosphorylation of ADP is mainly associated with glycolysis and biological oxidation involving the citric acid cycle and electron transport systems (Lehninger 1980). Thus, a decrease in ATPase activity through the lipophilic action of cypermethrin seems to be possible cause. It may therefore be inferred that if the uptake of this compound by fishes in natural environment, reaches tissue concentrations equal to that used in this investigation, the resulting disruption in ion concentration by ATPases may be sufficient to impair normal functioning of the organs such as gills, muscle and liver in fish.

References

APHA (1998) American Standard methods for the examination of water wastewater, 20th edn. APHA, Washington, DC

Chandravathy M, Reddy SLN (1995) *In vivo* alterations in the activities of ion-specific ATPases in the tissue of *Anabas scandens* associated with sub lethal lead nitrate toxicity and recovery responses during post-exposure period. J Environ Biol 16(4):301–304

Chhaya J, Thaker J, Mansuri AP, Kundu R (1997) Textile dyeing and printing industry effluent induced changes in activity of few ATPases in the gill and intestine of mudskipper, *Periophthalumus dipes*. Poll Res 16(2):93–97

David M (1995) Effect of feuvalerate on behavioral, physiological and biochemical aspects of freshwater fish *Labeo rohita.* PhD thesis, SK University, Anantapur, Andra Pradesh, India

Fenwick JC, Lam TJ (1988) Studies on effect of air exposure on gill Na^+-K^+ ATPases of the marble goby *Oxyeleotris marmorata* a facultative air-breathing fish. Fish Physiol Biochem 5:121–130

Finney DJ (1971) Probit analysis, 2nd edn. Cambridge University Press, London

Fu H, Steinebach DM, Vanden Hamer CJA, Balm PHN, Lock RAC (1990) Involvement of cortisol and metallothionein like protein

in the physiological responses of tilapia, *Oreochromis mossambicus* to sub lethal cadmium stress. Aquat Toxicol 16:257–270

Holcombe GW, Phipps GL, Tunner DK (1982) The acute toxicity of kelthane, dursha, disulfoton pydrin and permethrin to fathead minnows, *Pimephales promelas* and rainbow trout, *Salmo gairdners*. Environ Pollut 29A:167–178

Lehninger AL (1980) Biochemistry, 2nd edn. Kalyani Publication, Ludhiana

Lenio RL, McCormick JH, Jansen KM (1987) Changes in gill histology of fathead minnows and yellow perch transferred to soft water or acidified water with particular reference to chloride cells. Cell Tissue Res 250:389

Magare SR, Patil HT (2000) Effect of pesticide on oxygen consumption, red blood cell count and metabolites of a fish, *Puntius ticto*. Environ Ecol 18(4):891–894

Malla Reddy P, Harold Philip G (1988) Toxicity of a synthetic pyrethriod insecticide to a freshwater fish, *Labeo rohia*. Mendel 5(3):138–140

Pickering QH, Henderson C, Lenke AK (1962) The toxicity of organophorous insecticides to different species of warm water fish. Trans Am Fish Soc 91:178–184

Potts WTW, Parry G (1964) Osmotic and ionic regulation in animals. Pergamon Press, Oxford

Prashanth MS (2003) Cypermethrin induced physiological, biochemical and histopathological changes in freshwater fish, *Cirrhinus mrigala*. Ph.D. thesis, Kuvempu University, Dharwad

Raju DP (2000) Fenvalerate induced changes in the protein metabolism of freshwater fish *Tilapia mossambica* (Peters). PhD thesis, SK University, Anantapur, Andra Pradesh, India

Reichert WLAF, David C, Malins D (1979) Uptake and metabolism of lead and cadmium in cohosalmon *Onchornyncus kisutch*. Comp Biochem Physiol Comp Pharmacol 63:229–234

Renfro JLB, Schmidt Nielson D, Miller DB, Allens J (1974) Methyl mercury and inorganic mercury uptake, distribution and effect on osmoregulatory mechanisms in fishes. In: Vernberg FJ, Vernberg WB (eds) Pollution and physiology of marine organisms. Academic Press, San Diego, p 317

Sargent R, Bell MV, Killy (1980) The nature and properties of sodium ions plus potassium ion activated adenosine triphosphates and its role in marine salt secreting epithelia. In: Lahlou B (ed) Epithelia transport in the lower vertebrates. Cambridge University Press, London, pp 251–267

Schmidt Nielson B (1975) Osmoregulation. Effect of salinity and heavy metal. Fed Proc Fed Am Soc Exp Biol 33:2137–2146

Shivakumar R (2005) Endosufan induced metabolic alternation in freshwater fish *Catla catla*. PhD thesis, Karnataka University, Dharwad

Spehar RL, Carlson RW, Lemke AE, Mount DL, Pickering QH, Sankrski VM (1981) Effect of pollution on freshwater fish. J Water Pollut Control Fed 52:1703–1767