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ORIGINAL RESEARCH ARTICLE

Alterations in the Levels of Ions in Tissues of Freshwater Fish *Cirrhinus mrigala* Exposed to Deltamethrin

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ABSTRACT

The fish *Cirrinus mrigala* were exposed to lethal concentration of (8 µl/L) of deltamethrin for 1, 2, 3 and 4 days and to sublethal concentration (1/10 of lethal concentration, 0.8 µl/L) of deltamethrin for 1, 5, 10 and 15 days to know the levels of different ions namely sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}). All the ions in lethal concentration were found to decrease consistently under deltamethrin stress. During sublethal concentration there was a decrease in ionic level upto 10^{th} day of exposure period and in 15^{th} day there was an elevation seen in ionic concentrations. The current results clearly indicate that during sublethal concentration the fish gradually adopts and develops a resistant mechanism to deltamethrin stress.

Key words: *Cirrhinus mrigala*, deltamethrin, osmoregulation, pesticide

INTRODUCTION

During past few decades, many new broad spectrum pesticides are in use for crop protection. During the course of application to crops, some inevitable enter the water bodies through surface runoff, which gradually affect non target organisms like fishes. Deltamethrin is a α-cyano pyrethroid insecticide used extensively in pest control. In fish, it is a potent stress inducing agent (Rehman *et al*, 2006). Pyrethroids slow down the gating kinetics of Na channel of the axon (Kiss *et al*, 1991).

The inorganic ions maintain osmotic balance of a cell with that of external environment, and are important in carrying out of cellular metabolism. They are required to be present in proper proportion in every organism for carrying out protoplasmic activity (David *et al*, 2003). Any improper proportion of these ions in an organism will hamper with various physiological activities (Leone and Ochs, 1987; Baskin *et al*, 1981). Freshwater fishes live in hypotonic environment that is the water having less amount of salt as compared with the salts present inside the organism body. This leads to the loss of ions from the body of fish. To cope with this, freshwater fishes have adopted a special kind of mechanism called active transport mechanism. During the regulation of osmolarity of a system, sodium, potassium and calcium ions play a vital role in helping the fish maintain internal hypertonic environment.

There are limited reports available on possible effects of pyrethroid, deltamethrin on the activity of ions in fresh water fish. Hence, the present study was designed to evaluate the levels of sodium, potassium and calcium in different tissues of fresh water fish *Cirrhinus mrigala* under deltamethrin stress.

MATERIALS AND METHODS

Fresh water fish *Cirrhinus mrigala* weighing 20-30 g, were procured from Karnataka State Fisheries Department, Dharwad, Karnataka, India. The dermal adherents of fishes were cleansed by treating with potassium permanganate solution (0.5% w/v) for 2 minutes. Fishes were acclimatized to laboratory condition for 15 days and were fed *ad libitum* with commercial fish feed. The water was renewed daily and the quality of water determined and maintained as per APHA, 2005.

The commercial grade of deltamethrin (Decis, 30% EC) was obtained from Bayer Crop Science, India Ltd; Gujarat, India. The stock solution of the insecticide was prepared. The LC_{50} value for 96h

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was determined by following the method of Finney (1971) and was found to be 8µl/l. One tenth of LC_{50} concentration (0.8µl/l) was selected as sublethal concentration and used in present study. The fish were exposed to lethal concentration for 1, 2, 3 and 4 days and to sublethal concentrations, 1, 5, 10 and 15 days.

After the stipulated time gill, muscle and liver were isolated and weighed. The weighed organs were wet ashed in 50:50 (V/V) concentrated perchloric and nitric acid (Dall, 1967). After keeping the wet ash solutions for half an hour, until the organs were completely dissolved, they were evaporated at 100°C to 200°C temperature. The residues were dissolved in glass with distilled water and made up to 10 ml. It was filtered through Whatman No.1 filter paper. Further, appropriate dilutions were made prior to estimations and, the sodium, potassium and calcium ion were estimated with the help of flame photometer (Elico Pvt. Ltd., Model CL-22A). Standard solutions of sodium, potassium and calcium were prepared by using analytical grade chemicals. The values are expressed as µmol/g wet wt. of the organ. All statistical analyses were carried out using SPSS 10.1 and $p < 0.05$ was considered statistically significant.

RESULTS

Levels of sodium, potassium and calcium ions (µmol/g wet wt.) in the three target tissues namely gill, muscle and liver of freshwater fish, *Cirrhinus mrigala* on exposure to lethal concentration of deltamethrin for 1, 2, 3 and 4 days and sublethal concentration for 1,5,10 and 15 days are presented in (**Table 1, 2 & 3**).

A gradual decrease in the sodium level was observed in all the three tissues under lethal concentration of deltamethrin. Minimum percent decrease of (-2.833) was observed in liver on 1st day, while maximum percent decrease of (-45.707) was witnessed on 4th day of exposure in gills. Sublethal concentration recorded a continuous decrease in the sodium ion concentration in all the tissues up to day 10 and day 15 registered elevation in the sodium ion concentration. Sodium ion levels on day 1 and day 15 in liver showed almost equal rate of decrease -6.182% and -6.449% respectively. The values of gill and muscle on day 1 and day 15 differed with very less percent decrease (-23.69%) followed by gill (-20.556%) and muscle (-5.812%). Data presented in table shows values of percent decrease in exposure periods.

Table 1: Sodium Ion Content in the Different Organs of Fish *Cirrhinus mrigala Exposed* to Deltamethrin

Tissue	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Gill	57.901 A	49.143 E	44.312 G	33.821 H	31.435 I	53.352 B	49.934 D	45.998 F	50.979 C
±SD	0.0002	0.0001	0.0002	0.0003	0.0003	0.0004	0.0003	0.0003	0.0005
%Change		-15.124	-23.468	-41.587	-45.707	-7.885	-13.758	-20.556	-11.954
Muscle	44.251 A	38.970 C	33.600 G	25.910 H	24.398 I	41.388 B	37.896 E	34.750 F	38.968 D
±SD	0.0003	0.0001	0.0002	0.0002	0.0004	0.0004	0.0004	0.0003	0.0005
%Change		-11.933	-24.07	-41.448	-44.863	-5.812	-14.361	-21.47	-11.939
Liver	53.576 A	52.058 B	46.144 D	41.122 G	36.931 I	50.264 C	44.842 E	40.884 H	43.397 F
±SD	0.0002	0.0002	0.0003	0.0004	0.0003	0.0004	0.0003	0.0004	0.0002
%Change		-2.833	-13.87	-23.245	-31.067	-6.182	-16.301	-23.69	-18.999

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

The Potassium (K^+) ion levels showed gradual decrease from day 1 to day 4 in all the three tissues under lethal concentration. Among the three tissues, muscle exhibited maximum decrease of (-44.613%) followed by gill (-39.386%) and liver (-32.585). Lowest decrease value was noted in liver (-7.616%) on day 1 and highest decrease value was recorded in muscle on 4th day of exposure. Under sublethal concentration potassium ion concentration decreased continuously up to day 10 in all the three target tissues. While on day 15th potassium ion level showed elevated values, when compared with the data obtained on day 10th. Maximum decrease was recorded in liver (-44.613%) and data is presented in table. Muscle on first day of exposure to sublethal concentrations showed the least percent decrease of potassium ion level followed by gill and liver.

Table 2: Potassium Ion Content In The Different Organs Of Fish *Cirrhinus mrigala* Exposed To Deltamethrin

Tissue	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Gill	60.608 A	51.0857 E	48.838 G	42.580 H	36.736 I	57.240 B	53.824 C	50.725 F	53.442 D
±SD	0.0002	0.0002	0.0002	0.0003	0.0003	0.0004	0.0005	0.0003	0.0003
%Change		-15.711	-19.418	-29.744	-39.386	-5.555	-11.192	-16.307	-11.822
Muscle	68.979 A	62.245 C	57.495 F	51.435 H	47.310 I	65.789 B	60.968 D	56.173 G	60.281 E
±SD	0.0002	0.0003	0.0002	0.0002	0.0003	0.0003	0.0007	0.0005	0.0005
%Change		-9.761	-16.647	-25.443	-44.613	-4.624	-11.613	-18.565	-12.609
Liver	53.016 A	48.978 C	42.670 D	39.212 G	35.740 I	49.446 B	43.883 E	38.777 H	42.208 F
±SD	0.0002	0.0002	0.0002	0.0004	0.0003	0.0003	0.0003	0.0004	0.0003
%Change		-7.616	-19.514	-26.037	-32.585	-6.732	-17.226	-26.857	-20.386

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

Similar tendency as observed in sodium and potassium ion levels was observed in the calcium ion levels, which also showed gradual decrease in the ionic concentration from day 1 to day 4 on exposure to lethal concentration of deltamethrin. Maximum percent decrease was observed in gill (-38.832%) followed by liver (-33.351%) and muscle (-30.489%) on day 4. Minimum percent decrease was noted in liver (-5.053) followed by the muscle (-6.490) and gill (-10.828) on day 1. The sublethal dosage affected a depletion of maximum percent of calcium ions in the gills. In all the tissues from day 1 to day 10 a gradual decrease in the calcium ion level was noted. The day 15 showed slight increase in the ion concentration in all the three tissues. Maximum percent decrease was noted in liver (-4.789%) followed by muscle (-5.836%) and gill (-8.885%) on day 1.

Table 3: Calcium Ion Content In The Different Organs Of Fish *Cirrhinus Mrigala Exposed* To Deltamethrin

Tissue	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Gill	59.587 A	53.6775 C	47.192 E	40.791 H	36.448 I	54.292 B	48.322 D	42.059 G	44.478 F
±SD	0.0002	0.0003	0.0003	0.0003	0.0001	0.0003	0.0004	0.0003	0.0003
%Change		-10.828	-20.801	-31.543	-38.832	-8.885	-18.905	-29.415	-25.352
Muscle	72.411 A	67.711 C	62.093 F	56.052 H	50.333 I	68.184 B	64.022 E	59.861 G	65.164 D
±SD	0.0003	0.0002	0.0003	0.0002	0.0003	0.0004	0.0002	0.0003	0.0003
%Change		-6.49	-14.248	-22.591	-30.489	-5.836	-11.585	-17.33	-10.007
Liver	64.720 A	61.449 C	55.189 E	50.412 H	43.135 I	61.620 B	57.227 D	50.981 G	54.576 F
±SD	0.0002	0.0003	0.0002	0.0002	0.0003	0.0003	0.0002	0.0005	0.0003
%Change		-5.053	-14.725	-22.107	-33.351	-4.789	-11.577	-21.227	-15.672

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

DISCUSSION

Strict ion regulation is necessary for aquatic organisms if they also maintain water and ion homeostasis. The osmotic and ionic characteristics of the body fluids and tissues of freshwater organisms are largely influenced by the ambient medium. The maintenance of homeostasis in such a condition is very much dependent on the osmoregularity properties and it is a vital phenomenon to maintain the physiological balance between the external environment and internal milieu of an animal.

Alteration in osmotic regulatory mechanism under toxic stress condition may cause severe imbalance in biochemical composition of the tissue fluids followed by undesirable metabolic consequences. The principle components of osmoregulation are ions. Ions may be anionic or cationic in nature, based on the charge and these help in maintenance of perfect osmoregulatory of the cell. Osmo and ion regulation plays an important role in the cellular metabolism of an animal, and its imbalance leads to change in various physiological and biochemical activities (Moorthy *et al*., 1984). Freshwater fishes take up salts from their ambient medium to compensate the water loss through excretion. This obviously necessitates a high metabolic demand. The regulation between the energy and osmoregulation in aquatic animals is well documented by Tseng and Hwang (2008).

Sodium, potassium and calcium are not only important for the maintenance of osmoregulation

of body fluids (Baskin *et al*., 1981), but also for the transport of nutrients from the lumen of the digestive tract into interstitial cells (Crane, 1987) and uptake of neurotransmitters in the brain. ATPase enzyme complex helps in the uptake of ions from the external medium to the interior of the body of freshwater fishes.

Reports are available to indicate that the insecticides interfere in the transport processes in the biological membrane (Belzunces *et al*., 2012) and inhibit active transport of ions in several tissues (Iturri and Wolff, 1982). In fish gills form a major site for the ion transport and osmotic water movements hence, also for pesticides entry. They are the first organs to be exposed to pesticides as they are in constant touch with the polluted water. This affects the permeability characteristics and osmoregularity function of the gills thereby resulting in the decrease of these ions in gills tissue upon exposure to deltamethrin. In the present study the decrease in the levels of Na^+-K^+ and Ca^{2+} ions in the gill, muscle and liver exposed to lethal and sublethal concentrations of deltamethrin indicates changes in the permeable properties of the cell membrane of these organs and of deranged Na^+-K^+ and Ca^{2+} ionic pumps due to the 0probable consequences of tissue damage. Moorthy et al., (1984); Reddy et al (1991) offer a strong support for the present observations.

An appraisal of results in the present study suggests that the sodium content decreased as a function of time of exposure to deltamethrin. It is known that sodium content in tissue mainly depend on the permeability functional efficiency of bio-membrane and efficient functional role of Na^+ pump, which regulates ionic content of tissues. The level of Na+ signifies the tissue importance in the mobilisation of water transport, since sodium content in the membrane facilitates the water movement among the tissue (Javot and Maurel., 2002). It is known that any remarkable decrease in K+ level might be accompanied by serious disturbances in muscular irritability, myocardial function and respiration (Chebbi and David, 2010). The decrease in K+ content in the tissue of *Cirrhinus mrigala* exposed to deltamethrin might be attributed to the derangement in respiration at whole animal as observed in the present investigation.

The main reason for the decrease in sodium, potassium and calcium ion levels in the organs of fish, exposed to fenvalerate could be attributed to the suppressed activities of Na^+-K^+ , Mg^{2+} and Ca^+ ATPase (Panati *et al*, 2012). ATPase have been described as prominent energy linked enzymes in fishes (Hwang and Lee, 2007). Inhibition of these enzymes by deltamethrin influences the movement of ions by active transport. The suppression in ATPase activity also suggests a drastic decrease in the prolactin release, which might be particularly responsible for the hypocalcemia. Rapid induction of hypocalcemia by cadmium has been reported in rainbow trout (Giles 1984), Carp (Yamawaki et al., 1986) and Tilapia (Pratap *et al*., 1989).

It is quite evident that the fish, *Cirrhinus mrigala* under deltamethrin stress seldom undergoes total abolition of functional regulation of the ionic transport and water permeability and the imbalance in osmoregulation is compensated in harmonic fashion through the production of biochemically changed components like amino acids which go to rescue and compensate the imbalance ionic composition. Thus, inherent osmoregulation of freshwater fish is viewed in a nutshell but clear understanding on the basis of water permeability versus Na+ transport and the role of Ca2+ in the water transport mechanism still waits direct analyses.

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