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IMPACT OF ENDOSULFAN (35% EC) AND FENVALERATE (20% EC) ON ACTIVITY OF ACID PHOSPHATASE AND ALKALINE PHOSPHATASE IN A FRESHWATER FISH *LABEO ROHITA*

K. SUNEETHA* AND K. VEERAIAH

Faculty, Department of Biochemistry and Faculty, Department of Zoology, Acharya Nagarjuna University, Guntur, Vijayawada, Andhra Pradesh, India*

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ABSTRACT

The freshwater fish *Labeo rohita* was exposed to endosulfan and fenvalerate a synthetic pyrethroid. The LC_{50} values determined for endosulfan and fenvalerate at 24hrs were 0.6876 and 0.4749 µgl^{-1} respectively. The fish were exposed to lethal endosulfan 0.6876 and fenvalerate 0.4749 µgl^{-1} and sublethal concentrations of endosulfan 0.06876 and 0.04749 µgl^{-1} for 24hrs and 15days and changes in the activity of Acid phosphatase and Alkaline phosphatase were observed. The enzymes showed a decrease in activity on pesticide exposure.

KEY WORDS: Endosulfan, Fenvalerate, Acid phosphatase and Alkaline phosphatase.

INTRODUCTION

Agriculture is one of the main economies of not only the people of Andhra Pradesh but of the whole country. Pesticides are extensively used worldwide in agricultural practices to control pests and increase crop yield. In recent years, their use has increased considerably. As an agricultural developing nation India too relies predominantly on chemical pesticides to sustain a large population. India represents the largest manufacturer of basic pesticides in Asia and ranks 12th on the global spectrum. Of the total pesticide consumption in India, insecticides account for ~75%, followed by fungicides [~ 12%] and herbicides [~ 10%] (Indira *et al*., 2007). Indiscriminate use of pesticides and their untreated effluents affects fish and other aquatic animals (Wanee *et al.,* 2002).

Organochlorines (OCs) are the most widely used class of insecticides in the world and considered to be the most hazardous with respect to environmental pollution. They are persistent and non-biodegradable and their residues buildup in the aquatic food chain and remain there for a long period of time and thus show neurotoxicity to the animals (Adetola *et al.,* 2011). Endosulfan, a compound of cyclodiene subgroup of organochlorine pesticides, is used extensively to control insect pests in fruit crops, vegetables, oilseed and cereal crops (Tariq *et al.,* 2007). An extensive usage of endosulfan increases its frequency in the aquatic environments even at considerable distances from the point of its original application (Miles and Pfeuffer, 1997). Fenvalerate is one of the pyrethroid insecticide and most widely used in agricultural crops such as cotton, paddy, jowar, maize, soyabean, tomato, lady's finger, cauliflower, tobacco and tea. These pyrethroids account for approximately one fourth of the worldwide insecticide market (Casida and Quistad, 1998; Suneetha, 2016).

Fish are excellent subjects for the study of various effects of contaminants present in water samples since they can metabolize concentrate and store water borne pollutants. Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans (Ali *et al.,* 2007).

Phosphatases are good indicators of stress condition in the biological systems (Verma *et al.,* 1980). ALP is involved in carbohydrate metabolism, growth and differentiation, protein synthesis,

synthesis of certain enzymes, secretion activity, and transport to phosphorylated intermediates across the cell membranes (Vijayavel, and Balasubramanian, 2006). Thus, any alteration in the activity of ALP affects the organisms.

The present study has been made to investigate the changes in activities of Acid phosphatase and Alkaline phosphatase enzymes in the fresh water fish *Labeo rohita* induced by lethal and sublethal concentrations of Endosulfan and Fenvalerate.

MATERIALS AND METHODS

The freshwater fish *Labeo rohita* is an edible and commercially valuable fish. Live fish of size 6-7±1cm and 6-8 g weight were brought from a local fish farm and acclimatized at 28 ± 2^0C in the laboratory for one week. The stock solutions for Endosulfan 35% Emulsifiable Concentrate (EC) and Fenvalerate 20% Emulsifiable Concentrate (EC) were prepared in 95% acetone to yield a concentration of 100mg/100 mL which were further diluted with distilled water to get a working solution. The water used for acclimatization and conducting experiments was clear unchlorinated ground water. In each test ten fish were introduced in toxicant glass chambers with a capacity of ten liters. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The toxic tests were conducted to choose the mortality range from ten percent to ninety percent for 24 hrs in static tests. The concentration that produced fifty percent mortality in test species was noted. LC_{50} values were calculated by Finney's Probit analysis (1971) Finney, (1971).

Acid phosphatase activity was determined following the method of Bergmeyer (1956) (Bergmeyer, 1956) wherein Sodium p-nitrophenyl phosphate was used as substrate. Buffer/substrate solution (0.5M citrate buffer, 0.0055 M p-nitrophenyl phosphate, pH 4.8) was added to 10mL of homogenate. The reaction mixture was incubated for 30 min at 37 °C. 4 ml of 0.1M NaOH was then added to stop the enzymatic reaction. The absorbance was measured at 410nm. The activity of acid phosphatase was expressed as units per mg protein. Alkaline phosphatase activity was also determined with the similar method as of acid phosphatase excepting the Glycine buffer at a pH of 8.8 was used.

RESULTS AND DISCUSSION

The present investigation was undertaken to

Table 1. Changes in activity of acid phosphatase (expressed as µ moles PNP/mg of protein/30min wet. weight of tissues) in different tissues of *Labeo rohita* on exposure to sublethal and lethal concentrations of endosulfan and fenvalerate for 24 hr.

Organs	Control	Endosulfan - 24hr				Fenvalerate- 24hr			
		Sub-Lethal	% Change	Lethal	% Change	Sub-Lethal	% Change	Lethal	% Change
Brain	10.5± 0.015	10± 0.001	4.76	9.5± 0.005	9.52	9.8± 0.015	6.66	9.26± 0.002	11.8
Gill	9.21± 0.001	8.85± 0.002	3.90	8.32± 0.004	9.6	8.5± 0.003	7.7	7.91±0.021	14.11
Kidney	11.5± 0.008	11± 0.003	4.34	10.76± 0.002	6.43	10.87± 0.006	5.47	10.3±0.014	10.43
Liver	15.6± 0.469	13.51± 0.061	6.9	13.9± 0.0012	10.89	14.1±0.0015	9.61	12.93±0.011	17.11
Muscle	13.2± 0.005	12.44± 0.005	5.75	12.16± 0.015	7.87	12.32±0.001	6.66	11.54± 0.003	12.57

Values are the means of five observations: (±) indicates the standard deviation values are significant at $P > 0.05$

Table 2. Changes in activity of acid phosphatase (expressed as µ moles PNP/mg of protein/30min wet. weight of tissues) in different tissues of *Labeo rohita* on exposure to sublethal concentrations of endosulfan and fenvalerate for 15 days.

Organs		Endosulfan		Fenvalerate	
	Control	Sub-Lethal	% Change	Sub-Lethal	% Change
Brain	8.05± 0.05	7.06± 0.005	12.29	6.24± 0.01	22.48
Gill	7.02± 0.03	5.72± 0.005	18.51	5.32± 0.005	24.21
Kidney	10.31± 0.005	9.05± 0.005	12.22	8.5± 0.005	17.55
Liver	8.6± 0.06	6.03± 0.005	29.88	5.46± 0.005	36.51
Muscle	10.12± 0.001	8.54± 0.005	15.61	8± 0.005	20.94

Values are the means of five observations: (±) indicates the standard deviation values are significant at $P > 0.05$

Table 3. Changes in activity of Alkaline phosphatase (expressed as µ moles PNP/mg of protein/30min wet. weight of tissues) in different tissues of *Labeo rohita* on exposure to sublethal and lethal concentrations of endosulfan and fenvalerate for 24 hr.

Organs	Control	Endosulfan - 24hr				Fenvalerate- 24hr			
		Sub-Lethal	% Change	Lethal	% Change	Sub-Lethal	% Change	Lethal	% Change
Brain	15.3± 0.015	14.2± 0.001	7.18	13.5± 0.015	11.76	13.8± 0.01	9.80	12.2± 0.003	20.26
Gill	12.1± 0.01	11.2± 0.01	7.43	10.2± 0.01	15.70	10.3± 0.01	14.87	9.31±0.01	23.05
Kidney	13.54± 0.01	12.5± 0.02	7.68	11.5± 0.01	15.06	11.8± 0. 04	12.85	10.3±0.01	23.92
Liver	24.4± 0.06	20.6± 0.012	15.57	19.5± 0.02	20.08	19.1±0.015	21.72	17.5±0.021	28.27
Muscle	20.42± 0.01	18.4± 0.03	9.89	17.6± 0.03	13.80	18.2±0.01	10.87	15.4± 0.002	24.58

Values are the means of five observations: (±) indicates the standard deviation values are significant at $P > 0.05$

Table 4. Changes in activity of Alkaline phosphatase (expressed as µ moles PNP/mg of protein/30min wet. weight of tissues) in different tissues of *Labeo rohita* on exposure to sublethal concentrations of endosulfan and fenvalerate for 15 days.

Organs	Control	Endosulfan		Fenvalerate	
		Sub-Lethal	% Change	Sub-Lethal	% Change
Brain	13.7± 0.015	12.5± 0.005	8.75	11.35± 0.01	17.15
Gill	10.02± 0.03	9.51± 0.005	5.08	8.6± 0.005	14.17
Kidney	12.3± 0.04	11.20± 0.005	8.94	10.12± 0.03	17.72
Liver	22.3± 0.06	19.03± 0.03	14.66	18.04± 0.005	19.10
Muscle	21.2± 0.1	19.3± 0.05	8.96	17.5± 0.005	17.45

Values are the means of five observations: (±) indicates the standard deviation values are significant at $P > 0.05$

observe the effect of Endosulfan and Fenvlerate on the activities of acid and alkaline phosphatase. Acid and alkaline phosphatases are general enzymes present in almost all the tissues. They are hydrolytic enzymes concerned with the process of transphosphorylation and have an important role in the general energetics of an organism. They are associated with the transport of metabolites with metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrate, and with synthesis of proteins, Srivastava *et al.,* (1995).

In the present investigation a marked decrease in the activity of both the enzymes particularly fenvalerate treated tissues show more decrease when compared to endosulfan. This is in conformity with the report by Parthasarathi and Karuppasamy (1998) in liver, intestine and muscle tissues of C. punctatus when exposed to fenvalerate. The decrease in ALP activity has been reported in the fresh water crab, *Spiralothelphusa hydrodroma* treated with the pesticides, cypermethrin by Sreenivasan *et al.,* 2011.

CONCLUSION

The present work indicates that both endosulfan and fenvalerate caused alterations in the activities of Acid phosphatase and Alkaline phosphatase of fish *Labeo rohita*, but comparatively fenvalerate treated fish tissues showed more decrement in values this may be due to more pesticidal stress. Due to lipophilc nature of pyrethroids, biological membranes and tissues redily take up pyrethroids. Pyrethroids are several orders of magnitude more toxic to fish than the other pesticides (Oros *et al.,* 2005).

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