Acute Toxicity Induced Changes in Protease Activity in Intestine of *Sarotherodon mossambicus* Due to Pesticides

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Abstract
In the present era of chemicals no sector has left without use of chemicals that directly or indirectly pose threat to the biotic world. Most of the chemicals accumulate in aquatic bodies along with runoff rain water and other drainage systems, thus impact life therein. Therefore. It has become necessary to check their impact on life systems. In this light, the freshwater fishes *Sarotherodon mossambicus* about 10 cm long and 15 gm in weight were collected from local sources and stocked in glass aquaria in laboratory containing aerated tap water for one week for acclimation. Their appropriate groups were prepared and were exposed to predetermined LC_0, LC_{50} concentrations of organochlorine pesticide, endosulfan and organophosphorous pesticide, chlorpyriphos for 96 hr (acute exposure). The control groups were simultaneously run. The protease activities in the intestine of above mentioned groups of fishes were determined. Results indicated that the protease activities in the intestine of fishes were decreased due to exposure of fishes to endosulfan and chlorpyriphos. However, the decrease was more significant due to chlorpyriphos exposure than endosulfan.

Index Terms **- Pesticides, Toxicity, *Sarotherodon mossambicus*.**

I. INTRODUCTION

In the present chemical era no sector has left without using chemicals that directly or indirectly pose threat to biotic world. Though the very purpose of chemicals is served, it is imperative to check their impact on life systems, including aquatic life. Most of the aquatic animals are tabled by human being, hence it adds to the food value. Among thousands of chemicals, agrochemicals, during last six decades, have attracted attention of researchers. Pesticides, being pest controlling chemicals, are indiscriminately used worldwide and have impaired biota at large. Among hundreds of agrochemicals, endosulfan and chlorpyriphos became more popular due to their effectiveness. Endosulfan is a polycyclic chlorinated hydrocarbon, categorized under hazardous chemical group. [1]. Most of the rivers in India have shown presence of endosulfan in varied amount. Because of endosulfan runoff from the agricultural fields into the rivers, many fish kill episodes have been reported [2] [3].

Chlorpyriphos is a broad spectrum organophosphorous pesticide displaying insecticidal activity against wide range of insects and arthropod pests. Direct aquatic exposure to chlorpyriphos has also been shown to be an effective means of controlling certain aquatic insect and crustacean pests [4]. Reference [5] has reported it as high toxic to fishes. It brings about inhibition of acetyl cholinesterase in nerve cells in fish [6]. It may show some secondary effects on fishes which include oxidative stress, immunotoxicity, neurotoxicity, endocrine disruption, and gut microbiota dysbiosis [7 - 10].

Reference [11] – [13] have used different pesticides like thiotox, dichlorovos, carbofuron, sumithion, sevin and determined toxicity to different fish species to know the level of toxicity effluents. Toxic effect of endo & diazinon on amylase, protease and lipase in intestine of Channa striatus [14] and of UV treated sewage on amylase activity in liver and intestine of striped marsh frog tadpoles were reported [15]. Amylase, protease and lipase activities in stomach and intestine of fish *Anabas testudineus* exposed to arsenic and chromium were reported by [16]. Literature survey indicated that the reports on protease activity in intestine of fish *S. mossambicus* exposed to different pesticides were scanty; hence the present study was designed.

II. RESEARCH METHODOLOGY

The biochemical studies related to determination of enzyme activity were carried out in the fishes belonging to the controls, LC0, LC50 (acute toxicity) predetermined concentrations of endosulfan (35EC) and chlorpyriphos (20EC). The enzyme activity in the intestine of above mentioned groups of fishes was determined.

The enzyme activity was determined as follows.

Enzyme preparation:

To conduct the enzyme study, five fishes from each group, were sacrificed after exposing them to predetermined sub-lethal and lethal concentrations of endosulfan and chlorpyriphos for 96 h. After quick removal of intestine, blotting, weighing and homogenization was done with mortal and pastel. By using 0.9% chilled NaCl solution, tissue concentration was made to 10 mg/ml. Homogenate was centrifuged at 3000 r.p.m. for 10 minutes and aliquot of supernatant was used as enzyme source. The homogenate was stored in freezer until used.

Assay:

Protease: Protease activity was measured according to Reference [17]. The absorbance of reaction mixture was read at 660 nm against a control in which the enzyme was substituted by preheated supernatant. The assay consisted of 1 ml of 1% casein (Hammerstein) prepared in 0.1 M borate buffer of pH 7.6 and 0.5 ml of supernatant. The tubes were incubated at 37oC for 30 minutes. The reaction was terminated by adding 3 ml of 5% TCA. The precipitation of un-reacted protein was measured by centrifugation & thus the supernatants were obtained. 1 ml of supernatant was taken in test tube in which 4.5 ml of Lowry's reagent and 0.5 ml of diluted phenol reagent was added. After 30 minutes O.D. was read at 660 nm against blank [17]. The standard curve was obtained by using different tyrosine concentrations, keeping all other conditions constant. The total activity and specific enzyme activity were expressed in terms of µg tyrosine / gm tissue /hr and µg tyrosine /gm protein /hr, respectively.

III. RESULTS AND DISCUSSION

The predetermined LC0, LC50 concentrations of endosulfan (35EC) were 0.0014 ppm and 0.0042 ppm, respectively and that for chlorpyriphos (20EC) were 0.100 ppm and 0.140 ppm, respectively. Changes in the protease activity in intestine of fish *S. mossambicus* due to endosulfan and chlorpyriphos toxicity for 96 h are shown in Table.

Control of Acute Tests: Total protease activity in intestine of fish was 17.100 mg tyrosine / gm tissue / hr, while specific activity was o.1520 mg tyrosine / gm protein / hr.

Total as well as specific enzyme activity in the intestine of fish was significantly ($p < 0.001$) decreased in LC0 and LC50 concentrations of endosulfan and chlorpyriphos. In LC0 concentration of endosulfan, total enzyme activity was not significantly decreased but specific enzyme activity was significantly decreased (53.75). In LC50 concentration of endosulfan, both the total and specific enzyme activities decreased significantly with percent values of 36.84 and 70.13, respectively. In general, decrease in activity was more in LC50 group than LC0. In LC0 and LC50 concentrations of chlorpyriphos, there was significant decrease in total and specific enzyme activities. The percent decrease in LC0 concentration was 57.89 and 77. 76 while in LC50, it was 63.15 and 83.02, respectively. In general decrease in enzyme activity was more in LC50 group than LC0, but it was more in both the concentrations of chlorpyriphos than endosulfan (Table).

It is imperative that pesticides enter into fish body through water, sediments and food; and bioaccumulate to influence rate of development, growth and reproduction in fish [18]. Gastric glands in the wall of stomach of fish secrete digestive enzymes. Further, these digestive enzymes are secreted by columnar epithelial cells of intestinal villi and also by acinar cells of hepatopancreas. Therefore in fish, digestion of food takes place in stomach and anterior part of intestine where later plays crucial role in the initial absorption and metabolism of various organic pollutants (Yuen et al., 2007). Digestive enzymes like amylase and protease are directly related to carbohydrate and protein metabolism hence important in fish [16].

Reference [19] has reported disorders of various functional characteristics of the digestive tract of the fish upon exposure to various xenobiotics. Even at very low concentrations, xenobiotic substances adversely affect activities of digestive enzymes in fish. Therefore analysis of effects of pollutants on digestive enzyme activities is essential to highlight the risk of xenobiotic substances. Absorption of xenobiotic substances in intestine results impaired function of absorption of various energy sources [20].

The present study also reported considerable decrease in protease activity in intestine of fish S. mossambicus due to endosulfan and chlorpyriphos toxicity. Reference [16] has reported considerable decline in protease activity in the stomach of fish *A. testudineus* exposed to arsenic and chromium. In stomach of fish change in pH of gastric juice may bring about change in protease activity which leads to profuse secretion of neutral mucin and disruption of zymogen granules in gastric gland, that influence the secretion of proteolytic enzymes. Inhibition in protease activity has been reported by [21] in Zebra fish exposed to fenvalerate. They have interpreted that the decline in protease activity might be due to alteration of enzyme binding sites by fenvalerate.

Table: Effect of pesticides on the protease activity in intestine of the fish, *S. mossambicus* after **acute** exposure

(Total activity in mg tyrosine /gm tissue /hr)
(Specific activity in mg tyrosine /gm protein /hr)

Pesticide		**Endosulfan**		**Chlorpyriphos**	
Activity	**Control**	**LC_0 (0.0014)**	**LC_{50} (0.0042)**	**LC_0 (0.100)**	**LC_{50} (0.140)**
Total	17.100 ±2.545	16.900 ±1.272 -(01.16) N.S.	10.800 ±0.509 -(36.84)**	07.200 ±0.509 -(57.89)**	06.300 ±0.254 -(63.15)**
Specific	0.1520 ±0.0227	00.0703 ±0.0047 -(53.75)**	0.0454 ±0.0021 -(70.13)**	0.0338 ±0.0023 -(77.76)**	0.0258 ±0.0010 -(83.02)**

Values in parenthesis are percentages; * = P < 0.05; ** = P < 0.001; N.S. = Non-significant; ±S.D. of 5 animals.

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