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IMPACT OF ENDOSULFAN ON PHOSPHATASE ACTIVITY IN BRAIN AND MUSCLE OF FRESHWATER FISH *CHANNA STRIATUS* (BLOCH)

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ABSTRACT

Pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors. Among common pesticides, organophosphorus compounds are widely used in agriculture, medicine and industry. Excessive application of pesticides from agricultural fields contaminate aquatic medium, resulting in serious damage to non-target species, including fish. The changes in enzymatic system may alter the metabolic processes. More recently changes in enzymes concentrations are being employed in the evaluation of toxicological responses. The aim of the present study is to assess the acid phosphatase (ACP) and alkaline phosphatase (ALP) activity in brain and muscle of the fish *Channa striatus* exposed to sublethal concentrations of endosulfan 1/10th (high), 1/15th (medium) and 1/20th (low) of the 96 hour LC_{50} values for the period of 7, 14 and 21 days. The fish exposed to endosulfan showed a decrease the ACP and ALP activity for 7, 14 and 21 days in brain and muscle. However, no information is on record concerning the three different sublethal concentrations of endosulfan on the phosphatase activities of freshwater fish *Channa striatus*. The objective of the present work was to observe the effect of endosulfan on phosphatase activities in brain and muscle of freshwater fish, *Channa striatus.*

INTRODUCTION

Aquatic medium receiving sewage effluents represent an important point source of water pollution since their discharges consisting of complex mixture of chemicals. Chemicals like metals, polycyclic aromatic hydrocarbons, pesticides, organotins, volatile organic compounds, chlorobenzenes, phthalates and alkylphenols have been reported in wastewater, as well as certain pharmaceuticals and hormones (Abessa *et al.,* 2005; Gasperi *et al.,* 2008; Bolong *et al.,* 2009; Metcalfe *et al.,* 2010; Cazenave *et al.,* 2014). The importance of studying the effects of pollutants in aquatic organisms is a main issue for the environment but also regarding the potential impacts on human health (Malhao *et al.,* 2013). The large-scale application of pesticides to crops and forests may contribute to the presence of toxic substances in the environment. These chemicals can find their way into water reservoirs, streams and rivers, thus producing an adverse impact on the aquatic biota, which includes fishes (John and Prakash, 2003; Wang *et al.,* 2013).

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Due to rapid increase in the industrialization and human population, the pollution of aquatic ecosystem has become an universal phenomenon in the present day world (Belazutshi and Raghuprasad, 2008). The main sources of water pollution are industrial waste, domestic sewage, drainage and pesticides used for food production (Maruthanayagam and Sharmila, 2004). Aquatic medium contamination of the pesticides causes acute and chronic poisoning of fish and other organism (Shabnam and Badre Alam, 2012). The freshwater is polluted due to entry of excess sewage water, industrial effluents and large number of pesticides in natural and agricultural pest management. The pesticides like organophosphate, organochloride and carbamate are regularly used in agricultural pest management for food production but through their excessive and indiscriminate use in agricultural pest management and public health operations. The rapidly increasing use of insecticides in agriculture posses serious hazards to aquatic animals (Magar and Afsar, 2013). Enzymes play an important role in metabolism. They are exceeding efficient and very specific interms of nature of reaction catalyzed and the substrate utilized. They synthesis and final concentration of enzyme is under genetic control and is greatly influenced by very small molecules of substances. These cellular catalysts control the formation of biochemical intermediates essential to all physiological functions. Hence, changes in enzyme levels are one of the fundamental steps to assess the effects of toxicants (Magar and Afsar, 2013). Fishes are very sensitive to a wide variety of toxicants in water, various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides (Herger *et al.*, 1995) Due to accumulation of pesticides in tissues produces many physiological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma and Ramamurthi, 1982).

Cells naturally contain enzymes for their functions such that damages to cellular membrane lead to their escape into the blood where their presence or activities can easily be measured as an index of cell integrity (Coppo *et al.,* 2002; Abalaka *et al.,* 2011). Certain serum chemistry could be used to identify tissue damage (Patti and Kulkarni, 1993). Phosphatases are mainly localized at cell membrane. Any damage in the cells may result in alteration in phosphatases activity (Shabnam and Badre Alam, 2012). Phosphatases are good indicators of stress condition in the biological system (Gupta *et al*., 1975; Verma *et al*., 1980). Phosphatases are of two types, namely, acid phosphatase and alkaline phosphatase. Acid phosphatase, the lysosomal enzyme (De Duve *et al*., 1995) plays a vital role in autolytic degradation of tissues (Nath and Butler, 1971) capable of catalytic transphosphorylation. The lysosomal system has been shown to be very sensitive to change in the intra and extra cellular environment and subsequently to be involved directly or indirectly in controlling many physiological and pathological processes (James and Soni, 1994). The present investigation was to assess the phosphatase activity in brain and muscle of *Channa striatus* exposed to three different sublethal concentrations of endosulfan.

MATERIALS AND METHODS

The fish *Channa striatus* having mean weight 25-30 gm and length 22 – 24 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1%KMNO4 solution and then kept in plastic pools for acclimatization for a period of two weeks. They were fed on rice bran and oil cake daily. The endosulfan was used in this study and stock solutions were prepared. Endosulfan LC_{50} was found out for 96 h (6.82 µg/L) (Sprague, 1971) and $1/20^{th}$, $1/15^{th}$ and $1/0^{th}$ of the LC_{50} values were 0.34, 0.45 and 0.68 µg/L respectively taken as sublethal concentrations for this study. Forty fish were selected and divided into 4 groups of 10 each. The first group was maintained in free from endosulfan and served as the control. The other 3 groups were exposed to sub lethal concentration of endosulfan 10 litre capacity aquaria. The 2^{nd}, 3^{rd} and 4^{th} groups were exposed to endosulfan for 7, 14 and 21 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for ACP and ALP estimation. The phosphatase activities of the tissues were estimated by the method of Tennis Wood *et al.* (1976). The data so obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

RESULTS

The ACP and ALP activities in brain and muscle of *Channa striatus* exposed to low, medium and high sublethal concentration of endosulfan showed significant decrease when compared to control fish. The decrease the activities of ACP and ALP in brain and muscle of Channa striatus were more pronounced at 21 days of exposure period (Table 1, 2, 3 and 4).

Table 1. Acid phosphatase activity (µg inorganic phosphate liberated/mg/hr) in brain of *Channa striatus* exposed to sublethal concentrations of endosulfan

	7 Days	14 Days	21 Days
Control Brain	0.593 ± 0.03^{c}	0.544 ± 0.03^{d}	0.534 ± 0.03^{d}
Low concentration	0.524 ± 0.04^{b}	0.481 ± 0.02^{c}	0.463 ± 0.02^{c}
Medium concentration	0.473 ± 0.02^{a}	0.423 ± 0.03^{b}	0.371 ± 0.01^{b}
High Concentration	0.431 ± 0.03^{a}	0.350 ± 0.02^{a}	0.253 ± 0.01^{a}

All the values mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$)
Duncan Multiple Range Test (DMRT)

Table 2. Acid phosphatase activity (µg inorganic phosphate liberated/mg/hr) in muscle of *Channa striatus* exposed to sublethal concentrations of endosulfan

	7 Days	14 Days	21 Days
Control Brain	0.378 ± 0.02^{d}	0.352 ± 0.02^{d}	0.384 ± 0.02^{d}
Low concentration	0.341 ± 0.02^{c}	0.290 ± 0.02^{c}	0.263 ± 0.01^{c}
Medium concentration	0.303 ± 0.02^{b}	0.211 ± 0.01^{b}	0.201 ± 0.01^{b}
High Concentration	0.243 ± 0.01^{a}	0.112 ± 0.001^{a}	0.091 ± 0.008^{a}

All the values mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$)
Duncan Multiple Range Test (DMRT)

Table 3. Alkaline phosphatase activity (µg inorganic phosphate liberated/mg/hr) in brain of *Channa striatus* exposed to sublethal concentrations of endosulfan

	7 Days	14 Days	21 Days
Control Brain	0.540 ± 0.03^{c}	0.485 ± 0.03^{d}	0.498 ± 0.03^{d}
Low concentration	0.478 ± 0.03^{b}	0.430 ± 0.03^{c}	0.396 ± 0.03^{c}
Medium concentration	0.450 ± 0.03^{ab}	0.381 ± 0.02^{b}	0.301 ± 0.02^{b}
High Concentration	0.410 ± 0.03^{a}	0.310 ± 0.02^{a}	0.213 ± 0.01^{a}

All the values mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$)
Duncan Multiple Range Test (DMRT)

Table 4. Alkaline phosphatase activity (µg inorganic phosphate liberated/mg/hr) in muscle of *Channa striatus* exposed to sublethal concentrations of endosulfan

	7 Days	14 Days	21 Days
Control Brain	0.443 ± 0.03^{c}	0.434 ± 0.03^{d}	0.425 ± 0.03^{d}
Low concentration	0.420 ± 0.03^{bc}	0.398 ± 0.03^{c}	0.354 ± 0.02^{c}
Medium concentration	0.395 ± 0.03^{b}	0.321 ± 0.02^{b}	0.260 ± 0.01^{b}
High Concentration	0.352 ± 0.02^{a}	0.270 ± 0.01^{a}	0.170 ± 0.01^{a}

All the values mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$)
Duncan Multiple Range Test (DMRT)

DISCUSSION

The use of pesticides, which became essential in the lastdecades for intensive farming, results in a widespread presence of residues in water, soil and foodstuffs (Crepet *et al.,* 2013). Endosulfan is an insecticide which has been widely used in agriculture. The technical grade material consists of two isomers (alpha and beta). Under natural environmental conditions, endosulfan is metabolized through oxidation and the main metabolite in the environment is endosulfan sulfate. Most ecotoxicology research has been conducted with technical grade endosulfan to determine effects on non-target aquatic organisms like fish (Hoang *et al.,* 2011). Agricultural runoff after rain events increases the risk of pesticides and other agricultural products contaminating local water ways and inshore marine habitats. Although an increasing number of environmental contaminants enter aquatic habitats, the long-term effects on non-target organisms are largely unknown. Anthropogenic stressors, including the presence of pesticides, impact populations of numerous species and are known to contribute to changes in community structure within rivers and streams (Schafer *et al.,* 2007; Bauer *et al.,* 2013). Biochemical responses of aquatic organisms to contaminants usually represent the first measurable effects of contaminant exposure, and accordingly are advantageous for use in monitoring programs (Hinton, 1994). Phosphatases are important enzymes of animal metabolism, which play important roles in the transport of metabolites across the membrane (Vorbrodt, 1959). Alkaline phosphatase diagnosis is important in the bone disease and hepatobiliary disease thus employed to assess the integrity of plasma membrane and endoplasmic reticulum (Akanji *et. al.,* 1993; Wright and Plummer, 1974) while acid phosphatase diagnosis is intended to detect the carcinoma of the prostate.

Acid phosphatase is regarded as a key lysosomal enzyme and it plays a vital role in the autolytic degradation of tissues (Nath and Butler, 1971). The alkaline phsophatase enzyme is associated with transfer of phosphates and is linked with transportation of intermediate compounds in glycogenesis or glycogenolysis. ALP is a membrane bound enzyme found at bile pole hepatocytes and also found in pinocytic vesicle and golgi complex. It is present in all cell membranes where active transport occurs and hydrolase and transphophorylase are in function. Decrease in ALP activity may be taken as an index of hepatic parenchymal damage and hepatocytic necrosis (Onikienko, 1963). Alkaline phosphatase enzymes are membrane bound enzymes found at the bile pole of hepatocytes, pinocytic vesicles, Golgi complex and in all cell membranes where active transport takes place (Shakoori *et al*., 1992). The results of the present findings showed a significant decrease in ACP and ALP activities in brain and muscle of *Channa striatus* exposed to sublethal concentrations of endosulfan at 7, 14 and 21 days.

Decreased activities of enzymes like acid phosphatase and alkaline phosphatase indicate the reduction and the diversion of Kreb's cycle substrate. Uncoupling of oxidative phosphorylation substantiates the operation of the other compensatory pathway to tide over the toxic stress. Alteration of serum enzyme levels (Shenoy *et al*., 2001) can be monitored to evaluate the hepatocellular damage caused by various foreign compounds (Shah *et al*., 2002). The fish *Cyprinus carpio* hepatic ACP and ALP activities decreased significantly at 12, 24 and 48 hours of zinc chloride treatment (Ali *et al*., 1992). Acid phosphatase and alkaline phosphatase are known as "Inducible enzyme" and their activity goes up when there is a toxic impact on the enzymes that begin to counteract the toxic effect (Leland, 1983). Rahman *et al*., (2000) have suggested that the decrease in the activities of ALP and ACP in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis. El-Demerdash (2004) noticed a decreased liver ALP, ACP activities in liver and brain tissues of aluminium exposed rats. Thenmozhi *et al.,* (2011) reported that decrease the activities of liver and muscle of *Labeo rohita* exposed to malathion. Alkaline phosphatase activity was lowered in kidney and spleen of *cyprinus carpio* exposed to atrazine and chlorpyrifos (Wang *et al.,* 2013). The pesticide endosulfan affects the phosphatase activities in brain and muscle of *Channa striatus.* The pesticide contaminated fish not safe for human consumption.

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