Impact Of Novel Insecticide Chlorantraniliprole On Alkaline Phosphatase Activity In Freshwater Fish Cirrhinus Mrigala

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Abstract: Chlorantraniliprole is a novel insecticide introduced by DuPont Crop Protection (Tokyo, Japan) in 2007 and now it is widely used to control agricultural pests from the Order Lepidopteron, Coleopteran, Dipterans and Hemiptera species. Due to run off water and soil erosion these insecticides get introduced at nearest water bodies and thereby adversely affects non-target organism like fishes. Alkaline phosphatase is a membrane bound glycoprotein present in tissues of fish and is involved in active membrane transport and carbohydrate metabolism. The aim of present study was to determine the toxic effect at lethal concentration of Chlorantraniliprole on alkaline phosphatase activity on freshwater fish Cirrhinus mrigala. Prior to experimental protocol, fingerlings were acclimatized in glass aquaria for seven days. After acclimatization, fingerlings were exposed to LC_0 and LC_{50} concentration of Chlorantraniliprole (0.0025ppm and 0.01 ppm respectively) in twenty-liter test container for 96 hrs (acute toxicity). It was observed that the alkaline phosphatase activity in muscle, intestine and liver; significantly decreased at LC_0 and LC_{50} concentration as compared to the control group. From the observed results, it can be concluded that, 'selected insecticide Chlorantraniliprole interferes with alkaline phosphatase activity in selected test fish Cirrhinus mrigala and retards the normal activity.

Index Terms: Alkaline Phosphates, Chlorantraniliprole, Cirrhinus mrigala, Acute toxicity.

1. INTRODUCTION

Water is one of the most important renewable natural resource available on earth [1]. Water sustains the life and environment. About 71% of earth surface is covered by water and about 96.5% water is occupied by oceans. Water is of prime importance for all the sectors like: agricultural irrigation, industrial purpose, domestic purpose, recreational purpose, etc. It plays an important role in national socio-economic development. Unfortunately water pollution is a major universal problem. It directly and indirectly affects the economy of country, health of population and environment. There are three major factors viz. urbanization, industrialization and agriculture which contributes to rise in water pollution [2]. Indian economy is totally depends on agriculture [3]. It supplies food, raw material for various industries and about 60% employment in agriculture and alied industries. Agriculture, forestry and fisheries sector contribute to about 18.6% of GDP in Indian economy. But on other hand, about 70% water pollution is also contributed by agriculture sector. This is mainly due to excessive use of organic fertilizer and pesticides [4].

The aim of modern agricultural practices is to produce high yield. For this purpose numerous insectisides are applied to protect the plants. Every year farmers prefer the newer inecticide which is less toxic to environment and other non-target organsms. Chlorantraniliprole (3-bromo-4'-chloro-1-(3-chloro-2-pyridyl)-2'-methyl-6'-(methylcarbamoyl) pyrazole-5-carboxanilide) is a novel anthranilicdiamide insecticide and is widely used to control agricultural pests in the Order Lepidopteron, Coleopteran, Dipterans and Hemiptera species [5] & [6]. Chlorantraniliprole was synthesized in 2007 by DuPont Crop Protection (Tokyo, Japan) as an active compound in several insecticides (Rynaxypyr, Ferterra, Altacor, Dermacor X-100, Coragen and Prevathon). Chlorantraniliprole binds to target site and interrupts the normal contraction of the muscle by activating ryanodine receptors located in the sarcoplasmic reticulum of the muscle cells and endoplasmic reticulum of non-muscle cells [7]. This results into uncontrolled release of stored intracellular calcium from the sarcoplasmic reticulum, leading to Calcium depletion, which in turn causes impaired regulation of muscle contraction. This implicates the symptoms of rapid feeding cessation, lethargy, paralysis, as the muscles get locked in a contracted state and eventually death of pest [8]. Alkaline phosphatase is a multifunctional and group of enzyme [9]. It has low substrate specificity that hydrolyzes wide variety of phosphate ester at alkaline pH. The nature of alkaline phosphatase is ubiquitous in membrane bound glycoprotein. It is present in different isoforms in different tissues [10]. On the basis of tissue expression, alkaline phosphatase has four isoforms. There are placental, intestinal, liver/bone/kidney and germ cell alkaline phosphatase [11]. It is involved in active transport mechanism, bone calcification, metabolism of carbohydrate, nucleotides and phospholipids [12], [13]. Fishes are macro indicators of toxicants in aquatic bodies. They are more vulnerable and sensitive to toxicants. They respond to exposed toxicants by altering physiological, biological and behavioral functions. The freshwater fish Cirrhinus mrigala (white carp or mrigala) is widely distributed and has great importance in fisheries and aquaculture sector. It is an ideal experimental model for toxicological study due to high susceptibility to toxicants, fast growing and easily acclimatized in laboratory condition. Therefore, it was proposed to evaluate the effect of lethal concentration of Chlorantraniliprole on alkaline phosphatase activity of freshwater fish Cirrhinus mrigala.

2 MATERIALS AND METHODS

2.1 Experimental fish and laboratory condition

The healthy freshwater fish Cirrhinus mrigala were collected from government fish seed production and rearing center Dhom, Dist. Satara, Maharashtra, India. In the laboratory condition, fishes were acclimatized in clear and de-chlorinated

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water for seven days in well-aerated glass aquaria at 12 hours light and 12 hours dark photoperiod. Before the acclimatization fishes were offered a bath with 0.05% $KMnO_4$ solution to avert bruise and disease. The acclimatized fishes were fed daily by formulated feed (TAIYO Pet Products Pvt Ltd, India). During the span of acclimatization, the water was changed daily to discard remaining food particles and faecal matter. The dead fishes were removed immediately to avoid possible deterioration of water quality. The water quality parameters such as temperature, pH, dissolved oxygen, carbon dioxide, hardness, etc. were checked weekly.

2.2 Toxicity Test

The insecticide chlorantraniliprole (98%) was purchased from M/S Super Bio Tech Marketing Company, India. A well-acclimatized healthy fish measuring 6±8 cm in length and 9-11g in weight were selected for the study. The toxicity was carried out in 20 liter plastic trough. In each trough, ten fish were released. The trough was distinguished in three groups 1) Control group (Without any exposure of Chlorantraniliprole) 2) LC_0 Concentration group (Exposed to 0.0025ppm concentration of Chlorantraniliprole) and 3) LC_{50} concentration group (Exposed to 0.01ppm concentration of Chlorantraniliprole). After 24hrs, the experimental medium was replaced by fresh medium. The procedure was reapeated for 96 hours (acute toxicity).

2.3 Analysis of Alkaline Phosphatase Activity

The muscle, intestine and liver tissues were separated from sacrificed fishes and alkaline phosphatase activity was estimated by Linhardt and Waiter (1965) method using p-nitrophenyl phosphate as the substrate [14]. Homogenate of each tissue was prepared in 0.9% chilled saline solution and centrifuged at 3000 rpm for 10 minutes. For the assay, triplet set of test tubes were prepared for each tissue. Thereafter, 0.2 ml supernatant was added in each test tube. Then 0.8 ml 0.05M sodium citrate buffer containing 5 X 10^{-3}M p-nitrophenyl phosphate pH 7.6 was added. The blank was prepared by adding 0.2 ml distilled water and 1 ml substrate buffer and all test tubes were incubated at 37^0C for 30 minutes. Then 4 ml of 0.1 N NaOH was added for inhibition of reaction. The absorbance value was measured at 400 nm by spectrophotometer by using blank. The lysosomal alkaline phosphatase activity in term of µmols of p-nitrophenol /mg protein was calculated by the formula: Phosphatase activity = Optical density X 2.76 X1000 / amount of protein/mg tissue.

2.4 Statistical analysis

The observed data from each group were expressed in arithmetic mean ± standard deviation. The level of significance was calculated using unpaired student's t test.

3 RESULTS

The result of the impact of Chlorantraniliprole on the alkaline phosphatase activity in various organs viz. muscle, intestine and liver of the fish Cirrhinus mrigala in the control group, LC_0 concentration group and LC_{50} concentration group after acute exposure (96 hours) are illustrate in Table 1 and represented Fig.1. In the muscle of control group, the alkaline phosphatase activity was 3.69±0.17 µm of p-nitrophenol phosphate/mg protein in tissue. However, in LC_0 concentration group fish muscle exhibited 1.92±0.02 µm of p-nitrophenol phosphate/mg protein in tissue and in the LC_{50} concentration group fish showed 1.18±0.1µm of p-nitrophenol phosphate/mg protein in tissue of muscle. In the control group fishes, 13.54±0.72 µm of p-nitrophenol phosphate/mg protein in tissueof the intestine, while in the LC_0 concentration group fishes exhibited 6.67±0.17µm of p-nitrophenol phosphate/mg protein in tissueof intestine and in the LC_{50} concentration group fishes it was 3.69±0.26µm of p-nitrophenol phosphate/mg protein in tissue of intestine.The Liver of control group fish exhibited 13.44±0.32µm of p-nitrophenol phosphate/mg protein in tissue. While, in the LC_0 concentration group fish showed 6.24 ± 0.13µm of p-nitrophenol phosphate/mg protein in tissueof liver and in the LC_{50} concentration group was 3.36±0.01µm of p-nitrophenol phosphate/mg protein in tissueof liver. The amount of p-nitrophenol phosphate in the muscle, intestine and liver tissues after acute exposure of Chlorantraniliprole is lower in control group as compared to the LC_0 and LC_{50} concentration group. The difference was highly significant at $p<0.001$.

TABLE 1

EFFECT OF CHLORANTRANILIPROLE ON THE ALKALINE PHOSPHATASE ACTIVITY IN VARIOUS TISSUE OF THE FISH CIRRHINUS MRIGALA AFTER ACUTE EXPOSURE

Groups	µm of p-nitrophenol phosphate/mg protein in tissue		
	Muscle	Intestine	Liver
Control	3.69±0.17	13.54±0.72	13.44±0.32
LC_0	1.92±0.02***	6.67±0.17***	6.24±0.13***
LC_{50}	1.18±0.1***	3.69±0.26***	3.36±0.01***

Results expressed as arithmetic mean ± standard deviation, *** indicates p< 0.000

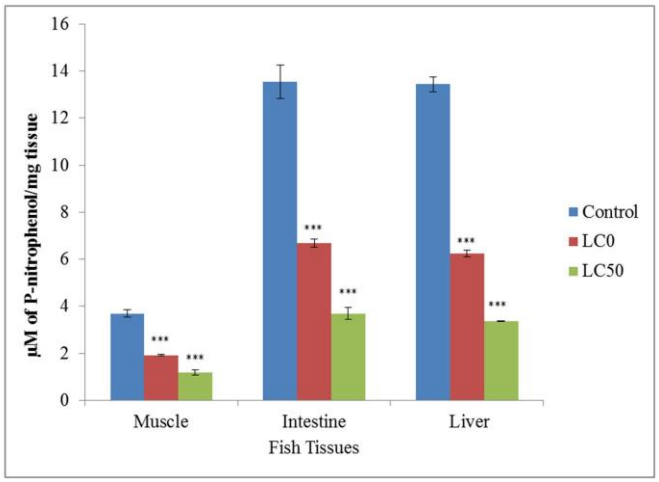

Fig. 1*. Alkaline phosphatase activity on muscle, intestine and liver of the fish Cirrhinus mrigala after acute exposure (96 hours) of chlorantraniliprole. Data expressed in arithmetic mean ± Standard deviation. Bars represent SD of six individual observations. *** indicates p<0.0001.*

4 DISCUSSION

In all living organism, enzymes are important class of proteins which act as catalyst and accelerates the rate of reactions

[15]. They offer a potential territory for checking of toxicants impacts and have pulled in a lot of consideration of researchers from different fields. The binding of the toxicant to specific enzymes leads to acceleration or inhibition of enzymatic activities and progression of various metabolic issues [16]. There are numerous toxicants that can bind to active site or near the active site of enzyme. They may impair substrate binding activity of enzyme [17]. Analysis of phosphatase activities is one of the important tools to evaluate toxicity stress of chemicals in aquatic organism. Alkaline phosphatase is a transmembrane glycoprotein of plasma membrane [18]. The plasma membrane act's as first barrier for toxicants [19]. Any damage to plasma membrane results in alternation in alkaline phosphatase activity [20]. In the present study, it was observed that on acute exposure (96 hours) of Chlorantraniliprole at LC_0 and LC_{50} concentration (0.0025ppm and 0.01 ppm respectively) there was significant decrease in the alkaline phosphatase activity of muscle, intestine and liver tissue of freshwater fish Cirrhinus mrigala. The alkaline phosphatase activity significantly decreased in the LC_0 concentration group fishes and LC_{50} concentration exposed group fish as compared to control group fishes which were never exposed to toxicants. The resultant decrease is attributed to damage of plasma membrane after exposure to Chlorantraniliprole [21]. The present observations are in agreement with finding of many workers. Deshpande et al. [22] observed that, exposure of sub lethal concentration synthetic pyrethroid, fenevalerate 20 EC and cypermethrine 25 EC leads to significantly decreased alkaline phosphatase in liver and intestine of freshwater fish Labeo rohita. Saad et al. [23] observed that, exposure of Chlorantraniliprole (LC50=0.009 mg/l) to 2nd and 4th instar larva of cotton leaf worm (Spodoptera littoralis) decreased the alkaline phosphatase activity. Srivastava et al. [24] demonstrated that, exposure of sublethal concentration of Azo dye and Eriochrome black T to freshwater fish Labeo rohita for 96 hours showed significant decreased alkaline phosphatase activity. Similar observation was reported by Priyatha and Chitra [25] after exposure of sub lethal concentration of acid orange (0.27 g/l) to freshwater fish Anabus testudineus. Magar and Shaikh [26] observed that, exposure to sublethal concentration of insecticide malathion (0.8 ppm) significantly decreased the acid phosphatase activity in gill, liver and kidney tissue of fresh water fish Channa punctatus. Parthasarathi and Karuppasamy [27] observed that, decrease in alkaline phosphatase activity in muscle cell is due to uncoupling of phosphorylation, increase glycogenolysis and altered function of mitochondria. Priyatha and Chitra [25] observed that, exposure of toxicant acid orange 7 inhibit the alkaline phosphatase activity and alter the membrane transport. This can lead to breakdown of glycogen as a result of dye intoxication.

5 CONCLUSION

It can be concluded that, the decreased alkaline phosphatase activity in muscle, intestine and liver tissue of the fish Cirrhinus mrigala exposed to predetermined values of toxicant, indicates that the Chlorantraniliprole act's on plasma membrane and alter's the membrane transport.

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