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IMPACT OF CHLORPYRIFOS ON ENZYMES ALKALINE PHOSPHATASE AND ACID PHOSPHATASE ACTIVITIES IN THE FRESHWATER FISH, *CIRRHINUS MRIGALA*

***Ronald Ross**

Department of Zoology, Annamalai University, Annamalainagar – 608 002, Tamil Nadu India

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ABSTRACT

Impact of pesticides is common pollutants of freshwater ecosystems where they induce adverse effects on the aquatic biota. Freshwater fish, Cirrhinus mrigala is an important carp species in Tamil Nadu region having good nutritional values. Fishes living in close association with may accumulate pesticides. In the present investigation, the toxic effects of the chlorpyrifos LC50 0.22 ppm on some enzymes (Alkaline phosphatase and acid phosphatase) of the freshwater carp fish, Cirrhinus mrigala were estimated. Increase in the gill alkaline phosphatase (ALP) level as observed in different sublethal concentrations when compared to control. The maximum increase in the gill alkaline phosphatase (ALP) was observed in the gill tissue of fish Cirrhinus mrigala exposed to 30% sublethal concentration of chlorpyrifes reared for 21 days. The results indicated the toxic nature of the pesticide chlorpyrifos. The maximum decrease in the kidney tissue acid phosphatase (ACP) was observed in the tissue of Cirrhinus mrigala exposed to 30% sublethal concentration of chlorpyrifes reared for 21 days.

Key words: Freshwater fish, Cirrhinus mrigala, chlorpyrifos, Alkaline phosphatases Acid phosphatases.

INTRODUCTION

Fishes are one of the most susceptible animals to pesticide pollution because of their anatomy and physiology. Fishes live in intimate contact with surrounding water through their gills and branchial surface comprises over half the surface area of the body. Only a few microns thick delicate gill epithelium separates the internal environment of fish from external aquatic environment which makes the fish very susceptible to aquatic pollutants. Therefore, contamination of water bodies by pesticides causes acute and chronic poisoning of fish and results in severe damage to vital organs (Singh *et al*., 2009; 2013). Pesticides are extensively used in intensive agricultural production and fish farms to control the pest population. These pesticides can reach natural waters either via transfer of the chemicals from the soil or by direct spraying on the target organisms. The pesticides affect non-target organisms, such as fish and prawn, which are of great economic importance to humans (Saravanan *et al*., 2010). Organophosphate pesticides are widely used in agriculture and public health and account for approximately 50% of the global insecticidal use (Shittu *et al*., 2012). Chlorpyrifos (CPF) is a broadspectrum organophosphate pesticide that is used heavily throughout the world for agriculture and domestic purposes (Ali *et al*., 2009). The toxic effects of CPF are increasingly threatening the health of humans and aquatic animals (Xing *et al*., 2012). Study of enzyme activities in the tissue and organs of aquatic organisms to assess the impact of pollutants is one of the emerging perspective in toxicological monitoring and remediation programmes (Oluah *et al*., 2005).

***Corresponding author:* Ronald Ross,**
Department of Zoology, Annamalai University, Tamil Nadu, India.

Enzyme analysis is widely applied for rapid detection to predict early monitoring of pesticide toxicity (Duta and Areids, 2003). However, Oluah, (1999) observed that increased or decreases in the values of ALT and AST indicate tissue damage in liver, kidney, muscle and gill. Alterations in alkaline phosphatase (ALP) activities in tissues, organs and serum have been reported in fish exposed to toxicants of varying concentrations (Jyothi and Narayan, 2000). There are a number of reports about the changes in the enzyme kinetics of organs and blood of fish exposed to toxicants (Svoboda, 2001; Velisek *et al*., 2006; Kumaran *et al.,* 2011). It was reported that the pesticides have caused either a significant increase or decrease or more effect in the enzyme activities. Atamanalp *et al.,* (2002a and 2002b) found a significant decrease in the activities of alkaline phospahtase (ALP) in the blood plasma of cypermethrin exposed rainbow trout. Exposure of fish that Indian major carp *Labeo rohita* by Adhikari *et al.,* (2004) to 0.139 ppm of cypermethrin for 45 days have resistence in altered enzyme activities and haematological changes in the fish. Acid phosphate (ACP) was unchanged while alkaline phosphatase (ALP) was depleted. In the present observation, the toxic effects of the chlorpyrifos on some enzymes (Alkaline phosphatase and acid phosphatase) of the freshwater carp fish, *Cirrhinus mrigala* were estimated.

MATERIALS AND METHODS

The freshwater carp fish, *Cirrhinus mrigala* were collected from Chidambaram area and were brought to the laboratory in large plastic troughs and acclimatized for one week.

Table 1. Levels of alkaline phosphatase (ALP) activities in different tissues of *Cirrhinus mrigala* exposed to sublethal concentration of Chlorpyrifos (µ mole/mg/hr)

Days	Exposure	Liver (µ mole/mg/hr)	Kidney (µ mole/mg/hr)	Gills (µ mole/mg/hr)	Muscles (µ mole/mg/hr)
7 days	Control	15.16 ± 0.15	6.53 ± 0.19	4.94 ± 0.12	8.86 ± 0.07
	Chlorpyrifos 10%	16.27 ± 0.10	6.42 ± 0.09	5.62 ± 0.12	9.33 ± 0.07
	Chlorpyrifos 30%	16.40 ± 0.11	7.59 ± 0.07	6.18 ± 0.06	10.74 ± 0.07
14 days	Control	15.39 ± 0.10	6.43 ± 0.12	4.69 ± 0.10	8.73 ± 0.14
	Chlorpyrifos 10%	17.40 ± 0.10	7.43 ± 0.09	5.89 ± 0.09	11.28 ± 0.09
	Chlorpyrifos 30%	18.32 ± 0.08	8.85 ± 0.08	6.43 ± 0.09	11.80 ± 0.10
21 days	Control	15.45 ± 0.13	6.38 ± 0.10	4.79 ± 0.13	8.67 ± 0.11
	Chlorpyrifos 10%	17.61 ± 0.08	8.47 ± 0.09	6.55 ± 0.06	12.08 ± 0.03
	Chlorpyrifos 30%	18.59 ± 0.12	9.34 ± 0.07	7.29 ± 0.06	12.30 ± 0.08

Values are mean ± SD (decrease or increase over control)

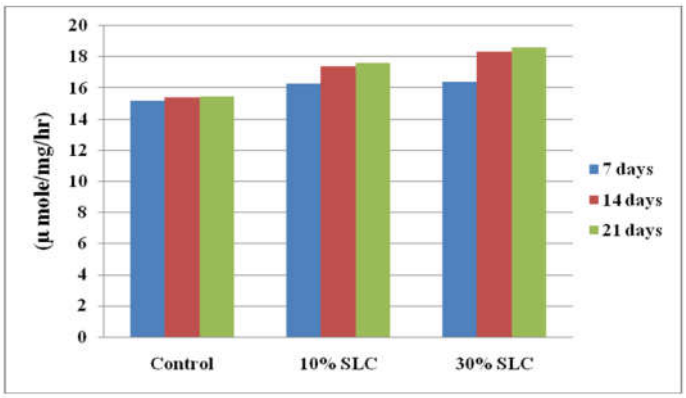

Fig. 1. Levels of alkaline phosphatase activities in liver of *Cirrhinus mrigala* exposed to sublethal concentration of chlorpyrifos

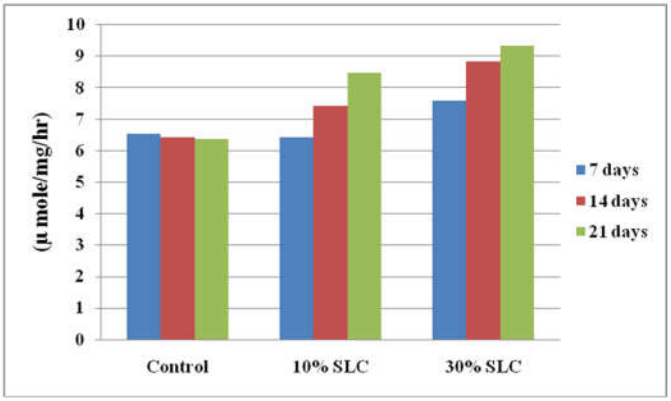

Fig. 2. Levels of alkaline phosphatase activities in kidney of *Cirrhinus mrigala* exposed to sublethal concentration of chlorpyrifos

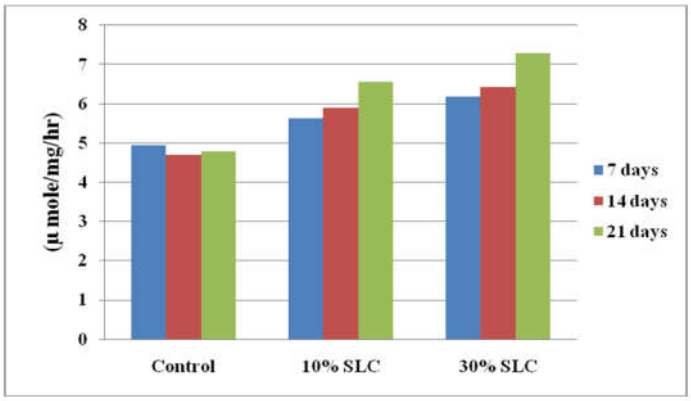

Fig. 3. Levels of alkaline phosphatase activities in gill of *Cirrhinus mrigala* exposed to sublethal concentration of chlorpyrifos

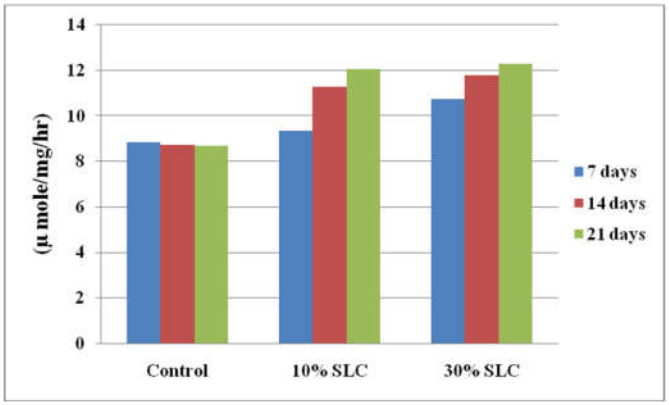

Fig. 4. Levels of alkaline phosphatase activities in muscles of *Cirrhinus mrigala* exposed to sublethal concentration of chlorpyrifos

Healthy, carp fish having equal size (length 10 to 15 cm) and weight (50 to 100 g) were used for experimentation. Stock solution of chlorpyrifos was prepared by dissolving appropriate amount of salt in distilled water. The physico-chemical characteristic of test water have analyzed regularly during the test periods following the standard method describe by APHA (1998). Batches of 10 healthy fishes were exposed to different concentrations of insecticide chlorpyrifos to calculate the medium lethal concentration LC_{50} value (0.22 ppm) using probit analysis Finney method (1971). The fishes (Four groups) were exposed to the two sub lethal concentrations ($1/10^{th}$ and $1/30^{th}$ mg/L) of chlorpyrifos for 7, 14 and 21 days respectively. Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as gill, kidney, liver and muscle were dissected and removed.

Acid and alkaline phosphataes were estimated following the procedure outlined by Tennis Wood *et al*. (1976), a modified method of Bessey *et al*. (1946). The tissues were homogenized in 0.25 M sucrose solution and centrifuged at 1000 X g for 10 minutes. The supernatants were filtered and the filtrates were used for enzyme analysis.

RESULTS

Liver alkaline phosphatase (ALP): Freshwater fish *Cirrhinus mrigala* treated with sublethal concentration of chlopyrifos on 10% and 30 % showed a increasing trend in the liver alkaline phosphatase (ALP) with compared to control (Table 1 and Fig. 1). The control alkaline phosphatase (ALP) values were recorded from 15.16 ± 0.15, 15.39 ± 0.10

Table 2. Levels of acid phosphatase activities in different tissues of *Cirrhinus mrigala* exposed to sub lethal concentration of chlorpyrifos

Days	Exposure	Liver	Kidney	Gills	Muscles
7 days	Control	8.31 ± 0.08	5.70 ± 0.08	5.58 ± 0.06	7.20 ± 0.06
	10% SLC	7.88 ± 0.07	4.91 ± 0.07	5.38 ± 0.08	6.85 ± 0.06
	30% SLC	7.39 ± 0.06	4.73 ± 0.08	5.28 ± 0.05	6.22 ± 0.06
14 days	Control	8.56 ± 0.06	5.89 ± 0.05	5.62 ± 0.05	7.2 ± 0.05
	10% SLC	7.87 ± 0.06	4.61 ± 0.04	4.82 ± 0.06	6.72 ± 0.02
	30% SLC	7.21 ± 0.06	4.42 ± 0.06	4.22 ± 0.06	6.57 ± 0.05
21 days	Control	7.33 ± 0.04	5.95 ± 0.04	5.32 ± 0.06	7.12 ± 0.07
	10% SLC	6.33 ± 0.05	4.23 ± 0.07	4.43 ± 0.05	5.82 ± 0.06
	30% SLC	5.39 ± 0.06	3.88 ± 0.05	4.09 ± 0.05	5.73 ± 0.06

Values are mean ± SD – or + indicate present decrease or increase over control

and 15.45 ± 0.13 (µ mole/mg/hr). The 10% sublethal concentration of liver alkaline phosphatase (ALP) values were recorded from 16.27 ± 0.10, 17.40 ± 0.10 and 17.61 ± 0.08 and the 30% sublethal concentration of liver alkaline phosphatase (ALP) values were recorded from 16.40 ± 0.11, 18.32 ± 0.08 and 18.59 ± 0.12 (µ mole/mg/hr) exposure period of 7, 14 and 21 days respectively.

Kidney alkaline phosphatase (ALP)

The fish *Cirrhinus mrigala* treated with sublethal concentration of chlopyrifos on 10% and 30 % showed a increasing trend in the kidney alkaline phosphatase (ALP) with compared to control (Table 2 and Fig. 2). The control alkaline phosphatase (ALP) values were recorded from 6.53 ± 0.19, 6.43 ± 0.12 and 6.38 ± 0.10 (µ mole/mg/hr). The 10% sublethal concentration of kinney alkaline phosphatase (ALP) values were recorded from 6.42 ± 0.09, 7.43 ± 0.09 and 8.47 ± 0.09 and the 30% sublethal concentration of kidney alkaline phosphatase (ALP) values were recorded from 7.59 ± 0.07, 8.85 ± 0.08 and 9.34 ± 0.07 (µ mole/mg/hr) exposure period of 7, 14 and 21 days respectively.

Gill alkaline phosphatase (ALP)

In fish *Cirrhinus mrigala* treated with sublethal concentration of chlopyrifos on 10% and 30 % showed a increasing trend in the gill alkaline phosphatase (ALP) with compared to control (Table 1 and Fig. 3). The control alkaline phosphatase (ALP) values were recorded from 4.94 ± 0.12, 4.69 ± 0.10 and 4.79 ± 0.13 (µ mole/mg/hr). The 10% sublethal concentration of gill alkaline phosphatase (ALP) values were recorded from 5.62 ± 0.12, 5.89 ± 0.09 and 6.55 ± 0.06 and the 30% sublethal concentration of gill alkaline phosphatase (ALP) values were recorded from 6.18 ± 0.06, 6.43 ± 0.09 and 7.29 ± 0.06 (µ mole/mg/hr) exposure period of 7, 14 and 21 days respectively.

Muscle alkaline phosphatase (ALP)

In *Cirrhinus mrigala* treated with sublethal concentration of chlopyrifos on 10% and 30 % showed a increasing trend in the muscle alkaline phosphatase (ALP) with compared to control (Table 1 and Fig. 4). The control alkaline phosphatase (ALP) values were recorded from 8.86 ± 0.07, 8.73 ± 0.14 and 8.67 ± 0.11 (µ mole/mg/hr). The 10% sublethal concentration of muscle alkaline phosphatase (ALP) values were recorded from 9.33 ± 0.07, 11.28 ± 0.09 and 12.08 ± 0.03 and the 30% sublethal concentration of muscle alkaline phosphatase (ALP) values were recorded from 10.74 ± 0.07, 11.80 ± 0.10 and 12.30 ± 0.08 (µ mole/mg/hr) exposure period of 7, 14 and 21 days respectively.

Increase in the gill alkaline phosphatase (ALP) level as observed in different sublethal concentrations when compared to control. The maximum increase in the gill alkaline phosphatase (ALP) was observed in the gill tissue of *Cirrhinus mrigala* exposed to 30% sublethal concentration of chlorpyrifes reared for 21 days.

Acid Phosphate

The highest activity of ACP in normal fish was observed in the liver (8.56 ± 0.06 (µ mole/mg/hr)), followed by muscle and kidney. The concentration was least in gills. The levels of ACP activity were found to be decreased in the fish exposed to sublethal concentrations of chlorpyrifos. The decrease was directly dependent on the concentration of chlorpyrifos and duration of exposure.

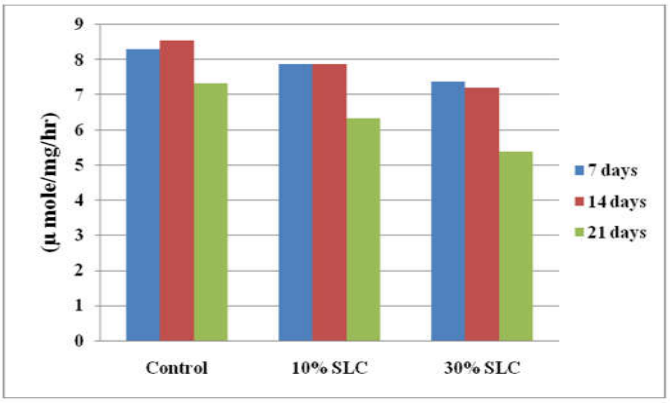

Fig. 5. Levels of acid phosphatase activities in liver of *Cirrhinus mrigala* exposed to sublethal concentration of chlorpyrifos

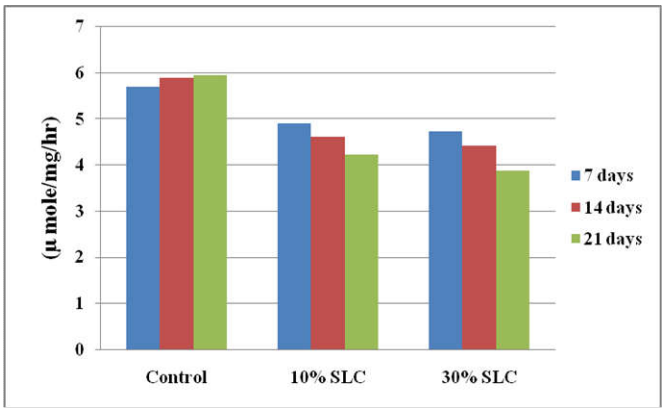

Fig. 6. Levels of acid phosphatase activities in kidney of *Cirrhinus mrigala* exposed to sublethal concentration of chlorpyrifos

Liver acid phosphatase (ALP)

Freshwater fish *Cirrhinus mrigala* treated with sublethal concentration of chlopyrifos on 10% and 30 % showed a decreasing trend in the liver acid phosphatase (ACP) with compared to control (Table 2 and Fig. 5). The control acid phosphatase (ALP) values were recorded from 8.31 ± 0.08, 8.56 ± 0.06 and 7.33 ± 0.04 (µ mole/mg/hr). The 10% sublethal concentration of liver acid phosphatase (ACP) values were recorded from 7.88 ± 0.07, 7.87 ± 0.06 and 6.33 ± 0.05 and the 30% sublethal concentration of liver acid phosphatase (ACP) values were recorded from 7.39 ± 0.06, 7.21 ± 0.06 and 5.39 ± 0.06 (µ mole/mg/hr) exposure period of 7, 14 and 21 days respectively.

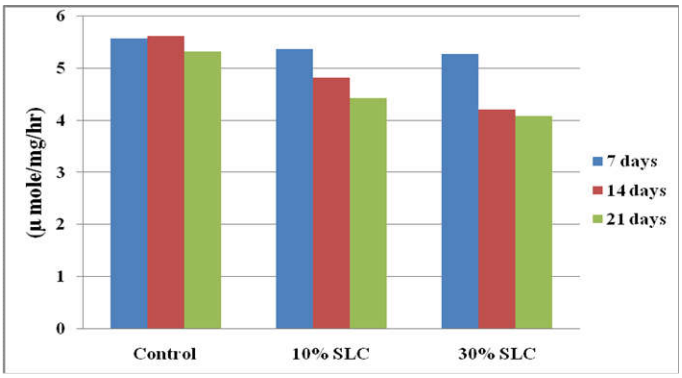

Fig. 7. Levels of acid phosphatase activities in gill of *Cirrhinus mrigala* exposed to sub lethal concentration of chlorpyrifos

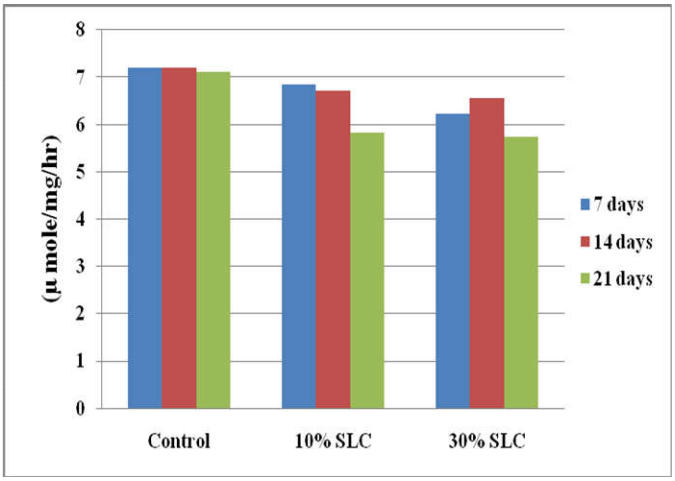

Fig. 8. Levels of acid phosphatase activities in muscles of *Cirrhinus mrigala* exposed to sublethal concentration of chlorpyrifos

Kidney acid phosphatase (ALP)

The fish *Cirrhinus mrigala* treated with sublethal concentration of chlopyrifos on 10% and 30 % showed a decreasing trend in the kidney acid phosphatase (ACP) with compared to control (Table 2 and Fig. 6). The control acid phosphatase (ACP) values were recorded from 5.70 ± 0.08, 5.89 ± 0.05 and 5.95 ± 0.04 (µ mole/mg/hr). The 10% sublethal concentration of kinney acid phosphatase (ACP) values were recorded from 4.91 ± 0.07, 7.43 ± 0.09 and 4.23 ± 0.07and the 30% sublethal concentration of kidney acid phosphatase (ACP) values were recorded from 4.73 ± 0.08, 4.42 ± 0.06 and 3.88 ± 0.05 (µ mole/mg/hr) exposure period of 7, 14 and 21 days respectively.

Gill acid phosphatase (ALP)

In *Cirrhinus mrigala* treated with sublethal concentration of chlopyrifos on 10% and 30 % showed a decreasing trend in the gill acid phosphatase (ACP) with compared to control (Table 2 and Fig. 7). The control acid phosphatase (ACP) values were recorded from 5.58 ± 0.06, 5.62 ± 0.05 and 5.32 ± 0.06 (µ mole/mg/hr). The 10% sublethal concentration of gill acid phosphatase (ACP) values were recorded from 4.38 ± 0.08, 4.82 ± 0.06 and 4.43 ± 0.05 and the 30% sublethal concentration of gill acid phosphatase (ACP) values were recorded from 4.28 ± 0.05, 4.22 ± 0.06 and 4.09 ± 0.05 (µ mole/mg/hr) exposure period of 7, 14 and 21 days respectively. Decrease in the gill acid phosphatase (ACP) level as observed in different sublethal concentrations when compared to control.

Muscle acid phosphatase (ALP)

In fish *Cirrhinus mrigala* treated with sublethal concentration of chlopyrifos on 10% and 30 % showed a decreasing trend in the muscle acid phosphatase (ACP) with compared to control (Table 4 and Fig. 10). The control alkaline phosphatase (ACP) values were recorded from 7.20 ± 0.06, 7.2 ± 0.05 and 7.12 ± 0.07 (µ mole/mg/hr). The 10% sublethal concentration of muscle acid phosphatase (ACP) values were recorded from 6.85 ± 0.06, 6.72 ± 0.02 and 5.82 ± 0.06 and the 30% sublethal concentration of muscle acid phosphatase (ACP) values were recorded from 6.22 ± 0.06, 6.57 ± 0.05 and 5.73 ± 0.06 (µ mole/mg/hr) exposure period of 7, 14 and 21 days respectively. The maximum decrease in the kidney acid phosphatase (ACP) was observed in the gill tissue of *Cirrhinusmrigala* exposed to 30% sublethal concentration of chlorpyrifes reared for 21 days.

DISCUSSION

In the present study, acute toxicity of chlorpyrifos caused 50% mortality of the freshwater fish, *Cirrhinus mrigala* was 0.22 ppm at 96 hrs. The LC_{50} value of chlorpyrifes for 96 hours was observed 0.22 ppm respectively. It was evident from the results that chlorpyrifos can be rated as highly toxic to fish. Similar trend was observed by (David *et al.,* 2002). It has been observed that control fish behaved in a natural manner i.e., they were active with well coordinated movements. They were alert towards slightest disturbance, but in the toxic environment the fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium may be due to inhibition of Ache activity, which items reserites in accumulation of acetyl choline in the cholinergic synapses, leading to hyper stimulation (Mushigeri and David, 2005). They slowly became lethargic, hyper excited, restless and secrete excess mucus all over their bodies. Mucus secretion in fish forms a barrier between the body and toxic media thereby probably reduces contact with the toxicant so as to minimize its irritation effect, or to eliminate it through epidermal mucus. Similar observations were reported by (Rao *et al.,* 2003). Opercular movements increased initially in all experimental fish but decreased steadily with the intensity of toxic level and exposure periods. The initial increase in opercular movements could possibly to increase the physiological activity under stressful conditions (Shivakumar and David, 2004). In the present study, sub lethal concentration of chlorpyrifos treated

fish showed abating of alkalin phospaate activity in accordance with concentration and time bound effect of pesticide. Alkaline phosphate is the brush border enzyme associated with maintenance of orthophosphate pool, the transfer of phosphyl group, the hydrolysin and stratification of metabolites moving across the membrane within the cells and between the extra cellular spaces (Borah and Yadav (1996). The reduction in alkaline activity might have been due to uncoupling of phosphorylation (Thenmozhi *et al.,* 2011). Enzyme analysis of organs such as liver, kidney, gill, heart and muscle in fish can provides information about the internal environment of the organism (Ghorpade *et al.,* 2002). It has been reported that the variation in enzyme activities in heavy metal treated fish is due to increased permeability of the cell as well as the direct effect of the heavy metal on the tissues (Allen and Rana, 2004). Dube and Hosetti, (2010) made similar observation in the fish *Labeo rohita* on exposure to sublethal concentrations of sodium cyanide. Due to the remarkable recession in the ACP and ALP activity, it may be assumed that the liver tissue of the experimental animal exhibited marked inhibition in the activity of phosphatase by sodium cyanide.

In the present study, acid phosphatase activities were observed from freshwater fish *Cirrhinus mrigala*, when treated with sublethal concentration of chlorpyrifos for 7, 14 and 21 days exposure. The ACP level was observed decreasing trend when compared to control. The maximum level of acid phosphatase was recorded in the kidney tissue and minimum level of acid phosphatase was recorded in the gill tissue of *Labeo rohita*. A similar finding was earlier reported by (Sudhanshu Tiwari *et al.,* 2012). The ACP value was decreased when compared to control (Marigouder *et al.,* 2009). They reported that the level of acid phosphatase activity were decrease due to heavy metals in chronic kidney damage. The activity of ACP can be used to indicate the tissue damage of liver and kidney (Hota and Radha, 1994). Sreenivasan *et al.,* (2011) was reported that the impact of cypermethrin on the variations in the acid phosphatase in gill tissue of *S. hydrodroma*. The acid phosphatase level was decreased when compared to control. Enzyme analysis of tissues such as gills, intestine, kidney, liver and muscles in fish can provide important information about the internal environment of the organism (Das *et al.,* 2004). The acid phosphatase activity were showed decreasing trend when compared to control. The maximum level of acid phosphatase in freshwater fish was observed in the liver and minimum was observed in the gill reported by (Palas Samanta *et al.,* 2014).

Conclusion

The present study revealed that the organophosphorus insecticide chlorpyrifos is potent to cause toxic responses, even structural alterations, in aquatic organism like fish. The results indicate that the usage of the chlorpyrifos in the agriculture fields may be a threat to aquatic fauna and flora as well as humans. Therefore, the information obtained may be useful for management and monitoring of agricultural insecticide contamination in aquatic ecosystem. It is also recommended that before using chlorpyrifos in any aquaculture processes, the estimated safe and dischargeable concentrations should be considered important to protect living organisms as well as fish.

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