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Toxic Effect Of Cypermethrin On Enzymes In Freshwater Fish *Oreochromis mossambicus* (Tilapia)

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Abstract : In agriculture, excessive use of various pesticides had increased in developing countries. These pesticides may be highly toxic, not only to aquatic organisms like fishes but also to humans. In recent years, synthetic pyrethroids were developed for major uses in various agriculture practices and public health purposes. We aim to find the toxic Effect of Cypermethrin on Freshwater Fish *Oreochromis mossambicus*(Tilapia). The fish *Oreochromis mossambicus were* exposed for 30 days to various sub lethal concentrations (1/10, 1/20, and 1/30) of cypermethrin. After completion of a 30 days exposure, the fish *Oreochromis mossambicus* was sacrificed and tissue samples of muscle, liver, and kidney were analyzed. Decreased value of ALP, ACP, and Increased value of AST, ALT was observed in all the sub lethal exposure of cypermethrin on treated freshwater fish *Oreochromis mossambicus* at 30 days on comparing with the control group. High variation of AST and ALT were observed in the liver at 1/10th concentration of cypermethrin and elevated variation of ALP and ACP was observed in the liver at 1/10th concentration of sub lethal level for 30 days exposure. While comparing with the control group, the effects of cypermethrin on experimental fish showed increased levels of AST, ALT in the tissues of muscle, liver, and kidney of *O. mossambicus,* and the level of ACP and ALP were found in a decreased manner. Increased levels of ALT and AST activity observed in freshwater fishes may be due to the reduction of metabolic activity and concentration of ACP and ALP in various tissues due to cellular necrosis. In the present study, we found that the effect of cypermethrin alters the activity of various enzymes in freshwater fish *Oreochromis mossambicus.* It is concluded from this study that exposure to cypermethrin affects the enzyme activities of fish.

Keywords: Cypermethrin, Tilapia, Alkaline phosphatase (ALP), Acid phosphate (ACP), Aspartate aminotransferase (AST), and Alanine aminotransferase

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1. INTRODUCTION

Developed countries are facing significant problems in environmental pollution[1]. Many agricultural and industrial activities have been involved in the contamination of freshwater bodies and also cause adverse effects on aquatic organisms and the health of human beings. The high persistence and accumulation of toxicological pollution affect aquatic organisms[3]. Fishes are sensitive to contaminants and pollutants of the water that may damage the organs of the fish and certain biochemical physiological processes. Excess sewage water pollutes the freshwater due to the entry of pesticides along with improper agricultural pest management and industrial effluents. The pesticides like organochlorine and organophosphate were regularly used in pest management but their indiscriminate use in pest management causes ill-effects to the aquatic organisms. The increased use of insecticides in the field of agriculture causes serious effects on aquatic animals. Various metabolic activities alter by changes in enzymes concentrations due to toxicological responses. The biochemical compositions of organisms are being changed under constant pollutant threats. Irreversible changes take place in the metabolic activities of aquatic organisms when exposed to pesticides. Pesticides are toxicants and many pesticides have been observed to produce biochemical changes in aquatic organisms like fish both at sub lethaland lethal levels. Organisms exposed to toxic stress result in disturbances in the physiological functioning of fishes. Fishes were highly exposed and significantly responds to the industrial and agricultural discharges. Temperature and level of oxygen in water are the various stress factors for fishes in aquatic mediums. Many authors observed the effect of pesticides on acid phosphatase activity in aquatic fishes[6,7]. Organophosphorus insecticides were used to control domestic and agricultural insect pests all over the world. The persistence of pesticides in the environment causes damage to aquatic life, especially to fish[8]. Pesticides affect the growth and nutritional value of non-target organisms, such as fish[9]. Agriculture and aquaculture were mainly affected by insecticides like cypermethrin[10]. Enzymes play a significant role in metabolism and food utilization. Enzymes play an important role in the autolytic process of tissues[11]. Analysis of enzymes is commonly used to predict pesticide toxicity[12]. Aspartate aminotransferase (AST)and Alanine aminotransferase (ALT) are the specific liver enzymes. Hepatotoxicity and Histopathologic changes can be assessed within a shorter time through analyzing the enzymes AST and ALT[13]. Decrease and increased value of AST and ALT indicates tissue damages in the kidney, liver, and gill[14]. Fish exposed to toxicants of different concentrations alter the activity of alkaline phosphatase (ALP) in tissues[15]. The present study aims to analyze the enzyme activity (AST, ALT, ALP, and ACP) of various tissues (Muscle, Liver, Kidney) in freshwater fish, *Oreochromis and mossambicus were* exposed for 30 days to various sub lethal (1/10,1/20,1/30) concentrations of cypermethrin.

2. MATERIALS AND METHODS

In the present study, fish *O.mossambicus*was exposed for 30 days to various concentrations (1/10,1/20 and 1/30) ofypermethrin, and the enzymes like ACP, ALP, AST, and ALT of the fish were analyzed. The insecticide cypermethrin was purchased from a local shop at Krishnagiri, Tamilnadu. *Oreochromis mossambicus*is widely distributed in Indian freshwater media. Fish *O.mossambicus* was acquired from the KRP Dam at Krishnagiri, Tamilnadu with well-aerated bags. The fishes was authenticated by a zoologist,and the studywas conducted at the Aquaculture laboratory of Government Arts College for Men, Krishnagiri. The fishes was acclimatized before they were used for the experiment. Fish tanks were cleaned with potassium permanganate solution to avoid fungal and other infections. Physically damaged, stressed and dead fish were removed from the tank. Fishes were fed with oil cake mixed with rice flour every day. Weight of 15 gm to 17gm and the length of 13 cm to 16 cm healthy fish were selected for the experiment. Fish were divided into four groups with ten numbers. Three groups were treated with cypermethrin (35% Ec.) of various sub lethal (1/30,1/20 and 1/10) concentrations. One group experimented as a control group. The LC50 value of cypermethrin was observed as 4.9 mg/l. Both treated and control fish were dissected after 30 days of the exposure period and tissues of muscle, liver, and thekidney was removed. Tissues were homogenized and centrifuged at 3500 rpm. The supernatant was used for enzymological analysis. The level of AST, ALT in the sample of *Oreochromis mossambicus* was analyzed by the methods of Reitman and frankel[16]. Alkaline Phosphatase and Acid Phosphatase level in tissues of *Oreochromis mossambicus*was determined by the method of Tenniswood[17]. The values were expressed in μmole/Protein/hr.

3. STATISTICAL ANALYSIS

The values are expressed as mean ± SD. Data were statistically analyzed using SPSS 20.0 software for Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT), which was applied to find out the significant difference between various treatment means and control means for the observed parameters. A probability value (P) of less than 0.05 was considered statistically significant.

4. RESULTS

In the present study, 96 hrs. LC50 value of cypermethrin was determined as 4.9 mg/l in the experimental fish *Oreochromis mossambicus*. Increased level of AST, ALT was observed in all the sub lethal exposure (1/10, 1/20, and 1/30) for 30 days in the tissues of muscle, liver, and kidney of experimental fish *Oreochromis mossambicus* when it is compared with a control group. Decreased levels of ACP and ALP were observed in the tissues of muscle, liver, and kidney of *Oreochromis mossambicus* for various exposure (1/10, 1/20, and 1/30) of cypermethrin for 30 days when it is compared with the control group. The liver shows a maximum increased level of AST, ALT and a maximum decreased level of ACP and ALP after 30 days of cypermethrin exposure.

Table – 1Changes of Aspartate aminotransferase level (µ mole/mg protein/hr.) in different tissues (muscle, liver, and kidney) of *Oreochromis mossambicus* exposed to the various sub lethal concentration of cypermethrin for 30 days.

Group	Exposure Period	Muscle	Liver	Kidney
Control	30 days	0.301 ± 0.04[a]	0.510 ± 0.04[a]	0.377 ± 0.05[a]
(1/30)Lower Concentration	30 days	0.324 ± 0.07[b]	0.538 ± 0.07[b]	0.413 ± 0.09[b]
(1/20)Medium Concentration	30 days	0.347 ± 0.03[c]	0.578 ± 0.06[c]	0.474 ± 0.06[c]
(1/10)Higher Concentration	30 days	0.378 ± 0.06[d]	0.628 ± 0.05[d]	0.495 ± 0.11[d]

Values are mean ± S.D., Sample Size (N) = 6. Values that do not share common superscript withinRow and column with superscripts a,b,c,d differ significantly at 5% (P<0.05) level between exposure and control groups.

Increased level of Aspartate aminotransferase was observed in tissues of liver, kidney, and muscle of experimental fish *Oreochromis mossambicus*in the various sub-lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. The high variation was observed in the tissues of the liver and kidney of experimental fish.

Table – 2Changes of Alanine aminotransferase level (µmole/mg protein/hr.) in different tissues (muscle, liver, and kidney) of *Oreochromis mossambicus* exposed to the various sub lethal concentration of cypermethrin for 30 days.

Group	Exposure Period	Muscle	Liver	Kidney
Control	30 days	0.531 ± 0.08[a]	0.842 ± 0.08[a]	0.521 ± 0.04[a]
(1/30)Lower Concentration	30 days	0.617 ± 0.06[b]	0.907 ± 0.06[b]	0.556 ± 0.03[b]
(1/20)Medium Concentration	30 days	0.675 ± 0.08[c]	0.956 ± 0.05[c]	0.574 ± 0.08[c]
(1/10)Higher Concentration	30 days	0.684 ± 0.04[d]	0.985 ± 0.07[d]	0.623 ± 0.04[d]

Values are mean ± S.D., Sample Size (N) = 6. Values that do not share common superscript withinRow and column with superscripts a,b,c,d differ significantly at 5% (P<0.05) level between exposure and control groups.

Increased level of Alanine aminotransferase was observed in tissues of the liver, muscle, and kidney of experimental fish *Oreochromis mossambicus*in the various sub-lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. The high variation was observed in the tissues of the liver and muscle of experimental fish.

Table – 3Changes in Acid phosphatase level (µ mole/mg protein/hr.) in different tissues (muscle, liver, and kidney) of *Oreochromis mossambicus* exposed to the various sub lethal concentration of cypermethrin for 30 days.

Group	Exposure Period	Muscle	Liver	Kidney
Control	30 days	0.442 ± 0.03[a]	0.829 ± 0.01[a]	0.591 ± 0.05[a]
(1/30)Lower Concentration	30 days	0.523 ± 0.06[b]	0.924 ± 0.07[b]	0.634 ± 0.04[b]
(1/20)Medium Concentration	30 days	0.538 ± 0.05[c]	0.943 ± 0.03[c]	0.701 ± 0.06[c]
(1/10)Higher Concentration	30 days	0.624 ± 0.02[d]	0.998 ± 0.05[d]	0.709 ± 0.06[c]

Values are mean ± S.D., Sample Size (N) = 6. Values that do not share common superscript within row and column with superscriptsa,b,c,d differ significantly at 5% (P<0.05) level between exposure and control groups.

Increased level of Acid phosphatase was observed in tissues of liver, kidney, and muscle of experimental fish *Oreochromis mossambicus*in the various sub-lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. The high variation was observed in the tissues of the liver and kidney of experimental fish.

Table – 4Changes of Alkaline phosphatase level (µ mole/mg protein/hr.) in different tissues (muscle, liver, and kidney) of *Oreochromismossambicus* exposed to the various sub lethal concentration of cypermethrin for 30 days.

Group	Exposure Period	Muscle	Liver	Kidney
Control	30 days	0.437 ± 0.04[a]	0.926 ± 0.02[a]	0.542 ± 0.03[a]
(1/30)Lower Concentration	30 days	0.523 ± 0.02[b]	0.973 ± 0.04[b]	0.657 ± 0.02[b]
(1/20)Medium Concentration	30 days	0.567 ± 0.05[c]	1.214 ± 0.06[c]	0.736 ± 0.07[c]
(1/10)Higher Concentration	30 days	0.623 ± 0.03[d]	1.347 ± 0.07[d]	0.812 ± 0.03[d]

Values are mean ± S.D., Sample Size (N) = 6. Values that do not share common superscript withinRow and column with superscripts a,b,c,d differ significantly at 5% (P<0.05) level between exposure and control groups.

Increased level of Acid phosphatase was observed in tissues of liver, kidney, and muscle of experimental fish *Oreochromis mossambicus*in the various sub-lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. The high variation was observed in the tissues of the liver and kidney of experimental fish.

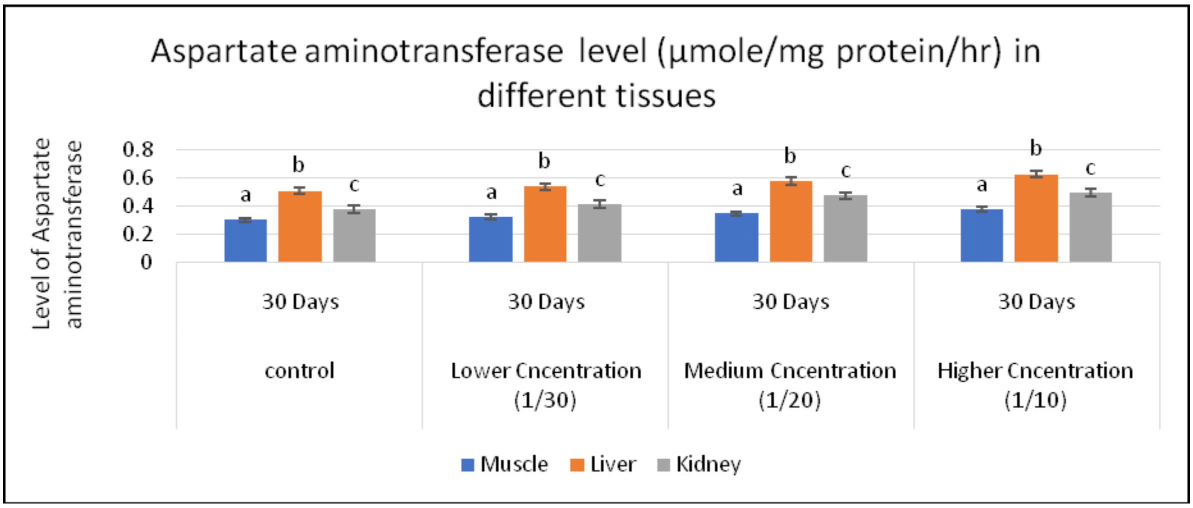

Fig.1.Changes of Aspartate Aminotransferase level (mole/mg protein/hr.)in different tissues (1.Muscle, 2.Liver and 3. Kidney) of *Oreochromis mossambicus* exposed to the various sub lethal concentration of cypermethrin for 30 days.

Fig.1 shows an increased level of Aspartate aminotransferase in tissues of the liver, kidney, and muscle of experimental fish *Oreochromis mossambicus* which was treated with various sub lethal concentrations of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with the control group. A highly increased level of AST activity was observed in the tissues of the liver and kidney of experimental fish.

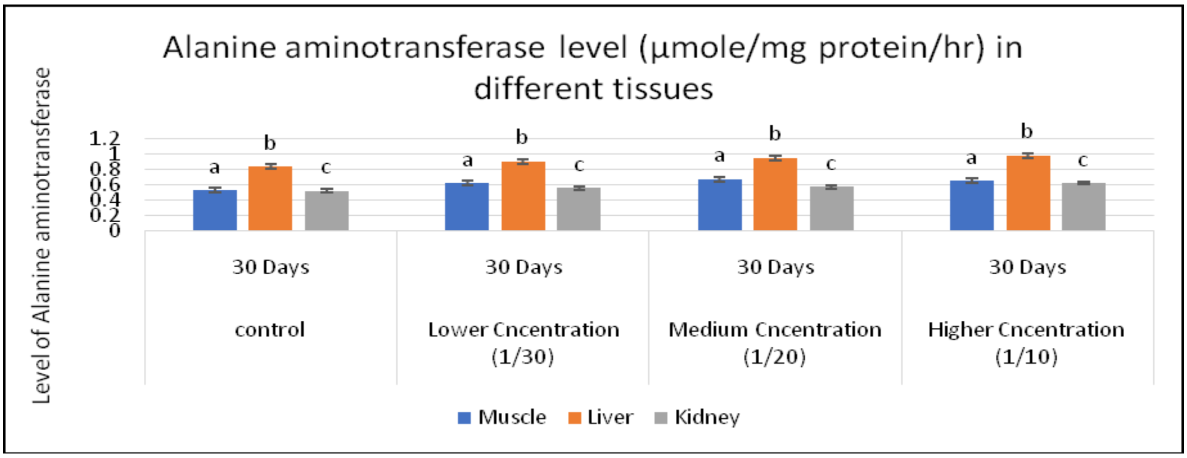

Fig. 2.Changes of Alanine aminotransferase level (µmole/mg protein/hr.) in different tissues (1.Muscle, 2.Liver, and 3. Kidney) of *Oreochromis mossambicus* exposed to the various sub lethal concentration of cypermethrin for 30 days.

Fig.2 shows an increased level of Alanine aminotransferase in tissues of the liver, muscle, and kidney of experimental fish *Oreochromis mossambicus* in the various sub-lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. A highly increased level of Alanine aminotransferase activity was observed in the tissues of the liver and muscle of experimental fish.

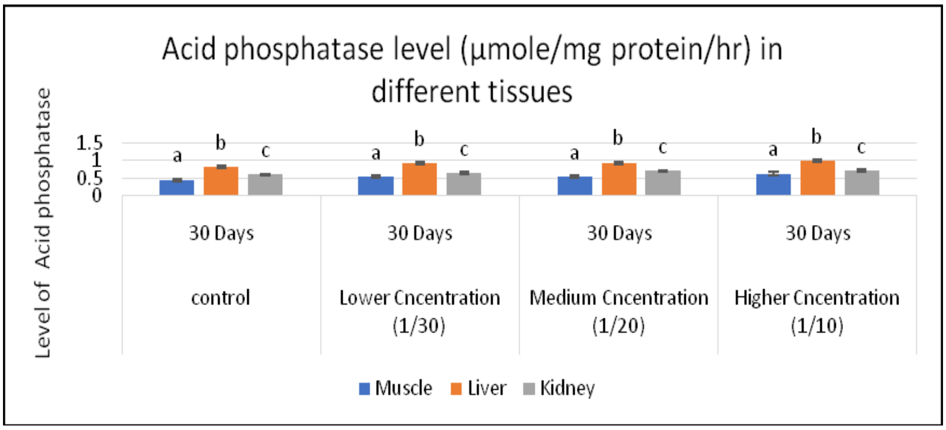

Fig.3. Changes in Acid phosphatase level (µmole/mg protein/hr.) in different tissues (1. Muscle, 2.Liver, and 3.Kidney) of *Oreochromis mossambicus* exposed to the various sub lethal concentration of Cypermethrin for 30 days.

Fig.3shows an increased level of Acid phosphatase in tissues of the liver, kidney, and muscle of experimental fish *Oreochromis mossambicus* in the various sub-lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. A highly increased level of ACP was observed in the tissues of the liver and kidney of experimental fish.

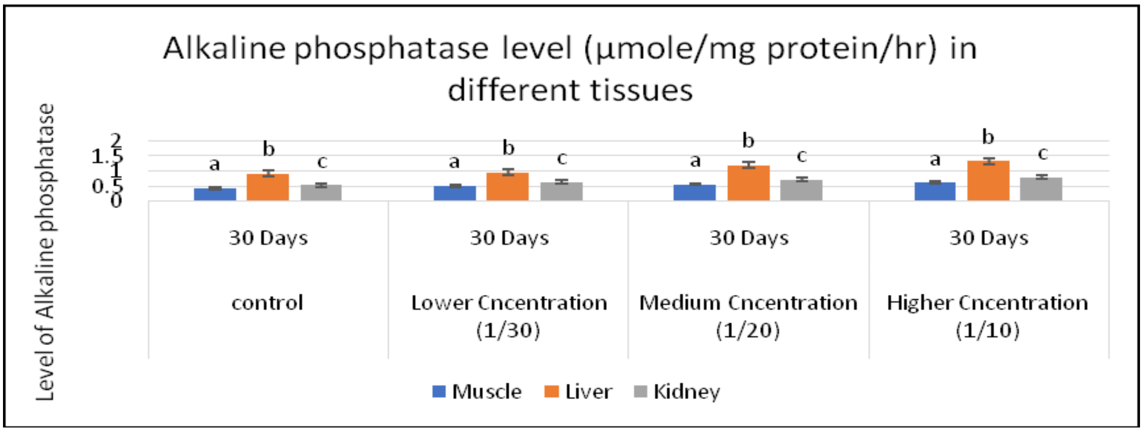

Fig.4.Changes in Alkaline phosphatase level (µmole/mg protein/hr.) in different tissues (1.Muscle, 2. Liver and 3. Kidney) of *Oreochromis mossambicus* exposed to the various sub lethal concentration of cypermethrin for 30 days.

Fig.4 shows an increased level of Alkaline phosphatase in tissues of liver, kidney, and muscle of experimental fish *Oreochromis mossambicus*in the various sub lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. A highly increased level of ALP activity was observed in the tissues of the liver and muscle of experimental fish.

5. DISCUSSION

During the experimental period of 30 days, the level of AST, ALT was increased and the level of ALP, ACP was declined in muscle, liver, and kidney of freshwater fish *Oreochromis mossambicus* at various sub-lethal concentrations (1/10,1/20 and 1/30) of cypermethrin. Similar observations were reported for freshwater fish *Channastriatus* treated with endosulfan pesticide[18]. Transamination is one pathway for the synthesis of amino acids, enabling protein metabolism during the energy demands of the organism under various conditions[19]. A similar reduction was also observed in *C. gariepinus* exposed to sub-lethal concentrations of cypermethrin to change enzyme activities[20]. Similar results were obtained when rainbow trout were exposed to *carbamazepine*. Li et al.,[21] and Bhatnagar et al.,[22] found the same observation as a decreased level of ALP, ACP in muscle and brain. Goelet al.,[23] reported the reduced activities of ACP, ALP in various organs of fish *Pontius conchonius* exposed to mercury. ALT activity of freshwater fishes exposed to paper mill effluent also showed changes when compared to control group fishes. [24] The decreased level of ALP activity was observed in the kidney and liver, recorded by Arellano et al.[25]. A reduced level of ALP is due to the inhibition of metabolic processes. The decreased level of ALP in the organ tissues may be attributed to a decline in the rate of synthesis caused by lowered metabolic demands[26]. Increased levels of ALT and AST activity were observed in freshwater fishes, maybe due to the decline of metabolic activity [27]. Shakoori et al.,[28] reported that decreased levels of ALP and ACP in the fish were due to increased necrosis in the hepatocytes. Stress-induced by doses of insecticide causes reduction of alkaline phosphatase activity[29]. Arsenic exposure in *Labeorohita* decreases the level of ACP[30]. Rahman et al.,[31] found the declining level of ACP and ALP in various tissues due to cellular necrosis. Increased levels of liver ALT, AST activities as observed in the present study were in agreement with the work of Reddy and Yellamma[32] who noticed cypermethrin-induced activities on ALT and AST of freshwater fish Tilapia. An increase in liver ALT and AST activities were also observed in Freshwater fish *Cirrhinusmrigala* exposed to sub-lethal concentration for 1, 7, 14, 21 days of cypermethrin[33]. Das and Mukherjee et al.,[34] observed the inhibition of succinate dehydrogenase and ATPase activities in the brain, kidney, and liver of the Indian major carp, *Labeorohita*. A significantly increased level of AST and ALT was observed in the liver of albino rats exposed to monocrotophos given orally for 90 days, experimented [35]. The present study confirms that the stress of pesticides stimulates enzyme activities. The present study concluded that enzymological alterations were induced in the muscle, liver, and kidney of freshwater fish *Oreochromis mossambicus* by cypermethrin depending on the toxicity of the chemical, dose of toxicants, and duration of exposure of the fish.

6. CONCLUSION

Fish *Oreochromis mossambicus* treated to various sub lethal concentrations of cypermethrin showed an increased level of AST, ALT, and decreased level of ACP and ALP at the end of the exposure period compared with control. Enzymological changes observed in the present experiment may potentially cause the survivability of the fish *Oreochromis mossambicus*. The present study suggest that when cypermethrin is used beyond the safe level, it is highly toxic to aquatic organisms like fish. The results provide awareness on safe level usage of cypermethrin and recommends the unsafe uses of cypermethrin should be restricted.

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8. AUTHOR CONTRIBUTION STATEMENT

Mrs.Kalpana Devi. K. K conceptualized and gathered the data about this work. Dr.Prakash Sahaya Leon. J analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology, results and contributed to the final manuscript.

9. CONFLICT OF INTEREST

There is no Conflict of interest.

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