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POTENTIAL EFFICACY OF *TRIBULU STERRTRI* AGINST TOXIC IMPACT OF CHLORPYRIFOS ON ENZYMOLOGICAL ALTERATION IN THE FRESH WATER FISH *ORIOCHROMMIS MOSSAMBICUS*

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ABSTRACT

Environmental pollution occurs when the environmental degradation crosses limit so that. It becomes lethal to living organisms. Pollution of water bodies forces them to acclimatize to various factors thus imposing a considerable amount of stress on their lives. Phosphatase is known to be sensitive to metal exposures and can be used to predict metal toxicity. The acid phosphatase (ACP) and alkaline phosphatase (ALP) enzyme activity brought a decrease in acid and alkaline phosphatase (ACP and ALP) in Liver and Kidney when a freshwater fish Oriochrommis mossambicus exposed to Chlorpyrifos concentration as compared to the control group. Tribulu sterrtri act as alter the acidic and alkaline phosphatase activity in the studied organs three groups of newly hatched spotted Oriochrommis mossambicus were held at three different temperatures in order to determine relationships between metabolic, digestive and growth response in rapidly developing larvae. Reduced glutathione showed a positive compensation (higher activity at a lower temperature) whereas glycolytic enzymes (pyruvate kinase and Lactate dehydrogenase) and aspartate aminotransferase (AST) showed a negative compensation (lower activity at a lower temperature). Citrate synthase was not affected by growth rate, indicating that the level of aerobic capacity was adequate in sustaining the high energy needs associated with rapid growth early in the life of the spotted Oriochrommis mossambicus. Hence, the results from present investigations may be useful in the assessment of environmental stress in the aquatic ecosystem.

KEY WORDS

Acid phosphatase, alkaline phosphatase, Tribulu sterrtri, Oriochrommis mossambicus, Chlorpyrifos

INTRODUCTION

Environmental pollution occurs when the environmental degradation crosses limit so that. It becomes lethal to living organisms. In India, pesticides constitute an important component in agriculture development and protection of public health since the tropical climate is very conducive to pest breeding. Kumar *et al.,* (2010). Contamination by pesticides in the aquatic ecosystem is a serious problem and fish are more frequently exposed to these pollutants and may be taken in through gills, skin and contaminated food. Lin et *al.,* (2001). fishes have an important role in the food chain; therefore, investigation of the effects of toxic pesticides such as chlorpyrifos on fish has a diagnostic significance in the evaluation of negative effects of pesticides to human health Tamizhazhagan et al (2017).

fish immune system, important for defense against a variety of harmful pathogens is very sensitive to homeostatic adjustments via endocrine regulation and is influenced by the biochemical profile of the nervous system. Insecticides can alter the immune functions of the body and result in immune-depression, uncontrolled cell proliferation, and alterations of the host defense mechanism against pathogens. Srivasta *et al.,* (2012).

A number of chemicals released into environment contaminate especially aquatic ecosystem through different ways. Most of these chemicals are in the form of pesticides, herbicides, rodenticides etc. Begum (2009). Widely spread pesticides are used to control agriculture pests but at the same time they are highly toxic to non-target natural population in the aquatic environment. This necessitated the need to understand and evaluate the biological effects of xenobiotic on aquatic ecosystem Bhattacharya (2009).

Organophosphorus (OP) compounds are one of the most widely used pesticides in the world replacing the organochlorine. OP pesticides have a common mechanism of action even though each of their chemical structure varies in their nature Tamizhazhagan (2015). They cause inhibition of the nervous tissue acetylcholinesterase (AChE) activity (Chandra Bazaz and Keshavnath (1993). AChE is an important enzyme that can be measured environmental bio-indicator in the animal body. Cholinesterase is involved in the signal transmission at neuromuscular junctions and is also intensely expressed in the organism nervous system. The main role of AChE is to catalyze the hydrolysis of acetylcholine into cholin and acetic acid at cholinergic synaptic sites Ramachandran et al., (1980).

MATERIALS AND METHODS

Collection and maintenance of the Experimental animal.

The freshwater fish *Oreochromis mossambicus* were collected from the VGM fish farm located in Kurinjipadi, Cuddalore district. The fishes were brought to the laboratory and transferred to the rectangular cement tanks (100 175) of 500 liters capacity containing chlorine free aerated well water, fishes of the same size and weight were used irrespective of their sex for the experiments.

Estimation of Marker Enzymes

Assay of Aspartate Amino Transferase

Tissue aspartate aminotransferase was assayed by using the diagnostic kit based on the method of Reitman and Frankel (1957). 0.5 mL of buffered substrate was added to 0.1 mL of serum and placed in a water bath at 37 °C. To the blank tubes, 0.1 mL distilled water was added instead of serum. Exactly an hour later, 2 drops of aniline citrate reagent and 0.5 mL of DNPH reagent were added and kept at room temperature for 20 min. Finally, 5.0 mL 0.4 N sodium hydroxide was added. A set of standards also treated in the same manner and read at 520 nm after 10 min.The results were expressed as IU/L of serum.

Assay of Alanine amino transferase

Alanine aminotransferase was assayed by using the diagnostic kit based on the method of Reitman and Frankel (1957). Procedure was same as that used for the assay of aspartate transaminase except the incubation time which was reduced to 30 min (60 min for AST). The results were expressed as IU/L.

Estimation of Alkaline phosphatase

Tissue alkaline phosphatase was estimated by using the diagnostic kit based on Kind and King's method (1954). The incubation mixture, contained 1.0 mL of buffered substrate 3.1 mL of deionised water and 0.1 mL of serum, was incubated at 37°C. Exactly after 15 min, 2.0 mL of color reagent was added to all the tubes. The control tubes received the enzyme after the addition of color reagent. 0.1 mL of standard and 0.1 mL of distilled water (blank) were also treated simultaneously and the color developed was read at 510 nm. The enzyme activity was expressed as IU/L of serum

Estimation of Acid Phosphatase

Tissue alkaline phosphatase was estimated by using the diagnostic kit based on King's method (1956). The incubation mixture, contained 1.0 mL of buffered substrate 0.1 mL of deionised water and 0.1 mL of serum, was incubated at 7 °C. Exactly after 15 min, 2.0 mL of color reagent was added to all the tubes. The control tubes received the enzyme after the addition of color reagent. 0.1 mL of standard and 0.1 mL of distilled water (blank) were also treated simultaneously and the color developed was read at 510 nm. The enzyme activity was expressed as IU/L of serum.

Estimation of Reduced glutathione (GSH)

Reduced glutathione was estimated by the method of Ellman (1959). This method was based on the formation of 2-nitro-5-thiobenzoic acid (a yellow colour compound) when 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) was added to compounds containing sulphydryl groups. 0.5 mL of supernatant was pipette out and precipitated with 2.0 mL of 5% TCA. 0.1 mL of supernatant was taken after centrifugation and 0.5 mL of Ellman's reagent and 3 mL of 0.3 M disodium hydrogen phosphate was added. The yellow color developed was read in a Spectrophotometer at 420 nm with a blank containing 3.5 mL of buffer. The amount of glutathione was expressed as mg/g of tissues.

Estimation of Glutathione peroxidase (GP_X)

The activity of GPx was measured by the method of Rotruck *et al.,* (1973). A known amount of enzyme preparation was allowed to react with H_2O_2 in the presence of GSH for a specified time period. Then the remaining GSH content was measured.

Assay of Glutathione - s –tranferase

The activity of GST was measured by the method of Habig (1974). Activity of glutathione – s- transferase was measured in tissues homogenate by following the increase in absorbance at 340 nm using 1-chloro-2, 4 dinitro benzene as substrate. 0.1 mL of phosphate buffer, 0.1 mL of CDNB added then 0.1 mL of tissues homogenate and 0.7 mL of water was added. This mixture was incubating at 37°C for 5minutes. After 5 minutes, add 0.5mL of 10% TCA solution and 0.1 mL of glutathione. GST activity read at 340nm.The activity was expressed as μg of CDNB-GSH conjugate formed/min/mg of protein.

Measurement of dehydrogenase activity

The tissue was isolated from animal in the cold room and 5 percent homogenate was prepared in 0.25 m sucrose solution and centrifuged at 2500 rpm for 15 minutes to remove cell debris. The supernatant was used for the enzyme assay.

RESULTS

The level of LPO enzyme activity in the gill tissue shows remarkable changes in the fingerlings of *Oreochromis mossambicus* exposed to sublethal concentration of chlorpyrifos and antidote *tribulus terrestris.* The per cent changes of group 2 over control are 73.40, 30.27, 30.97, 36.66 and 49.6 for 24, 48, 72, 96 and 120 hrs respectively. When group 3 exposed fish shows, the LPO response is gradually recovered. When fish is exposed to group 3, recovery occurs, and decreased level is observed than control. The increased per cent recoveries are -9.23, -8.71, -8.20, -7.93 and -7.88 for 24, 48, 72, 96 and 120 hrs respectively. The level of LPO activity is decreased in group 4 and a slight variation is observed when compared to group 3. The per cent changes are -2.41, -1.30, -0.86, -0.42 and -0.85 for 24, 48, 72, 96 and 120 hrs respectively. The recorded LPO content in muscle tissue for 4 groups are statistically significant at 1% and 5% level (Table 1).

The SOD activity in group 2 in gill tissue, the content is increased than in control (group 1). In the *Labeo rohita* fingerlings to group 3 and group 4, the SOD content is recovered when compared with group 2. The per cent increases are 25.00, 43.47, 44.00, 51.72 and 53.12 for 24, 48, 72, 96 and 120 hrs respectively. In the present investigation, fingerlings administrated with *chlorpyrifos* (group 2) result in an increased activity of the enzyme in liver tissue at all periods at significant (p

< 0.05) SOD values of 161.6, 165.61, 162.93, 168.60 and 170.61 for 24, 48, 72, 96 and 120 hrs respectively. In the administration of *chlorpyrifos* along with *tribulus terrestris* (group 3), the SOD response gradually recovers when compared to group 2, while in the *tribulus terrestris* administered fish, there are no markable changes occurring in the SOD content. The SOD contents in liver tissue are statistically significant in groups 2, 3 and 4 at 1% and 5% levels (Table 1). When *Labeo rohita* is exposed to group 3, the CAT content is recovered when compared to group 2. The per cent increases in group 2 are 36.18, 39.74, 45.28, 48.46 and 50.90 for 24, 48, 72, 96 and 120 hrs respectively. While in the fish exposed to group 4, the CAT response in gill tissue has been recovered without any changes. When compared with group 1, the slight variation is noticed in the percentage changes in group 4 are 1.31, 1.28, 1.88, 1.84 and 0.24 for 24 to 120 hrs respectively. (Table 1). In the present investigation, fingerlings when exposed to group 2, the administration of *chlorpyrifos* results in decreased content of the enzyme activity in gill tissue at all periods, the significant ($p < 0.05$) values are -42.55, -43.43, -49.57, -48.38 and -47.28 for 24, 48, 72, 96 and 120 hrs respectively. Administration of *chlorpyrifos* along with *tribulus terrestris* (group 3), the GSH response gradually recovers when compared to group 2. In the *tribulus terrestris* administered fish (group 4), (Table 2). When the *Oreochromis mossambicus* is exposed to *chlorpyrifos* along with *tribulus terrestris* (group 3), the LDH activity is recovered as to *chlorpyrifos* (group 2). The percent changes alone in group 3 are -6.20, -6.08, -7.79, -6.96 and -6.72 for 24, 48, 72, 96 and 120 hrs respectively.

The per cent decrease of succinic dehydrogenase (SDH) content at 24, 48, 72, 96 and 120 hrs for sublethal concentration of *chlorpyrifos* (group 2) and the decreased values are -5.79, -10.11, -16.75, -28.37 and -43.82 respectively. When compared to *chlorpyrifos* along with *tribulus terrestris* (group 3), the SDH activity is recovered when compared to *chlorpyrifos* (group 2). The level of ACP enzyme activity in the gill tissue shows an increase in the fingerlings exposed to sublethal concentration of *chlorpyrifos* (group 2). The per cent changes are 51.44, 53.14, 47.82, 51.32 and 52.84 for 24, 48, 72, 96 and 120 hrs respectively. In the fish exposed to *tribulus terrestris* alone (group 4), the ACP response in gill is recovered without any changes when compared with control. The percentage changes in *tribulus terrestris* (group 4) are -6.35, -5.71, -5.91, -3.17 and -2.59 for 24 to 120 hrs respectively. The level of ALP enzyme activity in the gill tissue shows an increase in the fingerlings exposed to sublethal concentration of *chlorpyrifos* (group 2). The percentage changes over control of group 2 are 21.80, 20.86, 27.28, 29.80 and 39.10 for 24, 48, 72, 96 and 120 hrs respectively. When compared to group 2, recovery is taken, without any changes (Table 4) The ACh enzyme activity in the gill tissue shows an increase in the fingerlings exposed to sublethal concentration of *chlorpyrifos* (group 2). In group 2, an increased ACh activity is observed and increased per cent changes are 99.69, 108.88, 124.47, 132.80 and 160.45 for 24, 48, 72, 96 and 120 hrs respectively (Table 5).

DISCUSSION

In the present investigation, sublethal concentration of chlorpyrifos exposed fish (group 2) fingerlings of *Oreochromis mossambicus* show an increase in lipid peroxidation and decrease in superoxide dismutase (SOD) and catalase (CAT) in gill, liver, kidney and muscle tissues as observed for 24, 48, 72, 96 and 120 hrs. The significantly increased activities of SOD and CAT enzymes observed in the present study reflect the antioxidant response against oxidative stress caused by *chlorpyrifos* exposure Vasantharaja et al (2012). The uptake from water occurs because of the very intimate contact with the medium that carries the chemicals in solution or suspension and also because fish have to extract oxygen from the medium by passing enormous volumes of water over their gills (APHA, 1995)

Saravanan *et al*. (2000) have reported that intraperitoneally applied microcystin (MC) significantly increases the SOD and CAT activities in various tissues of *Tilapia*. Sandbacka *et al*. (2000) have also reported that subchronic dietary exposure to MC increased SOD and CAT activities in *Tilapia*. Furthermore, they observe that the increase of enzyme activity in liver was higher than that in gill. Li *et al*. (2003) also register enhanced SOD and CAT activities in the hepatocytes of common carp (*Cyprinus carpio* L.), following exposure to MC-LR. Pinho *et al*. (2005) record that the increased SOD and CAT activities in the hepatopancreas tissue of the estuarine crab *Chasmagnathus granulatus* exposed to MCs were related to the production of ROS. Values for SOD activity in different freshwater fish species *Semotilus margarita*, *Catostomus commersoni, Salelinus namaycush, Oncorhynchus mykiss, Salelinus namaycush* (Dhanapakiam *et al*., 1998) are similar to those found in *Labeo rohita* during this study. The SOD variations observed for fish from sites can also be linked to gonad maturity and food availability to a certain extent and can modulate the metabolic status (Baskaran *et al*., 1991). An increased CAT and SOD activity in dichloros and $CuSO_4$ treated larvae further suggests that catalase functions to deal with H_2O_2 produced as a result of dismutation of O_2 by SOD Tamizhazhagan and Pugazhendy (2016). Organophosphate compounds have been reported to inhibit mitochondrial ATP production through the uncoupling of oxidative phosphorylation (Tilak *et al*., 2005) and this could lead to generation of reactive oxygen radicals (Singh *et al.,* 2009). The increase in lipid peroxidation observed in perchloroethylene (PER) administered animals might be a consequence of higher levels of superoxide radicals which are produced in significant amounts in response to PER exposure or inhibition of free radicals scavenging metalloenzymes that play a key role in the defence against ROS by transforming superoxide anions into hydrogen peroxide (Yim *et al*., 1995). In the present study, chlorpyrifos and *tribulusterrtri* (group 3) exposure induces a noticeable concentration-dependent decrease in GSH content in the gill, liver, kidney and muscle. Thus, it is possible that an increase in the activities of these enzymes contributes to the elimination of ROS from the cell induced by chlorpyrifos exposure. Although an increasing number of researchers have focused on the oxidative stress responses arising from xenobiotics, the reports on fish species are very limited and mainly examine the changes in the activities of related antioxidant enzymes Meenambal et al (2012).

In the present investigation, sublethal concentration of chlorpyrifos (group 2) exposed fish fingerlings of *Oreochromis mossambicus* shows an increase in (GSH-Px) activity and increase in CAT and SOD activity in gill, liver, kidney and muscle tissues which have been observed for 24, 48, 72, 96 and 120 hrs. CAT enzyme is an important antioxidant component sharing the same function with glutathione peroxidase (GSH-Px) for working primarily to degrade H_2O_2 to H_2O. Organic peroxides are the preferred substrate for GSH-Px in the presence of low H_2O_2 concentrations, but not at a high level of H_2O_2, which are catalyzed by CAT (Yu *et al.,* 1994).

Previous study has found that CAT plays a relatively more important role in detoxification in invertebrates than vertebrates (Livingstone *et al.,* 1992). Generally, any significant increase in SOD activity is accompanied by a comparable enhancement in CAT and or GSH-Px activities Tamizhazhagan et al (2016). Similar tendency is also found in our study. The high level of SOD activity is followed by the increased CAT activity accordingly in the haemocyte, serum, hepatopancreas and gill after LPS challenge taken together, our data reveal that the antioxidant enzyme activities like CAT and SOD vary in different tissues and cells against the LPS stress, suggesting that the enzyme activity along with their gene expression profile is tissue-specific as well as time-dependent under stress conditions.

Reactive oxygen species such as superoxide anions, hydroxyl radicals and hydrogen peroxide enhance the oxidative process and induce peroxidative damage to membrane lipids. Increased LPO may be one of the molecular mechanisms involved in the chlorpyrifos induced toxicity. The present results indicate that *tribulusterrtri* treatment results in decreased LPO in the liver and protects it from chlorpyrifos induced oxidative stress. A significant decrease in GSH content in the liver of fish after chlorpyrifos exposure indicated pro-oxidant conditions in the liver. A reduced GPx activity could indicate that its antioxidant capacity is surpassed by the amount of hydroperoxide products of lipid peroxidation (Remacle *et al*., 1992). The GST activity is involved in xenobiotic detoxification and excretion of xenobiotics and their metabolites, including MP (Hakim, 2006). Increased GST activity in tissues may indicate the development of a defensive mechanism to counteract the effects of chlorpyrifos and may reflect the possibility of a more efficient protection against herbicide toxicity Rao (2006). The increased GST activity in all tissues observed in the present study after exposure to *tribulusterrtri* suggests that the detoxification processes are increased and corroborates these assertions Prabakaran et al (2014). The considerable decline in the GSH tissue content during exposure to *tribulusterrtri* may be due to an increased utilization of GSH, which can be converted into oxidized glutathione and an inefficient GSH regeneration.

CONCLUSION

It is evident that chlorpyrifos presented in aquatic ecosystems can affect aquatic fauna in different ways. Alterations in physico-chemical properties of water, destruction of delicate balance in the environment, entry into the food chains and physiological damage to the vital tissues of aquatic fauna are the threatening issues of the modern-day pesticides. Long term exposure to these products causes countless abnormalities and reduces the life span of organisms. Finally, we conclude that chlorpyrifos is highly toxic to fish, and impose life threatening effect on fish at both lethal and sublethal concentrations. Altered enzymesdisorders observed in selecte dissues and enzymatical responses can be used as tools in bioassessment to monitor ecotoxicological risks associated with pesticides such as chlorpyrifos to various fish.

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Table 1. Changes in the level of lipid (mg/g wet wt. of tissue) content in the freshwater fish *Oriochromis mossambicus* fingerlings on the effect of chlorpyrifos and antidote tribulus terrtri exposed to 120 hrs sublethal concentrations

Tissues	Groups		Hours of exposure				
			24	48	72	96	120
Gill	Group 1 - Control		13.18 ± 0.03	13.12 ± 0.04	13.54 ± 0.06	14.19 ± 0.05	14.65 ± 0.05
	Group II - Chlorpyrifos		12.06** ± 0.04	12.46** ± 0.03	12.77** ± 0.07	13.18** ± 0.04	13.42** ± 0.05
		% COC	-8.49	-5.89	-5.68	-7.11	-8.39
	Group III - Chlorpyrifos + Tribulus terrtri		14.17** ± 0.02	14.29** ± 0.03	15.12** ± 0.04	15.85** ± 0.04	15.96** ± 0.05
		% COC	+7.51	+7.93	+4.28	+4.65	+8.14
	Group IV - Tribulus terrtri		13.41** ± 0.04	13.67** ± 0.05	13.22* ± 0.06	14.02** ± 0.07	14.38** ± 0.08
		% COC	-2.04	-3.14	-2.36	-1.19	-0.01
Liver	Group 1 - Control		22.13 ± 0.04	22.19 ± 0.05	22.27 ± 0.06	23.12 ± 0.04	29.42 ± 0.05
	Group II - Chlorpyrifos		16.18** ± 0.06	17.27** ± 0.05	17.88 ± 0.04	19.15 ± 0.06	19.85** ± 0.04
		% COC	-26.88	-22.17	-19.71	-31.89	-32.52
	Group III - Chlorpyrifos + Tribulus terrtri		14.11** ± 0.02	14.65** ± 0.06	15.43** ± 0.04	15.77** ± 0.04	17.12** ± 0.05
		% COC	-36.24	+81.63	-30.71	-43.91	-41.80
	Group IV - Tribulus terrtri		21.80** ± 0.04	21.87** ± 0.06	21.66** ± 0.05	27.45** ± 0.05	28.86** ± 0.06
		% COC	-30.28	-1.44	-2.73	-2.38	-1.90
Kidney	Group 1 - Control		17.55 ± 0.04	17.58 ± 0.05	17.46 ± 0.06	17.35 ± 0.05	17.69 ± 0.04
	Group II - Chlorpyrifos		16.22** ± 0.22	16.09** ± 0.03	16.02** ± 0.04	16.38** ± 0.05	16.12** ± 0.05
		% COC	-7.57	-5.68	-8.24	-5.59	-8.87
	Group III - Chlorpyrifos + Tribulus terrtri		15.49** ± 0.03	15.85** ± 0.04	14.89** ± 0.06	14.66** ± 0.04	15.96** ± 0.06
		% COC	-11.73	-9.84	-14.71	-15.50	-9.77
	Group IV - Tribulus terrtri		17.42** ± 0.02	17.39* ± 0.03	17.29** ± 0.05	17.26** ± 0.06	17.02** ± 0.08
		% COC	- 0.07	-1.08	-0.97	-0.51	-3.78
Muscle	Group 1 - Control		1.66 ± 0.01	1.68 ± 0.03	1.71 ± 0.03	1.74 ± 0.04	1.78 ± 0.05
	Group II - Chlorpyrifos		1.48** ± 0.02	1.52** ± 0.03	1.55** ± 0.34	1.49** ± 0.04	1.58** ± 0.03
		% COC	-10.84	-9.52	-9.35	-14.36	-11.23
	Group III - Chlorpyrifos + Tribulus terrtri		1.52** ± 0.03	1.56** ± 0.04	1.58** ± 0.05	1.62** ± 0.06	1.66** ± 0.07
		% COC	-8.43	-7.14	-7.60	-6.89	-6.74
	Group IV - Tribulus terrtri		1.64** ± 0.02	1.62* ± 0.03	1.69** ± 0.04	1.71** ± 0.05	1.73** ±
		% COC	-1.20	-3.57	-1.16	-1.72	-2.80

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

Table 2. Changes in the level of lipid peroxidation (nmole/mg protein) content in the freshwater fish *Oriochromis mossambicus* fingerlings on the effect of chlorpyrifos and antidote *tribulus terrtri* exposed to 120 hrs sublethal concentrations

Tissues	Groups		Hours of exposure				
			24	48	72	96	120
Gill	Group 1 - Control		0.094 ± 0.06	0.109 ± 0.08	0.113 ± 0.07	0.120 ± 0.09	0.125 ± 0.09
	Group II - Chlorpyrifos		0.125 ± 0.08	0.142** ± 0.09	0.148** ± 0.07	0.164** ± 0.06	0.187** ± 0.07
		% COC	+73.40	+30.27	+30.97	+36.66	+49.60
	Group III - Chlorpyrifos + Tribulus terrtri		0.068* ± 0.08	0.074* ± 0.07	0.083* ± 0.05	0.093* ± 0.04	0.108* ± 0.05
		% COC	-27.65	-32.11	-26.54	-22.5	-13.6
	Group IV - Tribulus terrtri		0.091** ± 0.08	0.119** ± 0.07	0.122* ± 0.09	0.129* ± 0.08	0.132* ± 0.06
		% COC	-3.19	-9.17	-7.96	-7.50	-5.60
Liver	Group 1 - Control		0.045 ± 0.05	0.049 ± 0.06	0.054 ± 0.05	0.062 ± 0.07	0.069 ± 0.08
	Group II - Chlorpyrifos		0.079 ± 0.07	0.087 ± 0.06	0.095 ± 0.09	0.116 ± 0.08	0.123 ± 0.07
		% COC	+75.55	+77.55	+75.92	+87.09	+78.26
	Group III - Chlorpyrifos + Tribulus terrtri		0.083** ± 0.04	0.089** ± 0.06	0.092** ± 0.05	0.108** ± 0.07	0.116* ± 0.09
		% COC	+84.44	+81.63	+70.37	+74.19	+68.11
	Group IV - Tribulus terrtri		0.038** ± 0.03	0.042** ± 0.05	0.049** ± 0.05	0.054** ± 0.06	0.058** ± 0.06
		% COC	-15.55	-14.28	-9.25	-12.90	-15.94
Kidney	Group 1 - Control		1.58 ± 0.22	1.75 ± 0.19	1.85 ± 0.42	1.89 ± 0.32	1.93 ± 0.40
	Group II - Chlorpyrifos		3.65 ± 0.22	4.17 ± 0.21	6.75 ± 0.22	8.32 ± 0.25	11.86 ± 0.29
		% COC	+131.01	+138.28	+264.86	+340.21	+514.50
	Group III - Chlorpyrifos + Tribulus terrtri		0.98** ± 0.16	1.42** ± 0.14	1.64** ± 0.15	2.12** ± 0.19	2.46** ± 0.17
		% COC	-37.97	-18.85	+11.35	-12.16	-27.46
	Group IV - Tribulus terrtri		1.47** ± 0.14	1.54** ± 0.13	1.91** ± 0.17	1.85** ± 0.19	1.90** ± 0.18
		% COC	- 6.96	-12.0	-3.24	-2.11	-1.55
Muscle	Group 1 - Control		1.80 ± 0.22	1.93 ± 0.26	2.12 ± 0.25	2.19 ± 0.19	2.35 ± 0.32
	Group II - Chlorpyrifos		5.71** ± 0.26	5.95* ± 0.29	1.1 ± 0.33	1.209 ± 0.03	1.220 ± 0.04
		% COC	+217.22	+208.29	+ 157.88	+159.44	+160.12
	Group III - Chlorpyrifos + Tribulus terrtri		0.413** ± 0.04	0.419* ± 0.06	0.425** ± 0.05	0.429** ± 0.06	0.432** ± 0.07
		% COC	-9.23	-8.71	-8.20	-7.93	-7.88
	Group IV - Tribulus terrtri		0.444* ± 0.05	0.453* ± 0.08	0.459* ± 0.08	0.464* ± 0.09	0.473* ± 0.08
		% COC	-2.41	-1.30	-0.86	-0.42	-0.85

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

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Table 3. Changes in the level of catalase (U/min/mg protein) activity in the freshwater fish *Oriochromis mossambicus* fingerlings on the effect of chlorpyrifos and antidote tribulus terrtri exposed to 120 hrs sublethal concentrations

Tissues	Groups		Hours of exposure				
			24	48	72	96	120
Gill	Group 1 - Control		0.152 ± 0.02	0.156 ± 0.04	0.159 ± 0.03	0.163 ± 0.06	0.165 ± 0.08
	Group II - Chlorpyrifos		0.207** ± 0.06	0.218** ± 0.05	0.231** ± 0.04	0.242** ± 0.03	0.249** ± 0.06
		% COC	+36.18	+39.74	+45.28	+48.46	+50.90
	Group III - Chlorpyrifos + Tribulus terrtri		0.167** ± 0.04	0.172** ± 0.05	0.179** ± 0.04	0.182** ± 0.02	0.187** ± 0.04
		% COC	+9.86	+10.25	+12.57	+11.65	+13.33
	Group IV - Tribulus terrtri		0.154** ± 0.02	0.158** ± 0.04	0.162** ± 0.03	0.166** ± 0.05	0.169** ± 0.07
		% COC	+-1.31	+1.28	+1.88	+1.84	+0.24
Liver	Group 1 - Control		0.136 ± 0.03	0.139 ± 0.02	0.144 ± 0.04	0.148 ± 0.05	0.152 ± 0.04
	Group II - Chlorpyrifos		0.177** ± 0.03	0.184** ± 0.05	0.192** ± 0.04	0.205** ± 0.05	0.217** ± 0.05
		% COC	+30.14	+32.37	+33.33	+38.51	+42.76
	Group III - Chlorpyrifos + Tribulus terrtri		0.155** ± 0.06	0.159** ± 0.07	0.162** ± 0.06	0.167** ± 0.05	0.172** ± 0.06
		% COC	+13.97	+14.38	+12.5	+12.83	+13.15
	Group IV - Tribulus terrtri		0.134** ± 0.02	0.137** ± 0.04	0.146** ± 0.03	0.151** ± 0.04	0.154** ± 0.05
		% COC	-1.47	-1.43	+1.38	+2.02	+1.31
Kidney	Group 1 - Control		0.603 ± 0.09	0.608 ± 0.08	0.612 ± 0.09	0.615 ± 0.09	0.619 ± 0.08
	Group II - Chlorpyrifos		0.457* ± 0.07	0.462* ± 0.06	0.469* ± 0.05	0.482* ± 0.07	0.495* ± 0.06
		% COC	-24.21	-24.01	-23.36	-21.62	-20.03
	Group III - Chlorpyrifos + Tribulus terrtri		0.635* ± 1.5	0.642* ± 1.7	0.651* ± 1.5	0.659* ± 1.8	0.673* ± 1.7
		% COC	+5.03	+5.59	+6.37	+7.15	+8.72
	Group IV - Tribulus terrtri		0.605* ± 0.09	0.614* ± 0.08	0.616* ± 0.09	0.618* ± 0.08	0.624* ± 0.09
		% COC	+0.33	+0.98	+0.65	+0.48	+0.80
Muscle	Group 1 - Control		0.376 ± 0.05	0.379 ± 0.04	0.382 ± 0.04	0.387 ± 0.05	0.394 ± 0.05
	Group II - Chlorpyrifos		0.526* ± 0.07	0.534* ± 0.05	0.541* ± 0.06	0.549* ± 0.07	0.562* ± 0.06
		% COC	+39.89	+40.89	+41.62	+41.86	+42.63
	Group III - Chlorpyrifos + Tribulus terrtri		0.412* ± 0.07	0.418* ± 0.06	0.424* ± 0.05	0.431* ± 0.04	0.439* ± 0.05
		% COC	+11.17	+10.29	+10.99	+11.36	+11.42
	Group IV - Tribulus terrtri		0.373** ± 0.06	0.374** ± 0.05	0.386** ± 0.07	0.389** ± 0.08	0.396** ± 0.09
		% COC	-0.79	-1.31	+1.04	+0.51	+0.50

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

Table 4. Changes in the level of reduced glutathione (µg/mg protein) content in the freshwater fish *Oriochromis mossambicus* fingerlings on the effect of chlorpyrifos and antidote tribulus terrtri exposed to 120 hrs sublethal concentrations

Tissues	Groups		Hours of exposure				
			24	48	72	96	120
Gill	Group 1 - Control		0.094 ± 0.001	0.099 ± 0.002	0.117 ± 0.008	0.124 ± 0.009	0.129 ± 0.008
	Group II - Chlorpyrifos		0.054** ± 0.001	0.056** ± 0.002	0.059** ± 0.002	0.064** ± 0.003	0.068** ± 0.004
		% COC	-42.55	-43.43	-49.57	-48.38	-47.28
	Group III - Chlorpyrifos + Tribulus terrtri		0.086** ± 0.003	0.089** ± 0.004	0.092** ± 0.003	0.098** ± 0.003	0.112** ± 0.007
		% COC	-8.15	-10.11	-21.36	-20.96	-13.17
	Group IV - Tribulus terrtri		0.091** ± 0.003	0.095** ± 0.003	0.120** ± 0.008	0.118** ± 0.008	0.122** ± 0.008
		% COC	-3.19	-4.04	-0.120	-4.83	-0.54
Liver	Group 1 - Control		0.044±0.001	0.049 ± 0.002	0.052 ± 0.003	0.055 ± 0.003	0.059± 0.003
	Group II - Chlorpyrifos		0.077** ± 0.002	0.084** ± 0.003	0.089** ± 0.004	0.099** ± 0.003	0.114** ± 0.008
		% COC	+75.00	+71.42	+71.15	+80.00	+93.22
	Group III - Chlorpyrifos + Tribulus terrtri		0.086** ± 0.002	0.089** ± 0.002	0.072** ± 0.003	0.077** ± 0.003	0.094** ± 0.004
		% COC	+95.45	+81.63	+38.46	+40.00	+59.32
	Group IV - Tribulus terrtri		0.048** ± 0.001	0.054** ± 0.002	0.056** ± 0.002	0.057** ± 0.003	0.063** ± 0.003
		% COC	+9.09	+10.20	+7.69	+3.63	+6.77
Kidney	Group 1 - Control		52.33 ± 1.12	54.45 ± 1.12	63.18 ± 1.18	67.25 ± 1.17	75.35 ± 1.19
	Group II - Chlorpyrifos		60.15** ± 1.36	64.33** ± 1.35	68.45** ± 1.36	78.27** ± 1.38	84.38** ± 1.39
		% COC	+14.94	+18.14	+8.34	+16.38	+11.93
	Group III - Chlorpyrifos + Tribulus terrtri		48.41** ± 2.15	57.42** ± 2.18	67.23** ± 3.11	75.14** ± 3.15	87.72** ± 3.17
		% COC	-7.49	-5.45	+6.41	+11.73	+16.37
	Group IV - Tribulus terrtri		54.15** ± 1.38	55.12** ± 1.39	58.45** ± 1.40	63.15** ± 1.38	69.43** ± 1.39
		% COC	+3.47	+1.23	-7.48	-6.09	-7.89
Muscle	Group 1 - Control		0.112 ± 0.008	0.117 ± 0.008	0.122 ± 0.010	0.125 ± 0.009	0.129 ± 0.009
	Group II - Chlorpyrifos		0.097** ± 0.001	0.110** ± 0.0008	0.116** ± 0.009	0.119** ± 0.008	0.124** ± 0.009
		% COC	-13.09	-5.98	-5.73	-4.80	-3.87
	Group III - Chlorpyrifos + Tribulus terrtri		0.096** ± 0.002	0.102** ± 0.006	0.115** ± 0.007	0.118** ± 0.008	0.122** ± 0.008
		% COC	-14.28	-12.82	-6.55	-5.60	-5.42
	Group IV - Tribulus terrtri		0.099** ± 0.002	0.113** ± 0.007	0.119** ± 0.008	0.122** ± 0.009	0.126** ± 0.008
		% COC	-11.60	-3.40	-2.45	-2.40	-2.32

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

Table 5. Changes in the level of glutathione peroxidase (µg/min/mg protein) activity in the freshwater fish *Oriochromis mossambicus* fingerlings on the effect of chlorpyrifos and antidote tribulus terrtri exposed to 120 hrs sublethal concentrations

Tissues	Groups		Hours of exposure 24	48	72	96	120
Gill	Group 1 - Control		0.054 ± 0.05	0.056 ± 0.07	0.059 ± 0.07	0.064 ± 0.08	0.068 ± 0.07
	Group II - Chlorpyrifos	% COC	0.094** ± 0.08 + 74.07	0.099** ± 0.09 +76.78	0.117** ± 0.05 +98.30	0.124** ± 0.08 +93.75	0.129** ± 0.07 +89.70
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.086** ± 0.04 +59.25	0.089** ± 0.07 +58.92	0.092** ± 0.05 +0.55	0.098** ± 0.06 +53.12	0.112** ± 0.07 +64.70
	Group IV - Tribulus terrtri	% COC	0.052** ± 0.07 -3.70	0.057** ± 0.06 -1.78	0.055** ± 0.05 -6.77	0.063** ± 0.04 -1.56	0.066** ± 0.08 -2.94
Liver	Group 1 - Control		0.044 ± 0.05	0.049 ± 0.06	0.052 ± 0.07	0.054 ± 0.07	0.059 ± 0.09
	Group II - Chlorpyrifos	% COC	0.077** ± 0.07 +75.00	0.084** ± 0.06 +71.42	0.089** ± 0.08 +71.15	0.094** ± 0.05 +74.07	0.098** ± 0.07 +66.10
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.028** ± 0.04 -36.37	0.033** ± 0.06 -32.65	0.037** ± 0.09 -28.84	0.042** ± 0.07 -22.23	0.046** ± 0.08 -22.03
	Group IV - Tribulus terrtri	% COC	0.041** ± 0.03 +6.81	0.043** ± 0.06 -12.24	0.049** ± 0.05 -5.76	0.057** ± 0.08 +5.56	0.061** ± 0.06 +3.38
Kidney	Group 1 - Control		0.223 ± 0.03	0.226 ± 0.0.04	0.229 ± 0.04	0.231 ± 0.05	0.239 ± 0.05
	Group II - Chlorpyrifos	% COC	0.412** ± 0.06 +84.75	0.422** ± 0.06 +86.72	0.428** ± 0.08 +86.89	0.434** ± 0.08 +87.86	0.442** ± 0.09 +84.93
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.324** ± 0.02 +45.29	0.329** ± 0.04 +45.57	0.334** ± 0.06 +45.85	0.338** ± 0.06 +46.32	0.403** ± 0.05 +68.61
	Group IV - Tribulus terrtri	% COC	0.220** ± 0.03 -1.34	0.224** ± 0.03 -0.88	0.235** ± 0.06 +2.62	0.247** ± 0.05 +6.92	0.251** ± 0.04 +5.02
Muscle	Group 1 - Control		0.177 ± 0.04	0.179 ± 0.06	0.184 ± 0.05	0.188 ± 0.07	0.192± 0.06
	Group II - Chlorpyrifos	% COC	0.221** ± 0.03 +24.85	0.227** ± 0.05 +26.81	0.235** ± 0.07 +27.71	0.239** ± 0.09 +27.12	0.245** ± 0.07 +27.60
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.145** ± 0.04 -18.07	0.149** ± 0.07 -16.75	0.153** ± 0.06 -16.84	0.156** ± 0.06 -17.02	0.161** ± 0.08 -16.14
	Group IV - Tribulus terrtri	% COC	0.170** ± 0.02 -3.95	0.174** ± 0.03 -2.79	0.178** ± 0.05 -3.26	0.183** ± 0.06 -2.65	0.188** ± 0.05 -2.08

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

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Table 6. Changes in the level of acid phosphatase (µmole/min/mg protein) activity in the freshwater fish *Oriochromis mossambicus* fingerlings on the effect of chlorpyrifos and antidote tribulus terrtri exposed to 120 hrs sublethal concentrations

Tissues	Groups		Hours of exposure				
			24	48	72	96	120
Gill	Group 1 – Control		0.173 ± 0.03	0.175 ± 0.04	0.184 ± 0.04	0.189 ± 0.03	0.193 ± 0.05
	Group II - Chlorpyrifos	% COC	0.262** ± 0.05 +51.44	0.268** ± 0.06 +53.14	0.272** ± 0.07 +47.82	0.286** ± 0.05 +51.32	0.295** ± 0.07 +52.84
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.215** ± 0.05 +24.27	0.218** ± 0.07 +24.57	0.221** ± 0.06 +20.10	0.226** ± 0.07 +19.57	0.230** ± 0.05 +19.17
	Group IV - Tribulus terrtri	% COC	0.162** ± 0.04 -6.35	0.165** ± 0.05 -5.71	0.173** ± 0.06 -5.91	0.183** ± 0.07 -3.17	0.188** ± 0.06 -2.59
Liver	Group 1 - Control		0.432 ± 0.09	0.437 ± 0.07	0.442 ± 0.08	0.443 ± 0.09	0.451 ± 0.08
	Group II - Chlorpyrifos	% COC	0.515** ± 0.09 +19.21	0.522** ± 1.50 +19.45	0.536** ± 1.70 +25.79	0.564** ± 1.13 +25.89	0.572** ± 1.16 +26.88
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.455** ± 0.07 +5.32	0.462** ± 0.07 +5.72	0.467** ± 0.05 +5.65	0.474** ± 0.05 +5.80	0.479** ± 0.06 +6.20
	Group IV - Tribulus terrtri	% COC	0.446** ± 0.07 +3.24	0.449** ± 0.07 +2.75	0.455** ± 0.06 +2.94	0.459** ± 0.05 +2.00	0.459** ± 0.07 +1.77
Kidney	Group 1 - Control		0.149 ± 0.02	0.154 ± 0.02	0.158 ± 0.03	0.162 ± 0.04	0.165 ± 0.05
	Group II - Chlorpyrifos	% COC	0.244** ± 0.05 +50.33	0.229** ± 0.05 +48.70	0.232** ± 0.04 +46.83	0.239** ± 0.04 +47.53	0.244** ± 0.06 +47.87
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.156** ± 0.04 14.69	0.164** ± 0.03 +6.49	0.168** ± 0.04 6.32	0.171** ± 0.03 +5.55	0.174** ± 0.04 +5.45
	Group IV - Tribulus terrtri	% COC	0.151** ± 0.05 +1.34	0.157** ± 0.04 +1.94	0.154** ± 0.03 +2.53	0.163** ± 0.03 +0.61	0.161** ± 0.04 -2.42
Muscle	Group 1 - Control		0.455 ± 0.05	0.459 ± 0.05	0.463 ± 0.03	0.466 ± 0.06	0.469 ± 0.07
	Group II - Chlorpyrifos	% COC	1.185** ± 0.02 +160.43	1.189** ± 0.03 +159.04	1.194** ± 0.07 +157.88	1.209** ± 0.03 +159.44	1.220** ± 0.04 +160.12
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.413** ± 0.04 -9.23	0.419** ± 0.06 -8.71	0.425** ± 0.05 -8.20	0.429** ± 0.06 -7.93	0.432** ± 0.07 -7.88
	Group IV - Tribulus terrtri	% COC	0.444** ± 0.05 -2.41	0.453** ± 0.08 -1.30	0.459** ± 0.08 -0.86	0.464** ± 0.09 -0.42	0.473** ± 0.08 +0.85

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

Table 7. Changes in the level of alkaline phosphatase (µmole/min/mg protein) activity in the freshwater fish *Oriochromis mossambicus* fingerlings on the effect of chlorpyrifos and antidote tribulus terrtri exposed to 120 hrs sublethal concentrations

Tissues	Groups		Hours of exposure				
			24	48	72	96	120
Gill	Group 1 – Control		0.133 ± 0.03	0.139 ± 0.04	0.143 ± 0.04	0.151 ± 0.03	0.156 ± 0.05
	Group II - Chlorpyrifos	% COC	0.162**± 0.05 +21.80	0.168** ± 0.06 +20.86	0.182**± 0.07 +27.28	0.196** ± 0.05 +29.80	0.217** ± 0.07 +39.10
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.145** ± 0.05 +9.02	0.152** ± 0.07 +9.35	0.162** ± 0.07 +13.28	0.176** ± 0.05 +16.55	0.185** ± 0.05 +18.58
	Group IV - Tribulus terrtri	% COC	0.122** ± 0.04 -8.27	0.135** ± 0.05 -2.87	0.147** ± 0.06 +2.79	0.159** ± 0.07 +5.29	0.163** ± 0.06 +4.48
Liver	Group 1 - Control		0.086** ± 0.03	0.088** ± 0.03	0.093** ± 0.06	0.096** ± 0.05	0.099** ± 0.06
	Group II - Chlorpyrifos	% COC	0.115** ± 0.04 +33.72	0.132** ± 1.5 +50.00	0.146** ± 1.7 +56.98	0.169** ± 1.13 +76.04	0.182** ± 1.16 +83.38
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.120** ± 0.02 +39.53	0.149** ± 0.05 +69.31	0.153** ± 0.04 +64.51	0.174** ± 0.05 +81.25	0.198** ± 0.05 +97.98
	Group IV - Tribulus terrtri	% COC	0.078** ± 0.04 -9.30	0.099** ± 0.06 -12.5	0.087** ± 0.06 -6.45	0.094** ± 0.08 -2.08	0.112** ± 0.07 +13.14
Kidney	Group 1 - Control		0.149 ± 0.02	0.164 ± 0.04	0.178± 0.06	0.182 ± 0.05	0.195 ± 0.07
	Group II - Chlorpyrifos	% COC	0.244** ± 0.06 +63.75	0.259** ± 0.08 +57.92	0.272**± 0.09 +52.80	0.267** ± 0.07 +46.70	0.284** ± 0.06 +45.64
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.166** ± 0.04 +11.40	0.174** ± 0.06 +6.09	0.188** ± 0.05 +5.61	0.191** ± 0.09 +4.94	0.224** ± 0.08 +14.87
	Group IV - Tribulus terrtri	% COC	0.135** ± 0.03 -9.39	0.157** ± 0.06 -4.26	0.174** ± 0.07 -2.24	0.176** ± 0.05 -3.29	0.181** ± 0.04 -7.17
Muscle	Group 1 - Control		0.255 ± 0.04	0.259 ± 0.06	0.268 ± 0.06	0.276 ± 0.08	0.289 ± 0.07
	Group II - Chlorpyrifos	% COC	0.385** ± 0.02 +71.12	0.429** ± 0.03 +65.63	0.454** ± 0.04 +69.40	0.479** ± 0.03 +73.55	0.488** ± 0.05 +68.85
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	0.313** ± 0.03 +22.74	0.349** ± 0.05 +34.74	0.365** ± 0.06 +36.19	0.383** ± 0.08 +38.76	0.392** ± 0.07 +35.64
	Group IV - Tribulus terrtri	% COC	0.244** ± 0.04 -4.31	0.253** ± 0.06 -2.31	0.259** ± 0.05 -3.35	0.264** ± 0.07 -4.34	0.273** ± 0.08 -5.53

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

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Table 8. Changes in the level of acetylcholine (μmole/mg protein/hr) activity in the freshwater fish *Oriochromis mossambicus* fingerlings on the effect of chlorpyrifos and antidote tribulus terrtri exposed to 120 hrs sublethal concentration

Tissues	Groups		Hours of exposure 24	48	72	96	120
Gill	Group 1 – Control		23.11 ± 0.04	23.18 ± 0.05	23.25 ± 0.06	23.56 ± 0.07	23.87 ± 0.05
	Group II - Chlorpyrifos	% COC	46.15** ± 0.03 +99.69	48.42** ± 0.07 +108.88	52.19** ± 0.03 +124.47	54.85** ± 0.06 +132.80	62.17** ± 0.04 +160.45
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	34.42** ± 0.04 +48.93	5.33** ± 0.05 +52.41	37.96**± 0.06 +63.26	48.17** ± 0.04 +104.45	51.75** ± 0.07 +116.79
	Group IV - Tribulus terrtri	% COC	20.14** ± 0.04 -12.85	21.49** ± 0.06 -7.29	22.35** ± 0.07 -3.87	22.67**± 0.08 -3.77	23.95** ± 0.09 -0.33
Liver	Group 1 – Control		19.14 ± 0.03	19.42 ± 0.04	19.64 ± 0.06	20.19 ± 0.04	28.39 ± 0.07
	Group II - Chlorpyrifos	% COC	35.19** ± 0.04 +83.85	35.89** ± 0.05 +84.80	42.11** ± 0.07 +114.40	51.45** ± 0.08 +154.82	61.19** ± 0.08 +115.53
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	25.39** ± 0.03 +32.65	27.12** ± 0.03 +39.64	32.49** ± 0.05 +65.42	38.17**± 0.07 +89.05	45.27** ± 0.06 +59.45
	Group IV - Tribulus terrtri	% COC	18.01±0.04 -5.90	18.13** ± 0.03 -6.64	20.14** ± 0.04 -3.92	22.14**± 0.04 -9.65	22.19** ± 0.06 -6.34
Brain	Group 1 - Control		34.04 ± 0.03	36.07 ± 0.04	37.12 ± 0.06	39.16 ± 0.08	40.72 ± 0.07
	Group II - Chlorpyrifos	% COC	62.46** ± 0.04 +83.49	72.35** ± 0.05 +100.58	80.76** ± 0.06 +117.56	95.12** ± 0.05 +142.90	122.85** ± 0.06 +201.69
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	68.37**± 0.05 +100.85	83.13** ± 0.06 +130.46	92.46**± 0.07 +149.08	112.14** ± 0.08 +186.36	134.48** ± 0.07 +230.25
	Group IV - Tribulus terrtri	% COC	32.12**± 0.04 -5.64	34.86** ± 0.05 -3.35	38.72** ± 0.06 -1.07	38.56** ± 0.07 -1.53	39.79** ± 0.05 -2.28
Kidney	Group 1 - Control		22.45 ± 0.04	22.51 ± 0.03	22.63 ± 0.06	22.74 ± 0.05	22.85 ± 0.06
	Group II - Chlorpyrifos	% COC	51.46** ± 0.06 +129.22	62.19** ± 0.07 +176.27	62.13** ± 0.08 +232.61	75.27** ± 0.04 +263.63	82.69** ± 0.06 +266.56
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	44.19**± 0.04 +96.83	44.37** ± 0.05 +97.11	58.49**± 0.06 +158.46	67.25** ± 0.08 +195.73	77.59** ± 0.05 +239.56
	Group IV - Tribulus terrtri	% COC	19.49**± 0.02 -13.18	20.17** ± 0.04 -10.39	22.09** ± 0.04 -2.38	22.56** ± 0.07 -0.79	23.45** ± 0.08 -2.62
Muscle	Group 1 - Control		27.05 ± 1.47	28.19 ± 1.45	28.74 ± 1.59	28.85 ± 1.73	29.19 ± 1.69
	Group II - Chlorpyrifos	% COC	34.24** ± 2.46 +29.45	36.49**± 2.58 +28.37	38.27** ± 4.56 +33.15	41.29** ± 2.58 +43.11	51.75** ± 4.58 +77.28
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	30.19** ± 1.38 +11.08	32.39** ± 5.29 +15.25	35.09** ± 4.32 +23.13	35.25** ± 8.16 +25.64	44.21** ± 4.89 +51.45
	Group IV - Tribulus terrtri	% COC	24.07** ± 1.35 -11.01	25.29** ± 3.32 -10.32	27.45** ± 3.38 -4.48	28.14 ± 4.69 -2.46	28.67** ± 4.86 -1.78

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Table 9. Changes in the level of acetylcholinesterase (µmole/mg protein/hr) activity in the freshwater fish *Oriochromis mossambicus* fingerlings on the effect of chlorpyrifos and antidote tribulus terrtri exposed to 120 hrs sublethal concentration

Tissues	Groups		Hours of exposure 24	48	72	96	120
Gill	Group 1 – Control		2.44 ± 0.06	2.49 ± 0.03	2.65 ± 0.06	2.73 ± 0.05	2.89 ± 0.05
	Group II - Chlorpyrifos	% COC	4.65** ± 0.04 +90.57	4.87**± 0.05 +95.58	5.25** ± 0.04 +98.11	6.87** ± 0.04 +151.64	7.85** ± 0.05 +171.62
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	5.12** ± 0.04 +109.83	5.45** ± 0.06 +118.87	7.18** ± 0.03 +170.94	8.45** ± 0.05 +209.52	9.17** ± 0.04 +217.30
	Group IV - Tribulus terrtri	% COC	2.12**± 0.04 -13.11	2.42** ± 0.05 -2.81	2.56** ± 0.07 -3.39	2.62** ± 0.08 -4.02	2.72** ± 0.06 -5.58
Liver	Group 1 - Control		1.69 ± 0.02	1.75 ± 0.04	1.89 ± 0.05	2.12 ± 0.04	2.46 ± 0.05
	Group II - Chlorpyrifos	% COC	2.46** ± 0.03 + 45.56	2.79** ± 0.03 +59.42	3.34** ± 0.05 +76.71	3.67** ± 0.07 +73.11	4.85** ± 0.06 +97.15
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	3.19** ± 0.04 +88.75	3.46** ± 0.06 +97.71	3.79** ± 0.07 +100.52	4.13** ± 0.04 +94.81	5.79** ± 0.05 +135.36
	Group IV - Tribulus terrtri	% COC	1.89** ± 0.04 -11.83	1.97** ± 0.03 -12.57	2.12** ± 0.04 -12.16	2.17**± 0.04 -2.35	2.24** ± 0.06 -8.94
Brain	Group 1 - Control		6.28 ± 0.05	6.77 ± 0.07	8.44 ± 0.06	9.12 ± 0.07	10.42 ± 0.05
	Group II - Chlorpyrifos	% COC	4.75** ± 0.04 -24.36	5.33** ± 0.06 -27.27	6.45** ± 0.08 -23.57	7.88** ± 0.09 -13.59	9.36** ± 0.07 -10.17
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	8.42**± 0.02 +34.07	8.96** ± 0.03 +32.34	10.89**± 0.04 +29.02	10.98** ± 0.05 +20.39	12.18** ± 0.05 +16.89
	Group IV - Tribulus terrtri	% COC	6.12**± 0.03 -2.54	6.40** ± 0.04 -5.46	8.13** ± 0.06 -3.67	9.03** ± 0.07 -0.98	10.22** ± 0.08 -1.91
Kidney	Group 1 - Control		2.68 ± 0.06	2.73 ± 0.05	2.85 ± 0.06	3.05 ± 0.04	3.25 ± 0.06
	Group II - Chlorpyrifos	% COC	2.15** ± 0.04 -19.77	2.48** ± 0.08 -9.15	2.59** ± 0.06 -9.12	2.95** ± 0.07 -3.27	3.14** ± 0.05 -3.38
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	2.96** ± 0.08 +10.44	2.84** ± 0.07 +4.76	2.47** ± 0.09 +13.34	3.14** ± 0.04 +11.37	3.17** ± 0.05 +16.30
	Group IV - Tribulus terrtri	% COC	2.41** ± 0.03 -10.07	2.56** ± 0.05 -6.22	2.67**± 0.04 -6.31	2.89** ± 0.08 -5.24	3.14** ± 0.09 -3.38
Muscle	Group 1 - Control		2.65 ± 1.65	2.45 ± 1.75	2.56 ± 1.79	3.11 ± 1.83	3.27 ± 1.89
	Group II - Chlorpyrifos	% COC	3.07** ± 1.34 +15.84	3.27** ± 5.23 +33.46	3.89** ± 4.32 +51.95	4.12** ± 8.16 +32.47	4.45** ± 4.89 +36.08
	Group III - Chlorpyrifos + Tribulus terrtri	% COC	4.18** ± 2.36 +57.73	3.85** ± 2.48 +57.14	3.76** ± 4.56 +46.87	4.85** ± 2.78 +55.94	5.14** ± 4.58 +57.18
	Group IV - Tribulus terrtri	% COC	2.28** ± 1.25 -13.96	2.15** ± 3.42 -12.24	3.26** ± 3.38 -27.34	3.65 ± 4.59 -17.36	4.12** ± 4.96 -25.99

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over contro

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