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Toxic Effect of Insecticides Mixtures on Antioxidant Enzymes in Different Organs of Fish, *Labeo rohita*

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Authors' Contribution
HN executed the research. SA supervisor and planned the research. Ka facilitated in conducting the research work in his laboratory. WH and SP help in compiling data. MB, S Maalik and S Mushtaq helped in writing the article.

Key words
Acute toxicity, Fish, Catalase, Glutathione S-transferase, Peroxidase, Superoxide dismutase, Pesticides mixture.

ABSTRACT

In this study insecticides toxic effects of mixture of organochlorine (endosulfan) and organophosphate (chlorpyrifos) investigated on the activity of antioxidant enzymes viz. superoxide dismutase, peroxidase, catalase and glutathione S-transferase in different organs (liver, gills, kidney, brain, heart and muscle) of fish, *Labeo rohita*. The LC_{50} of chlorpyrifos+endosulfan mixture was calculated as 1.95±0.02 μgL^{-1} for 96 h with the 95% confidence limits. The fish expose to the mixture (1:1) for 96-h. The results obtained from this study showed that superoxide dismutase, peroxidase and glutathione S-transferase activities in the liver, gills, kidney, brain, heart and muscle were significantly ($P<0.05$) increased compared to control. The superoxide dismutase activity in organs of fish followed the order: liver>brain>kidney>gills>heart>muscle. The mean peroxidase activity in *L. rohita* followed the pattern: liver>brain>gills>kidney>heart>muscle. The glutathione S-transferse activity followed the order: brain>muscle>liver>gills>kidney>heart. However, catalase activity was significantly ($P<0.05$) increased in liver, gills and kidney of pesticides mixture exposed *L. rohita* as compared to control while it was decreased in brain, heart and muscle.

INTRODUCTION

In present era, rapid development of industries and green revolution had led to serious environmental problems, water contamination being the most important (Samantha *et al*., 2005). The potentially harmful agrochemical chemicals such as pesticides are released in to the freshwater environment and had significantly unfavorable effects on non-target species like aquatic animals (John, 2007; Naz and Javed, 2012).

Endosulfan belong to organochlorine pesticides, is known be highly toxic to fish (Wan *et al*., 2005). Chlorpyrifos is widely used organophosphate pesticide, is a non-systemic insecticide designed to be effective by direct contact, ingestion and inhalation (Tomlin, 2006). This pesticide inhibits the AChE activity (Barata *et al*., 2004).

Fish are the keystone species which reflect the toxic effect of chemicals in water bodies (Slaninova *et al*., 2009). Pesticides may induce the production of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals (Kumar *et al*., 2011). The production of ROS in aquatic organisms exposed to pesticides is linked with the existence of toxicants and causes oxidative stress a possible mechanism of toxicity (Oropesa *et al*., 2008). The defensive mechanism of fish against free radicals consists of superoxide superoxide dismutase, catalase, peroxidase and glutathione S-transferase (Guven *et al*., 2008). Superoxide dismutase is primary enzyme, responsible for transformation of the O^{-2} into H_2O_2. Hydrogen peroxide further transformed into oxygen and water by catalase. Peroxidase also converts the group of peroxides, including hydrogen peroxide (Hermes, 2004; Maran *et al*., 2009). Glutathione-S-transferase is involved in the biotransformation process of toxic substances into less toxic so fish can excrete easily from the body. In present research work, toxic effect of endosulfan and chlorpyrifos on antioxidant enzymes of fish *Labeo rohita* was observed.

MATERIALS AND METHODS

Experimental fish

For semi-static acute toxicity bioassay, fingerlings of fish, *L. rohita* (90-day old; Average weight, 8.24±0.32) were

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obtained from local Fish seed Hatchery and transferred to the Fisheries Research Farm, University of Agriculture, Faisalabad, Pakistan. Fish were acclimatized to laboratory condition for 14 days. During acclimatization period, *L. rohita* were fed with commercial feed (3% of wet body weight). After acclimatization, fish were shifted to glass aquaria containing 70-liter water.

Test chemicals

Technical grade pesticides *viz*. chlorpyrifos (CPF) and endosulfan (END) were used as test chemicals. The methanol was used to prepare stock-1 solution while solutions of CPF+END mixture of required concentrations (10ppm and 1:1 ratio) were prepared by its further dilutions in deionized water.

96-h LC_{50} and lethal toxicity tests

The acute toxicity tests (96-h LC_{50} and lethal concentration) of pesticides mixture for *L. rohita* were performed with three replicates. Sixteen concentrations (0.00, 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60, 1.80, 2.00, 2.20, 2.40, 2.60, 2.80 and 3.00 µg/L) of CPF+END mixture were used. All aquaria were supplied with exceeding aeration for 2 h in order to get a uniform concentration of the insecticides, and then 10 fish were shifted into each aquarium. Observation on fish mortality was assessed at 24, 48, 72 and 96 h after the start of experiment and dead fish were removed instantaneously. Probit Analysis method was applied to calculate the LC_{50} and lethal concentration of CPF+END mixture for *L. rohita*.

Antioxidant enzymes

After determination of 96 h LC_{50} concentration, a group (n=20) of fish were separately, exposed to this concentration for 4-days. For enzymatic study fish (n=5) sampling was done after 24, 48, 72 and 96-h. To calculate the antioxidant enzymes *viz*. superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione S-transferase (GST) were obtained from gills, liver, kidney, brain, heart and muscle of fish. All the organs were, separately, homogenized in cold phosphate buffer (pH 6.5, 0.2 M) by the ratio of 1:4 (w/v) using a blender. After that homogenates were centrifuged at 10,000 rpm and 4°C for 15 min. Clear supernatants were obtained which were used for enzyme assay. The activity of SOD was checked according to the method of Giannopolitis and Ries (1977). CAT activity was measured by its ability to decrease the H_2O_2 concentration at 240 nm (Chance and Mehaly, 1977). The activity of POD was measured by following the procedure of Civello *et al*. (1995). The activity of GST was determined by following the method of Mannervik (1985).

Statistical analyses

Probit analysis method was applied to calculate the tolerance limits (LC_{50} and lethal concentration) of *L. rohita* for CPF+END mixture (Finney, 1971). ANOVA was performed on obtained data to find out the statistical differences among studied variables.

Table I.- Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione S-transferase (GST) (UmL^{-1}±SD) in gills of *Labeo rohita* during acute exposure of chlorpyrifos (CPF)+endosulfan (END) mixture.

Activity		Control	Treated
SOD	24 h	20.34±0.06b	41.79±0.07a
	48 h	20.30±0.08b	57.41±0.23a
	72 h	23.35±0.09b	73.17±0.12a
	96 h	20.37±0.16b	102.29±0.18a
CAT	24 h	185.90±2.01b	210.75±11.69a
	48 h	184.96±1.90b	218.09±11.05a
	72 h	185.97±2.11b	232.95±11.55a
	96 h	185.99±2.15b	244.67±12.35a
POD	24 h	1.46±0.07b	3.15±0.02a
	48 h	1.47±0.13b	4.25±0.05a
	72 h	1.48±0.09b	4.86±0.12a
	96 h	1.49±0.11b	5.95±0.02a
GST	24 h	118.72±1.58b	224.70±1.61a
	48 h	118.73±1.56b	262.48±1.99a
	72 h	118.87±1.33b	313.56±1.85a
	96 h	118.88±1.31b	380.67±1.66a

Means sharing similar letter in a row or in column are statistically non-significant (P>0.05).

RESULTS AND DISCUSSION

96-h LC_{50}

The LC_{50} concentration for 96 h was calculated as 1.95±0.02 μgL^{-1} with the 95% upper and lower confidence limits as 1.685 and 2.144 μgL^{-1}, respectively. The 96 h lethal concentration was estimated as 3.23±0.05 μgL^{-1} with the 95% upper and lower confidence limits as 2.906 and 3.858 μgL^{-1}, respectively. It was observed that the fish mortality increased with increasing the exposure concentration. Water contamination by toxic chemicals caused mass mortality of aquatic fauna like fish (Kumari 2005; Gupta *et al*., 2012). Mortality of fish due to insecticides contact primarily depends upon its tolerance to the chemical, its dose and time of exposure (Al-Rudainy and Kadhim, 2012). Several authors have been conducted experiments on pesticides toxicity (organochlorines, pyrethroids organophospates and carbamides) for many fish species (Ambreen and Javed 2015; Ghazala *et al*., 2014; Shrivastava *et al*., 2002; Shailkh and Yeragi, 2004; Visvanthan *et al*., 2009).

Table II.- SOD, CAT, POD and GST (UmL^{-1}±SD) in liver of *Labeo rohita* during acute exposure of CPF+END mixture.

Activity		Control	Treated
SOD	24 h	47.51±0.11b	90.40±0.12a
	48 h	47.52±0.17b	102.25±0.29a
	72 h	47.54±0.12b	117.32±0.21a
	96 h	47.55±0.12b	129.15±0.27a
CAT	24 h	232.50±2.58b	254.17±11.73a
	48 h	232.51±2.43b	269.70±11.65a
	72 h	232.53±2.48b	273.38±11.90a
	96 h	232.54±2.48b	291.05±12.53a
POD	24 h	1.95±0.03b	3.98±0.11a
	48 h	1.96±0.13b	4.67±0.02a
	72 h	1.97±0.18b	5.63±0.13a
	96 h	1.99±0.09b	6.32±0.06a
GST	24 h	176.67±1.75b	267.57±1.92a
	48 h	176.68±1.73b	301.83±1.47a
	72 h	174.73±1.65b	394.55±1.96a
	96 h	176.72±1.66b	425.35±2.30a

Means sharing similar letter in a row or in column are statistically non-significant (P>0.05).
For abbreviations, see Table I.

Table III.- SOD, CAT, POD and GST (UmL^{-1}±SD) in kidney of *Labeo rohita* during acute exposure of CPF+END mixture.

Activity		Control	Treated
SOD	24 h	25.78±0.07b	55.65±0.08a
	48 h	25.79±0.08b	74.49±0.021a
	72 h	27.83±0.11b	96.08±0.18a
	96 h	27.85±0.09b	117.25±0.23a
CAT	24 h	140.25±1.94b	164.25±10.13a
	48 h	140.26±1.96b	176.95±10.58a
	72 h	140.27±1.97b	183.33±10.47a
	96 h	140.28±1.99b	194.55±11.79a
POD	24 h	1.22±0.09b	3.00±2.65a
	48 h	1.23±0.04b	4.12±0.14a
	72 h	1.21±0.09b	4.74±0.16a
	96 h	1.24±0.12b	5.43±0.11a
GST	24 h	151.66±1.85b	234.66±1.85a
	48 h	151.67±1.83b	292.71±1.76a
	72 h	151.68±1.82b	361.42±2.27a
	96 h	151.69±1.80b	401.00±2.99a

Means sharing similar letter in a row or in column are statistically non-significant (P>0.05).
For abbreviations, see Table I.

Antioxidant enzymes activities

SOD activity

Tables I-VI show effect of insecticide mixture on SOD, CAT, POD and GST in gills, liver, kidney, brain, heart and muscles, respectively of *Labeo rohita*. Results of this study showed that SOD activity significantly (P<0.05) increased in liver, gills, kidney, brain, heart and muscle of CPF+END exposed *L. rohita* compared to control (Tables I-VI). The SOD activity increased in selected organs of fish as following order: liver>brain>kidney>gills>heart>muscle. The increased SOD activity under oxidative stress induced by CPF may be due to conversion of superoxide radicals into hydrogen peroxide which is further transformed by CAT into oxygen and water (Sanchez *et al*., 2005). SOD is also a first crucial antioxidant enzyme which plays an important role to cope with oxiredicals and comprises a basic defense against the lethal effects of reactive oxygen species (ROS) (Kohen and Nyska, 2002). Oruc (2010) also reported the significant increase in the SOD activity in fish exposed to chlorpyrifos. According to Kumar *et al*. (2011) SOD activity increased in bronchi and hepatic tissue of *Oreochromis mossambicus* after acute exposure to endosulfan.

Table IV.- SOD, CAT, POD and GST (UmL^{-1}±SD) in brain of *Labeo rohita* during acute exposure of CPF+END mixture.

Activity		Control	Treated
SOD	24 h	36.28±0.10b	68.74±0.09a
	48 h	26.29±0.13b	85.23±0.23a
	72 h	36.30±0.14b	99.76±0.15a
	96 h	36.31±0.17b	113.34±0.20a
CAT	24 h	126.67±1.79a	110.58±10.01b
	48 h	126.68±1.81a	96.66±10.02b
	72 h	126.69±1.82a	81.11±9.77b
	96 h	126.70±1.84a	72.23±9.42b
POD	24 h	1.75±0.07b	3.82±0.06a
	48 h	1.77±0.05b	4.44±0.13a
	72 h	1.78±0.10b	4.99±0.06a
	96 h	1.79±0.08b	6.06±0.09a
GST	24 h	211.45±2.30b	370.98±1.39a
	48 h	211.46±2.28b	406.17±1.06a
	72 h	211.44±2.31b	473.35±2.47a
	96 h	211.43±2.33b	521.75±1.79a

Means sharing similar letter in a row or in column are statistically non-significant (P>0.05).
For abbreviations, see Table I.

CAT activity

Tables I, II and III show that CAT activity significantly (P<0.05) increased in liver, gills and kidney of exposed *L. rohita* as compared to control while it was decreased in brain (Table IV), heart (Table V) and muscle (Table VI). The decreased in CAT activity changes the redox condition of the cells. Thus, increased level of CAT may be due to the removal of ROS from the cell generated by insecticides exposure (Stara *et al*., 2012). CAT activity in liver of common carp significantly increased in the presence of endosulfan (Salvo *et al*., 2012). According to Oruc and Usta (2007), CAT activity in muscle of *C. carpio* was inhibited after exposed to diazinon. Exposure of chloprpyrifos resulted in a significant reduction in CAT activity in the skeletal muscle and brain of *Heteropneustes fossilis* (Tripathi and Shasmal, 2010). Chlorpyrifos exposure caused reduction in gills, kidney and liver CAT activity of *Ctenopharyngodon idellus* (Kaur and Jindal, 2017).

Table V.- SOD, CAT, POD and GST (UmL^{-1}±SD) in heart of *Labeo rohita* during acute exposure of CPF+END mixture.

Activity		Control	Treated
SOD	24 h	14.53±0.04b	30.67±0.06a
	48 h	14.54±0.06b	41.20±0.19a
	72 h	14.55±0.05b	52.31±0.12a
	96 h	14.56±0.08b	66.28±0.16a
CAT	24 h	112.21±1.33a	92.17±5.81b
	48 h	112.22±1.35a	79.24±5.08b
	72 h	112.23±1.37a	69.32±4.84b
	96 h	112.24±1.38a	57.73±4.18b
POD	24 h	1.09±0.08b	2.94±0.04a
	48 h	1.09±0.43b	4.05±0.08a
	72 h	1.07±0.07b	4.29±0.09a
	96 h	1.1±0.26b	5.12±0.11a
GST	24 h	99.24±2.75b	187.10±2.99a
	48 h	99.26±2.71b	220.26±2.70a
	72 h	99.25±2.73b	264.18±2.86a
	96 h	99.23±2.77b	294.26±2.72a

Means sharing similar letter in a row or in column are statistically non-significant (P>0.05).
For abbreviations, see Table I.

POD activity

Exposure of CPF+END mixture significantly (P<0.05) increased the POD activity in all selected organs of *L. rohita* as compared to control (Tables I-VI). The mean POD activity was more pronounced in liver (Table II) of *L. rohita* followed by that of brain, gills, kidney, heart and muscle. The higher POD activity in liver may be due to its role in detoxification of toxicants. The production of ROS during the biotransformation of toxicants can harm the cell through oxidation (Van der Oost *et al*., 2003). The increased POD activity might be expected to scavenge the ROS by converting hydrogen peroxide (Hinton *et al*., 2008). Alteration in peroxidase activity in liver of tilapia exposed to, 4-D+azinphosmethyl mixture was reported by Oruc and Uner (2000). A duration-specific increase in peroxidase activity in gills, brain, liver and muscle of *L. rohita* exposed to acute concentration of endosulfan was observed by Ullah *et al*. (2016).

GST activity

Results showed a significant increase in liver, gills, kidney, heart, brain and muscle GST activity of insecticides exposed *L. rohita* compared to control (Tables I-VI). The GST activity increase in the following order: brain>muscle>liver>gills>kidney>heart. These results are also confirmed by Kumar *et al*. (2011) who reported the significantly increased GST activity in gills and liver of tilapia after acute exposure to endosulfan. The activity of GST increased under certain agrochemicals exposure (Dorval *et al*., 2003; Monteiro *et al*., 2006). Exposure of chlorpyrifos increased the GST activity in common carp and zebra fish (Nunes *et al*., 2018). The increase in GST activity was due to the increase metabolism of lipoperoxides produced through Fenton reaction or due to the biotransformation of toxicants, representing the adaptive mechanism of fish (Modesto and Martinez, 2010). According to the Gonçalves *et al*. (2018) GST activity can be changed in contaminated area containing the organic chemical.

Table VI.- SOD, CAT, POD and GST (UmL^{-1}±SD) in muscles of *Labeo rohita* during acute exposure of CPF+END mixture.

Activity		Control	Treated
SOD	24 h	7.75±0.03b	23.63±0.04a
	48 h	7.76±0.03b	32.78±0.11a
	72 h	7.77±0.03b	41.06±0.09a
	96 h	7.78±0.05b	53.72±0.08a
CAT	24 h	194.10±2.35a	169.55±10.16b
	48 h	194.11±2.36a	151.27±10.34b
	72 h	194.13±2.39a	143.75±10.34b
	96 h	194.16±2.44a	134.28±11.15b
POD	24 h	1.01±0.03b	2.88±0.04a
	48 h	1.02±0.03b	3.95±0.76a
	72 h	1.05±0.22b	4.02±0.10a
	96 h	1.06±0.05b	5.00±2.64a
GST	24 h	155.67±2.60b	314.45±2.48a
	48 h	155.66±2.12b	382.25±2.81a
	72 h	155.65±2.13b	457.50±2.39a
	96 h	155.64±2.15b	502.24±2.85a

Means sharing similar letter in a row or in column are statistically non-significant (P>0.05).
For abbreviations, see Table I.

CONCLUSION

This study showed that the exposure of insecticides in mixture form can lead to important alterations in antioxidant enzymes of fish. These enzymes can be used as valuable biomarker for identification of insecticides pollution in aquatic bodies. Results also indicated that the existence of pesticides in water bodies may be injurious to the health of aquatic animals especially for the fish.

Statement of conflict of interest

Authors have declared no conflict of interest.

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