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Effect of Triazophos on Esterase Activity and Protein Contents of Liver, Kidney, Brain, Blood and Muscles of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*

Ghazala,[1,2] Shahid Mahboob,[1,3]* K. A. Al-Ghanim,[3] Salma Sultana,[1] H. F. Alkahem Al- Balawi,[3] Tayyaba Sultana,[1] F. Al-Misned,[3] L. Ahmed[1] and Z. Ahmed[3]

[1]*Department of Zoology, Government College University, Faisalabad, Pakistan*
[2]*Department of Environmental Sciences, Govt. College, University, Faisalabad*
[3]*Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh, 11455, Kingdom of Saudi Arabia*

ABSTRACT

The objective of this study was to evaluate the effect of commercial formulation of triazophos on esterase activity in the liver, kidney, brain, blood and muscles of *Catla catla, Labeo rohita and Cirrhinus mrigala* fingerlings. Toxicity of triazophos was tested in the fingerlings of major carps following static bioassay and probit analysis. The fingerlings of *C. catla, L. rohita and C. mrigala* were exposed to three sub lethal concentrations: 1/5th (0.97, 0.48, 0.32), 1/10th (1.33, 0.66, 0.44) and 1/15th (0.12, 0.06 and 0.04 mg/L) part of LC_{50} of triazophos in three replicates for 8 weeks. The activity of cholinesterases (acetylcholinesterase and butyrylcholinesterase) was significantly inhibited even at the lowest concentrations of triazophos in brain, blood, gills, muscle, kidneys and liver. It has been concluded that cholinesterases in liver were more inhibited compared to those of brain, kidney and muscles.

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Authors' Contributions
SM conceived the study. G performed the experiments. SS and TS analyzed the data. LA and ZA performed statistical analysis. G and SM wrote the manuscript. KAAG, HFAAB and FAM helped in manuscript preparation.

Key words
Triazophos, esterase activity, major carps.

INTRODUCTION

Pesticides are important in protection of crops against different insects and play an important role in the yield of various cash crops, on one hand, but on the other hand, they are severely harmful for the human being and also contaminate soil, water, air and eatables for years. Effort is being made to minimize the application of pesticides and save crops from pests by genetic engineering, integrated pest management and use of biocontrol agents (Ghazala *et al*., 2014). In the meantime, however, the pesticides are still being used in bulk in developing and underdeveloped countries. Excessive use of these pesticides results in water pollution and toxicity to fish, which indirectly have adverse effects on human beings. Chronic exposure to lower doses of pesticides may have more severe effects compared with acute poisoning. Pesticide doses that are not lethal to fish, may adversely cause physiological and behavioral changes, that impairs both survival and reproduction in fish (Kegley *et al*., 1999). Metabolic changes, growth impairment, enzyme inhibition, decrease in the longevity and fecundity of the fish are few prominent biochemical changes induced by pesticide stress (Murty, 1986). The fish exhibit quick body movement, restlessness, convulsions, excess mucous secretion, respiratory problems, loss of balance and change in color when exposed to different pesticides (Haider and Inbaraj, 1986). The main biological function of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) is to regulate the transmission of nerve impulse through the neurotransmitter of acetylcholine (ACh). The protein contents have been shown to decrease in the brain, gills, muscle, kidney and liver function after pesticide stress (WHO, 1986a, b). In this paper, we evaluated the esterase activity in brain, liver, kidney, muscle and blood of *C. catla, L. rohita* and *C. mrigala* after exposure to triazophos.

MATERIALS AND METHOD

Fish and its maintenance

Healthy fingerlings of *C. catla*, *L. rohita* and *C. mrigala* (length 90±6 mm and 30.00±2.00 g body weights) were purchased from Fish Seed Hatcher, Faisalabad and maintained in 70 L glass aquaria in the laboratory and fed on commercial feed. The specimens were acclimatizatized for two weeks prior to pesticide exposure. Electrical conductivity, pH and temperature of

* Corresponding author: shahidmahboob60@hotmail.com
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aquarium water were maintained at 2.70–2.80 mS, 8.85–9.40 and 27±1ºC, respectively.

Procedure adopted

Technical grades of triazophos 90% [diethyl o-(1-phenyl-1h-1, 2, 4-triazol-3-yl) phosphoro-thioate)] was purchased from Ali Akbar Enterprises, Lahore, Pakistan. Triazophos was dissolved in methanol (Analytical grade, Merck) as $1/5^{th}$, $1/10^{th}$ and $1/15^{th}$ part of LC_{50} (predetermined). To confirm the solubility of pesticides in water, 1ppm concentration of each pesticide dissolved in relevant solvent and the test water sample was prepared and was confirmed with HPLC (Model L7400). Fish were exposed to these lower concentrations of pesticides in triplicates with 20 fish at each concentration for a period of 60 days. The fingerlings of *C. catla, L. rohita* and *C. mrigala* were exposed to the 3 sub lethal concentrations of 0.97, 0.48, 0.32; 1.33, 0.66, 0.44 and 0.12, 0.06 and 0.04 mg/ L, respectively of triazophos in three replicates for 60 days. The control fishes were exposed to carrier solvent alone. The fish were fed daily with commercial diet twice a day with an interval of 8 h. The water was replenished after every 4 days to maintain a continuous supply of pesticides to the fish. The fish were exposed to pesticide in a static bioassay system and were continuously observed.The fish were removed from each aquarium at the end of the experiment and anesthetized with MS-222 (Finquel®). They were dissected to remove the brain, gills, liver, kidney and muscle samples which were quickly removed, frozen in liquid nitrogen and were stored at -20°C. AChE and BuChE level were estimated according to the methodology of Ellman *et al.* (1961) and Kuster (2005). Total soluble proteins were determined by the Bradford (1976) standard method to assess enzymatic activity of the protein. The AChE and BuChE activity were expressed as a specific activity (normal substrate hydrolyzed/min mg protein)" (Ghazala, 2014).

The data collected in this study were statistically analyzed with the help of Minitab software. The differences among treatments were tested using ANOVA followed by the Tukey's HSD test.

RESULTS AND DISCUSSION

C. catla exhibit highly significant ($P<0.01$) difference in AChE activity among all the studied tissues (Tables I-II). The order of decrease in AChE activity was as follows: liver > kidney > blood > brain > gills > muscle. The maximum reduction in AChE activity in all the tissues was observed in fish exposed to 0.32 mg/L of triazophos (Table I). This reduction (90%) was recorded in the liver (Fig. 1). *Catla cata* data showed the least inhibition in blood against the exposure to 0.97 mg/L of triazophos (Fig. 1A).

The order of decrease in AChE activity in the different tissues of *L. rohita* was recorded as liver > gills > blood > kidney > muscle > brain. The maximum AChE activity was recorded in the liver and the minimum in fish brain (Table I). AChE activity was found to be inversely proportional to the level of triazophos in *C. catla* and *L. rohita* exposure concentrations. The differences in AChE activity was highly significant ($P<0.01$) in these two fish species (Table I). In *C. mrigala* the order of AChE activity was recorded as: liver > blood > muscles > brain > kidney > gills (Table I). A non-significant ($P<0.05$) difference was recorded in AChE of gills of *C. mrigala* exposed to 0.06mg/L and 0.04 mg/L concentration of triazophos. Reduction in the AChE activity was recorded in treated (04.74±0.023 µmol/min/g of protein) and control (132.66±0.127 µmol/min/g of protein) group of *C. mrigala*. The maximum inhibition in AChE activity was recorded in the brain of *L. rohita* and liver of *Catla catla* followed by *C. mrigala* (Fig. 1A) and muscles after exposure of *C. catla* with triazophos.

Different trends in BuChE activity were observed in the tissues of *C. catla, L. rohita* and *C. mrigala* after exposure with different concentrations of triazophos. Maximum BuChE activity was recorded in the liver of the major carps, whereas, the minimum BuChE activity was observed in the gills of *C. catla, L. rohita* and *C. mrigala.* In *C. catla* and *L. rohita* maximum BuChE inhibition was recorded in the blood. The differences were non-significant for BuChE activity in the two fish species exposed to the highest exposure concentrations of triazophos in this study (Table I). The different level of soluble protein contents was recorded in the various tissues of *C. catla, L. rohita* and *C. mrigala.* The maximum protein contents were recorded in control groups of all fish species and in all tissues except liver of *C. catla* (Fig. 1B).

Biomarkers are successfully used for monitoring of contaminants due to their high sensitivity and exhibit the first detectable signs of stress response after exposure to sub lethal in an organism (Stegeman *et al.,* 1992; Chamber and Boone, 2002). ChEs are widely used as a biomarkers in different organisms. The activity of these enzymes is affected after exposure of the organisms to toxic substances, *e.g.,* OP, carbamates, PAHs, halogenated aromatics and certain types of dioxins (Boer *et al.,* 1993). The brain AChE activity of fish is more affected after exposure to pesticides, particularly OP. The ChE activity in this experiment was inhibited to less than 50% of the normal level after exposure to triazophos. Poisoning to this level was accepted as a good indicator of intoxication of pesticides (Westlak *et al.,* 1981). In

Table I.- Effect of concentrations (mg/L=ppm) of triazophos on acetylcholinesterase (AChE) and butyrylcholinestase (BuChE) activities (µmol/min/g protein) of different organs of *Catla catla, Labeo rohita* and *Cirrhinus mrigala.*

Fish species	Treatments (mg/L)	Brain	Gills	Muscle	Kidney	Liver	Blood
AChE							
Catla catla	Control	34.55±0.03	14.24±0.14	13.80±0.12	36.39±0.05	121.99±0.06	22.40±0.23
	0.97	6.49±0.05	1.39±0.23	1.90±0.23	5.76±0.04	20.01±0.58	3.08±0.05
	0.48	10.69±0.05	4.44±0.25	3.99±0.05	7.09±0.05C	53.00±0.58	11.34±0.02
	0.32	17.99±0.05	7.98±0.05	6.23±0.13	27.27±0.16	98.00±0.29	19.67±0.01
Labeo rohita	Control	40.44±0.02	20.36±0.21	18.09±0.05	24.39±0.05	98.06±0.04	26.52±0.01
	1.33	00.75±0.03	4.98±0.05	3.98±0.05	2.90±0.06	3.25±0.14	6.23±0.02
	0.66	2.93±0.02	10.35±0.20	5.12±0.07	3.50±0.23	35.00±0.29	11.56±0.01
	0.44	4.20±0.06	15.77±0.04	6.01±0.12	7.17±0.1	57.10±0.38	11.56±0.01
Cirrhinus mrigala	Control	24.55±0.03	10.36±0.21	6.10±0.17	8.61±0.12	132.66±0.13	30.98±0.006
	0.12	0.87±0.04	1.67±0.04	1.98±0.05	1.01±0.12	4.74±0.02	3.87±0.03
	0.06	1.36±0.04	3.03±0.08	3.23±0.13	2.97±0.04	21.98±0.01	16.64±0.02
	0.04	4.68±0.04	3.72±0.41	5.34±0.20	4.05±0.03	78.00±0.23	24.67±0.02
BuChe							
Catla catla	Control	25.67±0.04	7.68±0.05	19.03±0.02	29.05±0.03	158.55±0.03	52.00±0.58
	0.97	2.77±0.04	1.45±0.26	5.56±0.04	7.09±0.05	19.70±0.06	2.61±0.06
	0.48	4.54±0.02	4.19±0.11	10.87±0.04	10.05±0.03	36.87±0.04	14.98±0.006
	0.32	13.28±0.05	6.01±0.12	13.29±0.05	21.34±0.07	87.46±0.04	24.98±0.006
Labeo rohita	Control	40.43±0.02	7.29±0.05	19.04±0.02	40.18±0.10	167.00±0.58	49.08±0.05
	1.33	2.92±0.04	2.78±0.05	4.38±0.05	9.03±0.02	13.56±0.04	2.51±0.06
	0.66	4.23±0.02	4.85±0.03	5.78±0.05	11.48±0.05	35.55±0.06	10.08±0.05
	0.44	10.38±0.05	5.01±0.12	12.04±0.02	17.20±0.12	79.98±0.01	20.48±0.05
Cirrhinus mrigala	Control	7.17±0.04	6.78±0.05	17.93±0.02	22.96±0.04	156.66±0.04	41.87±0.01
	0.12	00.45±0.03	1.04±0.02	2.56±0.04	5.45±0.26	7.90±0.06	6.56±0.02
	0.06	00.93±0.02	4.01±0.01	9.64±0.03	7.89±0.05	34.87±0.04	25.09±0.05
	0.04	2.72±0.03	5.74±0.02	14.98±0.05	17.05±0.03	98.56±0.04	30.25±0.14

Means with different letters for each fish in a column are highly significantly different (P<0.01). S.E. =standard error.

C. catla all the organs had AChE inhibition less than 50%, except in muscle samples exposed to the least concentration (0.97 mg/L) of triazophos, whereas in the other concentrations of triazophos variable inhibition was observed. The current findings are in line with the findings of Dembele *et al.* (2000), who reported the variable inhibition of AChE by chlorpyrifos, chlorfevinophos, diazinon and carbofuran in common carp (*C. carpio*). Chlorfevinifos being a potent inhibitor of ChE activity has caused 50% inhibition in common carp. In the muscle of *C. catla,* the severe inhibition of AChE was found with triazophos. Toni *et al*. (2010) reported a decrease in muscle AChE activities. The muscle AChE represents the largest pool of ChE in the body, it is also important to control the muscular function; the loss of muscular control can have many problems for fish, including the loss of swimming control and blockage of opercular movement. This may result in reduced oxygenation of the blood and consequently lead to hypoxia induced death (Zinkl *et al.*, 1987). In the brain, the highest inhibition of AChE activity was observed with the exposure of triazophos in *C. catla, L. rohita* and *C. mrigala*. Inhibition of ChE in either nervous system or muscle has been acknowledged as the adverse effect on the organisms because the target tissues of the enzyme activity are known to contribute in the neurotransmission (Padilla, 1995).

Liver as a major detoxifying organ may also have some sort of malfunctioning because of ChE inhibition after exposure to different pesticides. In *L. rohita* ChE was inhibited with OP and carbamates, whereas, the highest ChE inhibition was found after exposure to triazophos. In *C. mrigala,* the sequence of AChE activity was as follows: liver > blood > brain > gills > kidney > muscle (Table I). A non-significant variation was observed in the activity of AChE in the gills (3.03±0.07 and 3.72±0.413 µmol/min/g of protein) exposed to two different concentrations of triazophos. However, the rapid decline in the activity was observed in the liver of *C. mrigala* as 4.74±0.023 µmol/min/g of protein and 132.66±0.127 µmol/min/g of protein in the exposed and control groups. Kidneys were severely affected in

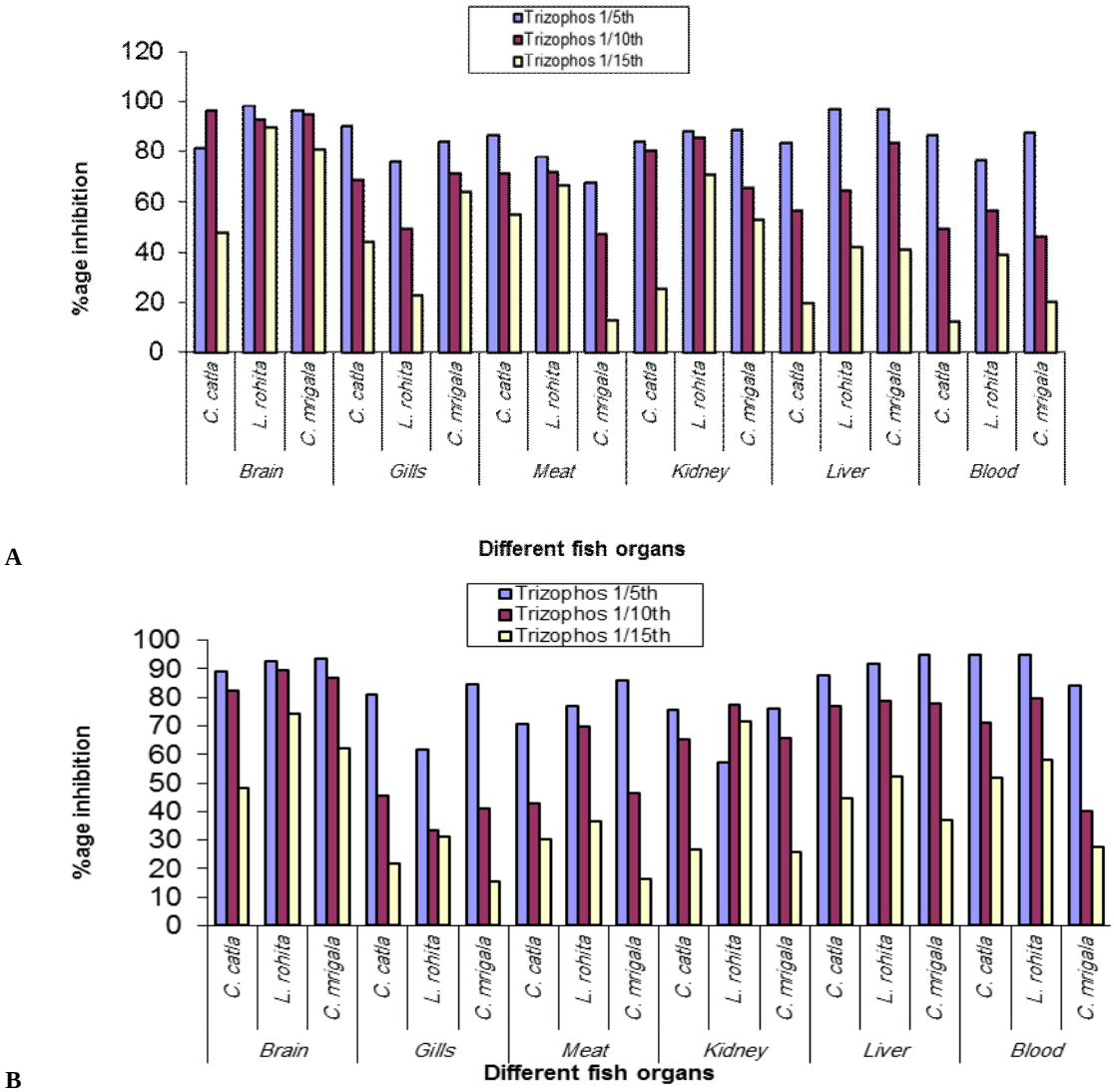

Fig. 1. Effect of various concentrations (inhibition %age) of triazophos on AChE (A) BuChE activity (B) in different organs of *C. catla, L. rohita and C. mrigala.*

L. rohita after exposure to triazophos. Salbego *et al.* (2010) determined the AChE activity in the brain and muscle of *Leporinus obtusidens* exposed to glyphosate for 90 days. The results disclosed the decrease in brain AChE, muscle protein content was inversely proportional to the concentration. The AChE and BuChE activities in blood were significantly reduced after 60 days of exposure and varied significantly from the control group. Although catfish brain seemed to be the most sensitive to the exposure of aldicarb, the fish with 90% inhibition of AChE was alive with moderate symptoms of intoxication (Everett *et al.*, 2000). In the current investigation, triazophos has shown 90% inhibition in brain AChE and BuChE. These findings are in agreement with Straus and Chamber (1995). The ChE activity reduction in gills can be attributed to suffocation and reduced respiratory activity as reported by the Chamber and Carr (1995), who reported the primary cause of AChE inhibition-induced death in mammals is generally related to respiratory failure, which may be a problem in fish as well.

The present investigation of ChEs in blood in different fish species showed reduced AChE and BuChE activities, after exposure to triazophos. In catfish, the inhibition of blood AChE was exhibited proportionally to the concentration of the inhabiting substance (Straus and Chamber, 1995). Our findings are in line with the results

reported by Straus and Chamber (1995). Different fish species may have different responses to toxic substances (Fernandz–Vega *et al.*, 2002). Recovery of esterase activity after pesticide intoxication requires different intervals. *In vivo* studies have also indicated a period of one week to recover brain esterase activity in fish after thiobencarb exposure (Babu *et al.*, 1989). In this study, no recovery pattern was observed in any of the sampled tissues. However, the result of the sub lethal toxicity assay showed the least variation from the control group. The difference in the rate of inhibition among different tissues could be due to variable molecular forms of these enzymes, such as substrate specific AChE and BuAChE and other enzymes called pseudocholinesterases that can hydrolyze ACh. It has also been known that the physiological regulation of each molecular form is widely varied for these enzymes (Massoulie *et al.*, 1993).

In the present study BuChE activity was found highest in the liver of experimental fish species. The minimum activity was observed in gills of *C. catla*, *L. rohita* and *C. mrigala*. The BuChE inhibition to the greatest extent was recorded in the blood of *C. catla* and *L. rohita* with non-significant difference followed by in the brain of *C. mrigala* with the highest exposure concentrations of triazophos in aquatic media. BuChE activity values in the brain were non-significantly different from those of *L. rohita* and *C. mrigala.* AChE is response for neurotransmission in all vertebrates. The role of BuChE remains ambiguous, whereas, in mammals malfunctioning of BuChE can increase the vulnerability to xenobiotics, including pesticides (Massoulie and Born, 1982).

CONCLUSIONS

AChE and BuChE activities were inhibited in all tested tissues of *C. catla*, *L. rohita* and *C. mrigala* after exposure to triazophos. Triazophos administration should be undertaken judiciously to minimize the health hazards to non-target organisms as well as human beings.

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