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Impact of organophosphates on blood serum enzymes of Indian major carps

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Abstract
The present study was carried out to envisage the impact of malathion, chlorpyrifos and dimethoate on the levels of blood serum enzymes of *Labeo rohita* and *Cirrhinus mrigala*. Increase upto 22.17%, 23.92%, 26.6% and 50.3% have been observed in the levels of LDH of *C. mrigala* exposed to D (0.001 ppm), C (0.001 ppm), M (0.001 ppm) and OPs in combination respectively. *L. rohita* fishes were exposed to D, C, M (0.001 ppm) and D+C+M exhibit increase in LDH level upto 18.95%, 20.6%, 25.6% and 44.8% has been observed respectively. Maximum increase i.e. 44.2% and 45% in levels of ALP was recorded in fishes exposed to D+C+M (0.001 + 0.001 + 0.001 ppm) in *L. rohita* and *C. mrigala* respectively. Decrease in ALP upto 23% and 33.1% have been observed in fishes exposed to D+C+M (0.001 + 0.001 + 0.001 ppm) in *L. rohita* and *C. mrigala* respectively.

Keywords: Organophosphates, pesticides, fishes, ALP, LDH, lipase, enzymes

1. Introduction
Recent years have witnessed marked intensive agriculture caused water pollution. Being highly efficient and less persistence, organophosphates has been extensively used as substitute for organochlorines and carbamates pesticides [1]. Due to extensive use of OPs they are widespread in the environment leading to soil and water pollution [2]. However, intensive agriculture caused water pollution has led to huge deterioration to the health of aquatic life [3]. The pollution caused due to pesticides application leads to environmental instability as it also targets the non target organisms too [4]. Being major source of protein, fishes are economically important and forms integral part of diet. But, directly or indirectly they are exposed to run off of agrochemicals [5]. Production of *Cirrhinus mrigala* and *Labeo rohita* is estimated approximately about 302,025 tonnes and 1,133,233 tonnes respectively among all carps [6]. Pesticide induced mortality remains one of the main reason for decrement in fish population. Analysis of blood serum enzymes may act as bioindicators of water pollution. LDH, ALP and lipase are among the most important enzymes that can be used to analyze the pollution induced stress among fishes [7]. Hence, the present study has been designed to analyze the effects of dimethoate, chlorpyrifos and malathion on the levels of blood serum enzymes.

2. Materials and methods
The present study was carried out in August, 2014 in Aquaculture laboratory of CCSHAU, Hisar.

2.1 Collection of fishes: Fishes viz. *Labeo rohita* and *Cirrhinus mrigala* of age six months having average size upto 4-6 inches *Labeo rohita* (F. Hamilton) and *Cirrhinus mrigala* (F. Hamilton) were procured from m local fish farm to the aquaculture laboratory, CCSHAU, Hisar. After disinfecting the fishes using dip treatment in $KMNO_4$ solution (4 ppm) for 5 seconds, they were stocked in well aerated water FRP tank of 400 liters capacity for acclimation for about 15 days.

2.2 Experimental set up: For carrying out the experiment, plastic tubs of capacity 100L were filled with chlorine free tap water and proper aeration was maintained continuously throughout the day. Tubs were then stocked with 5 fish (average B.W. and length. For each treatment, triplicates were maintained alongwith the control. Temperature (26-32° C) was maintained in laboratory. The technical grades of Dimethoate (D) 30 EC, Chlorpyrifos 30 EC (C) and Malathion (M) 50 EC were used at different concentrations to treat the fishes as mentioned in Table 1.

Table 1: Following treatments were given test fishes long with control.

S. No.	Treatments	Concentrations (ppm)
1.	Dimethoate (D)	0.0001, 0.0005, 0.001
2.	Chlorpyrifos (C)	0.0001, 0.0005, 0.001
3.	Malathion (M)	0.0001, 0.0005, 0.001
4.	Dimethoate+ chlorpyrifos + Malathion (D+C+M)	0.0001+ 0.0001+0.0001, 0.0005 + 0.0005 + 0.0005, 0.001 + 0.001 + 0.001

2.3 Collection of fish serum

Blood samples were collected in eppendorf tube from the caudal vein of experimental fishes that survive up to 60 days pesticide exposed period alongwith control. The blood samples were incubated at room temperature for coagulation and supernatant was collected. The collected supernatant was subjected to centrifugation for 10 min at 3,000 rpm. The harvested serum was then stored at -80 ^{O}C for assaying blood serum enzymes.

2.4 Enzyme assays

Blood samples for carrying out enzyme assay were collected in glass beaded sterile vial without any anticoagulant and serum was harvested by centrifugation at 3000 rpm for 20 minutes and then stored at -20^{o}C. Enzyme assays for ALP and LDH were analyzed with [2]EM 200^{TM} analyzer using commercially available [3]Transasia XL system pack kits procured from M/S Transasia Biomedical Limited, Mumbai. Lipase activity has been determined by using standard new calorimetric method as described by Worthington [8].

2.5 Statistical analysis

The reported data are the mean of triplicates. The data collected during experiment were subjected to one way ANOVA to analyze the significant differences among various treatments using OPSTAT software developed at CCS Haryana Agricultural University, Hisar. $P < 0.05$ probability level was used to analyze the significant differences among treatments.

3. Results and discussion

During the experiment, the morphological deformities like fin blackening, hemorrhage, fin erosion, descaling and deformities in body structure along with pigmentation in fishes exposed to pesticides have been observed. Change in fish behavior such as jerky and whirling movements, restless movement alongwith small resting periods, surfacing and engulfing air have been noticed in fishes exposed to the various pesticides. However, the deterioration level increased with the dose. It may also be noted that the detrimental effects were more prominent in *C. mrigala* exposed to pesticides alone as well as in combination. Pesticide exposure induced stress conditions for the fishes that led to the changes in LDH, ALP and lipase activity which have been indicated in Table 2, 3 and 4 respectively.

Table 2: Lactate dehydrogenase (IU/L) activity in blood serum of fresh water fishes, *Labeo rohita and Cirrhinus mrigala* exposed to pesticides

Treatments	*Labeo rohita*				*Cirrhinus mrigala*			
	Concentration (ppm)							
	0.0001	0.0005	0.001	Mean	0.0001	0.0005	0.001	Mean
Dimethoate	797.4 (1.62)	844.4 (7.61)	933.4 (18.95)	858.4	814.8 (3.17)	865.3 (9.56)	964.9 (22.17)	881.6
Chlorpyrifos	809.3 (3.13)	865.3 (10.27)	946.2 (20.6)	873.6	824.7 (4.42)	884.7 (12.02)	978.7 (23.92)	896.0
Malathion	829.7 (5.73)	870.7 (10.96)	985.3 (25.6)	895.2	842.8 (6.71)	885.3 (12.09)	999.8 (26.6)	909.3
D+C+M	835.4 (6.46)	1015.6 (29.43)	1136.3 (44.8)	995.8	846.7 (7.27)	1048.7 (32.78)	1186.7 (50.3)	1027.4
Control	784.7	784.7	784.7	784.7	789.8	789.8	789.8	789.8
Mean	811.3	876.2	957.2		823.7	894.8	984.0	

Values in parenthesis are per cent increase over control

Table 3: Effect of organophosphates on alkaline phosphatase (IU/L) activity in blood serum of *Labeo rohita and Cirrhinus mrigala*

Treatments	*Labeo rohita*				*Cirrhinus mrigala*			
	Concentration (ppm)							
	0.0001	0.0005	0.001	Mean	0.0001	0.0005	0.001	Mean
Dimethoate	55.27 (2.1)	57.27 (5.8)	60.27 (11.3)	57.6	56.67 (1.8)	59.87 (7.5)	62.87 (12.9)	59.80
Chlorpyrifos	60.13 (11.1)	62.73 (15.9	65.07 (20.2)	62.64	61.87 (11.1)	64.73 (16.3)	67.73 (21.7)	64.78
Malathion	63.07 (16.5)	66.87 (23.5)	73.27 (35.4)	67.73	64.07 (15.1)	68.67 (23.4)	75.8 (36.2)	69.51
D+C+M	65.67 (21.3)	69.67 (28.7)	78.07 (44.2)	71.13	67.87 (21.9)	72.87 (30.9)	80.73 (45.0)	73.82
Control	54.13	54.13	54.13	54.13	55.67	55.67	55.67	55.67
Mean	59.65	62.13	66.16	61.23	64.36	68.56		

Values in parenthesis are per cent increase over control

Table 4: Effect of OPs on lipase (IU/L) activity in blood serum of *Labeo rohita and Cirrhinus mrigala*

Treatments	*Labeo rohita*				*Cirrhinus mrigala*			
	Concentration (ppm)							
	0.0001	0.0005	0.001	Mean	0.0001	0.0005	0.001	Mean
Dimethoate	1.43 (5.9)	1.37 (9.9)	1.27 (16.4)	1.36	1.41 (6.6)	1.33 (11.9)	1.23 (18.5)	1.32
Chlorpyrifos	1.42 (6.6)	1.30 (14.5)	1.23 (19.1)	1.32	1.39 (7.9)	1.29 (14.6)	1.22 (19.2)	1.30
Malathion	1.39 (8.6)	1.29 (15.1)	1.21 (20.4)	1.30	1.37 (9.3)	1.27 (15.9)	1.20 (20.5)	1.28
D+C+M	1.29 (15.1)	1.23 (19.1)	1.17 (23.0)	1.23	1.30 (13.9)	1.19 (21.2)	1.01 (33.1)	1.17
Control	1.52	1.52	1.52	1.52	1.51	1.51	1.51	1.51
Mean	1.41	1.34	1.28		1.40	1.32	1.23	

Values in parenthesis are percent decrease over control

3.1 Lactate dehydrogenase activity in blood serum

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalyzes the inter-conversion of lactate and pyruvate depending on the bioavailability of NAD. Significant dose dependent increase in the levels of lactate dehydrogenase due to pesticide exposure has been observed as compared to control. In case of *Labeo rohita,* an increase of 25.6% has been induced due to exposure of fish to malathion at 0.001 ppm while dimethoate at 0.0001 ppm concentration induced an increase of 1.62% in lactate dehydrogenase activity. However, increase in LDH activity was more enhanced in case of *C. mrigala* where increase of 3.17% and 26.6% in the blood serum lactate dehydrogenase activity was induced by dimethoate (0.0001 ppm) and malathion (0.001ppm) respectively. The increase in Lactate dehydrogenase activity advocates the increased conversion of lactate to pyruvate, thereby increasing the rate of energy production to meet the energy needs of fish during stress conditions. When the fishes were exposed to the pesticides in combination (D+ C+ M), the toxicity enhanced marking the synergistic mode of pesticides' induced toxicity. Increase up to 50.3% and 44.8% in lactate dehydrogenase has been observed when *C. mrigala* and *C. mrigala* when exposed to D+C+M. Enhanced LDH activity due to pesticides' exposure in fishes may be indicated by the findings of several workers [9] [10] [3]. The increased LDH activity in fishes may point towards the increased production of lactate for gluconeogenesis in liver so that the emergency needs of increased energy demands can be met. This increase in LDH activity may be seen under the umbrella of adaptive mechanism for dealing with increased energy budget.

3.2 Alkaline Phosphatase activity in blood serum

In fish *C. mrigala*, an increase upto 1.8% and 36.2% of alkaline phosphatase activity in the blood serum has been observed due to dimethoate (0.0001 ppm) and malathion (0.001ppm) exposure respectively. However, in case of *Labeo rohita*, increase upto 16.5% and 2.1% of alkaline phosphatase activity in blood serum has been observed due to malathion exposure at 0.001 ppm and dimethoate at 0.0001 ppm respectively. Dose dependent significant increase in ALP activity has been observed in fishes exposed to pesticides as compared to control. However, increase upto 44.2% and 45% in ALP activity has been noted in fishes exposed to pesticides at 0.001 ppm in *L. rohita* and *C. mrigala* respectively. Increase in ALP can be stamped by the findings of Sancho *et al.* [3] who reported the stress induced increase in ALP level. Due to pesticide intoxication, the additional energy requirement leads to increased protein catabolism and increased dephosphorylation, leading to increase in ALP levels [11].

3.3 Lipase enzyme activity in blood serum

As indicated in Table 4, significant dose dependent reduction in the levels of lipase activity has been observed in fishes exposed to all the three pesticides alone as well as in combination. The reduction in lipase activity of blood serum upto 23.0% and 15.1% has been observed in *L. rohita* exposed to malathion in combination with dimethoate and chlorpyrifos at 0.001 ppm and 0.0001 ppm concentration respectively. However, in case of *C. mrigala* reduction upto 33.1% and 13.9% in fishes exposed to malathion in combination with dimethoate and chlorpyrifos at 0.001 ppm and 0.0001 ppm respectively. It may also be noted that the lipase activity was more reduced in case of *C. mrigala* as compared to *L. rohita* stamping the more sensitivity of *C. mrigala* towards pollution caused by pesticides. However, decrease in levels in lipid content due to organophosphate exposure has also been previously reported by Kumar and Ali [12]. The decreased levels of lipid (substrate) may be the probable cause of reduced lipase (enzyme) activity. Decreased level of lipase levels due to pesticide exposure may also be justified by the increased protein catabolism and reduced protein anabolism due to stress caused to fishes.

4. Conclusion

In both *L. rohita* and *C. mrigala*, the organophosphates at lower levels too cause changes in levels of blood serum enzymes. Increase in the levels of lactate dehydrogenase and alkaline phosphatase has been observed in fishes exposed to dimethoate, chlorpyrifos and malathion individually as well as in combination. Inhibitory effect on the activity of lipase has been noted in fishes exposed to dimethoate, chlorpyrifos and malathion individually as well as in combination even at low concentrations. But, *C. mrigala* can be marked as more sensitive fish as far as pesticide pollution is concerned. However, the present study leaves an avenue for the further study of specific metabolic pathways targeted due to pesticides pollution based on the present study.

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