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Assessment of organophosphates induced acetylcholinesterase inhibition in Indian major carps

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Abstract
The present study of 60 days was conducted to study the comparative inhibition of acetylcholinesterase activity due to the exposure of organophosphates *viz.* Malathion, dimethoate and chlorpyrifos when used unaccompanied as well as in combination on Indian major carps namely *Labeo rohita* and *Cirrhinus mrigala*. For this twelve concentrations were used. Dose dependent significant reduction in acetylcholinesterase activity of fishes exposed to pesticides has been observed. Reduction upto 24.1%, 12.6%, 14.3% and 18.4% in AChE activity has been observed in *C. mrigala* exposed to D+C+M (0.001 ppm), dimethoate (0.001 ppm), chlorpyrifos (0.001ppm) and Malathion (0.001ppm) respectively. While in *L. rohita,* decrease upto 24.7%, 13.7%, 15.1% and 19.2 has been detected in *C. mrigala* exposed to D+C+M (0.001 ppm), dimethoate (0.001ppm), chlorpyrifos (0.001 ppm) and Malathion (0.001ppm) respectively. However, Malathion induced maximum AChE inhibition and the effects were enhanced when Malathion was used in combination with chlorpyrifos and dimethoate that marks the synergistic mode of action of organophosphates. It may also be noted that pesticide induced enzyme inhibition was more in case of *L. rohita* as compared to *C. mrigala.*

Keywords: Organophosphate, Malathion, chlorpyrifos, carps, dimethoate, acetylcholinesterase

1. Introduction
Recent years have witnessed a marked increase in soil and water pollution. Pesticides are the major pollutants of soil and they are washed away that leads to water pollution [1]. Not only has the current use of agrochemicals, but leaching of persistent ingredients also contaminated the ecosystem [2]. Undoubtedly, agricultural productivity has increased under the umbrella of pesticides, yet they also cause toxicity to non- target organisms [3]. The pesticides induced toxicity has led to the ecosystem instability. Pesticides alter the biochemical and physiological metabolic processes of the non- target organisms that leads to the deterioration in their health. Organophosphates replaced organochlorines for their lesser persistence in agro-ecosystems. Organophosphates tend to inhibit cholinesterase enzyme irreversibly thus impairing the nervous transmission of that organism. Hence, acetylcholinesterase acts as sensitive biomarker for the presence of organophosphate pollutants [4]. OP pesticides may also interfere with the functioning of immune system and may induce immunotoxic effects via anti cholinergic or non- cholinergic pathways [5]. Malathion, dimethoate and chlorpyrifos are among widely used pesticides in India. Pedestaled upon the above facts, the study has been carried out to analyze the level of acetylcholinesterase inhibition caused due to malathion, dimethoate and chlorpyrifos individually as well as in combination in *Labeo rohita* and *Cirrhinus mrigala*.

2. Material and Methods:
The present study was carried out in August, 2014 in Dept. of Zoology, CCSHAU, Hisar.

2.1 Collection of test animal
Indian major carps *L. rohita* and *C. mrigala* of six month age (4-6 inches) were procured from the local fish farm situated in Ladwa, Hisar to the aquaculture laboratory of CCS Haryana Agricultural University, Hisar. Then the fishes were disinfected by dipping them in $KMNO_4$ solution (4 ppm) for 5 second and cultured in well aerated tank of 400 liters capacity for acclimation for about 15 days.

2.2 Procurement of chemicals
The technical grades of organophosphates *viz*. dimethoate (30 EC), chlorpyrifos (30 EC) and Malathion (50 EC) procured from Haryana Seed Company, Hisar were used for treating fishes at different concentrations as shown in Table 1.

Table 1: Following treatment were given test fishes along with control.

S. No.	Treatments	Concentrations (ppm)
1.	Dimethoate	0.0001, 0.0005, 0.001
2.	Chlorpyrifos	0.0001, 0.0005, 0.001
3.	Malathion	0.0001, 0.0005, 0.001
4.	Dimethoate+ chlorpyrifos + Malathion	0.0001+ 0.0001+0.0001, 0.0005 + 0.0005 + 0.0005, 0.001 + 0.001 + 0.001
5.	Control	No pesticides

2.3 Experimental set up
All the fishes were maintained under laboratories conditions (25.0± 1ºC LD 12:12) in properly aerated plastic tubs (100 L). The tubs were filled with 100 L of chlorine free tap water and five fishes were released in each tub. Triplicates for each treatment were maintained along with the control.

2.4 Analysis of physico-chemical characteristics of water
Physico-chemical parameters *viz.* temperature, pH, dissolved oxygen, alkalinity, free carbon dioxide of the water samples were analyzed as per recommendations of APHA [6].

2.5 Chemical analysis
Blood samples of treated fishes (that survive 60 days after treatment) along with control were collected from the caudal vein. The blood was left for half an hour in eppendorf tube at room temperature to allow coagulation. Then the blood was centrifuged for 10 min at 3,000 rpm and supernatant was collected. The serum was then stored at -20 ^{O}C for enzyme estimation. AChE activity has been determined by standard method as described by Ellman *et al.* [7].

2.6 Behavior and morphological study
The fishes were observed on daily basis to analyze the change in behavior and presence of morphological deformities.

2.7 Statistical analysis
The reported data are the arithmetic mean of triplicates and were subjected to Student t- test and one way ANOVA using OPSTAT software developed at CCSHAU, Hisar to analyze the significant difference among various treatments.

3. Results and Discussion

Table 2: The physico- chemical parameters of water used during experiment

Sr. No.	Parameters	Range
1.	Temperature	26-34 ºC
2.	Dissolved Oxygen	6.0-8.3 mg/l
3.	Free Carbon dioxide	1.5-3.2 mg/l
4.	Alkalinity	152-158 mg/l
5.	pH	6.7-7.5

3.1 Morphological changes
Observations like changes in body color and other apparently visible morphological aberrations like erosion, fin blackening, hemorrhage and body deformities at higher concentrations were observed in the fishes exposed to Malathion, dimethoate and chlorpyrifos. The detrimental effects of pesticides' exposure were marked by the presence of black patches on skin even at the slightest concentrations. The observations regarding morphological changes have previously been documented by Parikh *et al.* [8] and Dey and Saha [9].

3.2 Behavioral changes
The treated fish while swimming exhibited jerky and whirling movements even at the lowest concentration exposure. The treated fishes exhibited restless movement with small resting periods. Such respiratory problems and behavioral changes induced due to pesticide exposure have been documented by Marigoudar *et al*. [13] and Thenmoghi *et al.* [14]. In few cases, the pesticide exposure led to behavior like surfacing and engulfing air. The observations made during present study may be justified by the findings of Murthy *et al*. [12]. In general, the treated fishes tend to avoid the feed too and they abstained themselves from feeding in pesticide treated water. Cork screw pattern and sudden S jerks has also been observed during the experimental period in fishes. Dey and Saha [9] has also reported the occurrence of erratic jerky movements in malathion exposed fishes. Changes in behavior and occurrence of erratic and jerky movements can be seen under the umbrella of AChE inhibition.

3.3 Acetylcholinesterase enzyme activity in blood serum
The acetylcholinesterase activity in blood serum of fishes exposed to organophosphates alone as well as in combination as compared to the control fishes are shown in Table 3. Dose dependent significant reduction in levels of acetylcholinesterase activity has been observed even at the slightest exposure of pesticides in *L. rohita* as well as in *C. mrigala*. In *L. rohita* maximum (19.2%) and minimum (5.5%) decrease of acetylcholinesterase activity in blood serum has been observed in fishes exposed to malathion at 0.001 ppm and dimethoate at 0.0001ppm respectively. Inhibitory action of organophosphates on AChE activity is in concurrence with the findings of Joseph and Raj [15], Jindal and Kaur [16], Topal *et al.* [17] and Sharma *et al.* [18]. However, in case of *C. mrigala,* minimum (4.2%) and maximum (18.4 %) reduction of in the blood serum acetylcholinesterase activity was recorded in fishes exposed to dimethoate at 0.0001 ppm and malathion at 0.001ppm respectively. In case of both the carps, the toxic effects of pesticides were enhanced when used in combination that stamps the synergistic mode of toxicity of organophosphates at each concentration. Whereas, in fish *C. mrigala* the maximum reduction (24.1%) of acetylcholinesterase activity in blood serum in fishes exposed to malathion in combination with dimethoate and chlorpyrifos at 0.001 ppm concentration and minimum reduction of 16.5% has been recorded in fishes exposed to pesticides in combination at 0.0001 ppm. The acetylcholinesterase inhibition as observed in present study can be advocated by the findings of Rajni and Revathy [10] and Shoaib *et al.* [4]. Acetylcholinesterase enzyme is vital for various physiological functions such as orientation towards food, prey location and predator escaping [11]. Thus, the inhibition of above mentioned enzyme causes threat to fish survival in aquatic ecosystems. It may also be noted that more enzymatic inhibition due to OP exposure at all concentrations was observed in *L. rohita* as compared to that of *C. mrigala* that reflects the more sensitivity of former to the OP pollution.

Table 3: Effect of pesticides exposure on acetylcholinesterase (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	138.6 (5.5)	130.2 (11.2)	126.6 (13.7)	131.8	137.8 (4.2)	129.7 (9.8)	125.7 (12.6)	131.1
Chlorpyrifos	135.8 (7.4)	129.6 (11.7)	124.6 (15.1)	130.0	134.8 (6.3)	128.8 (10.4)	123.2 (14.3)	128.9
Malathion	128.4 (12.5)	121.4 (17.2)	118.6 (19.2)	122.8	127.7 (11.2)	120.7 (16.1)	117.3 (18.4)	121.9
D+C+M	122.2 (16.7)	115.5 (21.3)	110.5 (24.7)	116.1	120.1 (16.5)	114.7 (20.2)	109.2 (24.1)	114.7
Control	146.7	146.7	146.7	146.7	143.8	143.8	143.8	143.8
Mean	134.3	128.7	125.4		132.9	127.5	123.8	

Values in parenthesis are percent decrease over control

4. Conclusion

Malathion, dimethoate and chlorpyrifos induce inhibitory effect on AChE activity, while maximum inhibition was induced by Malathion. *L. rohita* has been found to be more sensitive to OP contamination as compared to *C. mrigala* as far as AChE inhibition is considered. Acetylcholinesterase enzyme can act as a sensitive biomarker for the presence of organophosphates in aquatic ecosystems.

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