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PESTICIDES' INDUCED ALTERATIONS IN BLOOD SERUM IONS OF INDIAN MAJOR CARPS

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ABSTRACT
The present study was conducted to analyze the impact of dimethoate, chlorpyrifos and malathion individually as well in combination at concentrations of 0.0001 ppm, 0.0005 and 0.001% on *Labeo rohita* and *Cirrhinus mrigala*. Dose dependent significant decrease in chloride and calcium while increase in potassium and sodium was observed. Maximum increase upto 21.7% and 48.8% in sodium and potassium respectively, while decrease upto 12% and 16% in calcium and chloride levels respectively was observed in *C. mrigala* exposed to pesticides in combination at 0.001 ppm. Maximum alterations in blood serum ions were found in the fishes exposed to pesticides in combination marking the synergistic mode of deterioration. *L. rohita* exposed to combination of pesticides at 0.001 ppm showed decrease in calcium (15.2%) and chloride (10.5%) concentrations and increase in sodium (21%) and potassium (54.1%) concentration in blood. However, when individually used malathion was found to be most altering OP followed by chlorpyrifos and dimethoate

INTRODUCTION

Recent years have seen marked increase in pesticides' induced soil and water pollution particularly due to highly intensive agriculture (Rani *et al*., 2017). In developing countries like India it has become vital to use the pesticides as approximately 30% of the total crop yield is damaged due to prevalence of pest (Das, 2013). However, organophosphates are most widely used pesticides that enjoy about 22% world market share of total pesticides (Lazonby and Waddington, 2015). Agrochemicals may enter aquatic ecosystems via atmospheric deposition, surface run off or leaching resulting in water pollution. Thus, the organophosphates' caused pollution in aquatic ecosystems, poses serious threat to fishes. The impact of pesticide pollution may be observed in the form of fish mortality, reduced fish production and bioaccumulation of pollutant in fish tissues, thus resulting in deterioration of human health (Adedeji and Okocha, 2012). These agrochemical may induce histological, physiological and biochemical alterations in fishes. However, the alterations induced by pesticides are directly linked to the amount of pesticide exposure. The pesticide induced alterations in biochemical parameters may act as sensitive parameter for analyzing the fish metabolism under stress conditions (Rani *et al*., 2015 and Rani *et al*., 2017). Chlorpyrifos, malathion and dimethoate are among the most widely used organophosphates for pest control. Calcium, sodium, potassium and chloride are among the essential mineral ions. Low levels of these ions may induce malfunctioning, decreased productivity, altered blood clotting, osteoporosis and anemia. Calcium plays vital role in neuromuscular excitability while sodium, potassium and chloride maintain acid base balance and osmotic pressure (Naveed *et al*., 2011). Thus, the changes in the levels of serum ions may act as indicator to assess the water pollution level caused by organophosphates. No formal attempts have been made previously to analyze the comparative toxicity of chlorpyrifos, malathion and dimethoate on Indian major carps. Hence, the present study dealt with the induced toxicity of above mentioned organophosphates individually as well as in combination on blood serum ions of *L. rohita* and *C. mrigala*.

MATERIALS AND METHODS

Procurement of test animal

The test animals *viz*. *Cirrhinus mrigala* and *Labeo rohita* approximately about 4 to 6 inches were procured from the local fish farm situated in Hansi and Hisar.

Experimental set up

After disinfecting fishes with $KMnO_4$ (0.1%), they were transferred to the tubs and acclimatized for one week at room temperature. Physico-chemical characteristics *i.e.* temperature, pH, dissolved oxygen, alkalinity and free CO_2 were maintained as per standards described by APHA, 1998.

Chemical analysis

The fishes were dissected after 60 days and blood samples were collected from caudal vein for ion estimations. Blood was centrifuged at 3000 rpm for five minutes and serum was harvested and stored at -20 C for further ion estimations. Sodium and Potassium ions were analyzed by flame photometric method (Richards, 1954). Chloride ion concentration was estimated by using standard Mercury

Thiocyanate method as described by Miller, 1984. Calcium ion concentration was analyzed via Arsenazo method as mentioned by Farrell, 1984.

Statistical analysis

The data reported are the mean of three replicates. The data so collected was subjected to ANOVA to analyze the significant differences among various concentrations.

RESULTS AND DISCUSSION

Sodium ions concentration

Table 1: Concentrations of pesticides to which fishes were exposed 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 (D+C+M)	0.0001+ 0.0001+0.0001, 0.0005 + 0.0005 + 0.0005, 0.001 + 0.001 + 0.001

In both the fishes, a dose dependent increase in sodium ion concentration was observed while the maximum increase was found at the concentration of 0.001%. In *C. mrigala* minimum increase of 7.9% in the blood serum sodium ions activity was induced by dimethoate at 0.0001 ppm and maximum was 19.2 % in malathion at 0.001ppm. However, in *Labeo rohita* maximum increase of sodium ions activity in blood serum 18.5% was induced by malathion at 0.001 ppm and dimethoate at 0.0001ppm induced minimum increase of 7.7% (Table 2). In *L. rohita* toxic effect of all the pesticides *i.e.* dimethoate, chlorpyrifos and malathion in combination with each other were enhanced at all the concentration levels. The observations can also be supported by the findings of Naveed *et al*. (2011) who reported time dependent increase in sodium concentration with the exposure of fish to pesticide. Sodium ion is vital for maintaining osmotic pressure and is also involved in heart contraction and nerve excitation in fishes Eisler and Edmunds (1996) has advocated stress induced increase in blood sodium concentration and decrease in liver ionic concentration. Swarnlata (l995) also suggested that increased urinary excretion of sodium due to renal tubular

Table 2: Effect of different pesticides on sodium ion (m Eq/ L) activity in *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	132.73	137.27	141.73	137.24	134.13	139.31	143.73	139.06
	-7.7	-11.4	-15		-7.9	-12.1	-15.7	
Chlorpyrifos	133.8	138.67	145.2	139.22	137.13	140.31	146.13	141.19
	-8.5	-12.5	-17.8		-10.3	-12.9	-17.9	
Malathion	134.73	139.31	146.13	140.06	136.27	140.8	148.15	141.74
	-9.3	-13	-18.5		-9.7	-13.3	-19.2	
D+C+M	134.8	140.2	149.2	141.4	137.67	141.67	151.27	143.53
	-9.4	-13.7	-21		-12.1	-14	-21.7	
Control	123.27	123.27	123.27	123.27	124.27	124.27	124.27	124.27
Mean	131.87	135.74	141.11		133.89	137.27	142.71	

Values in parenthesis are percent increase over control

Table 3: Effect of different pesticides on chloride (m mol/ L) ion activity in *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	98.73(1.0)	97.27(2.5)	95.13(4.6)	97.04	98.80(1.9)	97.73(3.0)	95.27(5.4)	97.27
Chlorpyrifos	97.20(2.5)	95.87(3.9)	93.13(6.6)	95.40	98.67(2.0)	96.67(4.0)	94.80(5.9)	96.71
Malathion	95.13(4.6)	93.67(6.1)	91.13(8.6)	93.31	97.13(3.6)	94.87(5.8)	91.87(8.8)	94.62
D+C+M	93.73(6.0)	91.27(8.5)	89.27(10.5)	91.42	94.20(6.5)	90.20(10.5)	88.67(12.0)	91.02
Control	99.73	99.73	99.73	99.73	100.73	100.73	100.73	100.73
Mean	96.91	95.56	93.68		97.91	96.04	94.27	

Values in parenthesis are percent decrease over control

Table 4: Effect of different pesticides on potassium ion (m Eq/ L) activity in *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	2.99(7.2)	3.11(11.5)	3.40(21.9)	3.16	3.07(6.2)	3.13(8.3)	3.67(27.0)	3.29
Chlorpyrifos	3.19(14.3)	3.30(18.3)	3.80(36.2)	3.43	3.19(10.4)	3.27(13.1)	3.93(36.0)	3.46
Malathion	3.27(17.2)	3.39(21.5)	4.10(47.0)	3.59	3.23(11.8)	3.71(28.4)	4.17(44.3)	3.70
D+C+M	3.37(20.8)	3.60(29.0)	4.30(54.1)	3.76	3.73(29.1)	3.84(32.9)	4.30(48.8)	3.96
Control	2.79	2.79	2.79	2.79	2.89	2.89	2.89	2.89
Mean	3.12	3.24	3.68		3.22	3.37	3.79	

Values in parenthesis are percent increase over control

Table 5: Effect of different pesticides on calcium ion (µg/ml) activity in *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	10.88(1.9)	11.11(3.8)	11.29(5.5)	11.10	10.97(2.0)	11.22(4.4)	11.37(5.8)	11.19
Chlorpyrifos	11.57(8.1)	11.79(10.2)	11.99(12.1)	11.78	11.62(8.3)	11.82(10.3)	12.02(11.8)	11.82
Malathion	11.68(9.2)	11.87(10.8)	12.07(12.8)	11.88	11.72(9.0)	11.92(10.9)	12.17(13.2)	11.94
D+C+M	11.77(10.0)	11.89(11.1)	12.33(15.2)	12.00	11.81(9.9)	11.99(11.5)	12.47(16.0)	12.09
Control	10.7	10.7	10.7	10.88	10.75	10.75	10.75	10.75
Mean	11.36	11.51	11.71		11.40	11.57	11.78	

Values in parenthesis are percent decrease over control

dysfunction or reduced intestinal absorption may result in decline of sodium ion concentration. However, it may also be noted that *C. mrigala* was found to be more sensitive towards pesticide exposure as far as sodium concentration is concerned.

Chloride ions concentration

Significant dose dependent decrease in blood chloride concentration has been observed. In *Labeo rohita,* maximum decrease of chloride ion in blood serum was 8.6% caused by malathion at 0.001 ppm and dimethoate at 0.0001 ppm induced minimum decrease of 1.0%. In *C. mrigala,* minimum decrease of 1.9% chloride ion activity in the blood serum was induced by dimethoate at 0.0001 ppm and maximum decrease of 8.8 % chloride ion induced by malathion at 0.001ppm (Table 3). However, when used in combination decrease upto 10.5% and 12% has been observed in *L. rohita* and *C. mrigala* respectively (Table 3). Hickman and Trump (1968) stated that pesticides induce stress on organism that lead to severe loss of water leading to increased GFR that may be the probable cause for chloride ion depletion. Rani *et al*. (2016) has also reported significant decrease in chloride ion concentration exposed to heavy metal pollutants.

Potassium ions concentration

An increase in 21.9%, 36.2%, 47% and 54% has been observed in fishes exposed to dimethoate, chlorpyrifos, malathion and in combination at 0.001% respectively in *L. rohita* (Table 4). However, when *C. mrigala* was exposed to dimethoate, chlorpyrifos, malathion and in combination at 0.001% significant increase in potassium ion concentration upto 27%, 36%, 44.3% and 48.8% was observed respectively. It may also be noted that the percent increase in potassium ion was observed in fishes exposed to the pesticides in combination marking their synergistic effects. The elevation in potassium ions in blood has previously been reported by Bano (1982) and Rani *et al*. (2016). Disturbed potassium regulation might be due to an impaired active reabsorption of potassium in renal tubules (Gill *et al*., l989).

Calcium ions concentration

Dose dependent significant increase in calcium ion concentration was observed in pesticide treated fishes (Table 5). The maximum increase of calcium ion in blood serum was 16.0% in fishes exposed pesticides in combination at 0.001 ppm concentration and minimum increase *i.e.* 9.9% was observed in fishes exposed to pesticides at 0.0001 ppm in combination. *C. mrigala* was found to be more sensitive than that of *L. rohita* to pesticide exposure. Increase in calcium ion due to stress conditions has also been previously reported by Fawole *et al*. (2007) and Rani *et al*. (2015). Esterogenic compound may lead to an increase in ion uptake as reported by McCormick *et al*. (2005) may also contribute to the rise in calcium ion concentration. Bansal *et al*. (l979) stated that an increase in blood serum calcium may be the result of calcium ions release into the blood from vital organs due to insecticides' induced toxicity.

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