The Bioscan 12(1): 81-83, 2017
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

COMPARATIVE ANALYSIS OF PROTEOLYTIC AND AMYLOLYTIC ACTIVITY IN PESTICIDES EXPOSED INDIAN MAJOR CARPS

MANJU RANI*[1], R. K. GUPTA[1], JYOTI YADAV[1] AND SANDEEP KUMAR[2]
[1]Department of Zoology, CCS Haryana Agricultural University, Hisar - 125 004, INDIA
[2]Department of Veterinary Biochemistry and Physiology,
COVS, Lala Laj pat Rai University of Veterinary and Animal Sciences, Hisar - 125 004, INDIA
e-mail: manjuranga24@gmail.com

KEYWORDS
Indian major carps
Enzyme activity
Dimethoate
Chlorpyrifos
Malathion

Received on :
27.01.2016

Accepted on :
22.02.2017

***Corresponding author**

ABSTRACT
The present study carried out during 2014 focused on the effects of different pesticides viz. dimethoate, chlorpyrifos and malathion singly as well as in combinations on proteolytic and amylolytic activity in two Indian major carps namely *C. mrigala* and *L. rohita*.Dose dependent significant reduction in proteolytic and amylolytic activity was observed in all treatments. The protease activity decrease upto 17%, 12.9%, 17% and 17.5% has been observed in *L. rohita* exposed to malathion, dimethoate, chlorpyrifos and dimethoate + chlorpyrifos + malathion at 0.001 ppm in combination as compared to control.However, a decrease in 15.4%, 9.3%, 14.2% and 14.5% in protease activity was observed in *C. mrigala* exposed to exposed to malathion, dimethoate, chlorpyrifos and dimethoate + chlorpyrifos + malathion at 0.001 ppm in combination respectively as compared to control.The reduction of 12.1%, 12.8%, 13.4%, 18.8% and 7.8%, 8.5%, 14.1% and 14.9% in amylase activity has been observed in *L. rohita* and *C. mrigala* respectively when exposed to dimethoate, chlorpyrifos, malathion and in combination at 0.001 ppm as compared to control.When the pesticides were used in combination the protease and amylase activity were reduced further marking the synergistic effects of pesticides.

INTRODUCTION

Pollution has become a matter of concern in last few decades. However, water pollution caused due to agricultural activities has led to immense detrimental effects on aquatic life. Fresh water fishes are economically important for being a major source of protein. But, directly or indirectly they are exposed to different pesticides (Despande *et al*., 2011). Carps comprising Indian major, common and Chinese are about 94% of the total production (Ramakrishna *et al*., 2013). Among carps, *C. mrigala* and *L. rohita* production is estimated to about 302,025 tonnes and 1,133,233 tonnes respectively (FAO, 2013).But, even the slightest of pesticide may induce disturbances in metabolism of fishes that may disturb the fish industry of India (McCarthy and Fuiman, 2008). Organophosphates are one of the widely used pesticides world wide. The OP compounds are widely used as insecticides, pesticides, chemical warfare agents, petroleum additives, and industrial plasticizers (Rao *et al*., 2003). Pesticide pollution may lead to the changes in morphology, behavior and physiology of the fishes (Dogan and Canan, 2007). However, the changes in biochemical parameters in fishes may act as important biomarker of pesticide induced toxicity. So, present study was carried out to analyze the effects of organophosphates namely dimethoate, chlorpyrifos and malathion on amylolytic and proteolytic activity in *Cirrhinus mrigala* and *Labeo rohita* at different concentrations individually as well as in combinations.

MATERIALS AND METHODS

Collection of test animal

Indian major carps *Cirrhinus mrigala* and *Labeo rohita* were procured from local fish farm to the aquaculture laboratory. The monitoring of various physico-chemical parameters of water used during the treatment period revealed ambient temperature in range of 26ºC-34ºC, dissolved oxygen range of 6.0-8.3 mg/l, pH remained around 6.7-7.5, alkalinity between 151-157 mg/l and free carbon dioxide between 1.5-3.2 mg/l. These conditions were maintained quite optimum as per APHA (1998) for normal fish activity and growth.

Experimental set up

The experiments were conducted under laboratories conditions (25.0 ± 1ºC) in plastic tubs (100l) with proper aeration. Tubs were then stocked with 5 fish (average B.W. and length). The technical grades of Dimethoate (D) 30 EC, Chlorpyrifos 30 EC (C) and Malathion (M) 50 EC were used for treating fishes along with the control. All the experiments were conducted in triplicates.

Estimation of enzymatic activity

Fishes were dissected and digestive tract was removed. It was homogenized in 5 volumes (v/w) of ice cold distilled water. The contents were centrifuged at 4ºC at 10000 rpm. The supernatants were taken for analysis. Proteolytic enzyme activity was estimated by Walter (1984) methodwith Bovine Serum Albumin as 1% substrate. Amylase enzyme activity was assayed as described by Sawhney and Singh (2000) in

which the increase in reducing power of buffered starch solutions was estimated.

Statistical analysis

The data were analyzed using OPSTAT software by applying ANOVA to figure out the significant differences between different treatments.

RESULTS

Both the fish species *L. rohita* and *C. mrigala* exhibited a dose dependent decrease in the specific protease and amylase activities with respect to control on exposure to the pesticides. All the three pesticides dimethoate, chlorpyrifos and malathion individually as well as in combination have been found to impose toxic effects on the fish, thereby reducing the specific protease as well as amylase activities as compared to the control. The data regarding the effects of malathion, dimethoate and chlorpyrifos on proteolytic and amylolytic activity has been depicted in Table 2 and Table 3 respectively. The toxicity induced reduction in proteolytic and amylolytic activity in both fishes has been observed.

Effects on protease enzyme activity

In the fish *C. mrigala* where a minimum reduction of 1.59 % in the body protease activity was induced by dimethoate at 0.0001 ppm and maximum 14.5 % was in malathion at 0.001ppm. Similar trend was seen in *Labeo rohita*. Maximumreduction *i.e.* 17.0% was caused by malathion (0.001 ppm) and dimethoate (0.0001) induced minimum reduction of 7.6% (Table 2). In fish *L. rohita* the toxic effects of all the pesticides *i.e.* dimethoate, chlorpyrifos and in combination were enhanced at all the concentration levels. The maximum reduction of protease was 17.5% when the fish exposed to malathion in combination with dimethoate and chlorpyrifos at 0.001 ppm concentration and minimum reduction was 2.3% in combination with dimethoate and chlorpyrifos at 0.0001 ppm. Similar trends of protease enzyme reduction were observed in fish C. *mrigala*. The maximum reduction of protease in blood serum was 15.4% when the fish exposed to malathion in combination with dimethoate and chlorpyrifos at 0.001 ppm concentration and minimum reduction was 1.9 % in malathion combination with dimethoate and chlorpyrifos at 0.0001 ppm.

Table 1: Different concentrations of pesticides used 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

Effects on amylase enzyme activity in body

In fish *C. mrigala*, minimum reduction of 7.1 % in the body amylase activity was induced by dimethoate at 0.0001 ppm and maximum 14.1 % was in malathion at 0.001ppm. Similar trend was seen in*Labeo rohita* maximum reduction of 13.4% was caused by (0.001 ppm) and dimethoate (0.0001) induced minimum reduction of 11.4%. The maximum reduction of amylase was 18.8% when the fish exposed to malathion combination with dimethoate and chlorpyrifos at 0.001 ppm concentration and minimum reduction was 13.4% in combination with dimethoate and chlorpyrifos at 0.0001 ppm. In both the fishes i.e. *L. rohita* and *C. mrigala* the toxic effect of all the pesticides i.e. dimethoate, chlorpyrifos and in combination with each other were enhanced at all the concentration levels. The maximum reduction of amylase was 14.9% when the fish exposed to in malathion combination with dimethoate and chlorpyrifos at 0.001 ppm concentration and minimum reduction was 8.5 % in malathion combination with dimethoate and chlorpyrifos at 0.0001 ppm.

DISCUSSION

Significant reduction in levels of protease and amylase has been observed when the fishes were treated with malathion, chlorpyrifos and dimethoate (alone as well as in combination). The reduction in amylase and protease activity in presence of pollutants has previously been advocated by Rani *et al.* (2015). The reduction of enzyme activity is the indicators of stress caused due to pesticide exposure. The protease and amylase enzymes are chemically proteins.The proteolytic activity reduction may be attributed to the alteration in protein value or proteins tertiary structure (Ganeshwade, 2011). This reduced proteolytic and amylolytic activity may represent the reduced growth rate and food utilization. These observations indicate that the pesticides' exposure even at low levels tend to inhibit growth of fishes due to induced stress. Reports by Khare(1993) stamps the pollutant induced rupture of cells and tissue deformities that affect the efficacy of digestive enzymes. Reduced efficacy of digestive enzymes may also interfere with the process of digestion.The fall in proteinlevel during exposure may be due to decreased anabolism of proteins Ganeshwade (2012). Significant reduction in the levels of proteins and glycogen has earlier been reported by Sreekala*et*

Table 2: Effect of different concentrations of different pesticides on protease activity (mg of tyrosin/ mg of protein/ h)

Treatments	*Labeo rohita*				*Cirrhinus mrigala*			
	0.0001ppm	0.0005 ppm	0.001ppm	Mean	0.0001 ppm	0.0005 ppm	0.001 ppm	Mean
Dimethoate	1.67(2.3)	1.62(5.3)	1.59(12.9)	1.63	1.59(1.9)	1.50(7.4)	1.47(9.3)	1.52
Chlorpyrifos	1.59(7.0)	1.51(11.5)	1.49(17.0)	1.53	1.49(8.0)	1.48(8.6)	1.39(14.2)	1.46
Malathion	1.58(7.6)	1.51(12.3)	1.42(17.0)	1.50	1.53(8.5)	1.43(11.7)	1.39(14.5)	1.43
D+C+M	1.6(7.8)	1.50(7.0)	1.41(17.5)	1.50	1.47(9.3)	1.41(13.0)	1.37(15.4)	1.42
Control	1.71	1.71	1.71	1.71	1.62	1.62	1.62	1.62
Mean	1.63	1.57	1.53		1.53	1.49	1.45	

Value in parenthesis are per cent increase/decrease over control C. D. at =0.05; For concentration in *L. rohita*: 0.011 and in *C. mrigala*: 0.012

Table 3: Effect of different concentrations of different pesticides on amylase activity (mg of maltose/ mg of protein/ h)

Treatments	*Labeo rohita*				*Cirrhinus mrigala*			
	0.0001 ppm	0.0005 ppm	0.001 ppm	Mean	0.0001 ppm	0.0005 ppm	0.001 ppm	Mean
Dimethoate	1.41(5.4)	1.38(7.4)	1.31(12.1)	1.37	1.39(1.4)	1.37(2.8)	1.30(7.8)	1.36
Chlorpyrifos	1.37(8.1)	1.33(10.7)	1.30(12.8)	1.33	1.37(2.8)	1.32(6.4)	1.29(8.5)	1.33
Malathion	1.32(11.4)	1.31(12.1)	1.29(13.4)	1.30	1.31(7.1)	1.27(9.9)	1.21(14.1)	1.30
D + C + M	1.29(13.4)	1.27(14.8)	1.21(18.8)	1.26	1.29(8.5)	1.22(13.5)	1.20(14.9)	1.24
Control	1.49	1.49	1.49	1.49	1.41	1.41	1.41	1.41
Mean	1.38	1.35	1.32		1.36	1.32	1.30	

Value in parenthesis are per cent increase/decrease over control C. D. at = 0.05; For concentration in *L. rohita*: 0.009 and in *C. mrigala*: 0.013

al.(2013). However, more reduction in enzymatic activity levels due to exposure to pesticides in combination may be due to the synergistic effects of pesticides on major carps. Both fish species showed concentration dependent decrease in activities of proteolytic and amylolytic enzymes activity. The maximum reduction of protease in blood serum was 15.4% when the fish exposed to malathion in combination with dimethoate and chlorpyrifos at 0.001 ppm concentration. The maximum reduction of amylase was 14.9% when the fish exposed to in malathion combination with dimethoate and chlorpyrifos at 0.001 ppm concentration and minimum reduction was 8.5 % in malathion combination with dimethoate and chlorpyrifos at 0.0001 ppm.

ACKNOWLEDGEMENT

The authors are immensely thankful to the Department of Zoology for providing the necessary requirements timely.

REFERENCES

APHA (American Public Health Association. 1998. Standard methodsfor the examination of water and waste water. *APHA, AWWA, WPFC, 16Ed, New York.*

Deshpande, A. M. and Satyanarayan, S. 2011. Toxicity evaluation of through fish bioassay raw bulk drug industry wastewater after electrochemical treatment. *Iranian J. Environmental Health Science and Engineering.* **8:** 373-380.

Dogan, D. and Canan, C. 2007. Hematological, biochemical, and behavioral responses of *Oncorhynchusmykiss* to dimethoate. *Fish Physiol. and Biochem*. **37(4):** 951-958.

FAO 2013. Fishery Information, Data and statistics unit, FAO Fisheries Department, Fishtat Plus: Universal software for fishery statistical time series.

Ganeshwade, R. M. 2011. Biochemical changes induced by dimethoate in the liver of freshwater fish *Punctiusticto*(Ham). *Biological forum-An International J.* **3(2):** 65-68.

Ganeshwade, R. M. 2012. Biochemical changes induced by dimethoate (Rogor 30% EC) in the gills of freshwater fish *Punctiusticto* (Hamilton). *J. Ecol. Nat. Environ*. **4(7):** 181-185.

Khare, P. 1993. Endosulfanrogar, mercuris chloride and lead nitrate induced histopathological and histochemical changes with stomach and intestine of *Mystus cavasius*. *Ph.D. Thesis, Baraktullah University, Bhopal*.

McCarthy, I. D. and Fuiman, L. A. 2008. Growth and protein metabolism in red drum (*Sciaenopsocellatus*) larvae exposed to environmental levels of atrazine and malathion. *Aqua Toxicol*. **88:** 220-229.

Ramakrishna, R., Shipton, T. A. and Hasan, M. R. 2013. Feeding and feed management of Indian major carps in Andhra Pradesh, India. FAO Fisheries and Aquaculture Technical Paper No.578. Rome, FAO. p. 90.

Rani, S., Gupta, R. K. and Tehri, K. 2015. Zinc and cadmium induced changes in the proteolytic and amylolytic enzyme activity in Indian major carps. *Bioscan*. **10(2):** 613-616.

Rao, V. J., Pavan, S. Y. and Madhavendra, S. S. 2003. Toxic effects of chlorpyrifos on morphology and acetylcholinesterase activity in the earthworm, *Eisenia fetida*. *Ecotoxicol. Environ. Safety*. **54:** 296-301.

Sawhney, S. K. and Singh, R. 2000. In Introductory Practical Biochemistry. *Narosa Publishing House*. p. 452.

Sreekala, G., Raghuprasad, S. G. and Zutshi, B. 2013. Biochemical markers and histopathology of the target tissues of *Labeo rohita* reared in freshwater lakes of Bangalore, Karnataka, *India. J. Res. Environ. Sci. Toxicol.* **2(2):** 42-52.

Walter, H. E. 1984. Methods of enzymatic analysis.*VerlagChemie, Weinchem*. p. 238.