Biolife 2(2):475-479
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

EFFECT OF PHENTHOATE ON PROTEIN LEVELS OF FRESHWATER FISH *LABEO ROHITA* (HAMILTON)

Somaiah, K[1], Sunita, K[2]* and Nagaraju, B[3]

[1]Department of Environmental Sciences, [2]Department of Zoology & Aquaculture, [3]Department of Biochemistry, Acharya Nagarjuna University, Guntur-522510, Andhra Pradesh, India.

E-mail: drsunitamichael@gmail.com

ABSTRACT

The organophosphorus pesticide Phenthoate has been widely used in agriculture for several crops such as paddy, cotton and vegetables to control serious insects and mites in many areas of India. These chemicals cause severe damage to aquatic ecosystem especially to fishes. The main aim and objective of present study is to evaluate the impact on proteins metabolism in fingerlings of *Labeo rohita* an economically important Indian major carp. The lethal concentration was found to be 2.1mg/l under laboratory condition and 1/10th of lethal concentration for 96hrs LC_{50} value is considered as sub lethal concentration. The exposure of fish to sub lethal concentration for 1, 4 and 8 days and investigated significant changes in the vital organs by following standard protocols. The results shows that the percentage of proteins degradation was minimum (7.62%) in gut and maximum percentage (11.76%) in liver for 1day, minimum reduction was (8.04 %) in brain and maximum was (13.64%) in liver for 4 days, minimum percentage depletion (8.36 %) in gill and maximum was (22.46%) in liver for 8 days compared to control. The total protein content levels rapidly induced might be due to the pesticide pollution and inhibition of metabolizing enzymes by toxicity. It is concluded that Phenthoate concentrations (low and high) is very toxic and causes alterations in vital organs of fish *L. rohita*.

Key words : Toxicity, protein levels, sub lethal concentration

.

INTRODUCTION

Water pollution is usually caused by various human sources, typically (point and nonpoint) industrial facilities and agrochemicals especially in aquatic ecosystem, has become a serious environmental problem now a days. These agrochemicals and industrial discharges may carried away effectively by rains, winds, rivers and floods into the large water bodies and change their physico-chemical properties with high toxicity. The water contamination cause damages to aquatic life especially to fishes which are very sensitive to wide range of toxicant in the water (Herger et al., 1995).

Different species of fish show active uptake and accumulation of many toxicants such as herbicides, pesticides, heavy metals and polychlorinated biphenyls from water bodies. Among all these agrochemicals, pesticides found to be extremely toxic to organisms and also to the food chain of aquatic ecosystems. The accumulation of pesticides produces some physiological, biochemical and as well as morphological responses in the freshwater fauna by influencing several activities of metabolites and enzymes reported by Ramamurthy et al. (1987).

The alterations of biochemical contents in different tissues of fish due to toxic impact of different agrochemicals and heavy metals have been reported by number of researchers (Nagrathnamma and Ramamurthy, 1982; Desai et al., 2002; Remia et al., 2008; Hadi et al., 2009; Ganeshwade, 2011). Organophosphates are most preferred insecticides in agriculture due to their effectiveness, less persistent life and easy detoxification in animal tissues which directly inhibit AchE (acetylechelenesterase) activity observed by (Rao et al., 2005) in fish and other aquatic organisms.

Phenthoate (alpha-ethoxycarbonylbenzyl O, O - diethyl phosphorodithioate) is commonly known as Phendol 50% EC (Emulsifiable Concentrate) and it is a broad spectrum, noncumulative, organophosphorus pesticide. It is widely used to protect vegetable crops and as well as controls the parasite on animals. *L. rohita* is one of the prime cultured freshwater fish in poly-culture and has tremendous economical importance. The main objective of present study was to assess the impacts of Phenthoate pesticide on the total protein content in the tissues of *Labeo rohita* exposed to sublethel concentraton for 1, 4 and 8 days. In order to investigate the percent changes caused by pesticide on protein catabolism was also evaluated along with standard deviation.

MATERIALS AND METHODS

Experimental design:

Healthy juvenile fresh water fish *L. rohita* measuring with 7.5 ± 1.5 cm in length and 8.5 ± 0.5 gm in body weight were collected from local fish farm at Buddam village in Bapatla mandal, Guntur district of Andhra Pradesh, India. The fishes were acclimatized to laboratory condition for two weeks in large plastic tubs using dechlorinated tap water (Tarsons Pvt. Ltd.), previously washed with 0.1% KMnO4 solution to free walls from microbial infection. Physico-chemical parameters of water were analyzed by following standard protocol suggested by American Public Health Associatin (APHA, 2005) having temperature of 28 ± 2^{o}C, pH 6.8 ± 0.05, Dissolved Oxygen 6.9-7.4 mg/L, salinity 0 ppt, and total hardness 170 mg/L as $CaCO_3$. During acclimatization, fishes were fed with fish pellets and rice bran on every day after renewing the water.

Preparation of Stock Solution:

Stock solution of Phenthoate was prepared by dissolving 1 gram of pesticide in 100 ml of acetone (stock solution) and the required quantity of Phenthoate was drawn from the stock solution to maintain the suitable concentration of 1 mg/L in the container. The fish were separated into several groups and each containing 10 individuals and pilot experiments were conducted to drive the LC_{50} determinations. These groups were exposed to different concentrations, for acute toxicity estimation, ranged from 0.4 , 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6 and 4.0 mg/L for 24, 48, 72 and 96 hours. During the whole experiment a control group was maintained with acetone for comparison.

The fish were not fed on the day before the beginning of the experiment. The concentration of the pesticide caused 50% mortality at 96 h exposure was taken as the LC_{50} value in the test organisms. Each day number of dead fish were counted and removed immediately from the test container. Percent mortality was carried out and the values were pooled up into probit scale. These values were determined and analyzed by using Finney's Probit analysis (Finney, 1971). The LC_{50} values obtained by the method given by the committee of aquatic toxicity (Anon, 1975). The 96 h LC_{50} value of Phenthoate to fish *L. rohita* was found to be 2.1 mg/L. Then the fish were exposed to sublethal concentrations of $1/10^{th}$ 96 h of LC_{50} value i.e., 0.21 mg/L for 1, 4 and 8 days respectively. A suitable control was also maintained to nullify any other effects that likely to affect the fish. Then the fishes were scarified immediately and isolated fresh (wet) tissue of vital organs such as brain, gill, liver, kidney, gut and muscle for estimation of the total proteins.

Estimation of total proteins:

The total protein content of the pesticide exposed tissue samples were estimated according to the modified standard method (Lowry et al., 1951). The Quantity of 5% homogenate of brain, gill, kidney, liver, muscle

and gut were isolated, precipitated with 5% TCA then centrifuged at 3000 rpm for 15minutes. The precipitate was dissolved in 1 ml of 1N NaOH solution and 0.2 ml of extract taken into test tube and mixed with 5 ml of alkaline copper solution. To this 0.5 ml of 50% Folin Phenol reagent was added after 30 minutes, the optical density was measured at 540 nm against the blank. The standard graph was plotted by using Lowry's method with bovine serum albumin (BSA) as standard solution. The values are expressed as mg/g wet weight of the tissue.

RESULTS AND DISCUSSION

The mean values obtained for total proteins in different tissues of fish and percent change over control along with standard deviation are given in Table 1 and 2 and are graphically represented in Figure 1 and 2. In one day control fish of *L. rohita,* the total protein content is in the order of liver > muscle > kidney > brain > gill > gut. During the exposure of lethal concentration of Phenthoate, percent change of total protein depletes in the order of liver > muscle > brain > kidney > gill > gut. During one day sublethal exposure maximum %change was observed in liver (11.76%) and minimum %change was shown in brain (5.03%) when compared with the control (Figure 1).

Table–1: Changes in the total protein (mg/g wet weight of tissue) content in different tissues of *Labeo rohita* on exposure to Phenthoate for 24 hours

Organs	Control (1 Day)	Lethal	% change (Control Vs. Lethal)	Sublethal (1 Day)	% change (Control Vs. Sublethal)
Brain	113.78± 0.005	95.02± 0.01	16.48 %	108.06± 0.005	5.03 %
Gill	110.02± 0.03	94.03± 0.005	14.53 %	102.99± 0.007	6.38 %
Kidney	116.6± 0.005	98.06± 0.004	15.90 %	106.20± 0.005	8.92 %
Liver	149.57± 0.01	110.04± 0.005	26.42 %	132.03± 0.05	11.76 %
Muscle	132.20± 0.003	109.02± 0.03	17.53 %	121.03±0.005	8.45 %
Gut	108.4±0.01	93.14± 0.003	10.38 %	100.15±0.005	7.62 %

Data are mean of five values ± SD.

Table-2: Changes in the total protein (mg/g wet weight of tissue) in different tissues of *Labeo rohita* on exposure to Phenthoate for 4 days and for 8 days

Organs	Control (4 days)	Sublethal (4 days)	% change (Control Vs. Sublethal)	Control (8 days)	Sublethal (8 days)	% change (Control Vs. Sublethal)
Brain	109.00 ± 0.006	100.24 ± 0.005	8.04 %	101.62 ± 0.005	92.81 ± 0.005	8.66%
Gill	106.24 ± 0.03	97.16 ± 0.007	8.55 %	97.26 ± 0.02	89.13 ± 0.03	8.36%
Kidney	109.35 ± 0.05	99.20 ± 0.005	9.28 %	95.34 ±0.01	83.53 ± 0.02	12.39%
Liver	138.05 ± 0.01	119.22 ± 0.04	13.64 %	117.43 ± 0.02	91.05 ± 0.04	22.46%
Muscle	127.43 ± 0.004	112.15 ± 0.015	11.99 %	118.45 ± 0.005	97.23 ± 0.03	17.91%
Gut	104.11 ± 0.03	93.14 ± 0.05	10.54 %	93.22 ± 0.006	85.61 ± 0.006	8.16%

Data are mean of five values ± SD.

exposure of pesticide mixture Monocrotophos and Fenvalerate. Suneetha (2011) and Tantarpale (2011) reported the changes in carbohydrate metabolism with the mixture of Endosulfan and Fenvalerate on *L. rohita.*

The decreased trend of protein content in various tissues of *L. rohita* in the present study may be due to metabolic utilization of keto acids in the synthesis of glucose or for the osmatic and ionic regulation as mention by Schmidt (1975), Vutukuru (2005), Venktramana et al., (2006), Muley (2007), Mamata Kumari (2007), Chezhian et al., (2010) and Murthy and Devi (1982). The present study revealed the reduction in protein levels in the tissues of *L. rohita* by following acute exposure of toxicant Phenthoate. Similar change was observed in *C. punctatus* exposed to technical grade malathion by Agrhari et al. (2006) and Tilak et al., (2003) explained the reduction of protein content of liver, brain and ovary *of* C. *punctatus* exposed to fenvalerate.

CONCLUSION

The present work indicates that Phenthoate causes alterations in the protein metabolism of fresh water fish *L. rohita*. Thus alteration in protein content in different tissues of the fish during the exposure to the chemical Phenthoate naturally affects the nutritive value of fish.

ACKNOWLDGEMENTS

The Authors are thankful to Dr. K. Veeraiah, Co-ordinator, Departments of Zoology and Dr. A.V.V.S. Swamy, Co-ordinator, Department of Environmental Sciences for providing laboratory facilities. One of the authors Mr. K. Somaiah is thankful to University Grants Commission, New Delhi, India for granting Rajiv Gandhi National Fellowship (RGNF) to carry out the research work.

REFERENCES

1. **Agrahari, S.K., Gopal and Pandey, K.C.** 2006. Biomarkers of Monocrotophos in behaviour of freshwater fish *Channa punctatus* (Bloch). J Environ Biol. 27: 453-457.
2. **Anon.** 1975. Committee on methods of toxicity tests with fish, macro invertebrates and amphibians, EPA, Oregon. p. 61.
3. **APHA.** 2005. Standard Methods for the Examination of Water and Wastewater. 21st Centennial edition, American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington DC, USA.
4. **Chezhian, A., Kabilan, N., Kumar, S.T., Senthamilselvan, D. and Sivakumari, K.** 2010. Impact of common mixed Effluent of spicot industrial Estate on histopathological and biochemical changes in estuarine fish *Lates calcarifer*. Curr Research J of Boil Sciences 2(3): 201-209.
5. **Desai, H.S., Nanda, B. and Panigrahi, J.** 2002. Toxicological effects on some biochemical parameters of freshwater fish *Channa punctatus* (Bloch) under the stress of nickel. J Environ Biol. 23(3): 275-277.
6. **Finney. D.J.** 1971. Probit analysis. Cambridge Univ Press, Cambridge, 333 pp.
7. **Ganeshwade, R.M.** 2011. Biochemical changes induced by Dimethoate in the liver of freshwater fish *Puntius ticto* (Ham). Biological Forum-An International Journal 3(2): 65-68.
8. **Hadi, A.A., Shokr A.E. and Alwan, S.F.** 2009. Effects of aluminium on the biochemical parameters of freshwater fish, *Tilapia zilli*. J Sci Appli. 3: 321-325.
9. **Herger, W., Jung S.J. and Peter H. 1995.** Acute and prolonged toxicity to aquatic organisms of new and existing chemicals and pesticides. Chemosphere. 31: 2707-2726.
10. **Hilmy, A.M., Shabana, M.B. and Daabees, A.Y.** 1985. Effect of cadmium upon *in vivo* and *in vitro* activity of protein and five enzymes in blood serum and tissue homogenate on *Mugil cephalus*. Comp Biochem Physio Comp Pharacol Toxicol. 81(1): 145-154.
11. **Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.** 1951. Protein

measurement with the Folin Phenol reagent. J Biol Chem. 193: 265-275.
12. **Mamata Kumari.** 2007. Biochemical changes induced by the pesticides abate in the liver of cat fish *Heteropneutes fossilis* (Bloch). Environ and Eco. 225(4): 1164-1166.
13. **Muley, D.V., Karanijikar, D.M. and Maske, S.V.** 2007. Impact of industrial effluents on the biochemical composition of freshwater fish *Labeo rohita.* J Environ Biol. 28(2): 243-249.
14. **Murthy, A.S. and Devi, A.P.** 1982. The effect of endosulfan and its isomers on tissue protein, glycogen and lipid in the fish *Channa punctatus*. Pesticidal Biochem Physiol. 17: 280-286.
15. **Nagratnamma and Ramamurthi, R.** 1982. Metabolic depression in the freshwater teleost *Cyprinus carpio* exposed to an organophosphate pesticide. Curr Sci. 51(B): 668-669.
16. **Radhaiah, V., Girija, M. and Jayantha Rao, K.** 1987. Changes in selected biochemical parameters in the kidney and blood of the fish, *Tilapia mossambica* (Peters) exposed to heptachlor. Bull Environ Contam Toxicol. 39: 1006-1011.
17. **Ramamurthy, R., Nagaratnamma, R. Jayasundermma B. and Rama Rao, P.** 1987. Histopathological lesions in the gill of freshwater teleost, *Cyprinus carpio* induced by methylparathion. Matsya. 13: 144-147.
18. **Rao, J.V., Ghousia, B., Pallela, R., Usman, P.K. and Nageswara Rao, R.** 2005. Changes in behavior and brain acetyl cholinesterase activity in mosquito fish, *Gambusia affinis* in response to the sub-lethal exposure to chlorpyrifos. Int J Environ Res Public Health, 2(3): 478-483.
19. **Rao, K.S.P. and Rao, K.V.R.** 1984. Changes in the tissue lipid profile of fish *Oreochromis mossambica* during methyl parathion toxicity-a time course study. Toxicol Lett. 23: 147-153.
20. **Remia, K.M., Logan Kumar S. and Rajmohan, D.** 2008. Effect of an insecticide (Monocrotophos) on some biochemical constituents of the fish *Tilapia mossambica. Poll. Res.*,27(3): 523-526.
21. **Schmidt, N. B.** 1975. Osmoregulation effect of salinity and heavy metal. Fed. Proc. 33: 2137-2146.
22. **Suneetha K.** 2011. Effects of Endosulfan and fenvalerate on carbohydrate metabolism of the freshwater fish, *Labeo rohita* (Hamilton). Int J Pharm Pharm Sci. 4(1): 262-268.
23. **Tantarpale. S.A.** 2011. Cypermethrin impact on total protein in muscle and liver of the freshwater fish *Channa striatus*. J Science Research Reporter 1(3): 155-158.
24. **Tilak, K.S. and Rao, D.K. 2003.** Chlorpyrifos toxicity of freshwater fish. J Aqua Biol. 8(2): 161-166.
25. **Tilak, K.S., Veeraiah, K. and Vardhan, K.S.** 2003. Toxicity and residue studies on fenvalerate to the freshwater fish *Channa punctatus* (Bloch). Bull Environ Contam Toxicol. 71: 1207–1212, (2003).
26. **Venktramana, G.V., Sadhya Rani, P.N. and Murthy, P.S.** 2006. Impact of malathion on the biochemical parameters of gobiid fish, *Glossogobius giurus* (Ham). J Environ Biol. 27(1): 119-122.
27. **Vutukuru, S.S.** 2005. Acute effects of hexavalent chromium on survial, oxygen consumption, Hematological parameters and some Biochemicals profiles of Indian major carp, *Labeo rohita*. Int J Environ Res Public health. 2(3): 456-462.

DOI:
https://dx.doi.org/10.5281/zenodo.7205394
Received: 3 April 2014;
Accepted; 20 May 2014;
Available online : 12 June 2014