The toxicity effect of pesticide Monocrotophos 36% E.C on the enzyme activity changes in liver and muscles of *Labeo rohita* (Hamilton, 1882)

TamizhazhaganV[1].Pugazhendy K*, SakthidasanV1, JayanthiC[2], Barbara Sawicka[3], Shuuduv Gerlee[4], RamarajanK[5], Manikandan P[5]

Department of Zoology, Annamalai University, Tamilnadu, India[1]

Department of Education, Annamalai University, Tamilnadu, India[2]

University of Life Sciences in Lublin, Poland[3]

Mongolian State University of Agriculture, school of Agro ecology, Ulaanbaatar[4]

Department of Microbiology, Annamalai University, Tamilnadu, India[5]

Abstract

This study was undertaken to find out the *Labeo rohita* fresh water fish enzyme changes in the fish muscles and liver. Toxicity was calculated probit analysis. Theresults 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 alkaline phosphate and acid phase were both cell were gradually decreased observed by in fish and other aquatic organism. The Monocrotophos affects not only fishes but also organisms in the food chain through the procedure of expenditure of one by the other those human begins affected various genetic disorders absolutely insecticides.

Key words: Monocrotophos, Labeo rohita, alkaline phosphate, Acid phosphates

Introduction

Water is one of the most essential needs for the survival of life on earth. Water covers 71% of the earth's surface.[1]Adversely human activities are directly or indirectly affect the environment. Developed and developing countries which are progressing rapidly in the field of agriculture, technology and industries are continuously releasing various kinds of harmful substances into the biosphere and thereby causing a severe threat to the environment[2-3]Any alteration in the chemical composition of natural aquatic environment usually induces changes in the biochemical aspects of the inhabitants particularly fishes[4]. The major sources of water pollution are domestic, agricultural and industrial wastes which are discharged into natural water bodies[5].Pesticides that are commonly used are categorized into three groups, inorganic, Natural organic and Synthetic Organic[25]. The inorganic pesticides include borates fluorides and mercurial Natural organic compounds including pyrethrum, rotenone and nicotine[6] and the synthetic organic compounds includes chlorinated hydrocarbon, organophosphates and cremates. The digestion in vertebrates is carried out by the intestinal entrecotes expressing brush border enzymes such as disaccharides, alkaline phosphates and transpeptidase[7-8]and[9]. 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[10]. 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 [11] in fish and other aquatic organisms

Materials and Methods

Collection and Maintains of fish

The fish abundant, inhabiting and tidal parts of rivers, and adjacent cultivate ponds[12]. Live specimen was caught from natural habitats cultural ponds. Laterthe collection fish were acclimatized to the laboratory condition.

For the experimental fishes with 9 – 10cms lengths were selected because the minimum length of the mature fish is 8 cm.

Enzyme Assay

Determination of the Activity of GPT

About One ml of the substrate was pipette out in the two test tubes marked as test and control. The test tubes were kept in the water bath at 37^0C for minutes. In one tube (Test) 0.2ml of sample was added. Both the tubes were incubated for 30 minutes at 37^0C. After incubation 0.2ml of sample was added to second tube (Control). For stranded graph, into a series of test tubes, standard pyruvate solution (0.1-0.5) was pipette out and made upto 0.1ml with phosphate buffer was taken. To all the tubes, two drops alanine citrate and 0.1ml of colour reagent were added. The tubes were incubated for 20 minutes at 37^0C. Then 10ml of 4N sodium hydroxide was added and incubated for 10 minutes at 37^0C. The colour developed was read at 520nm. The activity of enzyme is expressed as IU/L [26].

Determination of the Activity of GOT

To the tube mixed as test 1.0ml of buffered substrate and 0.2ml of sample was added. The control 1.0ml buffer substrate was added. Both were incubated at 37^0C for one hour. After incubation period, to the control tube 0.2ml of sample was added. Standard pyruvate and made up to 1.0ml with phosphate buffer. 0.1ml buffer was taken as blank. To all the tubes two drops of aniline citrate reagent was added, mixed, followed by 1.0ml of 2,4- dinitro phenyl hydrazine and incubated at 37^0 C for 20 minutes. Then 10ml of 0.4N sodium hydroxide was added and kept for 10 minutes at 37^0C. The brown colour developed was read at 520nm. The activity of the enzyme is expressed as UI/L[26]. s

Estimation of Alkaline Phosphatase of ALP

0.5ml borate buffer, 0.5ml of substance and 0.1ml supernatant were mixed, and incubated at room temperature for one hour. After incubation the enzyme reaction was arrested using 5.9ml of 0.05N NaOH and mixed well. The colour intensity was measured at 650nm. The mixture contain above all the sample was used as a blank. The Tyrosine was used as the standard[26].

Estimation of Acid Phosphatase of ACP

0.5ml sodium citrate buffer, 0.5ml of substance and 0.1ml supernatant were mixed, and incubated at room temperature for one hour. After incubation the enzyme reaction was arrested using 5.9ml of 0.05N NaOH and mixed well. The colour intensity was measured at 650nm. The mixture contain above all the sample was used as a blank. The Tyrosine was used as the standard[27].

Results

Aquatic toxicology is the study of effects of environmental contamination on aquatic organisms, such as the effect of pollution on the health fish or other aquatic organisms, Pesticide is pollutant. The pesticides capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose rate and persistence in the environment.The Aspartameamino acid transferese and Alanine transferese activity in the muscle of the control fish were GOT (15.21, 13.54, and 12.23) and GPT (1.93, 1.86 and 1.79mg/protein) for 10, 20 and 30 days respectively. In the experimental fishes, they GOT Table : 5 and GPT Table : 6 activity in the lower sublethal concentration and for higher sublethal concentration level was decreased for 10, 20, 30 day exposure periods, The decreased in enzyme activity of the muscles calculated was found to be statically insignificant and both concentrations of both experimental periods. In the liver control *Labeo rohita* GOT Table: 7 and GPT Table:8 activity (GOT 19.45, 18.23, 17.69 and 9.52, 7.23, 6.43 mg/protein) Table:1for 10, 20 and 30 day's exposure periods.

In the experimental fishes, the enzyme activity of the lower sublethal concentration and the fishes treated with higher sublethal concentration decreased for 10, 20 and 30 days of treated. The maximum decreased in the enzyme activity was found in the 30 days of treatment period in both the sublethal concentration of pesticide and the analyzed values were found to be statically insignificant in both the sublethal the acid phosphatese Table: 3 (ACP) Table: 3 activity in the muscle of the control fish was 0.20, 0.17 and 0.13 mg/protein for 10, 20 and 30 days of the treatment respectively. In the experimental fish, the ACP activity in the lower sublethal concentration 0.18, 0.14 and 0.11 mg/protein and higher concentration it was found 0.11, 0.06 and 0.03 mg/protein for 10,20 and 30 days exposure. The decreased in the enzyme activity muscles were statically significant in 30 days of experimental

periods. The ACP activity in the liver of the control fish was analyzed as 0.27, 0.21, and 0.16 mg/protein and in higher sublethal concentration, it was found to be 0.16, 0.10 and 0.06 mg/protein for days of experimental periods. The ACP activity was found to be statically insignificant in the 10 days of exposure period and statically significant in the 30 days of the exposure period in both the sublethal concentrations of pesticide.

The alkaline phosphatase (ALP) activity in the muscles Table: 5of the control fish were 0.42, 0.39 and 0.36 mg/protein for 10, 20 and 30 days respectively. The ALP of muscle in the lower sublethal concentration was 0.41,0.37 and 0.34 mg/protein for 10,20 and 30 days of exposure periods and in the higher sublethal concentration was 035, 0.32 and 0.30 mg/protein for 10, 20 and 30 days of exposure periods. The decrease ALP activity in the muscle was statically significant in 10, 20 and 30 days of exposure periods. The liver of fishes exposed to lower sublethal concentration 0.73, 0.68 and 0.l mg/protein for 10, 20 and 30 days of treatment periods. When the fish treated with higher sublethal concentration, it was 0.65, 0.58 and 0.54mg/protein for 10, 20 and 30 days of exposure periods. In the control fishes, the enzyme activity was found to be 0.76, 0.72 and 0.69mg/protein for 10, 20 and 30 days exposure periods. The values were found to be statically significant on 10, 20 and 30 days of exposure period in both the lower and higher sublethal concentration Monocrotophos.

Discussion

Pesticides destroy,prevent or repel pests such as insects, weeds and rodents but May causes a range of harmful health effects in humans, including cancer, short and long term injury to the nervous system, lung damage reproductive dysfunction and possible dysfunction of the endocrine (hormone) and immune system[28]. The ACP activity was found to be statistically insignificant in the 10 days of exposure period and statically significant in the 30 days of exposure period in both the sublethal concentration of pesticide. The intracellular distribution patterns of enzymes in liver tissues and reported that generally the enzyme, which was kept in latent state inside the membrane of lysosomes[13]. The effect of manganese in the cerebellum of fish increased the acid phosphates activity. The increased acid phosphatase activity Caviaprocellus was because of the pesticide chloropyrififosexposure[14]. In the present study, the decreased acid phosphates (ACP) activity was observed in both the lower and higher sublethal concentration of pesticides in the 10, 20 and 30 days of treatment. The effect of the pollutants in aquatic animals and stated that alkaline phosphatese (ALP) is a brush border enzymes, which splits various phosphorous esters at an alkaline pH and mediated transport[15]. The involvement of alkaline phosphatase in active transport[16], glycogen metabolism[17], protein synthesis and the synthesis of some enzymes and secretary activity were reported by [18]. Thus any alteration in the activity of alkaline phosphatase affects the organisms. In the present investigation, the activity of alkaline phosphatase (ALP) was found to decrease in the tissues of the test fishes when compared with control fishes. The maximum decrease was seen in higher sublethal concentration of pesticide for 30 days.

The inhibition of enzymes in the fish, Tilapia mossambia due to the exposure of the pesticide sevin was reported by[19] Decrease in GPT activity in the tissues of the same fish during methyl parathion exposure was reported by[20]. The reduction of GOT activity in the fish, *Sacobranchusfossillis* in response to thimidon toxicity was reported[21]. The decrease in tranminase enzymes activity has been reported [22] in the fish *Channapunctatus* treated with be pesticides, Quinalphos, Dichlorvos and suquin. The sublethal effects of the pesticide cypermethrin on enzyme activities in the freshwater fish *Cyprinuscarpio* and observed that the lactate dehydrogenase activity increased after and 8, 12 days of treatment [23]. In the present study the elevation in the GPT and GOT activity of the fish treated with pesticide might have been increased depending on anerobic carbohydrate metabolism cumulative effect or possibly to meet the increased energy demands under sustained and prolonged toxic stress[24] of pesticide monocrotophos.

Acknowledgement:

The authors are gratefully acknowledge the facility provide Department of Zoology, Annamalai University, Tamilnadu, India. Funds support of this research AdiDravidar Welfare department Tamilnadu.

Conflict of Interest:

The authors declare that they are no conflict of interest regarding this manuscript.

References

[1] CIA- The world fact book, Central Intelligence Agency.,www.cia.gov/ library/ publications/ the-world-fact book. 2008,10,109-116.
[2] Abbasi SA, Abbasi N, and Soni R., Heavy metal in the environment, 1st. Ed. Mital Publication, New Delhi, India 1998.
[3] APHA (American Public Health Association). Standard Methods of water and wastewater. 18th ed. American water works Association, Water Environment Federation Publication. APHA, Washington D.C. 1992.
[4] Edwards., C.A. Environmental pollution by pesticidesPleunum press, London. 1973 p. 250.
[5] De, A.K., Environmental Chemistry, 3rd edition. New Age International Pvt. Ltd. New Delhi. 1996.
[6] Bogan, R.H, Okey, R.W and Vargas, D.J., Pesticides in natural waters research, 1961, 14: 249.
[7] Maroux, S., Louvard. D., Baratti. J., The amino-peptidase from hog intestinal brush border. Biochem.Biophys.Acta., 1973,321, 282-295.
[8] Semenza, G Anchoring and biosynthesis of stalked brush border membrane protein: glycosidase and peptidases of enterocytes and renal tubuli. Ann. Rev. CellBiol., 1986, 2, 255-313.
[9] Ferraris, R. P., Villenas, S. A., Diamond, J Regulation of brush border enzyme activities and entrecote migration rates in mouse small intestine. Am. J. Physiol., 1992, 262, 1047-1059.
[10] Ramamurthy, R., Nagaratnamma, R. Jayasundermma B. and Rama Rao, P.,Histopathological lesions in the gill of freshwater teleost, *Cyprinuscarpio*induced by methylparathion. Matsya., 1987,13: 144-147.
[11] Rao, J.V., Ghousia, B., Pallela, R., Usman, P.K. and NageswaraRao, R., Changes in behavior and brain acetyl cholinesterase activity in mosquito fish, *Gambusiaaffinis*in response to the sub-lethal exposure to chlorpyrifos. Int J Environ Res Public Health.,2005,2(3): 478-483.
[12] RaveendranS.,Facultative Air – breathing, Hematology and nitrogen excretion in South – India catfish *Mystusgulio*(Hamilton) Ph.D thesis submitted to Bharathidasan University, Thiruchirappalli and Tamilnadu., 2000.
[13] Deduve,C.,B.G Pressman, R.Gianetto, R.Wattiaux and Applemans., Intracellular distribution patterns of enxzyme in fish liver tissue.Biochem.J., 1955,60:604-607.
[14] Sheeba L., A study on the effects of endosulfon an organochlorine compound in Caviaprocellus. Ph.D thesis University of Madras, Tamilnadu,India .,1999.
[15] Goldfisher, S.E. Esser and A.B. Novikoff, 1964.in: Uses of histological and histochemical assessment in the prognosis of the effect of aquatic pollution (ed.) D.E.Hinton, M.K.Kendall and B.B.Silver .Sect.528, Amer.Soc.testMat.Philadelphia., 194-208.
[16] Denielli, J.F., Structural factors in cell permeability and secretion. Symp.Soc.Exp.Biol, 1972, 6:1-15.
[17] Gupta, V and G.Rao.,Histological studies on the chloride plexes of the goat embryos II. Histological distribution of acid and alkaline phosphatase. ActaHistochem., 1974, 49: 60-63.
[18] Sumner, A.T.,The cytology and the histochemistry of the digestive gland cells of Helis.Quart.J.Microsc.Sci.,1965,106:173-192.
[19] Koundinya, P.R. and R.Ramamurthi., Effects of sumithion(Fenitrothion) on some selected enzyme system in the fish,Tilapiamossambica (Peters). Indian J.Exp.Biol.,1978, 16:801-811.
[20] Sivaprasad,R.K and R.Ramana., Effect of sublethal concentration of methyl parathion on selected oxidative enzyme and organic constituents in the tissue of freshwater fish Tilapia mossambica(Petres).Current .Sci., 1979,48:426-528.
[21] Verma, S.R., I.P Tonk and R.C Dalela., In viva enzymatic dysfunction induced by some aquatic pollutants in a fish, Sarcobranchusfossilis.J.Environ.Biol., 1980, 1:1.
[22] Ghosh, T.K., Toxic impact of three organophosphate pesticides on carbohydrate metabolism in a freshwater fish Channapunctatus.Adv.Bio.Sci., 1987,6:20.
[23] Sivakumari, R.,R.Manavalaramanujam, M.Ramesh and R.Lakshmi., Cypermethrin toxicity: Sublethal effects on enzyme activities in a freshwater fish, Cyprinuscarpio (Var.Communis).J.Environ.Biol., 1997,18(2): 121-125.
[24] Tamizhazhagan,V.,Pugazhendy,K.,2016 .The toxicity effect of Monocrotophos 36% E.C on the Biochemical changes *Labeo rohita* (Hamilton, 1882)**.**International Journal for Scientific Research & Development Vol. 3, Issue 1.802-808.
[25] Tamizhazhagan, V and Pugazhendy K. The toxicity effect of Monocrotophos 36% Ec on the Hematology, *Labeo rohita* (Hamilton, 1882).Int J Curr Pharm Res, 2015.7: 4, 92-95.
[26] Mohun HF and Cook IJ. I J Clin Path., 1957, 10, 364.
[27] Barrett,A.J: In: Dingle,J., T(Ed): Lysosomes: a Laboratory Handbook, Amsterdam: North-Holland Publ.Co. 1972, pp.46-135.
[28] Tamizhazhagan,V.,PugazhendyK.The toxicity effect of Monocrotophos 36 E.C% on the Histological changes in Gill of *Labeo rohita* (Hamilton, 1882).International journal for innovative research in multidisciplinary field. 2016. Vol- 2, Issue - 11,.435-439.

Table: 1 The GOT content of liver of *Labeo rohita* exposed to pesticide monocrotophos.

Concentration Monocrotophos	Exposure period(Days)	Amount of GOT content (μ mole pyruvate/mg/Protein)
Control 0 ppm	10	19.45±0.1201
	20	18.23±0.2105
	30	17.69±0.1105
Control 0.02 ppm	10	19.31±0.1150
	20	18.15±0.1124
	30	17.56±0.1105
Control 0.04 ppm	10	19.23±0.2533
	20	18.02±0.1325
	30	17.41±0.1872
Control 0.08 ppm	10	19.01±0.1314
	20	17.91±0.2740
	30	17.20±0.5543
Control 0.10 ppm	10	18.81±0.1314
	20	17.61±0.2740
	30	16.20±0.5543

Table: 2 The GPT content of liver of *Labeo rohita* exposed to pesticide monocrotophos.

Concentration Monocrotophos	Exposure period(Days)	Amount of GOT content (μ mole pyruvate/mg/Protein)
Control 0 ppm	10	9.52±0.1201
	20	7.23±0.2105
	30	6.45±0.1105
Control 0.02 ppm	10	9.35±0.1150
	20	7.01±0.1124
	30	6.21±0.1105
Control 0.04 ppm	10	9.11±0.2533
	20	6.95±0.1325
	30	5.85±0.1872
Control 0.08 ppm	10	8.94±0.1314
	20	6.75±0.2740
	30	5.41±0.5543
Control 0.10 ppm	10	8.71±0.1314
	20	6.35±0.2740
	30	5.12±0.5543

Table: 3 The ACP content of liver of *Labeo rohita* exposed to pesticide monocrotophos.

Concentration Monocrotophos	Exposure period(Days)	Amount of GOT content (μ mole pyruvate/mg/Protein)
Control 0 ppm	10	0.27±0.1011
	20	0.21±0.2325
	30	0.16±0.2105
Control 0.02 ppm	10	0.25±0.1754
	20	0.19±0.1625
	30	0.15±0.1741
Control 0.04 ppm	10	0.20±0.2424
	20	0.16±0.1125
	30	0.12±0.1742
Control 0.08 ppm	10	0.18±0.1254
	20	0.12±0.2740
	30	0.08±0.5453
Control 0.10 ppm	10	0.16±0.1756
	20	0.10±0.2142
	30	0.06±0.1414

Table: 4 The ALP content of liver of *Labeo rohita* exposed to pesticide monocrotophos.

Concentration Monocrotophos	Exposure period(Days)	Amount of GOT content (μ mole pyruvate/mg/Protein)
Control 0 ppm	10	0.76±0.1201
	20	0.72±0.2105
	30	0.69±0.1105
Control 0.02 ppm	10	0.25±0.1754
	20	0.19±0.1625
	30	0.15±0.1741
Control 0.04 ppm	10	0.73±0.1150
	20	0.68±0.1124
	30	0.65±0.1105
Control 0.08 ppm	10	0.69±0.2533
	20	0.64±0.1325
	30	0.59±0.5553
Control 0.10 ppm	10	0.65±0.1786
	20	0.58±0.2542
	30	0.54±0.1504

Table: 5 The GOT content of Muscle of *Labeo rohita* exposed to pesticide monocrotophos.

Concentration Monocrotophos	Exposure period(Days)	Amount of GOT content (μ mole pyruvate/mg/Protein)
Control 0 ppm	10	15.21±0.1325
	20	13.54±0.1452
	30	12.23±0.4210
Control 0.02 ppm	10	15.01±0.2154
	20	13.34±0.3521
	30	12.13±0.2145
Control 0.04 ppm	10	14.93±0.3562
	20	13.12±0.2415
	30	11.96±0.3562
Control 0.08 ppm	10	14.61±0.145
	20	13.01±0.3562
	30	11.81±0.4852
Control 0.10 ppm	10	14.48±0.2150
	20	12.97±0.3692
	30	11.62±0.2415

Table: 6 The GPT content of Muscle of *Labeo rohita* exposed to pesticide monocrotophos.

Concentration Monocrotophos	Exposure period(Days)	Amount of GOT content (μ mole pyruvate/mg/Protein)
Control 0 ppm	10	1.93±0.2150
	20	1.86±0.1240
	30	1.79±0.2130
Control 0.02 ppm	10	1.89±0.2140
	20	1.81±0.2150
	30	1.68±0.2514
Control 0.04 ppm	10	1.72±0.1208
	20	1.75±0.1542
	30	1.51±0.2415
Control 0.08 ppm	10	1.63±0.2451
	20	1.61±0.3562
	30	1.45±0.4752
Control 0.10 ppm	10	1.55±0.2451
	20	1.46±0.3562
	30	1.31±0.2581

Table: 7 The ACP content of Muscle of *Labeo rohita* exposed to pesticide monocrotophos.

Concentration Monocrotophos	Exposure period(Days)	Amount of GOT content (µ mole pyruvate/mg/Protein)
Control 0 ppm	10	0.20±0.1421
	20	0.17±0.1452
	30	0.13±0.1425
Control 0.02 ppm	10	0.18±0.2150
	20	0.14±0.2105
	30	0.11±0.3561
Control 0.04 ppm	10	0.16±0.3614
	20	0.12±0.4752
	30	0.09±0.2150
Control 0.08 ppm	10	0.13±0.3624
	20	0.09±0.2361
	30	0.06±0.4712
Control 0.10 ppm	10	0.11±0.2415
	20	0.06±0.1425
	30	0.03±0.1356

Table: 8 The ALP content of Muscle of *Labeo rohita* exposed to pesticide monocrotophos.

Concentration Monocrotophos	Exposure period(Days)	Amount of GOT content (µ mole pyruvate/mg/Protein)
Control 0 ppm	10	0.42±0.3521
	20	0.39±0.3214
	30	0.36±0.1205
Control 0.02 ppm	10	0.41±0.2153
	20	0.37±0.1321
	30	0.34±0.1324
Control 0.04 ppm	10	0.39±0.2632
	20	0.36±0.1254
	30	0.33±0.1785
Control 0.08 ppm	10	0.38±0.1321
	20	0.34±0.1452
	30	0.31±0.4125
Control 0.10 ppm	10	0.35±0.2416
	20	0.32±0.3652
	30	0.30±0.2351