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K Anusiya Devi
PG and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore 641029, Tamilnadu, India.

S Binukumari
PG and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore 641029, Tamilnadu, India.

J Vasanthi
PG and Research Department of Zoology, Nirmala College for Women, Red fields, Coimbatore-641029, Tamilnadu, India.

Correspondence
K Anusiya Devi
PG and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore 641029, Tamilnadu, India.

Effect of enzymes in the freshwater fish, *Labeo rohita* during acute and chronic sublethal exposure to the pesticide monocrotophos

K Anusiya Devi, S Binukumari and J Vasanthi

Abstract
Pesticides are widely used in modern agriculture to aid in the production of high quality food. However, some pesticides have the potential to cause serious health or environmental damage. Repeated exposure to sub-lethal doses of some pesticides can cause physiological and behavioural changes in fish that reduce populations, by causing abandonment of nests and broods, decreased immunity to disease and increased failure to avoid predators. Monocrotophos is one of the organophosphorus pesticide used in this study. The median lethal concentration (LC_{50}) of MC to fish *L. rohita* for 96 hour was found to be 45.1 ppm. In sublethal concentration ($1/10^{th}$ of LC_{50} 96 hour value, 4.51ppm) fishes were exposed for 24, 48, 72 and 96 hrs, 10 days, 20 days and 30 days. Organs of fishes were sacrificed and tested for enzymatical analysis. A significant decrease in ACP, ALP values and maximum increase in GOT, GPT and LDH values were observed throughout the study period when compared to the controls. It is essential for assessing the ecological risk of these pesticides.

Keywords: Enzymes; Monocrotophos; *Labeo rohita*; sub lethal

1. Introduction
Clean water is important for the development of fishery resources. Out of total global water, only 3% in the form of freshwater is suitable for human use (Kumar, 1974; Jhingran, 1975 and Anvar Batcha, 1997) [18, 15, 2]. Fish as a bioindicator species can play an important role in the monitoring of water pollution, as they respond with great sensitivity to changes in the aquatic environment (Lindstrom Seppa *et al*., 1981; Smolowitz *et al.,* 1991) [19, 30]. Pesticides affect growth and nutritional value of fish, when their concentration in water exceeds the critical maximum limit (Arunachalam *et al.,* 1980) [4]. Pesticides are not highly selective but are generally toxic to many macrophytes, non-target organisms such as fish (Ayoola, 2008; Franklin *et al.,* 2010) [6, 12].
Enzymes play significant role in food utilization and metabolism. The proteolytic enzymes participate in the breakdown of protein molecules into amino acids and these amino acids are in turn oxidized to give energy for body function (Saravanan *et al*., 2000) [26]. Enzymes are exceedingly efficient and very specific in terms of nature of reaction catalysed and the substrate utilized. The synthesis and final concentration of enzymes is under genetic control and is greatly influenced by very small molecules of substrate.

2. Material and methods
2.1. Collection and Maintenance of Fish
Fingerlings of the fresh water fish, *Labeo rohita* ranging in weight from 4g to 8g and measuring (4cm to 6cm in length) were procured from Aliyar Fish farm, Tamilnadu, India. The procured bulk samples of *Labeo rohita* were transported to the laboratory in well aerated polythene bags and acclimatized to the laboratory conditions under natural photo period for one week in large plastic containers at (26 ± 5 ^{0}C). The tank was previously washed with potassium permanganate to prevent any fungal infection. The fishes were maintained in dechlorinated tap water of the quality used in the test and water was renewed every day to provide freshwater rich in oxygen.

During the periods of acclimatization they were fed everyday with oil cake mixed with rice flour. Unhealthy fish and those with infections were removed. Feeding was stopped two days prior to the experiment to maintain same state of metabolic requirements. Fish belonging to both sexes were selected for the present investigation. All the precautions, laid down on recommendations of the toxicity tests to aquatic organisms were followed (Anon; 1975) [1]. The tap water free from contaminants was used as dilution water for the present study. The physico-chemical analysis of water used in the experiments was carried out using the method of APHA, (2005) [3]. Temperature 27.2 ± 0.9 (^{0}C), pH 7.1 ± 0.1, dissolved oxygen 5.4 ± 0.4 (mg /l), total hardness 180 ±1.9 (mg /l), salinity 0.3 ± 0.1 (ppt). Continuous artificial aeration was maintained throughout the acclimation and exposure periods.

2.2. Toxicant

Monocrotophos is one of the organophosphorus insecticides extensively used in agriculture and animal husbandry (Rao, 2004). Monocrotophos is a brownish yellow liquid with a sharp smell that irritates the eyes and skin. The IUPAC name is dimethyl (E)-1-methyl-2-(methyl-carbamoyl) vinyl phosphate. Molecular formula is C7H14NO5P and molecular weight is 223.2.

2.3. Determination of 96 h LC_{50} value of MC

The concentrations of the pollutant at which 50 percent of the test animals die during a specific test period of time is referred to as median lethal concentration (LC_{50}) (or) median tolerant limit. In aquatic toxicology the traditional LC_{50} test is often used to measure the potential risk of a chemical (Jack de Bruijin *et al*., 1991) [14]. Batches of 10 healthy fishes were exposed to different concentrations of pesticide, Monocrotophos, and the LC_{50} values calculated. One more set of fishes was maintained as control in tap water. To find the wide range of concentration 100-600 ml were chosen and the number of dead or affected fishes was counted at regular intervals upto 48 hrs. The level of the dissolved oxygen, pH, alkalinity and hardness were monitored and maintained constant. Appropriate narrow range of concentration was used to find the median lethal concentration, using a minimum of 6 fishes for each concentration and the mortality was recorded for every 24hrs upto 96hrs.It was found as for 48hrs, using probit analysis method (Finney, 1971) [11]. From the stock solution various sublethal concentrations were prepared for bioassay studies.

2.4. Sublethal toxicity

Seven groups of fishes were exposed to 1/10th of the pesticide 'Monocrotophos' for 24, 48, 72 and 96 hrs, 10 days, 20 days and 30 days. Another group was maintained as control. All the groups received the same type of food and other conditions were maintained similarly. At the end of each exposure period, fishes were sacrificed for further analysis.

2.5. Preparation of tissue samples

After each exposure period, tissues such as liver, gill, muscle, brain and kidney were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of enzymological parameters (ALP,ACP,GOT,GPT and LDH).

2.6. Collection of blood sample

Blood was collected from control and Monocrotophos treated groups by gill puncture. Plastic disposable syringe fitted with 26 gauge needle which was already moistured with heparin was used. The collected blood was expelled into separate heparinised plastic vials and kept immediately on ice. The blood was used for the estimation of GOT, GPT, LDH, ACP and ALP.

2.7. Estimation of alkaline phosphatase (ALP)

ALP both in blood and other tissues was determined by kind and king's method, (1954) [16]. This method is based on the principle that the Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate, and phenol at 100 pH. Phenol so formed react in alkaline medium with 4-amino antipyrine in the presence of the oxidizing agent potassium ferricyanide and formed orange–red coloured complex, which was measured colorimetrically at 510nm and expressed as IU/L.

2.8. Estimation of acid phosphatase (ACP)

ACP in blood and tissues was estimated by king's method, (1959) [17] using SPAN diagnostic reagent kit. The principle followed in this method was similar to that of ALP, except the fact that the reaction was carried at a lower pH namely 4.9.

2.9. Estimation of GOT and GPT

Quantitative estimation of GOT in the sample was done following the methods of Reitman and Frankle, (1957) [24]. Glutamate oxaloacetate aminotransferase (GOT) or Aspartate amino transferase (AST) catalyses the reversible interconversions between glutamate and aspirate and their 2-oxoanalogues.

Glutamate+oxaloacetate ----------> 2-oxoglutarate+Asparatate

The oxaloacetic acid was measured colorimetrically by a reaction with 2, 4 dinitrophenyl hydrazine after the addition of 0.4 N sodium hydroxide. The intensity of the colour developed was measured after incubating for 15 minutes at wave length 505nm (or) green filter.

2.9.1. GPT

The procedure adopted for the estimation of Glutamate pyruvate transaminase (GPT) (or) Alanine transaminase (ACT) was the same as for the GOT except that the substrate used here was alanine and the inoculation period allowed was 30minutes.The values in the both the cases were expressed as IU/L.

2.10. Estimation of LDH

Serum LDH was analysed using LDH test kit (AGAPPE Diagnostics, INDIA) based on SCE recommended method (Wei Bhaar, 1975) [33]. 1000 ml working reagent was added with 0.1ml sample, mixed and incubated at 37 ^{0}C or 1 minute. The change in absorbance per minute (OD/MIN) was measured during 3minutes at 340nm.

2.11 Calculation

LDH activity (V/L) = (OD/MIN) x 16030

2.12 Statistical analysis

The data were analysed statistically at t< 0.01.To test their significance the t values were calculated by Student's t-test.

3. Results

3.1. LC 50 value-96 h

In the present study the 96 h LC 50 value of MC to *L. rohita* was determined to be 45.1ppm.

3.2. Enzyme assays

The activity of the enzyme GOT, GPT and LDH was increased (p<0.01) in MC treated fish upto 30 days when compared to the control. GOT showed maximum increase of 200.00% in kidney after 96hrs exposure and minimum of 12.28% in gills after 24 hrs exposure and maximum increase of 363.35% in liver after 30 days exposure and minimum of 62.24% in blood after 10days exposure to Monocrotophos pesticide (Fig 1 & 2). GPT showed maximum increase of 151.02% in brain after 96hrs exposure and minimum of 11.76% in blood after 24 hrs exposure and maximum increase of 516.49% in gill after 30 days exposure and minimum of 65.68% in muscle after 10 days exposure to Monocrotophos pesticide (Fig 3 & 4). LDH showed maximum increase of 331.03% in liver after 96 hrs exposure and minimum of 4.59% in blood after 24 hrs exposure and maximum increase of 551.03% in liver after 30 days exposure and minimum of 51.54% in muscle after 10 days exposure in Monocrotophos pesticide (Fig 5 & 6). The enzymes like ACP and ALP was decreased significantly (p<0.01) when compared to the control group (Fig 7, 8, 9 & 10).

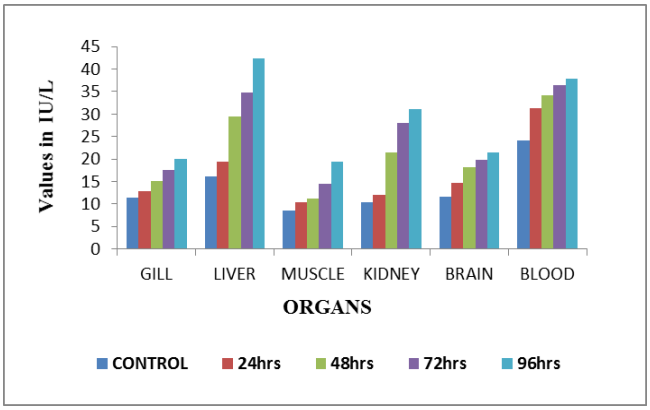

Fig 1: Changes in the GOT content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30days). Bars represent means and standard deviation of six individual observations. Significant at p<0.01(based on T-test) of short term exposure.

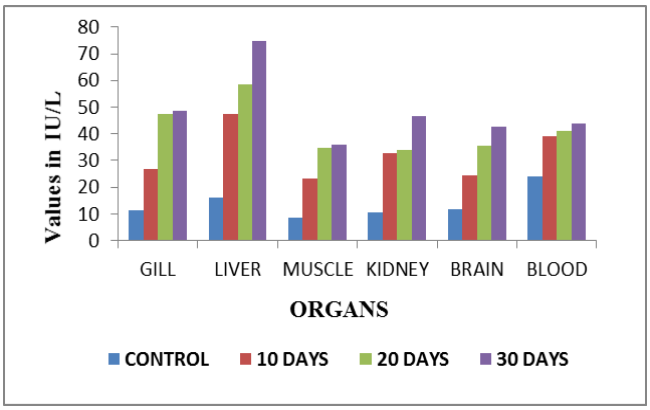

Fig 2: Changes in the GOT content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30days). Bars represent means and standard deviation of six individual observations. Significant at p<0.01 (based on T-test) of long term exposure.

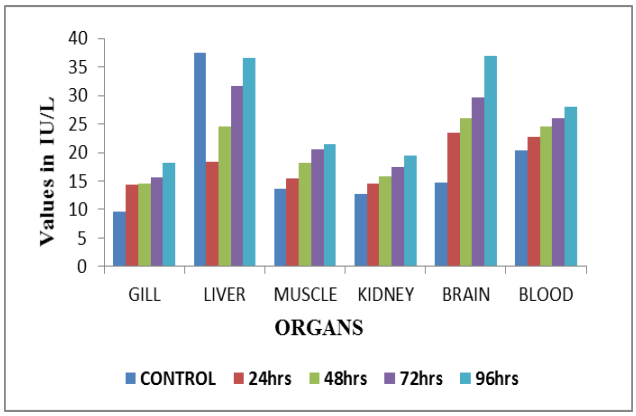

Fig 3: Changes in the GPT content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30days). Bars represent means and standard deviation of six individual observations. Significant at p<0.01 (based on T-test) of short term exposure.

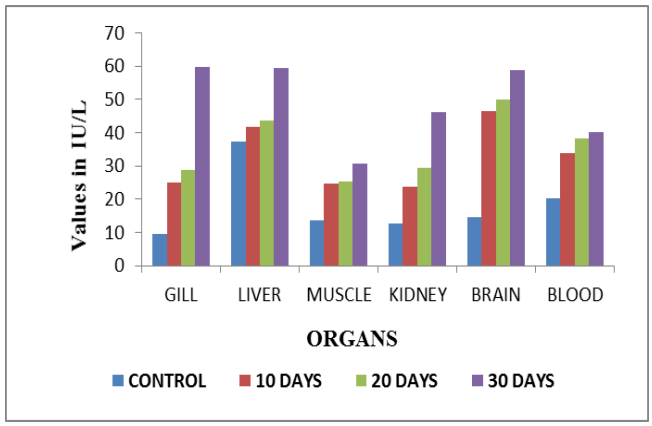

Fig 4: Changes in the GPT content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30days). Bars represent means and standard deviation of six individual observations. Significant at p<0.01 (based on T-test) of long term exposure.

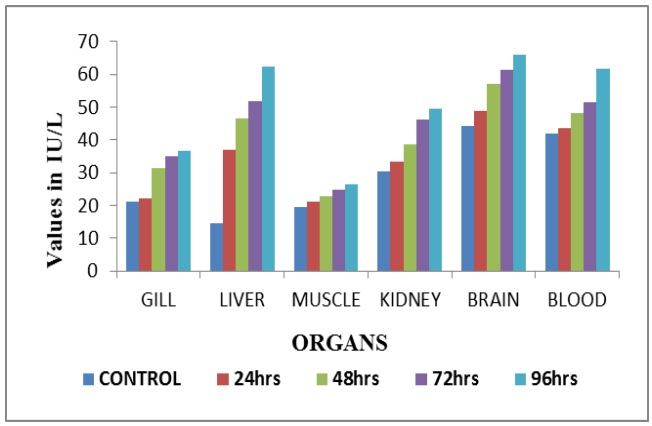

Fig 5: Changes in the LDH content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30days). Bars represent means and standard deviation of six individual observations. Significant at p<0.01 (based on T-test) of short term exposure.

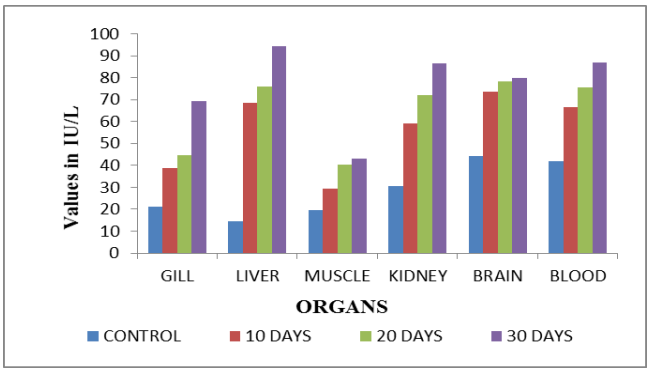

Fig 6: Changes in the LDH content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30days). Bars represent means and standard deviation of six individual observations. Significant at $p<0.01$ (based on T-test) of long term exposure.

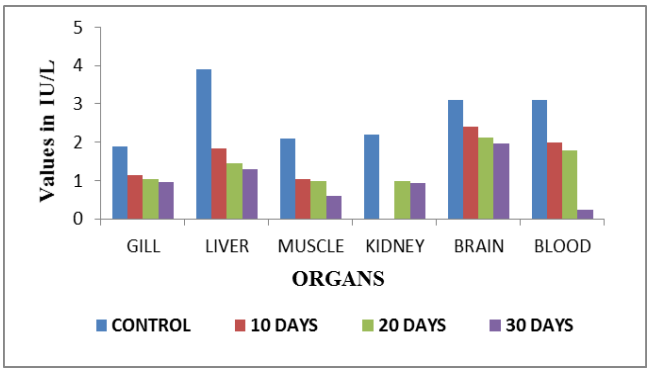

Fig 8: Changes in the ACP content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30days). Bars represent means and standard deviation of six individual observations. Significant at $p<0.01$ (based on T-test) of long term exposure.

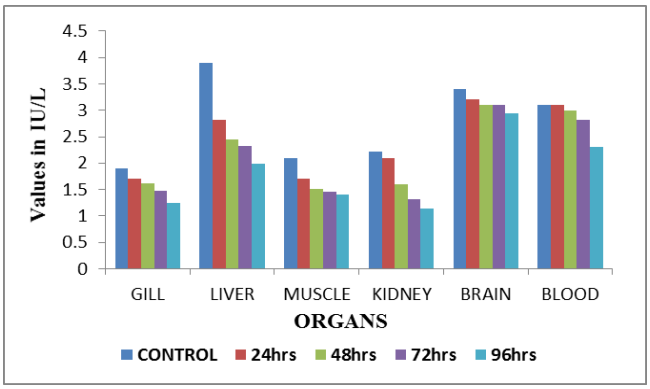

Fig 7: Changes in the ACP content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30 days). Bars represent means and standard deviation of six individual observations. Significant at $p<0.01$ (based on T-test) of short term exposure.

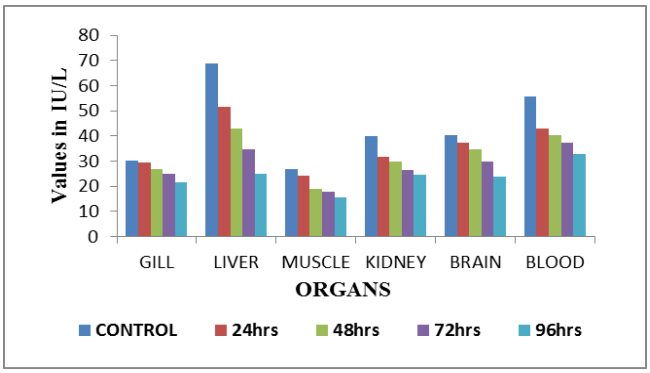

Fig 9: Changes in the ALP content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30days). Bars represent means and standard deviation of six individual observations. Significant at $p<0.01$ (based on T-test) of short term exposure.

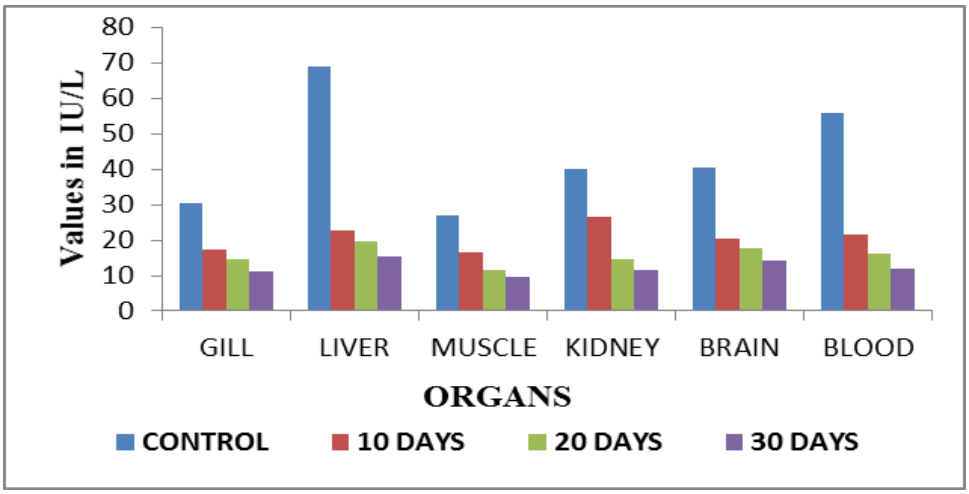

Fig 10: Changes in the ALP content in a fresh water fish *L. rohita* treated with sublethal concentration of MC (4.51 ppm; 30days). Bars represent means and standard deviation of six individual observations. Significant at $p<0.01$ (based on T-test) of long term exposure.

4. Discussion

4.1. GOT and GPT content in tissues

Transaminase forms an important group of enzymes mediating carbohydrate and lipid metabolism. The increase in GPT activity in present study may indicate anaerobic nature of carbohydrate metabolism in fish, possibly to meet the increased energy demands under sustained and prolonged pesticide stress as supported by Ramana Rao *et al*. (1990) [22] for study involving another toxicant. The increase in GOT and GPT activity may also be due to decrease in metabolic activity, disruption of enzyme system by blocking active sites and tissue damage. Similar observations were made by Bhatnagat and tyagi, (1995) [7]. Saqib *et al*., 2005 [25] also reported high levels of GOT and GPT was found in tissues with higher accumulation of pesticide residues. This possibly indicates a correlation between exposure of pesticides and increased level of the two enzymes. Radha krishnan nair and Jasmine, (2010) [21] reported the increased activity of GOT

and GPT in the intestine tissues of the fish, *Catla catla* on exposed to Triazophos. Increase in the duration of exposure also resulted in more tissue damage in an earlier study. This was due to increased activities of GOT and GPT (Shweta Agrahari *et al*., 2007) [28].

4.2. LDH level in tissues

In the present study there was a remarkable increase in LDH activity in the kidney as Asztalos *et al.* (1990) [5] reported earlier as significant increase in LDH activity in the fish, *Cyprinus carpio* exposed to insecticides. The increase in LDH level indicates metabolic changes that is the glycogen catabolism and glucose shift towards the formation of Lactate in stressed fish, primarily the muscle tissue (Simon *et al*., 1993) [29]. Chen *et al*. (2000) [8] observed a significant rise in serum LDH activity after liver infection. LDH level which indicates the energy demands are met by anaerobic respiration through increase in LDH activity. Moreover, several investigators have reported that the oxygen consumption and the activities of liver respiratory enzymes were decreased considerably with an elevation of LDH activities in stressed animals. They suggested that the stressed animals were meeting energy requirement through anaerobic oxidation (Das and Mukerjee, 2000) [9].

4.3. ACP and ALP activity in tissues

The decreased activities of ACP and ALP indicated disturbance in cell organelles like endoplasmic reticulum and membrane transport system. Such damage to cell organelles was reported in various studies. Shakoori *et al*. (1992) [27] have suggested the decrease of ACP and ALP activities are due to increased necrosis in the tissues like hepatocytes in the fish, *Gallus domestics* on exposed to Bifenthrin. The declining of ALP in exposed fish is due to the fall in the rate of synthesis of glycogen resulting from the low metabolic demands and the decrease in the metabolic transport (Edquist *et al*., 1992) [10]. Hong *et al.* (2000) [13] observed the elevated level of serum alanine transaminase and alkaline phosphate in the fish, *Nibea miichthioldes* on exposure to Carbofuran. The decrease in ACP activities in the liver reflects a possible decrease in biosynthetic activities and anaerobic capacity of fish (Tripathi *et al.*, 2003) [31]. Velisek *et al.* (2006) [32] reported the reduced serum alkaline phosphatase value in the rain bow trout, *Oncorhynchus mykiss* as a result of stress induced by low doses of cypermethrin. Similar findings of decreased ACP were reported in *Labeo rohita* on exposure to Arsenic by Nchumbeni *et al*. (2007) [20].

5. Conclusion

In the present study, it is concluded that MC has a profound influence on the enzymatical analysis of an Indian major carp *Labeo rohita* and MC is toxic to aquatic organisms. The results imply a better understanding on the toxicological endpoint of this specific pesticide and provide significant information on safe levels of the pesticide in the aquatic environment.

6. Conflict of Interest

None declared.

7. Acknowledgement

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8. References

1. Anon, Recommendations of the committee on methods for toxicity tests with fish, macro-invertebrates andamphibians EPA, Oregon. 1975, 61.
2. Anvar batcha SM. Studies on Hydrography and domestic pollution problems in the North Bank of River Cauvery. Journal of Environment and pollution. 1997; 5(1):69-71.
3. APHA, Standard Methods for the examination of water and waste water. 21st Ed. Washington DC, 2005.
4. Arunachalam S, Jeyalakshmi K, Aboubucker S. Toxic and sublethal effects of Carbaryl on a fresh water Cat fish, *Mystus vittatus.* Archives of Environmental Contamination Toxicology 1980; 9:307-316.
5. Asztalos B, Nemcsok J, Benedeczky I, Gabriel R, Szabo A, Refaie OJ. The effects of Pesticides on some biochemical parameters of common carp *Cyprinus carpio*. Arch. Environ. Contamin. Toxicol 1990; 19:275-282.
6. Ayoola SO. Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juvenile. Afr. J Agric Res. 2008; 3(12):825-834.
7. Bhatnagar MC, Meenakshi Tyagi. Pyrethroid induced alternations in transaminases in liver and muscle of *Clarias batrachus* (Linn). Proc. Acad. Environ. Biol 1995; 4(2):251-253.
8. Chen W, Kennedy DO, Kojima A, Matsui-Yuasa. Poly amines and thiols in the cytoprotective effects of L-cystein and L-methionine on Carbon tetrachloride induced hepatotoxicity. Aminoacids 2000; 18(3):19-327.
9. Das BK, Mukherjee SC, Murjani G. Histopathological studies on the myxoboliasis of *Cirrhinus mrigala*. Indian J fish. 2000; 47(1):61-64.
10. Edquist LE, Madej A, Forsberb M. Biochemical blood parameters in pregnant mink fed peb and fractions of peb. Ambio 1992; 21(8):577-581.
11. Finney DJ. Probit analysis, 3rd edition, (London: Cambridge University Press). 1971, 20.
12. Franklin RK, Loo HS, Osumanu HA. Incorporation of Bentazone with Exserohilum rostratum for Controlling Cyperus iria. Am. J Agri Biol Sci. 2010; 5:210-214.
13. Hong WB, Shan G Cai, Hu S. Studies on the toxicities of five types of organophosphates to the embryonic and development of Nibea miichthioldes. Marine sciences: Haiyang kexue dew (Marine sciences Haiyang kexue) 2000; 24(1):43-45.
14. Jack de Bruijin, Eddy Yedema, Willen Senior, Joop Heimeus. Lethal body burdens of four Organophosphorus pesticides in the guppy (Poecilia reticulata). Aquatic Toxicology. 1991; 20:111-122.
15. Jhingran UG. Fish and fisheries of India. Revised and enlarged second edition. Hindustan publishing corporation (India), 1975.
16. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed Phenol with Amino antipyrin J Clin path. 1954; 7:322.
17. King J. J. Med. Lab. Tech., Indian journal of biochemistry and biophysics. 1959; 16:265.
18. Kumar HD. Modern concept of Ecology, Vikas publication House Pvt. Ltd., (India), 1974.
19. Lindstrom-Seppa P, Koivussari U, Hanninin O. Extra hepatic metabolism in North-European freshwater fish. Comp. Biochem. Physiol 1981; 69:259-263.

20. Nuhumbeni Humtsoe, Reza Davoodi, Kulkarni BG, Bhavita Chewan. Effect of Arsenic on the enzymes of the rohu carp, *Labeo rohita* (Hamilton, 1822). Raffles Bull. Zool 2007; 14:17-19.
21. Radhakrishnan Nair C, Jasmine E. Toxic effect of Triazophos on transaminase activity in the selected tissues of freshwater fish *Catla catla*. Poll Res 2010; 29(1):73-76.
22. Ramana Rao KV, Surendranath P, Kodavanti PRS. Levels of Transminase in tissues of the peneaid prawn, *Metapenaeus monoceros* (Fabricius) following sub lethal kelthane exposure. Bull. Environ. Contam. Toxicol 1990; 44:114-120.
23. Rao JV. Effects of monocrotophos and its analogs in acetylcholinesterase activity's inhibition and its pattern of recovery on euryhaline fish, *Oreochromis mossambicus*. Ecotox. Environ Safe 2004; 59:217-222.
24. Reitman S, Frankel S. A colorimetric method for determination of serum Glutamic oxaloacetic and Glutamic pyruvate transaminase. Amer. J Clin Path. 1957; 28:56-63.
25. Saqib TA, Naqvi SN, Siddiqui PA, Azmi MA. Detection of pesticide residues in muscles, liver and fat of 3 species of *Labeo rohita* found in Kalri and Haleji lakes. J Environ Biol. 2005; 26:433-438.
26. Saravanan TS, Aneez Mohamed M, Harikrishnan R. Studies on the chronic effects of Endosulfan on blood and liver of *Oreochromis mossambicus*. J Ecol Res Biocon. 2000; 1:24-27.
27. Shakoori AR, Alam J, Aziz F, Sabir M. Toxic effect of bifenthrin (Talstar) on the liver of Gallus domestics. J Ecotoxicol Environ Monit. 1992; 21(1):1-11.
28. Shweta Agahai, Kashew Pandy C, Krishna Gopal. Biochemical alteration induced by Monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch). Pest. Biochem. Physiol 2007; 88(3):268-272.
29. Simon LM, Nemcsok J, Boross L. Studies on the effects of Paraquat on glycogen mobilization in liver of common carp (*Cyprinus carpio* L).Comparative Biochemistry and Physiology. 1993; 75(C):167-169.
30. Smolowitz RM, Hahn ME, Stegemann JJ. Immunochemical localization of cytochrome P450 1A induced by 3,3,4,4, Tetrachlorobiphenyl and 2, 3, 7, 8 Tertrachlorobenzofuran in liver and extra hepatic tissues of the teleost, *Stenotomus chrysops.* Drug Metabol. Disp. 1991; 19:113-123.
31. Tripathi G, Trinder P, Tiwana AJ. Biochemical effects of Pesticidal stress in non target species. J App Zoo Res. 2003; 16(2):245-249.
32. Velisek J, Wlasow T, Gomulka P, Svobodova Z, Dobsikova R, Novoton L *et al*. Effects of Cypermethrin on rainbow trout (*Oncorhynchus mykiss*). Veterinarni Medicina 2006; 51(10):469-476.
33. Wei Bhaar D. Normal ranges of alpha-HBDH, LDH, AP and LAP as measured with substrate-optimated test charges. Med. Welt 1975; 26:387-392.