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## Biochemical Alterations In Common Carp, *Cyprinus Carpio* (L) Exposed to Organ phosphorus Insecticide, Malathion

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### Abstract

The effect of exposure to sublethal concentrations of Malathion on biochemical activities in the serum of common carp, *Cyprinus carpio*, was studied during 96 h exposure. Alterations were observed in glucose, total protein, cholesterol, triglycerides, alanine aminotransferase (ALAT), aspartate aminotransferase (AAT), lactate dehydrogenase (LDH), activities in the serum of *C. carpio*. ALAT and AAT activity levels in Malathion treated fishes were significantly ( $p<0.05$ ) higher than the control fishes. The LDH activity in the *Carpio* after Malathion exposure was two-fold higher ( $p<0.05$ ) when compared to control. These results revealed that Malathion affects the intermediary metabolism of *C. carpio* and that the assayed enzymes can work as good biomarkers of Malathion contamination.

**Keywords:** Biochemical changes, *Cyprinus carpio*, Marker enzymes, Malathion

### 1. Introduction

The adoption of new technology in crop production and protection has increased the use of insecticides. Organophosphate and carbamate insecticides have been used more frequently because of their low potency and readily degradable nature as compared to organochlorine insecticides (Fowler and Mahan, 1980) [5]. Pesticides are used worldwide in agriculture and aquaculture to control the pest and insects (Enis Yonar *et al.*, 2012) [3]. Organophosphate pesticides like Malathion, dimethoate, monocrotophos and chlorpyrifos are widely used for paddy crop pests. The widespread use of synthetic organic pesticides over decades has led to their frequent exposure in the environment. Also acute and chronic exposures of humans to pesticides occur during their commercial production and their application. Synthetic pesticides are deliberately sprayed on crops or agricultural land to increase food production but these agrochemicals are not very selective in producing their effects. They are toxic to many non-target species and contaminate the environment (Singh *et al.*, 2006) [14]. Usage of pesticides in the ecosystem leads to development of various types of morphological, physiological, biochemical and behavioral changes in individuals. Potentially hazardous environmental toxicants like pesticides display a broad spectrum of biological effects, being toxic not only to target organisms but also to humans (Jamil *et al.*, 2007) [7].

Aquaculture apart from agriculture is common in India, where fish, the non-target organisms are directly exposed to pesticides used for the control of insects and pests. The pesticides affect the survival, growth rate, fecundity and reproductive activity of fish. Toxic substances even in very low concentration which is sublethal have been reported to interfere with basal metabolism and suppressed reproduction, steroidogenesis, lipid metabolism, degenerative changes in gonadotropin cells and reduction in interstitial cells size, gonadotropin levels act as reproductive biomarkers and also as endocrine disruptors (Singh and Vandana Singh, 2006) [15].

The exposure of fish to several types of chemical agents may induce changes in several haematological and biochemical parameters which are frequently used to evaluate fish health. Many works have been carried out on biochemical parameters (Ramesh and Saravanan, 2008) [11]. The aim of this study was to investigate the haematological, biochemical, and growth responses of common carp, *Cyprinus carpio* exposed to toxicant, Malathion.

## 2. Materials and Methods

The live healthy *Cyprinus carpio* were obtained from a commercial fish farm. The mean length of the fish was 6.78 cm (range 5.0 to 8.5) and weight was 5.73 gm (range 3.8 to 7.3). The fish (n=150) were acclimated for 4 weeks. The fishes were maintained at a constant water temperature of  $23 \pm 1^{\circ}\text{C}$  and a pH of 7.8. The fish were fed two percent total body mass twice daily, with conventional fish feed (rice bran and soya cake in 1:1 ratio) at the rate of 10 % body weight. The fish were divided into two batches control and experimental. The effect of pesticide on fish becomes consistent within 96 hours exposures, LC<sub>50</sub> 96 hours (0.129 mg/L) of cypermethrin was taken as lethal concentration for *Cyprinus carpio*. To study the biochemical responses 1/5th and 1/10th (0.02 mg/L and 0.01 mg/L) of LC<sub>50</sub> 96 hours was taken as sub lethal concentrations for further studies. Blood was collected by puncturing heart in vials. When blood collection the vials do not contain any anticoagulant for biochemical studies.

### 2.1. Biochemical analysis of fish blood

Analyses included estimation of plasma total protein, cholesterol and glucose, triglycerides. Among enzymes Lactate Dehydrogenase (LDH), Alanine amino tranferase (ALT) and Aspartate amino tranferase (AAT), were studied. Total proteins were estimated according to the method of Lowry *et al.* (1951)<sup>[9]</sup>. Cholesterol was estimated according to the method of Hanel and Dam (1955)<sup>[6]</sup>. Glucose activity was estimated according to Anthrone method of Roe (1955)<sup>[13]</sup>. Triglycerides was estimated according to the method of Van-Handel and Zilversmith (1957)<sup>[16]</sup>. Among enzymes LDH was measured by the method of Wacker *et al.*, (1956)<sup>[18]</sup>. ALT and AAT activities were determined according to the method of King (1965)<sup>[8]</sup>.

### 2.2. Statistical study

The results of static bioassay were analyzed using linear regression probit analysis (Finney, 1971)<sup>[4]</sup> using the statistical package (POLO- PC- LEORA software 1987). Biochemical results were tested by using two way ANOVA (analysis of variance). Post hoc test were carried out using Duncan multiple comparison test procedure. Significance was tested at  $p < 0.05$ .

## 3. Results

The LC<sub>50</sub> values range from 0.116 (120 h) to 0. 180 (24h) (Table 1). The 96h LC<sub>50</sub> value (0. 129mg/l) obtained using probit analysis (Table 2) is used for fixing the two incipient lethal level exposure concentrations of 0.0258 mg/l (1/5th 96h LC<sub>50</sub>) and 0.0129 mg/l (1/10th 96h LC<sub>50</sub>). The figure 1-6 indicates that the fish exposed to two sub lethal concentrations (0.0258and 0.0129 mg/l) of Malathion for 10, 20 and 30 days showed considerable variation over control.

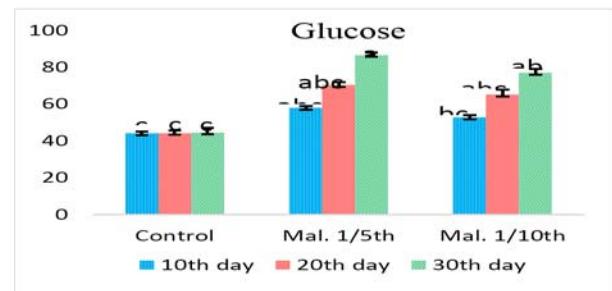
In the treated groups the decrease in the blood glucose values was found as compared to control group. In these exposed groups the blood glucose was found to range from  $57.9 \pm 1.3$  (10days),  $70.4 \pm 1.51$  (20days) and  $86.6 \pm 2.6$  (30days) for the concentration of 0.0258 ml/l,  $52.82 \pm 2.55$  (10days),  $65.23 \pm 1.36$  (20days) and  $77.01 \pm 2.11$  (30days) for the concentration of 0.0129 ml/l (Fig.1). In the treated groups the increase in the blood total protein values was found as compared to control group. In these exposed groups the blood total protein was found to range from  $2.9 \pm 0.36$  (10days),  $2.67 \pm 0.12$  (20days) and  $2.44 \pm 0.1$  (30days) for

the concentration of 0.0258 ml/l,  $3.24 \pm 0.20$  (10days),  $2.74 \pm 0.26$  (20days) and  $2.67 \pm 0.31$  (30days) for the concentration of 0.0129 ml/l (Fig.2).

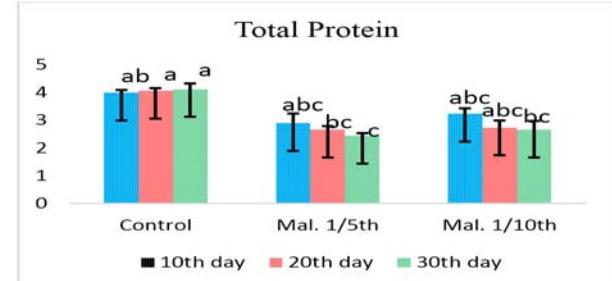
In the treated groups the increase in the blood cholesterol values was found as compared to control group. In these exposed groups the blood cholesterol was found to range from  $27.8 \pm 1.91$  (10days),  $28.13 \pm 1.17$  (20days) and  $30.27 \pm 1.44$  (30days) for the concentration of 0.0258 ml/l,  $27.53$  (10days),  $28.0 \pm 2.0$  (20days) and  $29.2 \pm 1.14$  (30days) for the concentration of 0.0129 ml/l (Fig.3).

In the treated groups the increase in the serum triglycerides level values was found as compared to control group. In these exposed groups the serum triglycerides level was found to range from  $28.01 \pm 2.0$  (10days),  $33.07 \pm 3.2$  (20days) and  $41.04 \pm 2.05$  (30days) for the concentration of 0.0258 ml/l,  $26.2 \pm 0.8$  (10days),  $30.4 \pm 2.42$  (20days) and  $36.93 \pm 2.91$  (30days) for the concentration of 0.0129 ml/l (Fig.4). In the treated groups the decrease in the serum LDH values was found as compared to control group. In these exposed groups the serum LDH was found to range from  $0.569 \pm 0.001$  (10days),  $0.505 \pm 0.003$  (20days) and  $0.495 \pm 0.004$  (30days) for the concentration of 0.0258 ml/l,  $0.524 \pm 0.004$  (10days),  $0.522 \pm 0.003$  (20days) and  $0.515 \pm 0.011$  (30days) for the concentration of 0.0129 ml/l (Fig.5).

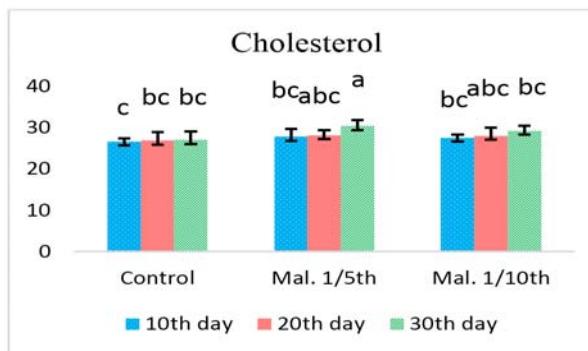
In the treated groups the increase in the serum ALAT level values was found as compared to control group. In these exposed groups the serum ALAT level was found to range from  $3.28 \pm 0.02$  (10days),  $3.35 \pm 0.02$  (20days) and  $3.46 \pm 0.03$  (30days) for the concentration of 0.0258 ml/l,  $3.25 \pm 0.04$  (10days),  $3.3 \pm 0.03$  (20days) and  $3.34 \pm 0.02$  (30days) for the concentration of 0.0129 ml/l. Also in the treated groups the increase in the serum AAT level values was found as compared to control group. In these exposed groups the serum AAT level was found to range from  $3.19 \pm 0.17$  (10days),  $3.2 \pm 0.26$  (20days) and  $3.32 \pm 0.10$  (30days) for the concentration of 0.0258 ml/l,  $3.13 \pm 0.15$  (10days),  $3.17 \pm 0.07$  (20days) and  $3.23 \pm 0.21$  (30days) for the concentration of 0.0129 ml/l (Fig.6, 7).



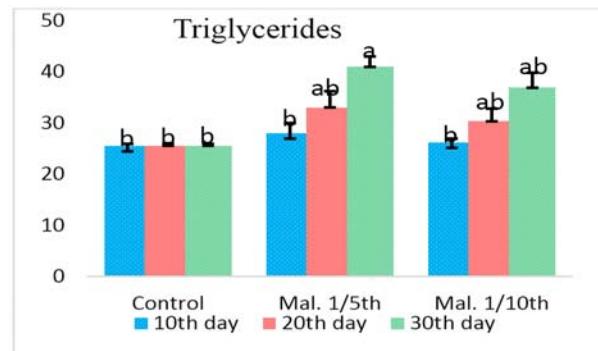
**Fig 1:** Mean Glucose (gm/dl) of *Cyprinus carpio* exposed to different concentrations of Malathion.



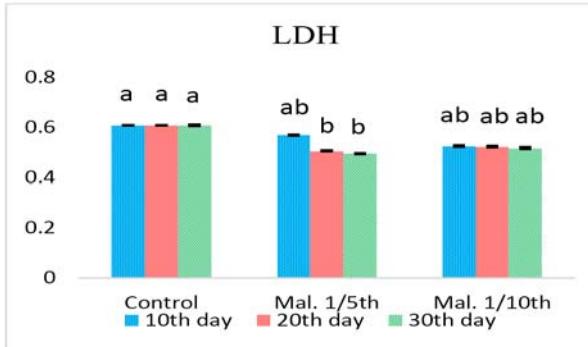
**Fig 2:** Mean Total protein (gm/dl) of *Cyprinus carpio* exposed to different concentrations of Malathion.



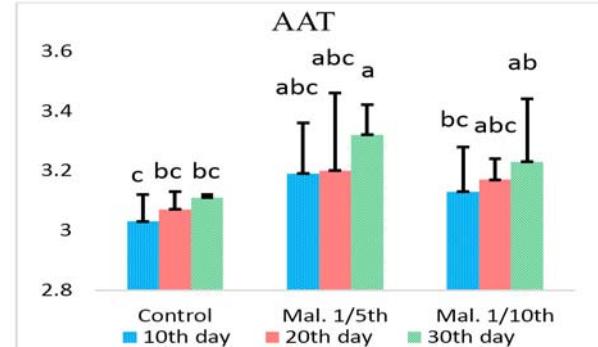
**Fig 3:** Mean Cholesterol (gm/dl) of *Cyprinus carpio* exposed to different concentrations of Malathion.



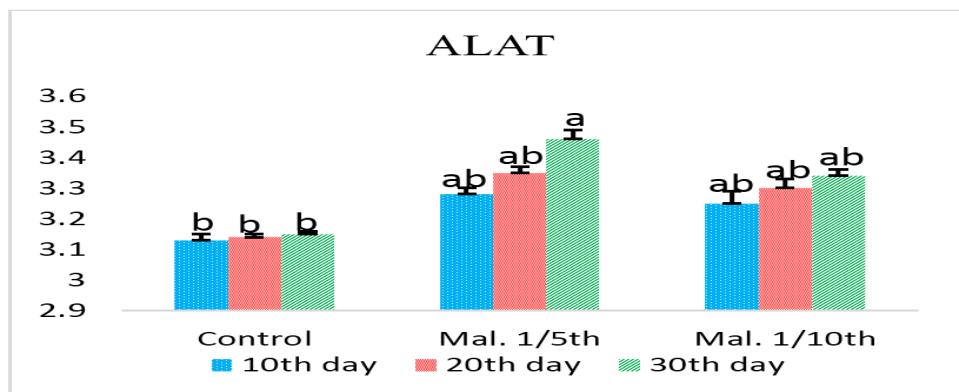
**Fig 4:** Mean Triglycerides (gm/dl) of *Cyprinus carpio* exposed to different concentrations of Malathion.



**Fig 5:** Mean LDH ( $\mu$ moles of pyruvate liberated/hr/mg protein) of *Cyprinus carpio* exposed to different concentrations of Malathion.



**Fig 6:** Mean AAT ( $\mu$ moles of pyruvate liberated/hr/mg protein) of *Cyprinus carpio* exposed to different concentrations of Malathion.



**Fig 7:** Mean ALAT ( $\mu$ moles of pyruvate liberated/hr/mg protein) of *Cyprinus carpio* exposed to different concentrations of Malathion.

**Table 1:** Log-dose/probit regression line analysis of the response of *Cyprinus carpio* exposed to Malathion for 96 hrs.

Dose (ml/L)	No.	Mor. %	Log Dose	Emp. Pro	Exp. Pro	Work Pro	Wt. Coef.	Weight w	Wx	Wy	Y
0.110	10	20	1.04	4.16	3.96	4.17	0.44	4.39	4.57	18.29	3.85
0.120	10	40	1.08	4.75	4.59	4.75	0.60	6.01	6.49	28.54	4.49
0.130	10	50	1.11	5.00	5.17	5.00	0.63	6.27	6.99	31.35	5.07
0.140	10	60	1.15	5.25	5.71	5.19	0.53	5.32	6.10	27.61	5.61
0.150	10	80	1.18	5.84	6.21	5.76	0.37	3.70	4.35	21.31	6.11
0.160	10	100	1.20	7.33	6.68	7.06	0.21	2.08	2.50	14.69	6.58

#### Statistics

SW= 27.770 SWX= 31.00 X Bar= 1.116 SWY= 141.790 Y Bar= 5.106

SWX \* X= 34.672 SWY \* Y= 738.268 SWXY= 159.187 b Value = 16.753

Regression Equation  $y = 16.753 x - 13.60$  If  $y = 5.0$  then  $x = 1.110$

This corresponds to dose of 0.129

Variance 0.0002 Chi-Square 2.05 (with 4 Deg. of freedom p)

Lower Limit 1.0823 Log Dose 1.1100 Upper Limit 1.1377

LCL= 0.120 UCL= 0.137

**Table 2:** LC<sub>50</sub> values (ml/l) of Malathion with their 95% confidential limits, Regression equation and Chi- square values of Cyprinus carpio exposed to pesticides for different durations.

Hrs. of exposure	LCL (ml/L)	LC <sub>50</sub> (ml/L)	UCL (ml/L)	Regression Equation	Chi-Square Value
24	0.172	0.180	0.187	y= 19.768 – 19.82 x	1.86
48	0.152	0.160	0.168	y= 18.858 – 17.71 x	3.09
72	0.138	0.146	0.154	y= 17.255 – 15.09 x	1.81
96	0.120	0.129	0.137	y= 16.753 – 13.60 x	2.05
120	0.109	0.116	0.122	y= 16.004 – 12.03 x	1.74

#### 4. Discussion

The toxicants present in sub-lethal concentrations in water might enter into the blood stream of the fish *Cyprinus carpio* through the gills or the mucus epithelium of the mouth and finally be distributed in different organs of the body which in turn affects various metabolic pathway. The significant increase in blood glucose which was dose dependent may be considered to be manifestation of stress induced by Malathion exposure. In agreement with our results, Luskova *et al.*, (2002)<sup>[10]</sup> reports significant increase in common carp *Cyprinus carpio* following the action of diazinon may be considered to be the manifestation of stress. Yaji *et al.*, (2011)<sup>[19]</sup> reported similar findings in fresh water fish *Oreochromis niloticus* exposed to cypermethrin.

In the present investigation there was an overall significant decrease (<0.05) in the protein content in all exposure periods in the toxicants. The quantity of protein is dependent on the rate of protein synthesis or on rate of its degradation. Renu Bala, (2013)<sup>[12]</sup> reported the decline in protein content in the present studies can be related to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanisms required during stress as many organisms will mobilize proteins as an energy source via the oxidation of amino acids or induce proteolysis to meet increased energy demand under the conditions of stress. The results presented in Fig 3 show a significant increased (<0.05) on exposure of Malathion compared to control in a dose dependent manner. The observed changes in the blood plasma cholesterol level in *Cyprinus carpio* in this study are indications that the pesticides affects lipid metabolism in the fish.

Destruction of cell membranes can leads to increased level of cholesterol in plasma. Similar changes have also been reported by Banaee *et al.*, (2013)<sup>[2]</sup> that the increase of blood triglycerides and cholesterol leads to liver dysfunction in fish *Cyprinus carpio* on the exposure of sublethal concentrations of chlorpyrifos. Disorder in triglycerides uptake by adipose tissues may leads to increase triglycerides in blood plasma. Banaee *et al.*, (2013)<sup>[2]</sup> observed similar findings in *Cyprinus carpio*, increase in stress hormones such as cortisol in blood of fish exposed to various insecticides, which stimulates lipid breakdown in adipose tissue. Also reported by Abdul Naveed *et al.*, (2011) the increase amount of triglycerides in blood plasma leads to affect the liver function on the exposure of triazophos in *Channa punctatus*.

LDH, AAT, and ALAT are found in heart, liver, skeletal muscle, kidney, pancreas, spleen, lung (gill), red blood cells and brain tissue. When disease or injury affect these tissues and the cells are destroyed, especially liver. According to Yaji *et al.*, (2011)<sup>[19]</sup> the significant decrease of LDH activity in *Oreochromis niloticus* blood serum suggest the liver dysfunction which may have affected the liver to synthesis triglycerides or slowed in metabolic rate as a result of cypermethrin exposure. Also observed the similar findings

by Abdul Naveed *et al.*, (2011) the significant decline of LDH activity in *Channa punctatus* blood plasma further suggest the decrease in the glycolytic process due to the lower metabolic rate as a result of triazophos exposure.

Amino transferases are widely acknowledged for their significance in protein metabolism by virtue of their ability to regulate both synthesis and degradation of aminoacids. In the present study, compared to control, ALAT and AAT were found to be significantly increased ( $p<0.05$ ) in all the exposure of fishes exposed to Malathion in a dose dependent manner. Venkateshwara Rao, (2006)<sup>[17]</sup> in fish *Oreochromis niloticus* suggest the increase in activity of aminotransferases in plasma may be due to liver damage, which results in the liberation of these intercellular enzymes and rise plasma aminotransferase levels. Therefore, the sum of these alterations can have a significant effect on energy metabolism. In conclusion, the present work indicates that agrochemicals causes considerable changes in intermediary metabolism and is likely to induce tissue damage in *Cyprinus carpio*. The causes for these alterations appear to be the result of high energy demands.

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