International Journal of Biology Research
ISSN: 2455-6548, Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 2; Issue 3; July 2017; Page No. 60-64

Effect of malathion on some haematological parameters in a fresh water fish, *Cyprinus carpio* L.

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Abstract
Malathion is an organophosphate (OP) insecticide widely used in developing countries. Because of its high acute toxicity. LC50 96 hr value of malathion to the freshwater fish *Cyprinus carpio* (Linn) was determined through static bioassays. The fish were reared in two sublethal concentration (0.025mg/l and 0.012mg/l) of the pesticide for 10, 20 and 30 days. Blood was collected from the treated and control fish and used for clinically important haematological analysis. Malathion toxicity resulted in a significant decrease in all the sub lethal concentrations and maximum -36.17% in RBC, Hemoglobin content (-43.37), PCV (-21.21), MCV (+23.42), MCHC (-28.14), MCH (-11.28), WBC (-34.69) and differential leukocyte counts such as lymphocyte, neutrophil and monocyte were significantly decreased ($p< 0.05$). The result of this study reveals that the organophosphate pesticide malathion adversely affects the hematology of the freshwater fish *Cyprinus carpio.* L, and calls for the limited and cautious use of highly toxic pesticides like malathion.

Keywords: malathion pesticides, Sub lethal toxicity, Hematology, *C. carpio*

Introduction
Blood is the most important and abundant body fluid. Its composition often reflects the total physiological condition. Blood of living organisms are very sensitive to changes and are widely used in Ichthyology research, aquaculture research as well as toxicology and biological monitoring. The blood parameters have been considered as diagnostic indices of pathological condition, findings are important for the assessment of systemic functions and overall health of animals. Furthermore, the findings also helps in diagnosing the structural and functional status of animals exposed to the toxicant (Atamanalp and Yanik, 2003; Talas and Gulhan, 2009; Suvetha *et al.*, 2010) [4, 14, 12]. A major part of the world's food is being supplied from fish source, so it is essential to secure the health of fishes. Ralio *et al*. (1985) [9] reported that the blood parameters of diagnostic importance are erythrocyte and leucocytes counts, haemoglobin and leucocye differential counts. Haematological study is important for toxicological research, environmental monitoring of fish and their health conditions during culture because fish generally are so intimately associated with the aquatic environment.

Malathion is an organophosphate pesticide and suited for the control of sucking and chewing insects on fruits and vegetables, it is also used to control mosquitoes, flies, household insects, animal parasites (ectoparasites), and head and body lice. Malathion, along with other phosphorodithioate insecticides (those containing two sulfur atoms bonded to phosphorous) must be oxidized before they have inhibitory potency and toxicity. Oxidation may occur in the environment or after malathion is absorbed into the body of a live organism.

The aim of this study was to investigate the effect of sublethal concentration of malathion on haematological parameters of *Cyprinus carpio.* L.

Materials and Methods
The live healthy *Cyprius 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 24 ± 1^0 C and pH of 6.2 – 7.4. The fish were fed two percent total body mass twice daily, with conventional fish feed (rice bran and ground nut oil cake in 1:1 ratio) at the rate of 10 % body weight. The fish were divided into batches control and experimental. The effect of pesticide on fish becomes consistent within 96 hours exposures, LC_{50} 96 hours (0.129 mg/ L) of malathion was taken as lethal concentration for *Cyprinus carpio*. To study the haematological responses $1/5^{th}$ and $1/10^{th}$ (0.025 mg/L and 0.012 mg/L) of LC_{50} 96 hours was taken as sub lethal concentrations for further studies. Blood was collected by puncturing heart in vials coated with 2% EDTA, as an anticoagulant.

Estimation of Haemoglobin (Hb)
Haemoglobin content was analysed and measured to 0.0200 of blood 5.000 of the Drabkin's reagents (200mg potassium ferric cyanide, 50mg potassium cyanide and 1.0g sodium carbonate dissolved in one liter distilled water) was added, mixed well thoroughly and allowed to stand for ten minutes to ensure the completion of the reaction. The solution was read at 540nm together with standard solution of Cyanmethaemoglobin against a blank containing 5.000 of the Drabkin's reagent. The haemoglobin content was expressed as gm/dl.

Total erythrocytes count (RBC)
Erythrocytes were counted by the method of Rusia and Sood, (1992) [11] using haemocytometer. The Neubaeur's counting chamber of the haemocytometer using counts, the total number of erythrocytes in millions per cubic millimeter of blood was calculated.

Total leucocytes count (WBC)
White blood cells were counted by the method of Rusia and Sood, (1992) [11] using haemocytometer. The Neubaeur's counting chamber using counts, the total number of leucocytes in thousands per cubic millimeter of blood was calculated.

Estimation of HaematoCrit (Ht) or PCV
Haematocrit in percentage was estimated by microhaematocrit method described by Nelson and Morris, (1989) [7] using microcentrifuge and a microhaematocrit reader and the concentration of the red cells was taken as the haematocrit value which was expressed in percentage.

$$Ht\ (\%) = \frac{L1}{L2} \times 100$$

Where,

L1 = is the height of the RBC column
L2 = is the total length of the column (RBC + Plasma + buffer coat) in millimeter and expressed in percent.

Differential leucocytes count (Leucogram)
The differential counts such as lymphocytes, monocytes and neutrophil were determined by blood film stained with May-Grunwald- Giesma stain.

Other blood indices
Heamatological indices such as Mean corpuscular haemoglobin content (MCHC), Mean corpuscular volume (MCV), Mean corpuscular heamoglobin (MCH), were calculated from the Hb content, RBC and Hcrt (%) using the following formula proposed by Johansson – Sjobeck and Larsson (1978) [5].

Mean Corpuscular Volume
To determine the average volume of a single red blood cell in cubic microns.

$$MCV\ (\mu m3) = \frac{\text{Haematocrit}\ (\%)}{\text{Erythrocyte}\ (x10^6/mm^3)} \times 10$$

Mean Corpuscular Haemoglobin
To determine average haemoglobin content of a single red cell in micro- micrograms.

$$MCH\ (pg) = \frac{\text{Haemoglobin}}{\text{Erythrocyte}\ (x10^6/mm^3)} \times 10$$

Mean Corpuscular Heamoglobin Concentration
To determine the haemoglobin content of 100 ml of packed cells as a percentage as opposed to the percentage of the haemoglobin of whole blood.

$$MCHC\ (\%) = \frac{\text{Haemoglobin}}{\text{Haematocrit}\ (\%)} \times 100$$

Statistical study
The results of static bioassay were analyzed using linear regression probit analysis (Finney, 1971) using the statistical package (POLO- PC- LEORA software 1987). Haematological results were tested by using two way ANOVA (analysis of variance) followed by Duncan's multiple comparison test procedure. Significance was tested at $p < 0.05$

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

Dose (mg/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_{50} values (mg/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 (mg/l)	LC_{50} (mg/l)	UCL (mg/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

Note: LCL= Lower Confident Limit, UCL= Upper Confident Limit
LC_{50} = Lethal Concentration for 50 percent of the exposed fish

Table 3: Haematological parameters under the influence of sublethal concentrations of malathion

parameters	Days	Sub lethal (1/5th)			Sub lethal (1/10th)		
		10	20	30	10	20	30
Hb	Control	4.14±0.03[a]	4.18±0.02[a]	4.15±0.02[a]	4.14±0.03[a]	4.18±0.02[a]	4.15±0.02[a]
	Exposed	3.63±0.07[a]	3.15±0.04[ab]	2.35±0.01[b]	3.91±0.06[a]	3.62±0.09[a]	3.0±0.05[ab]
	% change	-12.32	-24.64	-43.37	-5.56	-13.39	-27.71
RBC	Control	1.36±0.01[a]	1.4±0.02[a]	1.41±0.02[a]	1.36±0.01[a]	1.4±0.02[a]	1.41±0.02[a]
	Exposed	1.18±0.05[ab]	1.06±0.06[ab]	0.9±0.04[b]	1.22±0.09[ab]	1.16±0.04[ab]	1.02±0.02[ab]
	%change	-13.23	-24.28	-36.17	-10.29	-17.14	-27.66
WBC	Control	18.22±0.23[a]	18.46±0.50[a]	18.56±0.57[a]	18.22±0.23[a]	18.46±0.50[a]	18.56±0.57[a]
	Exposed	16.58±1.84[ab]	15.12±1.96[ab]	12.12±1.25[b]	17.00±1.79[a]	14.85±1.34[ab]	12.26±1.30[b]
	% change	-9.00	-18.09	-34.69	-6.69	-19.56	-33.94
PCV	Control	17.06±0.2[ab]	17.2±0.2[ab]	17.3±0.2[a]	17.06±0.2[ab]	17.2±0.2[ab]	17.3±0.2[a]
	Exposed	15.26±1.04[abcd]	14.8±1.05[bcd]	13.63±1.06[d]	16.6±1.06[abc]	15.2±1.02[abcd]	14.52±1.06[cd]
	% change	-10.55	-13.95	-21.21	-2.7	-11.63	-16.07
MCH	Control	30.44±1.69[a]	29.86±1.99[ab]	29.43±1.69[ab]	30.44±1.69[a]	29.86±1.99[ab]	29.43±1.69[ab]
	Exposed	30.76±2.15[a]	29.72±1.48[ab]	26.11±1.65[b]	32.05±1.75a	31.20±1.3[a]	29.41±1.46[ab]
	% change	1.05	-0.47	-11.28	5.29	4.49	-0.07
MCV	Control	125.44±2.47[bc]	122.86±2.91[c]	122.7±2.95[c]	125.44±2.471[bc]	122.86±2.91[c]	122.7±2.95[c]
	Exposed	129.32±2.05[bc]	139.62±3.91[abc]	151.44±4.66[a]	36.06±3.64[abc]	131.03±2.05[bc]	142.35±4.17[ab]
	% change	3.09	13.64	23.42	8.46	6.65	16.01
MCHC	Control	24.26±1.78[a]	24.30±1.65[a]	23.99±1.65[a]	24.26±1.78[a]	24.30±1.65[a]	23.99±1.65[a]
	Exposed	23.78±1.77[a]	21.28±1.27[ab]	17.24±0.95[b]	23.55±1.34[a]	23.82±1.16[a]	20.66±1.02[ab]
	% change	-1.98	-12.43	-28.14	-2.93	-1.98	-13.88

Values are means of three replicates mean ± SD. Column Values with different superscripts are significantly different ($p < 0.05$)

Table 4: Differential leucocytes count in common carp affected by sub lethal concentrations of malathion

parameters	Days	Sub lethal (1/5th)			Sub lethal (1/10th)		
		10	20	30	10	20	30
Lymphocyte	Control	74.6±3.51[a]	74.6±3.51[a]	74.73±3.52[a]	74.6±3.51[a]	74.6±3.51[a]	74.73±3.52[a]
	Exposed	67.16±4.14[abc]	62.8±3.43[bc]	60.12±2.49[c]	72.5±4.12[ab]	70.34±3.15[abc]	64.5±3.05[abc]
	% change	-9.97	-15.82	-19.55	-2.82	-5.71	-13.69
Neutrophils	Control	3.26±0.21[a]	3.26±0.21[a]	3.33±0.15[a]	3.26±0.21[a]	3.26±0.21[a]	3.33±0.15[a]
	Exposed	2.94±0.27[abc]	2.26±0.21[bc]	2.18±0.15[c]	3.02±0.19[ab]	2.98±0.13[abc]	2.84±0.21[abc]
	%change	-9.82	-30.67	-34.53	-7.36	-8.59	-14.71
Monocyte	Control	2.54±0.09[a]	2.56±0.02[a]	2.56±0.02[a]	2.54±0.09[a]	2.56±0.02[a]	2.56±0.02[a]
	Exposed	2.34±0.01[ab]	1.92±0.09[abc]	1.58±0.03[bc]	2.00±0.06[abc]	1.96±0.07[abc]	1.44±0.09[c]
	% change	-7.87	-25	-38.28	-21.26	-23.44	-43.75

Values are means of three replicates mean ± SD. Column Values with different superscripts are significantly different ($p < 0.05$)

Results

Acute toxicity

The LC_{50} values range from 0.116 (120 h) to 0.180 (24h) (Table 2). The 96h LC_{50} value (0. 129mg/l) obtained using probit analysis (Table 1) is used for fixing the two incipient lethal level exposure concentrations of 0.025 mg/l (1/5th 96h LC_{50}) and 0.012 mg/l (1/10th 96h LC_{50}).

Haematological Studies

The data in Table- 3 and 4 indicates that the fish exposed to two sub lethal concentrations (0.025 and 0.012 mg/l) of malathion for 10, 20 and 30 days showed considerable variation over control.

In the treated groups the decrease in the RBC values were found as compared to control group. In these exposed groups when malathion concentration 0.025mg/l was treated, the RBC was noticed differ from 1.18±0.05 (10days), 1.06±0.06 (20days) and 0.9±0.04 (30days), and for the concentration of 0.012mg/l it changed from 1.22±0.09 (10days), 1.16±0.04 (20days) and 1.02±0.02 (30days). In both concentration of malathion the range of percent change values were increased from -12.32% to -43.3% in 0.025 mg/l concentration and increased from -5.56% to -27.71% in 0.0129 mg/l concentration. PCV value was observed in the malathion treated groups that declined equally as compared to control group. In these exposed groups, the PCV was found to range from 15.26 ± 1.04 in 10days, 14.8 ± 1.05 within 20days and 13.63 ± 1.06 within 30days for the concentration of 0.025 mg/l. As for the malathion treated concentration of 0.012 mg/l the PVC value was observed as range to 16.6 ± 1.06 in 10 days, 15.2 ± 1.02 within 20 days and 14.52 ± 1.06 within 30 days. MCV values were above the control values. Positive at both the sub lethal exposure. MCH found (11.28%) decreased on 30th day of 0.025mg/l exposure while all the values were above the control values. MCHC values decreased in both concentrations on all exposure days with variable reduction. At 0.025mg/l concentration (1.98%) decrease recorded on 10th day, (12.43%) decrease on 20th day and (28.14%) decrease on 30th day. At 0.012mg/l exposure (2.93%) decrease on 10th day, (1.98%) on 20thday and (13.88%) decrease on 30th day than the control values.

Differential Leucocytes

Percentage value of lymphocyte was found to be increased in both concentrations of malathion throughout the exposure period. In both concentrations of malathion the range of percent change values were increased from -9.97% to -19.55% at 0.0258 mg/l concentration and increased from -2.82% to -13.69% at 0.0129 mg/l concentration. At 0.0258mg/l and 0.0129mg/l exposure of malathion the percent change values were increased from -9.82% to -34.53% and -7.36% to -

14.71%, respectively. The range of percentage increase in monocyte level of *C. caarpio* varied from -7.87% to -38.28% and from -21.26% to -43.75%, in the various sub-lethal concentrations (0.025mg/l and 0.012mg/l) of malathion.

Discussion

The blood parameters have been used as a sensitive indicator of stress in fishes that are exposed to different water pollutants and toxicants of various types. Sub lethal concentrations in the aquatic environment will not necessarily result in outright mortality of aquatic organism. In the present study the pesticides specific differences in haematological indices were evident. As far as values of RBC, Hb and PCV are concerned *Cyprinus carpio* showed a significant decrease in proportion to concentration of the pesticide exposure compared to control. Such species-specific differences are common among fishes and hence, the results are parallel with the earlier reported species-specific changes in the heamatological parameters of the fishes.

In the light of the present study, it is showed that the mean haemoglobin in the control was 4.14 ± 0.034 and a decrease in the concentration of haemoglobin in blood is usually caused by the effect of toxicant like malathion indicated anaemic conditions in fish due to stress-caused hemolysis and inhibition of aerobic glycolysis curtailing denovo synthesis of haemoglobin. Alireza *et al*., (2012) [3] in which insufficient amount of haemoglobin content could be influence energy balance of the body, reported similar findings and results in oxygen deficiency were observed in benny fish *Mesopotamichthyes sharpeyi*.

In the present study, when the fish *Cyprinus carpio* is exposed to toxicant have shown a decrease (27.66%) in the RBC count. The decrease in the number of circulating RBCs probably reflects the physiological functioning of haemopoietic system, which is considered the most sensitive indicator towards environment pollutants. Rafeek. A. Maniyar (2012) [8] reported similar results in *Cyprinus carpio* exposed to sub-lethal concentrations of monocrotophos with decreased RBCs. PCV appears to be positively correlated with RBC counts, hence a decrease in PCV is observed to this work. Zaki *et al.*, (2009) [15] reported a decreased RBC count, haemoglobin concentration and PCV value in the fish exposed to malathion. Adeyemo (2007) [1] reported decreased haemoglobin, RBC count and haematocrit values in *Clarius gariepinus* exposed to lead nitrate.

This present work observed significant decrease (p<0.05) of leukocyte count of common carp in during exposure to sub-lethal concentration of malathion. A low white blood cell count may be disfunction in hematological tissues (spleen and kidney) or certain infectious diseases. Lower than normal levels of lymphocytes (lymphopenia) can be an indicator of immune system deficiency. Poisonous substances treatment can also deplete the body's supply of lymphocytes, as can exposure to toxicants. Kalavathy *et al* ., (2001) [6] suggested that decrease in WBC count could be the result of autolysis caused due to haemolytic enzymes leaked out by the cells under toxicant stress. Significant decrease (<0.05) of monocytes count of *Cyprinus carpio* after the sub-lethal exposure of and organophosphate based pesticides lead to monocytopenia. Svoboda *et al*., (2001) [13] report monocytopenia in *Cyprinus carpio* after an effect of diazinon. Ajani *et al.*, (2012) [2] observed the significant decrease in monocytes count obtained in *Clarius gariepenius* exposed to sub-lethal concentrations of diazinon.

The erythrocyte indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) seem to be changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Similar response was noted in common carp and other freshwater fish exposed to acute toxic level of pesticides (Svobodoa *et al*., 2001; Rao, 2010) [13,10].

Thus from the present study it can be concluded that the exposure of fish to malathion pesticides resulted in significant alterations in haematological parameters. These alterations may suppress normal growth, reproduction, immunity and even survival of fish in natural environment. Furthermore, the haematological studies provide a rapid and sensitive method for predicting the effects of sub-lethal exposure on general health and well-being of fish.

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