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RESEARCH ARTICLE

Acute toxicity and blood profile of freshwater fish, *Clarias batrachus* (Linn.) exposed to Malathion

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Abstract

Effects of malathion on behavior and some haematological indices using *Clarias batrachus* as animal model was evaluated in this study. Experiments were conducted to evaluate the toxic effects of malathion in *C. batrachus* blood profile after 24, 48, 72 and 96 h exposed to sub-lethal concentrations of malathion (0.05, 0.25 and 0.5 ppm). *Clarias batrahus* (n=40) with average weight of 65.2 g and average length of 16.8 cm were divided into 4 groups at 10 fishes per group being acclimatized for 14 d. They were then exposed to various concentrations of malathion for 96 h. The 96 h LC_{50} was calculated to be 1 ppm. It was observed that with increase of exposure time, total erythrocyte (RBC), hemoglobin (Hb), hematocrit (Ht) and mean corpuscular volume (MCV) values decreased but leucocytes (WBC), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) values increased. It is believed that observed depression in hematocrit and hemoglobin values coupled with decreased and deformed erythrocytes are obvious signs of anemia. Resulted changes in erythrocytes and leukocytes after exposing to malathion are due to malfunction in hemopoiesis and decrease in non-specific immune system. In conclusion, long-term exposure to malathion at sub-lethal concentration induced biochemical alterations in *C. batrachus* and offers a simple tool to evaluate toxicity derived alterations.

Keywords: Malanthion, behavior, haematological indices, toxic effects, exposure time, biochemical alterations.

Introduction

Pesticides and insecticides can pollute water sources by two ways: through direct application of pesticides in aquatic systems and indirect uses such as erosion from agricultural lands and agricultural waste water infiltration and eventually washed into deep water environments and ecosystem (Dutta and Arends, 2003). Pesticides in the aquatic environment can negatively affect the ecosystem. Although the aquatic environment is not the actual target of such pesticide, but the widespread use of them had led to some serious problems including toxic residues in grass and toxicity of non-target organisms such as mammals, birds and fish (Saeed *et al.,* 2012; Shankar *et al.,* 2013). One of the main pesticide is organophosphorous insecticide malathion is a common active substance of them which is most widely used insecticides in agriculture for controlling the insects which, mainly affects the nervous system by stopping the activity of acetylcholine esterase and also it penetrate in to the interstitial fluid of fish, effects on fish reproduction and reduce the population (Dutta and Arends, 2003). Blood and tissue of living organisms are very sensitive to changes and are widely used in Ichthyology research. Hematological indices are vital for the evaluation of fish physiological status. Blood cell responses are important indicators of changes in the internal and external environment of animals.

In fish, exposure to chemical pollutants can either increase or decrease their haematological levels. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases (Golovina, 1996; Luskova, 1997). Fish live in very intimate contact with their environment and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components (Wilson and Taylor, 1993; Saravanan *et al.,* 2010). Blood is a liquid connective tissue which truly reflects physical and chemical changes occurring in organism; therefore, detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat. Blood provides an ideal medium for toxicity studies. The hematological parameters have been considered as diagnostic indices of pathological conditions in animals and could be used as potential biomarkers of insecticides (Parma *et al.,* 2007; Ololade and Oginni, 2010). Fish blood can serve as a valuable tool in detecting physiological changes taking place in animal. In this study, we evaluated several hematological and biochemical parameters on *C. batrachus* to establish this species as a model for toxicological studies. Also, we aimed to evaluate the effects of an acute intoxication with malathion on these parameters.

Materials and methods

Study area: Avalapalli dam in between Bangalore and Hosur is located at 13° 20' 58" to 13° 21'39" S latitude and 77° 06' 22" to 77° 06' 38" W longitude, about 23 miles North East of Bangalore is the study area. It supports agriculture and small-scale fishing and serves as a water source for the forest nursery nearby, the water is also used for drinking after recycling. The dam covers an area of 200 acres and has a catchment area of about 2.5 Km^2. It receives sewage and municipal wastes and used as huge public sanitary dump.

Experimental animal: The freshwater fish, *Clarias batrachus* from water bodies was used (Sankey tank: The tank has a well-maintained park and a corporation swimming pool at the Southern part and a nursery towards the North, adjacent to the tank. It also attracts large populations of migratory birds apart from small time fishing activities. The tank presently has no major source of pollution) as control fishes and polluted water body (Avalapalli dam) fishes were collected by using cast and gill net (mesh: 10 mm). Both (control) and polluted water (experimental) live fishes were brought to the laboratory and maintained in a separate glass aquaria (60"x30"x20") with respective of their tank water. The fishes were considered as normal on the basis of their external appearance and absence of symptoms of diseases. Fishes were transported in a container filled with pond water and then acclimatized under laboratory conditions for two weeks (14 d) prior to the commencement of the experiment. During the acclimatization period, the fish were fed 4% of their body weight with commercial feed pellets (40% crude protein, 4.22% fat, 5.88% crude fiber, 10.30% ash and 10.03% moisture) daily once and the water was renewed every other day. The mortality throughout the period of acclimatization was less than 10%.

Tested substance: Malathion is an insecticide. It is a colorless liquid and has a strong scent. The product used in the experiments was supplied by the Rallis India Ltd. The malathion [O, O, dimethyl, S-(1, 2, dicarbo ethoxyethyl) Phodophorodithiote] was dissolved in acetone and was added to test water to obtain the desired concentration. The stock solution of 1 mg/L was prepared separately and the dispersed degrees of concentration were prepared by adopting the dilution techniques as outlined in APHA (1971).

Toxicity tests: Experiments were carried out in 50 L aquaria. Tests were carried out in four batches: (i) Control-10 fishes were maintained in water without malathion; (ii) (iii) and (iv) Experimental-10 fishes were kept in each of the aquaria contaminated with different sub-lethal concentration of malathion (0.05, 0.25 and 0.5 ppm) lower than LC_{50} (1 ppm for 96 h). Environmental conditions were similar in all the four aquaria. Concentration of 0.5 ppm caused the smallest number of dead individuals (1 fish in 96 h of exposition). The concentration of 0.05 and 0.25 ppm was chosen because these were generally the smallest concentration used in toxicity tests with *C. batrachus.*

Behavior: Observations were made regarding the behavioral changes of fishes with respect to opercular movement, respiration, balance, grouping and preferred region in the experimental aquaria.

Hematological baseline values in C. batrachus: Blood was drawn from the posterior caudal peduncle from fishes supplemented with to 0.05, 0.25 and 0.5 ppm at 24, 48, 72 and 96 h after exposure to malathion respectively. At the same time, blood from control fish were also drawn in heparinized bottles for red blood cell count (RBC), haematocrit (PCV), hemoglobin (Hb) and white blood cell count (WBC). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were derived from the RBC, PCV and Hb as described by Wintrobe (1977) and Jain (1986). MCV was calculated in femtoliters = PCV/RBC×10, MCH was calculated in picograms = Hb/RBC×10, MCHC = (Hb in 100 mg blood /Hct) × 100 and color index = Hb%/RBC%.

Statistical analysis: All the data obtained in the study was calculated by differences in the means and are presented as mean with standard error of mean.

Results and discussion

The normal values of hematological parameters in control group can be seen in Table 1. Quantitative changes of hematological parameters in fish blood exposed to sub-lethal concentration of malathion (0.05, 0.25 and 0.5 ppm) after 24, 48, 72 and 96 h. Significant reduction in Ht, Hb, RBC and MCV levels were observed, whereas the WBC, MCHC and MCH levels showed an increment, with increase in the concentration and exposure period of malathion. Fishes exposed to the maximum concentration (0.5 ppm) of malathion showed decrease ($2.36\pm0.04 \times 10^6 mm^3$) in RBC for 96 h exposure, whereas a significant increase in WBC ($40.91\pm0.20 \times 10^3 mm^3$), MCH (16.00±0.43 pg) and MCHC (52.83±1.86%) values were observed (Figs. 1-4). MCHC showed a significant increase from 45.32 ± 0.90% (in 0.5 ppm for 24 h) to 52.83 ± 1.86% (in 0.5 ppm for 96 h) in treated fishes. However, fishes exposed to 0. 05 ppm to 0.5 ppm malathion for 24 h to 96 h showed a slight increase in their color index (Table 1). The toxic effects of malathion on fish are multidirectional and manifested by numerous changes in the physiological and chemical processes of their body systems. Significant variations were observed between various blood parameters with different concentrations of toxicants.

Table 1. Effect of sub-lethal concentrations of malathion on haematological parameters of *Clarias batrachus* (mean ± S.E).

Duration of exposure	Malathion conc. (ppm)	RBC ($x10^6/mm^3$)	WBC ($x10^3/mm^3$)	MCH (pg)	MCHC (%)	MCV (μm^3)	Hb (g%)	PCV (%)	Color index
Control	-	4.35±0.07	26.09±0.35	11.95±0.17	36.61±1.92	32.64±0.26	34.66±0.37	14.20±0.25	0.398±0.01
24 h	0.05	4.16±0.01	26.48±0.21	11.77±0.18	37.12±1.96	31.73±0.34	32.66±0.30	13.20±0.10	0.392±0.02
	0.25	3.94±0.12	29.62±0.10	12.70±0.29	41.74±1.37	28.75±0.53	31.53±0.03	11.33±0.31	0.404±0.01
	0.5	3.50±0.07	32.78±0.22	13.02±0.14	45.32±0.90	28.74±0.58	30.40±0.04	10.06±0.10	0.434±0.01
48 h	0.05	4.08±0.04	29.32±0.34	11.29±0.18	35.81±1.78	31.54±0.21	30.73±0.21	12.87±0.04	0.376±0.02
	0.25	3.66±0.03	32.15±0.36	12.13±0.21	44.40±0.54	27.32±0.41	29.60±0.03	10.00±0.07	0.403±0.10
	0.5	3.20±0.03	36.05±0.15	13.34±0.30	48.91±2.39	27.38±0.37	28.46±0.04	08.73±0.33	0.444±0.01
72 h	0.05	3.89±0.08	32.64±0.23	11.07±0.12	35.91±1.51	30.84±0.07	28.73±0.21	12.00±0.35	0.369±0.03
	0.25	3.26±0.09	35.17±0.49	12.69±0.09	48.70±1.81	26.07±0.28	27.60±0.06	08.50±0.25	0.423±0.02
	0.5	2.94±0.03	39.24±0.36	13.50±0.31	53.64±0.40	25.17±0.32	26.46±0.06	07.40±0.28	0.450±0.01
96 h	0.05	3.69±0.34	37.30±0.21	10.51±0.25	38.56±1.72	27.26±0.15	25.86±0.24	10.06±0.40	0.350±0.01
	0.25	3.06±0.08	39.53±0.13	12.12±0.29	51.74±0.79	23.43±0.37	24.73±0.07	07.17±0.11	0.404±0.01
	0.5	2.36±0.04	40.91±0.20	16.00±0.43	52.83±1.86	25.84±0.63	23.60±0.07	06.10±0.07	0.500±0.01

The data shows that changes in hematological indices of fish may be due to malathion (as it may probably apply to any other insecticide) and are predetermined both by the concentrations of the insecticides in the water and time of exposure and both these factors can cause reversible and irreversible changes in the homeostatic system of fish (Farkas *et al.,* 2002; Javeed, 2003; Parma *et al.,* 2007). Erythrocyte counts of fish exposed to 0.05 and 0.5 ppm, indicated a decrease as compared to control values at the end of the experiment. Reduction in hemoglobin was accompanied by lowest hematocrit value (Fig. 7). Increase in WBC count; reflect the occurrence of leucocytosis in the treated fish samples. This was perhaps, a typical defensive response of the fish against a toxic invasion and second most common probability may be leukemia (Sudha, 2012).

In blood parameters, a significant difference between control and each concentration was found. Fish blood reflects pathophysiological status and its parameters are important in diagnosis of the structural and functional status of fish exposed to toxicants (Sampath *et al.,* 1998; Ololade and Oginni, 2010). In the light of the present study, the mean value of PCV in compare to the control group, decreased progressively in groups of 0.05 and 0.5 ppm malathion exposed fishes respectively (Fig. 7). A decrease in the erythrocyte count or in the percent of haematocrit indicates the worsening of an organism state and developing anemia (Fig. 1 and 7). The study shows that mean hemoglobin in the control was 34.66±0.37 and a decrease in the concentration of hemoglobin in blood is usually caused by the effect of toxic malathion on gills, as well as decrease in oxygen, which also suggests anemia or confirms toxic impact of malathion in *C. batrachus* (Fig. 6). Mean corpuscular volume (MCV) decreased whereas, MCH and MCHC increased considerably in all treatments compared to control (Figs. 3-5). This is in agreement with the work of Shah (2006) following a short-term exposure of tench (*Tinca tinca*) to lead.

Fig. 1. Changes in red blood cell count of *C. batrachus* exposed to sub-lethal concentrations of malathion.

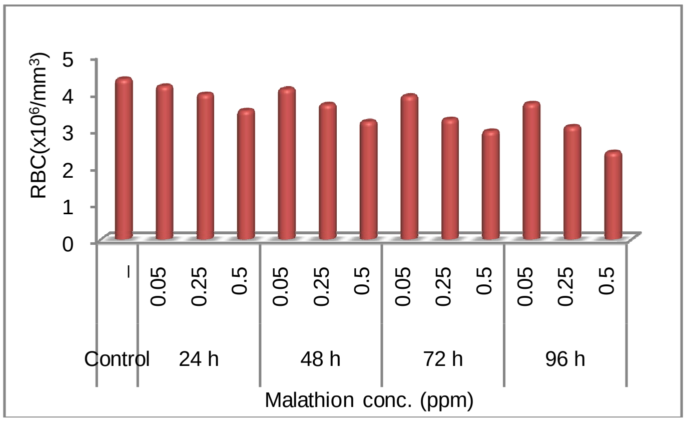
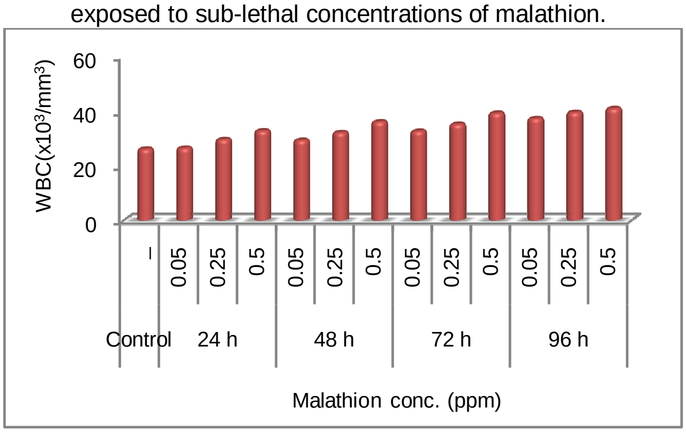


Fig. 2. Changes in white blood cell count of *C. batrachus* exposed to sub-lethal concentrations of malathion.



These alterations were attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in hemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, induced by exposure to lead (Shah, 2006). The decrease in RBC and Hb content indicates acute anemia.

Fig. 3. Changes in mean corpuscular hemoglobin of *C. batrachus* exposed to sub-lethal concentrations of malathion.

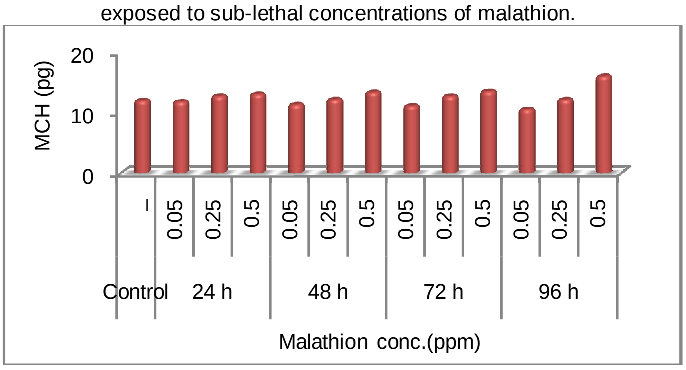


Fig. 4. Changes in mean corpuscular hemoglobin concentration (%) of *C. batrachus* exposed to sub-lethal concentrations of malathion.

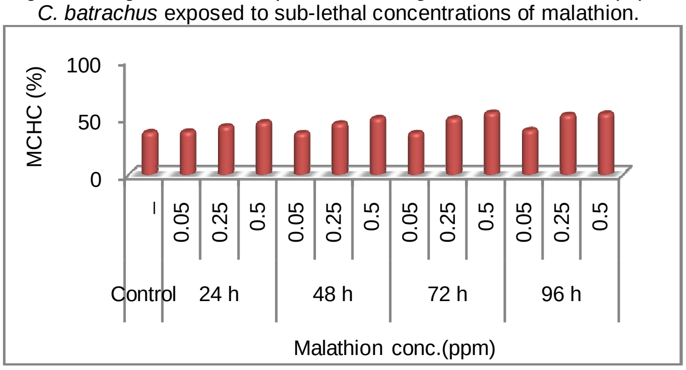


Fig. 5. Changes in mean corpuscular volume of *C. batrachus* exposed to sub-lethal concentrations of malathion.

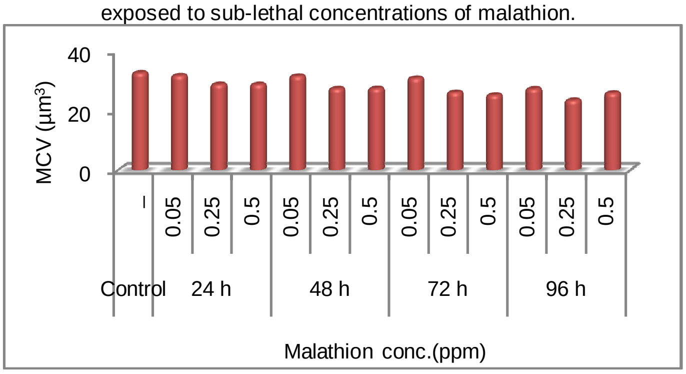


Fig. 6. Changes in hemoglobin concentration of *C. batrachus* exposed to sub-lethal concentrations of malathion.

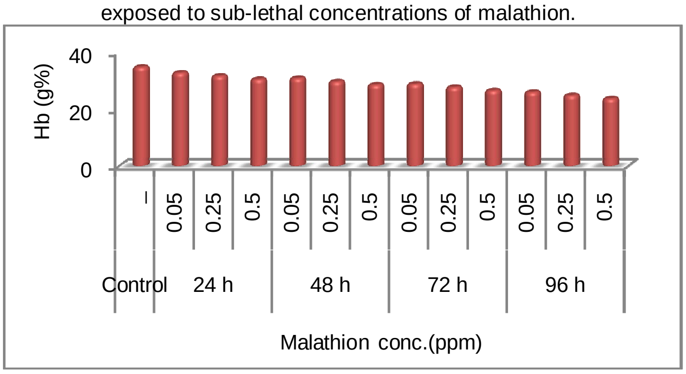
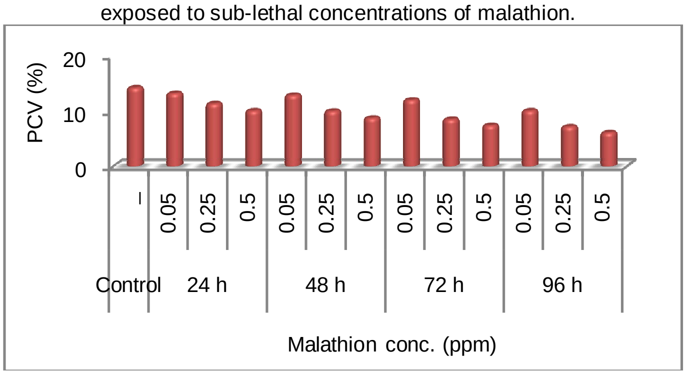


Fig. 7. Changes in packed cell volume of *C. batrachus* exposed to sub-lethal concentrations of malathion.



The anemia could be due to the destruction of RBC triggered by the influx of malathion into the erythrocytes and may also be of hemolytic type of RBC. Similar observations were also reported by Tilak *et al*. (2007), Saravanan *et al.* (2010) and Saeed *et al*. (2012). In the present investigation, haemolysis might have been one of the causes for reduction in Hb, RBC and PCV values. Another reason for RBC suppression could also damage the haemopoetic tissue. PCV appears to be positively correlated with RBC counts; hence a decrease in PCV is observed. Similar results have been reported for freshwater fishes exposed to pesticides (Balathakur and Bais, 2000; Venkataraman *et al.,* 2001).

Hematological findings after exposure to malathion revealed toxic manifestations with evident effects after, 24, 48, 72 and 96 h. Malathion belongs to an organophosphorus insecticide class which is easily degraded in natural environment, but they prove to be toxic for fish due to poor ability of the fish to metabolize these compounds (Mukhopaday and Dehadrai, 1980; Parma *et al.,* 2007; Tilak and Kumari, 2009; Saeed *et al*., 2012). The alterations of blood parameters were attributed to a decrease in the erythrocyte count indicate the developing anemia. The anemia could be due to the destruction of RBC production which triggered by the influx of malathion. The increase of WBC shows that, a typical defensive response of the fish against a toxic invasion.

Conclusion

Under the light of this study, it is concluded that malathion is moderately toxic to *C. batrchus*. Exposure to low concentrations of malathion results in significant hematological alterations and behavioral changes and these changes may be potentially disruptive for the survivability of the fish in their natural environment. This fact should be taken into consideration when it is used for pest control in the agricultural fields surrounding their natural freshwater reservoirs.

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