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Original Article

Acute and sublethal intoxication of malathion in an Indian major carp, *Labeo rohita*: haematological and biochemical responses.

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Abstract

This study aimed to determine the median lethal concentration (96-h LC_{50}), acute and sublethal effects of malathion, an organophosphorus pesticide on hematological and biochemical responses in an Indian major carp, *Labeo rohita*. In this study, the LC_{50} value of malathion for 96 h was found to be 3.4 ppm. During acute (3.4 ppm) and sublethal [1/10th of 96 h LC_{50} value (0.34 ppm) studies, all the hematological parameters except WBC were significantly decreased ($p<0.05$). Besides, when compared with the control group, a significant ($p<0.05$) decrease in biochemical activity was also observed in malathion treated fish during acute and sublethal exposure periods. These results suggest that the tested concentrations of malathion could have significant adverse effects on the hematological and biochemical parameters of fish, *Labeo rohita*. The changes in the parameters can be effectively used to determine the impact of malathion in the aquatic ecosystem.

Keywords: Malathion, *Labeo rohita*, Haematology, Biochemical, Acute study, Sublethal toxicity

Introduction

The possibility of being contaminated by a variety of chemicals in aquatic ecosystems that run through agricultural areas is high [1]. Water is one of the primary ways pesticides are transported from an application area to other locations in the environment. Upon entering the aquatic environment produce multiple changes in the organism by altering the growth rate, nutritional value, behavioral pattern and so on. It is exigent to find out the detrimental effects of pollutants especially pesticides on fish since they form an important link in the food chain and their contamination by pesticides imbalance the aquatic system. Fishes are an important source of protein and form a part of human food [2]. In India, non-target organisms are believed to be affected by the chemical formulations employed in agricultural practices and to find their way to freshwater bodies, ultimately polluting them [3]. To monitor the physiological and pathological changes in fishes, knowledge of hematological characters is an important tool [4]. Alterations of blood components are important biomonitoring tools in toxicological research because of the potentiality for rapid assessment of the chronic toxicities of a compound [41]. Generally, any unfavorable changes in water quality are reflected in the blood of the aquatic organisms by separating the blood cells with thin epithelial membrane [42]. Exogenous factors such as stress always induce major changes in blood composition [5]. For example, fluctuations in the levels of red blood cells (RBC), white blood cells (RBC), hemoglobin (Hb) concentration, and other basic components occurred in response to ecological and physiological conditions [6].

Likewise, exposure to pesticides causes severe alterations in the tissue biochemistry of fishes [7]. The biochemical alterations in organisms are considered as most sensitive and earliest events of any pollutant damage [43]. The most toxicants exert their effects at basic level of the organism by reacting with enzymes or metabolites and other functional components of the cell [44]. The measurement of biochemical changes in the tissue of fish under exposure to toxicants may be used to predict the toxic effect of pollutants. The pesticide, malathion (S-1,2-bis (ethoxycarbonyl) ethyl O, O-dimethyl phosphorodithioate) is a commonly used organophosphorus pesticide which once introduced into the environment, it may cause serious intimidation to aquatic organisms and cause severe metabolic disturbances in non-target species like fish and freshwater mussels [8].

Therefore, in the present investigation, an attempt has been made to study the effect of malathion on alterations in hematological and biochemical parameters of *Labeo rohita* with particular reference to the concentration of the pesticide and exposure duration.

Materials and Methods

Test pesticide

Technical grade malathion (CAS 121-75-5; 95%) manufactured by Kalyani Industries Ltd., Mumbai, India was taken for evaluation of its toxicity.

Fish and culture condition

Considering the limitations of the laboratory facilities, a static bioassay method was adopted [9]. Fingerlings of L.rohita (12±0.24 g in body weight and 6.4±0.02cm in body length) were collected from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Tamil Nadu, India. Under natural photoperiod conditions (12:12h light to the dark regime) before the experimentation, the fishes were acclimated to the laboratory condition for a month in 350-L fiberglass tanks containing aerated and dechlorinated water. The fishes were fed once in a day with rice bran and groundnut oil cake (ad libitum) during acclimatization and exposure periods. Each day, 80% of the water volume was renewed to assure water quality, and dead fishes (if occurred) were removed. The physicochemical characteristics of the test water were temperature (25.0±1.0°C), pH (7.0±1), salinity (0.5±0.02 ppt), and total hardness (16.1±0.5mg L^{-1}) [9]. To avoid prandial effects during the assay, the fishes were starved for 24h before experimentation.

Determination of LC_{50} for 96 h

Preliminary toxicity tests were carried out to find out the median lethal tolerance limit of fish to malathion for 96 h. For obtaining LC_{50} concentration, eight circular plastic tubs of 20 L capacity were used. 10 fishes were introduced in each tub with 20 L of water in each which was already received with different concentrations of malathion (1.4, 2.4, 3.4, 4.4, 5.4, 6.4, 7.4, and 8.4 ppm) for 96 h treatment. 20 fishes each in tubs with 20 L of water were also kept simultaneously as control. The mortality/ survival of fish in the experimental tubs was recorded after 96 h. The concentration at which 50% kill of fish occurred after 96 h treatment was taken as the median lethal concentration (LC_{50}) for 96 h. Five such preliminary toxicity tests were conducted for arriving at the median lethal concentration. LC_{50} for 96 h was calculated by probity analysis method [10].

Acute toxicity study for 96 h

For the determination of malathion toxicity study, five circular plastic tubs with 50 L capacity were taken and each was filled with 35 L of dechlorinated tap water. Then, LC_{50} 96 h concentration of malathion (3.4 ppm) was added to all tubs and mixed well. From the stock, 20 healthy fishes starved for 48 h were introduced into each tub. Parallel control of 40 fishes in different circular tubs was also maintained under similar conditions. At the end of 96 h, fish from the control and treated groups were taken for further analyses.

Sublethal toxicity study for 30 days

The sublethal toxicity experiment was carried out simultaneously with acute studies. For the study, five well-cleaned glass aquaria of 130 L capacity were taken and filled with 100 L of water and 50 fishes which were randomly selected from the stock was transferred into the aquaria. Then 1/10th of the 96 h LC_{50} concentration of malathion (0.34 ppm) was taken) and added to each aquarium [11]. The same control fish groups used in acute toxicity studies were also maintained for this study. Water was changed daily to avoid the accumulation of fecal matter and was renewed with the toxicant. The study was conducted for 30 days. 20 fishes were randomly selected from control and malathion treated glass aquarium and sacrificed without anesthetizing for further analysis upon completion of the stipulated exposure period of 30 days.

Hematology

At the end of 96 h and 30 d period exposure, blood was drawn from control and malathion treated fishes by cardiac puncture using a hypodermic syringe previously rinsed with heparin, an anticoagulant. Pooled blood was transferred into small vials, which is previously rinsed with heparin. The whole blood was used for the estimation of erythrocyte, leukocyte counts, hemoglobin, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Erythrocyte and leucocytes were counted by using a hemocytometer [12]. The hemoglobin content of the blood was estimated by the cyanmethaemoglobin method [13]. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were calculated from the average values of Hb% [14].

Biochemical study: protein, carbohydrate, and lipid

Estimation of total proteins

Total protein content was estimated by the method Lowry et al [15]. 100 mg of tissue was homogenized in 5 mL of cold distilled water. 5 mL of 0% TCA was immediately added to precipitate the protein. The precipitate was collected by

centrifugation at 3000 rpm for 15 minutes. The supernatant was discarded. The pellet was repeatedly washed with distilled water to remove the traces of TCA precipitated. Protein was re-dissolved in 0.1 N NaOH. .0.5mL of the solution was transferred into a test tube and 4 mL of alkaline copper sulphate (50 mL of 2% Na_2CO_3 and mL, 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartrate) reagent was added followed by 0.4 mL of diluted commercial Folin's reagent (diluted with distilled water in 1:1 ratio). The optical density of blue color developed was read at 750 nm after 30 minutes on the addition of the reagent using a spectrophotometer. Bovine serum albumin was used as a standard.

Estimation of carbohydrate

The gill, liver, kidney, and muscle glycogen were estimated using the anthrone reagent method [16]. The sample was homogenized by adding 0.5 mL of 60% KOH and 1 mL of 30% KOH, both prepared in water. The mixture was incubated in a boiling water bath for 30 minutes. 4 mL of ethanol was added to the homogenate, and it was kept in a refrigerator for 24 hours and then centrifuged at 3000 rpm for 20 minutes. The pellet was resuspended in 1 mL of distilled water where 0.25 mL was taken and mixed with 1.75 mL of anthrone reagent and kept in a boiling water bath for 15 minutes. The color developed was read at 620 nm spectrophotometrically.

Estimation of lipid

Total lipids were estimated by using sulpho phospho vanillin method [17]. The dry tissue powder of the fish fry sample was homogenized with chloroform: methanol (2:1) and centrifuged at 2500rpm for 15minutes. A known amount of the supernatant was taken in a test tube and the solvent was removed under vacuum. To the residue, 0.5 mL of concentrated sulphuric acid was added and was kept in a boiling water bath for 15minutes. After cooling to room temperature, 2.5 mL of the phosphoric acid vanillin reagent was added; thoroughly vortexed and the tubes were closed with cotton wool. Then they were incubated for 30minutes at room temperature. The optical density was measured at 520 nm in a Spectrophotometer (Chemito-2000). The values were calculated against cholesterol standard and were presented as mg/g dry weight of tissue powder.

Statistical analysis

The statistical analysis was made between control and malathion treated groups and the mean value of five individual observations was taken for each parameter. The standard error for the sample mean was calculated and the significance of sample means between control and malathion treated fishes were tested by using the student's t-test.

Results

To evaluate the potential toxicological effects of chemicals on aquatic organisms and to monitor water quality, aquatic toxicological tests are commonly used [18]. During the experiment, fishes showed marked behavioral changes such as erratic jumping movement, loss of movement coordination, hyperactivity, and increased gill mucous secretion [19,20].

Hematological and biochemical changes during acute and sublethal exposure periods

The changes in hematological and biochemical parameters of the fish *Labeo rohita* exposed to acute toxicity of malathion are presented in (Tables 1 and Table 2). During acute (3.4 ppm) treatment red blood corpuscle (RBC), hemoglobin, MCV, MCH, protein, carbohydrate, and lipid levels decreased whereas White blood corpuscle (WBC) increased in the pesticide-treated fishes. The graphical representations of the changes in the acute study are presented in (Figures 1-4).

Table 1. Changes in the hematological parameters (RBC, Hb, WBC, MCV, MCH) activity in Indian major carp, *Labeo rohita* treated with acute concentration of malathion (3.4ppm, 96 h).

Hematological parameters	Control	Malathion treated fish
RBC	2.16±0.58	1.52 ± 0.21 *(-29.63)
Hb	2.79±0.68	1.74±0.45 *(-37.63)
WBC	1.47±0.18	2.18±0.62 *(+48.3)
MCV	31.38±1.27	21.41±1.12 *(-31.77)
MCH	7.12±1.09	5.21±1.03 *(-26.83)

[1] Values are means±SE of five individual observations; (-) denotes percent decrease & (+) denotes percent increase over control in the parenthesis.

[2] *Values are significant at $p<0.05$.

Table 2. Changes in the biochemical parameters (protein, carbohydrate, lipid) activity in Indian major carp, *Labeo rohita* treated with acute concentration of malathion (3.4ppm, 96 h).

Biochemical parameters (mg/g wet tissue)	Control	Malathion treated fish
Protein		
Gill	2.64±0.30	1.42±0.10 *(-46.21)
Liver	3.23±0.08	1.62±0.13 *(-49.85)
Kidney	2.14±0.15	1.31±0.13 *(-38.79)
Muscle	1.34±0.10	0.82±0.05 *(-11.97)
Carbohydrate		
Gill	20.8±0.13	11.60±0.13 *(-44.23)
Liver	17.75±0.18	8.12±0.19 *(-54.25)
Kidney	19.0±0.12	11.81±0.19 *(-37.84)
Muscle	8.40±0.09	3.41±0.25 *(-59.4)
Lipid		
Gill	27.4±0.09	11.24±0.14 *(-58.98)
Liver	22.6±0.08	13.4±0.15 *(-40.71)
Kidney	31.2±0.14	11.1±0.14 *(-64.42)
Muscle	28.10±0.16	9.3±0.11 *(-66.9)

[1] Values are means±SE of five individual observations; (-) denotes percent decrease & (+) denotes percent increase over control in the parenthesis.

[2] *Values are significant at $p<0.05$.

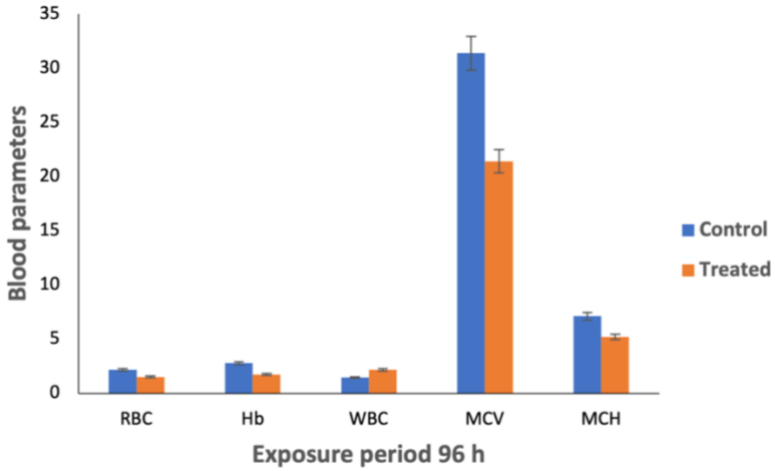

Figure 1. Hematological changes in gill, liver, kidney, and muscle of control and malathion treated fish (3.4 ppm; 96 h). The bar represents SE of the mean. Comparison of means (control and treated fish) was done by student's t-test.

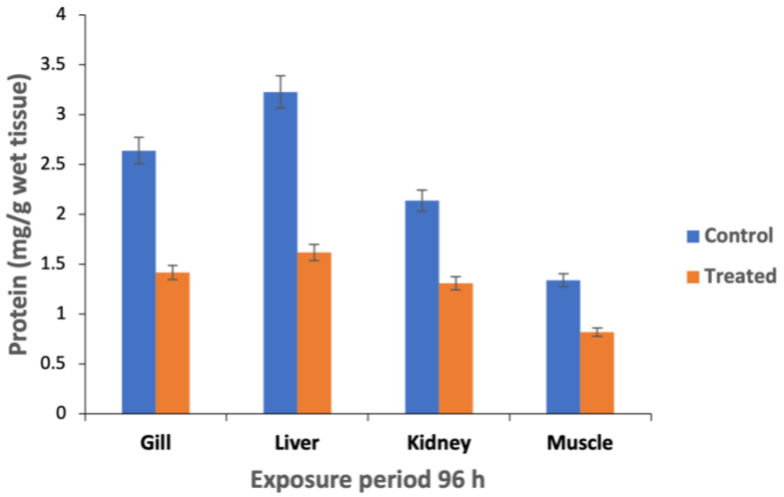

Figure 2. Changes in Protein metabolism of control and malathion treated fish (3.4 ppm; 96 h). The bar represents SE of the mean. Comparison of means (control and treated fish) was done by Student's t-test.

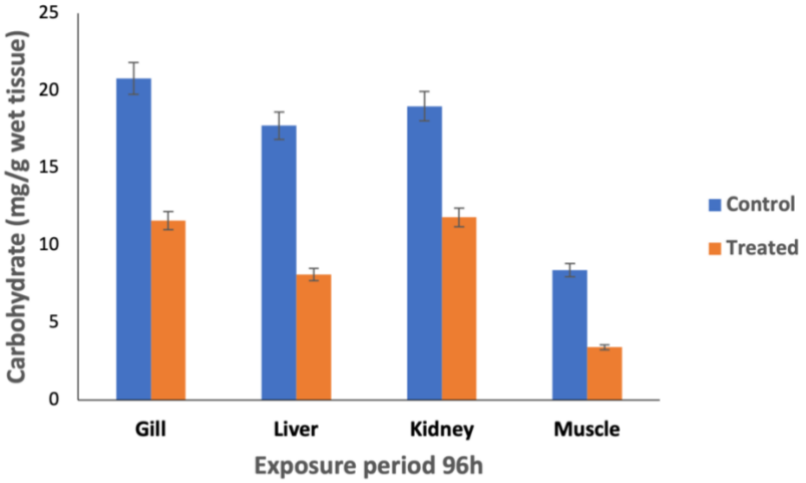

Figure 3. Changes in Carbohydrate metabolism of control and malathion treated fish (3.4 ppm; 96 h). The bar represents SE of the mean. Comparison of means (control and treated fish) was done by Student's t-test.

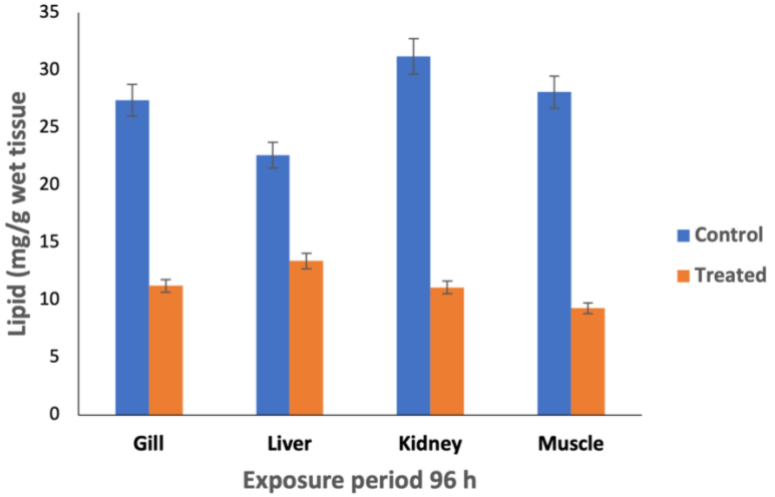

Figure 4. Changes in Lipid metabolism of control and malathion treated fish (3.4 ppm; 96 h). The bar represents SE of the mean. Comparison of means (control and treated fish) was done by Student's t-test.

Similarly, during the sublethal study using the concentration of 0.34 ppm malathion, all the parameters showed the same trend of changes as in the acute study which is presented in (Table 3 and Table 4). Here also WBC showed a remarkable increase than other parameters in the study. (Figure 5-8) denote graphical representations of the changes in the sublethal study.

Table 3. Changes in the hematological parameters (RBC, Hb, WBC, MCV, MCH) activity in Indian major carp, *Labeo rohita* treated with sublethal concentration of malathion (0.34ppm, 30 days).

Haematological parameters	Control	Malathion treated fish
RBC	2.16±0.58	1.01±0.25* (-53.24)
Hb	2.79±0.68	1.25±0.39* (-55.2)
WBC	1.47±0.18	2.82±0.71* (+91.84)
MCV	31.38±1.27	18.40±1.19* (-41.36)
MCH	7.12±1.09	2.23±1.02* (-68.68)

[1] Values are means±SE of five individual observations; (-) denotes percent decrease & (+) denotes percent increase over control in the parenthesis.

[2] *Values are significant at $p<0.05$.

Table 4. Changes in the hematological parameters (RBC, Hb, WBC, MCV, MCH) activity in Indian major carp, *Labeo rohita* treated with sublethal concentration of malathion (0.34ppm, 30 days).

Biochemical parameters (mg/g wet tissue)	Control	Malathion treated fish
Protein		
Gill	2.64±0.30	0.76±0.11* (-71.21)
Liver	3.23±0.08	1.18±0.04* (-63.47)
Kidney	2.14±0.15	0.62±0.13* (-71.03)
Muscle	1.34±0.10	0.52±0.09* (-61.19)
Carbohydrate		
Gill	20.8±0.13	7.90±0.18* (-60.66)
Liver	17.75±0.18	6.39±0.15* (-64.0)
Kidney	19.0±0.12	5.34±0.15* (-71.89)
Muscle	8.40±0.09	2.02±0.18* (-75.95)
Lipid		
Gill	27.4±0.09	8.10±0.13* (-70.44)
Liver	22.6±0.08	9.1±0.19* (-59.73)
Kidney	31.2±0.14	4.2±0.14* (-86.54)
Muscle	28.10±0.16	3.41±0.15* (-87.86)

[1] Values are means ± SE of five individual observations; (-) denotes percent decrease & (+) denotes percent increase over control in the parenthesis.

[2] *Values are significant at $p < 0.05$.

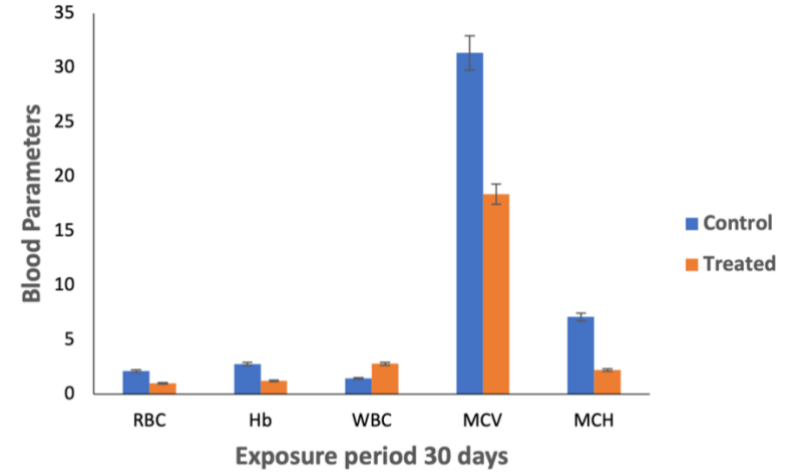

Figure 5. Hematological changes in control and malathion treated fish (0.34 ppm; 30 days). The bar represents SE of the mean. Comparison of means (control and treated fish) was done by Student's t-test.

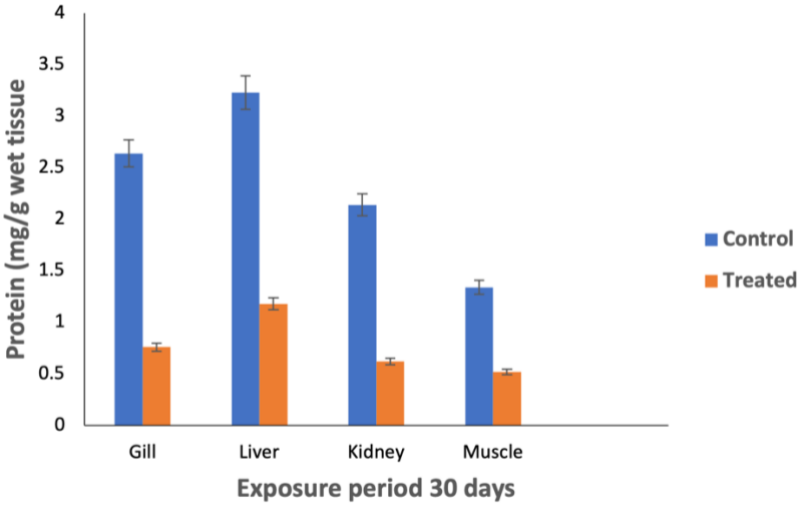

Figure 6. Changes in Protein metabolism of control and malathion treated fish (0.34 ppm; 30 days). The bar represents SE of the mean. Comparison of means (control and treated fish) was done by Student's t-test.

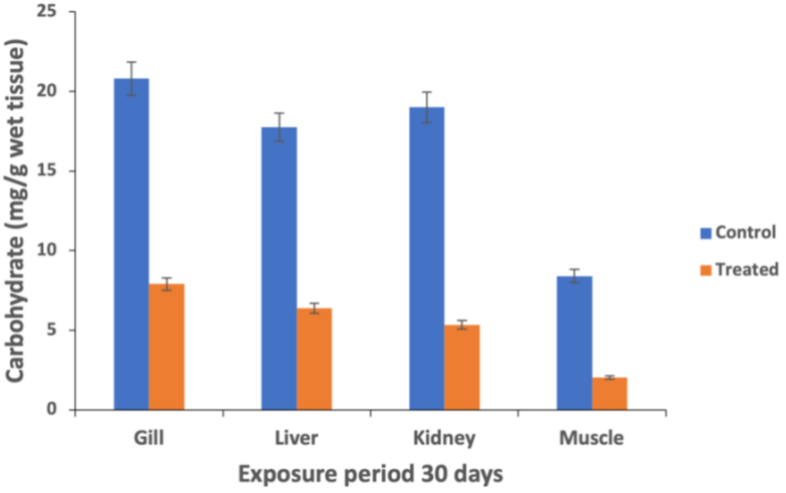

Figure 7. Changes in Carbohydrate metabolism of control and malathion treated fish (0.34 ppm; 30 days). The bar represents SE of the mean. Comparison of means (control and treated fish) was done by Student's t-test.

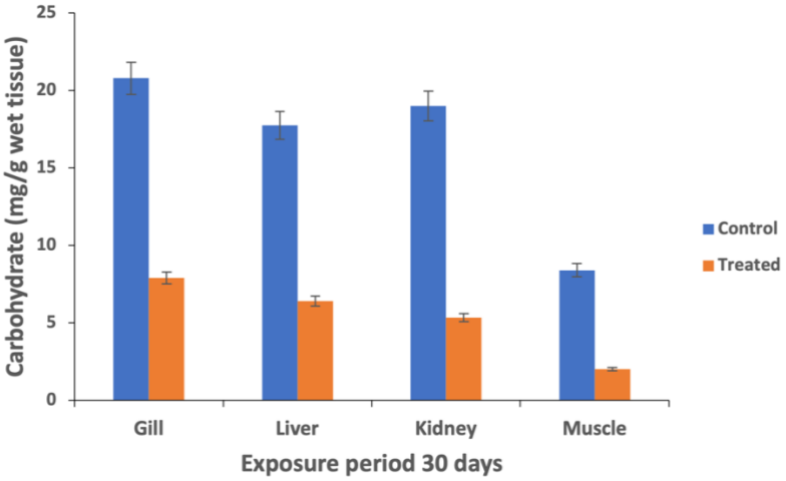

Figure 8. Changes in Lipid metabolism of control and malathion treated fish (0.34 ppm; 30 days). The bar represents SE of the mean. Comparison of means (control and treated fish) was done by Student's t-test.

Discussion

To ensure the sustainability of aquatic life from inadvertent exposure to contaminants and prevent human health effects from the consumption of contaminated fish, the evaluation of pesticides' risk to non-target organisms like fish is imperative [21]. The pesticide exposed fishes exhibited a reduction in hematological values indicated anemia which may be due to erythropoiesis, haemosynthesis, and osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in hematopoietic organs [22, 23].

In the present study, during acute and sublethal treatment, the significant decrease ($p<0.05$) in RBC count might have resulted from severe anemic state or haemolysing power of toxicant (malathion), particularly on the red cell membrane. Similarly, the significant decrease ($p<0.05$) in the hemoglobin content in the present study may be due to the rapid oxidation of hemoglobin to methemoglobin or the release of O_2 radicals brought about by the toxic stress of malathion. It is recognized that xenobiotics are capable of undergoing redox cycling can exert toxic effects by the generation of oxygen free radicals. A quick decrease in hemoglobin content in response to paraquat toxicity was observed in Cyprinus carpio by Matkovics et al. [24] and the authors suggested that it might presumably through methemoglobin formation and direct response of O_2-radicals.

Leucocytosis manifests in the form of leucocytosis with heterophilia and lymphopenia which are characteristics leucocytic response in animals exhibiting stress [1]. In the present study, the significant increase ($p<0.05$) in WBC count may be due to immunological reactions to produce antibodies to cope up with stress-induced by malathion which indicates hypersensitivity of leucocytes to malathion. The present result can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to lindane and malathion [25].

The results also revealed that after malathion exposure, other hematological indices including MCV and MCH experienced a considerable decrease ($p<0.05$). The alterations in MCV and MCH were ascribed to hemolysis and impairment in hemoglobin synthesis [26,27]. The MCH is a good indicator of red blood cell swelling [28,29]. Specifically, Oluah and Omerebel [30] found a decrease in MCV, and MCH after exposure of Clarias gariepinus to lead. Similar findings were recorded by Ololade and Ogini [31] after the exposure of Clarias gariepinus to Zinc.

Under conditions of stress, many organisms will mobilize proteins as an energy source for the oxidation of amino acids. In the present study, the significant decrease ($p<0.05$) of protein content may be due to stress-mediated immobilization of these compounds to fulfill an increased element for energy by the fish to cope with environmental conditions exposed by the toxicant [22]. The augmented proteolysis and possible utilization of their product for metabolic purposes may be the reason for the depletion in total protein content as reported by Ravinder et al [32]. On the other hand, Neff [33] has opined that a decline in protein content may be related to impaired food intake, increased energy cost of homeostasis, tissue repair, and detoxification mechanism during stress.

The results of the present study showed that the carbohydrate metabolism in gill, liver, kidney, and muscle is disrupted on exposure to malathion. Changes in carbohydrates have been suggested as a useful general indicator of stress in teleost. In the case of stress, carbohydrates are the first and immediate energy source to be utilized to a greater extent [34]. Hence, a reduction of total carbohydrates content in various tissues occurred on exposure to any kind of pollutant or toxicants. Carbohydrate reserves are depleted to meet energy requirements by all tissues under stressful conditions [35]. The carbohydrate content in the tissues in the present study significantly decreased ($p<0.05$) which may be due to rapid

utilization to meet the enhanced energy demands through glycolysis or hexose monophosphate pathway. Another reason for the decrease in glycogen content may be the inhibition of the enzyme glycogen synthetase. A decrease in tissue glycogen was observed in *Labeo rohita* on exposure to malathion and nuvan [36]. A similar reduction in tissue glycogen was observed when Sarorherodo mossambicus was exposed to DDT, malathion, and mercury [37]. The findings of the present study are also in agreement with those of Bakhshwan et al [38].

Lipids support as energy reserves to meet the metabolic requirement for more energy to mitigate toxic stress [39]. In the present investigation, the observed significant diminution ($p<0.05$) of the lipid profile may be due to increased lipid metabolism during stress conditions. A similar result was reported by Kumar and Gautam [40] in Channa punctatus with nuvan. The decline in the lipid levels may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol. A decrease in the lipid content of the gill, liver, kidney, and muscle tissues exposed to malathion recommends that lipid might have been directed for energy production for other metabolic functions in which these products play a key role during toxicant stress condition.

Conclusions

The study shows that non-target organisms in aquatic ecosystems such as *Labeo rohita* may be at risk of toxic effects of acute and sublethal concentrations of malathion. The present study declares that malathion is a highly toxic pesticide to *Labeo rohita* and the presence of malathion even at very low concentrations may cause adverse effects on aquatic organisms. Consistent biomonitoring and sensitization of stakeholders on responsible pesticide use are therefore imperative to forestall adverse ecological effects in aquatic ecosystems. To evaluate the toxic effect of malathion and other pesticides, the studied parameters in the experiment could be used as potential biomarkers. Further, ecological risk indices for this pesticide should be developed to safeguard aquatic life within the ambits of the United Nations Sustainable Development Goal 14 (sustaining life below water).

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Ethical statement

We declare that we do not need ethics approval to conduct the experimental work on major carp (*Labeo rohita*).

Conflict of interest

The authors declare that they have no conflict of interest.

CRediT author statement

JMS: Writing-Reviewing and Editing, Writing-original draft preparation, Data curation, and analysis;
SB: Conceptualization, Methodology, and Supervision.

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