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SUB LETHAL EFFECTS OF MALATHION (AN ORGANOPHOSPHATE) ON BIOCHEMICAL PARAMETERS OF FRESH WATER FISH *Labeo rohita* (HAMILTON)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author VY designed the study, wrote the protocol, revised manuscript and author MVR performed laboratory experiments and managed the analyses of the study. Author KT managed the literature searches, performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation was undertaken to study the toxic effect of the insecticide Malathion (an Organo phosphate- pesticide) on biochemical parameters of the fresh water fish *Labeo rohita* (Hamilton). The fish were exposed to sub lethal concentration of the insecticide Malathion and the variations were observed on different parameters i.e. proteins, carbohydrates and ninhydrine positive substances (FAA) at different time intervals i.e. 24, 48, 72 and 96 hrs of Malathion exposure from different tissues of fish *Labeo rohita* i.e. gill, liver, intestine, muscle and brain. The results revealed that biochemical constituents i.e. proteins, carbohydrates and ninhydrine positive substances were decreased in all the tissues of *L.rohita*. The toxic nature of insecticide Malathion was found to be more after 48 hrs of exposure as highest % of decease was found in biochemical constituents of all the tissues.

Keywords: Insecticide; malathion; *L. rohita*; proteins; carbohydrates; ninhydrine positive substances.

1. INTRODUCTION

The aquatic bodies across the globe are contaminated due to the heavy flow of industrial wastages and agricultural wastages such as insecticides and pesticides from different regions. Which has become a major problem not only for the survival of aquatic flora, fauna but also to the surroundings of nearby habitats [1]. The major entry routes of these insecticides polluting the aquatic bodies includes rain fall, runoff and atmospheric deposition. These insecticides ultimately find their way in to ponds, lakes and rivers [2,3,4] and cause toxic effects on non-target organisms such as fish [5-7]. Unsafe

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spraying and improper handling of the chemical pesticides may cause high risk of the health hazards to aquatic organisms especially fish [8,5].

The contamination of water by pesticides either directly or indirectly causes harmful effects on health, growth, survival and reproduction of aquatic animals especially fish which constitute an important source of food for human and animal consumption [9,10]. Fishes are highly sensitive to water contamination. Hence pollutants such as insecticides, herbicides may significantly affect some physiological and biochemical process when they accumulate in the tissues of fish [11-16].

Organophosphates are widely used throughout the world as an important group of pesticides, due to their insecticidal property, less toxicity, less persistence and rapid biodegradability in the environment [17]. Malathion (0, 0-dimethyl phosphoro dithioate of diethyl mercaptosuccinate) is a non systemic, wide spectrum organophosphate insecticide. When it enters in to the environment it causes deleterious effect on aquatic organisms and severe metabolic disturbances in non-target organisms like fish [4,18-22].

The alterations in biochemical parameters in different tissues of fish due to toxic effects of different heavy metals and pesticides have been reported by many researchers [22-31]. Extensive work has been done on the toxic effects of pesticides and herbicides on protein, carbohydrate, lipid content and histopathological studies of fishes [32,33].

In the present study, an attempt has been made to examine the sub lethal effects of Malathion on Biochemical constituents different tissues of fresh water fish *L. rohita* (Hamilton).

2. MATERIALS AND METHODS

The fresh water fish *Labeo rohita* were collected from local fresh water culture ponds by netting with the help of local fisher man located within the radius of 15 km from Kakatiya University, Warangal, Telangana State. The fish having average length 15 ± 1cm and weight about 50 ± 5g were brought to the laboratory and transferred in to plastic buckets (30X30X60 cm) containing water and disinfected with potassium permanganate and washed thoroughly prior to introduction of fish (to prevent fungal infection) then maintained at ambient room temperature (28 ± 2ºC) and were acclimatized to the laboratory conditions for about 10 to15 days prior to experimentation. They were regularly fed with commercial fish food and the medium (tap water) was changed daily to remove faeces and food remnants.

The fresh water fish, *Labeo rohita* were exposed to different concentrations of Malathion50% E.C (an technical grade). The healthy fish were grouped into five batches containing six each.

2.1 Toxicological Studies

The toxicity tests were conducted in accordance with standard method [34] (APHA, 1992). The pesticide Malathion was dissolved in acetone to yield a concentration of 100 mg/ml which were further diluted with distilled water for preparing required concentrations.

LC_{50} experiments: LC values usually refer to the concentration of a chemical in air but in environmental studies it can also mean the concentration of a chemical in water. For inhalation experiments, the concentration of the chemical in air that kills 50% of the test animals in a given time (usually four hours) is the LC50 value. For air borne and dissolved in water [35].

LC50: 100mg/ml in acetone is the estimated air concentration of a substance administered via inhalation route, ppm is used. Lower LC50 value indicates higher acute toxicity. The LC50 value for Copper sulphate, calculated by Finney's probit analysis Finney, (1964) method [36,35].

Acute toxicity test: The experiment was carried out at stocking density of 10 fish/ aquarium. The fish (four batches) were exposed to the sub lethal concentration. Concentration of each test media was increased gradually (0.5ppm to 1ppm), level of Malthion (an OP compound) concentration was maintained to 50% of toxicant concentration, while full toxicant concentrations were attained in 7hr of exposure, one batch was maintained as control i.e., without pesticide, and recorded the mortality rate of fishes [35].

2.2 Collection of Mortality Data

Fish mortalities were recorded at 24, 48, 72 and 96-hr of exposure, and dead fish were removed immediately from the test media [35].

2.3 Water Quality and Stocking Density

Water quality is vital for maintaining healthy, disease-free fish. Oxygen (6.0 ppm), temperature (28.5°C), pH (6.8–7.5), and salinity levels need to be closely monitored and controlled. Stocking density should be limited and filtration adequate to prevent build-up of toxic excretory products (ammonia and nitrate). Crowded conditions also increase cortisol levels, a cycle of 14 hours light, 10 hours dark is commonly used [35].

2.4 Preparation of Samples for Study

At the end of each exposure period of Malathion, respective fish batch were sacrificed and the tissues such as gill, liver, intestine, muscle and brain were dissected out and stored on ice jacketed containers for biochemical studies. The tissues were weighed to the nearest milligram and processed for further analysis. The tissue were ice-cold homogenized in 10% Tri Chloro Acetic acid (TCA) buffer (P^H 7.5) containing 0.9 % NaCl ice-cold centrifuged at 2000rpm for 15 minutes and clear supernatant and sediment was used for the analysis of total proteins, carbohydrates and ninhydrine positive substances (free amino acids--FAA). The protein sediment and supernatant (TCA precipitated and TCA soluble) were dissolved in 1N NaOH and protein content was determined through Lowry's method [37] described by Schacterle and Pollack [38], whereas carbohydrates were estimated by Corroll et al., (Anthrone) method [39] and ninhydrine positive substances (FAA) were estimated throughLee Takahashi method [40].

2.5 Statistical Analysis

Statistical analysis was performed by ANOVA to compare the results between the tissue components.

3. RESULTS

The Biochemical variations in the tissues of the fresh water fish *L. rohita* exposed to Malathion were presented in Tables 1, 2, 3 and 4 respectively. The total protein contents (structural and soluble) were decreased, whereas the carbohydrates and ninhydrine positive substances were found to be decreased in fish tissues exposed to Malathion at different time intervals i.e.24, 48, 72 and 96 h, without exposure of pesticide and were observed nearer to fish (control).

The results were presented in (Tables 1, 2, 3 and 4). The total protein content (Structural and soluble) were significantly ($p<0.05$) decreased in the gill of fish exposed to Malathion (Tables 1 and 2). It is observed that TCA soluble proteins were decreased and more pronounced in gill at 24, 48, 72 and 96 hrs of exposure. Whereas at 96hrs exposure the gill, liver, intestine, muscle and brain exhibited great reduction in TCA Soluble proteins (Table 1). A significant decrease was noticed TCA soluble proteins in muscle (3.95 mg) and low percentage of decrease in liver tissue (3.90 mg) compared to control (7.25 mg & 6.50 mg). Whereas in TCA precipitated protein (Structural) contents were significantly decrease at 24, 48, 72 and 96hrs of exposure (Table 2). At 96 hrs of exposure gill, liver, intestine, muscle and brain exhibited drastic reduction in TCA precipitated protein (structural) compared to control (8.36 mg & 12.50 mg). The high percentage of protein content was decreased in the brain tissue (4.98 mg) and low percentage of protein content (precipitated) was observed in muscle (7.60 mg).

The results presented in (Table 3) revealed that there is a drastic decrease in total carbohydrate content in different tissues of fish i.e. gill, liver, intestine, muscle and brain compared to control. In our findings Malathion exposed tissues at different time intervals i.e. 24, 48, 72 and 96 hrs the p value of carbohydrate content was found to be significant with $p<0.001$ in different tissues of fish. It can be concluded that there is significant variation observed between the various tissues of the fish.

The results presented in (Table 4) revealed that there is a drastic reduction in ninhydrine positive substances (FAA) at 24, 48, 72 and 96 hrs of exposure of Malathion in various tissues of fish at different time intervals with a significant p value of $p<0.005$ compared to control.

The free amino acids / ninhydrine positive substances were highly pronounced in muscle tissue followed by gill, liver, intestine and brain whereas which the fish is exposed to Malathion. The FAA were decreased in all tissues, we observed an increase in FAA during 48H of Malathion exposure, due to protein break down and release free amino acids. At 96H of Malathion exposure FAA were decreased in all tissues. High percentage decrease is observed in intestine, less percentage decrease is seen in muscle (Table 4).

Table 1. TCA soluble proteins of various tissues of *L. rohita* exposed to Malathion

Tissue/Dose	Control	24H	48H	72H	96H
Gill	6.25±0.28	5.85±0.27	5.65±0.30*	4.45±0.45*	3.85±0.19*
Liver	6.50±0.41	5.95±0.38	5.25±0.40*	4.65±0.32*	3.90±0.22*
Intestine	5.30±0.29	4..75±0.29	4.35±0.30	3.85±0.22*	3.10±0.35*
Muscle	7.25±0.41	6.52±0.42*	5.85±0.30	5.20±0.25*	3.95±0.16*
Brain	5.20±0.37	4.65±0.39*	4.16±0.30	3.85±0.17*	3.30±0.28

*The values are expressed mean 100mg±SE of wet weight of tissue; n=6; Statistically significant value to respective control value *P<0.001, **P<0.05, ***P<0.005*

Table 2. TCA precipitated proteins of various tissues of *L. rohita* exposed to Malathion

Tissue/Dose	Control	24H	48H	72H	96H
Gill	10.70±0.35	9.65±0.31	8.50±0.28*	7.50±0.25*	6.45±0.31*
Liver	9.50±0.42	8.50±0.38	7.55±0.33*	6.65±0.29*	5.68±0.25*
Intestine	10.75±0.28	9.70±0.22	8.55±0.24	7.45±0.19*	6.00±0.16*
Muscle	12.50±0.36	11.25±0.34*	10.15±0.28	9.20±0.22*	7.60±0.21*
Brain	8.36±0.27	7.55±0.24*	6.75±0.36	5.90±0.19*	4.98±0.16

*The values are expressed mean 100mg±SE of wet weight of tissue; n=6; Statistically significant value to respective control value *P<0.001,**P<0.05, ***P<0.005*

Table 3. Carbohydrates content of various tissues of *L. rohita* exposed to Malathion

Tissue/Dose	Control	24H	48H	72H	96H
Gill	5.19±0.36	4.52±0.34	4.14±0.28*	3.52±0.22*	2.98±0.26*
Liver	9.28±0.42	9.09±0.37	7.09±0.36*	6.35±0.29*	5.23±0.22*
Intestine	4.62±0.35	4.19±0.35	3.02±0.38	3.13±0.25*	2.86±0.31*
Muscle	4.34±0.37	4.10±0.43*	3.03±0.39	3.31±0.0.09*	2.66±0.12*
Brain	5.32±0.31	4.90±0.29*	4.24±0.24	3.69±0.30*	3.10±0.18

*The values are expressed mean 100mg±SE of wet weight of tissue; n=6; Statistically significant value to respective control value *P<0.001,**P<0.05, ***P<0.005*

Table 4. Free amino acids/Ninhydrine positive substances in various tissues of *L. rohita* exposed to Malathion

Tissue/Dose	Control	24H	48H	72H	96H
Gill	4.32±0.32	3.86±0.28	3.98±0.41*	3.20±0.50*	2.65±0.44*
Liver	4.20±0.41	3.82±0.21	4.08±0.28*	2.80±0.44*	2.50±0.28*
Intestine	3.96±0.19	3.48±0.32	3.09±0.30	2.76±0.28*	2.32±0.17*
Muscle	4.82±0.44	4.39±0.41*	3.88±0.28	3.48±0.51*	2.88±0.32*
Brain	3.42±0.37	3.17±0.44*	2.62±0.19	2.22±0.28*	1.98±0.44

*The values are expressed mean 100mg±SE of wet weight of tissue; n=6; Statistically significant value to respective control value *P<0.001,**P<0.05, ***P<0.005*

4. DISCUSSION

In the present investigation different tissues of *L. rohita* were exposed to sub lethal concentrations of Malathion affected the protein, carbohydrate and Ninhydrine positive substances at different time intervals. All these biochemical contents were significantly decreased on Malathion exposure.

The changes in biochemical parameters such as proteins, carbohydrates and ninhydrine positive substances are important to indicate the susceptibility of organ system to pollutants by altering their function [19]. Protein are the important organic substances involved in the architecture of the cell and play an important role in energy metabolism are also involved in the compensatory mechanism of stressed organisms [41,42,43]. The results of present study showed that when the fish were exposed to Malathion at different time intervals the protein contents were found to be decreased in various tissues. The percent decrease was found to be greater in all exposed tissues. The reduction of protein may be due to proteolysis and toxicity caused metabolic dysfunction under stress condition [18,26,31,42,44,45,46]. The results of the present findings showed a significant decrease in carbohydrate content in all the tissues of fish due to toxic effect of pesticide (Table 3). The reduction of carbohydrates suggests that the possibility of glycogenolysis and glycolytic pathway to provide excess energy in stress condition as reported [29,30,45,47,48].

The results showed a significant decreases in ninhydrine positive substance in all tissues of fish after Malathion exposure (Table 4). The high percentage of (66%) decrease is found in intestine and low percentage of FAA was found in muscle tissue (52.69%) of fish [29,30,48]. Increase in rate of degradation of amino acids [28,49] as shown in on present study (Table 4) on Malathion exposure which may enter in to TCA cycle through aminotransferases probably to cope up with high energy demands in order to meet the stress condition [50].

5. CONCLUSION

From the present study, it was concluded that the sub lethal effects of Malathion (an organophosphate) on biochemical parameters of fresh water fish *L. Rohita*. The various tissues exposed to different time intervals of malathion, affected on total protein, carbohydrate and ninhydrine positive substances levels of vital organs like gill, liver, intestine, muscle and brain of fish. Therefore the accumulation of pesticide in the water body primarily affects the non target organisms especially fish and get deposited in different tissues. These fish enter into the food chain and affect the humans and causes deleterious health effects. Hence the usage of the pesticides should be restricted to a minimal concentration to have a healthy ecosystem.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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