

## RESEARCH ARTICLE

### STUDIES ON THE IMPACT OF A MALATHION INSECTICIDE ON CERTAIN BIOCHEMICAL CONSTITUENTS OF A FRESHWATER FISH, *LABEO ROHITA*

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#### ABSTRACT

Malathion is an insecticide which is commonly used for the agricultural and non-agricultural purposes in India. Malathion is found effective for controlling mosquitoes, flies, household insects, animal parasites (ectoparasites) and head & body lice. The effect of insecticide Malathion is found to be highly toxic even to the non-targeted aquatic organisms including fish. The aim of the study, was to determine the effect of insecticide malathion on some biochemical characteristics (protein, carbohydrate and cholesterol in gill, liver, muscle and kidney) of the fish, *Labeo rohita*. Toxicity evaluation tests were conducted to determine LC<sub>50</sub> values. The 1/10<sup>th</sup> of 96 hrs, LC<sub>50</sub> value was selected as sublethal concentrations (0.5 ppm). All biochemical's parameters were found to be decreased in all tissues on comparison with control. The results indicated the toxic nature of the insecticide malathion.

**Keywords:** Malathion, *Labeo rohita*, biochemical and sublethal study.

#### 1. INTRODUCTION

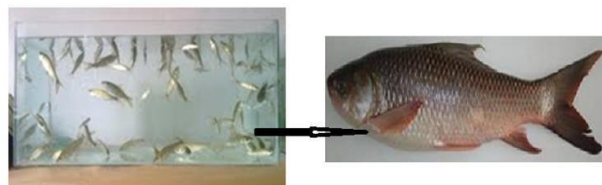
Pesticides are most commonly used in agriculture to aid in the production of high quality food. However, some pesticides have the ability to cause serious health and environmental damage (1). The exposure to the sub - lethal doses of some pesticides repeatedly can cause physiological and behavioural changes in fish that bring down populations increased susceptibility to increase and decreased efficiency to avoid predators (2). Malathion is one of the organophosphate insecticide. That was developed earlier (introduced in 1950). The over use of malathion on land may be washed along with the surface water which can adversely affect or kill the aquatic organisms and other higher organisms. The organisms of the aquatic habitat particularly fishes, are highly sensitive to this organo phosphate insecticide. The first indication of the stress is the biochemical changes occurring in the body of the organisms. A number of changes in biochemical parameters of aquatic organisms due to the pesticidal exposure have been reported by several investigators (3,4,5).

#### 2. MATERIALS AND METHODS

##### 2.1. Procurement and maintenance

A commercial formulation of malathion (Hi-Yield 55% EC 500gl-1) was purchased from a local market in Thudiyalur, Coimbatore district, Tamil Nadu and was used in this study. Bulk of sample of fishes (*Labeo rohita*) ranging in weight from 3-4gms measuring 3-5cm in length were procured from Aliyar reservoir. They were carried to the laboratory

in suitable polythene bags containing oxygenated water. The fishes were acclimated to the laboratory temperature (26±1.5) in large glass aquarium. Another group was maintained as control. The fishes were acclimatized to the laboratory conditions for two weeks in glass aquaria. The period of acclimation lasted for 2 weeks. Batches of 10 healthy fishes were exposed to different concentrations of insecticide malathion to calculate the median lethal concentration LC<sub>50</sub> value using probit analysis method (6).



**Collection and Maintenance of fresh water fish, *Labeo rohita*  
Morphological Characters of Experimental fish - *Labeo rohita***



**Toxicant - Malathion**

### 2.3. Evaluation of median lethal concentration (LC<sub>50</sub>)

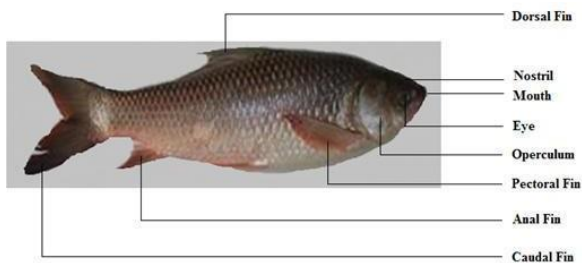
The concentration of the pollutant at which 50 percent of the test animals die during a specific test period of the concentration lethal to one half of the test population is referred to as median lethal concentration (LC<sub>50</sub>) or median reference limit in aquatic toxicology the traditional LC<sub>50</sub> test is often used to measure the potential risk of a chemicals (7). The fishes (Four groups) were exposed to the sublethal concentration (0.5 ppm) of malathion for 24, 48, 72 and 96hrs respectively.

### 2.4. Killing of Animals

The fish was caught very gently using a small dip net, one at a time with least disturbance. At the end of each exposure time, fishes were decapitated and tissues such as gill, liver, kidney and muscle were dissected and stored at 4°C until the analyses were performed. The tissues (10 mg) were homogenized in 80% methanol centrifuged at 3500 rpm for 15 min & the clear supernatant was used for the analysis of different parameters.

## 2.5. ESTIMATION OF BIOCHEMICAL PARAMETERS

### 2.5.1. Estimation of total protein



Total protein concentration was estimated by the method of Lowry *et al.* (1951) based on the following principle. In alkaline medium protein in the sample form a complex with copper ions. The amino acids containing aromatic groups, tyrosin and tryptophane present in copper protein complex react with Folin ciocalteu phenol reagent to give blue colour due to the reduction of phosphomolybdate. The intensity of the colour developed is proportional to the concentration of protein present in the sample. The value is expressed as mg/g of tissues.

### 2.5.2. Estimation of carbohydrate

Quantitative estimation of carbohydrate in the tissues was done following the method by Hedg's and Hofreiter (1962).

### 2.6. Principle

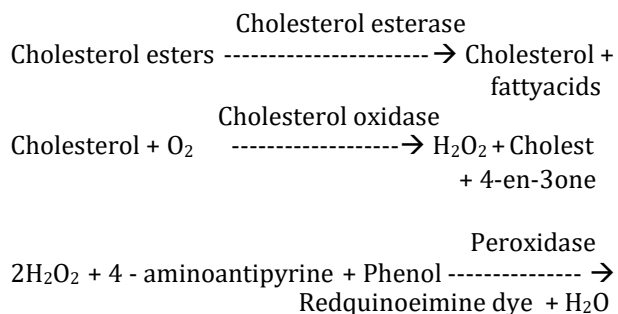
Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with

anthrone, a green coloured product with an absorption maximum at 630 nm. The value is expressed as mg/g of tissues.

### 2.7. Estimation of lipid

Estimation of Lipid was estimated by the method of Richmond, (1973) based on the following principle.

Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction cholesterol oxidase converts cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidatively occupies with 4-aminoantipyrine and phenol to produce red quinoeimine dye which has absorbance maximum at 510nm (500 - 530). The intensity of red colour is proportional to the amount of total cholesterol in the specimen. The value is expressed as mg/g of tissues.



### 2.6. Statistical analysis

Statistical analyses were performed using statistical package SPSS - 20, data are presented as mean  $\pm$  SD.

## 3. RESULTS AND DISCUSSION

The percentages of dead fishes for different Malathion doses of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 mg/L were determined for 24, 48, 72 and 96 hr (Table 1) was found in fingerling of *Labeo rohita*. Mortality of *Labeo rohita* increased with increasing concentrations of Malathion insecticide, while there was no mortality in the control. The various periods of Malathion exposure has revealed the depletion of the biochemical parameters like protein and glycogen in *Labeo rohita* (7). Protein plays a important role in almost every biological processes. Under stressed conditions, protein serve as the suppliers of energy for the metabolic pathways and biochemical reactions (8). The result of the present study showed that when the fish were exposed to malathion (0.5 ppm) the protein content were found to have decreased (Table 2). The present study revealed the reduction in protein levels in the tissues of *Labeo rohita* by following acute exposure of toxicant Malathion. Aruna *et al.* (9) observed that

the week of exposure to the sublethal concentrations of malathion showed a significant increase in total protein content in kidney of the fish, *Clarias batrachus* and the later periods of exposure showed a gradual decrease in the protein content. Similar

results were obtained in *Channa punctatus* when exposed to technical grade malathion. Remia *et al.* (3) observed that the proteolysis and increased metabolism under toxicant stress caused the reduction of protein.

**Table 1. Effect of Malathion on survival of *Labeo rohita* for 24, 48, 72 and 96 hr exposure time**

S. No.	Concentration (ml)	No. of exposed	No. of dead	Percent of mortality
Control	-	10	0	0
1	1.0	10	0	0
2	2.0	10	1	10
3	3.0	10	3	30
4	4.0	10	3	30
5	5.0*	10	5	50
6	6.0	10	6	60
7	7.0	10	6	60
8	8.0	10	8	80
9	9.0	10	8	80
10	10.0	10	10	100

**Table 2. Changes in the protein content in the tissues of *Labeo rohita* on short term exposure on short exposure**

Sample (mg/g wet tissue)	Exposure periods				
	Control	24hrs	48hrs	72hrs	96hrs
Gill	2.46 ± 0.38	1.98 ± 0.43	1.64 ± 0.04	1.32 ± 0.04	1.21 ± 0.08
% change		- 0.19	- 0.33	- 0.46	- 0.50
Liver	1.76 ± 0.07	1.65 ± 0.07	1.18 ± 0.09	1.12 ± 0.11	0.98 ± 0.04
% change		- 0.06	- 0.32	- 0.36	- 0.44
Kidney	2.01 ± 0.07	1.98 ± 0.04	1.47 ± 0.21	1.32 ± 0.10	1.00 ± 0.12
% change		- 0.01	- 0.26	- 0.34	- 0.50
Muscle	3.60 ± 0.31	3.21 ± 0.16	3.04 ± 0.15	2.94 ± 0.06	2.71 ± 0.10
% change		- 0.10	- 0.15	- 0.18	- 0.24

Values were expressed as mean ± S.D of three replicates using SPSS statistical package

**Table 3. Changes in the carbohydrate content in the tissues of *Labeo rohita* on short term exposure**

Sample (mg/g wet tissue)	Exposure periods				
	Control	24hrs	48hrs	72hrs	96hrs
Gill	12.42 ± 0.09	9.58 ± 0.05	7.84 ± 0.41	6.74 ± 0.37	4.98 ± 0.28
% change		- 0.22	- 0.36	- 0.45	- 0.59
Liver	18.62 ± 0.08	15.12 ± 0.22	11.42 ± 0.31	11.20 ± 0.24	10.45 ± 0.33
% change		- 0.18	- 0.38	- 0.39	- 0.43
Kidney	31.00 ± 4.03	26.42 ± 0.27	20.00 ± 3.27	12.40 ± 0.29	10.82 ± 0.13
% change		- 0.17	- 0.35	- 0.60	- 0.65
Muscle	30.41 ± 0.79	24.58 ± 0.45	19.68 ± 0.39	15.54 ± 0.29	13.42 ± 0.25
% change		- 0.19	- 0.35	- 0.48	- 0.55

Values were expressed as mean ± S.D of three replicates using SPSS statistical package

**Table 4. Changes in the cholesterol content in the tissues of *Labeo rohita* on**

Sample (mg/g wet tissue)	Exposure periods				
	Control	24hrs	48hrs	72hrs	96hrs
Gill	21.54 ± 0.35	15.38 ± 0.38	10.25 ± 0.36	9.40 ± 0.21	7.54 ± 0.39
% change		- 0.28	- 0.52	- 0.56	- 0.64
Liver	20.05 ± 2.13	16.45 ± 0.25	16.12 ± 0.17	14.00 ± 0.69	13.14 ± 0.22
% change		- 0.17	- 0.19	- 0.30	- 0.34
Kidney	37.12 ± 0.42	31.00 ± 3.45	26.24 ± 0.38	19.50 ± 0.37	11.00 ± 0.57
% change		- 0.16	- 0.29	- 0.47	- 0.70
Muscle	64.65 ± 0.20	56.75 ± 0.36	41.00 ± 4.53	34.10 ± 3.97	25.45 ± 0.37
% change		- 0.12	- 0.36	- 0.47	- 0.60

Values were expressed as mean ± S.D of three replicates using SPSS statistical package

Carbohydrates are stored in the form of glycogen in the tissues and organ like liver of fishes to supply energy needs during hypoxic condition and lack of food (10). The results of the present findings showed a significant decrease in carbohydrate content in all the tissues studied (Table 3). Arun Kumar and Jawahar Ali (11) reported that the sublethal concentrations of Malathin and glyphosate in the exposed shrimp *Streptocephalus dichotomus* showed a decrease in carbohydrate content. The decreased level of carbohydrates contents may affect the carbohydrate metabolism due to toxic effect (12).

Lipid play a crucial role in energy metabolism and providing energy to the metabolic processes (13). The results presented in Table 4 show a significant decrease in cholesterol content in the studied tissues of fish, *Labeo rohita*. Generally, the decrease in cholesterol contents in all tissues was found to be increased with the hours of exposure. The inhibition of cholesterol biosynthesis in the liver are the reduction in the absorption of dietary cholesterol might have resulted in the reduced cholesterol level (14). The reduction of lipids in various tissues have been studied by various authors. Mishra *et al.* (15) analysed the gradual depletion of lipid content of liver and muscle during the malathion exposure. Generally, the present results indicated the toxic nature of the insecticide malathion.

#### 4. SUMMARY AND CONCLUSION

In the present study, we have observed the sub lethal exposure of the Malathion proved to be toxic to fish *L. rohita*, which effects on the protein, carbohydrate and lipid levels of vital organs like gill, liver, kidney and muscle. Depletion of Protein, Carbohydrate and Cholesterol occurs after pesticidal exposure shows greater tendency for accumulation of pesticide Malathion in the body of the fresh water fish, *Labeo rohita*. Reducing the use of pesticides and introduction of natural remedies for pest encroachment could minimize pesticide pollution.

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