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EFFECT OF MALATHION (AN ORGANO PHOSPHATE) ON BIOCHEMICAL CONSTITUENTS OF FRESH WATER CAT FISH *HETEROPNEUSTES FOSSILIS* (BLOCH)

G.JAYAPRAKASH, N.RAJU, T.BHEEM RAO AND Y.VENKAIAH*

Department of Zoology, Kakatiya University, Warangal-506 009

***Corresponding Author: E Mail: venkaiahyanamala07@gmail.com; Mob. No.: +91-9849848705**

ABSTRACT

The present study was carried out to investigate the effect of malathion, an organophosphate compound (OP), on biochemical constituents of fresh water cat fish *H. fossilis*. The fish were exposed to sub lethal concentration of the toxicant malathion and the variations were observed in biochemical constituents in different tissues i.e. gill, liver, intestine, brain and muscle of the fish. The quantitative variations were observed in proteins, carbohydrates and ninhydrine positive substances at different time intervals i.e., 24, 48, 72 and 96 hrs. The results revealed that the components of proteins, carbohydrates and ninhydrine positive substances were found to be decreased significantly at 24, 48, 72 and 96hrs time interval of malathion exposure on different tissues of fish compared to control. The maximum decrease in proteins followed by ninhydrin positive substances (free amino acids) and carbohydrates was observed at 72hrs and 96hrs compared to 24hrs and 48hrs time interval in different tissues of fish *H. fossilis* on exposure to malathion.

Keywords: *Heteropneustes fossilis*, Carbohydrates, Ninhydrin positive substances, Malathion, Proteins

INTRODUCTION

Fresh water ecology is constantly under stress due to continuous use of agrochemicals especially the pesticides. Pesticides have been widely used all over the world to control the insects, pests, and other disease vectors. They ultimately find their way into aquatic habitats such as rivers, lakes and ponds. The environmental quality is determined by assessing the toxicity of different chemicals to fish and other aquatic organisms **[1]**. Pesticide toxicity to fish has been investigated in several studies **[2]**. Hence toxic studies are required on the physiology and metabolic activities of fish because of their importance in human nutrition.

For centuries pesticides have been used in agriculture to enhance food production by eradicating unwanted insets and controlling disease vectors **[3]**. Among these pesticides, organophosphate compounds (OPs) are commonly used as insecticides **[4].** Malathion (0, 0-dimethyl phospharodithioate of diethyl mercoptosuccinate) is a non systemic, wide spectrum organophosphate insecticide.

Once Malathion is introduced into the environment it may cause serious effects to aquatic organisms and is notorious for causing severe metabolic disturbances in non-target species like fish and fresh water mussels **[5].** Fishes are very sensitive to a wide variety of toxicants in water. Various species of fish show uptake and accumulation of many toxicants such as pesticides **[6].** Due to accumulation of pesticides in tissues, many physiological and biochemical changes may occur in fish and fresh water fauna by influencing the activities of several enzymes and metabolites **[7].**

The alterations in biochemical constituents in different tissues of fish due to toxic effects of different heavy metals and pesticides have been reported by many workers **[8-13]**. Extensive work has been done on the toxic effects of pesticides on protein, carbohydrate and lipid content of fish. The present study has been carried out to investigate the effect of Malathion on biochemical constituents in different tissues of the fresh water cat fish *Heteropneustes fossilis* (Bloch).

MATERIALS AND METHODS

In the present study, Fresh water fish *H. fossilis* was exposed to different concentrations of an insecticide and the variations in biochemical constituents of the fish were studied. The technical grade insecticide Malathion (50% E.C) was taken for this present study.

The fresh water fish *H. fossilis* (ranging in weight 50 to 100 gm and in length 20cm to 30 cm) were brought from local fresh water tanks

located within the radius of 15 km from the Kakatiya University, Warangal, Telangana State, India to the laboratory in well aerated polythene bag and acclimatized to the ambient room temperature (28±2^{o}C) in large plastic containers. During the period of acclimatization they were fed with oil cake mixed with rice flour. The period of acclimatization lasted for 15 days. The healthy fishes were grouped into five batches containing six and each were exposed to different concentrations of insecticide malathion to calculate the medium lethal concentration LC_{50} value using Probit analysis method **[14].** The Malathion was dissolved in acetone and diluted with water to the required concentrations. The fishes (five groups) were exposed to the sub lethal concentration (0.5 ppm to 1.0 ppm) of Malathion for 24, 48, 72 and 96 hrs respectively. Another group was maintained as control without pesticide. At the end of each exposure period, fishes were scarified and the tissues such as gill, liver, intestine, muscle and brain were dissected out and stored on ice-jacketed container for biochemical studies. The tissues were weighed to the nearest milligram and processed for further analysis.

The tissues were homogenized (10%) in 10% Tri Chloro Acetic Acid (TCA) centrifuged at 2000 rpm for 15 minutes and clear supernatant and sediment was used for the analysis of total proteins, carbohydrates and ninhydrine positive substances (FAA). The protein sediment and supernatant (TCA precipitated and soluble proteins) was dissolved in 1N NaOH and protein content was determined through the Lowry's reagent **[15]** described by Schacterle and Pollack (1973) **[16].** Ninhydrine positive substances were estimated by the method of Lee and Takahashi (1966) **[17]** and the total carbohydrate content in the tissues were estimated by the method of (Anthrone) Carroll *et al,* 1956 **[18].**

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance ANOVA to compare the results between the tissue components.

RESULTS

The results obtained from the quantitative estimates on the effect of Malathion on biochemical constituents of various tissues of cat fish *H. fossilis* are presented in **Tables-1, 2, 3 & 4 and Figures 1, 2, 3 & 4** respectively. In this experiment when the fish tissues i.e. gill, liver, intestine, brain and muscle after treatment with desired concentrations of the test chemical malathion at different time intervals, a drastic reduction was observed in total biochemical constituents of different

tissues of cat fish *H. fossilis* compared to control.

The results presented shows that the protein content was significantly decreased in the gill of fish exposed to Malathion **(Table 1, 2 and Figure 1, 2)**. It is observed that the TCA soluble proteins were decreased and this is more pronounced in the gill at 48, 72 and 96 hours of exposure, whereas at 96 hrs exposure gill, liver, intestine, brain and muscle exhibited greater reduction in TCA soluble proteins **(Table and Figure.1)** whereas in TCA precipitated protein contents were significantly decreased at 24, 48, 72 and 96 hrs of exposure of malathion (Table and figure.2). At 96 hrs exposure gill, liver, intestine, brain and muscle exhibited greater reduction in TCA precipitated proteins (structural proteins) compared to control. The reduction in soluble protein content and TCA precipitated protein content was found to be significant with $p<0.001$ in different tissues of fish.

The results presented in **Table 3 and Figure 3** revealed that there is a drastic reduction in total carbohydrate content in different tissues i.e. gill, liver, intestine, brain and muscle of cat fish *H. fossilis* compared to control. In our observations malathion exposed tissues with 24 , 48, 72 and 96 hrs treatment, the p value of carbohydrate content was found to be significant with $p<0.001$ in different tissues of cat fish *H. fossilis*. It can be concluded that there is a significant variation between the various tissues of cat fish *H. fossilis*.

The results presented in **Table 4 and Figure 4** revealed that the ninhydrine positive substances (free amino acids) were decreased at 24, 48, 72 and 96 hrs of exposure of malathion (Table and figure.4) whereas at 96 hrs exposure gill, liver, intestine, brain and muscle exhibited drastic reduction in free amino acid content. Various tissues of cat fish *H. fossilis* at different time intervals showed a significant p value with $p<0.05$ compared to control.

Table 1: TCA soluble proteins of various tissues of *Heteropneustes fossilis* exposed to varying periods of sub lethal concentration of Malathion

Tissue/Dose	Control	24 Hours	48 Hours	72 Hours	96 Hours
Gill	**3.66±0.21**	**3.66±0.21**	**2.04±0.62**	**1.83±0.23**	**0.87±0.19**
Liver	**5.29±0.32**	**4.16±0.54**	**3.70±0.81**	**2.54±0.61**	**1±0.25**
Intestine	**3.25±0.30**	**0.312± 0.05**	**0.254± 0.03**	**0.195± 0.03**	**0.08± 0.31**
Brain	**1±0.039**	**0.91±0.19**	**0.66±0.10**	**0.41±0.08**	**0.39±0.09**
Muscle	**3.3±0.46**	**1.70±0.33**	**1.66±0.73**	**1.65±0.61**	**0.95±0.21**

The values are expressed as mean ± SE mg/gm of wet weight of tissue.

Table 2: TCA precipitated proteins of various tissues of *Heteropneustes fossilis* exposed to varying periods of sub lethal concentration of malathion

Tissue/Dose	Control	24 Hours	48 Hours	72 Hours	96 Hours
Gill	15.37±0.57	13±0.48	10.04±0.19	10.29±0.57	6.2±0.97
Liver	14.16±0.79	12.25±0.88	11.5±0.89	8.04±1.44	7.66±0.56
Intestine	11.29±0.81	11.25±0.42	9.37±0.54	8.29±1.08	7.58±0.58
Brain	5.91±0.55	4.71±0.65	4.2±0.45	2.87±0.48	1.62±0.42
Muscle	10.7±0.78	10.95±0.93	7.25±1.20	7.2±0.64	4.16±0.32

The values are expressed as mean ± SE mg/gm of wet weight of tissue

Table 3: Carbohydrate content of various tissues of *Heteropneustes fossilis* exposed to varying periods of sub lethal concentration of malathion

Tissue/Dose	Control	24 Hours	48 Hours	72 Hours	96 Hours
Gill	0.73±0.210	0.52±0.416	0.29±0.47	0.28±0.06	0.22±0.25
Liver	2.56±0.415	1.62±0.072	1.51±0.30	1.44±0.14	1.00±0.24
Intestine	1.16±0.09	0.64±0.05	0.44±0.08	0.29±0.35	0.24±0.43
Brain	2.58±0.39	0.31±0.05	0.25±0.03	0.19±0.38	0.08±0.31
Muscle	0.64± 0.46	0.62±0.60	0.49±0.03	0.46±0.042	0.20±0.37

The values are expressed as mean ± SE mg/gm of wet weight of tissue

Table 4: Ninhydrine positive substances of various tissues of *Heteropneustes fossilis* exposed to varying periods of sub lethal concentration of malathion

Tissue/Dose	Control	24 Hours	48 Hours	72 Hours	96 Hours
Gill	2.41± 0.35	0.91± 0.26	0.87± 0.19	0.66± 0.15	0.29± 0.16
Liver	2.25± 0.75	0.916± 0.26	0.83± 0.10	0.58± 0.05	0.29± 0.13
Intestine	2.08±0.45	1.41± 0.59	4.35± 0.54	3.29± 0.58	2.58± 0.58
Brain	1.41±0.39	0.70±0.23	0.66±0.16	0.33±0.05	0.16±0.08
Muscle	2.66±0.55	1.33±0.23	0.87±0.19	0.45±0.11	0.12±0.08

The values are expressed as mean ± SE mg/gm of wet weight of tissue

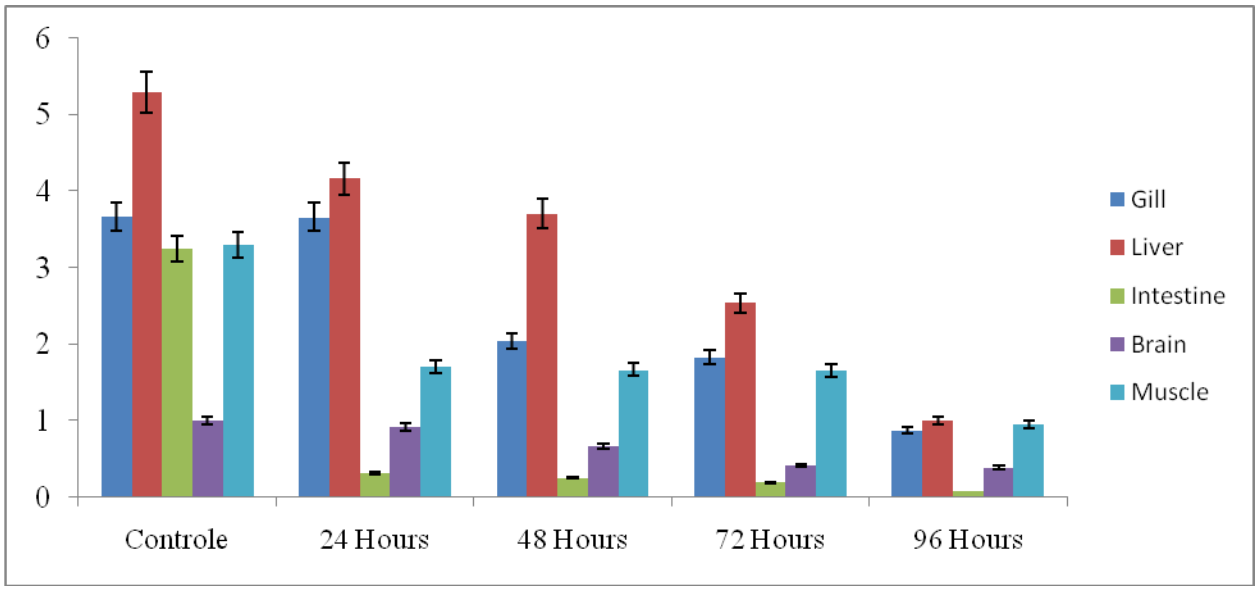

Figure 1: TCA soluble proteins of various tissues of *Heteropneustes fossilis* and the values are expressed as mean ± SE mg/gm of wet weight of tissue

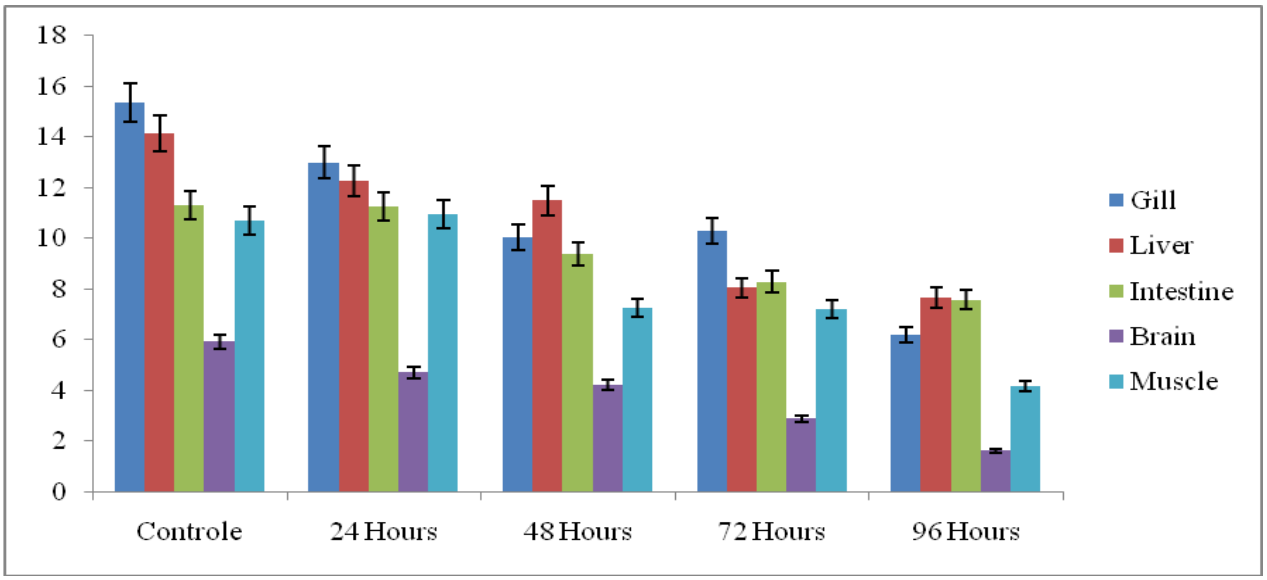

Figure 2: TCA precipitated proteins of various tissues of *Heteropneustes fossilis* and the values are expressed as mean ± SE mg/gm of wet weight of tissue

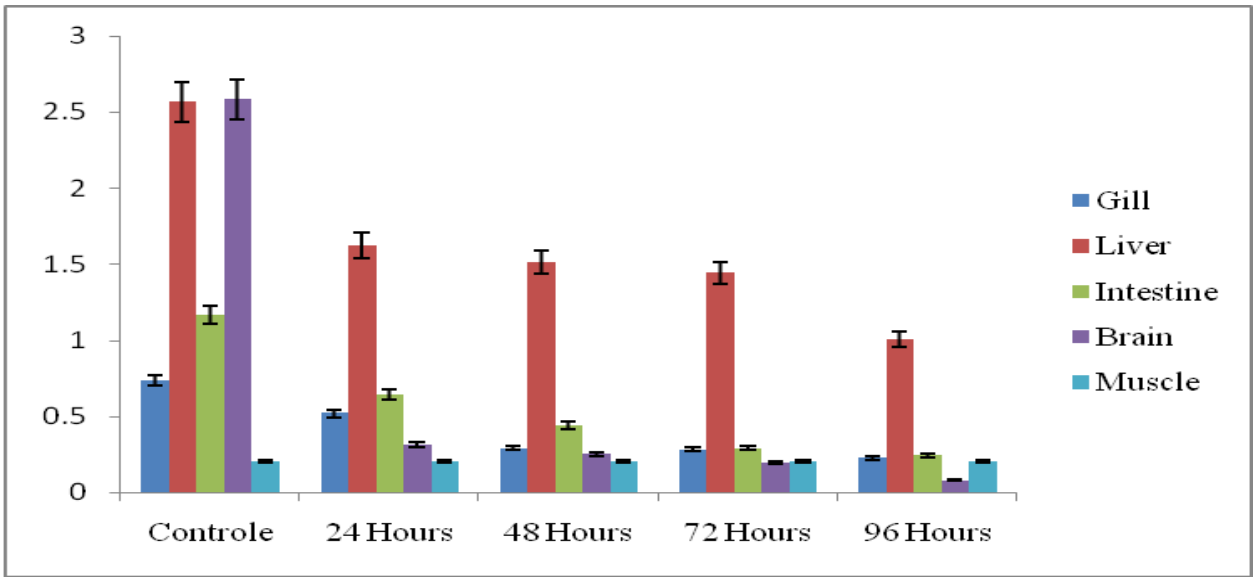

Figure 3: Carbohydrate content various tissues of *Heteropneustes fossilis* and the values are expressed as mean ± SE mg/gm of wet weight of tissue

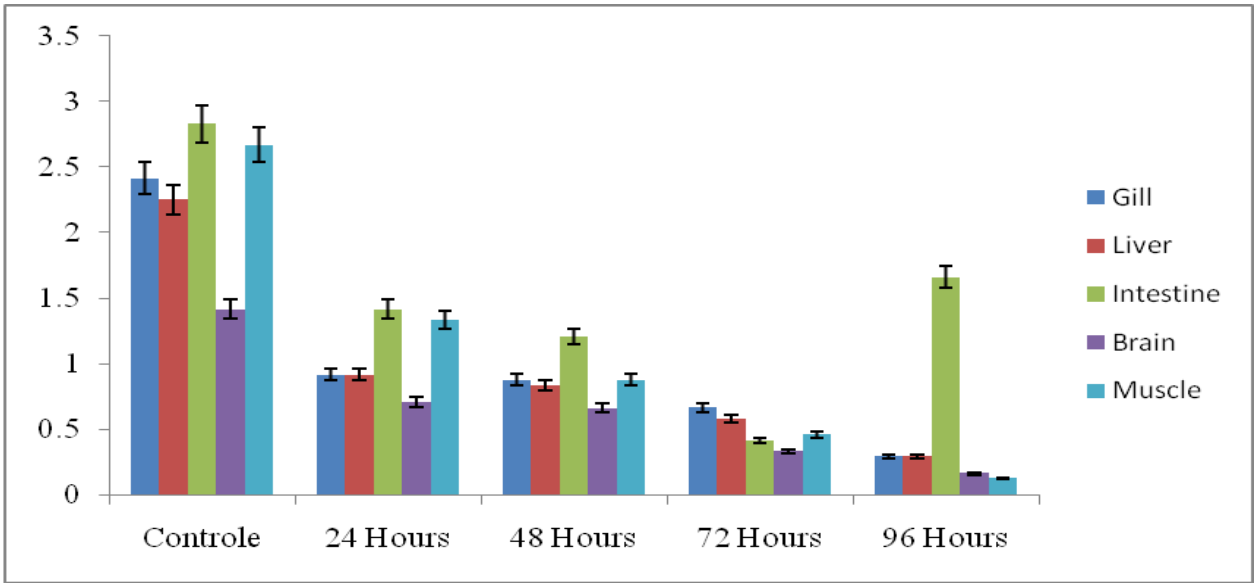

Figure 4: Ninhydrine positive substances of various tissues of *Heteropneustes fossilis* and the values are expressed as mean ± SE mg/gm of wet weight of tissue

DISCUSSION

The organophosphate compounds (OPs) that are mainly used as insecticides are the largest group of chemical used in control of pests including invertebrates and vertebrates **[4]**. The degree of toxicity produced by the poisonous substances is dose dependent upon environmental conditions such as temperature, pH, oxygen content and presence of residual molecules **[19].**

Due to Malathion intoxification, the normal functioning of cells with the resultant alterations in the fundamental biochemical mechanisms in test fish was noticed. Similar results were also noticed in freshwater *Labeo rohita* upon chronic exposure to the pesticide Malathion **[20].**

In our present investigation, the effect of Malathion on biochemical constituents of fresh water fish *H. fossilis* showed a considerable variation in different tissues of fish *H. fossilis* **[21].** Findings were correlated on pesticide treated molluscs. In the present study malathion caused reduction in the total structural and soluble protein content in various tissues of *H. fossilis* i.e., gill, liver, intestine, brain and muscle were found to be declined during the exposure period of 24, 48, 72 and 96 hrs. The studies on Malathion toxicity cause metabolic dysfunction in fish **[9, 22 and 23].**

Carbohydrates are less sensitive as compared to proteins towards the OP compounds. The results of the present findings showed a significant decrease in carbohydrate content

in all the tissues **(Table and Figure 3)**. The decrease in carbohydrates and ninhydrine positive substances (Free amino acids) was observed in various tissues of *H. fossilis* compared to control. The decrease in carbohydrate content is significant and may result in impairment of carbohydrate metabolism due to toxic effects **[21, 24-26].**

CONCLUSION

From the present study it is concluded that the Malathion exposure has a strong potential to alter the biochemical constituents in various tissues of *H. fossilis*. Further research has to be focused to evaluate the effect of alternative pesticides and related chemicals to reduce the adverse effects on physiological and biochemical aspects of fish. Therefore, the use of pesticides in the field may be a threat to a human, fauna and flora of the environment.

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