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IMPACT OF MALATHION (AN ORGANOPHOSPHATE) ON BIOCHEMICAL CONSTITUENTS (PROTEINS, CARBOHYDRATES AND FREE AMINOACIDS) OF FRESH WATER FISH *Channa punctatus* (Bloch)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author VY designed the study, wrote the protocol, and wrote the first draft of the manuscript and literature searches. Author MV performed the statistical analysis and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Pesticides are most hazardous chemicals causing high risk to the environment, fisheries and people. Organophosphate pesticides are increasingly used in recent years due to their biodegradability and short resistance time in the environment. The present study was carried out to determine the impact of Malathion an organophosphate pesticide on biochemical constituents i.e. proteins, carbohydrates, and ninhydrine positive substances (Free Amino acids) of fresh water fish *Channa punctatus* (Bloch). The fish were exposed to the toxicant Malathion at different time intervals i.e. 24, 48, 72 and 96hr and the quantitative variations were observed on biochemical constituents in different tissues of *C. punctatus* i.e. gill, liver, intestine, muscle and brain. The results revealed that the total proteins, carbohydrates and free aminoacids in different tissues of fish were found to be varied at different time intervals of Malathion exposure and a significant decrease was observed in all the constituents compared to control. The maximum decrease in proteins, carbohydrates and free aminoacids was observed at 96hrs and 72hrs followed by 48hrs and 24hrs in different tissues of fish *C. punctatus*. Thus our present investigation reports that the period of exposure of Malathion has affected the amount of biochemical constituents in *C. punctatus*.

Keywords: Organophosphates; Malathion; *C. punctatus;* proteins; carbohydrates; free aminoaicds.

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1. INTRODUCTION

Pesticides are used worldwide in agriculture and aquaculture to control the insect, pests and other disease vectors, which ultimately find their way into aquatic habitats such as rivers, lakes and ponds [1]. The environmental quality is determined by assessing the toxicity of different chemicals to fish and other aquatic organisms, as these chemical compounds ultimately enter the organisms through food web. Most of the chemicals used as pesticides are acutely toxic to many non-target organisms such as invertebrates, birds, mammals and fishes especially those inhabiting the marine environment [2-6]. However some pesticides cause serious health and environmental damage [7, 8]. The repeated use of pesticide cause chemical pollution resulting in potential health problems to live stock, especially to fish, birds, frogs and mammals [9, 10]. Extensive use of pesticides exposes organisms to sub lethal concentration causing physiological, biochemical and behavioural changes in the fish that reduce the fish population, like abandonment of nests and broods with decreased immunity to disease and increased failure to avoid predators [11, 12]. Hence it is necessary to study the effect of pesticides on fish which forms a part of human diet.

Among the pesticides, organophosphate compounds (OPs) are commonly used insecticides which maintain less toxicity, persistence and also rapid biodegradability in the environment [13-15]. Fishes are very sensitive to different pesticides in water which uptake and get accumulated. The toxic effects of organophosphate compounds on significant inhibition of alkaline phosphatises in various tissues of *Clarias batrachus* has been reported by [16]. Pesticide exposure causes severe alterations in the tissue biochemistry of fishes [17]. In general the toxic effects will be more when two (or) more toxicants act together in a synergistic manner [18-24].

In view of the paucity of information regarding the effect of Malathion on fresh water fish *C. Punctatus*, we have undertaken this investigation to study the impact of Malathion (an organophosphate) on biochemical constituents (proteins, carbohydrates and free aminoacids) of fresh water fish *C. punctatus* (Bloch).

2. MATERIALS AND METHODS

The fresh water fish *C. punctatus* were collected from local fresh water tanks located within the radius of 15 km from Kakatiya University, Warangal, Telangana State, by netting with the help of local fisher man. The fishes having average length 15 ± 1cm and weighed about 50±5gm 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 ± 2 ºC) and were acclimatized. 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 feaces and food remnants.

The fresh water fish, *C. punctatus* were exposed to different concentrations of Malathion of technical grade 50% E.C. The healthy fish were grouped into five batches containing six each. To calculate the medium lethal concentration LC_{50} value using probit analysis method [25].

2.1 Toxicological Studies

The toxicity tests were conducted in accordance with standard method [26]. The pesticide Malathion was dissolved in acetone to yield a concentration of 100mg/ml which were further diluted with distilled water for preparing required concentrations. The fish (four batches) were exposed to the sub lethal concentration (0.5ppm to 1ppm) of Malathion for 24, 48, 72 and 96 hrs separately and one batch was maintained as control i.e without pesticide, and recorded the mortality rate of fishes

2.2 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 homogenized in 10% Tri Chloro Acetic acid (TCA) buffer (ph 7.5) containing 0.9 % NaCl centrifuged at 2000 rpm for 15 minutes and clear supernatant and sediment was used for the analysis of total proteins, carbohydrates and free amino acids (FAA/ninhydrine positive substances). The protein sediment and supernatant (TCA precipitated and TCA soluble) were dissolved in 1N NaoH and protein content was determined through [27, 28], whereas carbohydrates were estimated by [29] and free amino acids (FAA/ ninhydrine positive substances) were estimated through [30].

2.3 Statistical Analysis

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

3. RESULTS

The results obtained from the present investigation on the impact of Malathion on biochemical constituents (protein, carbohydrate and free amino acids) of various tissues of fresh water fish *C. punctatus* are presented in Tables 1, 2, 3 and 4 and Figs. 1, 2, 3 and 4 respectively. In this study, when the fish tissues i.e. gill, liver, intestine, muscle and brain after treatment with different concentrations of the pesticide Malathion at different time intervals i.e. 24, 48, 72 and 96hrs a drastic reduction was observed in total biochemical constituents.

The results revealed that the total protein content was significantly decreased in all the tissues of the fish exposed to Malathion (tab.1, 2 & Figs. 1, 2). It is observed that the TCA Soluble proteins were decreased in all tissues at different time intervals of Malathion exposure, but at 96hr exposure, a great reduction was observed in brain, intestine, gill, liver and muscle (3.25, 3.29, 3.90, 4.85 and 5.92 mg/100mg) (tab.1 and Fig. 1). The TCA precipitated proteins (structural proteins) were also found to be declined at all the time intervals (24, 48, 72 and 96hr) of Malathion exposure compared to control but the reduction was found to be more pronounced at 96 hrs with 3.95, 6.95, 7.75, 8.50 and 9.90 mg/100mg in brain, gill, intestine, liver and muscle respectively (tab. 2 & Fig. 2). The reduction in TCA soluble and TCA precipitated protein content was found to be significant with $p<0.001$ in different tissues of fish.

The total carbohydrate content in different tissue of fish i.e. gill, liver, intestine, muscle and brain of fresh water fish *C. punctatus* were also found to be reduced on Malathion exposure at different time intervals compared to control (tab.3 & Fig. 3). In our observations among all the time intervals of Malathion exposure, a maximum reduction in carbohydrate content was noticed at 96hrs in all the tissues but maximum reduction was observed in intestine (2.92 mg/100mg) followed by muscle, gill, brain and liver (3.02, 3.10, 3.57 and 5.28 mg/100mg). 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 a significant variation between the tissues of fresh water fish *C. punctatus*.

The results presented in (tab. 4 & Fig. 4), revealed that there is a reduction in the content of free amino acids/ ninhydrine positive substances at 24, 48, 72 and 96hr exposure of Malathion compared to control. Though the reduction of FAA was found at all the time intervals, a major decrease was found at 96 hrs. The brain, intestine, liver, gill and muscle tissues exhibited a drastic reduction in free amino acid content with 2.50, 2.83, 2.89, 3.16 and 3.49 mg/100mg respectively at 96hr time interval and showed a significant p value with $p<0.005$.

Table 1. TCA soluble proteins of various tissues of *C. punctatus* exposed to malathion

Tissue/Dose	Control	24H	48H	72H	96H
Gill	6.60±0.42	5.95±0.25	5.30±0.41*	4.50±0.40	3.90±0.21
Liver	8.25±0.37	7.45±0.36*	6.*50*±0.30	5.80±0.29	4.85±0.32*
Intestine	5.60±0.30	5.10±0.30*	4.75±0.25	4.00±0.41*	3.29±0.28
Muscle	9.75±0.42	8.67±0.28	7.50±0.28*	6.80±0.30	5.92±0.36
Brain	5.35±0.31	4.80±0.33	4.50±0.39*	3.50±0.28	3.25±0.27*

*The values are expressed as mean±SE of mg/100mg wet weight of tissues; n=6 *P<0.001,**P<0.05, ***P<0.005*

Table 2. TCA precipitated proteins of various tissues of *C. punctatus* exposed to malathion

Tissue/Dose	Control	24H	48H	72H	96H
Gill	13.25±0.36	12.50±0.42*	10.75±0.32	8.25±0.51	6.95±0.32*
Liver	15.00±0.35	14.10±0.33*	12.00±0.43	10.50±0.28*	8.50±0.28
Intestine	12.95±0.42	11.65±0.27	10.50±0.40*	8.95±0.42	7.75±0.42
Muscle	17.50±0.35	15.80±0.42*	13.95±0.32	12.15±0.34*	9.90±0.32*
Brain	9.50±0.36	6.50±0.32*	6.00±0.46*	5.50±0.50	3.95±0.46

*The values are expressed as mean±SE of mg/100mg wet weight of tissues; n=6; *P<0.001,**P<0.05, ***P<0.005*

Table 3. Carbohydrates content of various tissues of *C. punctatus* exposed to malathion

Tissue/Dose	Control	24H	48H	72H	96H
Gill	5.28±0.33	4.69±0.44	4.28±0.33	3.75±0.51*	3.10±0.33
Liver	9.45±0.30	8.28±0.31*	7.42±0.42	6.48±0.36	5.28±0.30
Intestine	4.98±0.43	4.69±0.34*	4.10±0.27*	3.28±0.28*	2.92±0.42*
Muscle	5.12±0.33	4.55±0.28	4.09±0.32	3.48±0.32	3.02±0.32
Brain	6.12±0.32	5.52±0.32*	4.88±0.44	4.00±0.36*	3.57±0.27*

*The values are expressed as mean±SE of mg/100mg wet weight of tissues; n=6; P<0.001,**P<0.05, ***P<0.005*

Table 4. Free amino acids/Ninhydrine positive substances in various tissues of *C. punctatus* exposed to malathion

Tissue/Dose	Control	24H	48H	72H	96H
Gill	5.45±0.32	4.90±0.34	4.22±0.32*	3.90±0.52*	3.16±0.43*
Liver	4.95±0.28	4.22±0.42	4.08±0.42*	3.51±0.41*	2.89±0.32*
Intestine	4.83±0.30	4.29±0.28	3.82±0.38	3.41±0.28*	2.83±0.31*
Muscle	5.83±0.35	5.10±0.42*	4.65±0.34	3.92±0.43*	3.49±0.40*
Brain	4.49±0.37	4.12±0.32*	3.49±0.32	3.12±0.31*	2.50±0.42

*The values are expressed as mean±SE of mg/100mg wet weight of tissues; n=6; *P<0.001,**P<0.05, ***P<0.005*

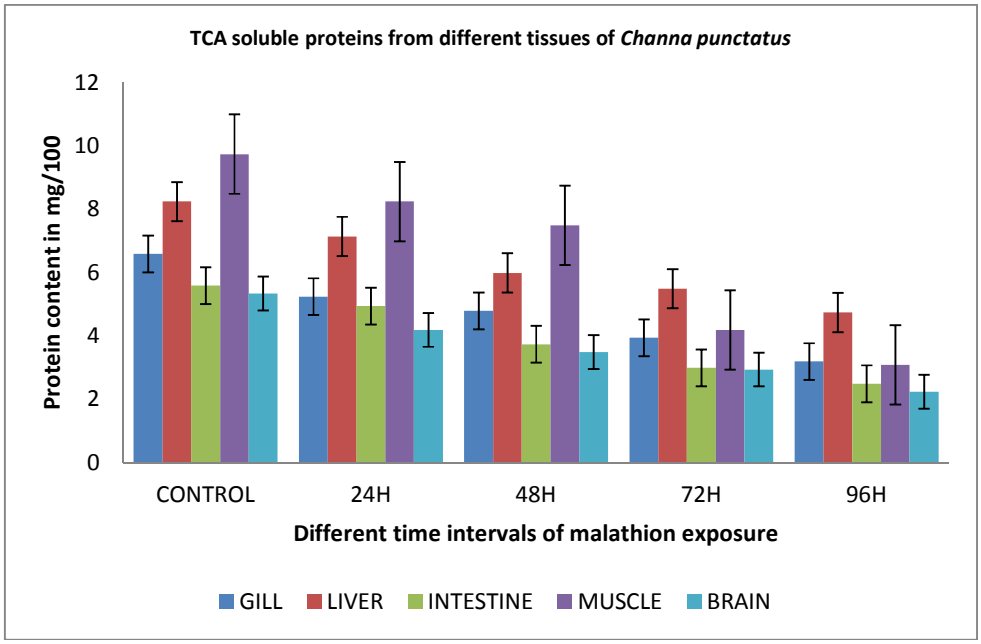

Fig. 1. TCA soluble proteins in various tissues of *C. punctatus*

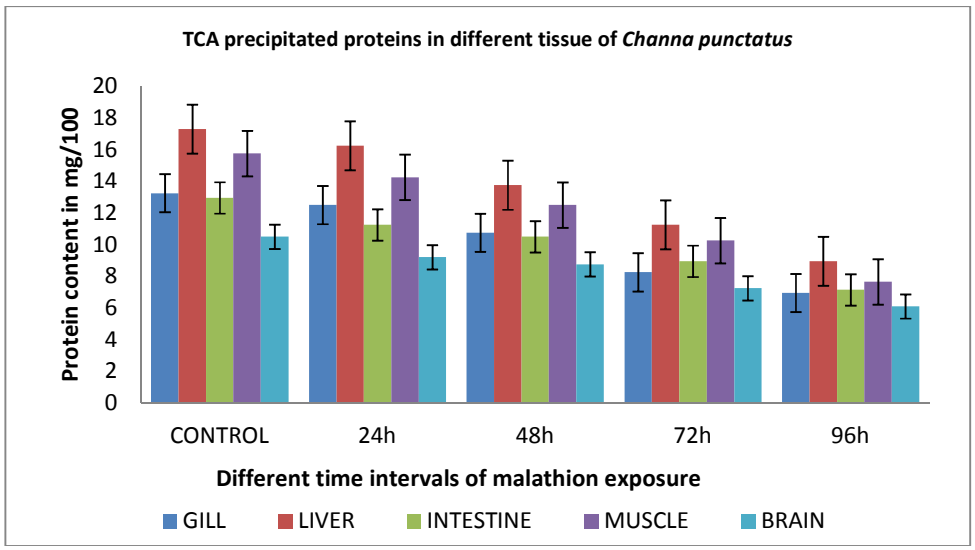

Fig. 2. TCA precipitated proteins in various tissues of *C. punctatus*

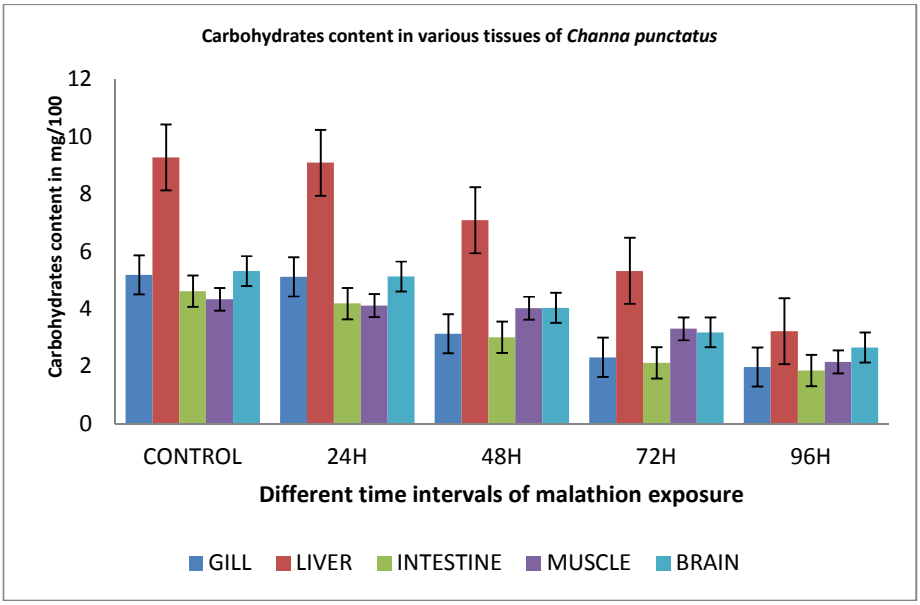

Fig. 3. Carbohydrate content in various tissues of *C. punctatus*

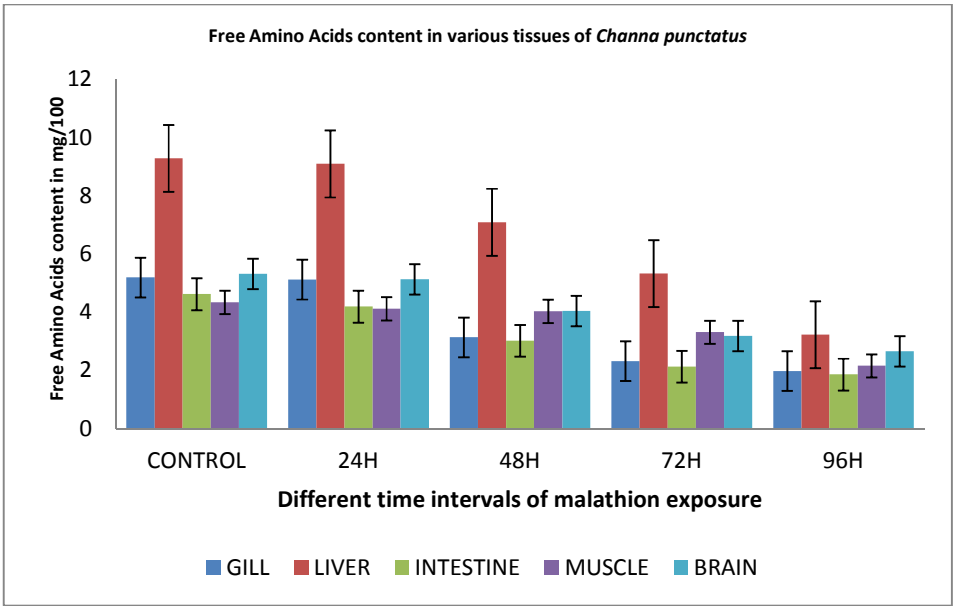

Fig. 4. Nynhydrine positive substances in various tissues of *C. punctatus*

4. DISCUSSION

Organophosphate pesticides have broad spectrum of harmful effects in the living world [31]. The pesticide Malathion (an organophosphate) is being extensively used as dust, emulsion and vapour to control wide variety of insect pests under different conditions and reported to have a low toxicity to mammals and relatively high toxicity to fish [32]. It ultimately finds a way into aquatic habitats such as runoff from agriculture lands, rivers, lakes and ponds [33-35].

Proteins are mainly involved in the architecture of cell [36-39,17]. During chronic exposure period of stress they are also a source of energy [40, 41]. During stress conditions fish need more energy to detoxify the toxicant and hence decline in protein constituent occurs in different fish tissues exposed to sub lethal

concentration of pesticides and insecticide in different tissues [39-40].

In the present study, Malathion caused a significant decrease in total structural, soluble protein content in various tissues of fish *C. punctatus* i.e. gill, liver, intestine, muscle and brain and were found to be declined during the exposure period of different time intervals i.e. 24, 48, 72 and 96hr. The Malathion toxicity causes metabolic dysfunction in fish [41-47].

The carbohydrates are less sensitive as compared to proteins towards the OP compounds. The results showed a significant decrease in the carbohydrate content in all the tissues of fish when exposed to Malathion. There was a significant decrease in the content of free amino acids/ ninhydrine positive substances in all the tissues on Malathion exposure as also reported by[17,48,49] in *Heteropneustes fossilis*. Thus extensive use of pesticides may accumulate and can cause major damage to the tissues of fishes.

5. CONCLUSION

From the present study we report that the sub lethal exposure of Malathion pesticide proved to be moderately toxic to fish *C. punctatus* as it effected the content of total proteins (soluble and structural), carbohydrates and free amino acids/ ninhydrine positive substances in vital tissues of gill, liver, intestine, muscle and brain which play major role in fish metabolism. Therefore, it can be concluded that the use of pesticides must be minimized otherwise they may pose serious threats to the aquatic flora and fauna as well as human life through the food chain.

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DISCLAIMER

Authors have declared that no competing interests exist. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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