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EFFECTS OF METHYL PARATHION ON BIOCHEMICAL CONSTITUENS IN DIFFERENT TISSUES OF FRESHWATER FISH, *H. molitrix*

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Abstract: Toxicity is the inherent property of a chemical molecule to produce injury on reaching a susceptible site on or in an organism. Today not only the animals and plants but Human beings are also continually being exposed to various chemical pollutants of the environment. Pesticides cause acute toxicity to a broad spectrum of aquatic organisms and thus it is imperative to understand its ecotoxicological implications. The Silver carp were exposed to three different concentrations of (1, 5 and 10 ppm) of the pesticide, Methyl parathion (which is an organophosphate insecticide) for four days. An attempt was made to interpret the impact of this Pesticide on the biochemical constituents of the various tissues of Hypophthalmichthys molitrix (Silver carp). A significant decline was observed in the total protein, carbohydrate and lipid contents of the different tissues of the fish, H. molitrix under the influence of methyl parathion toxicity.

Index Terms **- Pesticides, Methyl parathion, Biochemical parameters, Tissues, Silver carp, H. molitrix.**

I. INTRODUCTION

For the existence of all forms of life water is required which is available as only 2.5% of fresh water for the man's usage. Man has polluted much of this limited quantity of water with sewage, industrial wastes and wide array of synthetic chemicals, a recent analysis suggests that 70% of all available water in India is polluted (Chavan et al., 2005 and Mazher Sultana and Ganesan, 2012). Since the industrial revolution, industries have been booming, consequently millions of anthropogenic compounds have entered our environment. Persistent organic pollutants have been found even in remote areas of the world (Ballschmiter, et al., 2002). The untreated effluents released from the industries are primarily responsible for the water pollution problems. The Indian sector is ranked as the tenth biggest in the world in gross industrial output.

Toxicity is the inherent property of a chemical molecule to produce injury on reaching a susceptible site on or in an organism. Human beings, animals and plants are continually being exposed to various chemical pollutants in the environment. Whereas the toxicity of a chemical to plants, small animals, fishes or wildlife can be evaluated simply by exposing a small group of them under laboratory conditions to various levels of pollution, such an evaluation cannot ethically performed in human beings.

Biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed organisms. Toxicant affects biologically active molecules such as carbohydrates, proteins and lipids content of different organs in the fish O. mossambicus (Saradhamani and Binu Kumari, 2011). One of the advantages of using biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs including gill, liver, kidney, testes and ovary that are responsible for vital functions, such as detoxification, and reproduction.

Likewise, accumulation and biotransformation of xenobiotics in fish can also be ascertained through this carbohydrate, protein, lipid metabolism of fishes, when disturbed under the condition of toxic stress (Shaffi, 1978 and Hewitt et al., 2006).

Since insecticidal toxicity is harmful to physiological activities of the aquatic organisms like fishes, it is essential to investigate the effects of Methyl parathion on biochemical constituents. Therefore, to understand the toxic effect of Methyl parathion on Total proteins, carbohydrates and lipid contents of different tissues of the indigenous freshwater fish Hypophthalmichthys molitrix, a detailed analysis was undertaken and comprehended in the present study.

II. MATERIALS AND METHODS

Healthy H. molitrix, of both sexes with relatively same size ranging from (10–15 cm) and weight about (45–50 gm), oxygen packed in polythene bags were collected from culture ponds of Bharath Fish Farm, Poondi, Thiruvallur district, Tamil Nadu; were brought to the laboratory with minimal stress and released very carefully into the fish tanks half filled with bore well water. They were maintained in the stocking tank and acclimatized before experimentation.

The fishes were fed daily with pelleted feed at 5% body weight, twice (morning and evening) prepared by sieved rice bran, pounded groundnut oil cake, tapioca powder and mineral mixture. The feeding was stopped one day prior to experiment.

Maintenance of Fish

The fishes were maintained in the aquarium tanks of size 1'l x 2'b x 1'h throughout the period of study. Potassium permanganate (0.02%) was used as disinfectant to clean the tanks before and after experiments. The tanks were filled with water (2 litres per fish) and covered with mesh cloth to prevent the mosquitoes breeding in the water and also to prevent the fishes from jumping out of tank. During the period of study the room temperature fluctuated from 29˚C to 32˚C. The dissolved oxygen content of water used for the study was 4.8 to 5.4 ml / litre and salinity of 0.82 - 0.85 ppm. The pH of water was in the range of 7.2 - 7.4. Fishes were exposed to concentrations of 1, 5 and 10 ppm of Methyl parathion for 12, 24, 48 and 96 hrs. (Ashaduzzaman et al., 2016). Temperature, dissolved oxygen, conductivity and pH were measured during the experiment (APHA, 2005).

For these three treatment groups including control group and experimental group were subjected to test of toxicity. Each treatment was replicated thrice with 10 fish per tank with 60 L water capacity. Fishes were exposed to Methyl parathion at a nominal concentration of 1, 5 and 10 ppm for four days and toxicity tests were carried out. (Ashaduzzaman, 2016). Fish deaths were saved (24, 48, 72 and 96 hrs.) after the beginning and died fishes were taken instantly from the tank (Banaee et al., 2011).

Biochemical assay

After the test period, fishes were taken out of the water rapidly and held securely in a loom with a soft cloth covering the head for taking tissue samples (gills, muscle, liver, intestine and kidney).

Total Protein content of different tissues of the fish H. molitrix, exposed to Methyl parathion and control was determined by Lowry et al., method (1951). Similarly total carbohydrate content was determined by Selter et al. (1950).Total Lipid content was determined by Zlattis et al. (1953). Statistical analysis - The data obtained was subjected to mean ± SD and ANOVA for statistical significance.

III. RESULTS

BIOCHEMICAL CHANGES

The Total protein and standard deviation along with percent change over the control is tissues specific viz., gills, muscle, liver, intestine and kidney etc. of fish, H. molitrix affected by Methyl parathion and the calculated values were given in (Table – 1). All the tissues showed maximum decrease in total protein content in 10 ppm Methyl parathion treated fish (gills- 7.83 ± 1.204 mg/l, muscle-12.45 ± 3.002, liver- 6.16 ± 1.502, intestine -4.35 ± 0.036 and kidney -1.55 ± 0.008) as compared to control fish tissues, in gills (15.95 ±0.236 mg/l), muscle (23.58 ± 0.356), liver (14.98 ± 2.015), intestine (12.65 ±1.115) and kidney (7.25 ±0.208) respectively. Similarly in 5 ppm (10.15 ±0.549. 14.97 ± 0.016, 8.05 ±2.005, 6.72 ±0.205, 2.96 ± 0.075) and 1 ppm (12.25 ± 1.126, 17.32 ± 0.090, 10.62 ± 0. 108, 9.36 ± 0.421, 5.20 ± 0.250). Methyl parathion treated fish showed a significant decrease in the total protein content of the tissues as compared to control fish tissues.

Table 1: Changes in the Total protein content in different tissues of *H. molitrix* on exposure to Methyl parathion (1 ppm, 5 ppm and 10 ppm) after 96 hours and in control fish (Mean ±SD)

Tissue	Control	1 ppm	5 ppm	10 ppm
Gills Mean ± SD % Change	15. 95 ± 0.236	12.25 ± 1.126 23.20%	10.15 ± 0.549 36.36%	7.83 ± 1.204 50.90%
Muscle Mean ± SD % Change	23.58 ± 0.356	17.32 ± 0.090 26.55%	14.97 ± 0.016 36.54%	12.45 ± 3.002 47.20%
Liver Mean ± SD % Change	14.98 ± 2.015	10.62 ± 0. 108 29.16%	8.05 ± 2.005 46.26%	6.16 ± 1.502 58.87%
Intestine Mean ± SD % Change	12.65 ± 1.115	9.36 ± 0.421 26.01%	6.72 ± 0.205 46.88%	4.35 ± 0.036 89.33%
Kidney Mean ± SD % Change	7.25 ± 0.208	5.20 ± 0.250 28.27%	2.96 ± 0.075 59.17%	1.55 ± 0.008 78.62%

The values below the mean are percent changes over the Control values.

- Values are highly significant at p <0.005

The calculated values of total carbohydrate content and standard deviation value in the control and in affected tissues viz., gills, muscle, liver, intestine and kidney of fish, H. molitrix was given in the Table - 2. The carbohydrate content of the above tissues from the control fish was found to be as in Gill- 32.46 ± 0.140 mg/l, Muscle- 43.81 ±0.609 mg/l, Liver- 78.01±0.252 mg/l, Intestine- 34.63 ± 0.142 mg/l and Kidney- 8.71 ± 0.208 mg/l whereas the decreased total carbohydrate content of the affected fish was observed

to be as in Gills- 27.31 ± 0.208 mg/l , muscle- 38.12 ±0.201 mg/l, liver- 73.74 ± 0.244 mg/l, intestine - 32.92 ±1.072 mg/l, and kidney- 5.32 ±0.265 mg/l in 1 ppm concentration.

Table 2: Changes in the Total carbohydrate content in different tissues of *H. molitrix on exposure to* Methyl parathion (1 ppm, 5 ppm and 10 ppm) after 96 hours and in control fish (Mean ±SD)

Tissue	Control	1 ppm	5 ppm	10 ppm
Gills Mean ± SD % Change	32.46 ± 0.140	27.31 ± 0.208 15.87%	24.57 ± 0.234 24.31%	22.45 ± 0.250 30.85%
Muscle Mean ± SD % Change	43.81 ± 0.609	38.12 ± 0.201 12.99%	34.64 ± 1.729 20.93%	32.14 ± 0.741 26.64%
Liver Mean ± SD % Change	78.01 ± 0.252	73.74 ± 0.244 5.47%	70.65 ± 0.244 9.45%	65.33 ± 0.535 16.25%
Intestine Mean ± SD % Change	34.63 ± 0.142	32.92 ± 1.072 4.94%	26.69 ± 0.592 22.93%	22.21 ± 1.035 35.87%
Kidney Mean ± SD % Change	8.71 ± 0.208	5.32 ± 0.265 38.92%	3.29 ± 0.038 62.22%	1.78 ± 0.155 79.56%

The values below the mean are percent changes over the Control values.

- Values are highly significant at p <0.005

Whereas in 5 ppm Methyl parathion treated fish, H. molitrix, the decrease in total carbohydrate content was observed to be as 24.57 ±0.234 mg/l in Gills, 34.64 ±1.729 mg/l in muscle, 70.65 ± 0.244 mg/l in Liver, 26.69 ±0.592 mg/l in intestine, and 3.29 ± 0.038 mg/l in kidney respectively.

In 10 ppm Methyl parathion treated fish, H. molitrix the decrease was found in the tissues as gills- 22.45 ±0.250 mg/l, muscle - 32.14 ± 0.741 mg/l, liver - 65.33 ±0.535 mg/l, intestine- 22.21 ±1.035 mg/l, and Kidney-1.78 ± 0.155 mg/l as compared to total carbohydrate content of the control fish.

The total lipid content of gills, muscle, liver, intestine and kidney of control fish, H. molitrix was observed to be as 52.32 ±0.015 mg/l, 59.15 ±0.216 mg/l, 86.05 ± 0.073 mg/l, 31.75 ±0.046 mg/l and 13.68 ±0.543 mg/l, and for treated fish it was 48.05 ± 0.336 mg/l, 56.23 ± 0.365 mg/l, 77.44 ± 0.218 mg/l, 27.41 ± 0.352 mg/l and 10.52 ± 0.270 mg/l in1ppm; 43.55±0.154 mg/l, 49.81 ±1.053 mg/l, 72.29 ± 2.356mg/l, 24.59 ±0.083mg/l, 6.05 ±0.146mg/l, in 5ppm and mg/l, 32.03±0.072 mg/l, 42.18±1.035 mg/l, 65.52±2.016 mg/l, 22.24 ± 0.109 mg/l and 4.02±0.006 mg/l in 10 ppm Methyl parathion treated fish, H. molitrix tissues like gills, muscle, liver, intestine and kidney respectively. The decreased total lipid content of tissues in treated fish, H. molitrix was found to be very significant in all the tissues (Gill, Muscle, Liver, Intestine and Kidney) in the 10ppm (18.29 mg/l, 16.97 mg/l, 20.53 mg/l, 9.51 mg/l and 9.66 mg/l) Methyl parathion treated fish (Table 3).

Table 3: Changes in the Total lipid in content in different tissues of *H. molitrix on esposure to* Methyl parathion (1 ppm, 5 ppm and 10 ppm) after 96 hours and in control fish (Mean ±SD)

Tissue	Control	1 ppm	5 ppm	10 ppm
Gills Mean ± SD % Change	52.32 ± 0.015	48.05 ± 0.336 8.16%	43.55 ± 0.154 16.76%	32.03* ± 0.072 38.78%
Muscle Mean ± SD % Change	59.15 ± 0.216	56.23 ± 0.365 4.94%	49.81 ± 1.053 15.79%	42.18* ± 1.035 28.69%
Liver Mean ± SD % Change	86.05 ± 0.073	77.44 ± 0.218 10.01%	72.29 ± 2.356 15.99%	65.52* ± 2.016 23.86%
Intestine Mean ± SD % Change	31.75 ± 0.046	27.41 ± 0.352 13.67%	24.59 ± 0.083 22.55%	22.24* ± 0.109 29.95%
Kidney Mean ± SD % Change	13.68 ± 0.543	10.52 ± 0.270 23.10%	6.05 ± 0.146 55.78%	4.02* ± 0.006 70.40%

The values below the mean are percent changes over the Control values.

- Values are highly significant at p <0.005

The % change in the tissues were found to differ in 1 ppm, 5 ppm and 10 ppm methyl parathion treated fishes. The % change was significantly high in 10 ppm treated fishes than in 1 ppm, 5 ppm treated tissues of the fishes in the present study (Table 1, 2 and 3).

IV. DISCUSSION

Aquatic organisms may take up pollutants from sediments, water, suspended particle and food items. Multiple changes in the internal dynamics of the aquatic organism were found to be caused by introduction of small amount of many relative toxic materials. The concentration of individual and mixture of pollutants in water is considered hazardous to fish due to their ability to accumulate residues of toxicants in their tissues when exposed to concentration much lower than those which cause direct adverse effect (Sprague, 1971). The extent of pollution observed to limit the diversity of aquatic organisms and also pose a serious threat to the biotic life, especially fish. The use of biomarkers for pollution in fish is an effective strategy for monitoring the aquatic environment and diagnosing of negative impact (Lasheen et al., 2012).

According to Van der Oost et al. (2003) the aquatic contaminants causing biological changes in fish can be used to assess environmental risk, can be as called biomarkers. Saradhamani and Binu Kumari (2011) reported that biologically active molecules such as carbohydrates, proteins and lipids are affected by toxicants. The use of such biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed organisms.

The examination of specific target organs like, gill, liver, muscle, intestine and kidney that are responsible for vital functions, such as respiration, detoxification, metabolic activities and ultrafiltration can be used as biomarkers in environmental monitoring. Hewitt et al. (2006) indicated that the accumulation and biotransformation of xenobiotics in fish can also be ascertained through the metabolism of carbohydrate, protein, lipid in fishes disturbed under the condition of toxic stress. The liver can be considered a target organ and of great importance to fish, since it participates in processes such as the biotransformation and excretion of xenobiotics. Therefore, the liver can be studied in environmental monitoring due to its high sensitivity to contaminants (Thophon et al., 2003).

Nimaichandra et al., (2005) reported decrease in the glucose content of the liver, muscle and kidney tissue of Clarias batrachus on exposure to sodium arsenite in their studies. Similarly Neethirajan and Madhavan (2004) and Kale et al. (2010) observed a decrease in carbohydrate content of liver, muscle, and gill tissue of Mystus vittatus and C. mrigala on exposure to sumidon and sodium fluoride after acute and chronic toxicity. The present study also observed a decline in the glucose content of the gill, liver, muscle and intestine and kidney tissue of H. molitrix under the toxicity of methyl parathion. This decrease in protein indicates its metabolic utilization under methyl parathion toxic stress.

Ananth and Mathivanan (2013) in their studies on the fish, C. idella observed that there is a drastic change in the protein content of liver, kidney and brain when exposed to the heavy metal arsenic trioxide. Similarly Verma et al. (2005) also reported increased damages caused by the heavy metal accumulation in flora and fauna of aquatic system and found a drastic change in the biochemical constituents of M. dayanum under the influence of heavy metal toxicity.

Ali Alkaladi et al. (2015) reported in their studies that the Serum total protein and albumin levels were significantly decreased when O. niloticus (groups 2 and 3) was treated with ZnONPs only. But on addition of vitamin E and C to the diet of fish (groups 4 and 5) significantly improved all measured parameters as compared with groups (2 and 3) which was treated with ZnONPs only.

Tripathi and Yadav (2015) in their studies exposed Labeo rohita to sublethal concentration of Phenthoate and reported decline in total protein and glycogen in the tissues like, liver, muscle, kidney brain, gills and gut respectively. The present study also found a significant decrease in the total carbohydrate content of different tissues of the fish H. molitrix.

Shinde et al. (2002) indicated that the reduction protein content might be due to the impaired or low protein synthesis under the toxic stress condition and enhancement of photolytic activity in the organism, which is also supported by the studies of Reddy et al. (2011) in the freshwater Teleosts, Clarius batraus on exposure to sublethal concentration of chlorpyrifos. They observed the decline in total protein, amino acid and ammonia contents in gills, kidney, liver and muscle tissues. Nafisa et al. (2012) also reported high sensitivity of juvenile Killifish on exposure to very low i.e., 0.0025 ppm of chlorpyrifos and 0.039 ppm of methyl parathion.

Binukumari et al. (2016) reported a significant decrease in protein, carbohydrate and lipid in Labeo rohita when exposed to monocrotophos for 96 hrs. They stated that the assessment of ecological risk is essential to monitor the environmental pollution.

Fishes use glycogen, the storage form of carbohydrates for their immediate energy requirement during stress (Vutukuru, 2005). Liver and muscle are the two active sites where storage and metabolism of carbohydrate reserves take place. Generally, depletion in carbohydrates content is directly proportional to the exposure period of the toxicant.

In the present investigation, total sugar content in the gills, liver, muscle, intestine and kidney revealed a mixed trend in the different experimental groups at the different exposure. Similar observations of mixed trend in the sugar content were reported in the testes, liver, brain and kidney of Catla catla treated with heavy metal and fungicide (Sujatha, 2006). There was a decreased level of sugar content of the exposed fish in the muscle, liver, brain and kidney showing a state of recovery.

The lipid depletion may be caused by the stress condition in fishes due to the deposition of toxicants, fat is being utilized by the body which may leads to less cholesterol content in freshwater fishes (Gayathri and Mazher Sultana, 2010; Dheenadayalamurthy and Mazher Sultana, 2013).

Several researchers reported decreased total protein, glycogen and lipid in different tissues of fishes treated with malathion in Clarius batrachus (Kajare et al., 2000); carbaryl in Oreochromis niloticus (Matos et al., 2007); chlorpyrifos in Labeo rohita (Srinivasa et al., 2010 and chlorpyrifos in Channa bachua Padmini and Rajaram, 2016) respectively. The present results was observed to be in consistent with the studies of the above researchers.

V. CONCLUSION

H. molitrix was served as an important biological indicator of water quality, which is affected by the global environmental degradation. This fish serve as experimental model and hypotheses deduced from study on them can be extrapolated to human system to a certain extent. The Silver carp were exposed to three different concentrations of (1, 5 and 10 ppm) of the pesticide, Methyl parathion (which is an organophosphate insecticide) for four days. An attempt was made to interpret the impact of this Pesticide on the biochemical constituents of the various tissues of Hypophthalmichthys molitrix (Silver carp). A significant decline was observed in the total protein, carbohydrate and lipid contents of the different tissues of the fish, H. molitrix under the influence of methyl parathion toxicity. Further fishes form a major link in the food web of the aquatic ecosystem and their ability to produce large number of offspring and survive at high population densities, make them suitable for experimentation.

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