



RESEARCH ARTICLE

STUDIES ON THE TOXICITY OF METHYL PARATHION ON FRESH WATER FISH  
*OREOCHROMIS MOSSAMBICUS*

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ABSTRACT

Studies were made to evaluate the effect of sub lethal concentration of methyl parathion on biochemical changes of *Oreochromis mossambicus*. The fishes were caught from culture pond of Vallam fish farm near Thanjavur and acclimatized to the laboratory conditions. Experiments were conducted at Serfoji college lab. LC<sub>50</sub> values for 96 hours were determined for methyl parathion and were found to be 0.29 ppm. The fishes were exposed to 10% sub lethal concentrations of the 96 hours LC<sub>50</sub> value for 7, 14 and 21 days along with control. The changes in carbohydrate, protein and lipid content of ovary observed at different exposure periods. Significant depletion of biochemical constituents were observed in 21 days of exposure.

INTRODUCTION

Waste water from the textile industry is a complex mixture of many polluting substances ranging from organochlorine-based pesticides to heavy metals associated with dyes and the dyeing process (Barot and Bahadur, 2013). Inefficiencies in dyeing result in discharge of large amounts of the dyestuff and contamination of surface water with these effluents from the textile-dye and dyestuff industry represent a serious ecological problem (AbdelMoneim *et al.*, 2008). During textile processing, inefficiencies in dyeing result in large amounts of the dyestuff (varying from 2% loss when using basic dyes to a 50% loss when certain reactive dyes used) is being directly lost to the wastewater, which ultimately finds its way into the environment. Among all the chemical classes of dyes, azo dyes are considered to be recalcitrant, nonbiodegradable and persistent (Saratale *et al.*, 2011). Moreover, azo dyes as well as their breakdown products are cytotoxic or carcinogenic (Khehra *et al.*, 2006). Pollution due to pesticides needs considerable attention because of a toxicant, which lacks the capacity to dramatically increase the rate of mortality in exposed population, may still cause their ecological death after a long time of exposure, probably as a result of the cumulative effect of impaired metabolic functions (Ghosh and Shrotri, 1992). Such disrupted activities may also occur in non-target organisms.

These activities lead to changes in the composition of different tissues, which may serve as sensitive criteria on the effect of chronic toxicity of the pesticides (Colianese and Neff, 1982). Aquatic toxicologists assess the physiological aspects of fishes living in polluted waters (Gingerich and Weber, 1979). In India, *Oreochromis mossambicus* is found mostly in fresh water rivers, lakes and ponds, Tilapia (Pisces: Cichlidae) are among the most widely distributed exotic fishes in the world (Cononico *et al.*, 2005). Jubb (1961) recorded that the mean temperature for *Tilapia mossambicus* in its natural habitat was 25°C, with a temperature range of 21°C to 27°C.

In the present investigation to assess the Oxygen conception of *Oreochromis mossambicus* under different temperature, the results of the study are expected to help the oxygen consumption required for different size of fishes at different water temperature. Although there are considerable research activities in the field of pesticides, there is a wide variation in the amount of information available concerning the effect of particular pesticide on selected non-target organism. Changes in the protein level has been established in the fish *O. mossambicus* (Ganesan, *et al.*, 1989) that showed a steady decline in the liver protein of the fish treated with Endosulfan and was directly proportional to the period of exposure. They have reported an intensive proteolysis in the fish under the influence of the pesticide. The total protein in muscle and liver of *Channa punctatus* decreased after the period of exposure to Nuvacron (Sastry and Dasgupta, 1991). Protein which serves as

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an energy source during stress condition showed a decreasing trend in gill, liver, muscle and brain tissues of the *O. mossambicus* at the end of 96 hrs (Durairaj and Selvarajan, 1992). Tilak *et al.* (2003) Suggested that the decrease in the glycogen and total proteins in *C. punctatus* might be due to the stress when exposed to the pesticide fenvalerate. Jayantha Rao *et al.* (1987) observed a decline in protein content of renal tissue on exposure of fresh water teleost *T. mossambica* to heptachlor. Similarly Thenmozhi and Ramasamy (2006) noted a steady depletion of protein of Indian skipper frog *Rana cyanophlyctis* exposure to chronic phosphamidon. The decrease in protein synthesis is correlated to the decrease in amino acid incorporation and disaggregation of polysomes (Holbrook, 1980). Another biochemical component in aquatic organism that widely varies in relation to the impact of pesticides in carbohydrate. The tissue level carbohydrates represent the immediately available energy source at times of stress. The changes in the concentration of glycogen, protein and sugars may imply the organism and its different tissues. Depletion of total carbohydrate in muscle and liver tissues of *C. punctatus* due to penthoate was reported by Sambasiva *et al.* (1984), Ramalingam and Ramalingam (1982) noticed changes of carbohydrate metabolism on exposure to different kinds of toxicants viz., DDT, malathion and mercuric chloride in *S. mossambicus*. A significant decrease in total carbohydrate and glycogen in various tissues of air breathing fish *Anabas scandens* exposed to endosulfan was reported by Yasmeen *et al.* (1991).

Lipid, the storage reserve, is another vital biochemical component that gets varied under the influence of a number of limiting factors, one among them being the role of pesticides. Reduction in fat in *Dactus cucurbitae* treated with oxytetracycline and sulphanimide has been reported by Chinnarajan, (1972). Work pertaining to pesticide impact on lipids of non target organisms is rather meager. Decreasing total lipid under the stress of pesticide reflect on their immediate utilization to meet the energy demands (Harper *et al.*, 1977). Pesticides inhibit the metabolism of steroids. The increasing cholesterol is due to its non-utilization for the synthesis of steroid hormones causing accumulation in the tissue of a teleost fish (Kupfer, 1969). Due to the decrease in total lipid content under the stress of Methyl Parathion, the free fatty acids showed an increase in the tissues of fishes (Lehninger, 1978). Fluctuation in lipid content between muscle and liver is apparent in *Barbus chonchonius* exposed to Aldicarb for 15 and 30 days (Pant *et al.*, 1987). Murthy and Devi (1982) have also reported the changes in lipid and cholesterol in the fish *C. punctatus* under endosulfan exposure. Pesticides may be categorized as insecticides, herbicides defoliants, fungicides and odenticide (Benson, 1969). Pesticides vary in their chemical formulations as well as toxicity, environmental persistence and pathways of action. Among the pesticide, chlorinated hydrocarbons, organo-phosphates and carbamates are commonly used. The presence of pesticides in the aquatic system can obviously lead to multi-fold interaction with other forms of pollution. In India, scores of studies have been undertaken to estimate the acute toxicity level of various pesticides on aquatic fauna (Basak and Konar, 1977; Arora *et al.*, 1971; Sharma *et al.*, 1979 and Varma *et al.*, 1981). Sprague (1973) defined the acute toxicity as "stimulus severe enough to bring about response speedily usually within four days for fish". Though the acute toxicity tests, many workers emphasize more on sublethal toxicity than on acute toxicity.

The LC<sub>50</sub> data with reference to different formulations of pesticides on different species.

## MATERIALS AND METHODS

### Animal collection and maintenance

*Oreochromis mossambicus* (Order: Perciformes and Family: Cichlidae), selected for the present study, was collected from a vallam fish farm near Thanjavur District, India. Healthy fishes of comparable body weight ( $8 \pm 1.04$  g) and length ( $12 \pm 3.55$  cm) were selected for the study. The fishes were treated with 0.05% KMnO<sub>4</sub> solution for 2 min to clear any external infection. They were then transferred to 200 l capacity glass tanks filled with dechlorinated water, one week prior to the initiation of the experiment for acclimatization to laboratory conditions. A minimum of four fishes were introduced in each tank. The tanks were provided with continuous aeration and were maintained under normal day-night light duration. Feeding was carried out with oilcake during acclimatisation and stopped 24 h prior to experimentation.

The water was exchanged after every 24 h. Every effort was made to provide healthy conditions for fish and no mortality occurred during this period. Mercuric chloride (HgCl<sub>2</sub>) stock solution (1 mg ml<sup>-1</sup>) was prepared by dissolving analytical grade HgCl<sub>2</sub> in double distilled water. Test concentrations were prepared by diluting appropriate aliquots of the stock solution. The 96 h LC<sub>50</sub> tests were conducted to measure susceptibility and survival potential of fishes to HgCl<sub>2</sub>. The 96 h LC<sub>50</sub> of mercuric chloride was determined following the graphical method of Krouwer and Monti (1995) and confirmed using regression analysis. For this purpose, a preliminary bioassay was performed using HgCl<sub>2</sub> were selected in the experiment based on the 96h LC<sub>50</sub> value. A control group was also maintained without mercury exposure during the experimental period of 21 days. Twenty fishes per test concentrations were maintained and a minimum of 10 replicates were taken for each parameter. The data were analysed statistically using student's 't' test (Bailey, 1981).

### Bioassay test

Toxicity study was carried out by following the standard guidelines (APHA, 2005) to determine the lethal (LC<sub>50</sub>) level of chlorpyrifos using static renewal method. Ten fish each were accommodated in 45 liters of test solution in the aquarium. The experiment was conducted in triplicate. Dead fishes were removed immediately from the test medium to avoid deterioration. Three set of replicates were performed for each concentration. The 96 hr LC<sub>50</sub> value of the mortality in each exposure concentration of chlorpyrifos were recorded and tested by probit analysis program as described by (Finney, 1971). The total carbohydrate content was estimated by the technique of Roe (1955). Protein was estimated by the method of Lowry *et al.* (1951). The total lipids were extracted by the method of Folch *et al.* (1957) and quantitatively estimated following the procedure of Barnes and Blackstock (1973).

### Statistical analysis

The data collected on the different parameters of the control and experimental study were subjected to statistical analysis by using statistical software SPSS version 6.0. The statistical significance was tested at Standard Deviation.

## RESULTS AND DISCUSSION

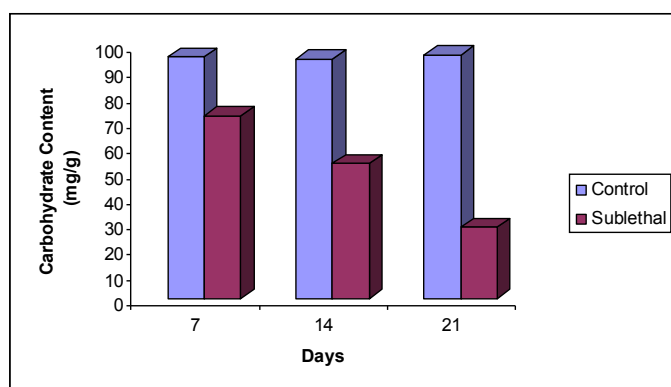
The total carbohydrate content of the ovary in control and experimental fishes are shown in Fig. 1 and Table 1. Exposure

of *O. mossambicus* to the pesticide reduced the level of carbohydrate in the ovary at all days of exposure. The percentage decreased over the control were -27.90 %, -57.11 % and -74.07 % respectively at 7, 14 and 21 days of exposure.

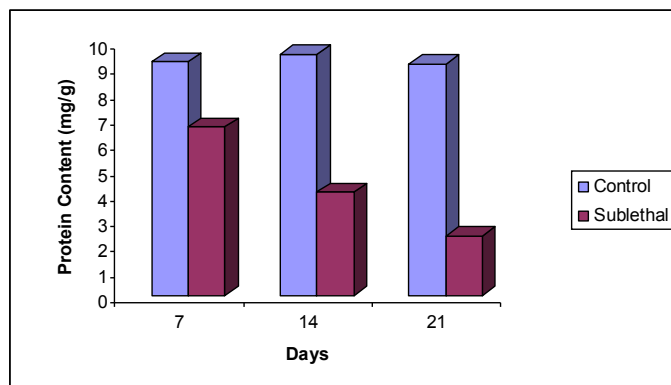
**Table 1. Biochemical changes in the ovary in response to prolonged exposure to methyl parathion**

Parameter	Experimental Group	Duration of Treatment (Days)		
		7	14	21
Carbohydrate	Control	9.21 ± 0.13	9.49 ± 0.68	9.14 ± 0.56
	Treated	6.64 ± 0.58	4.07 ± 0.22	2.37 ± 0.22
Protein	Control	95.42 ± 1.32	94.72 ± 1.52	96.31 ± 1.17
	Treated	72.14 ± 1.65	53.24 ± 1.19	28.16 ± 1.67
Lipid	Control	83.19 ± 0.70	82.89 ± 0.87	93.47 ± 0.76
	Treated	72.45 ± 0.92	59.18 ± 0.75	32.62 ± 1.05

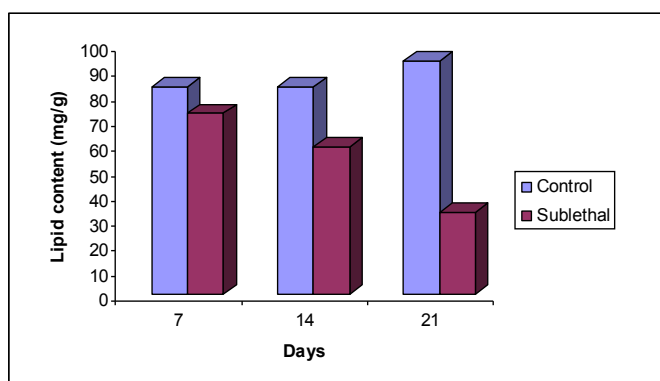
Values are the Mean ± SD of fish in each group



**Fig. 1. Carbohydrate level in the Ovary of *Oreochromis mossambicus* on the exposure to sub lethal concentration of methyl parathion**



**Fig. 2. Protein level in the Ovary of *Oreochromis mossambicus* on the exposure to sub lethal concentration of methyl parathion**



**Fig. 3. Lipid level in the Ovary of *Oreochromis mossambicus* on the exposure to sub lethal concentration of methyl parathion**

The carbohydrate of fishes comprised mainly glycogen and total free sugars and the fluctuations in the carbohydrate content may be due to accumulation and utilization of glycogen and total free sugars at different stages like gametogenesis and spawning. In fishes, generally the carbohydrate reserves may be rapidly utilized under unfavorable conditions and the great variation found in the tissues indicates that the level of mobilizable carbohydrate reserves may fluctuate widely and rapidly in response to fluctuations in the nutritional state of the animal. In the present study the percentage of carbohydrate content decreased in the ovary tissues of fishes exposed to sublethal concentrations of pesticides (Fig. 1). Tilak and Yacubu (2002) have observed that the fenvalerate exposed *Ctenopharyngodon idellus* showed a decrease in the carbohydrate content in the various tissues. The decrease in total carbohydrate level signifies its utility possibly to meet the higher energy demands of the fish reeling under pesticide toxicity. The synthesis and utilization of carbohydrate are therefore, altered in the organism subjected to pesticide stress.

Carbohydrates which supply the major portion of the metabolites for the energy requirements in a normal individual is oxidized for the energy requisites. Carbohydrates may be converted to glycogen or shunted in the metabolic pathway to supply the carbon chain for amino acids or converted into fat. At sub lethal concentration when the liver carbohydrate content decreased blood sugar level increased which suggests the breakdown of liver glycogen (glycogenolysis). The mobilization of glucose from the liver to the blood and its availability for utilization by the needy tissues for ensuring normal metabolic processes in the body appears inevitable when the fish is exposed to toxic medium. Muley *et al.* (2007) observed a fall in muscle carbohydrate level in *L. rohita* when exposed to Tannery, Electroplating and Textile effluents. Sastry and Dasgupta (1991) has shown that a high concentration of Nuvacon caused a decline in muscle carbohydrate level in *C. punctatus*. These observations are in conformity with the reports on the fall in muscle glycogen level in *C. punctatus*, when exposed to organophosphate pesticide, Dimethoate (Tripathi *et al.*, 2003). Studies in general have suggested that exposure to pesticide treatment interferences with the carbohydrate metabolism. A greater decrease of carbohydrate content indicates greater utilization of carbohydrate to cope with enhanced metabolism under stressful situation. Despite a continuous and rapid release of glucose by glycogenolysis in the liver, to meet the energy requirement for the increased muscular activity, a fall in the overall in fishes subjected to pesticide treatment. When the fish was exposed to a sublethal concentration for a period of 21 days, the protein content

decreased. The percentage over control after 7, 14 and 21 days of exposure were -24.39%, - 43.79% and -70.76% at sublethal concentration, which is found to be dependent upon duration of exposure (Fig.3). The lipid content decreased in the ovary at all the exposure periods, when the fish exposed to sublethal concentration (Fig. 4). The decreased of the lipid were -12.91 %, -28.60 % and - 60.92% after 7, 14 and 21 days of exposure. Proteins are mainly involved in the architecture of the cell. During chronic period of stress they are also a source of energy (Umminger, 1977). Behavioral responses of fish exposed to sub lethal concentrations of pesticides showed that they were under stress condition; fish needed more energy to detoxify the toxicants and to overcome stress. Since fish have a very little amount of carbohydrates, the next alternative source of energy is protein to meet the increased energy demand. The depletion of protein content in liver, muscle and gill tissues may have been due to their degradation and possible utilization of degraded products for metabolic purposes. Other workers such as Malla Reddy and Basha Mohideen (1988) have also reported decline in protein constituent in different fish tissues exposed to sub lethal concentrations of insecticides.

Protein is the most important constituent in living tissues, which is of considerable metabolic and structural value. Therefore, any change in this constituent indicates the stress inflicted on the metabolic functions required for maintaining a healthy physiological state. In this work the protein content of *O.mossambicus* at sub lethal concentrations decreased in all exposure periods. (Fig. 2 and Fig. 3). The depletion in tissue protein of *O.mossambicus* indicates rapid utilization of energy stores to meet the energy demands warranted by the environment. The observed depletion in tissue protein on treatment with sublethal doses of pesticides are suggestive of proteolytic activity, possibly to meet the excess energy demands under toxic conditions. Jeba kumar *et al.* (1990) recorded decrease in protein content of *Lepidocephalichthys thermalis* exposed to sub lethal concentrations of fenvalerate. A significant decrease was reported in the protein content of the liver and kidney in *L. rohita*, when exposed to 20% active ingredient EC. Fenvalerate (Annamani, 1986). A similar decrease in the total and soluble protein content was observed with fenvalerate in fish (Malla Reddy and Basha Mohideen 1988; Radaiah, 1988 and Tilak and Yacobu, 2002). Radaiah *et al.* (1988) noted decrease in the kidney of *T. mossambica* exposed to heptachlor. Manoharan and Sibbiah (1982) also noted decrease in total protein content of *Barbus stigma* exposed to endosulfan. The investigations of Koundinya and Ramamurthy (1978) revealed a decrease in protein content in *T. mossambica* exposed to different pesticides. Sastry and Siddique (1984) reported that the protein content was decreased in liver, muscle, kidney, intestine, brain and gill when *C. punctatus* was treated with quinaphos. Yeragi *et al.* (2000) observed the decreased levels of proteins in gills, testis, ovaries and muscles of marine crab *Uca marionis* exposed to acute and chronic levels of Malathion. Aruna Khare *et al.* (2000) observed that the sublethal concentrations of malathion showed a significant increase in the protein content in kidney of exposed fish during the first week and there after a gradual decrease in protein content was observed in the later periods of exposure.

### Conclusion

Long term exposure of organisms to pesticides means a continuous health hazard for the population. So, human population is at high risk by consuming these toxicated fishes.

This implies that one should take the necessary precaution in the application of pesticides to protect the life of fish and other aquatic fauna. It is likely that approaches using molecular biology techniques will revolutionize toxicological applications that are cheaper and do not require the use of animals to detect environmental stressors. Pesticide toxicity in fish has been studied by several workers who have shown that at chronic level, it causes diverse effects including behavior (Respiratory activity) and biochemical changes. With reports of toxicants usage and its adverse effects on non-target organisms like fish, it has become essential to formulate stringent rules against indiscriminate use of this pesticide. Since pesticide is present in the environment with other similar organophosphate compounds, additive responses to organophosphate compounds may induce lethal or sublethal effects in fish. It is, therefore, a matter of great public health significance to regularly monitor the pesticide residues in foods and humans in order to assess the population exposure to this pesticide. Besides, for a safe use of this pesticides more experimental work should be performed to determine the concentration and time of exposure that do not induce significant sub-lethal effects on fish.

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