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Full Length Research Paper

Biochemical changes induced by dimethoate (Rogor 30% EC) in the gills of fresh water fish *Puntius ticto* (Hamilton)

Ganeshwade R. M.

Padmabhushan, Dr. Vasantraodada Patil Mahavidyalaya, Tasgaon, District Sangli, Pin. 416312, India.
E-mail: rmganeshwade@gmail.com.

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The freshwater fish *Puntius ticto* was exposed to lethal (5.012 ppm) and sublethal (2.506 and 1.253 ppm) concentrations of dimethoate for 96 h and 60 days, respectively. Biochemical changes in the gills were analyzed after exposure period. Exposure of fish to lethal and two sublethal concentration of dimethoate showed decreased in the protein content in the gills after 60 days exposure. As compared to lethal exposure, significant change in the protein content was observed during the sublethal exposure. Glycogen, cholesterol and ascorbic acid contents in the gills also decreased after exposure.

Key words: Lethal and sublethal toxicity, dimethoate, *Puntius ticto*, biochemical changes.

INTRODUCTION

The rapid industrialization and green revolution introduced a large variety of chemicals into the environment. These chemicals create serious ecological problems particularly water pollution or aquatic pollution. Among these chemicals, pesticides are an integral part of present agricultural technology. These pesticides are injurious to non target organisms like fish. Fishes are very sensitive to a wide variety of toxicants in water; various species of fish shows uptake and accumulation of many contaminants or toxicants such as pesticides (Herger et.al., 1995). Accumulation of pesticides in tissues produces many physiological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma and Ramamurthi, 1982).

The alteration in biochemical contents in different tissues of fish due to toxic effects of different heavy metals and pesticides have been reported by number of workers (Gupta et al., 1987; Khan et al., 1992; James and Sampath, 1995; Das et al., 1999; Khare and Singh, 2002; Sobha et al., 2007; Hadi et al., 2009).

Organophosphorous pesticides are most preferred because of their low persistence in the environment as economically useful pesticide by agriculturists to eradicate insect pests (Raman et al., 1983). Dimethoate is one of the organophosphorous insecticide widely used against vegetables and fruit sucking aphids, mites, saw flies and boring insects on cereals, cotton, chili, tobacco and oil seeds. During rainy season along with running water, dimethoate insecticide enters the freshwater resources and results into aquatic pollution. Pesticides are also well known for causing more toxic effects in teleost (Scott, 1967; Jackson, 1968). The toxic effects may include both lethal and sublethal, which may change the growth rate, development, reproduction, histopathology, biochemistry, biochemistry, physiology and behavior (Rand and Petrocelli, 1985). Hence, an attempt is made to study the effect of dimethoate on biochemical changes in the gills of freshwater fish *Puntius ticto,* which is an esteemed food fish consumed in fresh condition by poor local people.

MATERIALS AND METHODS

The freshwater fish *P. ticto* (Average size 70.3 mm ± 12.72 and 4125 mg average wt.) were collected from freshwater resources around Aurangabad City of Maharashtra, India, and were acclimatized in aged dechlorinated and well aerated water for 2 weeks. The 12 h photo period was maintained throughout the experimental work. Twenty (20) individuals were used for the

Table 1. Biochemical contents in *the gills of P. ticto* to dimethoate toxicity exposure.

Parameter	Control	Lethal (5.012 ppm)	% Change	Sublethal (2.506 ppm)	% Change	Sublethal (1.253 ppm)	% Change
Protein	12.6509 ± 0.2397	11.5017 ± 0.1769	-9.0839	9.1474*** ± 0.1158	-27.694	9.616*** ± 0.1158	-24.037
Glycogen	0.5379 ±0.0124	0.2084*** ± 0.0172	-61.26	0.0992*** ± 0.0595	-81.56	0.1270*** ± 0.0119	-76.39
Cholesterol	8.7999 ± 1.0476	5.9741*** ± 0.5238	- 32.14	4.2253** ± 0.3025	-5 1.98	5.4474** ± 0.5236	-38.09
Ascorbic acid	2.5762 ± 0.2045	0.5724*** ± 0.1022	-77.28	0.3271*** ± 0.2045	-87.30	0.7769*** ± 0.1022	-69.34

The values are expressed in mg/100 mg dry weight (mean standard division). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

experiments. During acclimatization, they were fed on alternate days with pieces of live earthworms and starved for 24 h prior to the experimentation. Commercial graded dimethoate 30% EC was used as the pesticide toxicant in this experiment. Fishes were divided in to four groups, with the first group serving as control and other groups as experimental groups. The lethal concentration (LC_{50}) values were calculated by Probit analysis method (Finney, 1971). The experimental groups were administered with a LC (5.012 ppm) and sublethal concentrations (2.506 and 1.253 ppm) daily for 96 h and 60 days, respectively. Fishes from each group were sacrificed immediately at the end of exposure period and according to Food and Agriculture Organization (FAO) methods (Dybem, 1983), gills are taken out. Gills were put in Petri dish to dry at 120°C until reaching a constant weight. Dry gills were powdered and used for biochemical estimations. Biochemical contents were analyzed by following standard methods. Protein content was estimated by Follin phenol reagent method (Lowry et al., 1951), glycogen was estimated by using Anthrone reagent method (De Zawaan and Zandee, 1972), cholesterol content was analyzed by the method described by Kolmer et al. (1969) and ascorbic acid by the method described by Roe (1967).

RESULTS

Exposure of fish to lethal and two sublethal concentrations of dimethoate showed decreased protein content in the gills (that is, -9.0839 to -24.037%) after the exposure period of 60 days. As compared to lethal exposure, significant change in the protein content was observed during sublethal exposure (chronic toxicity). Glycogen content was decreased after lethal and sublethal exposure (-76.39 to -81.56%). When compared the results of two sublethal concentration exposure, results shows that increase in glycogen content is dose dependent. Cholesterol content was also decreased after lethal (-32.14%) and sublethal exposure (-38.09 to -51.98%). Ascorbic content was also decreased significantly after lethal (-77.28%) and sublethal exposure (-69.34 to -87.30%). The results obtained in the present study are shown in Table 1.

DISCUSSION

The gill is an important organ because of its direct contact with water, which allows the pesticides to enter through it and get accumulated in the fish body. It has been suggested that water pollutants damage the fish gill, presumably by causing breakdown of the gas exchange mechanism with consequent tissue hypotoxic conditions (Khare and Singh, 2002).

The pollutants act as one kind of stress and organism respond by developing necessary potential occurring in body give first indication of stress. During stress, an organism needs sufficient energy which is supplied from reserve material that is, protein, glycogen, cholesterol and ascorbic acid (Ganeshwade, 2011).

Decrease in the protein content was observed throughout the exposure period. The two sublethal exposure results show the decrease in protein content and it depend upon the concentration. The toxicity of dimethoate also showed a direct correlation with the concentration and time exposure. Similarly, this was also observed by Singh and Bhati (1994) and Khare and Singh (2002). Decrease in protein may be due to the impairment of protein synthesis or increase in the rate of its degradation to amino acids, which may be fed to tricarboxylic acid (TCA) cycle through aminotransferases probably to cope up with high energy demands in order to meet the stress condition. The decrease in protein content suggests an increase in proteolytic activity and possible utilization of its products for metabolic purpose. The fall in protein level during exposure may be due to increased catabolism and decreased anabolism of proteins. Decrease in protein content under toxicity stress has already being reported (James et. al., 1979; Natarajan, 1983; Khare and Singh, 2002).

Borah and Yadav (1995) have reported gradual decrease in protein and glycogen of gill in *Heteropneustes fossilis* under dimethoate toxicity. James and Sampath (1995) observed sublethal effects of mixtures of copper and ammonia on biochemical parameters in *H. fossilis* and showed concentration dependent significant reduction of protein and glycogen content in gill, liver and muscle. Ghousia and Vijayaraghavan (1995) reported decrease in protein content of dimethoate intoxicated fish *Clarias batrachus* indicating the physiological adaptability of these fish to compensate for pesticide stress. To overcome the stress the animals require high energy; this energy demand might have led to the stimulation of protein metabolism. Das et al. (1999) studied the effect of cypermethrin 25%

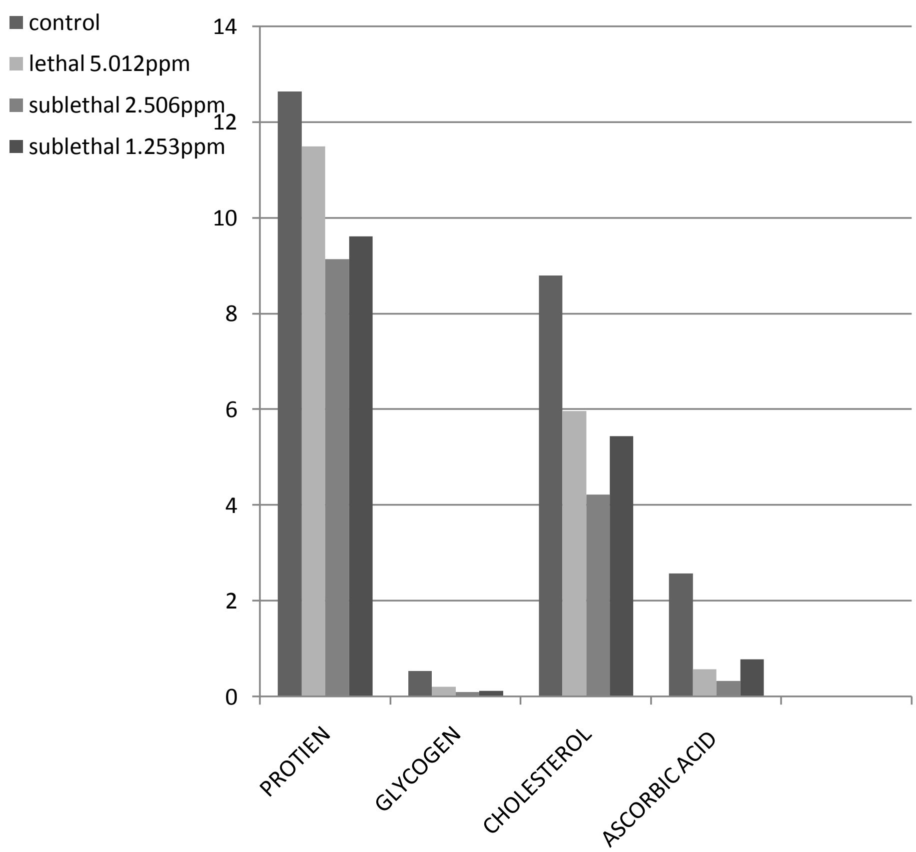

Figure 1. Biochemical contents in *the gills of P. ticto* to dimethoate toxicity exposure.

EC on biochemical composition and observed marked decrease in glycogen content in the gills of *Channa punctatus*. Susan et al. (1999) observed significant decrease in protein content in gills of *Catla catla* under sublethal concentration of pyrethroid fenvelerate. Rao and Ramaneshwari (2000) observed decrease in protein and carbohydrate content in the gill of *Labeo rohita, Mystus vittatus* and *C. punctata* under endosulfan and monocrotophos toxicity. Khare and Singh (2002) have reported the gradual decrease in protein content in the gills of *C. batrachus* under malathion toxicity.

During the present investigation, glycogen content was also decreased. A fall in the glycogen level in gills indicates its rapid utilization to meet the enhanced energy demands under dimethoate toxicity through glycolysis or hexos monophosphate pathway (Cappon and Nicholas, 1975) Figure 1. It could also be due to the prevalence of hypoxic or anoxic conditions, which normally enhances glycogen utilization (Dezwaan and Zandee, 1973). Enhanced utilization of glycogen and its consequent depletion in tissues may be attributed to hypoxia since it increases carbohydrate consumption. Under hypoxic conditions, the animal derives its energy from anaerobic breakdown of glucose, which is available to the cell by the increased glycogenolysis (Chandravathy and Reddy, 1995). Further, the decline in glycogen might be partly due to its utilization in the formation of glycoprotein and glycolipids, which are the essential constituents of various cells and other membranes (Vutukuru, 2005). The fish showed stress condition during exposure period as fast swimming, fast opercular movements, dashing with the walls of aquarium and reduced feeding in the present study. Thus, during such type of stress conditions, the glycogen reserves are depleted to meet energy demand (Rawat et al., 2002; Vutukuru, 2005; Tiwari and Singh, 2009). The toxicant exposed animals seemed to induce the glycogenolysis, possibly by increasing the activity of glycogen phosphorylase to meet the energy demand under stress condition or the toxicant might have an effect on glycogenesis by inhibiting the activity of carbohydrate metabolism.

Similarly, cholesterol level was also decreased in the gills after exposure period in *P. ticto.* Cholesterol is regarded as the major source for steroidogenesis during maturation and spawning periods (Armstrong, 1968; Sen and Bhattacharya, 1981). During present study, decrease in cholesterol content was observed; it might be possible that dimethoate causes general damage, blockage of enzyme system for steroidogenesis. The reduced cholesterol level may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol (Kanagaraj et al., 1993). Shakoori et al. (1996) reported that the cholesterol decrease may be due to utilization of fatty deposits instead of glucose for energy purpose. Remia et al. (2008) also reported decrease in protein and cholesterol content in the gills of *Tilapia mossambica* under monocrotophos stress.

Ascorbic acid plays a role directly related to homeostatic mechanism and is essential for wound healing and regeneration (Shah et al., 1971; Padhi and Patnaik, 1978). In the gills, the ascorbic acid content decreased; it may be due to shifting of ascorbic acid to other tissues like intestine, muscles, kidney and liver due to increase demand of energy and fatigue retardant suggested by Paratheswararao (1967) in the cichlid *Etroplus maculates*.

The present investigation showed a significant decrease in the glycogen and ascorbic acid content in the gill of *P. ticto* when exposed to lethal and sublethal concentration of dimethoate and slight decrease was found in the protein and cholesterol content. The results observed in the present study are in accordance with the findings of Valamathi and Azariah (2002) and Chezhian et al. (2010).

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