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Effect of sub-lethal concentration of endosulfan on lipid and fatty acid metabolism of spotted murrel, *Channa punctatus*

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Abstract

The spotted murrel, *Channa punctatus* were exposed to sub-lethal concentration of endosulfan (8.1 µg l^{-1}) for 12, 24, 48, 72 and 96 hr to elucidate the impact of pesticide on fatty acid composition of liver and muscle. After endosulfan exposure, fish from each control and experimental tanks were randomly sampled anesthetized, sacrificed and then the liver and muscle were dissected out for lipid and fatty acid (FA) profile. Results showed that total lipid, cholesterol, and triglyceride and FA in liver and muscle, and phospholipid in liver were significantly affected due to pesticide exposure. In liver and muscle tissues, 28.09 and 32.57% reduction of the total lipid, and 42.82 and 49.75% reduction in triglyceride and FA were observed at the end of 96 hrs of exposure. Reduction of total lipid, triglyceride and FA may be due to their mobilization for energy production to combat stress. In FA, oleic (25.46 to 22.48 % in liver and 25.75 to 21.87% in muscle) and linoleic acids (8.04 to 6.83% in liver and 9.88 to 8.09%) were reduced in both the tissues at the end of 96 hr of exposure. It may be concluded that exposure of fish to sub-lethal concentration of endosulfan had influenced the lipid and fatty acid metabolism of *Channa punctatus*.

Key words

Channa punctatus, Endosulfan, Fatty acid, Total lipid, Triglyceride

Introduction

Endosulfan, an organo-chlorine lipophilic pesticide, is being widely used in agriculture as well as in integrated agriculture-aquaculture farming systems to protect important food crops (Pullin and Shehadeh, 1980). Although it is phased out in many western countries, still it is being used in tropical and sub-tropical regions (EFSA, 2005), causing mass mortality of fish in various parts of the world (EJF, 2003). It also reaches adjoining water bodies via agricultural run-off (Nayak *et al.,* 1995), from sewage treatment plant (Aktar, 2009), accidental spills (Lambert, 1997) resulting in contamination of soil and water. Elevated residue levels of endosulfan in plant ingredients have also been reported (Lorenzatti *et al.,* 2004) and many of these plant ingredients are now increasingly used in aqua-feeds for sustainable aquaculture, thus exposing the aquatic animals to pesticide. It has been reported that exposure to endosulfan, even at sub-lethal doses, induces behavioral and biochemical changes in fish (Shafiq-ur-Rehman, 2006).

The spotted murrel, *Channa punctatus* is a commercially important fish available throughout India, inhabiting mostly in stagnant muddy pond waters, paddy fields, weedy derelict swamps, canals, lakes, reservoirs and beels (Chondor, 1999). Off late, the population of *C. punctatus* is dwindling rapidly and according to the International Union for Conservation of Nature, it is presently at the near-threatened category. Among various reasons that could be attributed for this happening, one reason is the effect of pesticide runoff from agricultural fields into water-bodies.

For fish, lipid is an important source of energy that undergoes rapid breakdown, re-synthesis and inter conversion in response to different stimuli (Chetty and Indira, 1994). Phospholipid helps in formation of lipoproteins, and enhances fat mobilization and also plays a vital role in absorption of fat-soluble vitamins from intestine. Cholesterol is also an important biological molecule that plays a significant role in membrane structure and integrity. The major circulatory lipids of fish are free fatty acids and tri-acyl-glycerols (TAG) and most of them are shuttled between tissues as free fatty acids or as TAG and phospholipids (Weber and Zwingelstein, 1995). Any alteration in lipid metabolism will significantly affect the health and energy metabolism in fish. In view of the above, the present experiment was undertaken to study the effect of sub-lethal endosulfan exposure on lipid and fatty acid metabolism in *C. punctatus.*

Materials and Methods

C. punctatus (35.6 ± 0.7 g), procured from the local fish market of Mumbai, India, were transported with proper oxygenation to the laboratory of the Central Institute of Fisheries Education, Mumbai, India. Fish were first given a prophylactic dip in salt solution (2%), for one minute, followed by oxy-tetracycline treatment (15 mg l^{-1}) for the first 3 days, and were then acclimatized in a 1000^{-1} tank with proper aeration for about 30 d, during which they were fed 40% crude protein diet. Feeding was stopped 24 hrs before starting the experiment.

Preparation of test solution : Technical grade endosulfan (99%) (Shroff Research Institute, Mumbai, India) consisting of α and β isomers (70:30) was used for the experiment. In our previous study (Sarma *et al.,* 2003), LC_{50} value of endosulfan pesticide for 96 hr for the test fish determined by probit analysis, (Trevors, 1986) was found to be 24.3 µg l^{-1}. Based on this value, the sub-lethal endosulfan dose of 8.1 µg l^{-1} was chosen in, present study.

Experimental design and estimation of lipid and fatty acid : The experiment was carried out in 60 l identical plastic tanks. A group of 36 fish were distributed in three tanks (replication) and were exposed to sub-lethal concentration ($1/3^{rd}$ of LC_{50} for 96 h = 8.1 µg l^{-1}) of endosulfan for varying periods (12, 24, 36, 48, 72, and 96 hr). Water was exchanged daily with fresh concentration of pesticide with minimum disturbance to the test animal. Round the clock, aeration was provided through a centralized pump. The fish were not fed during the experiment. The average water quality parameters were as follows: temperature 26-28°C, dissolved oxygen 6.5-7.1 mg l^{-1}, pH 7.2-7.5, alkalinity 48-58 mg l^{-1} and hardness 48-60 mg l^{-1}.

Sample preparation : Six fish (two from each replicate) were drawn at the beginning and end of 12, 24, 48, 72 and 96 hr. They were anesthetized using clove oil (50 µl l^{-1}), sacrificed, and then dissected to remove the tissues, liver and muscle. Lipid fraction from the muscle and liver was extracted using chloroform: methanol (2:1) mixture as per the method described by Folch *et al.* (1957). Total lipid (Marsh and Winstein, 1966), total phospholipid (Wanger *et al*., 1962), total cholesterol (Zlatkis *et al.,* 1953) and triglycerides and fatty acids (subtracting the value of cholesterol and phospholipid from the corresponding total lipids) were estimated from the extracted lipid. Different free fatty acids were identified using gas chromatography (SHIMADZU-QP5000) with Flame ionization detector.

Statistical analysis : All the data were subjected to one-way ANOVA using statistical software Statistical Package for the Social Sciences (SPSS) version 11.0. Duncan's multiple range test was used to determine the difference among treatment means at 5% level of significance.

Results and Discussion

Data pertaining to the effect of sub-lethal exposure of endosulfan on total lipid, total phospholipid, cholesterol, and triglyceride and fatty acid levels in liver and muscle of *C. punctatus,* at different periods of exposure are presented in Table 1. Total lipid and triglyceride and FA showed a significant decrease ($P<0.05$) with increase in exposure to endosulfan, in both tissues. In liver and muscle tissues, 28.09 and 32.57% reduction of total lipid and 42.82 and 49.75% reduction in triglyceride and FA were recorded, respectively, at the end of 96 hrs of exposure. In accordance with the present study, Bantu *et al.* (2013) reported that total lipid in liver and muscle decreased in *L. rohita* when exposed to sub-lethal concentration of chlorantraniliprole. Similarly, Sweilum (2006) also reported a gradual decrease in muscle lipid level with increase in pesticide concentrations in *Oreochromis niloticus* exposed to pesticide. Decline in total lipid content in the present study, may be attributed to utilization of lipid for meeting the energy demand under the pesticide stress (Bantu *et al.*, 2013). Similarly, gradual increase (50.06%, $P<0.05$) in total phospholipid was observed in liver tissues at the end of 96 hr. However, in muscles the level was not significantly affected. Elevation of phospholipid level in the present study may be attributed to prevention of tissue damages in the fish. It has been reported that milk phospholipids prevents tissue damage incurred from UV light (Dargitz *et al.,* 2012) and also phospholipid oxidation products have protective role in endotoxin-induced tissue damage (Bochkov *et al.,* 2002). Elevation of phospholipid may also be due to mobilization of fat soluble vitamins, which are required for activation or catalyzing essential bio-chemical reactions. Gimenez *et al.* (2011) also reported that fatty acids, vitamins (A, E and folate), zinc and magnesium play an important role in phospholipid metabolism in different organs of animal or human cells.

Cholesterol is another important biological molecule that play a significant role in membrane structure and fish physiology. In the present study, cholesterol level was drastically reduced by 66.1% within 12 hr of endosulfan exposure and at the end of 96 hr,

Table 1 : Lipid profile in liver and muscle tissues of *C. punctatus* exposed to sub-lethal concentration of endosulfan

Exposure	Total lipid		Phospholipid		Cholesterol		Triglyceride	
(hr)	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
Control	41.47 ± 0.92^{a} (0.00)	18.66 ± 1.73^{a} (0.00)	6.15 ± 0.22^{a} (0.00)	1.71 ± 0.06 (0.00)	7.67 ± 0.37^{a} (0.00)	1.90 ± 0.12^{a} (0.00)	27.84 ± 1.07^{a} (0.00)	15.05 ± 1.80^{a} (0.00)
12	35.47 ± 1.73^{b} (-14.83)	17.33 ± 1.34^{ab} (-7.08)	7.06 ± 0.58^{a} (14.84)	1.63 ± 0.12 (-4.78)	2.60 ± 0.50^{b} (-66.08)	1.58 ± 0.09^{a} (-17.37)	23.70 ± 1.32^{b} (-14.85)	13.49 ± 1.55^{ab} (-10.36)
24	35.59 ± 1.54^{b} (-14.55)	15.48 ± 1.21^{abc} (-17.03)	6.65 ± 0.07^{a} (8.21)	1.37 ± 0.07 (-19.91)	2.75 ± 0.28^{b} (-64.10)	1.68 ± 0.20^{a} (-11.80)	26.19 ± 1.43^{ab} (-5.93)	12.51 ± 1.020^{abc} (-16.85)
48	35.70 ± 0.78^{b} (-14.30)	14.15 ± 1.28^{bc} (-24.14)	6.21 ± 0.32^{a} (0.96)	1.30 ± 0.27 (-23.68)	4.02 ± 0.78^{bc} (-47.62)	2.88 ± 0.19^{b} (51.35)	25.47 ± 0.98^{ab} (-8.48)	9.97 ± 1.340^{bcd} (-33.75)
72	36.45 ± 1.04^{b} (-12.48)	13.28 ± 1.47^{bc} (-28.81)	9.14 ± 0.83^{b} (48.67)	1.62 ± 0.18 (-4.97)	4.06 ± 0.44^{bc} (-47.03)	2.90 ± 0.36^{b} (52.19)	23.59 ± 1.86^{b} (-15.26)	8.49 ± 1.64^{cd} (-43.58)
96	29.95 ± 0.73^{c} (-28.09)	12.58 ± 1.24^{c} (-32.57)	9.22 ± 0.29^{b} (50.06)	1.67 ± 0.72 (-2.31)	4.81 ± 0.44^{c} (-37.22)	2.95 ± 0.23^{b} (55.11)	15.96 ± 0.91^{c} (-42.82)	7.56 ± 1.38^{d} (-49.75)

Values with different superscripts differ significantly (P<0.05) (parameter wise); Units: Total lipid = mg lipid/ g of wet tissue; Phospholipid = mg lecithing of wet tissue; Cholesterol = mg cholesterol/g of wet tissue and Triglyceride = mg triglycerides and free fatty acid/g of wet tissue; Values are mean ± standard error (n= 6)

Table 2 : Free fatty acid profile in liver and muscle tissue of *C.punctatus* (n= 6) exposed to sub-lethal concentration of endosulfan

Exposure	Capric (%)		Lauric (%)		Myristic (%)		Palmitic (%)		Stearic (%)		Oleic (%)		Linoleic (%)		Arachidic(%)
(hr)	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control	6.80	8.38	5.95	7.55	12.62	14.10	22.089	20.15	7.43	6.86	25.46	25.75	8.04	9.88	1.51
12	7.79	8.10	7.18	7.08	13.36	14.25	22.26	18.76	8.06	6.30	25.37	25.13	9.20	9.28	-
24	6.67	7.64	6.24	7.06	13.21	13.96	23.63	21.29	7.34	6.03	21.10	24.36	7.03	9.17	-
48	7.71	7.65	7.39	7.54	14.20	16.22	19.79	19.08	6.07	6.96	22.98	24.51	8.84	10.67	-
72	6.84	7.87	6.62	8.40	12.83	15.04	24.36	17.98	8.22	6.77	24.25	21.47	8.46	8.44	-
96	6.00	7.48	5.92	7.17	5.73	14.54	27.70	20.54	10.85	5.75	22.48	21.87	6.83	8.09	-

37.2% reduction was observed in the liver tissue. In muscles, although the level decreased within 12 hr, thereafter the level increased significantly (P<0.05) and at the end of 96 hr of exposure, a 55.09% increase was recorded from the control. The present study showed that, re-synthesis of cholesterol might have been inhibited in the liver or transferred from liver tissue to serum or other organs. Several researchers (Borges *et al.* ,2007; Oner *et al.*, 2008; Fırat *et al.*, 2011) reported elevated cholesterol level in the serum of fish and attributed that the rise might be due to disruption in the hepatic cell owing to stress induced by toxicants and thereby releasing cholesterol into blood. Higher cholesterol level in muscles might be due to accumulation of cholesterol in the tissue.

Data pertaining to the effect of sub-lethal exposure of endosulfan on fatty acid profile in liver and muscles of *C. punctatus* at 12, 24, 48, 72 and 96 hrs of endosulfan exposure are presented in Table 2. Free fatty acid (FA) profile analysed by gas chromatography, identified following fatty acids-: capric (6:0), lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and arachidic (20:0). The complete FA profile could not be analyzed, in the present study, due to limitations in the library of gas chromatography. The FA profile in liver tissues did not show any clear-cut trend upon endosulfan exposure. The level of capric, myristic, oleic and linoleic acids decreased, whereas palmitic and stearic acid level increased at the end of 96 hrs of exposure. It was interesting to note that arachidic acid, which was present in control, was absent in pesticide treated groups. In muscles, percentage availability of capric, stearic, oleic and linoleic acids decreased whereas the lauric, myristic and palmitic acids remained almost same at the end of 96 hr of endosulfan exposure. In the present study, reduction in unsaturated fatty acids in liver and muscle could be due to their utilization for energy purpose. Similar to the present study, Montero *et al.* (1999) also reported reduction of oleic acid, arachidonic acid and n-3 HUFA (highly unsaturated fatty acid) in liver of *Sparus aurata* juveniles stocked at high stocking density and was attributed to meet increased energy demand.

The overall results demonstrated that sub-lethal exposure to endosulfan had a significant impact on lipid and fatty acid profile of *C. punctatus.* This study reiterates the importance of judicial use of pesticides, in order to avoid contamination of fresh water bodies, which are abode for aquatic animals.

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