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Influence of Alphamethrin on Oxidative Metabolism of the Freshwater Fish *Catla catla*

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In India, agriculture has aided development at the cost of environmental pollution caused by large-scale application of chemicals, fertilizers and pesticides (Waliszewski et al. 1999). These pesticides reach water bodies adjoining fields by rain or irrigation and pollute them. These freshwaters contain numerous organisms like phytoplankton, zooplankton, fishes and so on, So this pesticide pollution becomes a threat to an aquatic ecosystem (Tilak et al. 2002; Thakur and Bais 2000). Pyrethroids are good substitutes for organochlorine, carbamate and organophosphate pesticides (Elliot 1977). The lipophilicity of pyrethroids indicates that they are easily absorbed by the gill of fish, even at very low concentrations in aquatic media (Kumar et al. 1999). The knock down effect of pyrethroid compounds to insects, coupled with their extremely low toxicity to warm blooded animals make them suitable choices (Arshad et al. 1999).

Several workers reported that pyrethroids are more toxic to aquatic organisms like snails and fishes (Sahay et al. 1991; Mian and Mulla 1992; Singh and Sirvastava 1999). Srivastava (1999) also reported that the toxicities of cypermethrin and alphamethrin are significantly influenced by seasonal temperature variation. In case of cypermethrin and alphamethrin the toxicity increases 3 – 9 times due to variation in temperature between 15 and 25°C and suggested that use of synthetic pyrethroids should be avoided in water bodies or fields adjoining water bodies during the winter season. Also, Susan et al. (1999a) reported the bioaccumulation of fenvalerate, in whole body tissues of *Labeo rohita*, *Catla catla* and *Cirrihinus mrigala* (Ham.) exposed to sub-lethal and lethal concentrations of fenvalerate.

Earlier studies indicate that there is much information available on the toxicity and lethality of pyrethroids on insects and aquatic invertebrates. Very few reports are available on their mode of action and effect on non-target organisms which share the habitat. The present study is an attempt, to understand the long-term effect of alphamethrin on oxidative metabolism of *Catla catla.* Alphamethrin is an effective insecticide and *C. catla* is an important major food fish of the Indian capture/ culture fishery.

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MATERIAL AND METHODS

Live, healthy and disease free specimens of *Catla catla* (19-21 cm total length, 70-80 gram weight) of the same size were collected from Tara Matsya Breeding Centre, Maharajganj – 273 303 (U.P.) and acclimated to laboratory conditions in glass aquaria for 10 days. They were fed on artificial feed and starved 24h prior to experimentation.

The LC_{50} concentration of alphamethrin (40% of 24h LC_{50}) was determined by the method of Singh and Agarwal (1990). Three batches of 10 fish each were exposed to a sub-lethal concentration, 0.0024 mg/L (40% LC_{50} of 24h) for 15, 30 and 45 days and one batch of 10 fish in freshwater without any treatment was the control. No mortality was recorded during experimental periods. All the groups were kept in identical laboratory conditions and fed with artificial feed, (three times in a week). After feeding, water was changed and the predetermined quantity of pesticide was added to keep the treatment concentration constant through out the exposure period. Atmospheric and water temperature ranged from 30.5 - 31.5°C and 27.0 - 28.0°C, respectively, water pH was 7.3 - 7.5, while dissolved oxygen, free carbon dioxide and bicarbonate alkalinity ranged from 6.8 - 7.6 ppm, 4.4 - 6.5 ppm and 105.0 - 109.0 mg/L, respectively, upon measurement by of APHA (1998) methods.

For the biochemical estimation of enzyme activities, 10% tissue homogenates were prepared in cold 0.32M solutions and centrifuged at 1000g for 15 min to remove cell debris. A clear, cell-free extract was used for the estimation of the dehydrogenase. The activity of succinic dehydrogenase (SDH) was measured by the method of Nachalas et al. (1960). The homogenates (50mg/ml, w/v) was prepared in 0.5M potassium phosphate buffer (pH 7.6) in an ice bath and centrifuged at 4°C. The method of Srikanthan and Krishsnamurthy (1955) was employed for the determination of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activity. The cytochrome-c-oxidase and Mg^{2+} ATPase were measured by the methods of Oda et al. (1958) and Fritz and Hamrick (1966), respectively. The homogenates (50mg/ml, w/v) were prepared in 0.33M phosphate buffer (pH 7.4) in an ice bath and centrifuged at 4°C and the supernatant was used as the enzyme source. Protein content was measured by the method of Lowry et al. (1951). Tissues were removed and homogenates (5 mg/mL, w/v) were prepared in 10% TCA. Standard curves were prepared with different concentrations of bovine serum albumin. The statistical analysis was done the methods of Sokal and Rohlf (1973). Each assay was replicated six times, values are mean ±SE of six replicates and Student's 't' test was applied to determine significant ($P<0.05$) differences between treated and control groups.

RESULTS AND DISCUSSION

Table 1 and 2 show the biochemical changes in liver, brain, muscle and gills of *C. catla* exposed to the sub-lethal concentration of (40% of 24h LC_{50}) alphamethrin.

There was a negative correlation between exposure periods and SDH/ MDH activities, i.e., enzyme activities decreased with increased exposure period. LDH activity was significantly elevated in all tissues (brain, liver, muscle and gill). However alterations were more significant ($P<0.05$) at 30 and 45 days (Table 1). Maximum inhibition in SDH and MDH activity were observed in gill and muscle tissues, respectively. Maximum LDH activities were found in gill tissues.

Table 1. Changes in activities of succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) expressed as μ moles of formazon /mg protein/hr in selected tissue of *C. catla.*

Parameter	Tissue	Control	Exposure period (days)		
			15	30	45
SDH	Brain	0.97±0.03 (100)	0.68±0.06 * (69.76)	0.66±0.04* (68.32)	0.56±0.02** (57.69)
	Liver	0.67±0.03 (100)	0.48±0.02* (71.94)	0.46±0.02* (68.81)	0.41±0.01** (60.75)
	Muscle	0.35±0.01 (100)	0.30 ±0.01 (85.19)	0.24±0.02* (68.38)	0.21±0.001** (57.84)
	Gill	0.14±0.06 (100)	0.11±0.08* (79.43)	0.09±0.04* (65.95)	0.08±0.04** (56.03)
MDH	Brain	0.42±0.002 (100)	0.35±0.004 (83.90)	0.27±0.002** (63.94)	0.25±0.004** (59.62)
	Liver	0.36±0.001 (100)	0.25±0.002* (69.97)	0.24±0.002* (50.00)	0.20±0.001** (53.99)
	Muscle	0.44±0.001 (100)	0.03±0.001* (75.00)	0.02±0.002** (50.00)	0.02±0.004** (40.32)
	Gill	0.04±0.005 (100)	0.03±0.001 (76.19)	0.03±0.002* (66.67)	0.21±0.006** (50.00)
LDH	Brain	0.05±0.002 (100)	0.06±0.005* (126.53)	0.07±0.002** (144.89)	0.08±0.002** (157.14)
	Liver	0.49±0.006 (100)	0.62±0.004* (126.22)	0.68±0.005* (137.40)	0.70±0.002** (142.28)
	Muscle	0.16±0.004 (100)	0.19±0.004* (123.23)	0.21±0.002* (133.55)	0.22±0.004** (139.36)
	Gill	0.07±0.004 (100)	0.10±0.002* (135.21)	0.10±0.001** (143.66)	0.12±0.002** (164.78)

Values are mean ± SD of six replicates. Values in parenthesis are percent change with control taken as 100%. Data were analyzed through Student 't' test. *, Significant ($P< 0.05$), when treated groups were compared with controls. **, Significant when student 't' test was applied with control.

The sub-lethal concentration (40% of 24h LC_{50}) of alphamethrin on exposed *C. catla*, show depletion of cytochrome–c-oxidase and Mg^{2+} ATPase levels in liver, brain, and muscle at all exposure periods, (15, 30 and 45 days) (Table 2). In gills, depletion of cytochrome-c-oxidase and Mg^{2+} ATPase was significant at 30 and 45 days while nonsignificant depletion was recorded after 15 days (Table 2).

Table 2. Percent changes in activity of Mg^{2+}, ATPase (μ moles /mg protein/hr) and cytochrome-c-oxidase (μ moles of dye formazon formed /mg protein/hr) in different tissues of *C. catla* exposed to sub-lethal concentration of alphamethrin.

Parameter	Tissue	Control	Exposure period (days)		
			15	30	45
Cytochrome-c-oxidase	Brain	139.40±5.40 (100)	101.46±4.54* (72.78)	96.89±6.45* (69.51)	81.62±4.40** (58.55)
	Liver	160.41±4.50 (100)	126.98±3.60* (79.16)	112.43±6.85** (70.08)	99.64±2.84** (62.12)
	Muscle	83.56±4.50 (100)	66.24±3.45* (79.27)	57.72±2.45* (69.08)	56.64±4.50** (67.78)
	Gill	125.40±4.80 (100)	102.40±4.46 (81.66)	94.57±3.60 (75.42)	92.86±6.45* (74.05)
Mg^{2}+ ATPase	Brain	1.68±0.20 (100)	1.22±0±0.26* (72.62)	1.18±0.03* (70.24)	1.08±0.04** (64.29)
	Liver	1.55±0.03 (100)	1.23±0.04* (79.36)	1.17±0.04* (75.48)	1.05±0.10** (67.74)
	Muscle	1.36±0.20 (100)	1.06±0.24* (77.94)	1.01±0.05* (74.27)	0.96±0.02** (70.58)
	Gill	0.92±0.03 (100)	0.80±0.05 (86.96)	0.69±0.02* (75.00)	0.58±0.06** (63.04)

Values are mean ± SD of six replicates. Values in parenthesis are percent change with control taken as 100%. Data were analyzed through Student 't' test. *, Significant ($P< 0.05$), when treated groups were compared with controls. **, Significant when student 't' test was applied with control.

The biological activities of pyrethroids are closely related to their chemical structure (Elliot 1977). Very little literature is available on the mode of action of these pyrethroids (alphamethrin, permethrin, cypermethrin, delthmethrin and fenvelerate). However it was found that alphamethrin causes significantly altered oxidative metabolism in *C. catla* (Tables 1 - 2).

The specific activities of SDH, MDH, cytochrome oxidase and Mg^{2+} ATPase, which represent aerobic metabolism, were decreased in fish tissue during pesticide stress. This indicates that alphamethrin inhibits the conversion of succinate to fumarate or inhibits the oxidation process in the tri-carboxylic acid cycle (TCA cycle) resulting in low energy synthesis. The inhibition of SDH activity may be related to alteration in enzyme and substrate levels. A decrease in enzyme activity and enzyme substrate complex formation was reported. Singer et al. (1972) reported that the action of SDH was regulated by mitochondria. Inhibition of SDH activity might induce some structural changes in the mitochondrial membrane. Chatterjee et al. (1986) showed that synthetic pyrethroids induced membrane damage in mitochondria, as a result the mitochondrial enzymes were found to be altered. The same trend was also observed in the present study. Susan et al. (1999b) reported biochemical and enzymatic changes in tissue of *Catla catla* exposed to a sub-lethal fenvelerate concentration 0.016 mg/L for one day. During

exposure, GDH levels were highly elevated while a decreased enzymatic activity was observed in aspartate aminotransferases and alanine aminotransferase (AAT and AIAT) level. The pyrethroids, cause not only inhibition of acetylcholinesterase but also reduce the endogenous levels of 5-hydroxy tryptamine, epinephrine and dopamine in nervous tissue of freshwater fishes (Rao 1999). Malate dehydrogenase (MDH) activiy was significantly inhibited in alphamethrin-exposed fish (Table 2). These changes may be due to reduced substrate levels. The activity of LDH was also elevated in brain, liver, muscle and gill tissues of alphamethirn exposed fish. Ghosh and Chatterjee (1987) and Gupta (1986), reported elevation in LDH activity in different fish under pyrethroid exposure. It appeared that aerobic oxidation through the Krebs cycle was adversely affected in treated fish, as revealed by unequivocal inhibition in the activities of SDH and MDH in brain, liver, muscle and gills. Singh and Agarwal (1993) and Tripathi et al. (2002) supported such an assumption.

In this study the oxidative metabolism of treated fish, was altered due to cytochrome-c-oxidase. Neskovic and Vitorovic (1977) also reported, cytochrome-c-oxidase is responsible for reduced levels of oxidative metabolism. The present study clearly indicates that normal respiratory metabolism of different tissues of *Catla catla* was adversely affected by alphamethrin. Though we cannot avoid the use of synthetic pyrethroids, measures should be taken for the conservation of water quality and aquatic resources.

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