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Impact of alphamethrin on biochemical parameters of *Channa punctatus*

G. Tripathi* and H. Singh

Department of Zoology, J.N.V. University, Jodhpur-342 033, India

**Corresponding Author email : drgst@rediffmail.com*

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Abstract

Impact of alphamethrin (synthetic pyrethroid) on profiles of lactate dehydrogenase (LDH), catalase (CAT), DNA, RNA and protein in liver, brain, gill and skeletal muscle of the freshwater food fish *Channa punctatus* were investigated. Exposure of sublethal concentration of alphamethrin (0.018 ppm for 14 days) increased the activity of LDH in liver (1.8 fold), brain (1.4 fold), gill(1.6 fold), and skeletal muscle(2.2 fold) of the fish. However, it significantly decreased the activity of CAT in the tissues of liver (54%), skeletal muscle (52%), gill (51%) and brain (49%) of the fish. Similarly, DNA (skeletal muscle (36%), liver (30%), brain (28%) and gill (25%)) RNA (liver (42%), brain (32%), gill (35%) and skeletal muscle (45%)) and protein content (45%), brain (42%), gill (36%), and skeletal muscle (27%)) declined in different tissues of the fish exposed to alphamethrin. Maximum increase in the level of LDH was in skeletal muscle (2.2 fold) and minimum in brain(1.4 fold). Maximum reduction in CAT profile was in liver (54%), and minimum in brain (49%). Declines in DNA was maximum in skeletal muscle (36%) and minimum in gill (25%) whereas RNA and protein content were maximum in liver (42% and 45% respectively) and minimum in skeletal muscle (45% and 27% respectively). Alphamethrin was toxic to the freshwater fish due to its inducing effect on anaerobic enzyme (LDH) and inhibitory effect on antioxidant enzyme (CAT), DNA, RNA and protein. This reflected alphamethrin associated increase in anaerobiosis and decrease in oxidative defense and impairment in protein synthesizing capacity of *C. punctatus*. Further, induction in LDH and reduction in CAT and protein profile may be used as biomarker of alphamethrin toxicity in fish.

Key words

Alphamethrin, *Channa punctatus*, Antioxidative enzyme, Anaerobic enzyme

Introduction

Water pollution, due to industrial effluents and agricultural wastes, has become an inevitable and universal problem. Various pesticides including synthetic pyrethroids are extensively used in plant protection practices. Pyrethroids have a broad spectrum insecticidal activity and low mammalian toxicity (Verma *et al.,* 2001). They account for 30% of global insecticide consumption (Prama *et al*., 2007). Most of the pyrethroidal compounds enter the aquatic environment through run off water during agricultural operations, by drift during forest spray and by direct spraying of water bodies. They adversely effects fish and other non-target aquatic species (Joshi and Dharmlata, 2004; Joshi *et al*., 2007). *Pyrethrum crysogenthum* grows luxuriantly along the banks of river, ponds and lake. Hence, the possibility of entry of its decomposed flowers in surrounding water bodies cannot be ruled out. Alphamethrin (alpha-cypermethrin) is a type-II synthetic pyrethroid which is insoluble in water but soluble in acetone. Srivastava and Dumka (2001) reported that the toxicities of cypermethrin and alphamethrin are significantly influenced by seasonal temperature variation. Susan *et al*. (1999a) reported the bioaccumulation of fenvalerate in tissues of *Labeo rohita* and *Catla catla*. Srivastava *et al*. (2006) found reproductive impairment in *Channa punctatus* exposed to devicypermethrin.

Pyrethroids have been shown to be thousand times more toxic to fish than to mammals and birds at a comparable concentration (Verma *et al*., 2001). The hypersensitivity of fish to pyrethroid intoxication is partly due to species-specific differences in pyrethroid metabolism but principally because of the increased sensitivity of the piscine nervous system to the pesticide (Moore and Waring, 2001). Cypermethrin treated fish showed inhibition in phosphatase activity of liver and kidney of *Notopteus notopterus* (Gupta *et al*., 1998). Enzyme modulation by sublethal concentration of cypermethrin was studied in liver, heart and kidney of the fish *Cyprinus carpio*. The activity of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) showed marginal changes over that of their control values. Differences in activities of enzymes are cumulative toxic effect of cypermethrin (Shivakumari *et al.,* 1997). Cypermethrin induced biochemical changes have been reported in *Catla catla* (Singh and Singh, 2004), *Cyprinus carpio* (Velisek *et al*., 2006), *Cirrihinus mrigala* (Prashant *et al*., 2005) and *Channa punctatus* (Kumar *et al.*, 2007; Ansari *et al*., 2009). In view of the above the present study, aimed to elucidate the effect of alphamethrin on anaerobic (LDH) and antioxidant (catalase) enzymes and macromolecular content (DNA, RNA and protein) in liver, brain, gill and skeletal muscle of an economically important freshwater teleost *Channa punctatus*.

Materials and Methods

Experimental design : Adult and healthy fish, *Channa punctatus* (approximately weighing 160 ± 4 g, length 15-18 cm) were collected from local fish market of Jodhpur, India. This fish belongs to family Ophiocephalidae and order Ophiocephaliformes. Collections were done during resting phase (Nov-Feb) of the fish. The fish were acclimatized under laboratory condition at least two weeks prior to experimentation. Feeding was stopped during alphamethrin (98%) treatment. Fish were divided into three groups each containing six fish. The fish of group 1 were untreated and served as a control. The fish of group 2 were treated with acetone and served as vehicle control, Vehicle control was kept to check the effect of acetone on enzymes and other macromolecules. The fish of group 3 were treated with sublethal concentration of 0.018 ppm alphamethrin for 14 days. This sublethal concentration was obtained after a prelimnary experiment in laboratory.

Biochemical parameters : The specimens of *C. punctatus* were sacrificed by decapitation. Liver, brain, gill and skeletal muscle were removed and washed in 0.6% saline. They were properly cleaned and weighed rapidly. A 10% homogenate (w/v) was prepared in 0.1 m Tris –HCl or sodium phosphate buffer (pH 7.5). Homogenization was done at about 4°C. Homogenates were centrifuged at 12000 g for 15 min in a high speed refrigerated centrifuge (Remi Cooling Compufuge, (CPR 24) and the resulting supernatant was taken as the cytoplasmic fraction for assay of LDH and CAT. The procedure adopted for the assay of LDH was that of Foster and Moon (1986). However, catalase was assayed according to the method of Aebi (1984). LDH was assayed in a medium containing 100 mM Tris-HCl (pH 7.5). Optimum concentrations of substrate and coenzyme were used. The total volume of reaction mixture was 3 ml. The readings were taken using Cintra-5 UV-VIS Spectro photometer (GBC, Germany). The decrease in absorbance at 340 nm and 240 nm were recorded for LDH and CAT respectively.

The diphenylamine and orcinol method of Schneider (1975) were used for nucleotide (DNA, RNA) estimation. The absorbance was recorded at 595 nm and 695nm for DNA and RNA respectively. Since major amount of RNA in a cell is rRNA and RNA/DNA ratio reflects protein synthesizing status, it was imperative to include estimation of DNA, RNA and protein to demonstrate the effect of alphamethrin on protein synthesizing capacity of fish. The protein content was determined by Folin-Ciocalteau method of Lowry *et al.* (1951) and absorbance was taken at 650nm.

Statistical analysis : One way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) were performed using statistical package. The level of significance was kept at 0.05.

Results and Discussion

The LDH and CAT activities of liver, brain, gill and skeletal muscle of *C. punctatus* did not differ significantly between normal and control groups. The LDH activity increased significantly ($p<0.001$) in skeletal muscle (2.2 fold) followed by liver (1.8 fold), gill (1.6 fold) and brain (1.4 fold) in response to treatment of 0.018 ppm alphamethrin for 14 days (Table 1). In contrast, CAT activity significantly ($p<0.001$) decreased in the liver (54%), skeletal muscle (52%), gill (51%) and brain (49%) in comparison to control (Table 1 and 2).

The DNA and RNA contents did not differ significantly ($p<0.001$) between normal and control groups. However, treatment of alphamethrin for 14 days significantly ($p<0.001$) decreased the DNA, RNA and protein content in skeletal muscle (36 , 45 and 27%), liver (30, 42 and 45%), brain (28, 32 and 42%) and gills (25, 35 and 36%) as compared to their respective control. *C. punctatus* (Table 1 and 2).

Alphamethrin associated increase in LDH activity may be due to increased LDH synthesis and anaerobiosis (Radaiah and Rao, 1990). The tissue specific differences in activity of LDH may be assigned to the difference in uptake

of alphamethrin in different tissues (Singh and Srivastava, 1999). Das and Mukherjee (2002) also reported increase in LDH activity of brain and liver of *Labeo rohita* exposed to 0.014 ppm cypermethrin for 96 hrs. Begum (2005) documented an elevation in the activity of glutamate dehydrogenase in liver and brain of *C. punctatus* after exposure of cypermethrin (0.07mg l^{-1}) for 10 days. Likewise, Singh and Singh (2004) demonstrated an increase (1.2-1.7 fold) in LDH activity of liver, brain, gill and skeletal muscle of *C. carpio* exposed to alphamethrin (0.0024 mg l^{-1}) for 45 days. Elevated level of LDH in *C. punctatus* in response to alphamethrin indicated an increase in anaerobic respiratory activity and production of more lactate for completion of metabolic process.

The tissue specific differences in activity of CAT may be assigned to differences in uptake of pyrethroid (Sayeed, 2003). The alphamethrin dependent decrease in CAT might be due to binding of the toxicant to the enzyme molecule or inhibiting the enzyme synthesis as indicated in other cases (Sayeed *et al*., 2003; Tripathi and Verma, 2004). Alphamethrin induced reduction in CAT activity and specific of the fish indicated reduction in capacity of oxidative defenses due to pyrethroidal toxicity. Sayeed *et al*. (2003) also observed reduction in activity of catalase in liver, brain, kidney and gill of *C. punctatus* exposed to deltamethrin (0.75 mg l^{-1}) for 48 hr. However, the present findings are not in agreement with Atif (2005) who showed increased level of glutathione peroxidase in C. *punctatus* exposed to deltamethrin.

The reduction in DNA, RNA and protein content in different tissues of *C. punctatus* in response to alphamethrin treatment might be due to inhibitory effect of pyrethroid on DNA synthesis or increased degradation (Susan *et al*., 1999b). Our findings differ from the report of Das and Mukherjee (2002) who observed an increase in DNA and RNA content of liver, brain, kidney of the fish *Labeo rohita* in response to sublethal concentration (0.014ppm) cypermethrin for 96 hr. Kumar *et al*. (2007) also observed increasing effect on DNA and RNA profile of gill, brain, liver and kidney of *C. punctatus* when treated with cypermethrin (40-60µg l^{-1}) and l-cyhalothrin (0.8-1.6 µg l^{-1}) separately.

Begum (2005) reported reduction (30-40%) in protein content of liver of *C. punctatus* in response to sublethal concentration of cypermethrin (0.07 ppm). However, the present findings differ from the report of Tripathi *et al*. (2006) who showed increase in protein content of liver, heart, kidney and gill of the fresh water fish *Clarias batrachus* (Linn.) after exposure of cypermethrin (0.002 ppm) for 72 hr.

In retrospection of the above view it can be said that the synthetic pyrethroid alphamethrin was toxic to the freshwater fish due to its inducing effect on anaerobic enzyme (LDH) as well as inhibitory effect on antioxidant

Table 1 : Effect of alphamethrin on lactate dehydrogenase and catalase activities on different organs of *Channa punctatus*

	LDH				CAT			
	Liver	**Brain**	**Gill**	**Skeletal muscle**	**Liver**	**Brain**	**Gill**	**Skeletal muscle**
Normal	55.765 ± 3.036 [a]	20.246 ± 2.062 [c]	29.795 ± 2.015 [e]	90.187 ± 3.231 [g]	11.692 ± 1.316 [a]	10.997 ± 1.142 [c]	10.032 ± 1.141 [e]	8.457 ± 1.063 [g]
Control	52.650 ± 2.041 [a]	22.045 ± 1.006 [c]	27.658 ± 1.692 [e]	91.352 ± 5.012 [g]	11.926 ± 1.012 [a]	10.783 ± 2.421 [c]	9.846 ± 1.041 [e]	8.902 ± 1.104 [g]
Alphamethrin	93.638 ± 4.05 [b]	30.528 ± 2.014 [d]	44.600 ± 3.001 [f]	192.104 ±11.065 [h]	6.414 ± 1.201 [b]	5.548 ± 1.032 [d]	5.342 ± 1.052 [f]	4.352 ± 1.041 [h]

Values are mean of six replicates ± SE. Values with different letters in each parameter are significantly different

Table 2 : Effect of alphamethrin on DNA, RNA and protein content on different organs of *Channa punctatus*

	DNA				RNA				Protein			
	Liver	**Brain**	**Gill**	**Skeletal muscle**	**Liver**	**Brain**	**Gill**	**Skeletal muscle**	**Liver**	**Brain**	**Gill**	**Skeletal muscle**
Normal	9.489 ± 1.051[a]	6.759 ± 1.036 [c]	8.086 ± 1.071[e]	5.519 ± 1.072[g]	1.553 ± 0.052[a]	1.305 ± 0.067[c]	1.295 ± 0.071[e]	1.030 ± 0.014[g]	77.281 ± 4.038[a]	65.625 ± 3.065[c]	57.879 3.085[e]	88.341 ± 4.072[g]
Control	9.636 ± 1.042[a]	5.858 ± 1.086[c]	7.054 ± 1.067[e]	5.936 ± 1.081[g]	1.495 ± 0.042[a]	1.172 ± 0.012[c]	1.169 ± 0.052[e]	1.023 ± 0.01[g]	75.320 ± 4.054[a]	68.528 ± 3.112[c]	54.695 ± 3.093[e]	86.632 ± 4.084[g]
Alphamethrin	6.679 ± 1.036[b]	4.230 ± 1.015[d]	5.313 ± 1.028[f]	3.782 ± 0.249[h]	0.859 ± 0.014[b]	0.796 ± 0.052[d]	0.763 ± 0.031[f]	0.562 ± 0.008[h]	44.491 ± 3.033[b]	39.672 ± 2.162[d]	38.591 ± 2.063[f]	66.206 ± 3.143 [h]

Values are mean of six replicates ± SE. Values with different letters in each parameter are significantly different

enzyme (CAT), DNA, RNA and protein contents. This suggests alphamethrin associated induction in LDH and reduction in CAT and protein profile may be used as biomarker of alphamethrin toxicity in fish.

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