International Journal of Biology Research
ISSN: 2455-6548; Impact Factor: RJIF 5.22
Received: 27-04-2020; Accepted: 07-05-2020; Published 29-05-2020
www.biologyjournal.in
Volume 5; Issue 2; 2020; Page No. 04-08

Effect of cypermethrin on the organic constituents of *Channa punctatus*

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Abstract
Freshwater fish *Channa punctatus* was exposed to the synthetic pyrethroid cypermethrin in *in vitro* conditions. At 5.0 ppm or more concentration cypermethrin caused acute toxicity in this fish within 48 hours.
For studying the changes in the levels of organic constituents in this fish, three sublethal concentrations of cypermethrin viz. 0.10, 0.20 and 0.30ppm were selected and fish were exposed for a maximum period of 30 days. The effect of exposure was studied on organic constituents in muscle and liver after 5, 10, 15, 20, 25 and 30 days.
Exposure to cypermethrin caused significant changes in protein, total amino acids, glycogen, total fat and cholesterol. The results are discussed to study the effect of *in vitro* exposure of *Channa punctatus* to sub lethal concentrations of cypermethrin.

Keywords: pyrethroid, cypermethrin, organic constituents and *Channa punctatus*

1. Introduction
Cypermethrin is a synthetic pyrethroid which is commonly used as pesticide against a variety of pests. Pyrethroids possess moderate toxicity to mammals but they are highly toxic to fish and aquatic invertebrates (Miura and Takahashi, 1976, Coats *et al.*, 1979 and Srivastava *et al.*, 1997) [15, 2, 32]. Ford *et al.* (1989) [6] reported that synthetic pyrethroid acts as nerve poison. Chaudhari and Saxena (2016) [1] reported that pyrethroids cause genotoxicity in fresh water fish *Channa punctatus*.
Cypermethrin has been reported to cause alterations in the haematology and other biochemical activities of fish (Reddy *et al.*, 1991 [19], Saxena and Seth, 2002 [28], David *et al.*, 2004 [4], Prashanth, 2007 [22] and Saha and Kaviraj, 2009) [26]. Data from the studies of Singh and Agarwal (1993) and Mukhopadhyay *et al.* (2006) [17] revealed that the enzymatic activities in fish and other non-target organisms are also adversely affected due to exposure to cypermethrin. Prakash *et al.* (2010) 21[] reported the potential toxicity of cypermethrin in albino mice, leading to enzymatic alterations in testes as well as disruption of testosterone synthesis.
In the present investigations sub lethal concentrations of cypermethrin are used to study the changes in organic constituents of fresh water fish *Channa punctatus*.

2. Material and method
Live *Channa punctatus* used for present investigations were collected from local water bodies. Fish were brought from collection point to laboratory in plastic containers with water and maintained in glass aquaria. The healthy fish were about 10-15cm long and 40-60gm in weight. *Channa punctatus* were maintained in glass aquaria for acclimatization for 2-3 days and then exposed to various concentrations of cypermethrin in glass aquaria taking 10 fishes in each concentration.
Three different concentrations of cypermethrin viz. 0.10, 0.20 and 0.30ppm were administered to *Channa punctatus* for 5, 10, 15, 20, 25 and 30 days and their effect on organic constituents of muscle and liver was studied.
The organic constituents studied were protein, total amino acids, glycogen, total fat and cholesterol. Standard methods were used for biochemical studies and statistical analysis of data obtained.

3. Results and discussion
Three different concentrations of cypermethrin viz. 0.10, 0.20 and 0.30ppm were administered to *Channa punctatus* for 5, 10, 15, 20, 25 and 30 days and their effect on organic constituents of muscle and liver were studied and the data is presented in Tables 1 to 6.

Toxic effects of cypermethrin on *Channa punctatus*
Toxicity effects of cypermethrin are given in Table 1. Cypermethrin at 5.0ppm or more caused acute toxicity within 48 hours. At 3.0 ppm, only 50% fishes survived up to 30 days and at concentrations 0.30 ppm or below, no mortality was observed up to 30 days.

Protein concentration
The effect of exposure to cypermethrin on protein concentration in muscles and liver of experimental fishes is given in Table 2. Initial values of protein in muscles of *Channa punctatus* was 12.24 mg/100 mg wet weight. Exposure to cypermethrin significantly reduced protein concentration in muscles.
Initial values of protein in the control sample of the liver of *Channa punctatus* was 8.64 mg/100mg wet weight. Cypermethrin caused reduction in protein concentration in muscles and liver of *Channa punctatus*. Muscles contained more protein than liver in this fish. Effect was proportional to the time of exposure and concentration of cypermethrin. The concentration of 0.30ppm of cypermethrin and exposure of 30 days were found to be most effective. Maximum reduction in protein of muscles of *Channa punctatus* was 62.40 % and in liver it was 35.00% at 0.30ppm after 30days of exposure. 0.10ppm cypermethrin can be considered as safer concentration for maintaining

protein concentration of muscles in this fish as at this concentration after 5 days of exposure decrease in protein was not statistically significant.

Cypermethrin is reported to cause significant changes in protein metabolism of fish and other animals. Pisca *et al*. (1992) [20] reported significant effect of sublethal concentrations of cypermethrin on protein contents in muscles of *Cyprinus carpio*. Rajamannar and Manohar (1998) [23] also reported reduction in protein due to exposure to cypermethrin in *Labeo rohita*. Decrease in protein content caused by cypermethrin exposure was also reported by Singh and Agrawal (1994) [31]. Philip *et al*. (1995) [19] reported alterations in protein metabolism of *Cyprinus carpio* after exposure to cypermethrin. Das and Mukherjee (2003) 5[] studied toxicity of cypermethrin in *Labeo rohita* and reported decrease in serum proteins due to this exposure.

Depletion of tissue protein in fishes exposed to toxicants has been reported by several workers (Dubale and Awasthi, 1984 [5], Ram and Sathyanesan, 1985 [24] and Ghosh and Chatterjee, 1988). The reduction in proteins may be due to the increased energy cost of haemostasis, tissue repair and detoxification during stress (Neff, 1985) [18] or it could be due to altered enzyme activities (Lett *et al*.,1976) [14]. Similar findings have been reported by James *et al*. (1995) in *Heteropneustes fossilis* exposed to copper and mercury and by Virk and Sharma (2003) [33] in *Cirrhinus mrigala* due to exposure to nickel. The depletion of protein contents in the present investigations are parallel with the findings of previous workers.

Total amino acids

Changes in total amino acids in muscles and liver of *Channa punctatus* are given in Table 3. Initial value of total amino acids in the muscles was 0.93 mg/100mg wet weight. Exposure to cypermethrin caused moderate increase in total amino acids in muscles of *Channa punctatus*. Cypermethrin at 0.30ppm caused 19.32% increase in total amino acids in muscles of *Channa punctatus* after 30 days of exposure. Initial value of total amino acids in liver of *Channa punctatus* was 0.33 mg/100mg wet weight. Maximum increase in total amino acids in liver was 60.61% after exposure to 0.30ppm cypermethrin for 30days.

The findings of present investigations show that increase in total amino acids in liver was more prominent than increase in muscles in *Channa punctatus*. No significant change was recorded in total amino acids in muscles and liver of control group fishes which confirmed that total changes in exposed fishes were due to cypermethrin stress. When fish is exposed to any toxicant accumulation of amino acids may occur, which can be attributed to lesser use of amino acids (Seshagiri *et al*., 1987) [29] and their involvement in the maintenance of an acid base balance (Moorthy *et al*., 1984) [16].

The increase in total amino acids indicate the enhancement in proteolysis in muscles and liver or due to synthesis of amino acids to cope up with the demands from altered protein concentration in tissues (Kabeer *et al.*, 1980). However, except for a few amino acids all the other amino acids cannot be synthesized in fish tissues. Thus, it can be inferred that enhanced total amino acids portray increased proteolysis and a consequent rise in the total amino acids as a possible source of energy after glycogen depletion to meet the energy demands under cypermethrin stress.

Glycogen

Initial values of glycogen were recorded as 0.95 mg/100mg wet weight in muscles and 5.25mg/100mg wet weight in liver of *Channa punctatus*. These values show that glycogen in liver was much higher than in muscles. Exposure to cypermethrin caused reduction in glycogen contents in muscles and liver of *Channa punctatus*. These changes are given in Table 4.

In muscles this reduction was 58.14% in *Channa punctatus* after exposure to 0.30ppm cypermethrin for 30 days. This reduction was gradual during the exposure period. There was no significant change in the muscle glycogen in fishes of control group.

Liver glycogen in the fish was also decreased in the similar way. Maximum reduction of 59.13 % was recorded in *Channa punctatus* after 30 days exposure to 0.30ppm cypermethrin.

Jha and Jha (1995) reported the loss of glycogen in muscle and liver of freshwater fish *Anabas testudineus* due to intoxication by nickel chloride. The glycogen depletion observed in present studies may be associated with cypermethrin induced rapid glycogenolysis and inhibition of glycogenesis through activation of glycogen phosphorylase and depression of glycogen synthetase respectively or inhibition of glucose-6-phosphatase. The lowering of glycogen content can be correlated with increase in oxidative metabolism of these fishes. Philip *et al*. (1995) [19] also reported changes in glycogen contents in *Labeo rohita* due to cypermethrin stress. Some other workers (Sastry and Dasgupta, 1991; James *et al*., 1991 and Saxena and Seth, 2002) [28] have also reported similar changes in *Channa punctatus* and other fishes due to pesticidal stress.

Total fat

Total fat content in *Channa punctatus* was recorded as 0.59 mg/100mg wet weight in muscles and 2.42 mg/100mg wet weight in liver. Cypermethrin showed increasing effect on total fat in muscles and liver of this fish. Maximum increase in muscles was 32.14% in *Channa punctatus* exposed to 0.30ppm cypermethrin for 30 days. Fat content in liver was also increased by 72.12% in *Channa punctatus* exposed to 0.30ppm cypermethrin for 30 days. No significant change in fat content in muscles and liver was recorded in control group fishes during these 30 days. These changes are given in Table 5.

Katti and Sathyanesan (1983) reported elevated fat contents in liver of *Clarias batrachus* due to that exposure to lead nitrate. In the present studies total fat content was increased in muscles and liver of *Channa punctatus*. Increase in fat can be correlated with the changes in the activity of fat digesting enzymes like lipase which is responsible for breakdown of lipids into free fatty acids and glycerol. Since fat constitute very rich energy reserve, its increase indicates the changes in energy demands of fish during exposure to cypermethrin.

Cholesterol

Muscles and liver of experimental fish contained low cholesterol. The initial value of cholesterol was 0.17 mg/100mg wet weight in muscles of *Channa punctatus*. When fishes were exposed to sublethal concentrations of cypermethrin increase in muscle cholesterol was recorded. Maximum increase was 102.44% in *Channa punctatus* exposed to 0.30ppm cypermethrin for 30 days. During this period muscle cholesterol in control fishes remained almost unchanged. These changes are given in Table 6.

Initial value of cholesterol in liver was 0.44mg/100mg wet weight in control group of fishes. Exposure to sublethal concentrations of cypermethrin caused moderate increase in liver cholesterol. 22.09% increase was recorded in the liver of *Channa punctatus* due to 0.30 ppm cypermethrin after 30 days of exposure.

Gill and Pant (1983) reported the inducement of changes in cholesterol in *Puntius conchonius* due to cadmium toxicity. In present findings cypermethrin caused hypercholesterolemia in the muscles and liver of *Channa punctatus*. These changes may be linked to a general stress syndrome, increase in total fat and altered thyroid activity.

Table 1: Effect of *in vitro* exposure of cypermethrin in *Channa punctatus*

Concentration of Cypermethrin	Effect
10 ppm	Lethal effect observed within 24 hours
05 ppm	Lethal effect observed within 48 hours
03 ppm	50% fishes survived up to 30 days
0.3 ppm	No lethality observed up to 30days
0.2 ppm	No lethality observed up to 30days
0.1 ppm	No lethality observed up to 30days

Table 2: Changes in protein concentration (mg/100mg wet weight) in the muscles and liver of *Channa punctatus* exposed to cypermethrin

Exposure period in days	Tissue	Control	Concentration of cypermethrin in ppm		
			0.10	0.20	0.30
0	muscles	12.24±0.22[a]			
	liver	8.64±0.23[a]			
5	muscles	11.97±0.18	10.81±0.16	10.19±0.20	9.61±0.23
	liver	8.63±0.18	6.99±0.11	6.57±0.23	6.23±0.13
10	muscles	12.00±0.13	10.46±0.21	9.79±0.18	8.56±0.19
	liver	8.63±0.21	6.83±0.23	6.32±0.21	6.14±0.19
15	muscles	11.99±0.26	10.23±0.17	8.85±0.22	7.60±0.21
	liver	8.62±0.23	6.80±0.18	6.24±0.10	6.02±0.22
20	muscles	11.81±0.31	9.77±0.18	7.80±0.17	6.34±0.33
	liver	8.62±0.11	6.79±0.21	6.12±0.21	5.99±0.18
25	muscles	11.86±0.18	9.11±0.21	7.44±0.23	5.59±0.17

	liver	8.61±0.09	6.68±0.17	6.09±0.18	5.83±0.21
30	muscles	11.81±0.29	8.67±0.22	7.07±0.16	4.60±0.09
	liver	8.61±0.17	6.53±0.19	6.00±0.17	5.60±0.17

a = mean ± S.D.

Table 3: Changes in total amino acids (mg/100mg wet weight) in the muscles and liver of *Channa punctatus* exposed to cypermethrin

Exposure period in days	Tissue	Control	Concentration of cypermethrin in ppm		
			0.10	0.20	0.30
0	muscles	0.93±0.06[a]			
	liver	0.33±0.04[a]			
5	muscles	0.93±0.02	0.94±0.16	0.95±0.12	0.96±0.22
	liver	0.32±0.03	0.34±0.02	0.37±0.01	0.39±0.04
10	muscles	0.93±0.04	0.95±0.21	0.95±0.16	0.97±0.18
	liver	0.33±0.01	0.35±0.03	0.38±0.02	0.42±0.02
15	muscles	0.93±0.01	0.95±0.18	0.96±0.11	0.98±0.15
	liver	0.34±0.02	0.37±0.01	0.40±0.01	0.47±0.03
20	muscles	0.93±0.04	0.96±0.08	0.98±0.16	1.00±0.14
	liver	0.34±0.03	0.38±0.02	0.41±0.04	0.49±0.04
25	muscles	0.94±0.05	0.96±0.11	0.99±0.15	1.05±0.13
	liver	0.34±0.01	0.39±0.02	0.49±0.03	0.52±0.02
30	muscles	0.94±0.03	0.98±0.10	1.04±0.20	1.10±0.11
	liver	0.34±0.02	0.41±0.03	0.50±0.02	0.53±0.01

a = mean ± S.D.

Table 4: Changes in muscle glycogen (mg/100mg wet weight) in the muscles and liver of *Channa punctatus* exposed to cypermethrin

Exposure period in days	Tissue	Control	Concentration of cypermethrin in ppm		
			0.10	0.20	0.30
0	muscles	0.95±0.06[a]			
	liver	5.25±0.24[a]			
5	muscles	0.91±0.03	0.85±0.11	0.83±0.20	0.79±0.10
	liver	5.11±0.24	5.08±0.02	4.71±0.15	4.33±0.09
10	muscles	0.94±0.02	0.82±0.09	0.77±0.18	0.74±0.17
	liver	5.17±0.23	4.65±0.08	4.34±0.08	4.11±0.18
15	muscles	0.92±0.01	0.79±0.06	0.75±0.16	0.66±0.13
	liver	5.08±0.46	4.56±0.17	4.23±0.21	3.34±0.47
20	muscles	0.92±0.04	0.76±0.10	0.71±0.17	0.58±0.11
	liver	5.07±0.06	4.19±0.15	3.42±0.04	2.98±0.30
25	muscles	0.93±0.03	0.75±0.13	0.63±0.22	0.50±0.18
	liver	5.06±0.01	3.56±0.08	3.02±0.06	2.76±0.08
30	muscles	0.91±0.05	0.73±0.18	0.60±0.09	0.39±0.22
	liver	5.06±0.04	3.06±0.06	2.78±0.30	2.56±0.05

a = mean ± S.D.

Table 5: Changes in total fat (mg/100mg wet weight) in the muscles and liver of *Channa punctatus* exposed to cypermethrin

Exposure period in days	Tissue	Control	Concentration of cypermethrin in ppm		
			0.10	0.20	0.30
0	muscles	0.59±0.03[a]			
	liver	2.42±0.18[a]			
5	muscles	0.59±0.04	0.60±0.08	0.62±0.10	0.64±0.08
	liver	2.45±0.08	2.55±0.16	2.66±0.07	2.85±0.08
10	muscles	0.59±0.02	0.62±0.06	0.64±0.11	0.65±0.03
	liver	2.46±.0.05	2.68±0.08	2.96±0.08	3.08±0.06
15	muscles	0.59±0.03	0.63±0.07	0.65±0.09	0.68±0.08
	liver	2.47±0.03	2.92±0.06	3.12±0.04	3.27±0.06
20	muscles	0.59±0.04	0.66±0.09	0.67±0.06	0.69±0.10
	liver	2.45±0.10	3.07±0.12	3.21±0.09	3.40±0.07
25	muscles	0.59±0.02	0.68±0.05	0.68±0.11	0.75±0.02
	liver	2.46±0.06	3.17±0.09	3.27±0.13	3.94±0.07
30	muscles	0.59±0.03	0.69±0.04	0.74±0.05	0.77±0.06
	liver	2.49±0.09	3.21±0.05	3.86±0.07	4.16±0.05

a = mean ± S.D.

Table 6: Changes in Cholesterol (mg/100mg wet weight) in the muscles and liver of *Channa punctatus* exposed to cypermethrin

Exposure	Tissue	Control	Concentration of cypermethrin in ppm

period in days			0.10	0.20	0.30
0	muscles	0.17±0.01[a]			
	liver	0.44±0.02[a]			
5	muscles	0.17±0.04	0.19±0.01	0.20±0.06	0.22±0.06
	liver	0.43±0.03	0.43±0.07	0.44±0.06	0.45±0.06
10	muscles	0.17±0.05	0.19±0.02	0.24±0.02	0.25±0.07
	liver	0.43±0.02	0.43±0.08	0.45±0.03	0.49±0.03
15	muscles	0.17±0.03	0.20±0.01	0.23±0.01	0.27±0.05
	liver	0.43±0.04	0.44±0.06	0.49±0.04	0.49±0.02
20	muscles	0.17±0.03	0.21±0.05	0.24±0.06	0.29±0.02
	liver	0.43±0.02	0.48±0.05	0.50±0.03	0.51±0.02
25	muscles	0.17±0.05	0.24±0.04	0.28±0.09	0.31±0.07
	liver	0.43±0.03	0.49±0.07	0.51±0.02	0.51±0.03
30	muscles	0.17±0.04	0.28±0.05	0.32±0.04	0.34±0.04
	liver	0.43±0.02	0.49±0.06	0.51±0.05	0.52±0.02

a = mean ± S.D.

4. Conclusion

By observing these changes, it can be concluded that cypermethrin is showing toxicity in *Channa punctatus*. 0.30 ppm and lower concentrations of cypermethrin were safer for this fish than higher concentrations used as no mortality was recorded in this fish during 30 days.

Cypermethrin showed reducing trend in protein and glycogen levels studied in tissues of this fish, thus reducing the nutritive value of this fish. However, total amino acids, total fat and cholesterol exhibited increasing trend under all treatments.

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