

Effect of Lambda Cyhalothrin on the Activity of Enzymes Involved in Carbohydrate and Protein Metabolism

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ABSTRACT

An attempt has been made to study the toxic effects of a synthetic pyrethroid, lambda cyhalothrin in sub-lethal concentrations on the edible female catfish, *Clarias batrachus*. The fishes were exposed to the two different sub-lethal concentrations of the lambda cyhalothrin, a higher concentration of 5.768 ppm and a lower sub-lethal concentration of 2.884 ppm for a period of 45 days. Effect of lambda cyhalothrin on the activity of enzymes involved in metabolic pathways (carbohydrates and proteins) was analysed. The experimental analysis was carried out at regular intervals of 15, 30 and 45 days. A significant decline was also noticed in the total protein and protein bound sugar content of the various tissues under investigation in fishes of both experimental groups after 45 days of exposure to lambda cyhalothrin. The analysis showed initial increase in the activities of Glutamate dehydrogenase (GDH) and protease enzymes followed by a significant decline with the increase in the durations of exposure. Glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme activity decreases with increase in the duration of exposure indicating hampered metabolism leading to less NADPH production. A significant decline was also witnessed in the pyruvate content of the tissues of the pyrethroid-exposed fishes of both groups. Lactate content of the various tissues of the pyrethroid-exposed fishes showed a significant elevation after 45 days of exposure to the sub-lethal concentrations of the pyrethroid.

INTRODUCTION

Pesticides are the most important factor in improving agricultural production to sustain the greater supply of food, necessary to feed the growing population in developing countries like India. If the credits of pesticides include enhanced economic potential in terms of increased production of food and fiber, and amelioration of vector-borne diseases, then their debits have resulted in serious health implications to man and his environment. Uptake and accumulation of a pesticide by aquatic organisms seem to be more likely a function of habitat, habits, life cycle, and exchange equilibria than of food uptake; but they are also affected by many other factors, such as size of organism, pharmacokinetics and physical and chemical properties of the pesticide (Rosenberg, 1975).

In recent years, the application of pyrethroid pesticides for managing insect pests has increased significantly. In the aquatic environment, the aerobic and anaerobic breakdown

of pyrethroids often occurs at rates similar to those seen in soil (Laskowski, 2002).

Lambda cyhalothrin, a commonly used agricultural pyrethroid was registered by the USEPA in 1988. Lambda cyhalothrin is highly toxic to fishes and aquatic invertebrates. Recent studies have shown that pyrethroids may reach the aquatic bodies through sediment movement. Sediments contaminated by pyrethroids are of major concern today due to their wide spectrum aquatic toxicity (Gan et al., 2005). However, little is known about the toxicity of sediment associated pyrethroid residues to aquatic organisms despite the agricultural use of these compounds for more than two decades.

In recent times, aquaculture has been integrated into crop farming system as in the case of rice-paddy fields which are being used to produce a crop of fish. Aquaculture is the

answer to the growing fish demand and is one of the fastest growing sectors which provide an acceptable supplement or substitute to the wild fishes. Cat fishes which are extensively grown in rice paddy slurries provide additional source of income to the farmers (Teugels, 1984). The fishes raised in the paddy fields however face the threat of a direct exposure to toxicants used for pest control in the fields. However, fishes raised in commercial ponds may also possess the risk of exposure to various pesticides from the nearby fields which often pollute the pond (Nettleton et al., 1990). Catfishes being burrowers in habit are also likely to be exposed to the sediment bound pyrethroid compounds. Though the residence time of sediment-bound pyrethroids is usually some weeks but in case of repeated applications benthic organisms in particular may be chronically exposed. Hence, in the present study an attempt was made to assess the effect of lambda cyhalothrin on the activity of various intermediary metabolites at two different sub-lethal concentrations of lambda cyhalothrin on freshwater catfish, *Clarias batrachus*.

MATERIALS AND METHODS:

The technical grade synthetic pyrethroid, lambda cyhalothrin with 95% purity was obtained from Rallis India Ltd., Bangalore to test its effect on nutritionally important freshwater catfish, *Clarias batrachus*.

Healthy female catfishes, *Clarias batrachus* weighing 200-250 g and about 30-35 cms in length were chosen and sorted out into three groups of 25 fishes each. The fishes were examined carefully for any pathological symptoms and placed in dilute water containing 0.1 mg/l of potassium permanganate solution to avoid possibility of any dermal infection. They were maintained in tap water under ambient conditions of temperature and photoperiod. Group I served as the control while Group II and III were exposed to the two different sub-lethal concentrations of lambda cyhalothrin for a period of 45 days.

Group I: Control fishes maintained in dechlorinated toxicant free water.

Group II: Fishes maintained in higher sub-lethal concentration of the toxicant (5.768 ppm).

Group III: Fishes maintained in lower concentration sub-lethal concentration of the toxicant (2.884 ppm).

Control and experimental fish were fed minced goat liver daily, one hour before water renewal at 8:00 a.m. to remove nitrogenous waste and uneaten food. After water renewal, pesticide was added to maintain toxic concentration. Blood was collected from five fish per group on days 15, 30, and 45 by severing the caudal peduncle using a 5 ml syringe with minimal stress. Samples were transferred to EDTA vials; whole blood was used for hematological tests, and the rest was centrifuged at 10,000 rpm for 20 minutes. Plasma was stored at 20°C for biochemical and hormonal analysis.

ESTIMATION OF METABOLITES:

Assay of Specific Activity of Enzymes Involved in Carbohydrate and Protein Metabolism:

Specific activity of glutamate dehydrogenase (GDH):

Glutamate dehydrogenase was assayed in the tissues following the procedure of Delma Doherty (1970).

Specific activity of protease:

The activity of protease enzyme was assayed in the hepatic tissue following the procedure of Anson (1938).

Specific activity of malic enzyme:

The specific activity of Malic enzyme was assayed following the procedure Ochoa (1955).

Specific activity of glucose-6-phosphate dehydrogenase (G6PDH):

Glucose-6-phosphate dehydrogenase activity was assayed according to the method of Kornberg and Horecker (1955).

Estimation of pyruvate content:

Pyruvate content of the tissues was assayed following the procedure of Lu (1939).

Estimation of lactate content:

Lactate content of the tissues was assayed following the procedure of Harrower and Brown (1972).

RESULTS AND DISCUSSION:

The changes in the glutamate dehydrogenase (GDH) enzyme activity in the various tissues of the pyrethroid-exposed fishes are tabulated in table 1a & 1b.

The activity of glutamate dehydrogenase in the liver tissue of the experimental animal showed a significant elevation ($P < 0.05$) up the 30th day of exposure which was followed by a significant decline ($P < 0.05$) in the enzyme activity on 45th day of exposure to the two different sub-lethal concentrations of the pyrethroid. However, the values did not decline beyond the control values. Similar trend was witnessed in the muscle tissue of fishes of both experimental groups.

The ovarian tissue of the fishes exposed to the higher sub-lethal concentration of 5.768 ppm of lambda cyhalothrin showed a significant elevation ($P < 0.05$) of 35.52% in the GDH enzyme activity on the 30th day of exposure to the pyrethroid which was followed by a decline in the enzyme activity on the 45th day of exposure and the values reached near control levels. However, the fishes exposed to the lower sub-lethal concentration of 2.884 ppm showed a significant decline of 27.50% in the enzyme activity on the 45th day of exposure.

The heart and brain tissue of fishes of both experimental groups showed a similar trend of variation in the GDH activity. The initial significant increase ($P < 0.05$) in the GDH activity witnessed on the 30th day of exposure was followed by a significant decline ($P < 0.05$) on the 45th day when compared to the control group of fishes.

In the present study, the enhanced GDH activity in various tissues of the fishes exposed to the sublethal concentration of the pyrethroid might have resulted in efficient operation of oxidative deamination resulting in production of α -ketoglutarate. An enhancement in the activity of GDH was noticed in various tissues of *Clarias batrachus* exposed to trichlorfan (Shoba Rani and Janniah, 1991). Similar results were reported in the liver and muscle tissue of *Cyprinus carpio* when exposed to lead (Pugazhendy et al., 2005).

Table 2a & 2b depicts the changes in the protease enzyme activity of the fishes exposed to the two different sub-lethal concentrations of the pyrethroid for a period of 45 days.

In the liver tissue, a significant increase ($P < 0.05$) was witnessed in the protease activity on the 15th and 30th day of exposure to 5.768 ppm of lambda cyhalothrin in comparison to the control group of fishes. The increase was followed by a significant decline ($P < 0.05$) on the 45th day of exposure. The fishes exposed to the lower sub-lethal concentration of the pyrethroid also showed a similar trend.

The heart and brain tissues showed a similar trend of change in the protease enzyme activity in both the experimental groups. In both the tissues, the significant increase ($P < 0.05$) was witnessed in the enzyme activity only from the 30th day of exposure to the pyrethroid.

The decrease in the total protein level of all the tissues of the experimental animals exposed to sub-lethal concentrations of lambda cyhalothrin in the present study suggests that the high proteolytic activity corresponds to elevation of protease under stress conditions. Reddy et al. 1991 and Asztalos (1990) reported a significant elevation in the protease enzyme activity in the liver, brain and muscle tissue of fresh water fish, *Cyprinus carpio* subjected to fenvalerate and cypermethrin toxicity respectively.

The changes in the activity of malic enzyme in the various tissues of the pyrethroid-exposed fishes are tabulated (Table 3a & 3b).

All the tissues under study in the present investigation showed an overall significant decline ($P < 0.01$) in the malic enzyme activity in the experimental fishes when compared to the control group of fishes. The initial significant increase ($P < 0.05$) in the malic enzyme activity in the liver tissue observed on the 15th day of exposure was followed by a significant decline ($P < 0.05$) in the enzyme activity on the 30th and 45th day of exposure in both the groups of pyrethroid-exposed fishes. An overall decline of 23.71% and 18.69% was witnessed in the fishes exposed to the higher and lower sub-lethal concentration of the pyrethroid respectively at the end of the exposure period.

The muscle tissue of fishes of both experimental groups showed a different trend. A gradual and significant decline ($P < 0.05$) was witnessed in the enzyme activity throughout the exposure period in both groups of pyrethroid-exposed fishes in comparison to the control group of fishes. While the fishes exposed to the higher sub-lethal concentration of pyrethroid showed an overall decline of 73.41%, the fishes exposed to the lower sub-lethal concentration showed a decline of 47.35% in the enzyme activity.

The heart and brain tissues of the fishes exposed to the higher sub-lethal concentration of the pyrethroid showed an initial enhancement in the malic enzyme activity on the 15th day followed by a significant decline ($P < 0.05$) on the 30th and 45th day of exposure. However, in the fishes exposed to the lower sub-lethal concentration of the pyrethroid, a gradual and continuous decline was witnessed in the enzyme activity from the 15th day in both the tissues, while on the 30th and 45th day, a significant variation was noticed in the enzyme activity in comparison to the control group of fishes.

The ovarian tissue of fishes from both the experimental groups also showed a gradual and significant decline ($P < 0.05$) in the malic enzyme activity throughout the exposure period in comparison to the control group of fishes. While the fishes exposed to the higher sub-lethal concentration showed an overall decline of 50.17% in the enzyme activity, the fishes exposed to the lower sub-lethal concentration of the pyrethroid showed a decline of 36.84% at the end of the exposure period. Lorenzen et al. (1999) observed a significant decline in the malic enzyme activity in the embryos of herring gulls exposed to organochloride residues. Table 4a & Table 4b show the change in the glucose-6-phosphate dehydrogenase (G6PDH) activity of the fishes exposed to the two different sub-lethal concentrations of the pyrethroid for a period of 45 days.

The liver tissue of both groups of pyrethroid-exposed fishes showed a significant decline ($P < 0.05$) in the G6PDH activity when compared to the control group of fishes. Though the fishes exposed to higher sub-lethal concentration of the pyrethroid showed a significant decline ($P < 0.05$) in the enzyme activity throughout the period of exposure, the fishes exposed to the lower sub-lethal concentration of lambda cyhalothrin showed a significant decline only from the 30th day of exposure.

In the muscle tissue of fishes of both experimental groups, a significant decline ($P < 0.05$) in the enzyme activity comparison to the control group was witnessed only the 45th day of exposure. However, an overall significant decline 49.58% and 35.05% was witnessed in the glucose-6-phosphate dehydrogenase activity of the fishes exposed to the higher and lower sub-lethal concentrations of the pyrethroid respectively.

The ovarian tissue of the pyrethroid-exposed fishes also showed an overall significant decline ($P < 0.01$) in the enzyme activity. In comparison to the control group, an overall decline of 62.76% and 42.29% was witnessed in the G6PDH activity of the ovarian tissues of fishes exposed to the higher and lower sub-lethal concentration of the pyrethroid respectively.

In comparison to the control group, the heart and brain tissues of the pyrethroid-exposed fishes of both groups showed a similar pattern of decline in the G6PDH activity. An overall significant decline ($P < 0.01$) was witnessed in the enzyme activity of both the tissues in fishes of both experimental groups. A comparison of the enzyme activity at different durations of exposure also showed significant variation ($P < 0.05$) in the enzyme activity of the heart and brain tissues of fishes of both the experimental groups.

Increased levels of cortisol under the pyrethroid stress conditions in the present study could have also contributed to the decreased activities of G6PDH and malic enzyme since earlier observations have shown that corticosteroids are lipolytic action (Sheridan and Kao, 1998) the effect being exerted probably by the decline in the G6PDH and malic enzyme activity levels as witnessed in the lambda cyhalothrin exposed fishes in the present study.

The changes in the activity of pyruvate content of the various tissues analyzed in the present study depicted in Table 5a & 5b.

In concurrence with decline in the activity of the enzyme pyruvate dehydrogenase in the various tissues analyzed in the present study a significant ($P < 0.05$) was witnessed in the pyruvate content of the ovary, muscle, liver, heart and brain tissues of fishes exposed to both the sub-lethal concentrations of the pyrethroid in comparison to pyruvate content of the control group of animals. In both experimental groups, a decline of above 50% was witnessed in the pyruvate content of all the tissues of pyrethroid-exposed fishes.

The changes in lactate content of the various tissues of fishes exposed to the two different sub-lethal concentration of the pyrethroid are calculated. (Table 6a & 6b)

The ovarian, muscle, liver, heart and brain tissues of the experimental animals of both groups showed an overall significant elevation ($P < 0.01$) in the lactate content after 45 days of exposure to the sub-lethal concentrations of pyrethroid.

The fishes of the both the experimental groups witnessed the same trend in the changes of lactate content of the ovarian tissue. However, a significant variation ($P < 0.05$) was noted in the muscle lactate content of both groups of experimental fishes when compared to the control group.

The liver tissue of both groups of pyrethroid-exposed fishes showed a significant elevation in the lactate content ($P < 0.05$) in comparison to the control group. While the fishes exposed to the higher sub-lethal concentration of the pyrethroid showed an elevation of 70.32% of lactate content after 45 days of exposure to the pyrethroid, the fishes exposed to lower sub-lethal concentration witnessed an overall elevation of 67.63% in the lactate content.

The heart and brain tissues in fishes exposed to both the sub-lethal concentrations of the pyrethroid showed a significant enhancement ($P < 0.05$) in their lactate content on the 15th, 30th, and 45th day of exposure to the pyrethroid when compared to the control group of fishes. A comparison of the lactate content of these tissues at different durations of exposure also showed significant variation ($P < 0.05$) in both the experimental group of fishes.

CONCLUSION:

Thus, to reduce the environmental impact of pyrethroids such as lambda cyhalothrin, implementation of awareness programmes to farmers regarding the use pattern of these compounds is essential. Concrete efforts and feasible programme of action should be initiated to monitor the pesticide residues both in biotic and abiotic components of the environment. Usage of biopesticides should also be encouraged as an alternative to toxic chemicals.

TABLES:

Table 1a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on specific activity of glutamate dehydrogenase enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P Value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	7.54	0.000**	1.52 ^a ± 0.16	1.65 ^a ± 0.14 (+8.55)	2.06 ^b ± 0.26 (+35.52)	1.79 ^{ab} ± 0.23 (+17.76)	1.59 ^a ± 0.12
Muscle	89.16	0.000**	4.26 ^a ± 0.25	5.27 ^b ± 0.32 (+23.70)	6.47 ^d ± 0.20 (+51.87)	5.88 ^c ± 0.23 (+38.02)	4.64 ^a ± 0.12
Liver	92.85	0.000**	6.21 ^a ± 0.21	7.11 ^b ± 0.25 (+14.49)	8.49 ^d ± 0.23 (+36.71)	7.52 ^c ± 0.23 (+21.09)	6.76 ^b ± 0.15
Heart	56.03	0.000**	2.34 ^c ± 0.31	2.57 ^c ± 0.33 (+9.82)	3.42 ^d ± 0.23 (+46.15)	1.26 ^a ± 0.25 (-46.15)	1.84 ^b ± 0.17
Brain	148.00	0.000**	3.78 ^b ± 0.18	4.36 ^c ± 0.26 (+15.34)	5.66 ^d ± 0.21 (+49.73)	2.38 ^a ± 0.34 (-37.03)	3.42 ^b ± 0.19

Table 1b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on specific activity of glutamate dehydrogenase enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P Value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	9.3849	0.000**	1.60 ^{bc} ± 0.17	1.64 ^{bc} ± 0.15 (+2.50)	1.84 ^c ± 0.17 (+15.00)	1.16 ^a ± 0.25 (-27.50)	1.50 ^{ab} ± 0.24
Muscle	151.13	0.000**	4.26 ^b ± 0.16	4.68 ^c ± 0.15 (+9.85)	5.94 ^d ± 0.20 (+39.43)	3.22 ^a ± 0.25 (-24.41)	4.12 ^b ± 0.21
Liver	51.50	0.000**	6.52 ^b ± 0.20	6.74 ^b ± 0.30 (+3.37)	7.38 ^c ± 0.25 (+13.19)	5.29 ^a ± 0.22 (-18.84)	6.52 ^b ± 0.32
Heart	56.75	0.000**	2.19 ^b ± 0.23	2.56 ^b ± 0.18 (+16.89)	3.40 ^c ± 0.33 (+55.25)	1.35 ^a ± 0.33 (-38.35)	1.71 ^a ± 0.17
Brain	146.80	0.000**	3.66 ^b ± 0.20	4.15 ^c ± 0.24 (+13.38)	5.93 ^e ± 0.21 (+62.02)	4.92 ^d ± 0.25 (+34.42)	3.24 ^a ± 0.16

Values are Mean ± SD (n=6); the specific activity of enzyme was expressed as nanomoles of NADH oxidized/min/mg protein.

P value < 0.01-** denotes significance at 1% level (highly significant).

P value 0.011 to 0.05-* denotes significance at 5% level (significant).

P value > 0.05 - NS denotes non-significant.

Different alphabets in means between days in a row denote significance at 5% level.
 Means carrying at least one common superscript do not differ significantly ($P > 0.05$).
 Values in parentheses in experimental groups are % reduction (-) or % elevation (+) over control.

Table 2a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on specific activity of protease enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P Value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	28.65	0.000**	0.53 ^a ± 0.03	0.55 ^a ± 0.02 (+3.77)	0.61 ^b ± 0.02 (+15.09)	0.65 ^c ± 0.03 (+22.64)	0.55 ^a ± 0.02
Muscle	22.65	0.000**	0.56 ^{cd} ± 0.02	0.54 ^{bc} ± 0.03 (-3.57)	0.60 ^d ± 0.03 (+7.14)	0.47 ^a ± 0.03 (-16.07)	0.50 ^{ab} ± 0.02
Liver	119.10	0.000**	2.23 ^a ± 0.24	3.18 ^b ± 0.24 (+42.60)	4.97 ^d ± 0.23 (+122.80)	3.78 ^c ± 0.25 (+69.50)	2.90 ^b ± 0.20
Heart	8.58	0.000**	0.96 ^a ± 0.02	0.97 ^a ± 0.01 (+1.04)	1.40 ^b ± 0.24 (+45.85)	1.20 ^{ab} ± 0.21 (+25.00)	1.04 ^a ± 0.15
Brain	49.23	0.000**	2.42 ^a ± 0.20	2.61 ^{ab} ± 0.25 (+7.85)	4.07 ^d ± 0.26 (+68.18)	3.27 ^c ± 0.24 (+35.12)	2.82 ^b ± 0.19

Table 2b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on specific activity of protease enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	12.55	0.000**	0.51 ^a ± 0.03	0.54 ^{ab} ± 0.03 (+5.88)	0.59 ^{bc} ± 0.03 (+15.68)	0.61 ^c ± 0.02 (+19.60)	0.55 ^{ab} ± 0.02
Muscle	6.43	0.001**	0.55 ^b ± 0.02	0.56 ^b ± 0.02 (+1.81)	0.57 ^b ± 0.02 (+3.63)	0.53 ^{ab} ± 0.03 (-3.63)	0.51 ^a ± 0.02
Liver	41.15	0.000**	2.14 ^a ± 0.26	2.50 ^a ± 0.23 (+16.82)	3.50 ^b ± 0.25 (+63.55)	3.47 ^b ± 0.24 (+62.14)	2.53 ^a ± 0.20
Heart	6.03	0.001**	1.00 ^a ± 0.05	1.02 ^a ± 0.10 (+2.00)	1.35 ^b ± 0.29 (+35.00)	1.06 ^a ± 0.13 (+6.00)	0.98 ^a ± 0.05
Brain	6.97	0.000**	2.52 ^a ± 0.26	2.52 ^a ± 0.26 (+0)	3.13 ^b ± 0.27 (+24.20)	2.92 ^{ab} ± 0.27 (+15.87)	2.59 ^a ± 0.21

Values are Mean ± SD (n=6); the specific activity of enzyme was expressed as units/mg protein.

P value < 0.01 - ** denotes significance at 1% level (highly significant).

P value 0.011 to 0.05 - * denotes significance at 5% level (significant).

P value > 0.05 - NS denotes non-significant.

Different alphabets in means between days in a row denote significance at 5% level.

Means carrying at least one common superscript do not differ significantly ($P > 0.05$).

Values in parentheses in experimental groups are % reduction (-) or % elevation (+) over control.

Table 3a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on specific activity of malic enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P Value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	42.41	0.000**	5.82 ^d ± 0.39	5.10 ^{cd} ± 0.23 (-12.37)	3.94 ^b ± 0.75 (-32.30)	2.90 ^a ± 0.28 (-50.17)	5.04 ^c ± 0.28
Muscle	60.15	0.000**	4.74 ^c ± 0.33	3.68 ^b ± 0.54 (-22.36)	3.49 ^b ± 0.43 (-26.37)	1.26 ^a ± 0.42 (-73.41)	3.89 ^a ± 0.27
Liver	79.94	0.000**	12.44 ^c ± 0.48	13.37 ^d ± 0.38 (+7.47)	10.56 ^b ± 0.39 (-15.11)	9.49 ^a ± 0.46 (-23.71)	11.24 ^b ± 0.37
Heart	32.15	0.000**	2.58 ^{cd} ± 0.42	2.77 ^d ± 0.44 (+7.36)	1.81 ^b ± 0.23 (-29.84)	0.85 ^a ± 0.16 (-67.05)	1.89 ^b ± 0.30
Brain	22.93	0.000**	3.48 ^c ± 0.37	3.27 ^{bc} ± 0.31 (-6.03)	2.80 ^b ± 0.35 (-19.54)	1.95 ^a ± 0.25 (-43.96)	2.76 ^b ± 0.22

Table 3b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on specific activity of malic enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P Value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	51.40	0.000**	5.32 ^c ± 0.35	4.63 ^b ± 0.26 (-12.96)	3.73 ^a ± 0.23 (-29.88)	3.36 ^a ± 0.25 (-36.84)	4.61 ^b ± 0.23
Muscle	26.45	0.000**	4.35 ^d ± 0.32	3.54 ^{bc} ± 0.50 (-18.62)	3.27 ^b ± 0.35 (-24.82)	2.29 ^a ± 0.37 (-47.35)	3.93 ^{cd} ± 0.28
Liver	74.07	0.000**	12.41 ^c ± 0.35	13.30 ^d ± 0.36 (+7.17)	11.70 ^b ± 0.37 (-5.72)	10.09 ^a ± 0.36 (-18.69)	11.78 ^b ± 0.21
Heart	21.80	0.000**	2.49 ^c ± 0.32	2.39 ^{bc} ± 0.36 (-4.01)	1.85 ^b ± 0.20 (-25.70)	1.02 ^a ± 0.33 (-59.03)	1.69 ^b ± 0.42
Brain	30.90	0.000**	3.55 ^{ab} ± 0.42	4.51 ^c ± 0.43 (+27.04)	3.14 ^b ± 0.22 (-11.54)	2.38 ^a ± 0.35 (-32.95)	3.03 ^b ± 0.26

Values are Mean ± SD (n=6); the specific activity of enzyme was expressed as nanomoles of substrate converted/min/mg protein.

P value < 0.01-** denotes significance at 1% level (highly significant).

P value 0.011 to 0.05-* denotes significance at 5% level (significant).

P value > 0.05 - NS denotes non-significant.

Different alphabets in means between days in a row denote significance at 5% level.

Means carrying at least one common superscript do not differ significantly (P>0.05).

Values in parentheses in experimental groups are % reduction (-) or % elevation (+) over control.

Table 4a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on specific activity of glucose 6-phosphate dehydrogenase enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P Value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	62.45	0.000**	4.39 ^d ±0.43	3.51 ^c ±0.42 (-20.04)	2.27 ^b ±0.43 (-48.29)	1.23 ^a ±0.25 (-71.98)	3.59 ^c ±0.38
Muscle	10.76	0.000**	2.40 ^b ±0.33	2.39 ^b ±0.49 (-0.41)	2.28 ^b ±0.36 (-5.00)	1.21 ^a ±0.37 (-49.58)	2.07 ^b ±0.27
Liver	618.98	0.000**	23.68 ^c ±0.35	22.54 ^d ±0.45 (-4.81)	16.38 ^b ±0.41 (-30.82)	14.48 ^a ±0.42 (-38.85)	20.10 ^c ±0.28
Heart	105.36	0.000**	5.41 ^d ±0.40	4.88 ^{cd} ±0.40 (-9.79)	3.42 ^b ±0.40 (-36.78)	1.31 ^a ±0.42 (-75.78)	4.38 ^c ±0.29
Brain	307.56	0.000**	16.33 ^d ±0.43	14.53 ^c ±0.43 (-11.02)	13.67 ^b ±0.46 (-16.28)	8.42 ^a ±0.41 (-48.43)	14.35 ^{bc} ±0.35

Table 4b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on specific activity of glucose 6-phosphate dehydrogenase enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	53.11	0.000**	4.78 ^c ±0.52	4.32 ^c ±0.42 (-9.62)	2.46 ^a ±0.38 (-48.55)	1.78 ^a ±0.42 (-62.74)	3.42 ^b ±0.36
Muscle	5.50	0.002**	2.51 ^b ±0.46	2.50 ^b ±0.45 (-0.39)	2.29 ^{ab} ±0.35 (-8.76)	1.63 ^a ±0.46 (-35.05)	1.89 ^{ab} ±0.28
Liver	389.74	0.000**	23.41 ^c ±0.36	23.27 ^c ±0.44 (-0.59)	19.85 ^b ±0.40 (-15.20)	15.48 ^a ±0.43 (-33.87)	20.48 ^b ±0.36
Heart	50.87	0.000**	5.60 ^d ±0.46	4.84 ^c ±0.35 (-13.57)	4.12 ^b ±0.47 (-26.42)	2.40 ^a ±0.45 (-57.14)	4.32 ^{bc} ±0.27
Brain	215.63	0.000**	16.83 ^a ±0.38	15.33 ^c ±0.42 (-8.91)	14.51 ^b ±0.38 (-13.78)	10.54 ^a ±0.44 (-37.37)	14.80 ^{bc} ±0.32

Values are Mean ± SD (n=6); the specific activity of enzyme was expressed as μmoles of NADP+ reduced/minute/mg protein.

P value < 0.01-** denotes significance at 1% level (highly significant).

P value 0.011 to 0.05-* denotes significance at 5% level (significant).

P value > 0.05 - NS denotes non-significant.

Different alphabets in means between days in a row denote significance at 5% level.

Means carrying at least one common superscript do not differ significantly (P>0.05).

Values in parentheses in experimental groups are % reduction (-) or % elevation (+) over control.

Table 5a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.678 ppm) on pyruvate content in various tissues of *Clarias batrachus*.

Tissue	F Value	P Value	Control	Experimental days			
				15	30	45	Recovery
Ovary	74.35	0.000**	2.10 ^d ± 0.24	0.97 ^b ± 0.22 (-53.80)	0.58 ^a ±0.18 (-72.38)	0.45 ^a ± 0.18 (-78.57)	1.68 ^c ± 0.19
Muscle	45.69	0.000**	2.16 ^c ± 0.27	0.99 ^a ± 0.24 (-54.16)	0.91 ^a ± 0.19 (-57.87)	0.66 ^a ± 0.17 (-69.44)	1.42 ^b ± 0.17
Liver	100.42	0.000**	3.76 ^d ± 0.24	2.29 ^c ± 0.24 (-39.09)	1.66 ^b ± 0.22 (-55.85)	1.23 ^a ± 0.25 (-67.28)	2.55 ^c ± 0.23
Heart	92.87	0.000**	4.59 ^d ± 0.35	3.85 ^c ± 0.28 (-16.12)	3.06 ^b ± 0.24 (-33.33)	1.57 ^a ± 0.19 (-65.79)	2.75 ^b ± 0.36
Brain	78.63	0.000**	3.87 ^d ± 0.22	3.06 ^d ± 0.28 (-20.93)	2.20 ^b ± 0.21 (-43.15)	1.27 ^a ± 0.34 (-67.18)	2.90 ^c ± 0.27

Table 5b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on pyruvate content in various tissues of *Clarias batrachus*.

Tissue	F Value	P Value	Control	Experimental days			
				15	30	45	Recovery
Ovary	45.87	0.000**	2.11 ^d ± 0.23	1.58 ^c ± 0.21 (-25.11)	1.06 ^b ±0.23 (-49.76)	0.56 ^a ± 0.16 (-73.45)	1.78 ^{cd} ± 0.26
Muscle	26.86	0.000**	2.17 ^c ± 0.27	1.80 ^{bc} ± 0.25 (-17.05)	1.07 ^a ± 0.24 (-50.69)	0.94 ^a ± 0.24 (-56.68)	1.68 ^b ± 0.21

Liver	72.70	0.000**	3.62 ^d ± 0.26	2.76 ^c ± 0.22 (-23.75)	2.01 ^b ± 0.27 (-44.47)	1.31 ^a ± 0.29 (-63.81)	2.68 ^c ± 0.20
Heart	94.64	0.000**	4.62 ^d ± 0.23	4.18 ^c ± 0.17 (-9.52)	3.33 ^b ± 0.27 (-27.92)	2.22 ^a ± 0.24 (-51.94)	3.84 ^c ± 0.24
Brain	150.89	0.000**	3.76 ^d ± 0.140	3.22 ^c ± 0.18 (-19.36)	2.37 ^b ± 0.17 (-36.96)	1.34 ^a ± 0.23 (-64.36)	3.08 ^c ± 0.20

Values are Mean ± SD (n=6); Values are expressed as mg protein/ml enzyme.

P value < 0.01 - ** denotes significance at 1% level (highly significant)

P value 0.011 to 0.05 - * denotes significance at 5% level (significant)

P value > 0.05 – NS denotes non-significant

Different alphabets in means between days in a row denote significance at 5% level.

Means carrying at least one common superscript do not differ significantly (P>0.05)

Values in parentheses in experimental groups are % reduction (-) or % elevation (+) over control

Table 6a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on lactate content in various tissues of *Clarias batrachus*

Tissue	F Value	P Value	Control	Experimental days			
				15	30	45	Recovery
Ovary	26.98	0.000**	3.10 ^a ± 0.27	3.26 ^a ± 0.25 (+5.16)	4.02 ^b ± 0.20 (+29.67)	4.32 ^b ± 0.28 (+39.35)	3.29 ^a ± 0.25
Muscle	12.94	0.000**	2.13 ^a ± 0.29	2.45 ^{ab} ± 0.23 (+15.02)	2.89 ^{bc} ± 0.23 (+35.68)	3.17 ^c ± 0.32 (+48.82)	2.42 ^{ab} ± 0.33
Liver	20.64	0.000**	1.82 ^a ± 0.26	2.65 ^b ± 0.28 (+45.60)	2.78 ^{bc} ± 0.23 (+52.74)	3.10 ^c ± 0.22 (+70.32)	2.34 ^b ± 0.31
Heart	45.50	0.000**	2.49 ^a ± 0.24	2.99 ^b ± 0.09 (+20.08)	3.51 ^c ± 0.24 (+40.96)	4.02 ^d ± 0.25 (+61.44)	2.99 ^b ± 0.20
Brain	63.14	0.000**	3.02 ^a ± 0.20	3.67 ^b ± 0.19 (+21.52)	4.51 ^c ± 0.24 (+49.33)	5.00 ^d ± 0.26 (+65.56)	4.04 ^b ± 0.28

Table 6b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on lactate content in various tissues of *Clarias batrachus*

Tissue	F Value	P Value	Control	Experimental days			
				15	30	45	Recovery
Ovary	26.30	0.000**	3.14 ^a ± 0.24	3.43 ^a ± 0.20 (+9.23)	4.08 ^b ± 0.26 (+29.93)	4.32 ^b ± 0.14 (+37.57)	3.45 ^a ± 0.31
Muscle	5.83	0.000**	2.35 ^a ± 0.61	2.60 ^a ± 0.28 (+10.63)	3.00 ^b ± 0.22 (+27.65)	3.10 ^b ± 0.27 (+31.91)	2.38 ^a ± 0.24
Liver	27.80	0.000**	2.07 ^a ± 0.24	2.79 ^{bc} ± 0.21 (+34.78)	3.03 ^c ± 0.25 (+46.37)	3.47 ^d ± 0.24 (+67.63)	2.47 ^{ab} ± 0.29
Heart	39.06	0.000**	2.48 ^a ± 0.21	2.93 ^a ± 0.14 (+18.14)	3.33 ^b ± 0.16 (+34.27)	3.78 ^c ± 0.20 (+52.41)	2.95 ^b ± 0.23
Brain	103.80	0.000**	3.01 ^a ± 0.23	3.64 ^b ± 0.15 (+20.09)	4.24 ^c ± 0.13 (+40.86)	5.01 ^d ± 0.18 (+66.44)	3.81 ^b ± 0.18

Values are Mean \pm SD (n=6); Values are expressed as mg/g wet tissue.

P value < 0.01 - ** denotes significance at 1% level (highly significant)

P value 0.011 to 0.05 - * denotes significance at 5% level (significant)

P value > 0.05 – NS denotes non-significant

Different alphabets in means between days in a row denote significance at 5% level.

Means carrying at least one common superscript do not differ significantly (P>0.05)

Values in parentheses in experimental groups are % reduction (-) or % elevation (+) over control

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