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EFFECT OF LAMBDA CYHALOTHRIN ON THE ACTIVITY OF GLYCOLYTIC PATHWAY AND MITOCHONDRIAL OXIDATIVE ENZYMES

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ABSTRACT

The application of pyrethroids as an insecticide and anti parasitary preparations has markedly increased. Fishes are extremely susceptible to these substances. An important factor is the rapid absorption of their accessibility, thus also the risk for aquatic organisms. The present study is a contribution to the assessment of toxicity and effects of lambda cyhalothrin on a benthic species, *Clarias batrachus*. Effect of lambda cyhalothrin on the activity of glycolytic pathway and mitochondrial oxidative enzymes were studied. Three groups of 25 fishes each were selected for the study. Specific activity of pyruvate kinase at different durations of exposure to the pyrethroid showed significant variation. Specific activity of citrate synthase after 45 days of exposure, the enzyme activity of the hepatic tissue of fishes of both experimental groups remained above the control levels. Specific activity of α-Ketoglutarate dehydrogenase in the various tissues viz., ovary, muscle, liver, heart and brain of the pyrethroid-exposed fishes showed a significant decrease ($P<0.05$) by the end of the exposure period of 45 days when compared to the control group. In fishes exposed to the higher sub-lethal concentration of lambda cyhalothrin, the ovary, muscle, liver, heart and brain tissues showed a significant decline ($P<0.05$) in the SDH enzyme activity at all durations of exposure to the pyrethroid in comparison to the control of animals. In the present study, the synthetic pyrethroid lambda cyhalothrin showed a significant inhibition of the malate dehydrogenase activity of the various tissues of *Clarias batrachus* even at sub-lethal concentrations. The liver tissue of pyrethroid-exposed fishes of both groups showed a gradual and significant decline ($P<0.05$) in the ATP levels throughout the exposure period when compared to the control group of fishes.

INTRODUCTION

The impact of pesticides on growth, survival and fertility of fishes is an important concern today since pesticides hamper the normal physiological processes of the organisms. Fishes play a significant role in aquaculture, serving as an excellent source of protein for humans.

There has been a dramatic increase in the recent years in the use of pyrethroid pesticides to control insect pests. In the aquatic medium, the aerobic and anaerobic degradation of pyrethroids often continues at rates similar to that displayed in soil (Laskowski, 2002).

were measured using 5 wide mouth 2 L. flasks. Each flask Owing to the excessive use of synthetic pyrethroids today, the environment and water resources are being rapidly polluted thus endangering aquatic life directly and human life indirectly (Hill,

MATERIALS AND METHODS:

Freshwater female catfish, *Clarias batrachus* locally known as Magur, weighing 200-250 g and about 30-35cms in length were chosen and sorted out into three groups of 25 fishes. 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*.

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

1989). Synthetic pyrethroids are neuropoisons acting on axons in the peripheral and central nervous system by interacting with voltage dependent sodium channels as well as other ion channels (Bradbury and Coats, 1989). They also inhibit ATPase associated with the active transport mechanisms (Hutson, 1979) and hence affect the ion movement and osmoregulatory mechanisms of organisms and break down the critical concentration gradient leading to the death of organisms (Clark and Mastumara, 1982).

The present study is an attempt to assess the effect of lambda cyhalothrin on the activity of glycolytic pathway and mitochondrial oxidative enzymes at two different sub-lethal concentration of lambda cyhalothrin on freshwater catfish, *Clarias batrachus*.

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).

The control and experimental animals were fed daily with fresh pieces of minced goat liver. Food was given one hour prior to water renewal. Water was renewed daily at 8.00 hrs which facilitated the removal of nitrogenous waste excreted by the test fishes and for the removal of unconsumed food. After renewal of water the required quantity of the pesticide was added to maintain the toxic concentration of the water medium. At the end of every of 15-, 30- and 45-days blood was collected from five fishes each of the control and test groups by severing the caudal peduncle using 5 ml graduated hypodermic syringe fitted with 2.4 G x 1" needle causing minimum stress to the fishes.

ASSAY OF SPECIFIC ACTIVITY OF ENZYMES OF GLYCOLYTIC PATHWAY:

Specific activity of pyruvate kinase:

The activity of pyruvate kinase was assayed following the procedure of Cardenas and Dyson (1973)

ASSAY OF SPECIFIC ACTIVITY OF MITOCHONDRIAL OXIDATIVE

ENZYMES AND ESTIMATION OF ATP:

Specific activity of pyruvate dehydrogenase (PDH):

The specific activity of pyruvate dehydrogenase was assayed following the method of Hinman and Blass (1981)

Specific activity of citrate synthase:

The activity of citrate synthase was assayed by the method of Srere. (1963)

Specific activity of α-Ketoglutarate Dehydrogenase:

The activity of α-Ketoglutarate dehydrogenase was assayed by the method of Reed and Mukherjee (1969)

Specific activity of succinate dehydrogenase (SDH):

The activity of succinate dehydrogenase was assayed according to the method of slater and Boon (1952)

Specific activity of malate dehydrogenase (MDH):

The activity of malate dehydrogenase was assayed by the method of Mehler et al. (1948).

Estimation of ATP:

ATP concentration was measured by the method of Williamson and Corkey (1969)

RESULTS AND DISCUSSIONS:

The use of biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fishes. In toxicological studies of acute and chronic exposure, changes in concentration and activity of enzymes often directly reflect cell damage in specific organs (Casillas et al., 1983). Any impairment in the oxidative metabolic pathway leads to a depression in the metabolic rate of the animal favoring anaerobic metabolism over aerobic oxidation. Mitochondrial enzyme levels are an important determinant of aerobic capacity for ATP production. (Moyes et al., 1990).

Changes in the activity of pyruvate kinase from the various tissues analyzed in the present study are tabulated in table 1a & 1b.

In comparison to the second group of fishes, a significant decline ($p<0.05$) was witnessed in the pyruvate kinase activity of the muscle, liver, heart and brain tissues of fishes on the 15th, 30th , and 45th day of exposure to the higher sub-lethal concentration of the pyrethroid. In the ovarian tissues, though no significant change was witnessed in the enzyme activity on the 15th day of exposure, the 30th and 45th day of exposure showed a significant decline ($p<0.05$) in the enzyme activity in comparison to the control. A comparison of the enzyme activity at different durations of exposure to the pyrethroid also showed significant variation ($p<0.05$) in the enzyme activity of all the tissues.

The changes in the activity of citrate synthase in the various tissues of fishes exposed to the two different sub-lethal concentration of the pyrethroid are tabulated Table 2a and 2b.

The tissues showed a varied trend in the changes in the citrate synthase activity after exposure to the pyrethroid. The ovarian tissue of the fishes of both experimental groups showed a significant decline ($P<0.05$) in the citrate synthase activity on the 30th and 45th day of exposure to the pyrethroid. Though fishes of both experimental groups showed a decline the enzyme activity on the 15th day of study, it was found to be a non-significant change when compared to control group of fishes.

The muscle, heart and brain tissues of the fishes exposed to both the sub-lethal concentration of pyrethroid showed a significant decline ($P<0.05$) in the enzyme activity all throughout the exposure to the pyrethroid. In comparison to the control group, the experimental fishes showed a significant decline ($P<0.05$) on the 15th, 30th, and 45th day of exposure.

However, the hepatic tissue showed a varied trend in the changes in the enzyme activity. The experimental fishes of both groups, showed an initial significant increase ($P<0.05$) in the enzyme activity on the 15th day of exposure, which was followed by a decline on the 30th and 45th day. However, even after 45 days of exposure, the enzyme activity of the hepatic tissue of fishes of both experimental groups remained above the control levels.

The alterations in the α-ketoglutarate dehydrogenase enzyme activity in the various tissues of the pyrethroid-exposed fishes are tabulated (Table 3a & 3b).

The activity of α-Ketoglutarate dehydrogenase enzyme in the various tissues viz., ovary, muscle, liver, heart and brain of the pyrethroid-exposed fishes showed a significant decrease ($P<0.05$) by the end of the exposure period of 45 days when compared to the control group.

In fishes exposed to the higher sub-lethal concentration of the pyrethroid, the liver tissue showed an increase of 13.12% in the enzyme activity on the 30th day of exposure which was followed by a decline of 24.27% on the 45th day of exposure. However, in fishes exposed to the lower sub-lethal concentration of the pyrethroid, an increase of 6.88% was witnessed in the enzyme activity on the 15th day of exposure while the 30th day showed a significant decline of 5.19% in the enzyme activity when compared to the control group of fishes.

A similar trend was witnessed in the changes in the enzyme activity of the muscle tissue of pyrethroid-exposed fishes of both groups. An initial significant increase ($P<0.05$) in the enzyme activity on the 15th day of exposure was followed by a decline in the enzyme activity on the 30th and 45th day of exposure in both the experimental group of fishes. The ovarian tissue from fishes of both experimental groups also showed a similar trend of change in the activity of α-Ketoglutarate dehydrogenase. While the fishes exposed to the higher sub-lethal concentration of 5.768 ppm of lambda cyhalothrin showed and overall decline of 32.35% in the enzyme activity after 45 days of exposure to the pyrethroid, while the fishes exposed to the lower sub-lethal concentration of 2.884 ppm showed an overall decline of 17.58% at the end of the exposure period.

The heart and brain tissues of the pyrethroid exposed fishes of both groups also showed an overall significant ($P<0.01$) decline in the enzyme activity. In both tissues the enzyme activity was found to be significantly elevated ($P<0.05$) on the 15th day of exposure followed by a significant decline ($P<0.05$) on the 30th and 45th day of exposure to the higher sub-lethal concentration of the pyrethroid. Though a similar trend was witnessed in the heart and brain tissues of fishes exposed to the lower sub-lethal concentration of the pesticide, the heart tissue showed a significant change in the enzyme activity ($P<0.05$) only on the 45th day of exposure to the pyrethroid. However, the brain tissue showed significant variation ($P<0.05$) throughout the exposure period from the 15th day of exposure till the end of the exposure period of 45 days.

The alpha-ketoglutarate dehydrogenase complex is a key and arguably rate-limiting enzyme of the Krebs cycle (Huang et al., 2003) and showed a significant decline in activity in various tissues of the pyrethroid-exposed fishes. In correlation with results of the present study, a significant decline in the α-ketoglutarate dehydrogenase activity of the hepatopancreas and ovarian tissue of crab, Scylla serrate under conditions of naphthalene toxicity was also reported by Vijayavel and Balasubramanian (2006).

The changes in the succinate dehydrogenase (SDH) enzyme activity in the various tissues of the pyrethroid-exposed fishes are tabulated (Table 4a & 4b).

In fishes exposed to the higher sub-lethal concentration of lambda cyhalothrin, the ovary, muscle, liver, heart and brain tissues showed a significant decline ($P<0.05$) in the SDH enzyme activity at all durations of exposure to the pyrethroid in comparison to the control of animals. A comparison of the enzyme activity at different durations of exposure in the ovary, muscle, liver and heart tissues also showed significant variation ($P<0.05$). However, the enzyme activity of the brain tissue did not show any significant variation between the 15th and 30th day of exposure to the higher sub-lethal concentration of the pyrethroid.

On exposure to the lower sub-lethal concentration of the pyrethroid, only the ovarian, hepatic and cardiac tissues showed significant decline ($P<0.05$) in the enzyme activity from the 15th day of exposure to the pyrethroid, while in the muscle and brain significant decline ($P<0.05$) was witnessed only from the 30th day of exposure to the pyrethroid.

In both the experimental groups, the hepatic tissue showed the highest decline of 73.89% and 51.01% in the enzyme activity when exposed to the higher and lower sub-lethal concentrations of the pyrethroid respectively.

The activity of SDH may be taken as an indication of the level of operation of the TCA cycle (Bhagyalakshmi et al., 1984). The decline in the SDH levels of the liver, muscle, ovary, heart and brain tissues of *Clarias batrachus* in response to pyrethroid intoxication in the present study is in correlation with earlier findings of Singh and Srivastava (1982), who reported a significant decline in the activities of SDH in the brain, liver and muscle tissue of *Heteropneustes fossilis* exposed to endosulfan

Table 5a & 5b shows the changes in the malate dehydrogenase activity of the fishes exposed to the two different sub-lethal concentrations of the pyrethroid for a period of 45 days.

In fishes exposed to the higher sub-lethal concentration of the pyrethroid, lambda cyhalothrin, the MDH activity was found to be significantly elevated ($P<0.05$) in the liver and brain tissue on the 15th day of exposure which was followed by a significant decline ($P<0.05$). After 45 days of exposure the liver and brain tissues showed a decline of 54.06% and 34.90% respectively in the enzyme activity. A similar significant change ($P<0.05$) was witnessed in the MDH activity of the liver and brain tissues of fishes exposed to the lower sub-lethal concentration of pyrethroid.

In the ovarian tissue, significant elevation ($P<0.05$) was witnessed in the enzyme activity up to the 30th day of exposure, which was followed by a decline as witnessed by the enzyme activity on the 45th day of the exposure period. The MDH activity of the ovarian tissue still showed an elevation of only 26.95% over the control on the 45th day of exposure, in contrast to the elevation of 56.70% witnessed on the 30th day of exposure. Similarly, in fishes exposed to the lower sub-lethal concentration of the pyrethroid, an overall significant elevation ($P<0.05$) of 43% was witnessed in the enzyme activity up to the 30th day of exposure, which followed by a decline in the enzyme activity. Despite the decline witnessed, the enzymatic activity on the 45th day of exposure to the pyrethroid still remained significantly elevated ($P<0.05$) over the control values.

The cardiac tissue of fishes of both experimental groups also showed a similar trend of significant change ($P<0.05$) in the enzymatic activity like the ovarian tissue. The increase in the MDH activity witnessed up to the 30th day of exposure to the pyrethroid was followed by a significant decline, though the enzyme activity on the 45th day of exposure continued to remain significantly elevated beyond the control values in both groups of pyrethroid-exposed fishes.

The muscle tissue of fishes of both experimental groups, also showed an initial elevation in the MDH activity up to the 15th day of exposure which was followed by a significant decline ($P<0.05$) on the 30th and 45th day of exposure to pyrethroid. Also an overall significant change ($P<0.01$) was witnessed in the MDH activity of the muscle tissue of fishes of both experimental groups.

In the present study, the synthetic pyrethroid lambda cyhalothrin showed a significant inhibition of the malate dehydrogenase activity of the various tissues of *Clarias batrachus* even at sub-lethal concentrations. Endusulfan, an organochloride pesticide was reported to have decreased the malate dehydrogenase (MDH) activity in the muscle tissue of *Clarias batrachus* demonstrating a non-competitive enzyme inhibitory mechanism due to the enzyme-substrate-endosulfan complexing (Mishra and Shukla, 2003).

The changes in the level of ATP in the various tissues of the pyrethroid-exposed fishes are tabulated (Table 6a & 6b).

The liver tissue of pyrethroid-exposed fishes of both groups showed a gradual and significant decline ($P<0.05$) in the ATP levels throughout the exposure period when compared to the control group of fishes. An overall decline 64.68% and 52.60% was witnessed in the ATP levels of the hepatic tissue of the fishes exposed to the higher and lower sub-lethal concentrations of the pyrethroid respectively.

The muscle tissue from fishes of both groups again showed a similar trend of significant decline ($P<0.05$) in the ATP levels in comparison to the control group of fishes. A comparison made between the different days of exposure to the pyrethroid, also showed a significant variation ($P<0.05$) in the muscle ATP content in fishes of both experimental groups.

In the ovarian tissue, though the fishes exposed to the higher sub-lethal concentration of the pyrethroid showed significant decline in the ATP levels from the 15th day of exposure, a significant decline was witnessed in the ATP levels of the fishes exposed to the lower sub-lethal concentration only from the 30th day of exposure to the pyrethroid. However, both experimental groups showed an overall significant decline ($P<0.01$) in the ATP levels.

In the cardiac tissue of fishes exposed to the higher sub-lethal concentration of lambda cyhalothrin a significant decline ($P<0.05$) was witnessed in the ATP level from the 15th day of exposure in comparison to the control group of fishes. However, a different trend was witnessed in the fishes exposed to the lower sub-lethal concentration of the pyrethroid. A non-significant decline of 5.53% was witnessed in the ATP levels of the cardiac tissues of the fishes up to the 30th day of exposure. However, an overall significant decline ($P<0.01$) was witnessed in the ATP levels by the end of the exposure period of 45 days.

In the brain tissue of fishes of both experimental groups, an overall significant decline ($P<0.01$) was witnessed in the ATP levels in comparison to the control group of fishes. While the fishes exposed to the higher sub-lethal concentration of lambda cyhalothrin showed a decline of 63.49% in the brain ATP levels after 45 days of exposure to the pyrethroid, the fishes exposed to the lower sub-lethal concentration of pyrethroid showed an overall decline of 38.89%.

Tables:

Table 1a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.678 ppm) on activity of pyruvate kinase enzyme in various tissues of *Clarias batrachus*.

Tissue	F Value	P Value	Control	Experimental days			
				15	30	45	Recovery
Ovary	37.84	0.000**	$3.23^{c} \pm 0.34$	$3.79^{c} \pm 0.29$ (+17.33)	$2.46^{b} \pm 0.36$ (-23.83)	$1.61^{a} \pm 0.39$ (-50.15)	$2.52^{b} \pm 0.24$

Muscle	403.69	0.000**	19.64^{d} ± 0.78	24.31^{e} ± 0.41 (+23.77)	18.29^{c} ± 0.36 (-6.87)	14.10^{a} ± 0.29 (-28.20)	16.13^{b} ± 0.36
Liver	440.59	0.000**	33.70^{c} ± 0.45	36.53^{d} ± 0.43 (+8.39)	31.85^{b} ± 0.29 (-5.48)	27.35^{a} ± 0.40 (-18.84)	32.17^{b} ± 0.35
Heart	70.89	0.000**	3.17^{c} ± 0.39	4.22^{d} ± 0.32 (+33.12)	2.52^{b} ± 0.41 (-20.50)	1.19^{a} ± 0.28 (-62.46)	2.40^{b} ± 0.14
Brain	215.91	0.000**	6.25^{c} ± 0.38	7.97^{d} ± 0.45 (+27.52)	4.82^{b} ± 0.26 (-22.88)	2.82^{a} ± 0.25 (-54.88)	4.77^{b} ± 0.19

Table 1b: Effect of lambda cyhalothrin at lower sub-lethal concentration (5.678 ppm) on activity of pyruvate kinase enzyme in various tissues of *Clarias batrachus*.

Tissue	F Value	P Value	Control	Experimental days			
				15	30	45	Recovery
Ovary	30.96	0.000**	3.43^{c} ± 0.40	3.86^{c} ± 0.16 (+12.53)	2.37^{ab}±0.39 (-30.90)	1.95^{a} ± 0.40 (-43.15)	2.52^{b} ± 0.24
Muscle	283.40	0.000**	19.42^{c} ± 0.36	23.50^{e} ± 0.43 (+21.00)	20.99^{d} ± 0.32 (+8.08)	14.10^{a} ± 0.29 (-28.20)	16.13^{b} ± 0.36
Liver	475.10	0.000**	33.53^{d} ± 0.54	36.85^{e} ± 0.46 (+9.90)	30.18^{b} ± 0.29 (-9.99)	27.35^{a} ± 0.40 (-18.84)	32.17^{b} ± 0.35
Heart	51.07	0.000**	3.06^{c} ± 0.37	3.34^{c} ± 0.34 (+9.15)	2.05^{b} ± 0.19 (-33.00)	1.19^{a} ± 0.28 (-62.46)	2.40^{b} ± 0.14
Brain	169.35	0.000**	5.49^{d} ± 0.40	8.02^{e} ± 0.50 (+46.08)	4.15^{b} ± 0.37 (-24.40)	2.82^{a} ± 0.25 (-54.88)	4.77^{b} ± 0.19

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

Tissue	F Value	P value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	17.20	0.000**	3.51^{b} ± 0.11	3.44^{b} ± 0.16 (-1.99)	3.03^{a} ± 0.14 (-13.67)	2.92^{a} ± 0.18 (-16.80)	3.08^{a} ± 0.17
Muscle	578.37	0.000**	6.13^{d} ± 0.22	4.84^{b} ± 0.20 (-21.04)	2.20^{a} ± 0.15 (-64.11)	1.89^{a} ± 0.22 (-69.16)	5.42^{c} ± 0.19
Liver	258.53	0.000**	13.89^{b} ± 0.18	16.35^{d} ± 0.23 (+17.71)	15.41^{c} ± 0.18 (+10.94)	14.21^{b} ± 0.24 (+2.30)	12.91^{a} ± 0.17
Heart	52.75	0.000**	1.97^{b} ± 0.12	1.13^{a} ± 0.21 (-42.63)	0.98^{a} ± 0.13 (-50.25)	0.96^{a} ± 0.19 (-51.26)	1.28^{a} ± 0.13
Brain	330.22	0.000**	9.59^{e} ± 0.16	8.70^{d} ± 0.16 (-9.28)	7.01^{b} ± 0.12 (-26.99)	6.54^{a} ± 0.16 (-31.80)	8.27^{c} ± 0.22

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

Tissue	F Value	P value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	9.24	0.000**	3.48^{c} ± 0.10	3.22^{bc} ± 0.27 (-7.47)	3.08^{ab} ± 0.23 (-11.49)	2.81^{a} ± 0.19 (-19.25)	3.05^{ab} ± 0.15
Muscle	186.43	0.000**	6.38^{d} ± 0.19	5.86^{e} ± 0.23 (-8.15)	4.58^{b} ± 0.17 (-28.21)	4.03^{a} ± 0.51 (-36.83)	5.71^{c} ± 0.12
Liver	245.25	0.000**	13.42^{b} ± 0.18	15.99^{d} ± 0.25 (+19.15)	15.09^{c} ± 0.25 (+12.44)	14.85^{c} ± 0.19 (+10.65)	12.78^{a} ± 0.13
Heart	15.59	0.000**	1.94^{a} ± 0.32	1.56^{b} ± 0.23 (-19.58)	1.23^{ab} ± 0.21 (-36.59)	1.03^{a} ± 0.14 (-46.90)	1.59^{bc} ± 0.13
Brain	178.81	0.000**	9.68^{e} ± 0.19	8.83^{c} ± 0.15 (-8.78)	8.13^{b} ± 0.18 (-16.01)	7.22^{a} ± 0.16 (-25.41)	8.51^{c} ± 0.15

Values are Mean ± SD (n=6); the specific activity of enzyme was expressed as µmol citrate produced/min/µg protein at 32°C.
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 α-ketoglutarate 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	368.49	0.000**	$10.88^{d} \pm 0.23$	$12.24^{e} \pm 0.20$ (+12.50)	$10.19^{c} \pm 0.27$ (-6.34)	$7.36^{a} \pm 0.28$ (-32.35)	$9.19^{b} \pm 0.17$
Muscle	305.66	0.000**	$9.16^{c} \pm 0.24$	$10.49^{d} \pm 0.15$ (+14.51)	$8.67^{b} \pm 0.13$ (-5.34)	$6.48^{a} \pm 0.29$ (-29.25)	$8.33^{b} \pm 0.16$
Liver	167.73	0.000**	$6.55^{c} \pm 0.21$	$6.62^{c} \pm 0.17$ (+1.06)	$7.41^{d} \pm 0.15$ (+13.12)	$4.96^{a} \pm 0.20$ (-24.27)	$5.68^{b} \pm 0.16$
Heart	354.03	0.000**	$3.31^{c} \pm 0.23$	$4.79^{d} \pm 0.17$ (+44.71)	$2.91^{b} \pm 0.12$ (-12.08)	$1.06^{a} \pm 0.14$ (-67.97)	$2.16^{e} \pm 0.18$
Brain	558.13	0.000**	$4.89^{d} \pm 0.17$	$5.79^{e} \pm 0.10$ (+18.40)	$3.13^{b} \pm 0.15$ (-35.99)	$2.23^{a} \pm 0.13$ (-54.39)	$3.96^{c} \pm 0.17$

Table 3b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on specific activity of α-ketoglutarate 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	87.27	0.000**	$10.92^{bc} \pm 0.26$	$11.26^{c} \pm 0.15$ (+3.11)	$10.70^{b} \pm 0.43$ (-2.01)	$9.00^{a} \pm 0.25$ (-17.58)	$9.32^{a} \pm 0.14$
Muscle	80.59	0.000**	$9.34^{b} \pm 0.24$	$9.91^{c} \pm 0.20$ (+6.10)	$9.14^{b} \pm 0.25$ (-2.14)	$7.92^{a} \pm 0.23$ (-15.20)	$8.28^{a} \pm 0.16$
Liver	81.13	0.000**	$6.54^{d} \pm 0.12$	$6.99^{e} \pm 0.14$ (+6.88)	$6.20^{c} \pm 0.22$ (-5.19)	$5.58^{a} \pm 0.08$ (-14.67)	$5.92^{b} \pm 0.14$
Heart	18.59	0.000**	$3.02^{bc} \pm 0.52$	$3.34^{c} \pm 0.23$ (+10.59)	$2.98^{bc} \pm 0.11$ (-1.32)	$2.06^{a} \pm 0.16$ (-31.78)	$2.29^{a} \pm 0.11$
Brain	75.52	0.000**	$4.93^{b} \pm 0.14$	$5.28^{c} \pm 0.20$ (+7.09)	$4.81^{b} \pm 0.21$ (-2.93)	$3.90^{a} \pm 0.18$ (-20.89)	$3.94^{a} \pm 0.13$

Values are Mean ± SD (n=6); the specific activity of enzyme was expressed as nanomoles of potassium ferrocyanide liberated/hour/mg protein.

P value < 0.0-** 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 succinate 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	1673.88	0.000**	$32.23^{a} \pm 0.40$	$24.71^{c} \pm 0.44$ (-23.33)	$19.15^{b} \pm 0.33$ (-40.58)	$14.99^{a} \pm 0.38$ (-53.49)	$26.72^{d} \pm 0.44$
Muscle	943.52	0.000**	$33.63^{e} \pm 0.32$	$29.85^{d} \pm 0.22$ (-11.23)	$28.33^{c} \pm 0.34$ (-15.75)	$23.12^{a} \pm 0.35$ (-31.25)	$26.39^{b} \pm 0.31$
Liver	11108.74	0.000**	$56.04^{e} \pm 0.44$	$41.20^{c} \pm 0.40$ (-26.48)	$23.23^{b} \pm 0.38$ (-58.54)	$14.63^{a} \pm 0.41$ (-73.89)	$48.33^{d} \pm 0.38$
Heart	25.44	0.000**	$9.62^{c} \pm 0.37$	$9.24^{c} \pm 0.28$ (-3.95)	$8.45^{b} \pm 0.49$ (-12.16)	$7.56^{a} \pm 0.48$ (-21.41)	$8.18^{ab} \pm 0.35$
Brain	29.57	0.000**	$10.99^{c} \pm 0.30$	$9.98^{b} \pm 0.30$ (-9.19)	$9.80^{b} \pm 0.48$ (-10.82)	$9.04^{a} \pm 0.33$ (-17.74)	$9.15^{a} \pm 0.31$

Table 4b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on specific activity of succinate 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	1083.44	0.000**	$32.37^{d} \pm 0.28$	$28.91^{c} \pm 0.32$ (-10.68)	$24.98^{b} \pm 0.49$ (-22.82)	$18.55^{a} \pm 0.40$ (-42.69)	$25.42^{b} \pm 0.39$

Muscle	189.14	0.000**	32.97^{d} ± 0.38	32.74^{d} ± 0.38 (-0.69)	30.58^{c} ± 0.58 (-7.24)	27.23^{a} ± 0.47 (-17.40)	29.00^{b} ± 0.34
Liver	4300.41	0.000**	56.22^{e} ± 0.32	52.86^{d} ± 0.31 (-5.97)	39.19^{b} ± 0.65 (-30.29)	27.54^{a} ± 0.41 (-51.01)	45.94^{c} ± 0.36
Heart	9.08	0.000**	9.50^{b} ± 0.38	8.82^{a} ± 0.40 (-7.15)	8.74^{a} ± 0.32 (-8.00)	8.56^{a} ± 0.32 (-9.89)	8.43^{a} ± 0.24
Brain	18.45	0.000**	10.84^{d} ± 0.29	10.55^{cd} ± 0.44 (-2.67)	9.90^{b} ± 0.49 (-8.67)	9.14^{a} ± 0.35 (-15.68)	9.92^{bc} ± 0.26

Values are Mean ± SD (n=6); the specific activity of enzyme was expressed as nanomoles of succinate oxidized/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 (5.768 ppm) on specific activity of malate 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	1939.58	0.000**	19.93^{a} ± 0.19	20.05^{a} ± 0.18 (+0.60)	31.63^{d} ± 0.36 (+58.70)	26.95^{c} ± 0.24 (+35.22)	21.13^{e} ± 0.40
Muscle	4243.16	0.000**	39.78^{d} ± 0.16	40.07^{d} ± 0.24 (+0.72)	37.15^{c} ± 0.26 (-6.61)	25.11^{a} ± 0.36 (-46.93)	36.22^{b} ± 0.39
Liver	13047.54	0.000**	65.74^{d} ± 0.12	67.40^{e} ± 0.31 (+2.52)	42.26^{b} ± 0.34 (-35.71)	30.20^{a} ± 0.53 (-54.06)	58.52^{c} ± 0.30
Heart	312.76	0.000**	47.39^{a} ± 0.42	49.30^{b} ± 0.38 (+4.39)	54.73^{d} ± 0.33 (+15.48)	51.32^{c} ± 0.44 (+8.29)	48.80^{b} ± 0.39
Brain	2925.82	0.000**	50.19^{d} ± 0.27	53.24^{e} ± 0.42 (+6.07)	45.29^{b} ± 0.38 (-9.76)	32.66^{a} ± 0.31 (-34.90)	48.07^{c} ± 0.39

Table 5b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on specific activity of malate 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	462.04	0.000**	19.88^{a} ± 0.24	19.96^{a} ± 0.34 (+0.40)	28.43^{d} ± 0.52 (+43.00)	24.29^{c} ± 0.50 (+22.18)	21.80^{b} ± 0.39
Muscle	2555.69	0.000**	39.81^{d} ± 0.38	40.92^{e} ± 0.23 (+2.78)	36.26^{c} ± 0.37 (-8.91)	28.81^{b} ± 0.31 (-27.63)	25.93^{a} ± 0.31
Liver	4533.02	0.000**	65.81^{e} ± 0.30	68.14^{d} ± 0.35 (+3.54)	55.32^{c} ± 0.37 (-15.93)	39.74^{a} ± 0.42 (-39.61)	47.90^{b} ± 0.44
Heart	286.17	0.000**	47.29^{b} ± 0.40	50.68^{c} ± 0.53 (+7.16)	55.48^{d} ± 0.46 (+17.31)	50.33^{c} ± 0.78 (+6.42)	45.67^{a} ± 0.48
Brain	1434.84	0.000**	50.16^{d} ± 0.36	52.11^{e} ± 0.37 (+3.88)	47.82^{c} ± 0.31 (-4.66)	38.87^{a} ± 0.38 (-22.50)	42.92^{b} ± 0.33

Values are Mean ± SD (n=6); the specific activity of enzyme was expressed as nanomoles of NADH oxidized/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 6a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on levels of ATP in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	141.48	0.000**	8.39^{c} ± 0.32	7.77^{d} ± 0.37 (-7.38)	6.31^{b} ± 0.33 (-24.79)	4.20^{a} ± 0.37 (-49.94)	7.08^{c} ± 0.27
Muscle	241.83	0.000**	8.91^{d} ± 0.27	8.48^{d} ± 0.39 (-4.82)	6.41^{b} ± 0.45 (-28.05)	3.17^{a} ± 0.32 (-64.42)	7.16^{c} ± 0.30
Liver	206.47	0.000**	9.23^{d} ± 0.36	6.59^{c} ± 0.42 (-28.60)	5.05^{b} ± 0.45 (-45.28)	3.26^{a} ± 0.37 (-64.68)	7.16^{c} ± 0.30
Heart	87.78	0.000**	5.44^{d} ± 0.32	4.23^{c} ± 0.35 (-22.24)	3.03^{b} ± 0.28 (-44.30)	2.40^{a} ± 0.28 (-55.88)	3.26^{b} ± 0.34
Brain	119.54	0.000**	6.41^{a} ± 0.31	5.58^{c} ± 0.44 (-12.94)	4.90^{b} ± 0.37 (-23.55)	2.34^{a} ± 0.30 (-63.49)	5.25^{bc} ± 0.28

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

Tissue	F Value	P value	Control	15 Experimental Days	30 Experimental Days	45 Experimental Days	Recovery
Ovary	65.85	0.000**	8.74^{c} ± 0.36	8.35^{c} ± 0.48 (-4.46)	7.50^{b} ± 0.39 (-14.18)	5.42^{a} ± 0.35 (-37.98)	7.34^{b} ± 0.35

Muscle	136.49	0.000**	9.21^{d} ± 0.34	8.23^{c} ± 0.30 (-10.64)	7.13^{b} ± 0.40 (-22.58)	4.68^{a} ± 0.42 (-49.18)	8.37^{c} ± 0.36
Liver	148.22	0.000**	9.41^{e} ± 0.37	8.74^{d} ± 0.34 (-7.12)	6.46^{b} ± 0.45 (-31.34)	4.46^{a} ± 0.41 (-52.60)	7.41^{c} ± 0.39
Heart	14.27	0.000**	5.60^{b} ± 0.44	5.49^{b} ± 0.38 (-1.96)	5.29^{b} ± 0.36 (-5.53)	4.10^{a} ± 0.44 (-26.78)	4.80^{c} ± 0.26
Brain	37.65	0.000**	6.53^{c} ± 0.40	5.68^{b} ± 0.36 (-13.01)	5.51^{b} ± 0.46 (-15.62)	3.99^{a} ± 0.32 (-38.89)	5.23^{b} ± 0.28

Values are Mean ± SD (n=6); Values are expressed as nanomoles of ATP/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.

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