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Original Research Paper

Lambda Cyhalothrin Induced Changes in Protein Metabolism of Various Tissues in Freshwater Catfish *Clarias batrachus*

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ABSTRACT

The present study was undertaken to find out the effect of synthetic pyrethroid lambda cyhalothrin in liver, muscle, gills, kidney, testis and seminal vesicle of freshwater catfish *Clarias batrachus*. The fish were exposed to the pesticide for a period of 45 days at a sublethal concentration of 5.768 ppm. Biochemical analysis of total proteins and total free amino acids was carried out on the 15th, 30th and 45th day of exposure to find out changes in the biochemical constituents due to toxic stress caused to the fish. The results showed a significant decline in total proteins in all the tissues during different days of exposure to lambda cyhalothrin, while total free amino acids showed an increase in liver, kidney and testis, and a reverse trend in muscle, gills and seminal vesicle.

INTRODUCTION

Synthetic pyrethroids were introduced in India in 1976. Discovery of new synthetic pyrethroids have sparked exciting advancement in the control of insect pests (Bradbury & Coats 1989). Pyrethroids are manufactured as alternative to organochlorine, organophosphate and methyl carbamate insecticides. Synthetic pyrethroids are most widely used group of insecticides in the world as pest control agents in agriculture and public health programmes and preferred over other pesticides because of their short residual life (Anees 1975). However, the use of such pesticides in excess of the recommended dose, which ultimately enters the water bodies, has a fatal effect on fishes and other aquatic organisms (Metelev et al. 1983).

The toxicity of pyrethroid pesticides to aquatic organisms depends largely upon their solubility in water. All pyrethroids are not equally soluble in water. The gills of fish quickly absorb pyrethroids because of the lipophilic nature of the pesticide even at very low concentrations (Atamanalp et al. 2002). Pyrethroids are extremely toxic to fish and other aquatic fauna at acute sublethal levels (Hill 1989, Agnihotrudu 1998). The metabolism of pyrethroids like delmethrin, cypermethrin and cyhalothrin in mammals involves ester cleavage due to carboxyesterase by hydrolysis of the central ester bond and oxidation, whereas metabolism in fish is mostly oxidative (Demounte 1989). Fishes tend to lack the enzymatic machinery for the hydrolysis of these pyrethroids which is the obvious reason for the deleterious effect of these pesticide on fishes (Demounte 1989). Pyrethroids are metabolised and eliminated more slowly by fish as compared to mammals and birds, which may explain the higher toxicity of these compounds in fishes (Atamanalp 2002). Residual pyrethroids are developed which are less sensitive to the environmental breakdown. The environmental risk associated with lambda cyhalothrin runoff after agricultural application is yet to be assessed properly.

Proteins play an important role in energy metabolism and are required by organisms for tissue archi-

tecture maintenance. They are intimately related with all physiological processes, cell metabolism and intricately involved in subcellular functions (Neff 1985). *Clarias batrachus* is an important food fish and has great economic value in aquaculture, hence the present study was designed to evaluate effects of the synthetic pyrethroid lambda cyhalothrin on protein metabolism in *Clarias batrachus.* The static laboratory test may provide a conservative estimate of the potential for community level effects under field conditions.

MATERIALS AND METHODS

The technical grade synthetic pyrethroid, lambda cyhalothrin with 95% purity, used in the present study, was supplied by Rallis India Ltd., Bangalore for evaluation of its toxic effects on nutritionally important freshwater catfish, *Clarias batrachus.*

Healthy adult male catfish *Clarias batrachus* weighing 200-225 g and 30-35 cm were used as the experimental model to evaluate the toxicity of lambda cyhalothrin, a synthetic pyrethroid widely used for agricultural applications. The fish were procured from the local fish market at Maduravoyil, Chennai, Tamil Nadu, brought to the laboratory and acclimatized under laboratory conditions for a period of three weeks and fed *ad libitum.*

The fish were maintained in rectangular disinfected plastic tubs (64cm × 44cm × 29.5cm) filled with 20 litres of dechlorinated tap water. Feeding was stopped 24 hours before the commencement of the toxicity test to keep the animals more or less in the same metabolic state. The water quality was determined periodically for temperature, pH, salinity, dissolved oxygen, total hardness and alkalinity by standard methods (APHA 1998).

Preliminary toxicity tests were carried out to find the median lethal tolerance limit of experimental fish to lambda cyhalothrin for 96 hours. To determine 96 hour LC_{50} static renewable bioassay method was adopted. The LC_{50} bioassay test involved the exposure of five groups of fish to a range of five different concentrations of lambda cyhalothrin. Each group containing 10 fish was maintained in 20 litres of tap water and the survival rate was noted for a period of 96 hours. The concentration at which 50% survival/mortality occurred after 96 hours was taken as the median lethal concentration. The 96 hour LC_{50} was determined by Probit analysis method (Finney 1971). The LC_{50} concentration for 96 hour was found to be 28.84 ppm. One-fifth of the LC_{50} concentration was taken as the sublethal concentration. The fish were maintained for a period of 45 days at a sublethal concentration of 5.768 ppm. Group I served as control while Group II was exposed to sublethal concentrations of lambda cyhalothrin for a period of 45 days. At the end of every 15, 30 and 45 days six fish were sacrificed by cervical decapitation. The required tissues, i.e., liver, muscle, kidney, gills, brain, testis and seminal vesicle were dissected out and washed thoroughly with 0.9 N saline solution. Tissues were weighed and homogenized in tris 0.1 M HCL buffer using Potter Elevenhjem homogenizer. The homogenates of the tissues were centrifuged at 2500 rpm for 15 minutes in a refrigerated high speed centrifuge and clear supernatant was used for biochemical analysis. Total protein was estimated by the method of Lowry et al. (1951). Total free amino acids was estimated by ninhydrin method of Yemm & Cocking (1955).

The data collected on the different parameters were subjected to statistical analysis (Snedecor & Cochran 1989) by one way analysis of variance (ANOVA) followed by Duncan multiple range tests, and the statistical significance was tested at 1% and 5% levels. The percentage change in experimental groups over controls was calculated to determine % elevation (+) or % reduction (-) during the different days of exposure.

RESULTS

The total protein content of the liver, muscle, kidney and seminal vesicle in the experimental groups was significantly depleted ($P < 0.05$) during different days of exposure of the fish to the pesticide. A slight different trend in total protein content of the gills was observed showing a significant decline on the 45th day. The total protein content of the testis decreased significantly ($P < 0.05$) at the end of 45th day in the experimental groups, but significance was not pronounced on the 30th and 45th day of exposure in the animals (Table 1).

In the liver, the free amino acids increased from 15th day to 45th day in the animals exposed to the sublethal concentration. A similar trend observed in the liver was also seen in kidney and testis of the animals in experimental groups. The levels of free amino acids in kidney showed an elevation by the 15th day reaching to a maximum of on the 45th day. There was a significant elevation in free amino acid levels of the testis ($P < 0.05$) on the 30th day. There was an increase in the levels of free amino acids in liver, kidney and testis, whereas the muscle, gills and seminal vesicle showed a significant decrease ($P < 0.05$) throughout the period of exposure in the animals (Table 2).

DISCUSSION

Protein is the energy source to spare during chronic period of stress apart from carbohydrates and lipids. In the present study fish exposed to sublethal concentration of lambda cyhalothrin experienced greater stress during the process of detoxification of the pyrethroid which could have altered the metabolic status of the animal. The metabolic rate of the fish maintained at sublethal concentration of lambda cyhalothrin could have drastically changed than that of fish maintained in toxicant free water. The protein depletion in the fish exposed to lambda cyhalothrin is a physiological strategy played by the animal to adapt itself to the changed environment. This leads to degradative process like proteolysis and utilisation of degraded products for increased metabolism and to liberate extra energy during toxic stress (Arunachalam et al. 1980). Mehendale (1987) pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment of protein synthesis machinery. The quantity of protein is dependent on the rate of protein synthesis or on the rate of its degradation. The quantity of protein may also be affected due to impaired incorporation of amino acids into the polypeptide chain and inhibiting RNA synthesis (Tripathi & Verma 2004a). Reduction in binding of amino acids to tRNA leading to decreased protein synthesis, and hence protein depletion (Dhar & Banerjee 1983). Proteins share a major role as energy source when exposed to stress conditions (Umminger 1970). Metabolic functions in liver, muscle, kidney, gills, testis and seminal vesicle have hampered due to toxicity of lambda cyhalothrin. Detachment of ribosomes from endoplasmic reticulum and swollen mitochondria in hepatocytes was observed. The detachment of ribosomes from rough endoplasmic reticulum suggests reduction in protein synthesizing capacity of liver cells in fishes (Tripathi & Shukla 1990). Protein breakdown appears to take place at a faster rate than the rate of synthesis (Munro & Pelham 1985). Protein decline may be related to impaired food intake as a consequence of stress and increased energy cost for maintenance of homeostasis (Neff 1985). Other reasons, which could be attributed to the decline, result from inhibition of new peptide chain formation and dissociation of ribosomal subunits (Bradbury et al. 1987). It could be suggested that this pyrethroid reversibly block the key steps of transcription and translation in protein synthesis. Hepatotoxicity of malathion on protein metabolism has also been reported in *Cirrhinus mrigala* (Tiwari 2004). Depletion of protein in muscle could be due to mobilisation of protein from muscle to blood to compensate condition of acidosis (Palanichamy et

Table 1: Effect of lambda cyhalothrin at sublethal concentration (5.768 ppm) in total protein content in various tissues of *Clarias batrachus.*

Tissue	Control	Experimental Groups			F-value	P-value
		15 Days	30 Days	45 Days		
Liver	121.19±5.61^{d}	107.94±5.60^{c}(-10.93)	96.98±2.41^{b}(-19.98)	86.07±6.51^{a}(-28.98)	48.912	0.000**
Muscle	24.37±0.52^{d}	19.26±1.36^{c}(-20.97)	16.01±1.07^{b}(-34.30)	12.44±0.78^{a}(-48.95)	159.31	0.000**
Kidney	15.81±0.72^{d}	13.85±0.84^{c}(-12.40)	11.84±0.78^{b}(-25.11)	10.38±0.71^{a}(-34.35)	57.491	0.000**
Gills	18.55±1.95^{c}	16.10±1.62^{b}(-13.21)	14.32±1.57^{b}(-22.8)	13.69±0.84^{a}(-26.2)	11.834	0.000**
Testis	25.42±0.42^{c}	23.33±0.32^{b}(-8.22)	20.93±1.87^{a}(-17.66)	20.20±0.56^{a}(-20.54)	33.263	0.000**
Seminal Vesicle	13.58±1.74^{d}	11.67±1.34^{a}(-14.06)	12.21±0.16^{c}(-10.09)	11.69±0.52^{b}(-13.92)	3.756	0.024*

Table 2: Effect of lambda cyhalothrin at sublethal concentration (5.768 ppm) in levels of total free amino acids in various tissues of *Clarias batrachus.*

Tissue	Control	Experimental Groups			F-value	P-value
		15 Days	30 Days	45 Days		
Liver	43.46±2.36^{a}	48.20±0.92^{b}(+10.90)	51.24±1.02^{c}(+17.90)	56.68±0.80^{d}(+30.41)	91.224	0.000**
Muscle	37.10±3.12^{c}	35.27±2.80^{c}(-4.93)	29.99±1.22^{b}(-19.16)	24.35±1.92^{a}(-34.36)	34.761	0.000**
Kidney	3.43±0.26^{a}	4.70±0.36^{b}(+37.02)	5.58±0.42^{c}(+62.68)	6.21±0.05^{d}(+81.04)	92.485	0.000**
Gills	4.20±0.29^{d}	3.37±0.63^{c}(+19.76)	2.31±0.26^{b}(-45.00)	1.38±0.27^{a}(-67.14)	58.890	0.000**
Testis	12.89±0.67^{a}	13.86±0.16^{b}(+7.52)	14.4±0.09^{c}(+11.79)	14.86±0.09^{c}(+15.28)	29.225	0.000**
Seminal vesicle	10.04±0.68^{d}	8.25±0.03^{b}(-17.82)	8.74±0.25^{c}(-12.94)	7.23±0.10^{a}(-27.98)	60.599	0.000**

Values are mean ± SD (n = 6) : Values 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; P value > 0.05 → Not significant (NS)

Since P value is less than 0.01 there is significant difference between the different days of exposure in higher as well as lower concentration; Different alphabets in means between days in a row denote significance at 5% level; Means carrying atleast one common superscript do not differ significantly (P < 0.05); Values in parentheses in experimental groups are % reduction (-) or % elevation (+) over control.

al. 1986). All these biochemical mechanisms could have occurred due to energy diversification for the impending energy demands when the fish are under stress (Saravanan et al. 2000). Another reason for the decrease in protein content under toxic stress may be due to its utilization for repair of damaged cells, tissues and organelles.

The reduction in proteins may be due to direct utilisation of amino acids in gluconeogenesis to produce energy during stress instead of protein synthesis. According to Adams & Rinne (1982) any change in the environment alter synthesis and utilisation of proteins. This indicates that when the toxicant level is too high the fish cannot recover to synthesize protein at normal rate (Holbrook 1980). Tripathi & Verma (2004 a,b) also stated that toxicants may indirectly cease protein synthesis and may change expression of some proteins.

Hypoxic conditions in aquatic animals have also shown to induce stress proteins in many cells and tissues (Iwaki et al. 1993). Stress response and hypoxic conditions cause a reorganisation of metabolism such that the major energy requiring processes like protein synthesis shut down very rapidly. A depression in protein synthesis rate during stress and exposure to anoxic conditions in various tissue may also have freed amino acids for exploitation in gluconeogenic processes during anoxic conditions (Van Den Thillart & Van Waarde 1985). Similar mechanism may have occurred

in the tissues of fish exposed to lambda cyhalothrin. However, the exact mechanism of pesticide induced inhibition/decline in protein synthesis is yet to be investigated at the molecular level.

The present study showed an increase in the level of total free amino acids in liver and kidney tissues after exposure of fish to the pesticide. Increase in levels of free amino acids due to pesticidal stress was mainly a consequence of higher catabolic activity of protein to meet high energy demand by breakdown of proteins into free amino acids. The increase in amino acids has been reported in liver of fishes exposed to toxicants (Radhiah et al. 1987, Seshagiri Rao et al. 1987, Malla Reddy & Philip 1991). It is also suggested that increase of amino acids indicated activation of compensatory mechanism in the animal to encounter the toxic stress and increased protein breakdown.

The trend of free amino acid level also shows that protein synthesis is not stopped altogether but it is at a slower rate, but as far as liver and kidney are concerned the elevated levels of amino acids may be due to augmented proteolysis and shuttling of amino acids by breakdown of muscle proteins (Sesharigi Rao et al. 1983) to meet the increased energy demand created by lambda cyhalothrin induced stress which may result in replenishment of amino acids in liver and kidney and subsequent gluconeogenesis.

The free amino acids decreased in muscle and gills, which suggests their mobilisation into Kreb's cycle through transamination to cope up with the energy metabolism. The depletion of free amino acids in gills and muscle indicates enhanced amino acid catabolism. A decline in free amino acids was observed in different tissues of *Clarias batrachus* exposed to sublethal concentration of decis (Ravinder et al. 1988). Documented evidence shows that transamination and transdeamination reactions are prominent under stress condition (Kabeer Ahmed & Rao 1978) and the amino acids are channelised into the Kreb's cycle for gluconeogenesis for the production of energy by the processes.

Depletion in testicular and seminal vesicular proteins in the present study may have inhibited proteins involved in triggering spermatogenesis and cell cycle process in reproductive tissues. An increase in amino acids in testis as a result of protein breakdown could divert the amino acids to other oxidative pathways for their utilization in vital tissues to cope up with excess demand for energy rather than maintenance of reproductive process (Consten et al. 2001).

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