The Bioscan 6(3) : 421-423, 2011
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

# IMPACT OF CYPERMETHRIN (25%EC) ON FREE AMINO ACIDS AND PROTEASE ACTIVITY LEVELS IN THE FRESHWATER FISH *CIRRHINUS MRIGALA* (HAM.)

**P. NEELIMA*, L. CYRIL ARUN KUMAR[1], J.CHANDRA SEKHARA RAO AND N. GOPALA RAO**
Department of Zoology and Aquaculture, Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur – 522 510, A. P., INDIA
[1]Department of Zoology, VSR and NVR College, Tenali, Guntur – 522 510, A. P., INDIA
E-mail: neelima_chandini@yahoo.com

**KEY WORDS**
Cypermethrin
*Cirrhinus mrigala*
Enzyme activity
Muscle and Gill

**Received on :**
02.03.2011

**Accepted on :**
17.06.2011
*** Corresponding author**

**ABSTRACT**
Cypermethrin is a synthetic pyrethroid (SP) insecticide employed all over the world to control different pests of crops and fishes because of its low mammalian toxicity, degradability and persistence nature. Acute toxicity experiments were conducted to the fish *Cirrhinus mrigala* (Ham.) for the toxicant cypermethrin. Sub-lethal concentrations of 5, 10, 15 and 20% 96hr $LC_{50}$ were prepared and exposed the fish, *Cirrhinus mrigala* for 2, 4, 6 and 8 days for 96 hr to assess $LC_{50}$. Gill, liver and muscle tissues were dissected out at end of each day and free amino acids and protease activity was estimated. Dose as well as exposure period depended ($p \geq 0.05$) increase in the free amino acid levels and protease activity was observed. Maximum elevation of free amino acids and protease activity was observed in liver, followed by muscle and gill.

## INTRODUCTION

Indiscriminate use of different pesticides in agriculture to prevent crop damage from pests has increased over the years, especially in the developing countries (Santhakumar and Balaji, 2000). These pesticides, even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and alter the physico-chemical properties of water (Balchandra and Lomte, 2001). Among the pesticides pyrethroids are commonly used due to their high effectiveness, low toxicity to birds and mammals and easy biodegradability (Kale *et al.*, 1999). Cypermethrin is a highly potent and broad spectrum synthetic pyrethroid which is used extensively for pest control. Although it is non-persistence in the environment, the excess use of this pesticide may enter into natural waters through agricultural run-off and ultimately cause damage to non-target organisms such as fish (Stephenson, 1982; Prashanth and Neelagund, 2008; Singh *et al*., 2010).

Amino acids are considered one of the most reliable techniques for the detection of changes in protein synthesis in cells and therefore, the protein pattern can be used as a criterion for the differentiation between several organs exposed to some pollutants. When the proteins are denatured, it leads to alternation in the protease activity (Anilkumar *et al*., 2010). Hence, in the present study an attempt has been made to study the alternations in free amino acids and protease activity under the toxic effect of a synthetic pyrethroid, cypermethrin.

## MATERIALS AND METHODS

Fish *Cirrhinus mrigala* of size $6.5 \pm 0.5$ cm in length and $10 \pm 0.5$ g weight respectively were bought from a local fish farm in Tenali, Andhra Pradesh and acclimatized to laboratory conditions for one month and were stored in large glass aquaria. The holding water was changed daily. The average temperature of water was $24\text{-}26 \pm 1$ºC. The fish were fed with groundnut cake and twice a week with frog muscle and acclimated to laboratory conditions at $29 \pm 1$ºC. The physico-chemical parameters of water are given in Table 1. Commercial grade cypermethrin (25%EC) of liquid formations manufactured by Agro India Ltd. was purchased from local agro-chemical stores. Fish were exposed to 2.5, 3, 3.5, 4 and 4.5ppm of cypermethrin for 24, 48, 72 and 96hr and determined median lethal concentration ($LC_{50}$). Then fish were exposed to sub-lethal concentrations of cypermethrin for a period of 2, 4, 6 and 8 days. At the end of the each exposure period, fish were stunned to death and target organs such as gill, liver and muscle were dissected out and free amino acid levels in the tissues were estimated by the Ninhydrin method described by Moore and Stein (1957). Protease activity in the tissues was estimated using the Ninhydrin method described by Davis and Smith (1955). Statistical analysis was done according to Duncan's multiple range (DMR) test.

## RESULTS AND DISCUSSION

Observations revealed that, there was a gradual increase of

**Table 1: Physico-chemical parameters of water used for experiments are given below**

| S.No | Parameters | Values |
|---|---|---|
| 1 | Turbidity | 8 Silica units |
| 2 | Electrical conductivity 28ºC | 814 microohms/cm |
| 3 | pH value at 28ºC | 7.8 |
| 4 | Alkalinity | |
| | 1. Phenolphthalein | |
| | 2. Methyl orange | Nil470 |
| 5 | Total Hardness ( as $CaCO_3$) | 256mg/L |
| 6 | Calcium Hardness (as N) | 74mg/L |
| 7 | Sulphate (as $SO_4$) | Trace |
| 8 | Chloride (as Cl) | 36mg/L |
| 9 | Fluoride (as F) | 1.6mg/L |
| 10 | Iron (as Fe) | Nil |
| 11 | Dissolved Oxygen | 8.5-10ppm |
| 12 | Temperature | 24-26ºC |

* The sample of water used for experiments was clear and colourless

free amino acids levels and protease activity in all the tissues studied irrespective of the sub-lethal concentrations and exposure periods. Alterations in the free amino acids levels in *Cirrhinus mrigala* was given in Table 2. In the control fish, the gill free amino acids levels were in the range of 11.96±0.24-11.98±0.21 mg amino acid nitrogen/g wet wt. When the fish was exposed to sub-lethal concentrations, there was a increase in the gill free amino acids levels to 12.19±0.19, 12.21±0.09, 12.25±0.18 and 12.30±0.12 mg amino acid nitrogen/g wet wt. after 2, 4, 6 and 8 days of exposure periods respectively. Maximum elevation of 2.67% over control was observed in gill at 20% 96hr $LC_{50}$ after 8 days of exposure period. In similar manner, liver and muscle free amino acids levels were also found to increase as the concentration of the pesticide and exposure period increased. Liver showed free amino acids levels of 18.75±0.19 mg amino acid nitrogen/g wet wt. in control fish. These levels were found to increase to 19.12±0.17 mg amino acid nitrogen/g wet wt. (1.97% enhancement) over control. Muscle also showed an increase of free amino acids levels to 15.02±0.21 from the control valve of 14.52±0.18 mg amino acid nitrogen/g wet wt. i.e. 3.44% enhancement at highest concentration of 20% 96 $hLC_{50}$ after longest exposure period of 8 days. Among the tissues studied maximum increase in the free amino acids levels was observed in muscle (3.44%) followed by gill (2.67%) and (1.97%). Activity of protease enzyme under the sub-lethal concentrations of cypermethrin is given in Table 3. Protease activity in gill, liver and muscle of *Cirrhinus mrigala* exposed to sub-lethal concentrations of cypermethrin was observed to increase over the control fish. The observed control protease activity was 0.32±0.02–0.34±0.02 in gill, 0.42±0.01–0.45±0.01 in liver and 0.35±0.01–0.36±0.01 mm/g wet wt. (Table 3). The enhancement of protease activity was 34.37% after 2 days of exposure. It further increased to 52.94% at 20% 96hr $LC_{50}$ after 8 days of exposure period. Likewise liver and muscle also showed an enhancement of 35.5% and 66.66% of protease activity respectively after highest concentration of 20% 96hr$LC_{50}$ and longest exposure period of 8 days. Maximum enhancement of protease activity

**Table 2: Free amino acid levels (mg amino acid nitrogen/g wet wt) in the tissues of *Cirrhinus mrigala* on exposure to sub lethal concentrations of cypermethrin (25%EC)**

| Exposure Period(Days) | Tissues | Control | Sub lethal concentrations (%96hr$LC_{50}$) 5 | 10 | 15 | 20 |
|---|---|---|---|---|---|---|
| 2 | Gill | 11.96±0.24 | 11.98±0.22(0.17) | 12.04±0.28(0.67) | 12.13±0.24(1.42) | 12.19±0.19(1.92) |
| | Muscle | 18.72±0.19 | 18.75±0.21(0.16) | 18.79±0.22(0.37) | 18.85±0.21(0.69) | 18.89±0.18(0.91) |
| | Liver | 14.52±0.21 | 14.55±0.19(0.20) | 14.59±0.26(0.48) | 14.62±0.18(0.69) | 14.67±0.19(1.03) |
| 4 | Gill | 11.97±0.21 | 12.02±0.19(0.42) | 12.10±0.29(1.09) | 12.16±0.18(1.59) | 12.21±0.09(2.01) |
| | Muscle | 18.74±0.17 | 18.77±0.18(0.16) | 18.81±0.22(0.37) | 18.89±0.16(0.80) | 19.01±0.12(1.44) |
| | Liver | 14.54±0.23 | 14.59±0.24(0.34) | 14.65±0.28(0.76) | 14.72±0.21(1.24) | 14.81±0.18(1.86) |
| 6 | Gill | 11.96±0.19 | 12.07±0.21(0.92) | 12.14±0.25(1.51) | 12.20±0.23(2.01) | 12.25±0.18(2.42) |
| | Muscle | 18.74±0.18 | 18.80±0.20(0.32) | 18.87±0.20(0.69) | 18.97±0.19(1.23) | 19.07±0.12(1.76) |
| | Liver | 14.53±0.23 | 14.63±0.26(0.69) | 14.71±0.29(1.24) | 14.82±0.22(1.10) | 14.91±0.13(2.62) |
| 8 | Gill | 11.98±0.21 | 12.12±0.26(1.17) | 12.18±0.28(1.67) | 12.24±0.18(2.17) | 12.30±0.12(2.67) |
| | Muscle | 18.75±0.19 | 18.85±0.18(0.53) | 18.91±0.19(0.85) | 19.04±0.15(1.55) | 19.12±0.17(1.97) |
| | Liver | 14.52±0.18 | 14.70±0.19(1.24) | 14.78±0.21(1.79) | 14.89±0.17(2.55) | 15.02±0.21(3.44) |

**Table 3: Protease activity (mm/g wet wt) in the tissues of *Cirrhinus mrigala* exposure to sub lethal concentrations of cypermethrin (25%EC)**

| Exposure Period(Days) | Tissues | Control | Sub lethal concentrations (%96hr$LC_{50}$) 5 | 10 | 15 | 20 |
|---|---|---|---|---|---|---|
| 2 | Gill | 0.32±0.02 | 0.36±0.02(12.50) | 0.38±0.02(18.75) | 0.41±0.03(28.12) | 0.43±0.01(34.37) |
| | Muscle | 0.42±0.01 | 0.45±0.02(07.14) | 0.50±0.03(19.04) | 0.54±0.01(28.57) | 0.61±0.01(45.23) |
| | Liver | 0.35±0.01 | 0.40±0.03(14.28) | 0.46±0.04(31.42) | 0.50±0.02(42.85) | 0.53±0.01(51.42) |
| 4 | Gill | 0.32±0.02 | 0.38±0.02(18.75) | 0.41±0.02(28.12) | 0.43±0.02(34.37) | 0.46±0.02(43.75) |
| | Muscle | 0.43±0.01 | 0.48±0.02(11.62) | 0.52±0.01(20.93) | 0.56±0.03(30.23) | 0.65±0.01(51.16) |
| | Liver | 0.35±0.01 | 0.43±0.02(22.85) | 0.47±0.01(34.28) | 0.52±0.03(48.57) | 0.57±0.02(62.85) |
| 6 | Gill | 0.33±0.01 | 0.41±0.02(24.24) | 0.44±0.01(33.33) | 0.46±0.01(39.39) | 0.48±0.03(45.45) |
| | Muscle | 0.44±0.02 | 0.51±0.02(15.90) | 0.58±0.03(31.81) | 0.63±0.01(43.18) | 0.67±0.02(52.27) |
| | Liver | 0.35±0.02 | 0.46±0.03(31.42) | 0.51±0.01(45.71) | 0.54±0.01(54.28) | 0.58±0.03(65.71) |
| 8 | Gill | 0.34±0.02 | 0.43±0.03(26.47) | 0.47±0.02(38.23) | 0.49±0.02(44.11) | 0.52±0.02(52.94) |
| | Muscle | 0.45±0.01 | 0.58±0.01(28.88) | 0.63±0.02(40.00) | 0.60±0.02(33.33) | 0.70±0.03(55.55) |
| | Liver | 0.36±0.01 | 0.48±0.01(33.33) | 0.53±0.02(47.22) | 0.57±0.01(58.33) | 0.60±0.04(66.66) |

was observed in muscle (66.66%), followed by liver (55.55%) and gill (52.94%).

The free amino acids while acting as precursors for protein synthesis are involved in gluconeogenesis, synthesis of glycogen and keto acids. Hence, they contribute to a variety of metabolic pathways involving the anabolism or oxidation which determine the quantum of amino acid pool. In the present investigation the free amino acids levels were found to increase in the tissues studied. This increase in free amino acids levels suggests that tissues damage probably due to the increase proteolytic activity under toxic stress (Singh *et al*., 2010). This increase can also be attributed to the synthesis of amino acids in addition to their elevation by protein hydrolysis (Dubale and Punitashah, 1979; Reddy *et al*., 1991; Prashanth and David, 2006; Anita *et al*., 2010). Similar enhancement of free amino acids in *Labeo rohita* exposed to endosulfan was observed by Saravanan *et al*., (2010). The increase in protease activity under stress conditions clearly suggests that cypermethrin induces high protease activity which leads to the formation of higher free amino acid content is in agreement with Patil and David (2009). Among tissues studied liver is found to be affected more than other tissues as it is the metabolic center for detoxicfication as opined by Bashamohideen (1988). The overall increase in free acids level and protease activity in the present study might suggest the utilization of proteins under toxic stress conditions of the fish *C. mrigala*.

## ACKNOWLEDGEMENT

Authors are thankful to the Management, Principal and Head, Dept. of Zoology, V.S.R and N.V.R (autonomous) College, Tenali and Dept. of Zoology and Aquaculture, Acharya Nagajuna University, Nagarjuna Nagar, Guntur, A.P. for providing facilities and encouragement.

## REFERENCES

**Anilkumar, V., Janaiah, C. and Venkateshwarlu, P. 2010.** Impact of thiamethoxam on proteases, aminases and glutamate dehydrogenase in some tissues of fresehwater fish, *Channa punctatus* (Bloch). *An Int. J. Life Sci*. **5(1):** 135–137.

**Anita, S. T., Sobha, K., Veeraiah, K. and Tilak, K.S. 2010.** Studies on biochemical changes in the tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvelerate technical grade. *J. Toxicology and Envi. Health Sci.* **2(5):** 53–62.

**Balchandra, W. B. and Lomte, V. S. 2001.** Acute toxicity of pesticides carbaryl and endosulfan to freshwater bivalves, *Parreysia cylindrica*. *Poll. Res*. **20(1):** 25–29.

**Bashamohideen, M. D. 1988.** Toxicity evaluation of commercial grade malathion on the freshwater fish, *Cyprinus carpio*. *Env. Ecol.* **6:** 488–490.

**Davis, N. C. and Smith, E. L. 1955.** Assay of proteolytic enzymes. *Meth. Biochem. Anal.* **2:** 215–257.

**Dubale, M. S. and Punitashah. 1979.** Histopathological lesions induced by malathion in the liver of *Channa punctatus*. *Indian J. Exp. Biol.* **17(7):** 693–697.

**Kale, M. N., John Rathore, S. and Bhatnagar, D. 1999.** Lipid peroxidative damaga on pyrethroids exposureand alteration in antioxidant statusin rat erythrocytes: a possible involvement of reactive oxygen species. *Toxicol. Lett.* **105:** 197–205.

**Moore, S. and Stein, W. H. 1957.** A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Bio. Chem*. **211:** 907–913.

**Patil, V. K. and David, M. 2009.** Hepatotoxic potential of malathion in the freshwater teleost, *Labeo rohita* (Hamilton). *Vet. Archiv*. **79:** 179–188.

**Prashanth, M. S. and David, M. 2006.** Changes in nitrogen metabolism of the freshwater fish *Cirrhinus mrigala* following exposure to cypermethrin. *J. Basic Clin. Physiol. Pharmacol.* **17(1):** 63–70.

**Prashanth, M. S. and Neelagund, S. E. 2008.** Impact of cypermethrin on enzyme activities in the freshwater fish *Cirrhinus mrigala* (Hamilton). *Caspian J. Env. Sci.* **6(2):** 91–95.

**Reddy, P. M., Horald Philip, G. and Bashamohideen, M. D. 1991.** Fenvelerate induced biochemical changes in the selected tissues of freshwater fish, *Cyprinus carpio*. *J. Biochem. Inter*. **23(6):** 1087–1096.

**Santhakumar, M. and Balaji, M. 2000.** Acute toxicity on organophosphorus insecticide monocrotophos and its effects on behavior of an air-breathing fish, *Anabas testudineus* (Bloch). *J. Env. Biol.* **21(2):** 121–123.

**Saravanan, T. S., Rajesh, P. and Sundaramoorthy, M. 2010.** Studies on effects of chronic exposure of endosulphan to *Labeo rohita*. *J. Env. Bio.* **31(5):** 755–758.

**Singh, K. S., Singh,S. K. S. and Yadav, R. P. 2010.** Toxicological and biochemical alterations of cypermethrin (Synthetic pyrethroids) against freshwater teleost fish *Colisa fasciatus* at different seasons. *World J. Zoology*. **5(1):** 25–32.

**Stephenson, R. R. 1982.** Aquatic toxicology of cypermethrin. 1. Acute toxicity to some freshwater fish and invertebrates in laboratory test. *Aqua. Toxicol*. **2:** 175–185.