The Bioscan 5(1) : 135 - 137, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

IMPACT OF THIAMETHOXAM ON PROTEASES, AMINASES AND GLUTAMATE DEHYDROGENASE IN SOME TISSUES OF FRESHWATER FISH, *CHANNA PUNCTATUS* (BLOCH)

V. ANIL KUMAR*, C. JANAIAH AND P. VENKATESHWARLU[1]
Department of Zoology, Kakatiya University, Warangal - 506 009, A. P., INDIA
[1]Department of Zoology, Kakatiya Degree College,Warangal - 506 001, A. P., INDIA
E-mail: anilveludandi@yahoo.com

KEY WORDS
Thiamethoxam
Proteases
Aminases, GDH

Received on :
20.12.2009
Accepted on :
18.02.2010

***Corresponding author**

ABSTRACT
Sublethal concentration of Thiamethoxam (Organochloride) on the tissues (liver, brain, gill, muscle and kidney) enzyme parameters of teleost fish, *Channa punctatus* (Bloch) were analysed after 24, 48, 72 and 96 hrs of exposure. The increased neutral and alkaline proteases activities in the different tissues of *C. punctatus* indicate the damage caused due to impairment of energy supply and alkaline proteases activity indicates higher protein degradation. Therefore, the proteins are denatured leading to more activation of proteases. The activities of asparatate amino transferase (AAT) and alanine amino transferase (ALAT) were enhanced during the toxic exposure of thiamethoxam resulting in incorporation of amino acids into TCA cycle for energy production. The glutamate dehydrogenase (GDH) activity also enhanced levels of amino acids, transminases which might suggest the utilization of proteins under stress conditions of the fish.

INTRODUCTION

Thiamethoxam is one of the organochloride (OC) which is used for controlling insect pests from paddy fields and cotton fields. This pesticide may reach the surrounding fresh water bodies through irrigation or rain. Thiamethoxam causes severe destructive effects on aquatic fauna particularly fish fauna, which readily pass through cell membranes and changes the activities of several enzymes. OC compounds not only affect the respiratory system but also interfere with the cellular level of respiration (Betoulle *et al.,* 2000). Many researchers have worked the effects of following exposure of individuals to low levels of pesticide and other chemical toxins (Lee *et al.,* 1997; Chun-Yuv Yang *et al.,* 2003). According to Waliszewski *et al.,* 2003; Aronson *et al.,* 2000; Abdul Naveed (2003); Abdul Naveed *et al.,* (2004) most of pesticides may enter into the food chains and cause physiological damage. Recent studies reported that the impact of leather dyes on total proteins of *Cirrihinus mrigala* (Afaq and Rana, 2009). In the present study an attempt has been made to evaluate the effect of Thiamethoxam on the activities of proteases, transaminases and Glutamate dehydrogenase (GDH) in the tissues of liver, brain, gill, muscle and kidney of *C. Punctatus.*

MATERIALS AND METHODS

C. Punctatus weighing average of 82-120g and 25.5 ± 1.21cm in length, were procured from a local market, Warangal (A.P.) and kept in a cement tank 180 x 90 x 90 cm for one month for acclimatization under laboratory conditions for continuous water flow. The average temperature of water was 22 -24°C. The fish were fed *ad libitum* with ground nut cake along with the commercial pellets (1 – 1.5% body weight). Without discrimination, both the sexes of fish were used for the experiment. The physico-chemical parameters of water are given in Table 1. The LC_{50} of commercial grade Thiamethoxam 114.8 ppm was determined for 48 hr by the method of Bayne *et al.,* (1977). Batches of six fish were exposed to 24, 48, 72 and 96 hrs for sublethal concentration (38.26 ppm) along with control fish in separate tanks consisting of six liters of water. After the stipulated time intervals the fish were removed from the tanks and the tissues like liver, brain, muscle, gill and kidney were quickly isolated and kept in ice - jacketed petridishes for biochemical estimations.

For assaying neutral and alkaline proteases, 10% tissue homogenates were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 minutes. A clear cell free supernatant was used for the assay of proteases by the method of Davis and Smith (1955). Neutral protease activity was assayed at pH 7.0 using phosphate buffer and alkaline protease activity at pH 9.0 with carbonate bicarbonate buffer and 10mg of denatured haemoglobin protein was used as substrate. For the assay of glutamate dehydrogenase (GDH), 10% tissue homogenates were prepared in ice cold 0.25M sucrose solution and centrifuged at 3000 rpm for 15 minutes. A clear cell free supernatant was used for the assay of GDH by the method of Lee and Lardy (1965). In addition to substrate, buffer and enzyme, 0.1 micromoles of NAD^+ and 2 micromoles of INT were added to the reaction mixture.

For assaying AAT and ALAT activity by method of Reitman and Frankel (1957), 10% tissue homogenates were prepared in cold 0.25M sucrose solution and centrifuged at 3000 rpm for 15 minutes. The supernatant was used for the enzyme source.

Table 1: Physico-chemical parameters of water

Sl.No.	Parameters	Values
1	Temperature	22-24°C
2	pH	7.2-7.3
3	Electrical Conductivity (Milliohms/cm)	0.52
4	Calcium (mg/L)	5.0
5	Sodium (mg/L)	2.1
6	Bicarbonate (mg/L)	142
7	Total alkalimity (mg/L) as ($CaCO_3$)	69
8	Sulphate (mg/L)	7.1
9	Nitrates (mg/L)	3.4
10	Iodine (mg/L)	0.01
11	Chlorides (mg/L)	37.0
12	Dissolved Oxygen (mg/L)	9.2
13	Biological Oxygen demand (BOD)	1.6
14	Chemical Oxygen Demand (COD)	0.008
15	Free Carbon dioxide (mg/L)	10.0
16	Fluoride (mg/L)	0.03

RESULTS

Table 2 shows the enzyme activities of neutral and alkaline proteases, AAT and ALAT and GDH which were found to be increased in liver, brain, gill, muscle and kidney tissues of *C. punctatus* exposed to thiamethoxam for 24, 48, 72 and 96 hrs. The increased activities in *C.punctatus* evinced clearly that neutral proteases are more high in tissues of kidney, liver, brain, gill and muscle, whereas alkaline proteases were increased in kidney, brain, gill, muscle and liver tissues of *C.punctatus*.

The Asparatate amino transferase (AAT) and Alanine amino transferase (ALAT) activities also increased in the kidney, muscle, liver, brain and gill tissues of *C.punctatus*. Whereas the ALAT activity is more in the liver, kidney, gill, brain and muscle tissues of *C.punctatus*.

Glutamate dehydrogenase (GDH) activity is increased in the order of brain, liver, gill, kidney and muscle of *C.punctatus*.

DISCUSSION

During the toxic stress of thiamethoxam the activities of proteases (neutral and alkaline) increased in all the tissues of *C.punctatus*. Nemsok and Boross (1981) reported that the alteration in proteolytic activity is due to pesticides. The increased protein degradation will yield excess energy to overcome the toxic impact. This is further reported that through decreased protein content and increased levels of free amino acids in the tissues of *C.punctatus*. The enhanced tissue proteolysis strengthened in different fishes (Venkateshwarlu *et al.,* 1987; Shoba Rani *et al.,* 1989; Ganesh *et al.,* 2006) under induced toxicity of pesticides.

The enhanced activity of transaminases, asparatate amino transferase (AAT) and alanine amino transferase (ALAT) were observed under the toxic stress of Thiamethoxam showed that during the toxic period the animal can never take food. So the animal needs additional energy to overcome the toxicity (Webb, 2001; Schulman *et al.,* 2002). Hence, the activities of transminases may be enhanced along with proteolytic activities (Shoba Rani and Janaiah, 1991; Ganesh *et al.,* 2006). The elevated transaminases may indicate that fish can utilize the free amino acids from amino acid pool for energy production. Similar observations were made during toxicity of pesticides (Sajal ray *et al.,* 1988; Lydy and Linck, 2003; Dhanapakiam *et al.,* 2006). Abdul Naveed *et al.,* (2004) reported that if any malfunction occurs in energy yielding compounds, the cell

Table 2: Activities of proteases, aminases and GDH in tissue of control and Thiamethoxam exposed fish *Channa punctatus* (Bloch)

Parameters	Tissue	Control	Thiamethoxam treated 24 Hrs	48 Hrs	72 Hrs	96 Hrs
Neutral	Liver	0.37 ± 0.14	1.38* ± 0.40PC = 1.47	1.52* ± 0.41PC = 11.76	1.64 ± 0.50PC = 20.58	1.82 ± 0.26PC = 33.82
ProteasemM	Brain	1.23 ± 0.33	1.26* ± 0.25PC = 2.43	1.31* ± 0.29PC = 6.50	1.42* ± 0.031PC = 15.44	1.49 ± 0.89PC = 21.13
of Tyrosine	Gill	1.29 ± 0.11	1.34* ± 0.11PC = 3.87	1.39* ± 0.025PC = 7.75	1.47 ± 0.46PC = 13.95	1.49* ± 0.25PC = 15.50
equivalent /mg	Muscle	1.32 ± 0.29	1.35* ± 0.15PC = 2.27	1.36* ± 0.20PC = 3.03	1.39* ± 0.051PC = 5.30	1.48* ± 0.24PC = 12.12
protein / hr	Kidney	0.96 ± 0.01	1.09* ± 0.17PC = 13.54	1.19 ± 0.20PC = 23.95	1.24 ± 0.51PC = 29.16	1.29 ± 0.31PC = 34.37
Alkaline	Liver	1.36 ± 0.15	1.38* ± 0.10PC = 1.47	1.47* ± 0.20PC = 3.67	1.47* ± 0.23PC = 8.08	1.51* ± 0.45PC = 11.02
Proteases Mm	Brain	1.21 ± 0.50	1.24* ± 0.50PC = 2.47	1.29* ± 0.39PC = 6.61	1.36* ± 0.15PC = 12.39	1.39* ± 0.41PC = 14.87
ofTyrosine e	Gill	1.29 ± 0.68	1.32* ± 0.41PC = 2.32	1.38* ± 0.019PC = 6.97	1.46* ± 0.20PC = 13.17	1.48* ± 0.17PC = 14.72
quivalent / mg	Muscle	1.24 ± 0.10	1.26* ± 0.15PC = 1.61	1.28* ± 0.24PC = 3.22	1.36* ± 0.31PC = 9.67	1.39* ± 0.41PC = 12.09
protein / hr	Kidney	1.08 ± 0.10	1.10* ± 0.36PC = 1.85	1.17* ± 0.42PC = 8.33	1.21* ± 0.30PC = 12.03	1.27 ± 0.30PC = 17.59
AAT mM	Liver	3.04 ± 0.54	3.16* ± 0.24PC = 3.997	3.28* ± 0.19PC = 7.894	3.42* ± 0.74PC = 12.5	3.49* ± 0.26PC = 14.802
pyruvate	Brain	2.04 ± 0.62	2.10* ± 0.19PC = 2.941	2.18* ± 0.36PC = 5.862	2.27* ± 0.80PC = 11.27	2.31* ± 0.63PC = 13.235
formed/mg	Gill	2.18 ± 0.66	2.20* ± 0.35PC = 0.917	2.24* ± 0.41PC = 2.752	2.31* ± 0.36PC = 5.963	2.38* ± 0.45PC = 9.174
protein/ hr	Muscle	1.72 ± 0.29	1.83* ± 0.28PC = 6.395	1.94* ± 0.31PC = 12.79	2.04 ± 0.34PC = 118.60	2.14 ± 0.66PC = 24.41
	Kidney	1.09 ± 0.14	1.14* ± 0.22PC = 4.587	1.32 ± 0.36PC = 21.10	1.45 ± 0.24PC = 35.77	1.56 ± 0.38PC = 143.11
ALATmM	Liver	6.84 ± 1.06	6.92* ± 1.36PC = 1.16	7.24* ± 1.11PC = 5.84	7.39* ± 1.86PC = 8.04	7.84* ± 1.00PC = 14.61
pyruvate	Brain	5.03 ± 0.96	5.12* ± 0.39PC = 1.78	5.21* ± 0.40PC = 3.51	5.36* ± 0.71PC = 6.56	5.42* ± 1.14PC = 7.75
formed/mg	Gill	4.82 ± 0.79	4.86* ± 0.76PC = 0.829	4.93* ± 1.17PC = 2.28	5.07* ± 1.08PC = 5.18	5.23* ± 1.13PC = 8.50
protein/ hr	Muscle	4.89 ± 0.49	4.92* ± 0.89PC = 0.613	5.098* ± 1.08PC = 4.08	5.13* ± 0.78PC = 4.90	5.19* ± 0.72PC = 6.13
	Kidney	2.87 ± 0.82	2.93* ± 1.14PC = 2.09	3.01* ± 1.19PC = 4.81	3.11* ± 0.84PC = 8.36	3.22* ± 0.98PC = 12.19
GDHmM	Liver	0.43 ± 0.04	0.47* ± 0.01PC = 9.30	0.51 ± 0.03PC = 18.60	0.57 ± 0.04PC = 32.55	0.61 ± 0.01PC = 41.86
formazan	Brain	0.38 ± 0.01	0.41* ± 0.01PC = 7.89	0.48* ± 0.02PC = 13.15	0.51 ± 0.01PC = 34.21	0.57 ± 0.02PC = 50
formed/mg	Gill	0.31 ± 0.02	0.33* ± 0.01PC = 6.45	0.34* ± 0.05PC = 9.67	0.41 ± 0.07PC = 32.25	0.43 ± 0.01PC = 38.70
protein/hr	Muscle	0.35 ± 0.01	0.34* ± 0.05PC = 2.85	0.37* ± 0.01PC = 5.71	0.41 ± 0.02PC = 17.14	0.45 ± 0.04PC = 28.57
	Kidney	0.29 ± 0.021	0.30* ± 0.02PC = 3.44	0.35 ± 0.02PC = 20.68	0.37 ± 0.014PC = 27.58	0.39 ± 0.015PC = 34.48

Each value is mean ± S.D of six individual observations. Means are compared with Manu-Whitney is test of significance at $p < 0.05$ which is considered as statistically significant. PC denotes percent change over control. * Not significant.

switches over to the gluconeogenic process with the help of transaminases. The change in transaminases activities suggest a possible change in protein metabolism in the tissues of *C.punctatus* with the exposure of Thiamethoxam.

The enhancement in the activity of GDH is due to thiamethoxam toxicity was observed in liver, brain, gill, muscle and kidney tissues. According to David and Michael (2005); Satyanarayana (2005), the increased GDH activity may indicate with increased rapid utilization of amino acids and onset of detoxification mechanism (Prashanth, 2006; Ganesh *et al.,* 2006). This indicates higher oxidation of amino acids to combat the toxic effect of oc compound. The higher activity of GDH may result in efficient operation of oxidative deamination under toxic impact of Thiamethoxam.

The overall decrease in protein content in proteases and enhanced levels of amino acid transaminases and GDH might suggest the utilization of proteins under toxic stress conditions of the fish, *C.punctatus*.

ACKNOWLEDGEMENTS

One of the authors V.Anil Kumar thanks to the Head, Department of Zoology, Kakatiya University, Warangal for providing necessary facilities.

REFERENCES

Abdul Naveed. 2003. A review of environmental pollution and its impact on biotic fauna. Dissertation submitted to SRTM University, Nanded.

Abdul Naveed, Venkateshwarlu, P. and Janaiah, C. 2004. The action of sublethal concentration of Endosulfan and Kelthane on regulation of protein metabolism in the fish *Clarias batrachus*. *J. Nature. Environ. and pollut. Tech*. **3(4):** 539-544.

Afaq, S. and Rana, K. S. 2009. Impact of leather dyes on total protein of fresh water teleost, *Cirrhinus mrigala* (Ham). *Asian J. Exp. Sci.* **23(1):** 299-302.

Aronson, K. J., Miller, A. B., Woolocott, Ch. G., Stern, S. E. E., McCready, D. R., Lickley, L. A., Fish, F. B., Hiraki, G. Y., Holloway, C., Ross, T., Hanna, W. M., Sengupta, S. K. and Weber. 2000. Breast adipose tissue concentrations of polychlorinated biphenycls and other organochlorine and breast cancer risk. Cancer Epidemiol. Biomark. Preva., 55-63.

Betoulle, C., Duchiron, C. and De Schaux, P. 2000. Lindane increase invitro respiratory burst activity and intracellular calcium levels in rainbow trout (oncorhynchus mykiss) head, kidney, phagocytes. *J. Aquatic. Toxicology*. **48:** 211-221.

Bayne, B. L., Widdows, L. and Worral, C. 1977. Physiological responses of marine biota to pollutants J. Academic press, New York.

Chun-Yuv Yang, Yah – Tseng Tseng and Chih – ching change. 2003. Effects of air pollution on birth weight among children born between 1995 and 1997 in Kaohsiung, Taiwan. *J. Toxicol. Environ. Health*. **66:** 807-816.

David, L. Nelson. and Michael M.Cox., A. L. 2005. The biosynthesis of nucleotides. In Lehiningers Biochemistry 2nd edition. Worth Publishers. Inc. New York.

Davis, N. C. and Smith, E. L. 1955. Methods of biochemical analysis Glick D(ed) Interscience publishers, New York, p 247.

Dhanapakiam, P., Ramaswamy, V. K . and Mini Joseph, J. 2006. Changes in the level of transminases in Indian major carp, *Labeo rohita* exposed to sublethal concentration of tannery and distillery effluents. *J.Environ. Biol.* **27(3):** 567-570.

Ganesh, K., Janaiah, C., and Venkateshwarlu, P.2006. Specific activity levels of protease, aminases and GDH in fresh water fish *Clarias batrachus* on exposure to profenophos. *J. Aqua. Bio.* **21(2):** 196-199.

Lee, Y. L. and Lardy, H. A. 1965. Influence of thyroid hormones on L. gycerophosphate dehydrogenase in various organs of the rat. *J. Biol. Chem.* **240:** 1427.

Lee, B. W., Kelsey, K. T. and Hashimoto, D. 1997. The prevalence of pulmonary and upper respiratory tract symptoms and spiromeric test findings among newspaper pressroom workers exposed to solvents. *J. Occup. Environ. Med.* **39(10):** 960-69.

Lydy, M. J. and Linck, S. L. 2003. Assessing the impact of Triazine herbicides on organophosphate insecticide toxicity to the earth worm *Eisenia fetda. J. Archives. Environ. Contam. Toxicol.* **45:** 343-349.

Nemsok, J. and Boross, L. 1981. The effect of pesticides on the proteolytic enzymes activity of fishes. *Acta biologica. Szeged.* **27:** 3-7.

Prashanth, M. S. 2006. Impact of cypermethrin on protein metabolism in fresh water fish *Cirrihinus mrigala*. Nature. *Environ. Pollution. Tech.* **5(2):** 321-32.

Reitman. S., and Frankel, S. 1957. Colorimetric method for the determination of Serum glutamic oxylaectic and glutamic pyruvic transaminases. *J. Am. J. Clin. Pathol.* **28:** 56-63.

Sajal Ray., Chakrabarty, T., Bose, A. and Robin, S.1988. Effect of sublethal concentration of methyl parathion of enzymes. *J.Environ. Ecol.* **6(3):** 563-567.

Satyanarayana, U. 2005. Biochemistry books and allied (P) Ltd., 8/1 Chintamani Das Lane, Kolkata, 700009, India.

Schulman, L. J., Sargent, E. V., Naumann, B. D., Faria, E. C., Dolan, D. G. and Wargo, J. P. 2002. A human health risk assessment of pharmaceuticals in the aquatic environment. *J. Humn. Ecol. Risk. Assess.* **894:** 657-680.

Shoba Rani, V., Venkateshwarlu, P. and Janaiah, C. 1989. Alteration in aminotransferases of freshwater fish *Clarias batrachus,* after exposure to Trichlorfon. *J. Environ. and Ecol.* **7(4):** 930-932.

Shoba Rani, V. and Janaiah, C.1991. Ammonia metabolism in fresh water teleost, *Clarias batrachus* (Linn). On exposure to Trichlorfon. *J. Bull.Environ. contam. and Toxicol.* **46:** 731-737.

Venkateshwarlu, P., Shobha Rani, V. J., Janaiah, C. and Prasad, M. S. K. 1987. Effect of endosulfan on Carbohydrate metabolism in *Clarias batrachus* (Linn). *J. Comp. Anim. Physiol.* **5(2):** 79-84.

Waliszewski, S. M., Infanzon, R. M. and Hart, M. M. 2003. Difference in persistent organochloride pesticides concentration between breast adipose tissue and blood serum. *Bull. Environ. Contam. Toxicol.* **70:** 920-926.

Webb, S. F. 2001. A data – based perspective on the environmental risk assessment of human, Pharmaceuticals III-indirect human exposure. In Kummerer, K. (Ed), Pharmaceuticals in the environment. Springer. Berlin.pp. 221-230.