

## Impact of Azodrin on Protein Content in the Freshwater Fish *Catla Catla*

S. Janardana Reddy, D.Vineela and B. Kiran Kumar

Department of Fishery Science and Aquaculture, Tirupati-517502

### ABSTRACT

*Catla catla*, a freshwater fish exposed to lethal (3.654 ppm) and two sub lethal concentrations of azodrin (1.827 ppm and 0.913 ppm) for 96h and 60 days and protein content was observed from different tissues after the exposure period. Acute exposure (6.25 ppm) results in significant decrease in the level of protein in testis, ovary and brain and slight decrease in intestine, muscles, liver and gills; whereas increased protein level was observed in kidney. Chronic toxicity results showed decrease in the level of protein content in ovary, brain, intestine, muscles, gills and liver to 3.50 ppm and 1.50 ppm exposure; whereas in testis protein level was increased to 1.50 ppm and decrease protein content was observed in 3.50 ppm exposure.

### I. INTRODUCTION

It is well known that extensive usage of organophosphorus (OP) compounds in agriculture has resulted in a widespread distribution in the environment. The OP pesticides (OPs) have largely replaced organochlorine (OCl) compounds in the agricultural activities. OPs have been widely used to control agricultural pests, but these are harmful to non-target aquatic organisms when frequently used, due to contamination of aquatic environment through run-off (1). Although at present usage of several pesticides contribute to increased agricultural production and to eradicate vector borne diseases, their harmful effects on the non-target animals are not ruled out and also concentrated in them more readily than to the terrestrial organisms(2).

Aquatic animals inhabiting polluted water bodies tend to accumulate many chemicals in high concentrations even when the ambient environmental contamination levels are low (3) a potentially hazardous situation for the entire food chain. Once a toxicant enters an organism, several biochemical and physiological responses occur which may be adaptive or may lead to toxicity. The biochemical processes represent the most sensitive and relatively early events of pollutant damage. Thus, it is important that pollutant effects be determined and interpreted in biochemical terms, to delineate mechanisms of pollutant action, and possibly ways to mitigate adverse effects.

OPs are known to inhibit acetyl cholinesterase (AChE) enzyme, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolyzing the neurotransmitter acetylcholine to choline and acetate (3). Some Ops are highly soluble in water and can therefore easily contaminate aquatic ecosystems, thereby increasing the exposure risk of aquatic flora and fauna (4). Pesticides in water cause

damage to biotic life especially to fish. Fishes are very sensitive to a wide variety of toxicants in water. Various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides, polychlorinated biphenyls and heavy metals. Among these, pesticides have been found to be highly toxic not only to fishes but also to fish food organisms. Pesticides produce many physiological and biochemical changes in the freshwater fauna by influencing the activities of several enzymes and metabolites(5,6,7). It has also been reported that acute and chronic toxicities of pesticides caused biochemical alterations in organs (8, 9, 10, 11 &12).

The fish serves as bioindicator of water quality and the impact of pesticides can be well understood by analyzing the biochemical parameters of different tissues of it. As protein budget of a cell can be taken as an important diagnostic tool in the evaluation of its physiological state.

The alterations in biochemical contents in different tissues of fish due to toxic effects of different heavy metals and pesticides have been reported by many workers (13,14,15,16,17&7) Extensive work has been done on the toxic effects of pesticides on protein, carbohydrate and lipid contents of fishes, but very little work have been done on biochemical changes in *Catla catla*. Therefore the present work has been an attempt to assess the extent of alteration in protein content in *Catla catla* under azodrin toxicity.

### II. MATERIALS AND METHODS

The freshwater fish *Catla catla* were collected from the freshwater sources around Aurangabad city. Fishes were acclimatized in aged, dechlorinated and well aerated water for two weeks. During acclimatization they were fed on alternate days with pieces of live earthworms. The LC<sub>50</sub> values are

determined by following the guidelines given on committee of toxicity tests with aquatic organisms (18). The  $LC_{50}$  values are calculated by Probit Analysis Method (19). The acclimatized fishes were exposed to lethal concentration (5.012 ppm) for 96h and two sublethal concentrations (3.50 ppm and 1.50 ppm) for 60 days. Simultaneously a control group of healthy fishes were maintained under identical conditions. The fishes were sacrificed immediately at the end of exposure period and different tissues viz. gill, liver, gonads, brain, kidney, intestine and muscles were processed for the biochemical estimations. Protein content was estimated by Follin phenol reagent method (20).

### III. RESULTS AND DISCUSSION

In present study, an attempt has been made to examine the sub lethal toxic effect of organophosphate pesticide azodrin on protein metabolism in terms of tissue proteins in fish (**Table-1**).

Acute exposure (3.654ppm) resulted in significant decrease in the level of protein in testis, ovary and brain and least decrease in intestine, liver, gills and muscles. Whereas increased protein level was observed in kidney. The two sublethal exposure (1.827 ppm and 0.913 ppm) results show that there is decrease in the level of protein content in ovary, brain, intestine, muscles, gills and liver. Whereas in testis protein level increases at 1.50 ppm and decreases in 3.50 ppm exposure. Sub lethal exposure result when compared, we find, the protein level increases with decrease in the pesticide concentration i.e. increased protein level in ovary, testis, intestine, gills, brain and liver, whereas decreased amount of protein in muscles and kidney were observed at low concentration (1.50 ppm) (**Table-1**). Decrease in protein content after exposure to azodrin may be attributed to the improvement of protein synthesis and or increase in the rate of its degradation to amino acids which may be fed to TCA cycle through aminotransferases probably to cope up with high energy demands in order to meet the stress condition. The decrease in protein content suggests an increase in proteolytic activity and possible utilization of its products for metabolic purpose. Depletion of protein as a result of toxicity stress has already been reported by a number of workers (21, 22, 23, 24 and 25) reported decrease in protein content of muscles after DDT treatment in the fish *Clarias batrachus*. Saxena et al., (26) observed decreased level of protein in gonads of *Channa punctatus* after fenitrothion and carbofuran exposure. Reddy et al., (27) observed decreased level of protein in brain, liver and muscles of fenvalerate exposed fish *Cyprinus carpio*. Singh and Bhati (28) reported progressive decrease in the protein content with increase in exposure time in liver of *Channa punctatus* under 2, 4-D stress. Similar

results were observed during present investigation. The changes in protein content may be due to damage caused to hepatic tissue and increased proteolysis. Ghousia and Vijayaraghavan (30) reported decrease in protein content of Dimethoate intoxicated fish (*Clarias batrachus*) indicated physiological adaptability of the fish to compensate for pesticide stress. To overcome the stress the animals require high energy, this energy demand might have led to the stimulation of protein catabolism. Rajyashree (29) also observed decline in protein level in liver, muscles, gills and brain during carbamide exposure of *Labeo rohita*. Das et al., (8) observed marked decrease in the protein content of various tissues like kidney and muscles and slight increase in the protein content of brain and gills in cypermethrin treated fish, *Channa punctata*. Susan et al., (30) have also reported a significant decrease in protein content under sublethal concentrations of pyrethroid fenvalerate in the gills of *Catla catla*.

The survival ability of animals exposed to stress mainly depends on their protein synthetic potential. The degradation of protein suggests the increase in proteolytic activity and possible utilization of their products for metabolic purposes and cause damage to tissues. (31). Similar trend was also observed by Ghosh et al. (32).

The decreased trend of protein content in various tissues of *Catla catla* in the present study may be due to metabolic utilization of keto acids in the synthesis of glucose or for the osmotic and ionic regulation as reported by Vutukuru (33), Venktrama et al. (34), Mamata Kumari (35), Chezhan et al.(36) and Murthy and Devi (37). The present study revealed the reduction in protein levels in the tissues of *L. rohita* by following acute exposure of toxicant Phenthoate. Similar changes were also recorded in *C. punctatus* exposed to malathion by Agrhari et al. (38) and Tilak et al., (39) have also explained the reduction of protein content of liver, brain and ovary of *C. punctatus* exposed to fenvalerate.

The fall in protein level during OP exposure may be due to increased catabolism (40,5,6) and decreased anabolism of proteins (17). The reducing trend of protein content may be attributed to metabolic utilization of ketoacids to gluconeogenesis pathway for the synthesis of glucose or for the maintenance of osmotic and ionic regulations (41). The alteration in protein value in liver may also be related to some structural changes in the liver, the arrangement of hepatic cords leading to the alterations of liver metabolism. Decrease in protein content could possibly be due to protein breakdown and suggests decrease in protein is due to damage of hepatic tissue and an intensive proteolysis. (42, 43, 6, 7). Thus, a decrease in the protein content during exposure to azodrin naturally affects the nutritive value of fish.

## REFERENCES

- [1] Joseph B., Raj SJ, "Impact of pesticide toxicity on selected biomarkers in fishes", *Int.J.Zool.Res*, Vol.7: Page-212-222, 2011.
- [2] Edwards C A, "In Environmental pollution by pesticides", *Plenum press*: pp-542, 1973.
- [3] Colombo, J.C., Bilos, C., Campanaro, M., Presa, MJR., Catoggio, J.A., 1995. Bioaccumulation of polychlorinated biphenyl and chlorinated pesticides by the Asiatic clam, *Corbicula fluminea*. Its use as sensitive organism in the Rio de la Plata Estuary. *Argentina Environ. Sci. Technol.* 29, 914-927.
- [4] Fulton M H., Key P B, "Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticides exposure and effects", *Environ. Toxicol. Chem*, Vol. 20, pp-37-45, 2001.
- [4] Agdi K., Bouaid A., Esteban A M., Hernando P F., Azmani A., Camara C., (2000) Removal of atrazine and four organophosphorus pesticides from environmental waters by diatomaceous earth remediation method. *J. Environ. Monit*, Vol-2(5): Page-420-423.
- [5] S. Janardana Reddy and D.C.Reddy, "Effect of Phosalone toxicity on Detoxification Enzymes and Lipid Peroxidation of Indian Major Carp", *Aquacult.* Vol. 10(2), pp-147-159, 2009.
- [6] S. Janardana Reddy, "Dimethoate Toxicity on Haematological and Biochemical Indices of Prawn, *Macrobrachium rosenbergii*," *Arch.cell.Biol*, Vol.12 (1), pp-111-117. 2012.
- [7] S. Janardana Reddy and D.Vineela, "Impact of azodrin on haematological and behavioural responses of carp fish", *Electronic Journal of Biosciences*, Vol 01(3), pp-54-62, 2013.
- [8] Das, L. V., Jeewaprada, P. N. and Veeraiah, K, "Toxicity and effect of cypermethrin on biochemical constituents of freshwater teleost *Channa punctata*," *J. Ecotoxicol. Environ. Monit.* Vol. 9(3), pp-197-203, 1999.
- [9] Finney, D. J, "Probit Analysis, 3rd Edn. Cambridge Univ. Press", *London and New York*, 1971.
- [10] Rawat, D. K., Bais, V. S. and Agrawal, N. C., "A correlative study on liver glycogen and endosulfan toxicity in *Heteropneustes fossilis*", *J. Environ. Bo*, Vol. 23(2), pp- 205-207, 2002.
- [11] Sanjoy Deka and Rita Mahanta, "A Study on the Effect of Organophosphorus Pesticide Malathion on Hepato-Renal and Reproductive Organs of *Heteropneustes fossilis* (Bloch)", *The Science Probe*, Vol. 1, no. 1, pp, 1-13, 2012.
- [12] P. Giridhar, S.R.K. Neeraja, P. Indira, "Effect of Organophosphorus Nuvan on Some Aspects of Carbohydrate Metabolism in Fresh Water Fish *Labeo rohita* (Hamilton)", *Int. J. Pharm. Sci. Rev. Res*, 2015. Vol. 31(2), pp- 80-84, 2015.
- [13] Kamble, S. B, "Effect of pesticide on fresh water fish *Lepidocephalichthys thermalis* (C&V) from Marathwada region," Ph.D. thesis, *Dr. Babasaheb Ambedkar Marathwada University*, Aurangabad, 1983.
- [14] Gupta, A. K., Rai, V. and Agrawal, S. M, "Effect of starvation on total protein and free amino acids in some organs of the fish *Channa punctatus*". *Geobios*, Vol.14, pp-108-110, 1987.
- [15] James, R. and Sampath, K, "Sublethal effects of mixtures of copper and ammonia on selected biochemical and physiological parameters in the catfish, *Heteropneustes fossilis* (Bloch)", *Bull. Environ. Contam and Toxicol*, Vol.55(2), pp- 187-194, 1995.
- [16] Choudhary and Gaur, "Biochemical effect of cadmium toxicity on a hill stream teleost *Garra mullya* (Skyles) during egg maturation I total protein, GSI, HIS", *Poll. Res*, Vol. 11(3), pp-157-161, 2001.
- [17] Khare, A. and Singh, S, "Impact of malathion on protein content in the freshwater fish *Clarias batrachus*," *J. Ecotoxicol. Environ. Monit*, Vol.12 (2), pp-129-132, 2002.
- [18] Anon, "Recommendations of the committee on methods for toxicity tests with fish, macro invertebrates and amphibians", *EPA, Oregon*, Vol. 61, 1975.
- [19] Probit analysis, 3rd ed, *Cambridge University Press*, pp-0-80.
- [20] Lowry, O. M., Rosenbrough, N. J., Farr, A. C. and Randall, R. F, "Protein estimation with Folin Phenol reagent" *J. Biol. Chem*, Vol. 193, pp- 265-275, 1951.
- [21] Swami, K. S., Rao, K. S. J., Reddy, K. S., Moorthy, R. S., Shetty, G. L. and Indira, K, "The possible metabolic diversion adopted by the freshwater mussel to counter the toxic metabolic effects of selected pesticides," *Indian. J. Anim. Physiol*, Vol.1, pp-95-106. 1983.
- [22] Balint, T., Szegletes, T., Szegletes, Z., Halasy, K. and Nemcsok, "Biochemical and subcellular changes in carp exposed to the organophosphorus methidathion and pyrethroid deltamethrin," *Aquat.Toxicol*, Vol.33 (3-4), pp- 279-295, 1995.

- [23] Rao, L. M. and Ramaneshwari, "Effect of sublethal stress of endosulfan and monocrotophos on the biochemical components of *Labeo rohita*, *Mystus vittatus* and *Channa punctata*", *Ecol. Env. Cons*, Vol. 6, no.3, pp- 289-296,2000.
- [24] Shinde, V. R., Veeresh, M. U. and Kulkarni, R. S, "Ovarian changes in response to heavy metals exposure to the fish *Notopterus notopterus* (Pallas)," *J. Environ. Biol*, Vol.23, no.2,pp- 137-141,2002.
- [25] Yogana Bano., Shaikh, A. A. and Hameed, T, "Effect of sublethal concentration of DDT on muscle constituents of an air breathing cat fish *Clarias batrachus*," *Proc. Indian. Acad. Sci*, Vol. 90, no.1, pp-33-37, 1981.
- [26] Saxena, P. K., Singh, V. P., Kondal, J. K. and Soni, G. L., "Effect of some pesticides on in vitro lipid and protein synthesis by the liver of the freshwater teleost, *Channa punctatus*," *Environ. Pollut*, Vol.58, no.4,pp- 273-280, 1989.
- [27] Reddy, P. M., Philip, G. H. and Bashamohideen, M, "Fenvalerate induced biochemical changes in the selected tissue of freshwater fish, *Cyprinus carpio*", *Biochem. Int*, Vol. 23,no.6,pp- 1087-1096, 1991.
- [28] Singh, S. and Bhati, D. P. S, "Evaluation of liver protein due to stress under 2,4-D intoxication in *Channa punctatus* (Bloch)", *Bull. Environ. Contam. Toxicol*. Vol.3,pp- 149-152, 1994.
- [29] Rajyashree, M, "Carbamide induced alterations in some metabolic aspects of fish, *Labeo rohita*", *J. Ecotoxicol. Environ. Monit*, Vol.6, no.1, pp-41-44,1996.
- [30] Susan, A. T., Veeraiah, K. and Tilak, K. S, "Biochemical and enzymatic changes in the tissues of Catla catla exposed to the pyrethroid fenvalerate" *J. Ecobiol*, Vol.11, no.2, pp-109-116, 1999.
- [31]. Mastan B.K. and Rammayya,P.J. (2010) Biochemical Profile of *Channangachua* (Ham) exposed to sublethal doses of Dichlorovos (DDVP). The Internet Journal of Toxicology, 8: 27-32.
- [32]. Ghosh, D., Datta, S., Bhattacharya s and Mazumder S., (2006). Perturbation in the catfish immune responses by arsenic: organ and cell specific effects. *Comp. Biochem. Physiol.*, 143: 455-463.
- [33]. Vutukuru, S.S. 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, Hematological parameters and some Biochemicals profiles of Indian major carp, *Labeo rohita*. *Int J Environ Res Public health*. 2(3): 456-462.
- [34]. Venktramana, G.V., Sadhya Rani, P.N. and Murthy, P.S. 2006. Impact of malathion on the biochemical parameters of gobiid fish, *Glossogobius giurus* (Ham). *J Environ Biol*. 27(1): 119-122.
- [35]. Mamata Kumari. 2007. Biochemical changes induced by the pesticides abate in the liver of cat fish *Heteropneutes fossilis* (Bloch). *Environ and Eco*. 225(4): 1164-1166.
- [36]. Chezhian, A., Kabilan, N., Kumar, S.T., Senthamilselvan, D. and Sivakumari, K. 2010. Impact of common mixed Effluent of spicot industrial Estate on histopathological and biochemical changes in estuarine fish *Lates calcarifer*. *Curr Research J of Boil Sciences* 2(3): 201-209.
- [37]. Murthy, A.S. and Devi, A.P. 1982. The effect of endosulfan and its isomers on tissue protein, glycogen and lipid in the fish *Channa punctatus*. *Pesticidal Biochem Physiol*. 17: 280-286.
- [38]. Agrahari, S.K., Gopal and Pandey, K.C. 2006. Biomarkers of Monocrotophos in behaviour of freshwater fish *Channa punctatus* (Bloch). *J Environ Biol*. 27: 453-457.
- [39]. Tilak, K.S., Veeraiah, K. and Vardhan, K.S. 2003. Toxicity and residue studies on fenvalerate to the freshwater fish *Channa punctatus* (Bloch). *Bull Environ Contam Toxicol*. 71: 1207-1212, (2003).
- [40] Ghosia, B. and Vijayaraghavan, S, "In vivo toxicity of Dimethoate on proteins and transamines in the liver tissue of freshwater fish, *Clarias batrachus* (Linn)," *Bull. Contam. Toxicol*, Vol.54,pp- 370-375, 1995.
- [41] Schmidt, N. B, "Osmoregulation effect of salinity and heavy metal,". *Fed. Proc*, Vol.33, pp- 2137-2146, 1975.
- [42] Rao, K. S. P. and Rao, K. V. R, "Changes in the tissue lipid profile of fish *Oreochromis mossambica* during methyl parathion toxicity-a time course study", *Toxicol. Lett*, Vol.23, pp-147-153, 1984.
- [43] Hilmy, A. M., Shabana, M. B. and Daabees, A. Y, "Effect of cadmium upon in vivo and in vitro activity of protein and five enzymes in blood serum and tissue homogenate on *Mugil cephalus*", *Comp. Biochem. Physiol. Comp. Pharmacol. Toxicol*, Vol.81, no.1, pp- 145-154, 1985.

**Table-1: Variations in Protein content in various tissues of *Catla catla*, exposed to lethal and sub lethal concentrations of Azodrin.**

Tissues	Control	Exposure Concentrations		
		Lethal (3.654ppm)	Sub-lethal (1.827 ppm)	Sub-lethal (0.913ppm)
<b>Testis</b>	18.6084 ± 0.1753	8.6263 ± 0.0945 (-53.64)	10.1895 ± 0.2316 (45.24)	10.6526 ± 0.1158 (42.75)
<b>Ovary</b>	16.0549 ± 0.3063	6.9474 ± 0.1158 (-56.72)	10.9999 ± 0.2895 (-36.70)	17.2105 ± 0.895 (7.19)
<b>Kidney</b>	25.7925 ± 0.2316	40.5263 ± 0.5789 (57.12)	32.9548 ± 0.5789 (27.76)	28.9473 ± 0.2316 (12.23)
<b>Liver</b>	23.9629 ± 1.0942	21.5368 ± 0.4632 (-10.12)	18.2368 ± 0.2895 (-23.89)	20.3017 ± 0.3537 (-15.27)
<b>Muscle</b>	11.9968 ±0.0884	10.1562 ± 0.0579 (-15.34)	8.4629 ± 0.0579 (-29.45)	7.3168 ± 0.1523 (-39.01)
<b>Gill</b>	12.7542 ± 0.2397	11.5034 ± 0.1769 (-9.80)	9.3314 ± 0.1158 (-26.83)	9.8167 ± 0.11 (-23.03)
<b>Brain</b>	31.6518 ± 0.6127	12.5873 ± 0.1158 (-60.23)	17.6574 ± 0.2895 (-44.21 )	25.3259 ± 0.2316 (-19.98)
<b>Intestine</b>	10.4625 ± 0.3063	9.2105 ± 0.1158 (-11.96)	9.9893 ± 0.2316 (-4.52)	10.6130 ± 0.1158 (1.43)

Mean values are significant at P<0.05. (n=10)