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**Original Article** 

# EFFECT OF DELTAMETHRIN ON SOME ASPECTS OF PROTEIN METABOLISM IN FRESH WATER FISH *LABEO ROHITA* (HAMILTON)

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### ABSTRACT

**Objective:** The contamination of water by pesticides may effect on non - target organisms like fish. So an attempt was made on sublethal effect of deltamethrin on some aspects of protein metabolism in the fish *Labeo rohita*.

**Methods:** The fish fingerlings were exposed to sublethal concentration of Deltamethrin for five exposure periods i.e. 1, 7, 15, 20 and 30 day. In this investigation the levels of total proteins, protease activity and free amino acids were estimated in the brain, liver, gill, kidney and muscle of fish.

**Results:** The levels of total proteins declined on 1<sup>st</sup> day exposure and continued its declination up to 15<sup>th</sup> day. From 15<sup>th</sup> day onwards their levels gradually elevated and came nearer to control on 30<sup>th</sup> day exposure period. In contrast to this the levels of protease activity and free amino acids followed an opposite tend.

Conclusion: In the present study the shifts in protein metabolism might to compensate with situation shown by the fish for its survival.

Keywords: Deltamethrin, Sublethal, Labeo rohita, Total proteins, Protease activity and Free amino acids.

## INTRODUCTION

Pesticides are the biological toxicants which are required by man to kill insects, pests and also man's fight against the spread of diseases [1]. Now pesticides usage became an indispensible and integral part of world agriculture. Modern agriculture practices even though contributed to enhance crop production but also widely polluted aquatic environment [2]. Agriculture practices along with pest control programmers, the surface runoff and aerial spraying forming the major source for translocation pesticides into aquatic ecosystems [3-5]. The contamination of water by pesticides may effect on non - target organisms like fish [6-8]. The fish is a good indicator and highly sensitive in such ecosystem where the water gets contaminated with toxic chemicals. So an attempt was made on sub lethal effect of deltamethrin on some aspects of protein metabolism in the fish *Labeo rohita*.

#### MATERIALS AND METHODS

### **Test Chemical:**

The pesticide selected for the present investigation was synthetic pyrethroid Deltamethrin. It is widely used on diverse agricultural crops to control pests of crops, flies and mosquitoes. It has been widely used because of its high photostability, degradability, non - persistent nature and low mammalian toxicity. Its commercial name was Decis. Commercial grade was used and its effective concentration was 2.8%.

#### **Experimental design**

Fresh water fish *Labeo rohita*, weighing 10±2 gm were procured from local fisheries department and stored in spacious aquaria. The water in aquaria was aerated twice day, the fish were fed daily with ground nut cake and rice bran. The physic-chemical properties of water used for experiments had pH 7.4± 0.2, dissolved oxygen 6-7 ml /lt, hardness 160 ppm and temperature °28±1 Before experimentation has been executed, the fish were acclimated to the laboratory conditions for a period of 10 days. Later groups of 10 fish were exposed to different concentration of Deltamethrin ranging from 0.02  $\mu$  l to 0.2  $\mu$ l. The mortality was observed during 96 hrs exposure period. The LC50 / 96 hrs was determined from the percent and probit mortality versified by Dragstedt and Behrens method as given by Carpenter [10]. After determination of LC <sub>50</sub>/96

hrs (00.1µg/lt), the fish were exposed to sublethal concentration of Deltamethrin (1/10<sup>th</sup> of LC<sub>50</sub>/96hrs i.e. 0.01 µg/lt) for five exposure periods i.e. 1, 7, 15, 20 and 30 day.

#### Methods

In the present investigation the levels of total proteins, protease activity and free amino acids were estimated in the brain, liver, gill, kidney and muscle of fish. Each experiment was carried out in the organs of six individuals and the mean of six values were taken in to consideration. The total proteins were estimated by Folin phenol reagent method described by Lowry et *al* [11], protease activity were estimated using Ninhydrin method described by Davis and Smith [12] and free amino acids were estimated by the Ninhydrin method described by Moore and Stein [13].

# RESULTS

In the present investigation the levels of total proteins, protease activity and free amino acids were estimated in the brain, liver, gill, kidney and muscle of fish, on 1, 7, 15, 20 and 30 days of exposure to sublethal concentration of Deltamethrin besides control levels were presented in tables 1, 2 and 3. Where as The levels of total proteins declined relative to controls in all organs of fish at first day exposure and continued its declination up to 15 day exposure periods. From 15 day onwards their levels gradually elevated and came nearer to control at 30 day exposure period. Mean and standard deviation are a pool of six individual measurements. The percent change in the protease activity at different periods was calculated in relation to the protease activity in the control medium. The differences between control and exposure period days were found to be statistically significant (P < 0.01).

### DISCUSSION

Proteins are complex nitrogen containing macromolecules. They are the basic building block of animals [14]. The survival ability of animals exposed to stress majorly depends on their protein synthetic potentials. The proteins are the major source of energy during chronic conditions besides carbohydrates [15]. Young [16] reported protein budget of a cell can be taken as an important diagnostic tool in the evaluation of its physiological standards. Pesticides are known to interfere in protein synthesis and degradation there by altering the dynamic equilibrium [17-31].

In the present study relative to controls the levels of total proteins declined on first day exposure and continued its depletion up to 15 day exposure period. Whereas the levels of protease activity and free amino acids initially elevated on 1 day exposure period and continued its elevation up to 15 day exposure period. The decline in total protein levels followed by elevation in the levels of protease activity and free amino acids at initial exposure periods may indicates the high energy demand associated with imposed deltamethrin stress.

To overcome this animal tends to mobilize the proteins by stimulating the protease activity.

Seshagiri Rao *et al.*, [32] observed an increase in free amino acid level in the organs of the fish *Sarotherodon mossambicus* which could be due to degradation of proteins by proteolysis or due to decreased protein synthetic potentials in the pesticide induced pathological condition also supports the present trend in protein metabolism.

S. No.	Organs	Control	Exposure period in days					
			1 day	7 day	15 day	20 day	30 day	
1.	Brain	81.5	73.8	68.2	65.0	71.3	76.2	
	SD	6.72	5.78	4.38	3.58	5.13	6.32	
	PC		-9.44	-16.31	-25.76	20 day 71.3 5.13 -12.51 74.2 5.25 -22.05 95.6 8.01 20.29 86.5 6.16 19.90 115.6 10.79	-6.50	
2.	Gill	95.2	82.5	76.4	64.8	20 day 71.3 5.13 -12.51 74.2 5.25 -22.05 95.6 8.01 20.29 86.5 6.16 19.90 115.6 10.79	83.5	
	SD	7.78	6.09	5.74	4.48	5.25	6.91	
	PC		13.34	19.74	-31.93		12.28	
3.	Kidney	121.0	110.0	90.5	78.8	95.6	112.0	
	SD	11.7	9.74		8.01	10.12		
	PC		9.09	25.20	34.87	<b>20 day</b> 71.3 5.13 -12.51 74.2 5.25 -22.05 95.6 8.01 20.29 86.5 6.16 19.90 115.6 10.79	7.43	
4.	Liver	108.0	97.5	88.3	73.2	5.25 -22.05 95.6 8.01 20.29 86.5 6.16 19.90 115.6	99.0	
	SD	12.03	7.17	6.89	5.63	6.16	8.12	
	PC		9.72	18.24	32.22	19.90	8.33	
5.	Muscle	133.5	120.6	105.0	90.5	115.6	121.8	
	SD	12.0	11.18	9.75	8.32	10.79	11.96	
	PC		9.66	21.34	32.20	13.40	-8.76	

SD - Standard Deviation; PC - Percent change

Mean and standard deviation are a pool of six individual measurements. The percent change in the total proteins at different periods was calculated in relation to the total proteins in the control medium. The differences between control and exposure period days were found to be statistically significant (P < 0.01).

Table 2: Protease Activity (mg/gm wet wi	.) in the organs of fish <i>Laheo rohitha</i> on	exposure to sublethal concentration of Deltan	nethrin.
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S. No.	Organs	Control	Exposure period in days					
			1 day	7 day	15 day	20 day	30 day	
1.	Brain	0.142	1 day 7   0.142 0.169 0   0.014 0.018 0   19.01 2   0.205 0.230 0   0.016 0.018 0   12.19 1   0.180 0.206 0   0.017 0.018 0   0.300 0.352 0	0.180	0.192	0.164	0.136	
	SD	0.014	0.018	0.020	0.022	0.164 0.016 15.49 0.228 0.017 11.21 0.202 0.019 12.22 0.341 0.025	0.013	
	PC		19.01	26.76	35.21	15.49	-4.22	
2.	Gill	0.205	0.230	0.245	0.264	0.228	0.195	
	SD	0.016	0.018	0.019	0.028	0.017	0.015	
	PC		12.19	19.51	28.78	11.21	-4.87	
3.	<b>Kidney</b> 0.180 0.206	0.206	0.224	0.233	0.202	0.170		
	SD	0.017	0.018	0.017	0.020	0.202 0.019	0.021	
	PC		14.44	24.44	29.44	12.22	-5.55	
4.	Liver	0.300	0.352	0.376	0.385	$\begin{array}{c} 0.164\\ 0.016\\ 15.49\\ 0.228\\ 0.017\\ 11.21\\ 0.202\\ 0.019\\ 12.22\\ 0.341\\ 0.025\\ 13.66\\ 0.236\\ 0.024 \end{array}$	0.284	
	SD	0.028	0.031	0.032	0.036		0.019	
	PC		20.00	25.33	28.33	13.66	-5.33	
5.	Muscle	0.220	0.240	0.265	0.281	0.236	0.210	
	SD	0.023	0.025	0.027	0.028	0.024	0.019	
	PC		9.09	20.45	27.72	7.27	-4.54	

SD - Standard Deviation; PC - Percent change

Some of the observations were also supports the present trend in the decline of total proteins and elevation in free amino acid are as follows. Bhavan and Geraldine [33] reported the decline in soluble proteins in tissues of prawn *M. malcomsonii* on exposure to sublethal concentration of carbaryl. Magar and Shaik [34] observed decline in protein content and elevation of free amino acids in tissues of fish *Channa punctatus* on exposure to sublethal concentration of malathion. Pratap and Singh [35] reported significant decrease in total proteins and elevation in free amino acid levels in *Chnanna punctatus* on exposure to sublethal doses of apigenin. Ram Yadav and Ajay Singh [36] reported decline in protein content and elevation in free amino acids in snail *Lymnea acuminata* exposed to plant pesticide. Furthermore Singh and Singh [37] reported decline in total proteins in the fish *Trichogaster fasciatus* on exposure to pesticide dipterex. Fahmy [38] reported significant decrease in total proteins in various

tissues of *Oreochromis niloticus* on exposure to malathion. Arun Kumar and Jawahar Ali [39] observed decrease in protein content in the tissues of shrimp *Streptocephalus dichotomus* on exposure to sublethal concentration of malathion and glyphosate. Vidya and Nair [40] observed protein content decreased in tissues of *Etroplus suratensis* on exposure to sublethal concentration of  $\lambda$ - cyhalothrin. Binakumari and Vasanthi [41] reported decline in protein content in the tissues of fresh water fish *Labeo rohita* on exposure to pesticide dimethoate. Nagaraju and Venkataratnamma [42] observed protein depletion in tissues of fresh water fish *Labeo rohita* exposed to sublethal concentration of profenofos. Shivanagouda *et al* [43] observed decline in protein content in tissues of marine fish *Mugilcephalus* on exposure to sublethal concentration of carbaryl. Suneel Kumar [44]. reported significant decrease in total proteins in *Channa punctatus* on exposure to lethal concentration of nuvan. All these studies correlate with the decline in total proteins and elevation in levels of protease activity and free amino acids.

In later half of exposure the total proteins gradually elevated and came nearer to control at 30 day exposure period.

Table 3: Free amino acids (mg/gm wet wt.) in the organs of fish Labeo rohita on exposure to sublethal concentration of Deltamethrin.

S. No.	Organs	Control	Exposure period in days				
			1 day	7 day	15 day	20 day	30 day
1.	Brain	8.13	8.26	8.40	8.62	8.34	8.00
	SD	0.54	0.59	0.73	0.81	0.78	0.49
	PC		1.59	3.32	6.02	2.58	-1.59
2.	Gill	6.88	6.95	7.18	7.30	7.13	6.70
	SD	0.49	0.61	0.48	0.62	0.38	0.57
	PC		1.01	4.36	6.10	3.63	-2.61
3.	Kidney	7.58	7.70	7.84	7.89	7.61	7.42
	SD	0.68	0.42	0.46	0.49	0.37	0.32
	PC		1.58	3.43	5.27	0.39	-2.11
4.	Liver	11.26	11.40	11.67	11.82	11.38	11.02
	SD	0.75	0.62	0.86	0.75	0.34	0.27
	PC		1.24	3.64	4.97	1.06	-2.31
5.	Muscle	9.32	9.49	9.63	9.76	9.47	9.20
	SD	0.62	0.71	0.81	0.87	0.68	0.52
	PC		1.82	3.32	4.72	1.60	-1.28

SD - Standard Deviation; PC - Percent change, The values were found to be significant (P<0.001).

Mean and standard deviation are a pool of six individual measurements. The percent change in the free amino acids at different periods was calculated in relation to the free amino acids in the control medium. The differences between control and exposure period days were found to be statistically significant (P < 0.01). Whereas the levels of protease activity and free amino acids elevated in all organs of fish at first day exposure period, relative to controls. Their levels elevated up to 15 day exposure periods. From 15 day onwards their levels gradually decreased and came nearer to control on 30 day exposure period. The values were found to be significant (P<0.001).

Whereas the levels of protease activity and free amino acid levels goes on decreasing and came nearer to control on 30 day exposure period. Pratibha *et al* [45] reported decrease in soluble and insoluble protein in tissues of fish *Channa punctatus* at initial exposure period and elevation in protein content in later exposure periods also coincides with the present study. Dixon and Sprague [46], Kito *et al* [47], and Pampatwar *et al* [48] reported an increase in protein content may also help to fortify the organs for developing resistance to the imposed toxic stress and synthesis of enzymes necessary for detoxification. In the present study the shifts in protein metabolism might to compensate with situation shown by the animal for its survival.

## **CONFLICT OF INTERESTS**

**Declared None** 

#### REFERENCES

- 1. Gold B, Leuschen T, Brunk G, Gingell R. Metabolism of a DDT metabolite via a chlorohepoxide, Chem Bio Interact 1981;35:159.
- Pandey AC, Pandey AK and Das P. Fish and fisheries in relation to aquatic pollution In:Environmental Issues and management. S.R. Verma., A.K. Gupta and P. Das 9(Eds) Nature Conservators. Muzaffarnagar 2000;P:87-112.
- Glotfelly DE, Majewski MS and Seiber JN. Distribution of several organophosphorus insecticides in a foggy atmosphere. Environ Sci Technol 1990;24:353-57.
- Roche H, Tidov A and Persic A. Organochlorine pesticides and biomarker responses in two fishes *Oreochromis niloticus* (Linnaeus, 1758) and *Chrysichthys nigrodigtatus* (Lakebed, 1803) and an invertebrate, *Macro brachium vollenhoveniil* (Herkot, 1857), from the lake Taabo (Cote'd Ivoire). J Applied Sci 2007;7:3860-69.
- 5. Joseph B and Raj SJ. Impact of pesticide toxicity on selected biomarkers in fishes. Int J Zool Res 2011;7:212-22.
- 6. Burkpile DE, Moore MT and Holland MT. Susceptibility of five non target organisms to aqueous diazinon exposure. Bull Environ Contam Toxicol 2000;64:114.
- 7. Saxena KK and Gupta P. Impact of carbamates on glycogen contents in the muscles of fresh water fish *Channa punctatus*. Poll Res 2005;24:669-70.

- 8. Dutta HM, Misquitta Doyglas and Khan Sanaaullah. The effect of endosulfan on the testis of blue gill fish *Lepomis macrochirus*. A Histopathological study. Archives. Environ Contam Toxicol 2006;51(1):149-56.
- 9. Finney D. Probit analysis:2 Edn. Cambridge University Press. London;1964.
- Carpentor PL. In:Immunology and Serology. 3<sup>rd</sup> eds. W.B. Saunders Company. Philadelphia. London. Toronto. P.254;1975.
- Lowry OH, Rosenbrough NJ, Farr AL and Randall R. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
- 12. Davis NC and Smith EL. Assay of proteolytic enzymes. Meth Biochem Anal 1955;75(20):177-82.
- Moore S and Stein WH. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J Biol Chem 1954;24:907-13.
- Jauncey K. Carp(Cyprinus carpio) nutrition a review cited in recent advances in aquaculture, edited J.G. Muir and Roberts, West View Press, INC5500, Colorado1982;PP:222.
- Umminger BL. Physiological studies on super cooled kill fish *Fundus heteroclitus*. III. Carbohydrate metabolism and survival at subzero temperature. J Exp Zool 1970;173:159-74.
- Young UR. In:Mammalia protein metabolism(H.N. Mumo. Ed) Academic Press. New York, 1970;4:485.
- Ganesan RM, Jebakumar SRD and Jayaraman J. Sublethal effect of organochlorine insecticide endosulfon on protein, carbohydrate and lipid contents in their tissues of *Oreochromis* mossabicus. Proc Indian Sci (Anim Sci) 1989;98:51-55.
- Kamble GB and Muley DV. Effect of acute exposure of endosulfon and chloropyrifos on the biochemical composition of the fish *Sarotherondon mossambicus*. Indian J Environ Science 2000;4(1):97-102.
- Shoba Rani A, Sudharshan R, Reddy TN, Reddy PUM and Raju TN. Effect of Sodium arsenate on certain aspects of protein metabolism in freshwater teleost fish, *Tilapia mossambica*. J Env Biol 2001;22(2):101-04.
- Bhavan SP and Geraldine P. Carbaryl induced alterations in biochemical metabolism of the prawn *M. molcolmsonii.* Journ Env Biol 2002;23(2):157-62.

- John Sushma N, Sivaiah U and Jayantha Rao K. Impact of aluminium acetate on protein metabolism of albino mice. J Ecobiol 2007;20(4):361-67.
- Mahananda MR, Sahoo S and Mohanty BP. Effect of insecticide malathion on muscle protein and brain acetylcholinesterase activity in *Labeo rohita*. J Ecotoxicol Environ Monit 2008;18(1):77-84.
- 23. Vijay kumar M, Butchiram MS and Tilak KS. Effect of quinolphos an organophosphorus pesticide on nucleic acids and proteins of the fresh water fish *Channa Punctatus*. J Ecotoxicol Environ Monit 2009;19(1):07-12.
- 24. Visvanathan P, Maruthannayagam C and Govinda Raju M. Effect of malathion and endosulfan on biochemical changes in *Channa punctatus.* J Ecotoxicol Environ Monit 2009;19(3):251-57.
- 25. Abdul Naveed, Janaiah C and Venkateshwarlu P. The effects of lihocin toxicity on protein metabolism of the fresh water edible fish, *Channa punctatus* (Bloch). Journal of Toxicology and Environmental Health Sciences 2010;Vol. 3(1) pp. 018-023.
- 26. Sreenivasa V and Indirani R. Impact of dimethoate on biochemical constituents in the fish *Oreochromis mossambicus*. J Ecotoxicol Environ Monit 2010;20(2):151-56.
- Sindhe SCS, Jagadeesh Naik M, and Indira P. Effect of fungicide ziram on some aspects of protein metabolism in the fresh water teleost *Labeo rohita*. J Ecotoxicol Environ Monit 2010;20(3):257-61.
- Janardhan Reddy S, Loknatha O, Kiran Reddy T and Reddy DC. Effect of heavy metals on physiological parameters of the Indian major carp *Catla catla*. J Ecotoxicol Envrot Monit 2011;21(5):453-63.
- Nagaraj G, Sarma ALN. Survival and biochemical contents of *Catla catla* exposed to mercuric chloride and their ecological implications. J Ecobiol 2011;28(4):307-20.
- 30. Rekha P, John J. Study on the changes in the levels of protein metabolism in  $\lambda$  cyhalothrininduced hepatotoxicity in fresh water *Tilapia* (*Oreochromis Mossambicus*). Advances in Applied Science Research 2011;2 (3):57-62.
- Lesley Sounderraj SF, Sekhar P, Senthil Kumar P and Nancy Lesley. Effect of Systemic Pesticide Phosphamidon on Haematological Aspects of Common Frog *Rana tigrina*. Int J Pharma Biol Archi 2011;2(6):1776-1780.
- Seshagiri RK, Srinivas M, Kashi Reddy B, Swamy KS, Chetty CS. Effect of benthiocarb on protein metabolism of teleost, Sarotherodon mossambicus. Ind J Environ Health 1987;29:440-450.
- Bhavan PS, Geraldine P. Manifestation of carbaryl toxicity on soluble protein and histopathology in the hepatopancreas and gills of the prawn, *Macrobrachium malcolmsonii*. J Environ Biol 2009;30(4):533-538.
- 34. Magar RS, Afsar Shaikh. Biochemical changes in proteins and amino acids in *Channa punctatus* in responses to sublethal

treatment with the insecticide malathion. Trends in Life Sciences 2012;Vol:1, No:3

- 35. Pratap B and Singh A. In vivo Effects of Apigenin isolated from *Jatropha gossypifolia* plant on the Biochemical Profile of Fish. Global Journal of Pharmacology 2013;7 (2):166-171.
- Ram Yadav P and Ajay Singh. Toxic effects of Selected Plant Pesticides against Freshwater Snail *Lymnaea acuminate*. Int J Trad Nat Med 2013;2(3):149-163.
- Singh SP and Singh S. Metabolic effects of an organophosphorus dipterex in *Trichogaster fasciatus*. Ind J Biol Res 2011;Vol:1(1):P 61-66.
- Fahmy GH. Malathion toxicity:Effect of some metabolic activities in *Oreochromis niloticus*, the Tilapia fish. Int J Bio Biochem and Bioinf 2012;2(1):52-55.
- Arun Kumar MS and Jawahar Ali A. Toxic impact of two organophosphorus pesticides on acetylcholinesterase activity and biochemical composition of fresh water fairy shrimp *Streptocephalus dichotomous.* Int J Pharma Biosci 2013;(4)2:(B/P):966-972.
- Vidhya V, Nair CR. Lambda-Cyhalothrin Induced Protein Alternations in *Etroplus suratensis* (Bloch). Global Journal of Pharmacology 2013;7 (2):166-171.
- Binukumari S, Vasanthi J. The Toxic Effect of Pesticide Dimethoate 30% EC on the protein metabolism of the fresh water fish, *Labeo rohita*. Int J Curr Microbiol App Sci 2013;(2013) 2(12):79-82.
- 42. Nagarju B and Venkata Ratnamma V. Effect of profenos an organophosphate on protein levels in some tissues of fresh water fish *Labeo rohita (Hamilton)*. Int J Pharma Pharma Sci 2013;Vol:5, Suppl:1, 276-79.
- 43. Shivanagouda N, Sanagoudra U, Bhat G. Carbaryl Induced Changes in the Protein and Cholesterol Contents in the Liver and Muscle of Marine Benthic Fish, *Mugil cephalus*. American Journal of Biochemistry 2013;3(2):29-33.
- 44. Suneel K. Acute toxicity evaluation of nuvan in liver of *Channa* punctatus (Bloch). Adv Res Agri Vet Sci 2014;Vol:1,No:3, 35-38
- Pratiba R, Varsha Z, Dinesh D, Neeta L. Evaluation of impact of phosphamidon on protein status of fresh water fish *Channa punctatus*. Indian Journal Sci Res 2012;3(1):123-26.
- 46. Dixon DG, Sprague JB. Copper bioaccumulation and hepato protein synthesis during acclimation to copper by juvenile rainbow trout. Aquatic Toxicol 1981;1:69-81.
- Kito H, Osc Y, Sato T and Ishikawa T. Formation of metalloprotein in fish. Comp Biochem Physiol 1982;73C (1):129-34.
- Pampatwar DV, Ambore NE and Kadam MS. Effect of activity level on total amino acid content in liver of *Channa gaucha* and *Labeo rohita* a comparative study. J Aqua Boil 2007;22(1):125-27.