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EFFECT OF CYPERMETHRIN ON PROTEIN METABOLISM OF THE NILE CARP (*LABEO NILOTICUS*) SELECTED FROM EL-NZHA AIRPORT CHANNEL, ALEXANDRIA

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ABSTRACT

The Nile carp (Labeo niloticus) was collected from El-Nzha airport channel was exposed to sublethal concentrations (0.0064 ppm) of cypermethrin (α-cyano-3 phenoxy benzyl 1.3,2 (2-dimethyl, 2-2 dichloro vinyl) cyclo propane carboxylate) for 192 hours. Total protein content decreased in all the tested tissues whereas the free amino acid content was greatly increased. The increased in GDH - NAD dependent, asparate amino-transferases (AAT) and alanine amino transferase activities were more than the controls. These results are discussed.

Key Words: *Labeo niloticus*, cypermethrin, proteins, GDH, aminotransferases.

INTRODUCTION

Pesticide usage, while desirable for pest control has resulted in unprecedented chemical pollution (Soymeal, 2002) affecting the non-target organisms (Pimental, 1971; Ware, 1980). The biochemical responses of non-target organisms exposed to different pesticides have been well documented (Coppage and Mathews, 1974; Corbett, 1974; Abbassy, 1992) Exposure to chemical pollutants elicits many molecular and biochemical changes in fish which preceded cellular and systematic sysfunctions so that early warning signs of distress may be detected (Palmes, 1976; Klump and Person, 2002). Since aquatic environment is the ultimate sink for all pollutants, aquatic toxicity testing has become an integral part of the process of environmental hazard evaluation of the toxic chemicals (Barakat, 2002). Generally, the potential impact of pollutants is more on the aquatic organisms, because in the hydrosphere pesticides and such other substances are transported to a greater distance and hence many more non-target organisms are likely to be exposed to them than in the terrestrial environment (Murty, 1986; Klump and Person, 2002).

In Egypt, the recently introduced photostable synthetic pyrethroids (Daltamenthrin, Cypermethrin, Fenvalerate and Permethrin) are used extensively as possible replacements of some of the organochlorine, organophosphate and carbamate insecticides. Cypermethrin [(rs-α-cyano-3-phenoxybenzyl)-2-2-dimethyl cyclopropane carboxylate)] is one of the promising synthetic pyrethroid which is used to control insect pests. The natural and synthetic pyrethroids are found to be more toxic to fish organophosphates and carbomates (Bradbury *et al*., 1985; Chova, 1996). Though, the toxic mechanism of pyrethroids is not yet fully elucidated, yet it is suggested that pyrethroids definitely act on the nervous system (Chatterjee *et al*., 1986; Bainey and George, 2000). The lipophilicity of pyrethroids indicate that they are absorbed strongly by the gills of the fish even at very low concentration in water (Clark *et al*., 1985; Walker, 2002). The favourable toxicological properties of cypermethrin are related to their ability to penetrate rapidly and interact with the site of action in fishes (Chatterjee *et al*., 1986; Bult, 2001). Since synthetic pyrethroid exposure is very common, it is imperative to assess the extent of biochemical changes caused by cypermethrin in vital organs of *Labeo niloticus* an important freah water culture fish. The present study is undertaken to understand the toxic potential of cypermethrin on protein metabolism of *L. niloticus.* In particulate El-Nzha airport branch where agricultural and aqua-cultural practices are developing competently.

MATERIALS AND METHODS

Labeo niloticus of approximately same size and weight (10±2.8 gram) were collected from the depth of 2-3 m of El-Nzha airport Branch beside Agriculture Road, Alexandria. This branch is a part of the Nile river and this channel is the main fresh water resource for drinking, fishing and drainage (Fig. 1). The work was done through June and July 2001, and the fish acclimated to laboratory conditions (28±2^{o}C) in plastic tubs for one week. They were initially fed with rice bran and groundnut cake and latter subjected for staving for a period of 24 hours prior to experimentation. The physico-chemical parameters of the test water was as follows: temperature 28±2 ^{o}C, pH 8.1±0.4, dissolved oxygen 8±2 ppm, total hardness 160 (mg/l). alkalinity 452 (mg/l as $CaCO_3$) and chloride 1.8

(mg/l). The LC_{50} concentration of cypermethrin (technical grade) was determined by the method of Finney's probit analysis. Three batches of 10 fish each were exposed to sub-lethal concentration, 0.0064 ppm ($1/_{10}^{th}$ of 48 hr. LC_{50}) for 24,96 and 192 hours (8 days) and one batch of 10 in fresh water (acetone added) served as the control. The Mortality was not recorded in the exposed condition, water in the toxicant chambers was changed and the predetermined quantity of the pesticide was added to the water of each experimental toxicant chamber to keep the pesticide concentration constant through out the exposure periods.

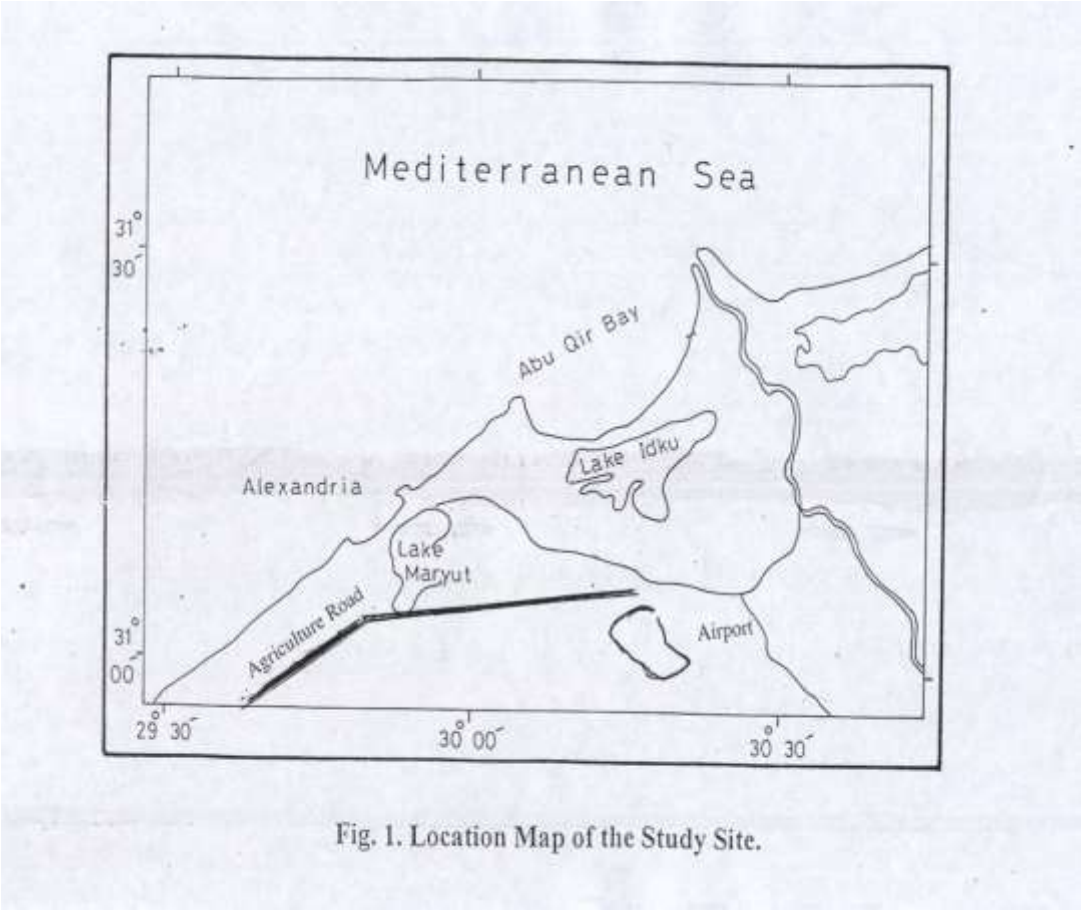

Fig. 1. Location Map of the Study Site.

Total protein was estimated by the method of Lowry *et al*., (1951), free amino acids content was estimated by the method of Moore and Stein (1954). For the estimation of enzyme activities 10% tissue homogenates were prepared in ice cold 0.25 sucrose solution and centrifuged at 2000 rpm for 15 min to remove the cell debris. A clear cell free extract was used for the estimation of glutamate dehydrogenase and aminotransferases. The activiy of glutamate dehydrogenase was estimated by the method of Lee and Lardy (1965) with slight modification as described by Prameelamma *et al*., (1975). The activities of aminotransferases (aspartate and alanine, AAT and ALAT) were determined by the method of Reitman and Frankel (1957). The student t' test was employed for analysing the data.

RESULTS AND DISCUSSION

The results summarized in Tables 1 and 2 indicate the biochemical changes in brain, liver, muscle, gill and kidney of *Labeo niloticus* exposed to sub-lethal concentration ($1/_{10}$ of 48 hr LC_{50}) of cypermethrin. Gradual depletion in total protein content was observed in all the tissue i.e. brain, liver, muscle, gill and kidney at the three exposure periods (1^{st}, 4^{th} and 8 days). The percent decrease was maximum in gill (57.7) followed by muscle (54.4), brain (49.6), kidney (49.5) and liver (42.5). The free aminoacid levels on the other hand were increased. The percent increase was maximum in liver (79.5) followed by gill (49.5), kidney (44.9), brain (43.9) and muscle (38.8) (Table 1).

Table 1. Changes in the total protein, free aminoacid levels in selected tissues of *Labeo niloticus* exposed to 0.0064 ppm of cypermethrin (values are mean ± SD of five individual observations).

Tissue	Parametre	Control	Exposure periods, hours		
			24 hours	96 hours	192 hours
Brain	Total protein	98.92 + 0.4183	74.19 ± 0.2738 (24.7)	56.31 ± 0.2738 (41.5)	48.45 ± 0.224 (49.6)
	Free aminoacids	4.84 + 0.3162	5.828 ± 0.4821 (20.4)	6.273 ± 0.3354 (38.8)	6.274 ± 0.500 (43.9)
Liver	Total protein	122.69 + 0.57	100.72 ± 0.432 (22.8)	79.04 ± 0.3646 (34.2)	68.64 ± 0.4183 (42.5)
	Free aminoacids	12.72 + 0.3346	17.54 ± 0.4560 (34.9)	19.417 ± 0.3346 (67.1)	20.678 ± 0.3974 (79.5)
Muscle	Total protein	134.22 + 0.2738	100.13 ± 0.3746 (25.4)	64.9 ±1.2612 (51.5)	60.48 ± 1.1362 (54.5)
	Free aminoacids	6 .43 + 0.3162	9.8 ± 0.4472 (16.2)	10.49 ± 0.3162 (27.6)	11.298 ± 0.522 (38.8)
Gill	Total protein	6.424 + 0.178	47.22 ± 0.0637 (26.5)	34.00 ± 0.3807 (46.4)	26.58 ± 0.4092 (57.7)
	Free aminoacids	4.72 + 0.3354	5.768 ± 0.4582 (22.2)	6.454 ± 0.4582 (39.1)	7.12 ± 0.3354 (49.5)
Kidney	Total protein	59.68 + 0.1946	46.252 ± 0.3805 (21.5)	36.24 ± 0.1997 (38.4)	29.58 ± 0.1969 (49.5)
	Free aminoacids	9.76 + 0.4582	11.702 ± 0.300 (19.9)	12.108 ± 0.200 (39.5)	12.158 ± 0.3162 (44.9)

Values in parentheses are percent deviation over control. Values are significant at $P < 0.05$ level).

The specific activities of the enzymes glutamate dehydrogenase and aminmotransferases of asparate aminotransferase (AAT) and alamine amino-transferase were elevated (Table 2). The percent increase was highest in kidney (1213.8) followed by muscle (754.4), brain (518.9), gill (444.7) and liver (179.3): Highest percent elevation of AAT was observed in kidney (72.5)followed by liver (69.6), gill (66.1) , muscle (62.3) and brain (59.5). The percent elevation of ALAT was also highest in kidney (78.1) followed by liver (70.3), gill (49.8), brain (42.1) and muscle (39.6) (Table 2).

The biological activities of pyrethroids are closely related to their chemical structure (Elliott, 1977; Klump and Person, 2002). Effect of pyrethroids on different organisms were found to depend on the optical and geometrical configuration of their acidic and alcoholic components (Elliott and James, 1978; Sahoo, *et al.,* 2002). The biochemical changes associated with the action of pyrethroids are little known (David and Somasundaram, 1985; Ghosh and Chatterjee, 1987; Gupta, 1986; Walker, 2002). However, the present findings focused that the synthetic pyrethroid cypermethrin is capable of inducing significant alterations in the protein metabolism of *L. niloticus*.

The decline in the total proteins was suggested to be a consequence of an intensive proteolysis contributing to increase of free aminoacids to be fed into the tricarboxylic acid cycle as ketoacids (Ramalingam and Ramalingam, 1982). The depletion of proteins could be attributed to the inhibition of enzymes involved in protein synthesis. The total ninhydrin positive substsances (FAA) showing an elevation following the intoxication of cypermethrin can be considered to bear a testimony to protreolysis as a consequence of which the levels of aminoacids rise. Though the elevation of free aminoacids can also be a consequence of impairment of incorporation of aminoacids into proteins (Cavanagh and Chen, 1971), the concomitant reduction in proteins support the view that cypermethrin exerts a proteolytic effect.

Table 2. Changes in the specific activities of Glutamate dehydrogenase (GDH) (μ moles of formazon/mg protein/hour) and Aminotransferases of Aspartate Amino-transferase (AAT) and Alanine aminotransferase (ALAT) (μ moles of pyruvate formed/mg protein/hour) in selected tissues of *Labeo niloticus* exposed to 0.0064 ppm of cypermethrin.

Tissue Parameter Control			Period of Exposure hours		
			24 hours	96 hours	192 hours
Brain	GDH	0.0652 ± 0.0057	0.146±0.0610 (123.9)	0.348±0.0334 (402.8)	0.432±0.020 (518.9)
	AAT	9.288 ± 0.1213	11.624±0.0938 (25.1)	14.123±0.1459 (55.17)	14.49±0.1284 (59.5)
	AlAT	5.408± 0.110	6.472±0.0237 (19.6)	7.034±0.0632 (35.6)	7.335±0.2059 (42.1)
Liver	GDH	0.66± 0.02	1.424±0.048 (115.7)	1.824±0.046 (139.3)	2.916±0.112 (179.3)
	AAT	17.4± 0.1720	22.595±0.941 (32.6)	26.280±0.1757 (55.1)	28.70±0.1082 (69.6)
	ALAT	9.216± 0.1951	11.943±0.1392 (29.6)	14.197±0.1082 (62.5)	14.509±0.1688 (70.3)
Muscle	GDH	0.104± 0.0071	0.746±0.0316 (617.3)	1.122±0.005 (750.0)	1.162±0.005 (754.4)
	AAT	7.664 ± 0.2479	9.840±0.1338 (28.4)	11.653±0.2154 (54.8)	12.166±0.1213 (62.3)
	ALAT	3.84± 0.1019	4.638±0.0237 (20.8)	4.674±0.0524 (32.8)	4.858±0.0769 (39.6)
	GDH	0.144± 0.0167	0.660±0.0316 (358.0)	0.762±0.0264 (429.1)	0.828±0.0273 (444.7)
Gill	AAT	5.072 ± 0.1729	6.216±0.1035 (22.5)	7.277±0.0632 (46.2)	8.092±0.1005 (66.1)
	ALAT	3.328± 0.0769	4.132±0.0769 (24.2)	4.498±0.0964 (41.2)	4.7222±0.0522 (49.8)
	GDH	0.088± 0.0229	1.04±0.0632 (1081.8)	1.392±0.0522 (1362.1)	1.456±0.0223 (1213.8)
Kidney	AAT	6.824± 0.1042	8.680±0.0867 (27.2)	10.636±0.1245 (64.2)	10.777±0.1442 (72.5)
	ALAT	2.32± 0.0632	3.099±0.0955 (33.6)	4.616±0.0955 (62.9)	5.226±0.0593 (78.1)

Elevation in the specific activity levels of GDH, AAT and ALAT, in different tissues brain, liver, muscle, gill and kidney of *Labeo niloticus* can be considered a response to the stress induced by cypermethrin to generate ketoacids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and or energy production necessary to meet the excess energy demand under the toxic manifestations. GDH catalyses the reversible determination of glutamate to α-ketaglutarate ammonia., AAT catalyses reversible transamination of glutamate and pyruvate to α-ketaglutarate and alanine. Thus, the amino transferases with GDH contribute some precursor substances such as α-ketaglutarate, pyruvate, oxalocetate, glutamate and various synthetic and oxidative metabolism.

The elevation in GDH activity could be attributed to the mitochondrial permeability or to the lysosomal damage or to the induced synthesis of the enzyme (Johnson and Benington, 1970; Sahoo *et al*., 2002). Since GDH is also a mitochondrial enzyme any alteration in the organization of mitochondria may lead to the alteration in the enzyme activity.

The present findings clearly indicate that normal respiratory metabolism of different tissues of exposed *L. niloticus* were adversely effected by the synthetic pyrethroids. Further, the relative toxic potentiality of the insecticides differed in relation to tissues, biomolecules and the duration of exposure. Though the pesticides use cannot be avoided, measures should be taken for the conservation of the water quality and also the aquatic resources.

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