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BIOCHEMICAL CHANGES IN PROTEINS AND AMINO ACIDS IN *CHANNA PUNCTATUS* IN RESPONSES TO SUBLETHAL TREATMENT WITH THE INSECTICIDE MALATHION.

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ABSTRACT
Malathion is commonly used insecticide for agricultural and non-agricultural purpose in India. In present study the toxic effect of insecticide malathion on biochemical characteristics (protein and amino acid) of fresh water fish *Channa punctatus* were estimated. Fish was exposed to $1/5^{th}$ LC_{50} (0.8 ppm) of pesticide malathion up to 96 hours. The biochemical results revealed decline in protein content whereas amino acids content were increased after the sublethal exposure to malathion.

KEY WORDS: *Channa pucntatus*, Malathion, sublethal.

INTRODUCTION

The rapid industrial and agricultural growth throughout the world in general and in India particular due to alarming rise in human population has caused tremendous environmental contamination. The aquatic environment is affected by different types of chemical toxic to the organisms that originate from both natural and anthropogenic source. The pesticide has been one of the major sources of aquatic pollution in India. The effluents from pesticides are so often disposed into water reservoirs such as lakes, ponds, rivers and oceans without any pretreatment or partial treatment.

The next massive introduction of a new group of pesticides after organochlorine insecticides was of organophosphorus insecticides. They replaced the organochlorine due to their less persistent life and easy detoxification in animal tissues (Quarishi, 1977). Although other groups of insecticides with a shorter life and comparatively very low mammalian toxicity are available (e.g. pyrethroids), organophosphorus (Op) compounds are still used frequently in agricultural may affect fish population as they enter the water through irrigation or rain (Li, 1975).

One of the main factors causing pollution of the environment is the irrational use of organophosphorus insecticides (Al-Haj *et al.*, 2005). Among organophorophorus pesticides malathion is considered relatively safe for the use in mammals. Its rapid degradation by carboxylesterases competes with the cytochrome p450 formation of malaxon, the toxic metabolite. However, impurities in commercial formulations are potent inhibitors of carboxylesterare, allowing a dramatic increase in malaxon formation (Buratti, *et al.*, 2005).

Malathion (*O*-dimethyl-S1-2-di(ethoxycarbonyl)-ethylphosphorodithioate) is an organophosphorous insecticide widely used in agriculture and houses for the control of diseases vectors. It is a major source of environment poisoning in developing countries (WHO, 2003). Toxicological tests have shown that malathion affected central nervous system, immune system, adrenal gland, liver and blood. Proteins are most abundant intracellular macro-molecules and constitute over half the dry weight of most organisms. They occupy a central position in the architecture and functioning of living matter. They are intimately connected with all phases of chemical and physical activity, that constitute the life of the cell. Therefore they are, essential to cell structure and cell function. The interplay between enzymatic and non-enzymatic proteins governs the metabolic harmony (Lehinger, 1984). They are also involved in major physiological events to maintain the homeostasis of the cell. Therefore, the assessment of the protein content can be considered as a diagnostic tool to determine the physiological process of the cell (Kapil and Ragothaman, 1999; Munshigeri, 2003). All proteins are macromolecules because of their very high molecular weights. These are the polymers i.e. chain-like molecules produced by joining a number of small units of amino acids called monomers. The amino acids are therefore, regarded as building blocks of proteins. Amino acids are essential intermediates in protein synthesis and its degradation products appear in the form of different nitrogenous substances. In present study attempt has been made to study the protein and amino acid content in muscle, gill and liver of *Channa punctatus* under sublethal treatment of malathion.

MATERIALS AND METHODS

The fish *Channa punctaus* were collected from local river Godavari Dist-Nanded and brought to laboratory. These fishes were observed for any pathological symptoms and then placed in 0.1% potassium per magnate ($KmNo_4$) for two minutes so as to avoid any dermal infection. The fishes were then washed with water and acclimatized to laboratory conditions for two weeks in glass aquaria of 100L. The physico-chemical parameters of water analyzed by following standard method suggested by APHA (1998). During acclimatization the fishes were provided with a diet consisting of live earthworms on alternate day. Food supply was withdrawn 24 hrs prior to experimentation. A commercial grade of

pesticide Malathion 50% EC was used for bioassay test. A stock solution of toxicant was prepared and few concentrations from stock solution were prepared as per the dilution technique (APHA, 1998).

For experimentation, laboratory acclimatized fishes were divided into two grows of 10 fishes per aquarium. Group 'A' served as control kept in tap water. Group 'B' was exposed to sub lethal i.e. ($1/5^{th}$ of LC_{50} of 96 hours i.e. 0.8 ppm) concentration of malathion solution. Experiments were carried out up to four days. Water was renewed during every 24 hours in order to provide fresh oxygenated water and also to maintain the concentration of malathion. The fishes were scarified immediately at the end of 24, 48, 72 and 96 hours in both groups. Tissues like muscle, gill and liver were excised rapidly and processed for the biochemical estimation. The protein content was estimated by the method of Lowry *et al.,* (1951). Total amino acid content was estimated according to Ninhydrin method (Rosen, H., 1957).

Table 1 - Levels of Protein content in different tissues of *Channa punctatus* exposed to sub-lethal concentration of malathion

Sr. No	Tissue	Control	Experimental			
			24 hrs	48 hrs	72hrs	96 hrs
1	**Muscle**	140.85 ± 2.03	144.50±2.88	138.16±2.73	133.56±1.8*	136.16±2.31*
2	**Gill**	94.45±1.25	89.83±0.96*	85.81±2.88	79.25±0.99*	69.81±2.01
3	**Liver**	108.73±1.91	102.85±1.44*	98.80±2.18*	93.08±1.11	88.20±0.80*

- The values are expressed as mg/g wet wt. of tissue
- Values are mean ± SD of six replicates * p<0.05, ** P<0.01, *** p>0.01, significant when student's test was applied between control and experimental groups.

Table 2 - Levels of Total Free Amino Acid content in different tissues of *Channa punctatus* exposed to sub-lethal concentration of malathion

Sr. No	Tissue	Control	Experimental			
			24 hrs	48 hrs	72hrs	96 hrs
1	**Muscle**	40.46±1.13	39.28±1.53	40.87±1.35	37.31±1.57	42.19±1.72
2	**Gill**	37.08±2.85	41.03±1.38*	43.14±1.27*	40.29±2.03	46.16±2.41*
3	**Liver**	31.33±2.86	34.29±1.10	36.03±2.41	39.35±0.97*	44.91±1.79

- The values are expressed as mg/g wet wt. of tissue
- Values are mean ± SD of six replicates * p<0.05, ** P<0.01, *** p>0.01, significant when student's test was applied between control and experimental groups

RESULTS

The variation in free amino acid content due to exposure of malathion are given in table (6) and fig. (16-20). The total free amino acid contents varied from 37.31 to 42.19 in muscle, 66.14 to 76.16 in kidney, 48.35 to 53.41 in Heart, 53.18 to 64.70 in stomach of malathion exposed fish. Overall increased values were noted during 96hrs in all organs of exposed fish. FAA content in muscle fluctuated during 24, 48, 72, and 96 hours values. In kidney during 24 and 48 hours values decreased whereas sudden increased in FAA content during 72 and 96 hours. In heart FAA content increased during 24 hours then values decreased during 48, and 72 hours. Increase in FAA content during 96 hours was observed. FAA content in stomach decreased up to 48 hours and again increased during 72 and 96 hours compared to control. The free amino acid content expressed as mg/gm weight of tissue.

After the completion of sublethal treatment of malathion during 24 hours, 48 hours, 72 hours and 96 hours the biochemical parameters were studied in both control and treated group. Data of the biochemical analysis is given in table -1 and table -2. Fish exposed to insecticide malathion showed initial increase in muscle protein during 24 hours and then goes on decrease up to 96 hours in treated group compared with control. Protein content in gill and liver showed decreasing trend in 96 hours in treated group compared with control group. The variation in free amino acid content due to exposure of malathion are given in table (2) . The total free amino acid contents varied from 37.31 to 42.19 in muscle, 40.29 to 46.16 in gill, 34.29 to 44.91 in liver of malathion exposed fish. Overall increased values were noted during 96hrs in all organs of exposed fish. Values of protein and amino acid were expressed in mg/gm wet.wt of tissue.

DISCUSSION

The contamination of water by widely utilized organophosphorus insecticide such as malathion is a potential problem for fishes. In present study attempt has been made to study acute effect of malathion on tissues like muscle, gill and liver of fresh water fish *Channa punctatus* with some aspect to nitrogen metabolism.

PROTEIN

Protein is most characteristic organic compound found in the living cell while the protoplasm of the cell is composed of protein. They play vital role in the process of interaction of cellular medium. During present investigation increase in muscle and stomach protein content of *Channa punctatus* had been observed during 24 hours and 48 hours respectively after exposure to sub-lethal concentration of malathion. Proteins are building blocks of animal body. Rajeshwar Rao *et al.,* (1983) suggested that incline in protein content might be due to pesticidal stress and the energy was derived from carbohydrate metabolism.

Another reason for increase in protein content is due to increase in protein synthesis by increase the enzyme activity involved in protein synthesis. Toxic stress like malathion treatment to the fish causes increase in the rate of protein synthesis. In experimentation, after 96 hours the decline in protein content was observed. This fall attributed to the constantly increasing contact of the pesticide with the bio-system which ultimately resulted in protein breakdown. Kabeer (1979) showed increase in protein content in fish, *Tilapia mossambica* treated with methyl parathion and malathion. Shivaprasad Rao *et al.,* (1980) reported incline in protein level due to increased level of protease and synthetic potentiality of proteins in various tissues under pesticidal impact.

Even though most of the workers found that, there was reduction in protein contents in various tissues of the animal under different stress conditions. The decrease in protein content may be due to reduced protein synthesis and increased proteolysis. Joseph, (1987) observed the effect of copper on biochemical composition of *Cyprinus carpio* and found that total protein content of the brain, liver and muscle were declined.

Kumar and Gopal (2001) showed protein level in different organ viz liver; brain, kidney and muscle were found decreased. They concluded degradation of the protein into free amino acid, which is used for different metabolic activity during stress condition. Venkataramana *et al.,* (2006) showed impact of malathion on cardic muscle of gobiid fish, *Glossogobius giuris* (Ham.). They concluded along with glycogen, cholesterol and protein showed significant decrease at high concentration (0.5 ppm) when treated for longer duration (96 hr). Yaganabano *et al.,* (1981) and Bano (1982) showed decrease in the total protein content of selected tissue and cholesterol content of serum and liver in *Clarius batrachus* exposed to eldrin. Ramalingam and Ramalingam (1982) stated that proteins expected to involve in the compensatory mechanism of stressed organisms similar observation were made by Jagdeesen and Mathivanan (1999).

Chandravathy and Reddy (1994) suggested that decline in the muscle protein content might be due to reduced protein synthesis, increased proteolysis and also due to utilization for metabolic process under lead toxicity. Rao *et al.,* (1987) and Baskaren *et al.,* (1989) reported reduction in protein content could be due to its utilization to mitigate the energy demand when the fish is under stress. Palanichmy *et al.,* (1986) worked on sublethal effect of selected pesticides on protein, carbohydrate and lipid content of muscle and liver of *Oreochromis mossambicus* and observed decrease in protein content. Ramalingam and Ramalingam (1982) worked on sublethal level of DDT, malathion and mercury on tissue protein of *Sarotherodon mossambicus.* They observed that decline in total protein. Jagadessan and Mathivanan (1999) reported that the depletion of protein level induces to diversification of energy, to meet the impending energy demands during the toxic stress. They stated that the liver synthesized a greater amount of protein, which is needed ostensibly repair of damage of organelle and tissue regenerator.

AMINO ACID

Amino acids are essential intermediates in the process of protein synthesis and its degradation products appear in the form of different nitrogenous substances. Amino acid and some nitrogenous compounds play an important part during osmotic stress hence increase or decrease in free amino acid content provide valuable information during stress phenomenon at the tissue level. James *et al.,* (1979) showed possible reason behind increase in F.A.A. content. They concluded that increase in concentration of F.A.A. attributed to stepped up proteolysis or increase synthesis of free amino acid by transaminase reaction. Seshagiri Rao *et al.,* (1987), studied effect of bethiocarb on protein metabolism of fresh water teleost fish, *Sarotherodon mossambicus* and observed an increase in free amino acid level in the organs of the fish which could be due to degradation of proteins by proteolysis or due to decreased protein synthetic potentials in the pesticide induced pathological condition.

Durga *et al.,* (2002) worked on effect of cypermethrin on protein metabolism of the fish *Labeo rohita* and observed that total protein level decreased in all the tissues tested whereas the free amino acid levels were increased. During present investigation total free amino acid content showed a significant increase in the tissue of *Channa punctatus* exosed to sublethal concentration of malathion. The increased F.A.A. level suggests tissue damage probably due to the increased proteolysis activity under pesticide toxic stress. However, the elevated levels of F.A.A. can be utilized for energy production by feeding them in to the TCA cycle through aminotransferase reactions. The increase in the levels of F.A.A. can also be attributed to the synthesis of amino acids in addition to their elevation by protein hydrolysis. Another possibility for increased F.A.A. level might be their increase due to transamination and amination of keto acid.

Increment in free amino acid level was the result of breakdown of protein for energy requirement and imparted incorporation of amino acids is protein synthesis (Singh *et al.,* 1996). It also attributed to lesser use of amino acids and their involvement in the maintained of acid base balance (Moorthy *et al.,* 1984). Stress condition induced elevation in the trans amination pathway (Natarajan, 1985). Kamble *et al.,* (1984) have reported an increase in total free amino acid contents in the hepatopancreas, muscle and gills of fresh water crab, *Barytelphusa guarini,* when exposed to sublethal concentration of hildan, an organochlorine insecticide.

In present investigation an increase in F.A.A. has been observed during 96 hours under treatment of malathion. The increase in F.A.A. levels of tissues indicates stepped up proteases activity and fixation of ammonia into keto acid (Rao, 1987; Ali, 2003). Tripathi *et al.,* (2003) reported that the enhanced F.A.A. may be due to depletion of reserved glycogen so that the fish can try to yield metabolic energy by glueoneogenesis process. Similar findings were observed by Anupama (1989) in various animals during different toxic conditions. Mali *et al.,* (2005) reported remarkable changes in free amino acid content of fresh water female crab, *Barytelphusa guerini* exposed to cadmium sulphate. They reported that F.A.A. content in leg muscle, gill, hepatopancreas and heart in female crab *Barytelphusa guerini* was initially increased at 48 hours while slightly decreased at 72 hours in all tissues. Finally the slight increase was observed followed by negligible increase. It is concluded that malathion poisoning leads to alteration in protein and amino acid content in *Channa punctatus*. Decrease in protein value might be due to proteolysis which leads into increase in free amino acid level in fish body. Fish with low protein value is not used for nutritional food purpose.

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