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Impact of Acetamiprid Toxicity on Biochemical Biomarkers (Protein and Carbohydrate) in some Tissues of the Fish *Oreochromis mossambicus*

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ABSTRACT

The study analyze the effect of pesticide-acetamiprid on protein content and carbohydrate content of liver, brain and gill tissues of *Oreochromis mossambicus*. Acetamiprid is an neonicotinoid pesticide and is used by the farmers to protect their crops. Pesticides may enter water bodies as a result of spray drift and leaching from the soil in concentrations, which may exert adverse effects on fish populations. Lethal concentration (LC_{50}) of acetamiprid for *O. mossambicus* has been calculated by probit analysis and recorded as 5.99 ppm at 96 h. Chronic exposure shows decreased protein and carbohydrate content in liver, brain and gill tissues during all the exposure periods when compared with the control. This significant decrease in protein and carbohydrate content was observed due to toxic effect of acetamiprid. Long term exposure of organisms to pesticides means a continuous health hazard for the fish population and it is on high risk by consuming these toxicated fishes.

Key words: Tilapia, acclimatized, proteolysis, pesticide, disorder, sub-lethal, LC_{50}

INTRODUCTION

The intensive development of agriculture in recent years cum rapid growth of industrialization in our country brought out great increase in manufacture and utilization of fertilizers, pesticides, petrochemical products, detergent and other synthetic chemicals and agricultural wastes pose a serious threat to the water ecosystem and aquatic life (Allison *et al*., 1963; Ganeshwade, 2012). Pesticides are basically poisons and therefore, toxic to living organism at particular dose. All pesticides (natural and synthetic) have the potential to cause harm during their manufacture or refinement, at the time of application to crops, as residues that persist on food, in the disruption of the natural balance that exists between pests and their natural enemies.

Pesticides become part of the water column and fish ingest the pesticides, usually through their gills, although sometimes through their scales. The pesticides diffuse into their organs and fat tissues and sequestered there causes severe alterations in the tissue biochemistry and histology of fishes (Muthukumaravel *et al*., 2013). Fish is considered as the most important and vital link in the food chain of ecosystem and the inland fisheries are important sources of protein in a nation's diet, a thorough understanding of pesticide effects on fishes would be really vital for fish conservation and fisheries development. Many pesticides have been reported to produce a number of biochemical changes in fish both at lethal and more often, at sublethal level changes in ion

concentrations, organic constituents, enzyme activity, endocrinal activity and osmoregulation in fish has been attributed to pesticides. *Oreochromis mossambicus* showed a declining trend of protein, carbohydrate and lipid content in the tissues like brain, gill, kidney, liver and muscles upon exposure to organochlorine and carbamate pesticides (Ganesan *et al*., 1989) and there is an increase in chromatid break and chromosomal exchange due to carbamate pesticides (Rita and Milton, 2006). Reduction in total serum protein content induces proteinaemia and may be correlated with reduced protein synthesis by liver (Sharma *et al*., 2009). Protein synthesis can be disturbed either by affecting t he nucleic acid metabolism or structure, or in the protein forming system itself. Toxic agents acting directly upon ribosomes, RNA, enzymes or coenzymes may also have a drastic influence on protein synthesis. Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism (Yeragi *et al*., 2003; Remia *et al*., 2008; Yiu *et al*., 2008; Fahmy, 2012). Proteins are important biomolecules involved in a wide spectrum of cellular functions (Prashanth, 2006). A number of workers have reported decline in protein level of various organs and tissues under toxic stress of various chemicals. The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress (Remia *et al*., 2008). Dalela *et al*. (1981) observed a decrease in protein content in *Mystus vittatus* under pesticide exposure and reported that the depletion of protein may be due to the excretion of proteins by kidney due to kidney failure or impaired protein synthesis as a result of liver disorders. Begum and Vijayaraghavan (1996) have reported that the protein content of muscle tissues of fish *Clarias batrachus* was significantly depleted during 192 h of dimethoate exposure. Protein synthesis can be disturbed on many levels by a variety of mechanisms, either by affecting the nucleic acid metabolism or structure, or in the protein forming system itself. Decreased concentrations of TP are common in many disease states and may result from impaired synthesis (liver disease), reduced absorption or protein loss (Bernet *et al*., 2001). The impact of these pesticides on aquatic organisms is due to the movement of pesticides from various diffuse or point sources. These pesticides are posing a great threat to aquatic fauna especially to fishes, which constitute one of the major sources of protein rich food for mankind (Sharma and Singh, 2007). In this study, the fish, *O. mossambicus* was investigated to evaluate the impact of acetamiprid on protein and carbohydrate content in various tissues.

MATERIALS AND METHODS

Specimens of *O. mossambicus* were obtained from local vicinity and introduced into large glass tank disinfected with potassium permanganate and washed thoroughly prior to introduction of fish (to prevent fungal infection). Fishes with same weight were acclimatized for about 20 days before the commencement of the experiment. They were fed on commercial fish food which was given daily at morning hours. The LC_{50} of acetamiprid was calculated by the log-dose/Probit regression line method (Finney, 1971) and was recorded. Sublethal or safe level concentrations were derived from 96 h LC_{50} as per the procedure given by APHA (2005) to observe various responses of the test fishes on prolonged exposure to acetamiprid. In the present study 1/15 of the 96 h LC_{50} were selected as sublethal concentration and the fishes were exposed to this concentration for a period of 0, 7, 14, 21 and 28 days. A control batch corresponding to each test group in triplicate was simultaneously experimented to compare the toxicated values of protein and carbohydrate content which was estimated by the method of Koller (1984) and Trinder (1969) and the mean values with standard deviations are tabulated using MS-Excel software. Fresh concentrations were supplied daily to maintain a constant toxic media. At the end of each exposure period, fishes were sacrificed and tissues such as liver, brain and gill were dissected and were used for the analysis.

RESULTS AND DISCUSSION

The changes in biochemical parameters such as carbohydrates, proteins and lipids are important to indicate the susceptibility of organ systems to pollutants by altering their function. Alterations in biochemical components as response to environmental stress are authenticated by many authors. Venkataramana *et al*. (2006) studied the toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *O. mossambicus*. Tilak *et al*. (2001) studied the toxicity and effect of chloropyriphos to the freshwater fish *Labeo rohita* (Hamilton).

The result of the present study showed significant decrease in protein content in the tissues studied. The protein content in all the control tissue samples showed an increasing trend with increasing exposure period and in treated tissue samples there is a decreasing trend with increasing exposure period. The protein content of control liver tissue was 95.5±1.00 mg L^{-1} and it reduced gradually to 20.6±0.57 mg L^{-1} for acetamiprid at 28th day and in brain and gill tissues there is a decreasing trend from 29.5±0.57 to 15.3±0.57 and 86±1.73 to 44.0±1.00 mg L^{-1} (Table 1). The per cent decrease was found to be greater in all exposures in liver tissue. The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress (Remia *et al*., 2008).

Dalela *et al*. (1981) observed a decrease in protein content in *Mystus vittatus* under pesticide exposure and reported that the depletion of protein may be due to the excretion of proteins by kidney due to kidney failure or impaired protein synthesis as a result of liver disorders. Reddy and Philip (1991) studied a decrease in protein content and an increase in free amino acid and protease activity levels in *Cyprinus carprio* exposed to Malathion. Singh and Srivastava (1992) observed similar effects on the total serum proteins of the fish (*Heteropneustes fossilis*) due to aldrin toxicity suggested that pesticides may affect the protein content in two ways either they inhibit the RNA synthesis resulting in low RNA and low protein content, or they may absorb amino acid into polypeptide chain. A number of workers have reported decline in protein level of various organs and tissues under toxic stress of various chemicals. Begum and Vijayaraghavan (1996) have reported that the protein content of muscle tissues of fish (*Clarias batrachus*) was significantly depleted during 192 h of dimethoate exposure. Kumar and Saradhamani (2004) observed significant decrease in protein content in all the tissue of the fish (*Cirrihinus mirgala*) upon exposure to the pesticide avaunt. Proteins can be expected to be involved in the compensatory mechanism of stressed organisms. The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress (Remia *et al*., 2008). Such a decrease in the protein content of fish when it is under chronic stress of acetamiprid could be due to the degradation of proteins as a source of

Table 1: Showing the protein content of Liver, Brain and Gill of control and treated (acetamiprid) fish *O. mossambicus* in different exposure periods

	Li (mg L^{-1})		Br (mg L^{-1})		Gi (mg L^{-1})	
Duration (days)	C	A	C	A	C	A
0	92.0±1.00	51.6±1.52	26.0±2.00	25.7±1.77	81.0±1.73	67.6±0.57
7	93.3±2.08	45.5±1.00	27.0±2.00	24.0±0.05	83.0±2.64	64.6±0.57
14	94.0±2.00	31.6±1.52	27.8±1.00	22.9±0.10	83.8±2.30	63.0±1.00
21	94.8±2.00	27.6±1.52	29.0±1.50	22.0±1.00	85.0±1.00	48.0±1.00
28	95.5±1.00**	20.6±0.57*	29.5±0.57**	15.3±0.57*	86.0±1.73**	44.0±1.00*

Values are expressed as Means±SD for n = 3 C: Control, A: Acetamiprid treated, Li: Liver, Br: Brain, Gi: Gill tissue. *Significant decrease, **Significant increase

Table 2: Showing the Carbohydrate content of Liver, Brain and Gill of control and treated (acetamiprid) fish *O. mossambicus* in different exposure periods

	Li (mg L^{-1})		Br (mg L^{-1})		Gi (mg L^{-1})	
Duration (days)	C	A	C	A	C	A
0	11.8±0.15	5.30±0.36	1.3±0.10	0.50±0.10	1.8±0.20	1.60±0.10
7	12.0±2.00	4.33±0.41	1.43±0.05	0.43±0.01	2.5±0.10	0.96±0.05
14	13.0±2.08	2.50±0.50	1.5±0.10	0.20±0.10	2.8±0.15	0.80±0.10
21	13.8±2.00	2.00±1.00	1.6±0.10	0.16±0.05	3.2±0.30	0.60±0.10
28	14.6±1.00**	1.13±0.32*	1.7±0.20**	0.13±0.05*	4.0±0.30**	0.16±0.05*

Values are expressed as Means±SD for n = 3 C: Control, A: Acetamiprid treated, Li: Liver, Br: Brain, Gi: Gill tissue. *Significant decrease, **Significant increase

energy to meet the extra energy demand for its maintenance at the time of stress (Joseph and Raj, 2011). Sandhya *et al*. (2006) reported that reduction in protein content could be due to its utilization to mitigate the energy demand when the fish are under stress.

Carbohydrate is an essential component of living cells and sources of energy for animals. The results of the present findings showed a significant decrease in carbohydrate content in all the tissues studied. The Carbohydrate content of control liver tissue was 14.6±1.00 mg L^{-1} and it reduced gradually to 1.13±0.32 mg L^{-1} for acetamiprid at 28th day and in brain and gill tissues there is a decreasing trend from 1.7±0.20-0.13±0.05 and 4.0±0.30-0.16±0.05 mg L^{-1} (Table 2). The reduction in carbohydrate ranged from 5.30-1.13 mg L^{-1} for acetamiprid treated liver, 0.50-0.13 mg L^{-1} for acetamiprid treated brain and 1.60-0.16 mg L^{-1} for acetamiprid treated gill in 0, 7th, 14th, 21st and 28th day exposure time. Thenmozhi *et al*. (2011) reported that the decrease in carbohydrates contents may result in impairment of carbohydrate metabolism due to toxic effect. The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition. Many workers reported a similar trend of decrease in carbohydrate (Venkataramana *et al*., 2006; Saradhamani and Selvarani, 2009). On the other hand, some other workers (Janeczek *et al*., 2007; Puoci *et al*., 2008; Logaswamy and Remia, 2009) reported that sublethal concentration of certain organophosphate pesticides caused glycogenolysis, which produced hyperglycemia in the African food fish *Tilapia* and the Indian catfish, *Heteropneustes fossilis*. Remia *et al*. (2008) reported that the elevation of carbohydrates might be due to the stress induced by the insecticides as physiology of organisms with the help of corticosteroids.

CONCLUSION

The changes in biochemical's parameters such as proteins and carbohydrates are important to indicate the susceptibility of organ systems to pollutants by altering their function. The results of the present study clearly indicate the toxic nature of the insecticide acetamiprid on the biochemical constituents of *O. mossambicus*. The significant decrease in proteins and carbohydrates in the acetamiprid treated fishes will naturally affect the nutritive value of the fish. Long term exposure of organisms to pesticides means a continuous health hazard for the population. From the present study, it is assumed that reduction in protein content induces proteinaemia and may be correlated with reduced protein synthesis, which reflect changes in the normal activities of the animal. Therefore it is necessary to monitor the aquatic system and assess the toxic effect of aquatic organisms particularly fish.

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