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Effect of Deltamethrin on Energy Metabolic Activities and Ionic Balance of *Heteropneustes fossilis*.

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Abstract—Deltamethrin, a synthetic pyrethroid contaminating aquatic ecosystems as a potential toxic pollutant, is investigated in the present study. The impact of exposure of the freshwater fish *Heteropneustes fossilis* to two sub lethal concentrations (0.07mg/L and 0.14 mg/L) of deltamethrin for 30 days on the physiological activities of endocrine gland Ultimobranchial gland and energy metabolic activities viz. Na^+/K^+ ATPase, Ca^{2+} and Mg^{2+} ATPase and inorganic ions Na^+, K^+, Ca^{2+} and Mg^{2+} in brain, kidney, gills, muscle and intestine was assessed. Significant ($p<0.01$) decrease was found in Na^+/K^+ ATPase, Ca^{2+} and Mg^{2+} ATPase activities in fish exposed to higher concentration. Ionic levels in vitals tissues were significantly decreased after exposure to the two sub lethal concentrations. Ultimobranchial gland, Brain and intestine were the most affected tissues.

Keywords- Endocrine gland, energy, metabolites, ultimobranchial gland, ionic balance, ATPase, deltamethrin, lethal concentration

Introduction:

Deltamethrin is recommended by the World Health Organization for application to walls and mosquito nets, and is also used for other in-home insect control, and for agriculture. Deltamethrin is a synthetic parathyroid insecticide that kills insect on contact and through digestion. It is known to be more suitable for agricultural use because of their improved potency and stability as well as low mammalian toxicity [1]. These were found to be highly effective in controlling mosquitoes, midges and other agricultural pests [2]. Pyrethroids have been reported to be extensively toxic to fish [3]. They are lipophilic in nature and enter the fish body via gills [4]. Adverse effects of deltamethrin on fish have been reported with reference to hematological and biochemical variables [5], [6]. ATPases exist in all cell membranes and regulate the ionic concentrations inside the cells. Ca^{2+} and Mg^{2+} ATPases are involved in the regulation of Ca^{2+} and Mg^{2+} ions, which play a significant role in many metabolic pathways and a crucial role in a variety of pathological and toxicological processes. Therefore, in the present study the effect of deltamethrin on endocrine gland, Na^+ / K^+ ATPase, Ca^{2+} and Mg^{2+} ATPase activities as well as on ions levels in brain, kidney, gills, muscle and intestine of *Heteropneustes fossilis* has been investigated.

Materials and Methods:

Deltamethrin [cyano - (3 - phenoxy-phenyl) methyl, 2- (2,2- dibromoethyenyl)- 2, 2-dimethylcyclopropane carboxylate] was procured from Hoechst Schering Agro Evo Limited Ankleshwer, India. Healthy specimens of freshwater *H. fossilis (weight.* 30-35 g, length 12-15 cm) were purchased from commercial dealer. The fishes were kept in dechlorinated tap water at a temperature of 20-23 °C under constant day/night cycle in large 50L glass aquaria provided with a filter and continuous aeration for two weeks prior to the beginning of the experiments. They were fed daily with commercially available dried flakes (Tetra ^{R} brand) at 2% body weight for 30 days prior to the experiment. Physico-chemical characteristics of the water used was analyzed for pH 6.9 ± 0.02; temperature 23°C; electrical conductivity 268.24 ± 16.59 umho/cm; dissolved oxygen 8.8 ± 2.5 mg/L; alkalinity 90 ± 10.5 mg/L as $CaCO_3$ and hardness 118 ± 12 mg/L ' as $CaCO_3$, all aquaria were cleaned and water was changed on alternate days. Only healthy fish of either sex was used in the experiment. A static bioassay test was conducted according to Standard Method [7] to determine the LC_{50} for 96 hr of deltamethrin to *H. fossilis* the recorded value was 0.42 mg/L. For biochemical studies fish were exposed in two separate groups (each contained 30 fish) to two sub lethal concentrations 0.14 mg/L (l/3rd of LC_{50}) and 0.07 mg/L (1/6th of LC_{50}). Control groups with 30 fish were maintained in tap water containing 2 ml acetone. Fish were dissected after 30 days of exposure-and the vital tissues *viz.* brain, kidney, gills, muscle and intestine were removed in cold.

Enzyme activity was determined in reaction mixtures A and B in absence and presence of ouabain [8]. Reaction mixture A for total ATPase activity contained 0.2 ml of 200 mM KC1, 0.2 ml of 1 M NaCl, 0.1 ml of 100 mm $MgCl_2$, 1 ml of 200 mm tris buffer at pH 7.4, 0.2 ml of distilled water and 0.1 ml of tissue homogenate. The mixture was pre-incubated at room temperature for 5 minutes and then incubated for 15 minutes at ambient temperature after adding 0.2 ml of 25 mm ATP di-sodium salt for Mg^{2+} ATPase activity, reaction mixture B contained 0.1 ml of 100 mm $MgCl_2$,1 ml of 200 mM tris buffer pH 7.4, 0.1 ml of tissue homogenate, 0.16 ml of water, 0.2 ml of 10 mm ouabain and pre-incubated for 5 minutes. The reaction was initiated by adding 0.2 ml of 1 M NaCl and 0.2 ml of 25 mm ATP disodium salt and incubated for 15 minutes at ambient temperature. The reaction in both sets was terminated by adding 1 ml of 10% trichloroacetic acid (TCA) and centrifuged at 3000 X g for 5 minutes. The supernatant was used for inorganic phosphate estimation [9]. To 0.5 ml of supernatant 3 ml of distilled water, 0.5 ml of 2.5 % ammonium molybdate in 5N $H_2SO_4$and 0.2 ml of 1,2,4 aminonaphthol sulphonic acid (ANSA) were added. The mixture was vortexes and optical density was read at 600 nm after 10 minutes. The difference in the inorganic phosphate (Pi) liberated in the two reaction mixtures gave the activity of Na^+/K^+ ATPase. The ATPase activity was expressed as µmole Pi liberated/mg protein/hr. Ca^{2+} and Mg^{2+} ATPase activity was assayed by the method of [10]. The levels of sodium, potassium, calcium and magnesium ions were estimated in brain, kidney, gills, muscle and intestine of *H. fossilis*. The tissues were added with 5 ml of nitric acid and were left for overnight. The dissolved tissues were then heated at low temperature till evaporation; 2 ml of digestion mixture (nitric acid, sulphuric acid and perchloric acid 6:1:1) was added and again heated until it became colorless. It was diluted 10 ml. and the concentration of sodium, potassium, calcium and magnesium ions in tissues was measured with the help of an Atomic Absorption

Spectrophotometer (SP-500). Histopathology of the vital tissues and endocrine glands were studies using compound and electron microscope.

Results and Discussion:

The Endocrine gland Ultimobranchial gland is responsible for regulation of calcium and ionic balance. And it is examined by compound and electron microscopy after exposed to the lower conc. (0.085mg/l) and higher conc. (0.17 mg/l) of Deltamethrin. The Ultimobranchial gland exhibited mild histological changes at 0.085 mg/l of deltamethrin. Decreased in the staining response of the cytoplasm & nuclear volume of U. G cells. At higher concentration exposure severe changes were observed viz. nuclear volume exhibited further decreased with degeneration and vacuolation in the cells The AF positive cell exhibited increased granulation, but in the AF negative cells the nuclear volume increased at lower concentration. Significant changes observed in the Ultimobranchial gland at higher concentration of deltamethrin.

Exposure to both concentrations of deltamethrin adversely affected the activity of Na^+/K^+ ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase (Table 1). However, inhibition was greater with the higher concentration (0.14 mg/L). Endocrine gland, intestine, brain and gills were the most affected tissues of the fish. Na^+/K^+ ATPase activity decreased with increasing concentration of deltamethrin in gills> brain> kidney> intestine> muscle. On the other hand, Na^+/K^+ ATPase activity of gills significantly increased with the lower concentration. The levels of Na^+ and K^+ decreased maximally in intestine and gills on exposure to 0.14 mg/L but at lower concentration, significant decrease was noted only in intestine. Significant inhibition in the order brain>muscle>gills>intestine>kidney was noted in Ca^{2+} ATPase activity at higher concentration (Table 2). However, the enzyme activity was elevated in gills at lower concentration (0.07 mg/L). The level of Ca^{2+} decreased in intestine>muscle>gills>brain>kidney. At lower concentration significant decrease was observed only in intestine. Mg^{2+} ATPase activity in brain and muscle decreased with higher concentration but increased in the kidney with lower concentration of deltamethrin (Table 3). The concentration of Mg^{2+} decreased in most of the tissues. At lower concentration significant decrease was observed only in intestine. Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase are the membrane bound enzymes, which serve to concentrate nutrients within the cell to maintain proper level of electrolytes and to maintain correct osmotic pressure of intracellular fluids. Deltamethrin present in the ambient medium being lypophilic in nature comes in direct contact with gills and ruptures the chloride cells membrane through which insecticide enters blood and reaches the target tissues. The ATPases are localized in the chloride cells of the gills and are primarily used as specific markers for damage of ions transport in fish. At the basolateral membrane the ions enter the chloride cells from the water by passive diffusion and are actively transported to the blood by high ATPase activities [11]. The other mode of action is direct effect of insecticide on enzyme protein or primary lethal lesion in gills of fish exposed to the toxicant. Therefore, inhibition in enzyme activities and decrease in the levels of ions occur in the exposed fish. Insecticides bind with the food particle consumed by fish and reach the intestine. The membrane of villi is disrupted by the action of deltamethrin. Inhibition in the activity of Na^+/K^+ ATPase can cause disruption the structure of the plasma membrane and/or that of mitochondria resulting in metabolic depression in the animals itself [12].

Hence, inhibition in the activities of Na/K^{+} ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase in gills of the exposed fish indicates disruption in its cellular ionic regulation and salt uptake as the pyrethroids are efficiently absorbed across gills [13]. Reference [14] shows the mechanism of the ATPase and osmoregulation inhibition in the gill of coastal teleost *Salmo gairdneri* exposed to chromium. In their model, chromium blocked the active transport system of the gill epithelial as well as chloride cells, glomerular and epithelial cells of the tubules and thus altered the osmoregulatory mechanism of the fish. Because ion-dependent ATPases are known to regulate the influx and efflux of ions across the membrane to maintain the physiological requirement of the cell, inhibition of Na^{+}/K^{+} ATPase in gills probably disturbed Na^{+} and K^{+} pump, resulting in uncontrollable entry of Na^{+} into cells along the concentration gradient and the water molecules along the osmotic gradient. This process may cause swelling of the cell and finally membrane rupture [15]. Similarly, insecticide DDT and parathion have previously been shown to reduce Ca^{2+} uptake by sarcoplamic reticulum and to bring about a considerable reduction in Ca^{2+}/ Mg^{z+} ATPase or 'calcium pump' activity in flounder sarcoplasmic reticulum [16].

It is well established that pesticides reach the muscular tissue of fish *via* blood by diffusion through the skin. Present results show that parathyroid stress affects the activity of membrane ATPase system blocking the normal distribution of the essential ions into muscle cells. This may cause severe effect on the normal functioning of the muscle. Alteration in ATPase activity reflects change in membrane permeability. The stimulation in Na^{+} / K^{+} ATPase, Ca^{2+} ATPase and Mg^{+} ATPase may be attributed to change in cell metabolism, ionic imbalance or membrane alteration. A marked decrease in the concentration of Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} which play a vital role in different enzyme systems and acid-base balance of fish observed in all the vital tissue of fish *viz.* brain, kidney, gills, muscle and intestine indicate a disturbed ionic balance and complete failure of osmoregulation. It may be probably a consequence of gill and kidney damage frequently reported in pesticide and metal intoxicated fish species [17]-[19]. The deltamethrin induced injuries are apparently of such serious nature that normal mechanisms of regulation are incapable of restoring the ionic balance. Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} ions are crucial for maintaining the integrity and stability of gill epithelial cell membrane [20] as well as to the development of action potential in muscle and nerves cells [21] pronounced alternation of tissues and plasma concentration severely affects these processes. It is very interesting to record that the maximum decrease of ions was noticed in intestine. Reference [22] shows that genesis of muscle action potential and hence beat to beat regulation of muscle activity; depend upon the flux of ions through hydrophobic channels in sarcolemma of intestine. They also suggested that Mg^{2+} plays an important role in both Na^{+} and K^{+} ion in intestinal cell. Furthermore, intestine sarcolemma has also shown to posses a remarkable ability to bind a considerable amount of calcium [23] it is likely this may be an important source of calcium during calcium pump activity involving calcium activated ATPase. Present study focused that even at sublethal concentration of deltamethrin in water might produce dysfunction of several physiological and biochemical consequences in fish. Further inhibition of ATPase and reduction of major cations, recapitulates disruption in the functional activities of the cell, leading to damaged membrane transport system.

Table 1: Na^+/K^+ ATPase activity in different tissues of *H. fossilis* exposed to 0.07 mg/L & 0.14 mg/L of deltamethrin for 30 days.

Enzyme activity (μ mole/Pi/mg protein/h)			
Tissue	**Control**	**0.07 mg/L**	**0.14 mg/L**
Brain	17.4 ± 0.06	21.6 ± 0.05 (11.9%)	12.3 ± 0.18** (-38.5%)
Kidney	9.2 ± 0.23	9.0 ± 0.15 (-3.3%)	6.2 ± 0.08* (-33.3%)
Gills	21.3 ± 0.15	23.2 ± 0.02* (14.2%)	9.0 ± 0.15*** (-55.6%)
Muscle	8.2 ± 0.21	7.9 ± 0.15 (-3.6%)	5.4 ± 0.12* (-34.1%)
Intestine	22.0 + 0.65	24.3 ± 0.19 (15.7%)	13.5 ± 0.04* (-30.9%)

Each value represents the mean ± SD of five observations, * = p <0.05; ** = p <0.01; * * * = p <0.001.

Table 2: Ca^{2+} ATPase activity in different tissues of *H. fossilis* exposed to 0.07 mg/L & 0.14 mg/L of deltamethrin for 30 days.

Enzyme activity (µ mole Pi/mg protein/h)			
Tissue	**Control**	**0.07 mg/L**	**0.14 mg/L**
Brain	11.1 ± 0.06	10.2 ± 0.04 (-9.0%)	7.2 ± .08* (-38.5%)
Kidney	12.0 ± 0.19	9.1 ± 0.25 (-26.32%)	7.0 ±0.02* (-37.2%)
Gills	13.3 ± 0.08	15.3 ± 0.24* (13.9%)	8.3 ± .02* (-37.2%)
Muscle	5.0 ± 0.23	5.5 ± 1.50 (8.6%)	2.9 ± .24* (-46.9%)
Intestine	12.9 + 0.38	11.4 ± 0.32 (-10.0%)	10.6 ± 0.26* (-22.1%)

Each value represents the mean ± SD of five observations, * = p <Q.Q5; ** = p <0.01.

Table 3: Mg^{2+} ATPase activity in different tissues of *H. fossilis* exposed to 0.07 mg/L & 0.14 mg/L of deltamethrin for 30 days.

Enzyme activity (µ mole/Pi/mg protein/h)			
Tissue	**Contronl**	**0.07 mg/L**	**0.14 mg/L**
Brain	5.0 ± 0.05	4.4 ± 0.12 (-12.5%)	2..9 ± 0.18** (-35.4%)
Kidney	13.6 ± 0.08	14.9 ± 0.23 (11.2%)	9.0 ± 0.02* (-23.2%)
Gills	4.3 ± 0.22	3.8 ± 0.52 (-4.8%)	2.9 ± 0.06* (-31.7%)
Muscle	6.7 ± 0.03	5.3 ± 0.35 (-19.6%)	3.4 ± 0.03* (-53.12%)
Intestine	13.2 ± 0.34	12.5 ± 0.05* (-6.2%)	7.4 ± 0.05* (-42.8%)

Each value represents the mean ± SD of five observations, * = p <0.05; ** = p<0.01

Table 4: The level of different ions in different tissues of *H. fossilis* exposed to 0.07 mg/L & 0.14 mg/L of deltamethrin for 30 days.

Ions	Brain	Kidney	Gills	Muscle	Intestine
Na^{+}					
C	104.5 ± 2.04	62.9 ± 0.38	23.8 ± 0.25	47.4 ± 0.05	66.5 ± 0.08
E1	103.8 ± 1.25 (-0.9%)	61.8 ± 0.05 (-1.7%)	22.2 ± 0.24 (-7.4%)	45.2 ± 0.26 (-4.7%)	51.5 ± 1.25 (-22.8%)
E2	72.8 ± 1.34* (-33.5%)	48.2 ± 0.08** (-27.6%)	10.0 ± 0.2** (-60.5%)	32.3 ± 0.15* (-32.5%)	22.5 ± 0.3** (-33.9%)
K^{+}					
C	198.8 ± 0.28	33.5 ± 0.23	33.1 ± 0.25	94.4 ± 2.15	130.5 ± 0.06
E1	193.8 ± 0.15 (-3.7%)	38.6 ± 0.06 (3%)	36.5 ± 0.06 (-1.6%)	86.1 ± 2.36 (-6.9%)	87.3 ± 0.25* (-29.3%)
E2	135.5 ± 0.07* (-32.5%)	22.2 ± 0.09** (-37.4%)	20.5 ± 0.06** (-43.2%)	70.4 ± 0.35* (-22.12%)	30.6 ± 0.2** (-75.2%)
Ca^{2+}					
C	48.3 ± 0.39	49.4 ± 0.50	24.9 ± 1.05	17.2 ± 0.27	38.7 ± 0.07
E1	43.2 ± 0.26 (-6.6%)	42.1 ± 0.52 (-14.7%)	22.6 ± 0.31 (-9.2%)	15.2 ± 0.08 (-11.6%)	23.4 ± 0.06 (-39.5%)
E2	22.3 ± 0.3** (-51.8%)	28.7 ± 0.20** (-42.3%)	12.3 ± 0.45** (-50.6%)	5.9 ± 0.06* (-65.6%)	12.2 ± 0.1** (-68.7%)
Mg^{2+}					
C	94.5 ± 0.08	124.6 ± 1.15	49.2 ± 0.43	34.3 ± 0.05	170.8 ± 1.05
E1	87.3 ± 0.15 (-3.5%)	118.1 ± 0.52 (-5.2 %)	46.7 ± 0.15 (-5%)	30.9 ± 0.46 (-9.9%)	100.5 ± 0.5* (-41.1%)
E2	67.2 ± 0.3** (-30.1%)	69.4 ± 0.85** (-44.3%)	24.3 ± 0.06** (-50.4%)	14.3 ± 0.02** (-58.3%)	58.5 ± 0.8** (-65.7%)

Each value represents the mean ± SD of five observations, values are * = $p < 0.05$; ** = $p < 0.01$. mg/ gm wet wt.
O Control, E1 = 0.085 mg/L; E2 = 0.17 mg/L

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