JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Impact of deltamethrin on membrane associated Na^+/K^+, Ca^{2+} and Mg^{2+} ATPase in freshwater fish *Heteropneustes fossilis*

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Abstract—Synthetic pyrethroid deltamethrin contaminating aquatic ecosystems as a potential toxic pollutant, is investigated in the present study. The 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. Deltamethrin effect on Na^+/K^+ ATPase, Ca^{2+} and Mg^{2+} ATPase 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 of deltamethrin. The ATPases in brain and intestine was found more affected in the fish.

Keywords- ATPase, deltamethrin, lethal concentration, pollutant, aquatic, ecosystem

1. INTRODUCTION

Deltamethrin is a synthetic parathyroid insecticide that kills insect on contact and through digestion. World Health Organization recommended deltamethrin for application to control mosquito in-home and pest in agriculture. 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 Na^+ / K^+ ATPase, Ca^{2+} and Mg^{2+} ATPase activities in brain, kidney, gills, muscle and intestine of *Heteropneustes fossilis* has been investigated.

II. MATERIALS AND METHODS

Deltamethrin [cyano - (3 - phenoxy-phenyl) methyl;2- (2,2- dibromoethyenyl)- 2,2-dimethylcyclopropane carboxylate] 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 changed on alternate days. Only healthy fish of either sex used in the experiment. A static bioassay test conducted according to Standard Method [7] to determine the LC_{50} for 96 hr of deltamethrin to *H. fossilis*the. The recorded value of deltamethrin LC_{50} for *H. fossilis* 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_{5O}). Control groups with 30 fish were maintained in tap water containing 2 ml acetone. Fish were dissected after 30 days of exposure for further biochemical analysis. Na^+ / K^+ ATPase, Ca^{2+} and Mg^{2+} ATPase activities assayed by the method of [10].

III. RESULTS AND DISCUSSION

Exposure 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). 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). 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 *Salmogairdneri*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. It may be probably a consequence of gill and kidney damage frequently reported in pesticide and metal intoxicated fish species [17]-[22]. Furthermore, intestine sarcolemma has also shown to possess 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: Alternation in Na^+/K^+ ATPase activity in different tissues of *H. fossilis* *exposed to* 0.07 mg/L and 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: Alternation in Ca^{2+} ATPase activity in different tissues of *H. fossilis exposed to* 0.07 mg/L and 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: Alternation in Mg^{2+} ATPase activity in different tissues of *H. fossilis exposed to* 0.07 mg/L and 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	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

Acknowledgment- Author thanks CSIR-IITR, Lucknow and CRM Jat PG College Hisar for financial support and laboratory facilities during this research.

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