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Research Article

DELTAMETHRIN INDUCED ALTERATION IN Na^+-K^+, Mg^{2+}, Ca^{2+} ASSOCIATED ATPases ACTIVITY IN THE FRESHWATER FISH *CIRRHINUS MRIGALA*

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ABSTRACT

Deltamethrin is a synthetic pyrethroid, contaminating aquatic ecosystems as a potential toxic pollutant; it affects the physiological function of the aquatic fauna. In this present study, the impact of exposure of the freshwater fish *Cirrhinus mrigala* to both lethal and sub lethal concentrations (8 µl/L and 0.8 µl/L) of deltamethrin on the activities of Na^+-K^+ATPase, Ca^{2+} and Mg^{2+} associated ATPases in gills, muscle and liver was assessed. As a result significant ($p<0.05$) decrease was found in Na^+-K^+, Ca^{2+} and Mg^{2+} ATPase activities of the fish. The present studies conclude that the deltamethrin alters the membrane permeability of ATPases, resulting in the breakdown of the active transport mechanism and causes the alterations in physiology of whole organisms.

Keywords: ATPase, *Cirrhinus mrigala* , Deltamethrin, Lethal concentration, Pyrethroid.

INTRODUCTION

Fishes are extremely valuable resource and recently, it is evident that these are becoming scarce. One consequence of this scarcity is the increasing concern for fish survival and a growing interest in identifying the levels of various chemical pollutants, which are not safe for fish and other aquatic life. The frequent uses of pesticides in agriculture practices as well as pest control leads to the soil and water pollution. Thus, pesticide continuously reaching the aquatic ecosystems getting enriched in the aquatic food chain (Chebbi and David, 2009) and fish being intoxicated, it is more likely to affect humans at the top of food chain (David *et al.,* 2013). Therefore it is desirable to study the effect of these pesticides on fish species.

Pyrethroids have been reported to be extensively toxic to fish. They are lipophilic in nature and enter the fish body via gills (Thatheyus and Selvam, 2013). Deltamethrin is a synthetic pyrithroid, it's known to be more suitable for agricultural use because of their improved potency and stability as well as low mammalian toxicity (Sandhia and Kumaran, 2013).

These were found to be highly effective in controlling mosquitoes, midgets and other agricultural pests; hence, the World Health Organization (WHO) has recommended the deltamethrin for the home and agricultural insect control (WHO, 1989). Adverse effects of deltamethrin on fish have been reported with reference to hematological and biochemical variables (Srivastav *et al.,* 1997; Kumar *et al.,* 1999; David *et al.,* 2013).

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. ATPases require Na^+-K^+, Mg^{2+} and Ca^{2+} ions for their activity and are involved in the cleavage of ATP to ADP/AMP and inorganic phosphate with the liberation of energy (Begum, 2011). In addition to its fundamental importance to ion transport, ATPase activity could be used as an indicator of physiological changes. Osmotic regulations in freshwater fish are intimately bound to control ionic concentration as well as cell and body volume (Kumosani, 2004). These membrane enzymes which carry out ion transport with

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parallel energy production are well characterized and variation in their activity can be used to measure the toxic impact of chemicals (Cotou *et al.,* 2001). Therefore, in the present study the effect of deltamethrin on Na^+-K^+ATPase, Ca^{2+} and Mg^{2+} ATPase activities in gills, muscle and liver of *Cirrhinus mrigala* has been investigated.

MATERIALS AND METHODS

The mean length of 15-20 cm and weight of 20-30 g of healthy fresh water fish, *Cirrhinus mrigala* were procured from Karnataka Fisheries Board, Dharwad, Karnataka State, India and brought to the laboratory in a plastic container. Procured healthy fishes were selected and treated with potassium permanganate solution (0.5% w/v) for 5 min to remove any dermal adherent. All the fishes were acclimated for one week period at room temperature (26 ± 2^{o}C) with food ad libitum under standard laboratory condition. 12h photoperiod was maintained and the water was aerated twice in a day.

The deltamethrin of commercial grade (Decis, 30% EC) was obtained from Bayer Crop Science India Ltd., Gujarat, India. Fishes were exposed to both lethal (8 µl/l) for 1, 2, 3, 4 days and sub lethal concentration (0.8 µl/l) of deltamethrin for days 1, 5, 10, and 15. Simultaneously control group was also maintained.

Estimation of Na^+-K^+, Mg^{2+}, and Ca^{2+} associated ATPases activities (ATPase phosphorylase E.C. 3.6.1.3)

Activities of Na^+-K^+, Mg^{2+} and Ca^{2+} ATPases were estimated separately in the organs by the method described by Watson and Beamish (1981) with slight modification. 1% tissue homogenate (W/V) were prepared in ice-cold 0.25 M sucrose solution containing 5 mM EDTA and 0.1 M imidazole. The homogenates were centrifuged at 2500 rpm for 10 min and the supernatants were taken as crude enzyme extract for the assay of the ATPase enzyme activities. After due standardization of enzyme kinetic parameters, three sets of incubation mixtures were prepared, in a total volume of 2 ml, the first set consisted of 100 mM disodium adenosine triphosphate (prepared in 20 mM tris-HCL buffer at pH 7.5), 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, and 0.3 ml of enzyme extract. The second set consisted of 100 mM disodium ATP (prepared 2 mM tris HCl buffer at pH 7.5), 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 1 mM ouabain (potent inhibitor of Na^+-K^+ ATPase) and 0.3 ml of enzyme extract and the third set consisted of 100 mM disodium ATP (prepared in 20 mM tris-HCl buffer at pH 7.8), 5 mM Cadmium and 0.3 ml of enzyme extract. All the three incubation sets were incubated at 37°C for exactly 15 min and then the reaction was arrested by adding 2 ml of cold 10% TCA. The inorganic phosphates liberated were estimated by the method of Fiske and Subba Rao (1925). The absorbance was measured at 660 nm. Endogenous blanks were prepared to find out the endogenous inorganic phosphates. Another blank was prepared without using the co factor to deduct the sodium salt stimulated activity as the co-factor used was disodium salt of ATP. The first set gave the total ATPase activities of Na^+-K^+ and Mg^{2+}, whereas the second set gave only the Mg^{2+} ATPase activity as ouabain inhibits Na^+-K^+ stimulated ATPase. Hence, the Na^+-K^+ activity was derived by subtracting the Mg^{2+} ATPase from total of Na^+-K^+ and Mg^{2+} ATPase activities. The third set directly gave the Ca^{2+} ATPase activity. All these three ATPase activities are expressed as µMPi liberated/mg protein/h.

Statistical analysis

Averages of six individual estimations were taken and the mean values of control and experimental fishes were subjected to statistical analysis. Mean, ±SD, percent changes, one-way ANOVA were performed.

RESULTS

Activities of Na^+-K^+, Ca^{2+} and Mg^{2+} ATPase (µM Pi formed/mg protein/h) in the three target tissues namely gill, muscle and liver of freshwater fish, *Cirrhinus mrigala* on exposure

to lethal concentration of deltamethrin for 1, 2, 3 and 4 days and sub lethal concentration for 1, 5, 10 and 15 days are presented in table 1, 2 and 3. Na^+-K^+ ATPase activity showed a concurrence with that of the ionic strength of Na^+-K^+, which exhibited a decrease value in the lethal concentration. Fluctuations in the activity were observed in sub lethal concentration up to day 10 and finally day 15 showed elevation in all the three target tissue such as gill, muscle and liver (Table 1). Ca^{2+}ATPase activity showed a gradual decrement in lethal concentration and variations at sublethal concentration up to day 10 and enhancement on day 15 in gill, muscle and liver (Table 2). Mg^{2+}ATPase did not differ in exhibiting the trend of decrease in both lethal and sub lethal concentrations (Table 3), simultaneously showing an increase on day 15 of sub lethal concentration, which was also observed in Na^+-K^+ and Ca^{2+} ATPase.

Table 1: Alterations in Na^+-K^+ATPase activity levels (µ moles of Pi formed / mg protein / hr) in different tissues of freshwater fish *Cirrhinus mrigala* exposed to lethal and sublethal dose of deltamethrin. Values in parentheses indicate percent change over control.

Tissue	Control	Exposure Period in Days							
		Lethal				Sub lethal			
		1	2	3	4	1	5	10	15
Gill	9.933 ^{A}	8.982 ^{C}	7.916 ^{G}	6.704 ^{H}	5.565 ^{I}	9.023 ^{B}	8.797 ^{E}	8.353 ^{F}	8.918 ^{D}
±SD	0.0844	0.0004	0.0003	0.004	0.059	0.069	0.066	0.0897	0.0569
% Change		-9.573	-20.302	-32.5	-43.968	-9.159	-11.434	-15.908	-10.218
Muscle	7.498 ^{B}	7.170 ^{C}	6.654 ^{E}	5.635 ^{G}	5.181 ^{I}	7.539 ^{A}	5.937 ^{F}	5.550 ^{H}	6.670 ^{D}
±SD	0.0004	0.0008	0.0005	0.0006	0.0005	0.0006	0.0006	0.0003	0.0004
% Change		-4.38	-11.256	-24.844	-30.902	-2.895	-20.819	-25.987	-11.042
Liver	6.613 ^{A}	6.329 ^{C}	5.674 ^{E}	5.046 ^{H}	4.467 ^{I}	6.492 ^{B}	5.701^{D}	5.271 ^{G}	5.594 ^{F}
±SD	0.0005	0.0002	0.0004	0.0004	0.0004	0.0005	0.0007	0.0003	0.0005
% Change		-4.293	-14.194	-23.69	-32.446	-1.835	-13.79	-20.138	-15.409

Means are ± SD (n=6) for a parameter in a row followed by the same letter are not significantly different (P≤0.05) from each other according to Duncan's multiple range test.

Table 2: Alterations in Ca^{2+}ATPase activity levels (µ moles of Pi formed / mg protein / hr) in different tissues of freshwater fish *Cirrhinus mrigala* exposed to lethal and sublethal dose of deltamethrin. Values in parentheses indicate percent change over control.

Tissue	Control	Exposure Period in Days							
		Lethal				Sub lethal			
		1	2	3	4	1	5	10	15
Gill	8.789 ^{A}	7.508 ^{B}	6.714 ^{D}	5.709 ^{G}	4.697 ^{I}	6.799 ^{C}	6.094 ^{E}	4.681 ^{H}	5.777 ^{F}
±SD	0.0004	0.0003	0.0003	0.0004	0.0004	0.0002	0.0003	0.0004	0.0003
%Change		-14.569	-23.603	-35.043	-46.56	-22.64	-30.66	-46.737	-34.27
Muscle	5.366 ^{A}	4.609 ^{C}	3.614 ^{E}	2.726 ^{G}	1.782 ^{I}	4.986 ^{B}	4.322 ^{D}	2.230 ^{H}	3.312 ^{F}
±SD	0.6364	0.0555	0.0214	0.0004	0.00004	0.0003	0.2205	0.0005	0.0003
% Change		-14.11	-32.641	-49.201	-66.777	-7.076	-19.443	-58.435	-38.26
Liver	2.653 ^{A}	2.047 ^{C}	1.412 ^{G}	0.951 ^{H}	0.435 ^{I}	2.347 ^{B}	1.958 ^{D}	1.481 ^{F}	1.884 ^{E}
±SD	0.0004	0.0004	0.0001	0.0004	0.0004	0.0003	0.0005	0.0006	0.0006
%Change		-22.828	-46.789	-64.162	-83.592	-11.54	-26.185	-44.187	-28.997

Means are ± SD (n=6) for a parameter in a row followed by the same letter are not significantly different (P≤0.05) from each other according to Duncan's multiple range test.

Table 3. Alterations in Mg^{2+} ATPase activity levels (µ moles of Pi formed / mg protein / hr) in different tissues of freshwater fish *Cirrhinus mrigala* exposed to lethal and sublethal dose of deltamethrin. Values in parentheses indicate percent change over control.

Tissue	Control	Exposure Period in Days							
		Lethal				Sub lethal			
		1	2	3	4	1	5	10	15
Gill	8.514 ^{A}	7.587 ^{C}	6.944 ^{F}	6.271 ^{H}	5.415 ^{I}	8.276 ^{B}	7.484 ^{D}	6.805 ^{G}	7.260 ^{E}
±SD	0.0003	0.0005	0.0004	0.0004	0.0008	0.0004	0.0006	0.0005	0.0005
% Change		-10.881	-18.441	-26.34	-36.398	-2.798	-12.097	-20.075	-14.726
Muscle	5.318 ^{A}	4.819 ^{C}	4.223 ^{F}	3.584 ^{H}	2.944 ^{I}	5.053 ^{B}	4.456 ^{E}	4.079 ^{G}	4.679 ^{D}
±SD	0.0005	0.0235	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0005
% Change		-9.38	-20.582	-32.6	-44.639	-4.983	-16.206	-23.307	-12.018
Liver	2.945 ^{A}	2.440 ^{C}	1.988 ^{F}	1.630 ^{H}	1.112 ^{I}	2.792 ^{B}	2.241 ^{D}	1.901 ^{G}	2.234 ^{E}
±SD	0.0006	0.0006	0.0004	0.0005	0.0005	0.0005	0.0005	0.0003	0.0004
% Change		-17.126	-32.495	-44.65	-62.23	-5.179	-23.894	-35.449	-24.12

Means are ± SD (n=6) for a parameter in a row followed by the same letter are not significantly different (P≤0.05) from each other according to Duncan's multiple range test.

DISCUSSION

ATPase is membrane bound group of enzymes responsible for the movement of different ions across the membrane. In fish various toxicants and ions enter into the body by absorption and adsorption by the gill surface and then followed by diffusion. An interaction with the membrane may disrupt the osmotic and ionic regulation of the gill tissue by affecting the membrane permeability, mainly due to inactivation of the ATPase in the bronchial epithelial cells (Parvez *et al.*, 2006). The observed changes in the levels of ATPases in *C. mrigala* could be attributed to pathological changes in tissues such as the liver and gills, which are involved in the exchange of ions between the fish and the surrounding water, and to the reduction of Na^{+}-K^{+}-ATPase activity, which plays a central role in whole body ion regulation under toxicant exposure (Begum, 2011). Previous studies (De Boeck *et al.*, 2001; Monteiro *et al.*, 2005) reported that the disturbance in osmoregulation was associated with increased epithelial permeability and inhibition of active ion uptake and subsequently to the decline of ATPase activity. Some of them are reported that, the pyrethroid causes the disturbances in neural transmission and such a disturbance might lead to perturbations in ATPase system (Nicolas *et al.*, 2007).

Na^{+}/K^{+}–ATPase, Mg^{2+}ATPase and Ca^{2+}ATPase (ecto-ATP-ase) are membrane enzymes ubiquitous in animal cells that involve adenosine triphosphate (ATP) as a substrate for their functioning (Rodrigez and Pena, 1995; Unnisa and Devaraj, 2007). Na^{+}/K^{+}–ATPase (EC 3.6.1.3) plays a key role in the active transport of monovalent cations (Na^{+} and K^{+}) across the membrane (Jorgensen *et al.*, 2003). The activities of Na^{+}-K^{+}, Mg^{2+} and Ca^{2+} ATPases decreased in gill, muscle and liver of the fish on exposure to both lethal and sub lethal concentration of deltamethrin. The decrease in these activities indicates the demolition of cellular ionic regulation in the organs of the fish as reported by Marigoudar (2012). Also, he has reported that, the disruption may be due to the effect of deltamethrin on passive movement of ions i.e. the permeability characteristics. The decrease in activities may also be due to interaction of pesticide with Mg^{2+} and Na^{+}-K^{+} ATPases thereby inducing inhibition. The inhibition of Na^{+}-K^{+}-ATPase in the gills probably disturbs the Na^{+}/K^{+} pump, resulting in the erratic entry of Na^{+} into the cell along a concentration gradient and the water molecule along the osmotic gradient. This process may cause swelling of the cell and finally membrane rupture (Oruc *et al.*, 2002).

Mg^{2+}ATPase have a unique role in energy synthesis through oxidative phosphorylation in mitochondria and is presumed to be present in all types of cells (Siraj *et al.*, 2010). It is responsible for the transepithelial regulation of Mg^{2+} ions, which are essential to the integrity of the cellular membrane, intracellular cements and the stabilization of branchial permeability (Parvez *et al.*, 2006). In most cases Mg^{2+}-ATPase is taken

as an index of general ATPase activity because of its abundant distribution and dual localization in mitochondria and cytosol (Lehninger and Albert, 1988). In the present study, the Mg^{2+}-ATPase activity showed progressive inhibition in liver, muscle and gill tissue. Significant inhibition was detected in Mg^{2+}-ATPase activity. Shwetha and Hosetti, (2012) have suggested that the decrease in Mg^{2+}ATPase activity might be due to the damage of the mitochondria membranes, which may interfere with the conversion of oxidative energy to phosphate bond energy. Since Mg^{2+} ATPase is involved in oxidative phosphorylation (Vesna *et al.,* 2008). Daya *et al.,* (2000) has reported that uncoupling agents increase the hydrolysis of ATP and inhibits the phosphorylation. This mechanism may be operating in the pesticide treated fish and impair the energy producing system.

Table 2 represents Ca^{2+}-ATPases in the tissues of *C. mrigala* exposed to deltamethrin. Significant inhibition was observed in all the tissues such as gills, liver, muscle respectively. The inhibition of Ca^{2+}-ATPase activity may be due to the inhibition of oxidative phosphorylation (Tiwari *et al.,* 2002) and degradation products of lipid peroxidation on the enzyme molecule (Ardelt *et al.,* 1994; Daya *et al.,* 2000). Ca^{2+} in the membrane assists the cross-linking of skeletal proteins and binds to anionic sites in the lipid bilayer and alters membrane fluidity (Curry, 1992). A decrease in Ca^{2+}-ATPases has been reported by several researchers (Okolie and Audu, 2004; Sandhia and Kumaran, 2013; Sureshkumar, 2013). Unnisa and Devaraj (2007) and Shwetha and Hosetti, 2012 observed that cyanide specifically inhibits the activity of Ca^{2+}ATPase in fish. Since this enzyme is directly involved in the oxidative phosphorylation the action of pesticide on this system correlates with toxicity of pesticide. The inhibition is due to phosphorylation of active site of the enzyme as in the case of acetylcholinesterase inhibition. Since Na^{+}-K^{+} ATPase is considered as a marker enzyme to understand the physiological impairment of the cell (Martine and Arivoli, 2008). The inhibition reveals the disruption of ionic movement in neuronal and glial cells. Such alterations in ionic balance depolarize the nerve and due to depolarization the nerve cells increase in the releasing of neurotransmitter, which in turn inhibits Na^{+}-K^{+} ATPase activity. The same was drawn by Vesna *et al.,* (2008)

In support to the findings of the present study, it has been reported that exposure to fenithrothon decreased ATPase activity of *Anguilla anguilla* (Sancho *et al.,* 1997). Oruc *et al.,* (2002) reported increased lipid peroxidise formation in three fish species exposed to azinphosmethyl, which could disturb the anatomical integrity of the biomembrane and diminish its fluidity leading to inhibition of several membrane bound enzymes including Na^{+}-K^{+} ATPase. It is reported that pyrethroids, cypermethrin, and phorate impair the stability of the cell membrane by damaging its structural lipid by peroxidation decomposition, which may lead to subsequent cell necrosis and functional dearrangements (Kakko *et al.,* 2000).

Greater degree of decrease in Na^{+}-K^{+} and Ca^{2+} levels and the activities of Na^{+}-K^{+} , Mg^{2+} and Ca^{2+} ATPase in the fish exposed to the lethal concentration of deltamethrin indicates severe disruption in the cellular ionic regulation. High concentration of deltamethrin might have greatly altered the permeability characteristics of the membranes of the organs by interacting with the membrane proteins readily to serve alterations in the acute transport through destabilizing the membrane bound enzymes and related hormonal and energy producing process. Further, the progressive decrease in the ionic levels and progressive suppression of Na^{+}-K^{+}, Mg^{2+} and Ca^{2+} ATPase activities in the organs of fish, over time of exposure to the lethal concentration deltamethrin indicate the increase in the binding of the deltamethrin to the active sites of membrane bound enzymes as the degree of inhibition is dependent on the concentration of deltamethrin available to the active sites on enzyme molecule. The drastic decrease in the rate of oxygen consumption and oxidative metabolic cycles in the organs of fish from day 1 to day 4 in the lethal concentrations also lend support for the study of decrease in the Na^{+}, K^{+} and Ca^{2+} ionic levels and activity of associated ATPase activities.

In sublethal concentration of deltamethrin the Na^{+}-K^{+} and Ca^{2+} levels significantly decreased with ion-competent inhibition of Na^{+}-K^{+}, Mg^{2+} and Ca^{2+} associated ATPases activities in the organs of fish at 1^{st} day and 10^{th} day of exposures. The significant elevations in the ionic levels and enzyme activities on 15^{th} day indicates that, a prolonged exposure of the sub acute concentration of deltamethrin could not elicit inhibitory effect either on the uptake of

ions or on the activities of ATPase and instead it stimulated the uptake. Possibly the inhibition of ATPase activity is dependent on the functional groups of the enzyme and the amount of deltamethrin available for the competitive replacement of the substrate. Further requirement of chloride cells has been proposed as a fundamental and physiologically significant response of freshwater fish to increase the capability to take up Na^+-K^+ and Ca^{2+} from water (Leino *et al.,* 1987). Even the secretion might be increased to induce particularly hypercalcemia by remobilization of Ca^{2+} from exchangeable Ca^{2+} stores. All these factors could be an active operation for the elevation in ion levels and ATPase activities in the gills, muscle and liver of the fish. The increased ionic level may be helpful to the animal to prevent the entry of toxic deltamethrin by maintaining cation concentration gradient (Vasilets and Schwarz, 1993).

CONCLUSIONS

Thus, from the present study, it is evident that both lethal and sublethal doses of deltamethrin in freshwater fish significantly inhibited Na^+-K^+ATPase, Mg^{2+}ATPase and Ca^{2+} ATPase enzymes. Thus toxic potential of deltamethrin was cleanly illustrated by the increased inhibition or decreased activity levels of Na^+-K^+ATPase, Mg^{2+} and Ca^{2+}ATPase activity in different tissues of the freshwater fish *Cirrhinus mrigala*. Further conclude that greater degree of decrease in associated ATPase activities of exposed fish to both lethal and sublethal concentration of deltamethrin indicates severe disruption in the cellular ionic regulation it might have greatly altered the permeability characteristics of the membrane and also it can destabilizing the membrane bound enzymes and related hormonal and energy producing process.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest associated with this article.

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REFERENCES

Ardelt, B.K., Borowitz, J.L., Maduh, E.U., Swain, S.L. and Isom, G.E., 1994. Cyanide-induced lipid peroxidation in different organs: subcellular distribution and hydroperoxide generation in neuronal cells. *Toxicology*, 89(2): 127-137.

Begum, G., 2011.Organ-specific ATPase and phosphorylase enzyme activities in a food fish exposed to a carbamate insecticide and recovery response. *Fish Physiol. Biochem.*, 37(1): 61-69.

Chebbi, S.G. and David, M., 2009. Neurobehavioral responses of the freshwater teleost, C*yprinus carpio* (Linnaeus) under quinalphos intoxication. *Biotech. Anim. Husband.*, 25(3-4): 241-249.

Cotou, E., Castritsi-Catharios, I. and Moraitou-Apostolopoulou M., 2001. Surfactant- based oil dispersant toxicity to developing nauplii of Artemia: effects on ATPase enzymatic system. *Chemosphere.*, 42: 959-964.

Curry, S.C., 1992. Hydrogen cyanide and inorganic cyanide salts. In: Sullivan JB Jr., Krieger GR, (Ed.) Hazardous materials toxicology–clinical principles of environmental health. Baltimore, Williams and Wilkins, pp. 698-710

David, M., Sangeetha, J., Harish, E.R., Srinivas, S.S. and Naik, V.R., 2013. Alterations in the levels of Ach and associated AChE in the tissues of fresh water fish *Cirrhinus mrigala* exposed to deltamethrin. *Int.. Pharma. Biol. Arch.*, 4(6): 1237-1241.

Daya, S., Walker, R.B. and Anoopkumar-Dukie, S., 2000. Cyanide-induced free radical production and lipid peroxidation in rat brain homogenate is reduced by aspirin. *Metabolic Brain Dis.*, 15(3): 203-210.

De Boeck, G., Vlaeminck, A., Balm, P.H., Lock, R.A., De Wachter, B. and Blust R., 2001. Morphological and metabolic changes in common carp, *Cyprinus carpio*, during short-term copper exposure: interactions between Cu^{2+} and plasma cortisol elevation. *Environ Toxicol Chem.*, 20: 374-380.

Fiske, C. H. and Subba Rao, Y., 1925. The colorimetric determination of phosphorous. *Biol. Chem.*, 66: 375-400.

Jorgensen, P.L., Hakansson, K.O. and Karlish, S.J.D., 2003. Structure and mechanism of Na^+/K^+– ATPase: functional sites and their

interactions, *Annu. Rev. Physiol.,* 65: 817-849.

Kakko, I., Toim ela, T. and Tähti, H., 2000. Piperonyl butoxide potentiates the synaptosome ATPase inhibiting effect of pyrethrin. *Chemosphere.,* 40: 301-305.

Kumar, S., Lata, S. and Gopal, K., 1999. Deltamethrin induced physiological changes in freshwater cat fish *Heteropneustes fossilis*, *Bull. Environ. Contam. Toxicol.*, 62: 254-258.

Kumosani, T.A., 2004. Na^+-K^+-ATPase activity from various organs of Tilapia (*Oreochromis mossambicus*): A Bioindicator for environment monitoring. Proc. 2nd *Saudi Sci. Conf. Fac. Sci.*, KAU(Jeddah), pp. 29-41.

Lehninger, L. and Albert, 1988. The molecular basis of cell structure and function. In: Biochemistry Edn. 2nd. Kalyani Publishers, Ludhiana, New Delhi.

Leino, R.L., Wilkinson, P. and Anderson, J.G., 1987. Histopathological changes in the gills of pearl dace, *Semotilus margarita* and fathead minnows, *Pimephales promelas,* from experimentally acidified Canadian lakes. *Can. J. Fish Aqua. Sci.*, 44: 126-134.

Marigoudar, S.R., 2012. Cypermethrin induced some pathophysiological and biochemical changes in the freshwater teleost, *Labeo rohita* (Hamilton). Ph.D Thesis, Karnatak University, Dharwad, India.

Martine P.D.P. and Arivoli, S., 2008. Biochemical study of freshwater fish *Catla catla* with reference to mercury chloride. *Iran. J. Environ. Health. Sci. Eng.*, 5(2): 109-116.

Monteiro, S.M., Mancera, J.M., Fernandes, A.F. and Sousa M., 2005. Copper induced alterations of biochemical parameters in the gill and plasma of *Oreochromis niloticus*. *Comp Biochem Physiol.*, 141: 375-383.

Nicolas, D., Axel, D. and Jean-Marie, D., 2007 The sublethal effects of pesticides on beneficial Arthropods. *Annu. Rev. Entomol*., 52: 81-106.

Okolie, N.P. and Audu, K. 2004. Correlation between cyanide induced decreases in ocular Ca^{2+}- ATPase and lenticular opacification. *J.* Biomed. *Sci.*, 3(1): 37-34.

Oruc, E.O., Uner, N. and Tamer, L., 2002. Comparison of Na^+- K^+-ATPase activities and malondialdehyde contents in liver tissue for three fish species exposed to azinphos methyl. *Bull. Environ. Contam. Toxicol.*, 69: 271-277.

Unnisa, Z. A. and Devaraj, N.S., 2007. Effect of methacrylonitrile on membrane bound enzymes of rat brain. *Indian J. Physiol. Pharmacol.*, 51 (4): 405-409.

Vasilets, L.A. and Schwarz, W., 1993. Structure-function relationships of cation binding in the Na^+-K^+–ATPase. *Biochem. Biophys. Acta.*, 1154: 201-222.

Vesna, V., Tatjana, M., Marijana, P. and Danijela, K., 2008. Na^+-K^+-ATPase as the target enzyme for organic and inorganic compounds. *Sensors*, 8: 8321-8360.

Watson, T.A. and Beamish, F.W.H., 1981. The effects of zinc on branchial adenosine triphosphatase enzymes *in vitro* from rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.*, 68C: 167-173.

WHO, 1989. Allethrin, bioallethrin, S-bioallethrin, environmental health criteria 87, International programmes on chemical safety, Geneva, Switzerland, pp. 2-20.