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RESEARCH ARTICLE

EFFECT OF DELTAMETHRIN AND NEEM-BASED FORMULATION ACHOOK ON ACTIVITIES OF PHOSPHATASES IN TISSUES OF ZEBRAFISH *DANIO RERIO*

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ABSTRACT

The present study is aimed to investigate the changes in activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) in liver, ovary and muscle of Zebrafish after exposure to 96 h LC_5, LC_{10} and LC_{20} of synthetic pyrethroid Deltamethrin and neem based formulation Achook. It was found that the activities of ACP and ALP in treated fishes was significantly reduced ($p<0.001$) in response to treatments of both the pesticides compared with controls. The activity of ACP was reduced to 91, 96 & 92% of controls (100%) in liver, ovary and muscle, respectively for Deltamethrin whereas 96, 98 & 97% for Achook treated fishes after LC_5 exposure for 4 days. Also, the activity of ALP was reduced to 65, 60 & 57% of controls (100%) in liver, ovary and muscle respectively after 16 days exposure to LC_{20} of Deltamethrin. The reduction in ALP activity was 75, 65 & 67% of controls in liver, ovary and muscle, respectively due to Achook at the same concentration and exposure period as that of Deltamethrin. There was a concentration and time dependent inhibition in the activities of both ACP & ALP enzymes after 4, 8, 12 & 16 days of exposure to both the pesticides. The natural pesticides may not be treated safe to the fish and should be used with great cautions.

INTRODUCTION

Pollution of the aquatic environment is a serious and growing problem. Increasing amount of industrial, agricultural and commercial chemicals into the aquatic environment having led to various deleterious effects on the aquatic organisms. Also, it is reported that the drainage waters discharged into the lake are high in solids, nutrients, pesticides, heavy metals and organics. The aquatic ecosystem is the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Rahman *et al.,* 2002). Pesticides are useful to control economically important crops, but are found very much hazardous to the aquatic flora and fauna and in turn, the entire food chain including human beings (Paul and Simonin, 2006).
Synthetic pyrethroids, the newest major class of insecticides are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleo-resin extract of genus *Chrysanthemum* flowers. Because of their beneficial qualities, synthetic pyrethroids, such as Deltamethrin, have attracted farmers and health departments to use them in pest control. Type-II pyrethroids including Deltamethrin are potentially toxic to fish and least toxic to mammals. They are reported to alter the biochemical constituents in different tissues of fish (Anita Susan *et al.,* 2010; Sharma and Ansari, 2011). All pyrethroids are potent neurotoxicant and due to their lipo-philicity, biological membranes and tissues readily take up them (Oros *et al.,* 2005). Cost-effective, non-toxic, bio-degradable, eco-friendly and botanical soft-pesticides are the need of present day agriculture as an alternative to hazardous and synthetic pesticides. The neem (*Azadirachta indica* A. Juss) is a tropical evergreen tree, native to Indian sub-continent which is a natural source of insecticides and agrochemicals along with a number other properties. Azadirachtin (a tetranotriterpenoid) is one of a major component of neem, which have pesticidal property (Anon, 1992). However, the disturbance in the total protein level due to different neem extracts have been reported (Ibrahim *et al.,* 1992; Mahdi *et al.,* 2003; Winkaler *et al.,* 2007). Recently, various pesticides were found toxic to adult, embryo and fingerlings of Zebrafish (Ansari and Sharma, 2009; Ansari and Ahmad, 2010; Ahmad and Ansari, 2011; Ansari and Ansari, 2011; Ahmad *et al.,* 2011) and cause skeletal deformities (Kumar and Ansari, 1984) and reduced reproductive ability (Sharma and Ansari, 2010).

Fish, among the group of non-target aquatic organisms, represent the largest and most diverse group of vertebrates. A number of characteristics make them excellent experimental models for toxicological research, especially for the contaminants which are likely to exert their impact on aquatic systems. Cells contain enzymes that are necessary to their function. When the integrity of a cell is disrupted, enzymes escape into plasma/serum, where their activity can be measured as a useful index of cell integrity. Thus, by estimating the enzyme activities in an organism, we can easily identify disturbance in its metabolism. Hence, a need was felt to investigate the changes in activities of acid phosphatase

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(ACP) and alkaline phosphatase (ALP) in the liver, ovary and muscle of Zebrafish (*Danio rerio*) after sub-lethal exposure of Deltamethrin (synthetic pyrethroid) and Achook (neem pesticide). Zebrafish was used as the test species as per recommendation of the International Organization for Standardization and the Organization for Economic Co-operation and Development (OECD, 1992).

MATERIALS AND METHODS

For the present experiment Zebrafish were procured from our stock aquarium. The water of the stock aquarium was aerated continuously through stone diffusers connected to a mechanical air compressor. Water temperature ranged between 25±2°C and the pH was maintained between 6.6 and 8.5. Fish were fed twice daily alternately with raw chopped goat liver and brine shrimps. The diet was supplemented with *Drosophila* flies once daily. For the present study, matured adult fishes were exposed to different concentrations *viz.,* 96-h LC_5, LC_{10} and LC_{20} of Deltamethrin and Achook for 16 days continuously. Fifty fishes for each concentration of the pesticides were used. In these aquaria water was replaced daily with fresh treatment of pesticides. Each experiment was accompanied by its respective control.

After the expiry of the exposure periods (4, 8, 12 & 16 days), required number of exposed fishes were taken out from experimental and control groups. Activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) in the liver, ovary and muscle of Zebrafish were estimated according to the method proposed by Andersch and Szcypinski (1947) later modified by Bergmeyer (1967) using p-nitrophenylphosphate as substrate. The activities of phosphatases have been expressed as µM substrate hydrolyzed/30 minutes/mg protein. Two way ANOVA was employed to test the significance of the data.

RESULT AND DISCUSSION

In the present investigation we observed significant ($p<0.001$) alterations in activities of ACP and ALP enzymes in liver, ovary and muscle of Zebrafish exposed to Deltamethrin and Achook pesticides at different concentrations and exposure periods. The activity of ACP was reduced to 91, 96 & 92% of controls (100%) in liver, ovary and muscle, respectively for Deltamethrin whereas 96, 98 & 97% for Achook treated fishes after LC_5 exposure for 4 days. The 8 days exposure of LC_{10} reduced the ACP activity to 84, 73 & 85% in liver, ovary and muscle, respectively for Deltamethrin while 86, 84 & 89% for Achook treatment. Further increase in concentration caused drastic inactivation of the enzyme activity. At the LC_{20} exposure for 16 days ACP activity remained only 58, 59 & 60% in liver, ovary and muscle, respectively for Deltamethrin whereas 66, 65 & 64% for Achook (Table 1). Also, from results it is evident that in the liver, ovary and muscle of Zebrafish LC_5 and LC_{10} of Deltamethrin causes greater decrease in activity of ALP than Achook (Table 2). The ALP activity was reduced to 65, 60 & 57% of controls (100%) in liver, ovary and muscle respectively after 16 days exposure to LC_{20} of Deltamethrin. The reduction in ALP activity from the control was 75, 65 & 67% in liver, ovary and muscle respectively due to Achook at the same concentration and exposure period as that of Deltamethrin. There was a concentration-dependent inhibition in the activities of both ACP & ALP enzymes. Thus the result showed that Deltamethrin is more toxic than Achook.

In the treated group of fishes abnormal behaviour such as restlessness, sudden quick and jerky movements were observed at low concentration of pesticides whereas, increased opercular movements accompanied with surface to bottom movements and loss of equilibrium was observed in the fishes exposed to high concentrations. Similar observation has been reported by Rahman *et al.* (2002) in some fishes. The organ most associated with the detoxification and biomarker process is liver and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in the water (Camargo and Martinez, 2007). Fish is a good indicator of changes in water because under stress conditions biochemical changes such as alterations in enzyme activities and metabolic products occur in their body. Enzymes are relatively fragile substances with a tendency to undergo denaturation and inactivation under suitable conditions (Lopez *et al.,* 2003). The majority of insecticides are biotransformed in metabolites by liver through various enzyme systems (Roy, 2002) and as a consequence of this process, liver undergoes different levels of damages. Lysosomal enzymes, both acid alkaline phosphatase participate in degradation of proteins, carbohydrates and lipids (Pipe *et al.,* 1993; Xue and Renault, 2000). These enzymes are released by the lysosomes for the hydrolysis of foreign material; hence it has a role in certain detoxification functions. Das *et al.* (2004) have been reported the changes in phosphatase activity in fishes due to exposure to industrial effluents.

ALP is composed of several isoenzymes that are present in practically all tissues of the body, especially in cell membranes. Any damage in hepatic cells may result in alteration in ALP activity. The concentration-dependent inhibition observed in this investigation is in agreement with the earlier report of Sastry and Sharma (1980). More reasonably this can be explained that like acetylcholinesterase the ALP has serine residue at its active site and the organophosphate compounds are generally inhibitors of serine containing enzymes (Bell *et al.,* 1970). Similarly, Das and Mukherjee (2003) reported depletion of alkaline phosphatase due to sub-lethal exposure of *Labeo rohita* fingerlings to cypermethrin. In our earlier experiment we also observed inhibition in protein content (Sharma and Ansari, 2011). The inhibition in protein level may also be due to the decrease in ALP activity as it plays an important role in protein synthesis (Pilo *et al.,* 1972). The decreased activity in these enzymes in intoxicated Zebrafish as observed in present investigation may be due to the labilization of the lysosomal membrane releasing the enzyme. Similar observations were also made by Abou-Donia (1978) and Tayyaba *et al.* (1981) after treatment with pesticides. Zebrafish, exposed to diazinon is known to affect the nervous tissue (brain) by inhibition of acetyl-cholinesterase (AChE) and phosphatases activity (Ansari *et al.,* 1987). The inhibition of ACP in liver, ovary and muscle indicate that these pesticides may cause considerably functional impairment in the lysosomal metabolism due to the direct action of the pesticides on the enzyme system. Acute toxicity data for Deltamethrin in fish have been published as a report of the WHO (1990) and classified as highly toxic to

Table 1. Effect of Deltamethrin and Achook on ACP (µM substrate hydrolyzed/30 minutes/mg protein) in Zebrafish

Tissue	Period (days)	Exposure Concentrations (µg/l)						
		Control	Deltamethrin			Achook		
		(0.00)	LC_{5} (0.016µg/l)	LC_{10} (0.025µg/l)	LC_{20} (0.043µg/l)	LC_{5} (0.025µg/l)	LC_{10} (0.17µg/l)	LC_{20} (0.35µg/l)
Liver	4	18.59±0.19 (100)	16.94±0.07 (91)	15.79±0.10 (85)	14.59±0.33 (78)	17.82±0.38 (96)	16.52±0.58 (89)	14.88±0.58 (80)
	8	18.44±0.36 (100)	16.66±0.34 (90)	15.54±0.23 (84)	12.66±0.37 (69)	16.96±0.26 (92)	15.93±0.39 (86)	13.30±0.30 (72)
	12	17.66±0.34 (100)	15.67±0.16 (89)	13.86±0.12 (78)	11.30±0.33 (64)	15.84±0.29 (90)	14.60±0.43 (83)	12.06±0.51 (68)
	16	17.58±0.32 (100)	14.50±0.26 (82)	13.39±0.20 (76)	10.20±0.17 (58)	14.83±0.18 (84)	14.04±0.80 (80)	11.64±0.50 (66)
Ovary	4	17.46±0.34 (100)	16.80±0.11 (96)	14.73±0.18 (84)	13.43±0.33 (77)	17.15±0.04 (98)	16.55±0.14 (95)	15.08±0.05 (86)
	8	17.16±0.16 (100)	14.50±0.26 (84)	12.54±0.30 (73)	11.68±0.27 (68)	16.12±0.12 (93)	14.44±0.08 (84)	12.67±0.21 (74)
	12	16.86±0.12 (100)	12.59±0.22 (75)	11.30±0.34 (67)	10.59±0.28 (63)	15.36±0.14 (91)	13.82±0.10 (83)	12.11±0.18 (72)
	16	16.38±0.20 (100)	11.46±0.27 (70)	10.24±0.14 (63)	9.63±0.28 (59)	14.17±0.15 (86)	11.10±0.06 (68)	10.72±0.17 (65)
Muscle	4	18.80±0.14 (100)	17.29±0.17 (92)	16.69±0.20 (89)	15.48±0.30 (82)	18.18±0.09 (97)	17.67±0.12 (94)	17.12±0.05 (91)
	8	18.42±0.26 (100)	16.28±0.21 (88)	15.64±0.27 (85)	14.73±0.18 (80)	17.28±0.07 (94)	16.43±0.09 (89)	15.36±0.12 (83)
	12	17.52±0.32 (100)	14.52±0.25 (83)	13.46±0.29 (77)	12.50±0.25 (71)	16.12±0.10 (92)	15.19±0.17 (87)	13.36±0.05 (76)
	16	17.43±0.24 (100)	13.68±0.23 (78)	12.50±0.29 (72)	10.47±0.27 (60)	15.75±0.15 (90)	14.63±0.13 (84)	11.23±0.18 (64)

Values are mean ± SD of six individual observations; Values are significant at $p<0.001$ (two-way ANOVA).

Table 2. Effect of Deltamethrin and Achook on ALP (µM substrate hydrolyzed/30 minutes/mg protein) in Zebrafish

Tissue	Period (days)	Exposure Concentrations (µg/l)						
		Control	Deltamethrin			Achook		
		(0.00)	LC_{5} (0.016µg/l)	LC_{10} (0.025µg/l)	LC_{20} (0.043µg/l)	LC_{5} (0.025µg/l)	LC_{10} (0.17µg/l)	LC_{20} (0.35µg/l)
Liver	4	15.67±0.16 (100)	14.33±0.25 (91)	13.17±0.34 (84)	12.35±0.34 (79)	15.18±0.10 (97)	14.15±0.10 (90)	13.71±0.47 (87)
	8	15.50±0.26 (100)	13.42±0.30 (87)	12.06±0.06 (78)	11.40±0.41 (74)	14.58±0.13 (96)	13.61±0.19 (88)	12.74±0.15 (82)
	12	14.38±0.20 (100)	12.33±0.35 (85)	11.17±0.16 (77)	10.23±0.26 (71)	13.14±0.07 (91)	12.30±0.12 (85)	11.32±0.17 (79)
	16	14.16±0.37 (100)	11.30±0.14 (80)	10.20±0.20 (72)	9.21±0.19 (65)	12.80±0.12 (90)	10.78±0.13 (76)	10.70±0.20 (75)
Ovary	4	13.86±0.12 (100)	12.20±0.14 (88)	11.20±0.14 (81)	10.19±0.21 (73)	13.35±0.12 (96)	12.82±0.09 (92)	11.64±0.19 (84)
	8	13.39±0.20 (100)	11.65±0.15 (87)	10.65±0.08 (80)	9.75±0.12 (73)	13.05±0.03 (95)	12.30±0.15 (90)	10.68±0.10 (78)
	12	14.49±0.22 (100)	11.39±0.40 (79)	10.09±0.09 (70)	9.58±0.20 (66)	12.78±0.10 (88)	11.76±0.11 (81)	10.33±0.17 (71)
	16	14.22±0.17 (100)	10.29±0.23 (72)	9.42±0.39 (66)	8.55±0.27 (60)	11.75±0.19 (83)	10.70±0.13 (75)	9.27±0.12 (65)
Muscle	4	14.47±0.19 (100)	13.49±0.27 (93)	12.33±0.26 (85)	11.28±0.20 (78)	14.23±0.07 (98)	13.26±0.20 (91)	12.72±0.13 (88)
	8	15.28±0.13 (100)	13.21±0.14 (86)	12.10±0.07 (79)	10.21±0.16 (67)	14.13±0.11 (92)	13.16±0.13 (90)	11.35±0.27 (74)
	12	15.23±0.26 (100)	12.37±0.27 (81)	10.35±0.34 (68)	9.48±0.26 (62)	13.25±0.13 (87)	12.18±0.08 (80)	10.75±0.33 (70)
	16	15.18±0.28 (100)	12.02±0.07 (79)	9.20±0.16 (60)	8.70±0.20 (57)	12.83±0.16 (84)	11.78±0.23 (78)	10.15±0.07 (67)

fish, being in the range of $LC_{50}<100$ µg/l. The plant based pesticidal toxic effects on the environment are not too detrimental (Das and Mukherjee, 2003). But, some studies have shown that plant toxins at low concentrations are very toxic to all groups of aquatic fauna (Goktepe *et al.,* 2004). The toxicity of two neem based pesticides Nimbecidine and Neemgold on a fresh water loach, *Lepidocephalichthys guntea* has been reported (Mondal *et al.,* 2007). Recently, Achook a neem based pesticide was found toxic to adult Zebrafish (Ansari and Sharma, 2009) and causes alterations in reproductive ability (Sharma and Ansari, 2010). Our results indicate the inert ingredients do have a toxic effect on the aquatic fishes and changes the phosphatases activities which in turn will affect the overall health of fish. We, therefore, recommend that the commercial formulations of neem pesticides should be redesigned. These pesticides should be used with great caution and in sustainable way so that, it may not be hazardous to aquatic environment and human beings.

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