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ALPHAMETHRIN TOXICITY: EFFECT ON THE REPRODUCTIVE ABILITY AND THE ACTIVITIES OF PHOSPHATASES IN THE TISSUES OF ZEBRAFISH, *DANIO RERIO*

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ABSTRACT

Alphamethrin, a synthetic pyrethroid used extensively for the control of insect pests and public health programmes. In the present study, the adult Zebrafish, their embryos and fingerlings were used as a model to investigate the toxic effects of Alphamethrin. The 24 to 96-h LC_{10}, LC_{50} and LC_{90}, slope and chi-square values were calculated for the adults and fingerlings exposed to Alphamethrin. The 24-h LC_{50} value of Alphamethrin was 1.29 µg l^{-1} and for 96-h 0.17 µg l^{-1} for adults. The 72-h LC_{50} for the embryo was 0.024 µg l^{-1} and for fingerlings the 96-h LC_{50} value of Alphamethrin was 0.020 µg l^{-1}. The results show significant decrease in fecundity and hatchability in comparison to the control group. The present study was also aimed to investigate the changes in the activities of enzymes acid phosphatase (ACP) and alkaline phosphatase (ALP) in the gill and liver of Zebrafish after exposure to 20 %, 40 %, 60 % and 80 % of the 24-h LC_{50} values of Alphamethrin. It was found that the activities of ACP and ALP in treated fishes were significantly reduced ($p < 0.05$) in response to treatment of pesticide as compared to control. The toxicity was concentration as well as time dependent.

Key words: Alphamethrin, Embryo, Fingerling, Phosphatases, Toxicity, Zebrafish.

INTRODUCTION

Due to rapid increase in the industrialization and human population, the pollution of aquatic ecosystem has become an universal phenomenon in the present day world (Belazutshi and Raghuprasad SG, 2008). The main sources of water pollution are industrial waste, domestic sewage, drainage and pesticides used for food production (Maruthanayagam and Sharmila G, 2004). Aquatic contamination of the pesticides causes acute and chronic poisoning of fish and other organism. The early life stages of fish, like eggs and larvae are particularly sensitive to contaminant (Fiumann LA, 1993). The reduced fitness and growth of the fish occurs at sublethal levels depending on exposure time, toxicity and concentrations of the chemical substances involved (Lanno RP and Dixon DG, 1994).

The synthetic pyrethroids, a new generation insecticides are synthesized derivatives of naturally occurring pyrethrins, taken from pyrethrum, the oleo-resin extract of genus *Chrysanthemum* flowers. They are more effective than the organophosphate pesticides, replacing them in many agricultural, commercial and residential applications. Due to lipophilic nature, biological membranes and tissues readily take them (Oros DR et al. 2005). They are potent neurotoxicant and interfere with nerve cell function by interacting with voltage-dependent sodium channels as well as other ion channels, resulting in repetitive firing of neurons and eventually

causing paralysis (Shafer TG and Mayer DA, 2004). Fish sensitivity to pyrethroids may be explained by their relatively slow metabolism and elimination of these compounds (David M et al. 2003). Alphamethrin, a synthetic pyrethroid is extensively used not only as an ectoparasiticide in animals, but also used in agricultural crop production and public health programme. It is non toxic to birds but highly toxic to fish and aquatic invertebrates (Paul EA and Simonin HA, 2006). This is mainly because it is metabolized and eliminated more slowly by the fish than mammals (WHO, 1992). Moore A and Waring CP (2001) reported that even a low level of cypermethrin in the aquatic ecosystem might have a significant long-term effect on Atlantic salmon populations through disruptions of reproductive functions. The pesticides may damage the vital organs (Joshi N et al. 2007), causes skeletal deformities (Kumar K and Ansari BA, 1984) and reduced reproductive ability (Sharma DK and Ansari BA, 2010; Singh PK and Ansari BA, 2010; Ahmad MK and Ansari BA, 2011) in fishes.

Enzymes are biochemical molecules that control metabolic processes of organisms, thus a slight variation in enzyme activities would affect the organism (Roy SS, 2002). Biochemical changes induced by pesticidal stress lead to metabolic disturbance, vital enzymes inhibition, retardation of growth and reduction of fecundity and longevity of organisms (Fatima E et al. 2006; Lissandra G et al. 2006). 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 (Coppo JA et al. 2002). Thus, by estimating the enzyme activities in an organism, we can easily identify disturbance in its metabolism. Liver is an established organ in fishes and plays an important role in uptake, accumulation, biotransformation and excretion of xenobiotics (Thophon S et al. 2003). The gills which participate in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to the changes in the quality of the water are considered the primary target of the contaminants (Camarago MM and Martinez CB, 2007).

Zebrafish was selected for the present study because they are model organisms for developmental toxicology research and also recommended by International Organization for Standardization and the Organization for Economic Co-operation and Development (OECD, 1992). A promising alternative approach to classical acute fish toxicity testing with live fish is the fish embryo toxicity test (FET) (Lammer E et al. 2008), which has been used for the exact evaluation of chemical toxicity to fish. Therefore, the present study was undertaken to investigate the toxic effects of Alphamethrin on the reproductive ability, embryo, fingerling and changes in the activities of phosphatases (ACP and ALP) in the gill and liver of Zebrafish, *Danio rerio.*

MATERIALS AND METHODS

Toxicity test

For bioassay, adult Zebrafish of similar age were procured from the general culture to determine the 24, 48, 72 and 96-h LC_{50} values using five different concentrations *viz.,* 0.06, 0.12, 0.24, 0.48 and 0.96 µg l^{-1} of Alphamethrin previously diluted in acetone. The tests were conducted in glass aquaria containing 10 litre dechlorinated water. Two replicates of ten fishes for each concentration of pesticides were performed. Water was changed daily with fresh treatment of pesticide. A fish was considered dead when its gill movements ceased and it did not respond to gentle prodding. Dead fish was removed carefully from aquaria to avoid deterioration.

Reproductive ability

For the study of reproductive ability adult fishes (10 females and 20 males) were procured from the general stock and exposed for one month to four different concentrations of Alphamethrin (96-h LC_5 0.02, LC_{10} 0.03, LC_{15} 0.04 and LC_{20} 0.05 µg l^{-1}). Each set of experiment was accompanied with a control group having no pesticide. After one month continuous stress of the pesticide the adult Zebrafish were brought back to the normal water for breeding. Three matured females along with six males placed in 25 litre glass aquaria to breed in laboratory by the method of Ansari BA and Kumar K (1986). The eggs were counted and average fecundity was established. Unfertilized eggs were detected by their milky appearance and discarded. The hatched and dead

embryos were recorded till 72-h and survival of fingerlings up to one week was observed.

Embryo and Fingerlings toxicity

For this study Zebrafish were bred in the laboratory to obtain the fertilized eggs. Five concentrations 0.015, 0.020, 0.025, 0.030 and 0.035 µg l^{-1} of Alphamethrin for embryo and fingerlings of Zebrafish were selected with three replicates for each concentration. The stock solution was prepared by serial dilution of the pesticides in acetone. Acetone alone in the same amount served as control. Water was changed after 24-h with fresh treatment of pesticide. For embryo toxicity tests lots of 100 fertilized eggs were separated in 500 ml beakers with 250 ml dechlorinated water. At every 24-h until the end of the test dead eggs was counted and removed. The dead embryos became white due to coagulation or precipitation of protein. At the end of the incubation period of 72-h the total hatched eggs were counted.

To perform the fingerlings toxicity, the eleutheroembryonic stage *i.e.,* 5-days old fingerlings of Zebrafish were used. Three replicates of ten fingerlings for each concentration were placed in 500 glass beakers having 250 ml of dechlorinated water. Five concentrations *viz.,* 0.015, 0.020, 0.025, 0.030 and 0.035 µg l^{-1} were selected. Mortality of fingerlings was recorded after 24, 48, 72 and 96-h exposure periods. The susceptibility of the adults, embryos and fingerlings of Zebrafish to Alphamethrin pesticide were determined by StatPlus® version 2009 computer software programme to calculate LC_{50} (with 95% confidence limits), slope and chi-square values.

Biochemical estimations

For this study, matured adult fishes were exposed to different concentrations *viz.,* 20 %, 40 %, 60 % and 80 % of the 24-h LC_{50} value of Alphamethrin for 21 days continuously. 50 fishes for each concentration of the pesticide were used. In these aquaria water was replaced daily with fresh treatment of pesticides. The experiment was accompanied by the control.

After the expiry of the exposure periods (7, 14 and 21 days), required number of exposed fishes were taken out from experimental and control groups. Activities of acid and alkaline phosphatases in the gill and liver of Zebrafish were estimated according to the method originally proposed by Andersch MA and Szcypinski AJ (1947) later modified by Bergmeyer (1967) using p-nitrophenylphosphate as substrate. The activities of phosphatases enzyme (ACP and ALP) has been expressed as µ mole substrate hydrolyzed/30 minutes/mg protein. Analysis of variance (ANOVA) was employed to test the significance of the data.

RESULTS AND DISCUSSIONS

The exposure of adult fish to different concentrations of pesticide showed behavioral changes. These changes may be related to the consequent alteration in the physiological process. They became restless, aggregated at one corner of the aquarium, showed erratic and jerky swimming, frequent surfacing, secreted mucous from whole body and loss of equilibrium. At high concentrations the pectoral and pelvic fins were expanded and they rolled vertically prior to death. During the toxicity test it was observed that the mortality increases with the increase in concentrations and the LC_{50} decreases with the increase in the exposure period (Table 1). It shows that toxicity of Alphamethrin is concentration as well as time dependent. The 24-h LC_{50} value of Alphamethrin was 1.29 µg l^{-1} which decreased to 0.17 µg l^{-1} after 96-h of exposure. The slope values shown in the table are steep which indicate that slight increase in concentration may cause more mortality. The LC_{50} values of the pesticide showed a significant ($p<0.05$) negative correlation with the exposure time. The chi-square values were not significant, indicating that the fish populations used in the experiments were homogeneous.

During the experiment a significant ($p<0.05$) reduction in fecundity, viability, hatchability was observed (Table 2). It is revealed from the present experiment that the average numbers of eggs laid by Zebrafish are 331 under normal conditions whereas this number remarkably reduced to 205 after one month stress of 0.05 µg l^{-1} (LC_{20}) Alphamethrin. A significant ($p<0.05$) decrease in hatchability up to 53.46 % was observed. Results shows that the survival of the hatched fingerlings was not affected after 72-h.

The results of the toxicity of Alphamethrin to embryos and fingerlings of Zebrafish are illustrated in

Table 3. With increase in concentrations of pesticide the number of dead embryos in treated groups increased to 16 % at 0.015 µg l^{-1} and at highest concentration *i.e.,* 0.035 µg l^{-1} it was increased to 91 % (Table 3). It is clear that at lowest concentration of pesticide the hatching was 252 (84 %) which sharply decreased to 27 (9 %) at highest concentration of pesticide as compared to the control group. The 72h LC_{50} value for embryo was calculated to be 0.024 µg l^{-1}. Mortality of fingerlings was observed at 24 to 96-h at different concentrations (0.015, 0.020, 0.025, 0.030 and 0.035 µg l^{-1}). After exposure of the pesticide the fingerlings of Zebrafish also showed behavioral changes, they aggregated at one corner of the test aquarium, swimming fast at the water surface throughout the experiment. For fingerlings the 24-h LC_{50} value of Alphamethrin was 0.030 µg l^{-1}, while for 48-h and 72-h it was 0.027 µg l^{-1} and 0.023 µg l^{-1} respectively which further decreased to 0.020 µg l^{-1} after 96-h of exposure (Table 3). The number of dead fingerlings increases with increase in concentrations of pesticide. This shows that the effect of Alphamethrin is concentration as well as time dependent. Here also the slope value was steep.

In the present investigation we observed a significant ($p<0.05$) alterations in activities of phosphatases (ACP and ALP) in the gill and liver of Zebrafish exposed to Alphamethrin at different concentrations and exposure periods. The activity of ACP was reduced to 85 and 92 % of controls (100 %) in gill and liver, respectively after 20 % of 24-h LC_{50} for 7 days. After 14 days exposure to 40 % of 24-h LC_{50} of this pesticide reduced the ACP activity to 71 and 72 % in gill and liver respectively. Further increase in concentration caused drastic inactivation of the enzymatic activity. At the 80 % of 24-h LC_{50} of Alphamethrin for 21 days ACP activity remained only 41 % in the gill and 49 % in the liver (Tables 4 and 5). The ALP activity was reduced to 88 and 92 % treated in the gill and liver respectively after 20 % of 24-h LC_{50} of Alphamethrin for 7 days. The 21 days exposure of 80 % of 24-h LC_{50} of this pesticide reduced the activity of ALP up to 40 and 46 % in the gill and liver respectively (Tables 6 and 7).

During the present investigation it is found that Alphamethrin was highly toxic to Zebrafish, *Danio rerio* and the 96-h LC_{50} value was found to be 0.17 µg l^{-1}. Behavioral changes due to alphamethrin exposure were observed as erratic, jerky, abrupt swimming, frequent surfacing and gulping. Similar observations were also made by Polat H et al. (2002) and Baser S et al. (2003) after exposure of fish to synthetic pyrethroids. It was found that 96-h LC_{50} value of Alphamethrin for Tilapia, *Oreochromis niloticus* was 5.99 µg l^{-1} (Sarikaya R, 2009). It is also reported that Zebrafish is sensitive to Deltamethrin with 96-h LC_{50} value of 0.121 µg l^{-1} (Ansari BA and Sharma DK, 2009) and for Lambda-Cyhalothrin it was 0.119 µg l^{-1} (Ansari BA and Ahmad MK, 2010b). Ansari S and Ansari BA (2011) observed that Dimethoate was toxic to the adult, embryos and fingerlings of Zebrafish and caused a significant reduction in fecundity, viability, hatchability and survival of fingerlings.

However, fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure. According to Peterson RE et al. (1993) early developmental stages of fishes are more sensitive to chemical exposure. The embryo and fingerling toxicity tests are valuable for assessing potential impacts on growth, reproduction and survival of fish in polluted environment and are important tools for good environmental monitoring (Kristensen P, 1994). In the present study it has been observed that increasing Alphamethrin concentration had significant effects on hatchability which may be due to inhibition of some hatching enzymes. Von Westerhagen's review (1988) mentions numerous studies which show that contaminants may reduce oocytes volume, as indicated by reduction in oocyte diameter. *Carassius auratus gibelo* exposed to Deltamethrin (2 µg l^{-1} for 14 days) exhibited symptoms of induced hepatic, gonadal and renal toxicity. Decreased number of spermatozoa is a severe reaction that affects the reproductive potential of animals (Staicu et al. 2007). Svobodova Z et al. (2003) reported that 96-h LC_{50} value of Deltamethrin to *Cyprinus carpio* juveniles was 0.00145 mg l^{-1}.

During the development sensitivity may change with some compounds showing higher sensitivity in embryos whereas others are more toxic to larvae (Gaikowski MP et al. 1996). Recently, Ansari BA and Ahmad MK (2010a) reported that the embryo of Zebrafish is sensitive to Lambda-Cyhalothrin than neemgold a neem based pesticide.

Hassan SM et al. (2008) reported that the toxicity of Quillaja saponin extrated from the bark of the tree, *Quillaza saponaria* using Zebrafish embryo and found that at higher concentrations the embryo exhibited shrinkage of the chorion, decreased hatching time and embryonic mortality. Sisman T (2010) studied early life stages toxicity in Zebrafish and significant differences were observed in the spawning success after exposure to the pesticides. According to Ansari BA and Kumar K (1986) exposure to long-term sub-lethal concentrations of Malathion affects the embryonic stage and fingerlings of Zebrafish. Also, Rahmi A et al. (2005) reported the toxicity of Cypermethrin for the early stages of fish. Very recently we have reported that the chorion of Zebrafish provides no protection to the developing embryo exposed to neem pesticide Azacel (Ahmad MK and Ansari BA, 2011).

Enzymes are relatively fragile substances with a tendency to undergo denaturation and inactivation under adverse conditions (Lopez LE et al. 2003). The majority of insecticides are biotransformed in metabolites by liver through various enzyme systems (Roy SS, 2002) and as a consequence of this process, liver undergoes different levels of damages. According to Kumar K and Ansari BA (1986) the activity of phosphatases was decreased in the liver of Zebrafish treated with Malathion. Phosphatases are mainly localized at cell membrane. Any damage in the cells may result in alteration in phosphatases activity. The dose dependent inhibition in the activities of acid and alkaline phosphatases observed in this investigation is in agreement with the report of many other workers (Joshi UM and Desai AK, 1981; Sherekar PV and Kulkarni KM, 1987). Inyang IR et al. (2011) observed the reduction in ACP and ALP in the gill and liver of *Clarias gariepinus* treated with Diazinon. The metabolic pathways of fish are affected by various pollutants, organic and inorganic chemicals due to the alteration of cellular enzymatic activities. The decreased activities of ACP and ALP indicate disturbance in the structure and integrity of cell organelles like endoplasmic reticulum and membrane transport system. Das PC et al. (2004) have reported the changes in phosphatase activity in fishes due to exposure to industrial effluents. Similarly, Das BK and Mukherjee SC (2003) reported depletion of alkaline phosphatase due to sub-lethal exposure of *Labeo rohita* fingerlings to Cypermethrin.

Table 1. ***Toxicity of Alphamethrin against Zebrafish.***

Treated Period (h)	Effective Concentrations (µg l^{-1})	95% Confidence limits of LC_{50} (µg l^{-1})		Slope	Chi-square Values
		LCL	UCL		
24	LC_{10} 0.05 LC_{50} 1.29 LC_{90} 33.75	0.55	1.87	12.62	0.34
48	LC_{10} 0 .04 LC_{50} 0.64 LC_{90} 10.03	0.49	0.98	8.45	0.11
72	LC_{10} 0.03 LC_{50} 0.29 LC_{90} 3.03	0.18	0.54	6.08	0.09
96	LC_{10} 0.02 LC_{50} 0.17 LC_{90} 1.12	0.11	0.26	4.25	0.02

†Batches of ten fishes were exposed to five different concentrations of Alphamethrin (diluted in acetone). Mortality was recorded every 24-h. Each set of experiment was replicated two times. The control groups were treated with acetone simultaneously. The LC_{50} values of the pesticide showed a significant ($p<0.05$) negative correlation with exposure time. LCL and UCL denote the lower and upper confidence limits respectively for the LC_{50} values.

Table 2. ***Effect of Alphamethrin on the fecundity, viability, hatchability and survival of Zebrafish.†***

Concentrations (µg l^{-1})	Average number of eggs laid/ female	Average number of viable eggs	Hatchability after 72 h	Survival of fingerlings after one week
0.00	331	318	302 (94.97)	292 (96.69)
0.02	302	279	245 (87.81)	222 (90.61)
0.03	269	243	192 (79.01)	157 (81.77)
0.04	242	206	146 (70.87)	101 (69.18)
0.05	205	159	74 (46.54)	40 (54.05)

†Fishes were exposed to four different concentrations for one month under pesticidal stress of Alphamethrin ranging from 0.02 to 0.05 µg l^{-1}. Data in parentheses are percent values. All the data were found significant ($p<0.05$) when Student's t-test was applied between treated and control.

Table 3. ***Toxicity of Alphamethrin to Zebrafish embryo and 5-day-old fingerlings. †***

Concentrations (µg l^{-1})	Embryo toxicity		Fingerling toxicity			
			Number of dead fingerlings			
	Number of dead embryos	Total hatching in 72 h	24 h	48 h	72 h	96 h
0.00	05 (1.67)	295 [98.33]	NIL	NIL	NIL	NIL
0.015	48 (16.00)	252 [84.00]	03	05	07	09
0.020	86 (28.66)	214 [71.33]	04	06	09	13
0.025	157 (52.33)	143 [47.66]	09	11	17	19
0.030	218 (72.67)	82 [27.33]	15	18	21	23
0.035	273 (91.00)	27 [9.00]	19	23	26	29

†300 eggs were used in three batches of 100 each for embryo toxicity. 30 fingerlings were used in three batches of 10 each for fingerling toxicity.
Data in parentheses '( )' shows the % mortality and '[ ]' shows the % hatching of the Zebrafish embryo.

Summary of probit analysis of Table 3.

Test stage	Exposure Duration (h)	Effective Concentrations (µg l^{-1})			Confidence limits of LC_{50} (µg l^{-1})		Slope	Chi-square Values
		LC_{10}	LC_{50}	LC_{90}	LCL	UCL		
Embryo	72	0.016	0.024	0.038	0.021	0.026	1.45	9.39
Fingerling	24	0.017	0.030	0.056	0.028	0.036	1.60	1.92
	48	0.015	0.027	0.049	0.024	0.030	1.59	1.16
	72	0.013	0.023	0.041	0.020	0.025	1.58	1.11
	96	0.012	0.020	0.036	0.018	0.022	1.54	0.94

Table 4.

Effect of Alphamethrin on ACP (µM substrate hydrolyzed/30 minutes/mg protein) in the gill of Zebrafish. †

Concentrations (µg l^{-1})*	Treatment Period (days)			Summary of computation for ANOVA					
	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control (0.00)	13.51±0.23 (100)	12.50±0.32 (100)	11.63±0.18 (100)	Variations due to concentrations	2	100.56	50.28	459.93	0.05
(0.26)	11.54±0.25 (85)	9.68±0.49 (77)	8.48±0.29 (73)	Variations due to operations	4	452.50	113.13	1034.8	0.05
(0.51)	9.95±0.59 (74)	8.84±0.56 (71)	7.13±0.07 (61)	Interaction	8	4.44	0.55	5.08	NS
(0.77)	8.43±0.34 (62)	6.96±0.44 (56)	5.79±0.12 (50)	Residual	75	8.20	0.11		
(1.03)	7.25±0.12 (54)	6.56±0.17 (52)	4.73±0.14 (41)	Total	89	565.69			

†Values are mean ± SD of six individual observations and significant at p < 0.05 (two-way ANOVA). NS = Not Significant.

***The concentrations used were 20% (0.26), 40% (0.51), 60% (0.77) and 80% (1.03) of 24-h LC_{50} value.**

Table 5. ***Effect of Alphamethrin on ACP (µM substrate hydrolyzed/30 minutes/mg protein) in the liver of Zebrafish. †***

Concentrations (µg l^{-1})*	Treatment Period (days)			Summary of computation for ANOVA					
	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control (0.00)	13.43±0.22 (100)	12.45±0.31 (100)	11.51±0.19 (100)	Variations due to concentrations	2	103.35	51.67	290.14	0.05
(0.26)	12.31±0.65 (92)	10.97±0.66 (87)	9.09±0.73 (79)	Variations due to operations	4	336.33	84.08	472.12	0.05
(0.51)	10.27±0.18 (76)	9.08±0.62 (72)	7.79±0.11 (68)	Interaction	8	3.35	0.42	2.35	NS
(0.77)	9.23±0.13 (69)	7.99±0.67 (64)	6.45±0.23 (56)	Residual	75	13.36	0.18		
(1.03)	8.36±0..25 (62)	7.57±0.24 (60)	5.69±0.15 (49)	Total	89	456.39			

† Values are mean ± SD of six individual observations and significant at p<0.05 (two-way ANOVA).
NS = Not Significant.
****The concentrations used were 20% (0.26), 40% (0.51), 60% (0.77) and 80% (1.03) of 24-h LC_{50} value.***

Table 6: ***Effect of Alphamethrin on ALP (µM substrate hydrolyzed/30 minutes/mg protein) in the gill of Zebrafish. †***

Concentrations (µg l^{-1})*	Treatment Period (days)			Summary of computation for ANOVA					
	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control (0.00)	12.65±0.15 (100)	11.46±0.22 (100)	10.62±0.19 (100)	Variations due to concentrations	2	180.10	90.05	519.50	0.05
(0.26)	11.18±0.51 (88)	8.74±0.45 (76)	6.73±0.47 (63)	Variations due to operations	4	413.61	103.40	596.55	0.05
(0.51)	10.02±0.52 (79)	7.50±0.51 (65)	5.23±0.91 (49)	Interaction	8	20.96	2.62	15.12	0.05
(0.77)	8.65±0.49 (68)	6.19±0.49 (54)	4.77±0.13 (45)	Residual	75	13.00	0.17		
(1.03)	6.37±0.45 (50)	5.38±0.37 (47)	4.25±0.66 (40)	Total	89	627.67			

†Values are mean ± SD of six individual observations and significant at p<0.05 (two-way ANOVA).
****The concentrations used were 20% (0.26), 40% (0.51), 60% (0.77) and 80% (1.03) of 24-h LC_{50} value.***

Table 7: ***Effect of Alphamethrin on ALP (µM substrate hydrolyzed/30 minutes/mg protein) in the liver of Zebrafish. †***

Concentrations (µg l^{-1})*	Treatment Period (days)			Summary of computation for ANOVA					
	7	14	21	Source of variations	df	Sum of squares	Mean of squares	F	P<
Control (0.00)	12.78±0.11 (100)	11.24±0.14 (100)	10.63±0.15 (100)	Variations due to concentrations	2	234.28	117.14	534.95	0.05
(0.26)	11.81±0.74 (92)	9.08±0.04 (81)	7.38±0.49 (70)	Variations due to operations	4	273.27	68.32	311.99	0.05
(0.51)	10.71±0.54 (84)	7.70±0.50 (69)	5.82±0.54 (55)	Interaction	8	13.26	1.66	7.57	0.05
(0.77)	9.63±0.62 (75)	6.79±0.59 (60)	5.46±0.47 (51)	Residual	75	16.42	0.22		
(1.03)	8.77±0.39 (69)	6.24±0.56 (56)	4.94±0.41 (46)	Total	89	537.24			

†Values are mean ± SD of six individual observations and significant at p<0.05 (two-way ANOVA).
****The concentrations used were 20% (0.26), 40% (0.51), 60% (0.77) and 80% (1.03) of 24-h LC_{50} value.***

CONCLUSIONS

It is concluded from the present study that Zebrafish and its early life stages are sensitive to low levels of Alphamethrin in aquatic environment and significantly affect its population. These results also indicate that even low concentrations of Alphamethrin have a toxic effect on fishes and changes the phosphatases activities which in turn will affect the overall health of the fish. It is suggested that the use of pesticide in aquatic ecosystem should be minimized to prevent their effects on developing embryo and fingerlings. Therefore these pesticides should be used with great caution and in a sustainable way so that it may not be hazardous to aquatic biota and human beings. In addition, potential risk from Alphamethrin metabolites should be investigated to get a more complete picture in terms of toxicity.

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