Investigation of Acute Toxicity Diazinon, Deltamethrin, Butachlor and pretilachlor on Zebra Cichlid (Cryptoheros nigrofasciatus)

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ABSTRACT

Background: The presence of pesticide due to the huge demand for agricultural purposes is very prevalent in surface waters of Iran. These pesticides could finally accumulate in aquatic ecosystems and have been proved to have toxic effects on aquatic animals. The aim of this study was to assess the acute toxicity of Diazinon, Deltamethrin, Butachlor and Pretilachlor on Zebra Cichlid (Cryptoheros nigrofasciatus).

Methods: Fish samples were exposed to different concentrations of Diazinon (60%) (0, 2, 4, 8, 16 and 32 ppm), Deltamethrin (2.5%) (0, 0.02, 0.04, 0.10, 0.20 and 0.40 ppm), butachlor (60%) (0, 2, 4, 8, 16 and 32 ppm) and pretilachlor (50%) (0, 5, 10, 20, 30 and 40 ppm) for 96 h within the 100 L glass aquaria and cumulative mortality of Zebra Cichlid fish was calculated in 24-h interval.

Results: The very low LC50 obtained for diazinon (5.06±0.37 ppm), deltamethrin (0.15±0.39 ppm), butachlor (8.93±0.26 ppm) and pretilachlor (20.72±0.58 ppm) indicated that these are highly toxic chemicals.

Conclusion: Our results demonstrate that deltamethrin and pretilachlor had the lowest and highest rate of mortality on the Zebra Cichlid respectively.

Keywords: Butachlor, Deltamethrin, Diazinon, LC50, Pretilachlor, Zebra Cichlid.

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INTRODUCTION

Increased use of pesticides results in contamination of natural ecosystems especially the aquatic ecosystem [1]. These toxic substances may accumulate in the food chain and cause serious ecological and health problems. Chemical pesticides with persistent molecules (long half-life periods) pose a threat to aquatic life forms and also to the human population consuming the affected fish.

Presence of pesticide in surface waters was reported in Europe and North America since 50 years ago, and since then many documents have been proved the toxic effects of these pollutants for aquatic environment [2, 3].

Organphosphorus pesticides (OPs) are widely used in agriculture, and the aquatic environments near the fields are influenced by of OPs such as diazinon (O, O-diethylO-(2-isopropyl-4-methyl-6-pyrimiinyl) phosphorothioate) [4].

Diazinon is an organphosphorus pesticide extensively used in agriculture and is a moderately persistent substance [5, 6]. The toxicity of diazinon is due to blocking of acetyl cholinesterase (ACHE) activity, which could be harmful for non-target aquatic species close to agricultural fields [6].

The pyrethroids including deltamethrin are widely used as pediculicides and are among the most potent insecticides known [7]. Pyrethroids have been proved to be extremely toxic to fish and some aquatic arthropods, such as shrimps [8, 9]. The toxicity of Pyrethroids on amphibians, birds and mammals have been reviewed before [10].

Acute toxicity of a pesticide refers to the chemical's ability to cause damage to an animal from a single exposure, generally of short duration. Many workers have employed the acute toxicity tests of pesticides on fish to

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acquire rapid estimates of the concentrations that cause direct, irreversible damage to test organisms [11, 12].

Butachlor was first introduced in Japan in 1973 and has been used for the control of grass and broadleaf weeds in transplanted rice paddies [13].

Pretilachlor a herbicide of clorstamyd group selectively inhibits synthesis of long-chain fatty acids and is effective for a range of slim and broad-leaved weeds in rice fields [14].

The present study was performed to determine acute toxicity of diazinon, deltamethrin, butachlor and pretilachlor as potential dangerous organic pesticides to assess the effects of these chemicals on the freshwater fish zebra cichlid.

MATERIALS AND METHODS

The selected fish species for present study was zebra cichlid. Test chambers were glass aquaria of 100 L. All fish were acclimated for a week in these aquaria before assays with continuous aeration. Water temperature was regulated at 27^0C by aquarium heaters. The fish were feed twice a day with formulated feed and the dead fish were immediately removed to avoid possible water deterioration [15].

Nominal concentrations of active ingredients tested were 0, 2, 4, 8, 16 and 32 ppm of commercial dose (60%) for diazinon and 0, 0.02, 0.04, 0.10, 0.20 and 0.40 ppm of commercial dose (2.5%) for deltamethrin and 0, 2, 4, 8, 16 and 32 ppm of commercial dose (60%) for butachlor and 0, 5, 10, 20, 30 and 40 ppm of commercial dose (50%) for pretilachlor. In total, 20 groups (5 for diazinon, 5 for deltamethrin, 5 for butachlor and 5 for pretilachlor) of Twenty-one zebra cichlid were exposed for 96h in aerated glass aquaria with 100 l of test medium. During acute toxicity experiment, the water in each aquarium was aerated and the temperature was 27^0C. No food was provided to the specimens during the assay and test media was not renewed. Mortality rates were recorded at 0, 24, 48, 72 and 96 hours. Acute toxicity tests were carried out according to Hotos GN and Vlahos N. Salinity [16]. The nominal concentration of diazinon, deltamethrin, butachlor and pretilachlor estimated to result in 50% mortality of zebra cichlid within 24 h (24-h LC50), 48 h, 72 h, and 96 h was assessed by probit analysis using Finney's method and the maximum-likelihood procedure (SPSS 2002, SPSS Inc., Chicago, Illinois, USA). The LC50 value was obtained by fitting a regression equation arithmically and also by graphical interpolation using logarithms of the diazinon, deltamethrin, butachlor and pretilachlor concentrations versus probit value of percentage of mortality. After the acute toxicity test, the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) were determined for each measured endpoint [16].

RESULTS

No fish died during the acclimation period before exposure, and no control fish died during acute toxicity tests. The mortality of zebra cichlid for diazinon doses of 0, 2, 4, 8, 16 and 32 ppm and 0, 0.02, 0.04, 0.10, 0.20 and 0.40 ppm for deltamethrin and 0, 2, 4, 8, 16 and 32 ppm for butachlor and 0, 5, 10, 20, 30 and 40 ppm for pretilachlor were examined during the exposure times at 24, 48, 72 and 96 h (table 1, 2, 3, 4). The mortality of zebra cichlid increased significantly with increasing concentrations from 2 ppm to higher concentrations for diazinon and 0.04 ppm to higher concentrations for deltamethrin and 4 ppm to higher concentrations for butachlor and 10 ppm to higher concentrations for pretilachlor.

Table 1. Cumulative mortality of zebra cichlid Fish (n=21 in each concentration) acute exposure to diazinon.

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
2	0	0	0	0
4	3	5	8	12
8	8	10	13	17
16	14	17	20	21
32	17	18	21	21

Table 2. Cumulative mortality of zebra cichlid Fish (n=21 each concentration) acute exposure to deltamethrin.

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
0.02	0	0	0	0
0.04	0	0	0	0
0.10	3	3	6	7
0.20	7	10	12	15
0.40	19	20	21	21

Table 3. Cumulative mortality of zebra cichlid Fish (n=21 each concentration) acute exposurer to butachlor.

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
2	0	0	0	0
4	0	0	3	5
8	6	9	10	14
16	8	13	15	17
32	14	17	18	21

Table 4. Cumulative mortality of zebra cichlid Fish (n=21 each concentration) acute exposure to pretilachlor.

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
5	0	0	0	0
10	0	0	0	0
20	5	7	7	13
30	14	15	17	18
40	21	21	21	21

Median lethal concentrations are presented in tables 5, 6, 7 and 8. Because mortality (or survival) data were collected for each exposure concentration at various exposure durations (24, 48, 72, or 96 hours), the data could be plotted; the straight line of best fit was then drawn through the points. These were time–mortality lines and the LT50 (median lethal survival time) was estimated for each concentration.

Table 5. Lethal Concentrations (LC10-99) of diazinon depending on time (24-96h) for zebra Cichlid.

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{10}	1.70±0.20	1.64±0.19	2.03±0.28	1.95±0.37
LC_{20}	6.78±0.20	4.37±0.19	3.81±0.28	3.01±0.37
LC_{30}	10.45±0.20	7.75±0.19	5.10±0.28	3.79±0.37
LC_{40}	13.58±0.20	10.63±0.19	6.19±0.28	4.45±0.37
LC_{50}	**16.50±0.20**	**13.32±0.19**	**7.22±0.28**	**5.06±0.37**
LC_{60}	19.43±0.20	16.01±0.19	8.25±0.28	5.68±0.37
LC_{70}	22.56±0.20	18.90±0.19	9.34±0.28	6.33±0.37
LC_{80}	26.22±0.20	22.27±0.19	10.63±0.28	7.11±0.37
LC_{90}	31.31±0.20	26.94±0.19	12.41±0.28	8.18±0.37
LC_{99}	43.38±0.20	38.05±0.19	16.64±0.28	10.72±0.37

Table 6. Lethal Concentrations (LC10-99) of deltamethrin depending on time (24-96h) for zebra cichlid.

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{10}	0.11±0.32	0.10±0.33	0.08±0.38	0.07±0.39
LC_{20}	0.16±0.32	0.14±0.33	0.11±0.38	0.10±0.39
LC_{30}	0.19±0.32	0.17±0.33	0.13±0.38	0.12±0.39
LC_{40}	0.22±0.32	0.19±0.33	0.15±0.38	0.13±0.39
LC_{50}	**0.25±0.32**	**0.21±0.33**	**0.17±0.38**	**0.15±0.39**
LC_{60}	0.27±0.32	0.24±0.33	0.19±0.38	0.16±0.39
LC_{70}	0.30±0.32	0.26±0.33	0.21±0.38	0.18±0.39
LC_{80}	0.33±0.32	0.29±0.33	0.23±0.38	0.20±0.39
LC_{90}	0.38±0.32	0.33±0.33	0.26±0.38	0.23±0.39
LC_{99}	0.49±0.32	0.43±0.33	0.34±0.38	0.29±0.39

Table 7. Lethal Concentrations (LC10-99) of butachlor depending on time (24-96h) for zebra Cichlid.

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{10}	6.60±0.24	3.50±0.22	2.84±0.20	2.09±0.26
LC_{20}	12.35±0.24	8.26±0.22	5.74±0.20	4.44±0.26
LC_{30}	16.51±0.24	11.70±0.22	9.09±0.20	6.13±0.26
LC_{40}	20.06±0.24	14.63±0.22	11.95±0.20	7.58±0.26
LC_{50}	**23.37±0.24**	**17.37±0.22**	**14.62±0.20**	**8.93±0.26**
LC_{60}	26.69±0.24	20.11±0.22	17.30±0.20	10.28±0.26
LC_{70}	30.24±0.24	23.05±0.22	20.16±0.20	11.73±0.26
LC_{80}	34.40±0.24	26.48±0.22	23.51±0.20	13.43±0.26
LC_{90}	40.16±0.24	31.24±0.22	28.16±0.20	15.78±0.26
LC_{99}	53.84±0.24	42.54±0.22	39.19±0.20	21.36±0.26

Table 8. Lethal Concentrations (LC10-99) of pretilachlor depending on time (24-96h) for zebra cichlid.

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{10}	17.44±0.73	15.78±0.64	15.73±0.72	12.54±0.58
LC_{20}	20.35±0.73	18.80±0.64	18.45±0.72	15.35±0.58
LC_{30}	22.44±0.73	20.99±0.64	20.42±0.72	17.37±0.58
LC_{40}	24.23±0.73	22.85±0.64	22.10±0.72	19.10±0.58
LC_{50}	**25.91±0.73**	**24.59±0.64**	**23.67±0.72**	**20.72±0.58**
LC_{60}	27.58±0.73	26.34±0.64	25.24±0.72	22.33±0.58
LC_{70}	29.37±0.73	28.20±0.64	26.92±0.72	24.06±0.58
LC_{80}	31.47±0.73	30.38±0.64	28.88±0.72	26.09±0.58
LC_{90}	34.37±0.73	33.41±0.64	31.61±0.72	28.90±0.58
LC_{99}	41.27±0.73	40.60±0.64	38.08±0.72	35.56±0.58

Toxicity testing had two statistical endpoints: 1: Hypothesis Testing: is there a statistically significant difference between the mean response in the treatments and mean response in controls or reference samples. (LOEC: Lowest Observed Effect Concentration; NOEC: No Observed Effect Concentration). 2: Point Estimates: what toxic concentration would cause a specific effect on the test population? (LC50: the median Lethal Concentration). Statistical endpoints of acute toxicity of diazinon, deltamethrin, butachlor and pretilachlor for zebra cichlid during different times after exposure are shown in figures 1, 2, 3 and 4, respectively.

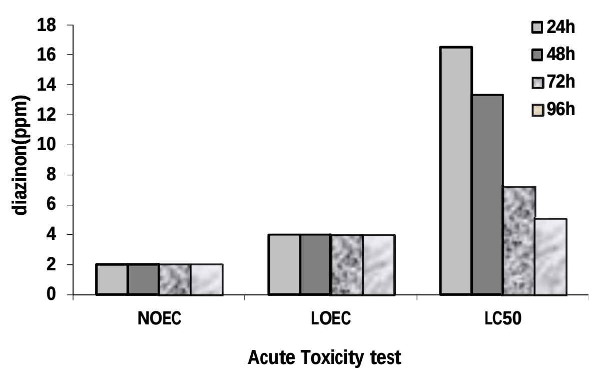

Figure1. Statistical endpoints of acute toxicity in zebra cichlid Fish exposed to diazinon in different times (24h, 48h, 72 h and 96 h respectively).

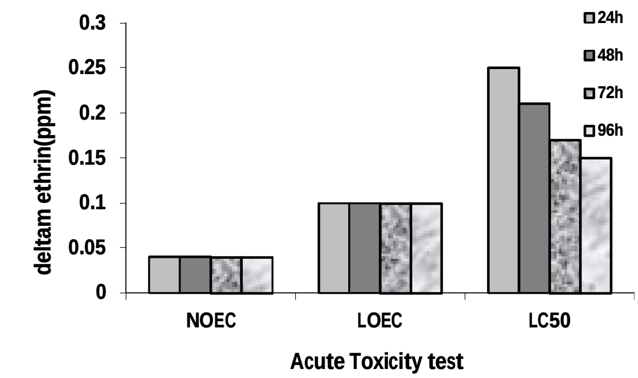

Figure 2. Statistical endpoints of acute toxicity in zebra cichlid Fish exposed to deltamethrin in different times (24h, 48h, 72 h and 96 h respectively).

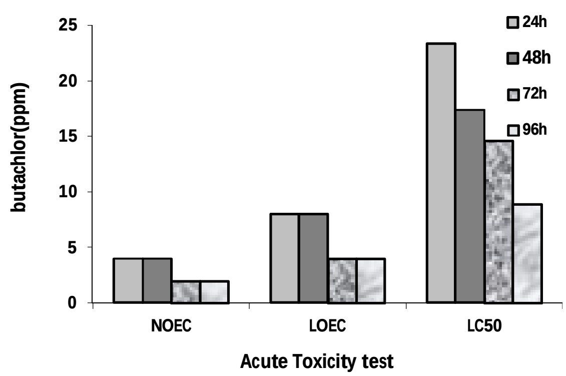

Figure 3. Statistical endpoints of acute toxicity in zebra cichlid Fish exposed to butachlor in different times (24h, 48h, 72 h and 96 h respectively).

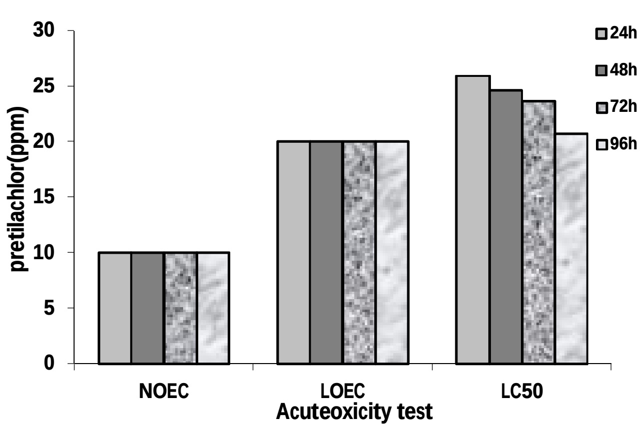

Figure 4. Statistical endpoints of acute toxicity in zebra cichlid Fish exposed to pretilachlor in different times (24h, 48h, 72 h and 96 h respectively).

DISCUSSION

The results of the present study indicate that diazinon, deltamethrin, butachlor and pretilachlor varied in their acute toxicity effects on zebra cichlid. The toxicity of deltamethrin, diazinon, butachlor and pretilachlor on zebra cichlid increased with increasing concentrations and exposure time.

Occurrence of pesticides in high concentrations in agricultural wastewaters and their toxicity to aquatic organisms especially fish species have been reported by many researchers [17]. Contamination of aquatic environment with pesticides via rainfall runoff is quite a possibility [18]. Fishes are sensitive to aquatic contamination and there are serious concerns about their potential adverse effects on human and wildlife populations. In addition we found that diazinon, deltamethrin, butachlor and pretilachlor are lethal substrates to zebra cichlid.

Previous studies indicated the high toxicity of deltamethrin to fish species and our results are in agreement with these reports. Boateng J.O. et al. reported that young fish are more susceptible to toxins and different species respond differently to concentrations of chemicals [19]. Mittal PK, Adak T. and Sharma V. estimated that LC50 of deltamethrin for P. reticulate was 0.016 ppm[20]. Viran R, Erkoc F. and Kocak O. reported LC50 of deltamethrin in guppies as 5.13 mg/L [8]. Mestres R. and Mestres G. found 96-h LC50 values were as follows: Salmo gairdneri, 0.39 mg/L; Cyprinus carpio, 1.84 mg/L; and Sarotherodon mossambica, 3.50 mg/L [21]. LC50 value of deltamethrin in Tilapia, Oreochromis niloticus was reported as15.47 µg/l [19].

Although due to its adsorption by sediments, deltamethrin is thought to be less toxic in field conditions, these data are useful for potential ecosystem risk assessment [8].

Previous studies have used a variety of methods to detect the acute and chronic toxicity of butachlor by preparing various water conditions. This makes comparisons between fish species difficult. For example, the reported 96 h LC50 values for Abramis brama was 1.21 ppm [18], for Leptodactylus magna astacus 0.0019 ppm [13], and 48 h

LC50 values for Hipophthalmichthys molitrix was0.37 ppm [22] which were quite different.

The toxicity of pretilachlor for zebra cichlid (Cryptoheros nigrofasciatus) increased with increasing concentrations and exposure time. The reported methods for detection of acute and chronic toxicity of pretilachlor have been varied too and the 96-h LC50 values for Misgurnus anguillicaudatus have been reported 14.57 ppm (18) and for Lutjanus argentimaculatus 11.86 ppm [14].

The 96-hLC50 values of diazinon in different fishes were reported from tens to several tens of mg l^{-1} [23]. Value of diazinon 96h LC50 was 0.8 mg l^{-1} for guppy (Poecilia reticulata) and for zebra fish (Brachydanio rerio) was 8 mg l^{-1} (24, 25). Different factors have been suggested to cause selective toxicity of diazinon on different fishes: different detoxification systems, variable absorption, and different inhibition of acetyl cholinesterase [24,25, 26].

CONCLUSION

Our results demonstrated that deltamethrin and pretilachlor had the lowest and highest rates of mortality in Zebra Cichlid respectively, and the mortality rate increased with increasing concentrations of toxins with time.

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