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Acute Toxicity Test of Two Pesticides Diazinon and Deltamethrin, on Swordtail Fish (*Xiphophorus helleri*)

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Abstract: Pesticides and chemicals used in agriculture may finally inter aquatic environment and accumulate in the food chain and may cause serious ecological and health problems. The aim of the present study was to investigate acute effects of diazinon and deltamethrin as potential dangerous agricultural chemicals to assess and compare mortality rates of these chemicals on Swordtail Fish (*Xiphophorus helleri*) in the form of LC_{50}. Fish were exposed to different concentrations of diazinon and deltamethrin (between 0-64 ppm for diazinon and 0-8 ppm for deltamethrin) for 96 h in 120 L glass aquaria. Although the observed significant difference between acute toxicity of the two pesticides, a very low $LC_{50\text{-}96h}$ was obtained for deltamethrin (2.87 ppm) and diazinon (14.3 ppm) indicates that deltamethrin and diazinon are highly toxic to *X. helleri*. Further studies are recommended to investigate the effects of these harmful chemicals on fish physiology and histology and their accumulation in fish tissues. Although these chemicals are thought to be less toxic in field conditions due to their adsorption to sediments, these data are useful when assessing potential ecosystem risks.

Key words: Fish · LC_{50} · Diazinon · Deltamethrin · Pollution · Toxicity

INTRODUCTION

In recent years there is a growing concern over accumulation and persistence of pesticides in the aquatic environment comprises a threat to biological life including human beings [1, 2]. Chemical pesticides with persistent molecules (long half-life periods) pose a threat to fish and also to the human population consuming the affected fish.

Presence of pesticide in surface waters was reported in Canada, North America and Europe since 50 years ago and since then many documents have been demonstrated the toxic effects of these pollutants to aquatic environment [3-6]. Organophosphorus pesticides (OPs) are largely used in agriculture and the aquatic environment near to fields Is under influence of OPs such as diazinon [O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimiinyl) phosphorothioate] [5].

Diazinon is a contact organophosphorus pesticide extensively used in agriculture and possesses moderately persistence constitution [7,8]. The toxicity of diazinon is due to blocking of acetyl cholinesterase (AChE) activity, which causes deleterious impacts on non-target aquatic species close to agricultural fields [8].

The pyrethroids including deltamethrin are widely used as pediculicides and are among the most potent insecticides known [9, 10]. Pyrethroids have been proved to be extremely toxic to fish and some aquatic arthropods, for example shrimp [10-12]. The toxicity of Pyrethroids on mammals, birds and amphibians have been reviewed by Bradbury and Coats [11].

Acute toxicity of a pesticide refers to the chemical's ability to cause injury to an animal from a single exposure, generally of short duration. The acute toxicity tests of pesticides to fish has been widely used to acquire rapid estimates of the concentrations that cause direct, irreversible harm to test organisms [13].

Swordtail (*Xiphophorus helleri*) is a small, hardy and easily raised species of ovoviparous fish with a short generation time (1-1.5 months), in which it is easy to distinguish females from males, as the secondary sex characteristic of the former is a "sword," a set of lengthened rays at the ventral margin of the caudal fin. These distinct advantages [14] make it an ideal species for toxicological studies on environmental poisons. The present study was performed to investigate the mortality effects and toxicity test of some agricultural

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poisons and also to determine and compare acute toxicity of diazinon and Deltamethrin as potential dangerous organic pesticides to assess mortality effects of these chemicals to the freshwater Swordtail Fish *Xiphophorus Helleri*.

MATERIALS AND METHODS

The selected fish species for present study was *Xiphophorus Helleri*. Lethal experiments were conducted using young Swordtail Fish. Test chambers were glass aquaria. All samples were acclimated for a week in these aquaria before assays with continuous aeration. Water temperature was regulated at 27°C by using heater. Fish were fed twice daily with formulated feed and dead fish were immediately removed to avoid possible water quality deterioration [15].

Nominal concentrations of active ingredient tested were 0, 4, 8, 16, 32 and 64 ppm for diazinon and 0, 0.4, 1, 2, 4 and 8 ppm for Deltamethrin. Groups of seven Swordtail Fish were exposed for 96h in aerated glass aquaria with 120 L of test medium. During acute toxicity experiment the temperature was 27°C. Feeding was not provided to the specimens during the assay and test media was not renewed. Mortality rates were recorded at time 0, 24, 48, 72 and 96 h. Acute toxicity tests was carried out according to Hotos and Vlahos [16]. The nominal concentration of diazinon and deltamethrin estimated to result in 50% mortality of Swordtail Fish within 24 h (24-h LC_{50}), 48 h, 72 h and 96 h were attained by probit analysis by Finney's (1971) method [17] and using the maximum-likelihood procedure (SPSS 2002, SPSS Inc., Chicago, Illinois, USA). The LC_{50} value is obtained by fitting a regression equation arithmetically and also by graphical interpolation by taking logarithms of the diazinon and dentinol concentrations versus probit value of percentage mortality.

The 95% confidence limits for LC_{50} are estimated by using the formula LC_{50} (95% CL) = LC_{50}±1.96 [SE (LC_{50})]. The SE of LC_{50} is calculated from the formula:

$$SE(LC50) = 1/b\sqrt{pnw}$$

Where: b=the slope of the chemical/probit response (regression) line; p=the number of chemical used, n = the number of animals in each group, w = the average weight of the observations [16]. After the acute toxicity test, the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) were determined for each measured endpoint.

RESULTS

There was no recorded mortality in fish during the acclimation period before exposure and in control group during acute toxicity tests. No fish died during the acclimation period before exposure and no control fish died during acute toxicity tests. The mortality of Swordtail Fish for diazinon and deltamethrin doses was examined during the exposure times at 24, 48, 72 and 96 h (Table 1, 2). There was no recorded mortality in fish during the acclimation period before exposure and in control group during acute toxicity tests. For diazinon there was 100% mortality at 64 ppm within the 96h after exposure (table 1). For deltamethrin 100% mortality was observed at 4 ppm within few hours after exposure (Table2).

Median lethal concentrations of 10%, 30%, 50%, 70% and 90% tests are presented in Table 3. Because mortality (or survival) data are collected for each exposure

Table 1: Cumulative mortality of Swordtail Fish (n=7, each concentration) exposed to acute diazinon

	No. of mortality			
Concentration (ppm)	24h	48h	72h	96h
0.00	0	0	0	0
4.00	0	1	1	1
8.00	0	0	1	1
16.0	1	1	2	4
32.0	0	1	3	7
64.0	4	7	7	7

Table 2: Cumulative mortality of Swordtail Fish (n=7, each concentration) exposed to acute Deltamethrin

	No. of mortality			
Concentration (ppm)	24h	48h	72h	96h
0.00	0	0	0	0
0.40	0	0	0	0
1.00	0	0	0	0
2.00	0	0	0	0
4.00	7	7	7	7
8.00	7	7	7	7

Table 3: Lethal Concentrations (LC_{1-99}) of diazinon (mean±standard error) depending on time (24-96h) for Swordtail Fish

	Concentration (ppm) (95 % of confidence limits)			
Point	24h	48h	72h	96h
LC_1	3.81±0.62	-	-	-
LC_{10}	29.7±0.62	15.3±0.47	1.10±0.33	5.25±0.56
LC_{30}	48.6±0.62	28.9±0.47	25.1±0.33	10.6±0.56
LC_{50}	61.6±0.62	38.3±0.47	41.7±0.33	14.3±0.56
LC_{70}	74.7±0.62	47.7±0.47	58.3±0.33	18.0±0.56
LC_{90}	93.5±0.62	61.3±0.47	82.3±0.33	23.4±0.56
LC_{99}	119.±0.62	80.1±0.47	115.±0.33	30.8±0.56

Table 4: Lethal Concentrations (LC_{1-99}) of Deltamethrin (mean±standard error) depending on time (24-96h) for Swordtail Fish

	Concentration (ppm) (95 % of confidence limits)			
Point	24h	48h	72h	96h
LC_1	2.03±0.17	2.03±0.17	2.03±0.17	2.03±0.17
LC_{10}	2.41±0.17	2.41±0.17	2.41±0.17	2.41±0.17
LC_{30}	2.68±0.17	2.68±0.17	2.68±0.17	2.68±0.17
LC_{50}	2.87±0.17	2.87±0.17	2.87±0.17	2.87±0.17
LC_{70}	3.06±0.17	3.06±0.17	3.06±0.17	3.06±0.17
LC_{90}	3.33±0.17	3.33±0.17	3.33±0.17	3.33±0.17
LC_{99}	3.70±0.17	3.70±0.17	3.70±0.17	3.70±0.17

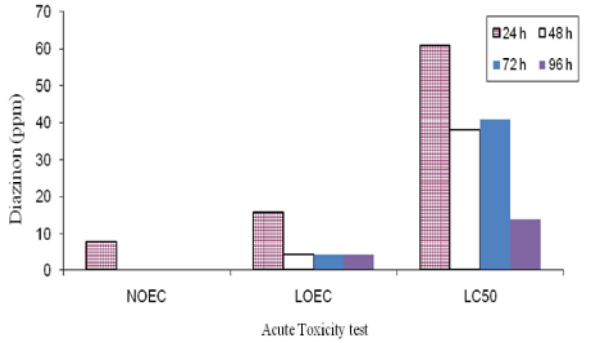

Fig 1: Acute toxicity testing statistical endpoints in Swordtail Fish exposed to crude Diazinon in different times (24h, 48h, 72 h and 96 h respectively)

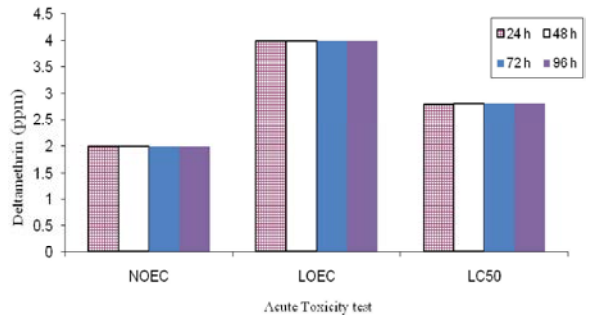

Fig 2: Acute toxicity testing statistical endpoints in Swordtail Fish exposed to crude Deltamethrin in different times (24h, 48h, 72 h and 96 h respectively)

concentration in a toxicity test at various exposure durations (24, 48, 72, or 96 hours), data can be plotted in other ways; the straight line of best fit is then drawn through the points. These are time-mortality lines.

Toxicity Testing Statistical Endpoints are in tow part: 1- Hypothesis Testing: is there a statistically significant difference between the mean response in the treatments and mean response in control or reference sample? LOEC: Lowest Observed Effect Concentration; NOEC: No Observed Effect Concentration. 2- Point Estimates: what toxicant concentration will cause a specific effect on the test population? LC_{50}: the median Lethal Concentration. Our result for Toxicity Testing Statistical Endpoints is in Fig 1-2.

DISCUSSION

The obtained results of the present study indicate that both chemicals diazinon and deltamethrin varied in their acute toxicity to Swordtail Fish (*Xiphophorus helleri)*. The toxicity of deltamethrin and diazinon on young Swordtail fish increased with increasing concentration 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 [4, 6, 8]. Contamination of aquatic environment with pesticides via rainfall runoff is very possible [18]. Fishes are sensitive to aquatic contamination, although the sensitivity of *Pangasius* spp. found to be lower than other species [1]. Serious concerns remains due to their potential to cause adverse effects on human and wildlife populations. In addition we found that both diazinon and deltamethrin are lethal substrates to *P. Hypop hthalmus*.The 96h LC_{50} was calculated to be 2.52±0.77ppm for diazinon and 0.10±2.71 ppm for deltamethrin and here we report deltamethrin to be highly toxic to fish.

The 96h LC_{50} values of diazinon on different fishes reported from tenths to several tens of mg l^{-1} [19, 20]. Value of diazinon 96h LC_{50} was 0.8 mg l^{-1} for guppy (*Poecilia reticulata*) but for zebra fish (*Brachydanio rerio)* was 8 mg l^{-1} [20,21]. Different factor have been suggested to cause selective toxicity of diazinon on different fishes: different detoxification, absorption and different inhibition of acetylcholinesterase [20, 22].

Previous studies indicate the high toxicity of deltamethrin to fish species and our results are in good agreement with these reports. Boateng *et al*. [23] reported that young fish are more susceptible and different species respond unlike to concentrations of chemicals: Mittal *et al*. [24] estimated deltamethrin toxicity to *P. reticulate* to be LC_{50}=0.016 ppm. Viran *et al*. [10] reported LC_{50} value of deltamethrin in guppies as 5.13 mg/l. Mestres and Mestres [25] found 96-h fish LC_{50} values as follows: *Salmo gairdneri*, 0.39 mg/l; *Cyprinus carpio*, 1.84 mg/l; and *Sarotherodon mossambica*, 3.50 mg/l. LC_{50} value of deltamethrin in Tilapia, *Oreochromis niloticus* as15.47 µg/l was reported by Boateng *et al*. [23].

Although deltamethrin is thought to be less toxic in field conditions due to its adsorption to sediments, these data are useful to assessment of potential ecosystem risks [10].

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