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Toxicity response of *Poecilia reticulata* Peters 1859 (Cyprinodontiformes: Poeciliidae) to some agricultural pesticides

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Abstract. *Hedayati A, Tarkhani R, Shadi A. 2012. Mortality response of* Poecilia reticulata *Peters 1859 (Cyprinodontiformes: Poeciliidae) to some agricultural pesticides. Nusantara Bioscience 4: 6-10.* This research was performed 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 guppy *Poecilia reticulata*. LC_{50} of 24 h, 48 h, 72 h, and 96 h was attained by probit analysis by Finney's and using the maximum-likelihood procedure (SPSS). The 24-96 h LC_{50} for the diazinon were 40.9±0.98, 33.2±0.84, 23.2±0.74 and 16.8±0.57 ppm respectively. The 24-96 h LC_{50} for the deltamethrin were 0.297±0.13, 0.236±0.16, 0.204±0.47 and 0.195±0.06 ppm respectively. In the present study, LC_{50} values indicated that deltamethrin was more toxic than diazinon to this species. LC_{50} values obtained in the present study were different from the corresponding values that have been published in the literature for other species of fish..

Key words: fish, LC_{50}, diazinon,deltamethrin, pollution, toxicity, guppy.

Abstrak. *Hedayati A, Tarkhani R, Shadi A. 2012. Respon kematian* Poecilia reticulata *Peters 1859 (Cyprinodontiformes: Poeciliidae) terhadap beberapa pestisida pertanian. Nusantara Bioscience 4: 6-10.* Penelitian ini dilakukan untuk menentukan dan membandingkan toksisitas akut diazinon dan deltametrin sebagai pestisida organik dengan potensi berbahaya untuk menilai efek kematian dari bahan-bahan kimia ini pada guppy air tawar *Poecilia reticulata*. LC_{50} 24 jam, 48 jam, 72 jam dan 96 jam dilakukan dengan analisis probit Finney dan menggunakan prosedur maximum-likelihood (SPSS). Nilai LC_{50} 24-96 jam untuk diazinon adalah 40,9±0,98, 0,84±33,2, 23,2±0,74 dan 16,8±0,57 ppm. LC_{50} 24-96 jam untuk deltametrin adalah 0.297±0,13, 0,236±0,16, 0,204±0,47 dan 0,195±0,06 ppm. Dalam penelitian ini, nilai LC_{50} menunjukkan bahwa deltametrin lebih beracun dari diazinon untuk spesies ini. LC_{50} yang diperoleh dalam penelitian ini menunjukkan hasil yang berbeda dibandingkan dengan nilai LC_{50} pada spesies ikan lainnya.

Kata kunci: ikan, LC_{50}, diazinon, deltametrin, polusi, toksisitas, guppy.

INTRODUCTION

Increased use of pesticides results in contamination of natural ecosystems especially the aquatic ecosystem (Stalin et al. 2008). 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 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 (Tinoco-Ojanguren and Halperin 1998; Capel et al. 2001; Miller et al. 2002; Galloway and Handy 2003). Organo-phosphorus pesticides (OPs) are largely used in agriculture, and the aquatic environment near the fields is under influence of OPs such as diazinon [O,O-diethyl O- (2-isopropyl-4-methyl-6-pyrimiinyl) phosphorothioate] (Tinoco-Ojanguren and Halperin 1998).

Diazinon is a contact organophosphorus pesticide extensively used in agriculture and possesses moderately persistence constitution (Larkin and Tjeerdema 2000; Bazrafshan 2007). The toxicity of diazinon is due to the blocking of acetyl cholinesterase (AChE) activity, which causes deleterious impacts on non-target aquatic species close to agricultural fields (Larkin and Tjeerdema 2000).

The pyrethroids including deltamethrin are widely used as pediculicides and are among the most potent insecticides known (Smith and Stratton 1986; Viran et al. 2003). Pyrethroids have been proved to be extremely toxic to fish and some aquatic arthropods, for example shrimp (Bradbury and Coats 1989; Srivastav 1997; Viran et al. 2003). The toxicity of pyrethroids on mammals, birds and amphibians have been reviewed by Bradbury and Coats (1989).

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 have been widely used to acquire rapid estimates of the concentrations that cause direct, irreversible harm to tested organisms (Parrish 1995; Pandey et al. 2005).

The acute toxicity data can provide useful information to identify the mode of action of a substance and also help to do comparison of dose response among different chemicals. The 96 h LC_{50} tests are conducted to measure the vulnerability and survival potential of organisms to particular toxic chemicals. Substances with lower LC_{50} values are more toxic because lower concentrations results 50% of mortality in organisms.

Guppies are from common fresh water fishes which are capable of tolerating a wide range of fluctuations in water quality and are good model fish for ecotoxicological studies. The present study was performed 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 guppy *Poecilia reticulata*.

MATERIALS AND METHODS

Healthy, unsexed *P. reticulata* (guppy) were selected for the present study (Figure 1). Lethal experiments were conducted using 70 healthy guppy. Test chambers were glass aquaria of 120l. All samples were acclimated for a week in these aquaria before assays with continuous aeration. Water temperature was maintained at 27^{o}C by using a heater. Fish were feed twice daily with formulated feed and dead fish were immediately removed to avoid possible water quality deterioration (Gooley et al. 2000).

Nominal concentrations of active ingredient tested were 0, 5, 15, 30, and 50 ppm of commercial dose (60%) for diazinon and 0, 0.03, 0.04, 0.06, 0.10, 0.20, 0.30 and 0.40 ppm of commercial dose (2.5%) for deltamethrin. Groups of seven guppies were exposed for 96 h in aerated glass aquaria with 120l of test medium. During acute toxicity experiment, the water in each aquarium was aerated and the temperature was 27^{o}C. No food was provided to the specimens during the assay and test media were not renewed. Mortality rates were recorded at 0, 24, 48, 72 and 96 h. Acute toxicity tests were carried out according to Hotos and Vlahos (1998). The nominal concentration of diazinon and deltamethrin estimated to result in 50% mortality of guppy within 24 h (24-h LC_{50}), 48 h, 72 h, and 96 h was attained by probit analysis by Finney's (1971) method (Finney 1971) and using the maximum-likelihood procedure (SPSS 2002). The LC_{50} value is obtained by fitting a regression equation arithmically 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} was estimated by using the formula LC_{50} (95% CL) = LC_{50}±1.96 [SE (LC_{50})]. The SE of LC_{50} was 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 (Hotos and Vlahos 1998). After the acute toxicity test, the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) were determined for each measured endpoint.

RESULTS AND DISCUSSION

A number of fish died during the acclimation period before exposure, and no control fish died during acute toxicity tests. The mortality of *P.reticulata* for diazinon doses, 5, 15, 30, and 50ppm for diazinon and 0.03, 0.04, 0.06, 0.10, 0.20, 0.30 and 0.40 ppm for deltamethrin were examined during the exposure times at 24, 48, 72 and 96 h (Table 1 and 2). Significantly increased mortality of *P.reticulata* was observed with increasing concentrations from 2 ppm to higher concentrations.

For diazinon there was 100% mortality at 30 and higher concentrations within the 96 h, whereas 100% mortality for 0.30 ppm deltamethrin was 72 h and for 0.40 ppm were 48 h after exposure (Table 2).

Table 1. Cumulative mortality of Guppy Fish (n=21, each concentration) exposed to acute commercial diazinon.

Concentration (ppm)	No. of mortality 24 h	48 h	72 h	96 h
0	0	0	0	0
5	0	0	0	0
15	0	0	5	6
30	6	11	16	21
50	15	18	21	21

Table 2. Cumulative mortality of Guppy Fish (n=21, each concentration) exposed to acute commercial deltamethrin.

Concentration (ppm)	No. of mortality 24 h	48 h	72 h	96 h
0.00	0	0	0	0
0.03	0	0	0	0
0.04	0	0	0	0
0.06	0	0	0	0
0.10	0	0	0	0
0.20	0	6	10	12
0.30	15	19	20	21
0.40	18	20	21	21

Median lethal concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% tests were presented in Table 3. Because mortality (or survival) data are collected for each exposure 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. The LT_{50} (median lethal survival time) can be estimated for each concentration.

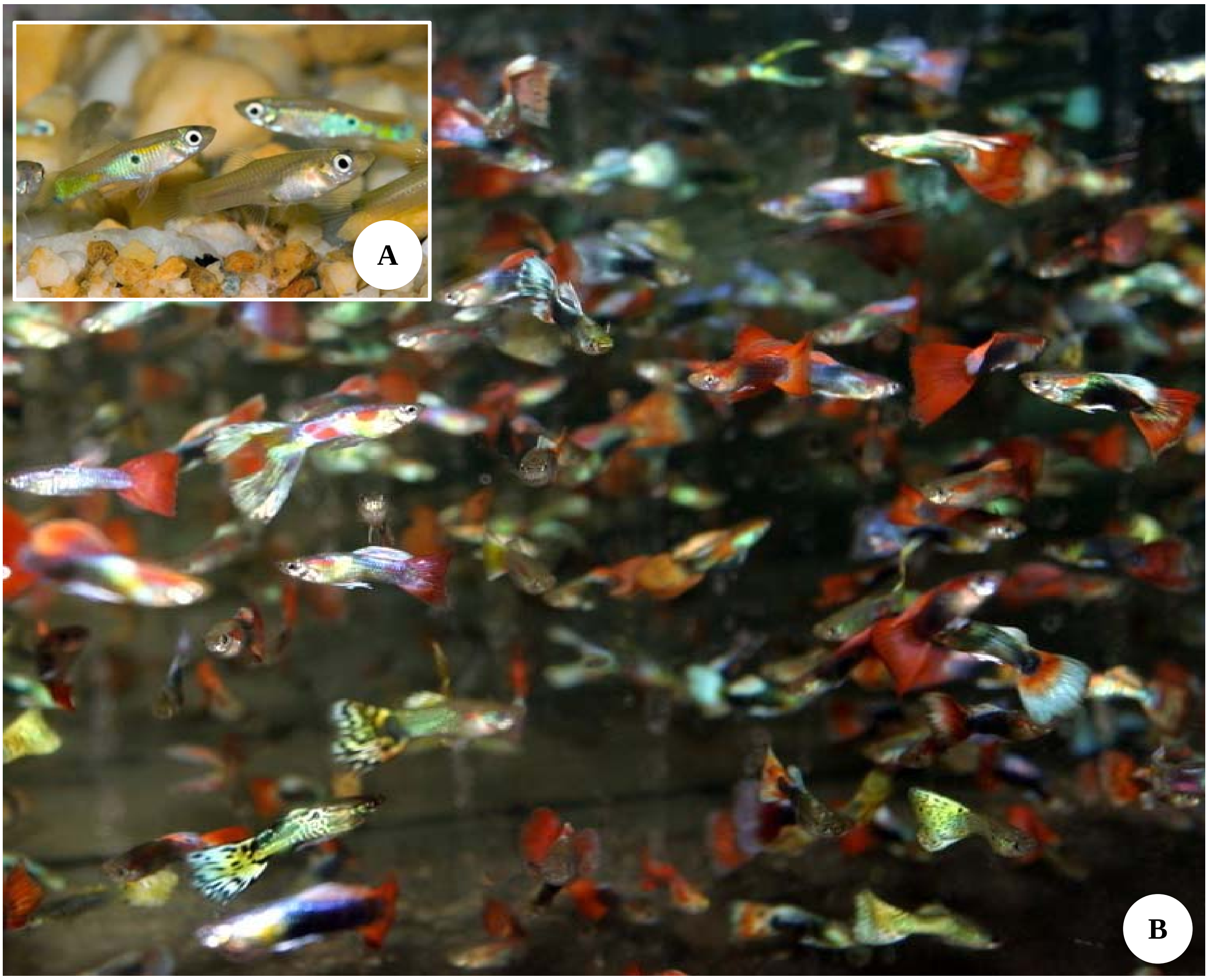

Figure 1.A. Wild-common guppy used in this research, B. Clumps of various ornamental guppies (*Poecilia reticulata*). (photo: from several sources)

Table 3. Lethal Concentrations (LC_{1-99}) of commercial dose diazinon (mean±Standard Error) depending on time (24-96 h) for Guppy.

Point	Concentration (ppm) (95% of confidence limits) 24 h	48 h	72 h	96 h
LC_1	9.97±0.98	5.92±0.84	1.07±0.74	9.06±0.57
LC_{10}	23.8±0.98	18.1±0.84	11.0±0.74	12.5±0.57
LC_{20}	29.7±0.98	23.3±0.84	15.1±0.74	14.0±0.57
LC_{30}	33.9±0.98	27.0±0.84	18.2±0.74	15.1±0.57
LC_{40}	37.5±0.98	30.2±0.84	20.7±0.74	16.0±0.57
LC_{50}	**40.9±0.98**	**33.2±0.84**	**23.2±0.74**	**16.8±0.57**
LC_{60}	44.3±0.98	36.2±0.84	25.6±0.74	17.7±0.57
LC_{70}	47.9±0.98	39.4±0.84	28.1±0.74	18.6±0.57
LC_{80}	52.1±0.98	43.1±0.84	31.2±0.74	19.6±0.57
LC_{90}	57.9±0.98	48.2±0.84	35.4±0.74	21.1±0.57
LC_{99}	71.8±0.98	60.5±0.84	45.3±0.74	24.6±0.57

Table 4. Lethal Concentrations (LC_{1-99}) of commercial dose deltamethrin (mean±Standard Error) depending on time (24-96 h) for Guppy fish.

Point	Concentration (ppm) (95 % of confidence limits) 24 h	48 h	72 h	96 h
LC_1	0.141±0.13	0.107±0.16	0.151±0.47	0.142±0.06
LC_{10}	0.211±0.13	0.165±0.16	0.174±0.47	0.166±0.06
LC_{20}	0.241±0.13	0.189±0.16	0.184±0.47	0.176±0.06
LC_{30}	0.262±0.13	0.207±0.16	0.192±0.47	0.183±0.06
LC_{40}	0.280±0.13	0.222±0.16	0.198±0.47	0.190±0.06
LC_{50}	**0.297±0.13**	**0.236±0.16**	**0.204±0.47**	**0.195±0.06**
LC_{60}	0.314±0.13	0.250±0.16	0.209±0.47	0.201±0.06
LC_{70}	0.333±0.13	0.265±0.16	0.216±0.47	0.207±0.06
LC_{80}	0.354±0.13	0.282±0.16	0.223±0.47	0.215±0.06
LC_{90}	0.384±0.13	0.307±0.16	0.233±0.47	0.225±0.06
LC_{99}	0.454±0.13	0.364±0.16	0.257±0.47	0.248±0.06

Toxicity Testing Statistical Endpoints are in two parts: 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 Figure 2 and 3.

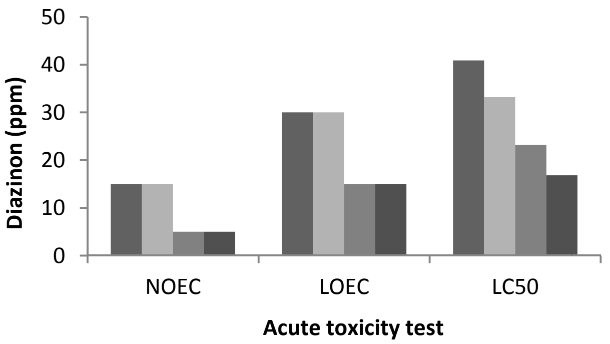

Figure 2. Acute toxicity testing statistical endpoints in Guppy Fish exposed to crude Diazinon in different times (24 h, 48 h, 72 h and 96 h respectively).

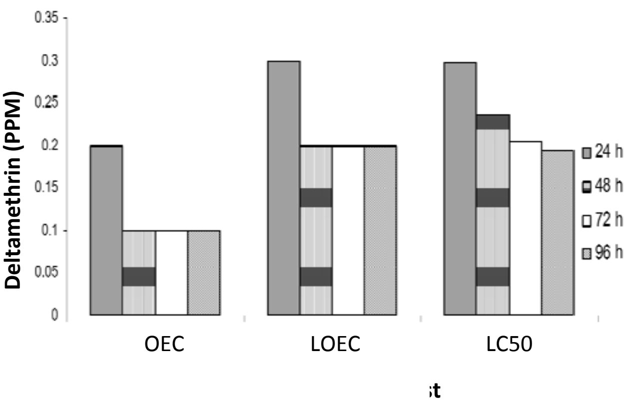

Figure 3. Acute toxicity testing statistical endpoints in Guppy Fish exposed to crude deltamethrin in different times (24 h, 48 h, 72 h and 96 h respectively).

Discussion

The results of present study indicate that both chemicals diazinon and deltamethrin varied in their acute toxicity to *P. reticulata***.** The toxicity of deltamethrin and diazinon on *P. reticulata* 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 (Larkin and Tjeerdema 2000; Capel et al. 2001; Galloway and Handy 2003). Contamination of aquatic environment with pesticides via rainfall runoff is very possible (Willis and McDowell 1982). Fishes are sensitive to aquatic contamination,and 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.reticulata*. The 96 h LC_{50} was calculated to be 16.8±0.57ppm for commercial dose diazinon and 0.195±0.06 ppm for deltamethrin and here we report deltamethrin to be highly toxic to fish.

The 96 h LC_{50} values of diazinon on different fishes reported from tenths to several tens of mg/L (Tsuda et al. 1997; Adedeji et al. 2008). Value of diazinon 96 h LC_{50} was 0.8 mg/L for guppy (*Poecilia reticulata*) but for zebra fish (*Brachydanio rerio)* was 8 mg/L. Different factor have been suggested to cause selective toxicity of diazinon on different fishes: different detoxification, absorption and different inhibition of acetylcholinesterase (Adedeji et al. 2008; Oh et al. 1991).

Previous studies indicate the high toxicity of deltamethrin to fish species and our results are in good agreement with these reports. Boateng et al. (2006) reported that young fish are more susceptible, and different species respond differently to concentrations of chemicals: Mittal et al. (1994) estimated deltamethrin toxicity to *P. reticulate* to be LC_{50} = 0.016 ppm. Viran et al. (2003) report LC_{50} value of deltamethrin in guppies as 5.13 mg/L. Mestres and Mestres (1992) 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 (Mestres and Mestres 1992). LC_{50} value of deltamethrin in tilapia, *Oreochromis niloticus* as15.47 μg/L was reported by Boateng et al. (2006). 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 (Viran et al. 2003).

CONCLUSION

LC_{50} values indicated that deltamethrin is more toxic than diazinon to this species. LC_{50} obtained in the present study were different from the corresponding values that have been published in the literature for other species of fish.. Although the LC_{50} under a defined set of environmental conditions can provide useful information, the numeric value could not be used in the field, so subsequently we used some biomarkers for better understanding of agricultural pesticides toxicity.

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