Investigation of LC50, NOEC and LOEC of Glyphosate, Deltamethrin and Pretilachlor in Guppies (*Poecilia Reticulata*)

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ABSTRACT

Background: The presence of pesticides is very prevalent in surface waters of Iran. These toxic substances may accumulate in the food chain and cause serious ecological and health problems. The aim of this study was to determine the acute toxicity of glyphosate, deltamethrin and pretilachlor as potential dangerous organic pesticides to assess lethal effects of these chemicals agents to the Guppy (*Poecilia reticulata*).

Methods: Fish samples were exposed to different concentrations of glyphosate (41%) (0, 2, 5, 10, 15 and 25 ppm), deltamethrin (2.5%) (0, 0.01, 0.02, 0.04, 0.10 and 0.30 ppm) and pretilachlor (50%) (0, 2, 4, 8, 16 and 32 ppm) for 96 h and cumulative mortality of the guppies was calculated with 24 h intervals.

Results: $LC_{50\text{-}96h}$ was 12.01±1.00, 0.08±0.47 and 8.24±0.42 for glyphosate, deltamethrin and pretilachlor respectively. The very low LC_{50} obtained for glyphosate (12.01±1.00 ppm), deltamethrin (0.08±0.47 ppm) and pretilachlor (8.24±0.42 ppm) indicate that glyphosate, deltamethrin and pretilachlor are highly toxic to guppies.

Conclusion: Our results demonstrated that deltamethrin and glyphosate had the lowest and highest rate of mortality on the guppy respectively.

Keywords: Deltamethrin, Glyphosate, Guppy, LC_{50}, Pretilachlor.

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INTRODUCTION

There is growing concern over aquatic pollution because of its detrimental effects on biological life including human beings. Increased use of pesticides results in contamination of natural ecosystems especially the aquatic systems [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 60 years ago, and since then many documents have proved the toxic effects of these pollutants on aquatic environments [2, 3].

Deltamethrin is a pyrethroid insecticide. Pyrethroids are synthetic compounds that mimic the pyrethrins that are isolated from chrysanthemum flowers. Deltamethrin is a broad-spectrum insecticide that works by interfering with nerve cells' ability to send normal signals by jamming tiny gates on the cells that open and close rapidly to carry the message. Deltamethrin can be found in a wide variety of products used in agriculture, gardens, and lawns, indoors, and even in pets [4, 5].

Glyphosate is a non-selective herbicide registered for use on many food and non-food crops as well as non-crop areas where total vegetation control is desired. Applied at lower levels, it serves as a plant growth regulator [6].

Acute toxicity of pesticides refers to the chemical’s ability to cause damage to animals from a single exposure, generally of short duration. Many workers have used the acute toxicity tests of pesticides on fish to acquire rapid estimates of the concentrations that causes direct, irreversible harm to test organisms [7, 8].

Pretilachlor is a systemic herbicide belonging to chloroacetamide Name: 2-chloro-2', 6'-diethyl-N-(2-propoxyethyl)-acetanilide [9, 10]. Since herbicides halt cell division via preventing the synthesis of long chain fatty acids in different plant cells, destruction of plant and algae in the contaminated water environments

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damage directly the food chain and fauna of the habitats. In addition to the toxic effects on plants, this herbicide is poisonous for animal, including fishes [11, 12].

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

MATERIALS AND METHODS

The selected fish species for present study was guppy. Test chambers were glass aquaria of 120 L. All fish were acclimated for a week in these aquaria before assays with continuous aeration of water and the temperature was regulated at 25^{0}C by aquarium heaters. The fish were fed twice a day with formulated feed and the dead ones were immediately removed to avoid possible water deterioration [13].

Nominal concentrations of active ingredients tested were 0, 2, 5, 10, 15 and 25 ppm of commercial (41%) glyphosate and 0, 0.01, 0.02, 0.04, 0.10 and 0.30 ppm of commercial (2.5%) deltamethrin and 0, 2, 4, 8, 16 and 32 ppm of commercial (50%) pretilachlor. In total 15 groups (5 for glyphosate, 5 for deltamethrin and 5 for pretilachlor) of seven guppies were exposed for 96h in aerated glass aquaria with 120 L of test medium. During acute toxicity experiment, the water in each aquarium was aerated and the temperature was 25^{0}C. 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 h. Acute toxicity tests were carried out according to Hotos GN et al. [14]. The nominal concentration of glyphosate, deltamethrin and pretilachlor 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 method and using the maximum-likelihood procedure (SPSS 2002, SPSS Inc., Chicago, Illinois, USA). The LC_{50} value was obtained by fitting a regression equation arithmically and also by graphical interpolation by taking logarithms of the glyphosate, deltamethrin 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 [14].

RESULTS

No fish died during the acclimation period before exposure, and no control fish died during acute toxicity tests. The mortality of guppies for glyphosate doses 0, 2, 5, 10, 15 and 25 ppm and 0, 0.01, 0.02, 0.04, 0.10 and 0.30 ppm for deltamethrin and 0, 2, 4, 8, 16 and 32 ppm for pretilachlor were examined during the exposure times at 24, 48, 72 and 96 h (table 1, 2, 3). The mortality of guppies increased significantly with increasing concentrations from 10 ppm to higher concentrations for glyphosate and 0.04 ppm to higher concentrations for deltamethrin and 4 ppm to higher concentrations for pretilachlor.

Median lethal concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% test are presented in tables 4, 5 and 6. Because the mortality (or survival) data were collected for each exposure concentration in a toxicity test at various exposure durations (24, 48, 72, or 96 hours), data could be plotted in other ways; the straight line of best fit was then drawn through the points. These are the time–mortality lines. The LT_{50} (median lethal survival time) could be estimated for each concentration.

Table 1. Cumulative mortality of guppy fish (n=7 each concentration) exposed to acute Glyphosate.

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
2	0	0	0	0
5	0	0	0	0
10	1	2	2	3
15	3	5	5	5
25	7	7	7	7

Table 2. Cumulative mortality of guppy fish (n=7 each concentration) exposed to acute Deltamethrin.

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
0.01	0	0	0	0
0.02	0	0	0	0
0.04	1	1	2	3
0.10	3	3	3	4
0.30	7	7	7	7

Table 3. Cumulative mortality of guppy fish (n=7 each concentration) exposed to acute Pretilachlor.

Concentration (ppm)	No. of mortality			
	24h	48h	72h	96h
Control	0	0	0	0
2	0	0	0	0
4	0	1	2	3
8	2	3	4	4
16	4	5	5	6
32	7	7	7	7

Table 4. Lethal Concentrations (LC_{10-99}) of glyphosate depending on time (24-96h) for guppy.

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{10}	9.99±1.27	8.07±1.25	8.05±1.25	7.05±1.00
LC_{20}	11.77±0. 27	9.64±1.25	9.64±1.25	8.75±1.00
LC_{30}	13.06±0. 27	10.77±1.25	10.77±1.25	9.98±1.00
LC_{40}	14.15±0. 27	11.74±1.25	11.74±1.25	11.02±1.00
LC_{50}	**15.18±0. 27**	**12.64±1.25**	**12.64±1.25**	**12.01±1.00**
LC_{60}	16.21±0. 27	13.55±1.25	13.55±1.25	12.99±1.00
LC_{70}	17.30±0. 27	14.51±1.25	14.51±1.25	14.03±1.00
LC_{80}	18.60±0. 27	15.64±1.25	15.64±1.25	15.26±1.00
LC_{90}	20.37±0. 27	17.21±1.25	17.21±1.25	16.97±1.00
LC_{99}	24.60±0. 27	20.94±1.25	20.94±1.25	19.24±1.00

Table 5. Lethal Concentrations (LC_{10-99}) of deltamethrin depending on time (24-96h) for guppy.

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{10}	0.05±0.66	0.05±0.66	0.03±0.51	0.02±0.47
LC_{20}	0.06±0.66	0.07±0.66	0.05±0.51	0.04±0.47
LC_{30}	0.08±0.66	0.08±0.66	0.07±0.51	0.06±0.47
LC_{40}	0.09±0.66	0.09±0.66	0.08±0.51	0.07±0.47
LC_{50}	**0.10±0.66**	**0.10±0.66**	**0.09±0.51**	**0.08±0.47**
LC_{60}	0.11±0.66	0.11±0.66	0.10±0.51	0.09±0.47
LC_{70}	0.12±0.66	0.12±0.66	0.12±0.51	0.10±0.47
LC_{80}	0.13±0.66	0.14±0.66	0.13±0.51	0.11±0.47
LC_{90}	0.15±0.66	0.15±0.66	0.15±0.51	0.13±0.47
LC_{99}	0.19±0.66	0.19±0.66	0.20±0.51	0.18±0.47

Table 6. Lethal Concentrations (LC_{10-99}) of pretilachlor depending on time (24-96h) for guppy.

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{10}	6.69±0.67	3.78±0.50	1.76±0.42	1.25±0.42
LC_{20}	9.20±0.67	6.37±0.50	4.61±0.42	3.65±0.42
LC_{30}	11.01±0.67	8.24±0.50	6.67±0.42	5.38±0.42
LC_{40}	12.56±0.67	9.84±0.50	8.43±0.42	6.86±0.42
LC_{50}	**14.00±0.67**	**11.33±0.50**	**10.08±0.42**	**8.24±0.42**
LC_{60}	15.42±0.67	12.83±0.50	11.72±0.42	9.62±0.42
LC_{70}	16.99±0.67	14.43±0.50	13.48±0.42	11.10±0.42
LC_{80}	18.80±0.67	16.30±0.50	15.54±0.42	12.83±0.42
LC_{90}	21.31±0.67	18.90±0.50	18.40±0.42	15.23±0.42
LC_{99}	27.27±0.67	25.06±0.50	25.18±0.42	20.93±0.42

Toxicity Testing Statistical Endpoints were 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) and NOEC (No Observed Effect Concentration)? 2- Point Estimates: what toxicant concentrations will cause a specific effect on the test population? LC_{50}(the median Lethal Concentration). Acute toxicity testing statistical endpoints of glyphosate, deltamethrin and pretilachlor for guppy during different times after exposure are shown in figures 1, 2 and 3, respectively.

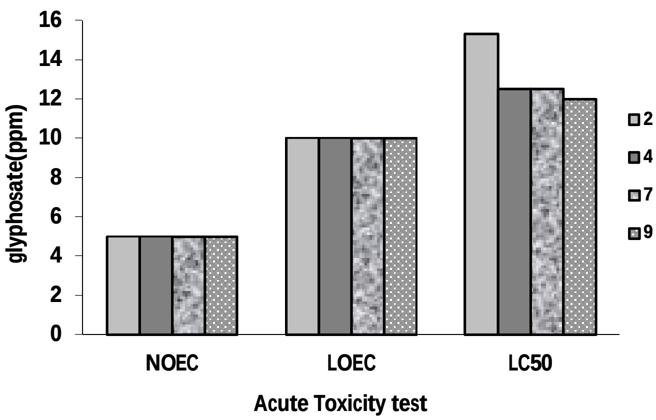

Figure1. Acute toxicity testing statistical endpoints in guppy fish exposed to glyphosate in different times (24h, 48h, 72 h and 96 h respectively).

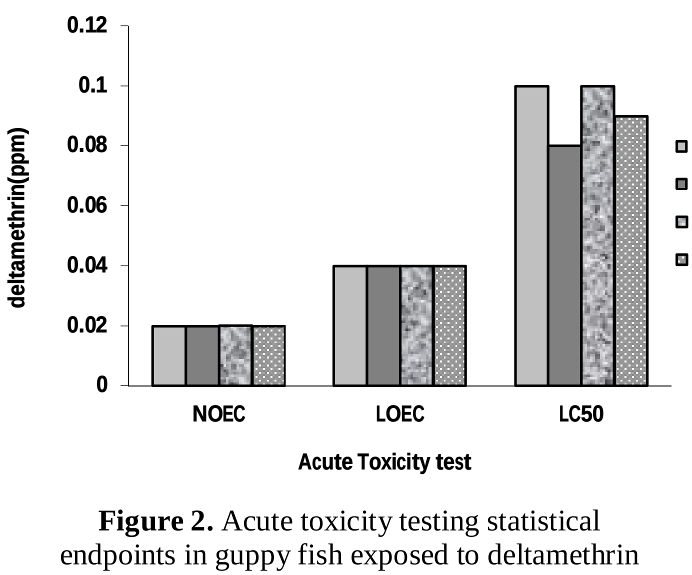

Figure 2. Acute toxicity testing statistical endpoints in guppy fish exposed to deltamethrin in different times (24h, 48h, 72 h and 96 h respectively).

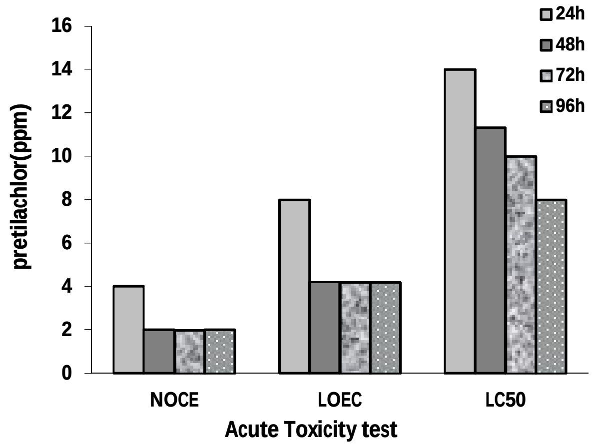

Figure 3. Acute toxicity testing statistical endpoints in guppy fish exposed to pretilachlor in different times (24h, 48h, 72 h and 96 h respectively).

DISCUSSION

Plant based pesticides are developed to replace deleterious chemical pesticides. Even though chemical pesticides are target specific and effective, their impact on the environment is mostly harmful. Plant based pesticides contain active substances with low half-life periods and their effects on the environment are less detrimental [1]. Increased use of chemical pesticides results in the excess inflow of toxic chemicals, mainly into the aquatic ecosystem. The aquatic flora and fauna are affected by the toxic substances which eventually enter their systems or bring about external damages [11]. Several species of fish are susceptible to deleterious effects when exposed to pesticides and other environmental stressors [3].

The results of the present study indicate that glyphosate, deltamethrin and pretilachlor varied in their acute toxicity to guppies. The toxicity of glyphosate, deltamethrin and pretilachlor on guppies increased with increasing concentration and exposure time.

Occurrence of pesticides in high concentrations in agricultural waste waters and their toxicity to aquatic organisms especially fish species have been reported by many researchers [15]. Contamination of aquatic environment with pesticides via rainfall runoffs is very possible [16]. 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 glyphosate, deltamethrin and pretilachlor are lethal substrates for guppies.

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, and different species respond differently to various concentrations of chemicals [17]. Mittal PK, Adak T, and Sharma V. estimated deltamethrin toxicity for *P. reticulate* to be LC_{50}=0.016 ppm. [18]. Larkin DJ and Tjeerdema R. reported LC_{50} value of deltamethrin in *Clarias gariepinus* as 5.13 mg/L [6]. Mestres R and Mestres G. 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 [19]. LC_{50} value of deltamethrin in Tilapia, *Oreochromis niloticus* as15.47 µg/l was reported by Boateng J.O et al. [17].

Although deltamethrin is thought to be less toxic in field conditions due to its adsorption by sediments, these data are useful for potential ecosystem risk assessment [6]. Fishes are sensitive to aquatic contamination and serious concerns remains due to their potential for causing adverse effects on human and wildlife populations.

In our study, the toxicity of pretilachlor on guppy (*Poecilia reticulata*) increased with increasing concentrations and exposure time. We used a variety of methods to detect the acute and chronic toxicity of pretilachlor by preparing various water concentrations. This makes comparisons between fish species difficult. For example, it has been reported that 96 h LC_{50} values for the *Misgurnus anguillicaudatus* is 14.57 ppm [16] and for *Lutjanus argentimaculatus* 11.86 ppm [12].

The $96hLC_{50}$ values of glyphosate on different fishes were reported from ten to several tens of mg l^{-1} [20]. The value of glyphosate 96h LC_{50} is 13.25 mg l^{-1} for Tilapia and for *Cichlasoma dimerus* is 24.30 mg l^{-1} [21]. Different factors have been suggested to cause selective toxicity of glyphosate on different fishes: dissimilar detoxification, absorption and different levels of inhibition of acetyl cholinesterase [22, 23].

CONCLUSION

Our results demonstrate that deltamethrin and glyphosate had the lowest and highest rates of mortality on the guppy respectively; however the rate of mortality was increased with increasing the concentration of toxins with time.

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REFERENCES

1. Stalin SI, Kiruba S, Manohar S. Comparative Study on the Toxicity of a Synthetic Pyrethroid, deltamethrin and a Neem Based Pesticide, Azadirachtin to *Poecilia reticulata* Peters 1859 (Cyprinodontiformes: Poeciliidae) Turkish Journal of Fisheries and Aquatic Sciences. 2008; 8:1-5.
2. Miller G, Sweet L, Adams G, Omann M, Meier P. In vitro toxicity and interactions of environmental contaminants (Arochlor 1254 and mercury) and immunomodulatory agents (lipopolysaccharide and cortisol) on thymocytes from lake trout (*Salvelinus namaycush*). Fish Shellfish Immunol. 2002; 13:11–26.
3. Capel D, Larson J, Winterstein T. The behavior of thirty-nine pesticides in surface waters as a function of scale. Hydrol. Process. 2001; 15: 1251–1269.
4. Tinoco-Ojanguren R, Halperin D. Poverty, production, and health: inhibition of erythrocyte cholinesterase via occupational exposure to organophosphate insecticides in Chiapas, Mexico. Arch. Environ. Health. 1998; 3: 29–35.
5. Bazrafshan ES, Naseri A, Mahvi M. Performance evaluation of electrocoagulation process for deltamethrin removal from aqueous environments by using iron electrons, Iranian Journal of Environmental Health Science and Engineering. 2007; 4: 127–132.
6. Larkin DJ, Tjeerdema R. Fate and effects of glyphosate and deltamethrin. Rev. Environ. Contam. Toxicol. 2000; 166: 49–82.
7. Smith TM, Stratton G. Effects of synthetic pyrethroid insecticides on nontarget organisms. Res. Rev. 1986; 97: 93–119.
8. Viran R, Erkoc F, Kocak O. Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). Ecotoxicology and Environmental Safety. 2003; 55: 82–85.
9. Srivastav AK, Srivastava K, Crivastav S. Impact of Pretilachlor on serum calcium and inorganic phosphate of freshwater catfish, *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol. 1997; 59: 841–846.
10. Bradbury SP, Coats R. Comparative toxicology of the pyrethroid insecticides. Rev. Environ. Contamin. Toxicol. 1989; 108: 133–177.

11. Parrish PR. Acute toxicity tests. In Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment, 2nd, ed. G. M. Rand. pp. 947±973.Taylor & Francis, Washington DC. 1995; 145: 210–228.
12. Pandey S, Kumar R, Sharma S, Verma M. Acute toxicity bioassays of mercuric chloride and Pretilachlor on air-breathing fish *Lutjanus argentimaculatus* (Bloch). Ecotoxicology and Environmental Safety. 2005; 61:114–120.
13. Gooley GJ, Gavine F, Dalton W, Bretherton M, Samblebe M. Feasibility of aquaculture in dairy manufacturing wastewater to enhance environmental performance and offset costs. Final Report DRDC Project No. MAF001. Marine and Freshwater Resources Institute, Snobs Creek. 2000; pp. 84.
14. Hotos GN, Vlahos N. Salinity tolerance of *Mugil cephalus* and *Chelon labrosus*, Pisces: Mugilidae/fry in experimental conditions. Aquaculture. 1998; 167: 329–338.
15. Galloway T, Handy R. Immunotoxicity of organophosphorous pesticides. Ecotoxicology. 2003; 12: 345–63.
16. Lima A. Effects of pesticides and 50% pretilachlor on the mortality of *Misgurnus anguillicaudatus*. Aquat. Toxicol. 2000; 40: 150–160.
17. Boateng J.O, Nunoo F, Dankwa E, Ocran M. Acute Toxic Effects of Deltamethrin on Tilapia, *Oreochromis niloticus* (Linnaeus, 1758). West Africa Journal of Applied Ecology. 2006; 9:1-5.
18. Mittal PK, Adak T, Sharma V. Comparative toxicity of certain mosquitocidal compounds to larvivorous fish, *Poecilia reticulata*. Ind. J. Malariol. 1994; 31: 43–47.
19. Mestres R, Mestres G. Deltamethrin: uses and environmental safety. Rev. Environ. Contamin. Toxicol. 1992; 124: 1–18.
20. Tsuda T, Kojima M, Nakajima A, Aoki S. Acute toxicity, accumulation and excretion of organ phosphorus insecticides and their oxidation products in killifish. Chemosphere. 1997; 35: 939-949.
21. Keizer JD, Gostino G, Vittozzi L. The importance of biotransformation in the toxicity of xenobiotics to fish.1.Toxicity and bioaccumulation of glyphosate in tilapia and *Cichlasoma dimerus*. Aquat. Toxicol. 1991; 21: 239-254.
22. Adedeji OB, Adedeji A, Adeyemo A, Agbede S. Acute toxicity of glyphosate to the African catfish (*Clarias gariepinus*). African Journal of Biotechnology. 2008; 7: 651-654.
23. Oh HS, Lee SK, Kim H, Roh K. Mechanism of selective toxicity of glyphosate to killifish (*Oryzias latipes*) and loach (*Misgurnus anguillicaudatus*). Aquat. Toxicol. Risk Assess. 1991; 14: 343-353.