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Toxicity Evaluation and Mortality Responses of *Pangasius hypophthalmus* During Experimental Exposure to Some Agricultural Pesticides

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Abstract: 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 Tra catfish (*Pangasius hypophthalmus*) in the form of LC_{50}. Fish samples (7 fish in each test group) were exposed to different concentrations of diazinon and deltamethrin (between 0-40 ppm for diazinon and 0-2 ppm for deltamethrin) for 96 h in 120 L glass aquaria. Although significant difference was observed between acute toxicity of two chemicals, the very low LC_{50} obtained for deltamethrin (0.10 ppm) and diazinon (11ppm) indicated that deltamethrin and diazinon are highly toxic to *P. hypophthalmus*. Further studies are recommended to investigate the effects of these chemicals on the physiological and histological impacts and their accumulation in vital tissues of fishes. 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

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 pesticides in surface waters were 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 [1-6].

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 known potent insecticides [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 [13,14].

The acute toxicity data can provide useful information to identify the mode of action of a substance and also help to 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.

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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 Tra Catfish *Pangasius hypophthalmus*.

MATERIALS AND METHODS

The selected fish species for present study was *Pangasius hypophthalmus* (Siluroidei, Pangasiidae). Lethal experiments were conducted using 140 young Tra Catfish. 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 regulated at 27°C by using heater. Fish were feed twice daily with formulated feed during the acclimation period only and dead fish were immediately removed to avoid possible water quality deterioration [15].

Nominal concentrations of active ingredient tested were 0, 0.05, 0.1, 0.5, 1, 2, 3, 5, 10, 20 and 40 ppm for diazinon and 0, 0.05, 0.06, 0.07, 0.08, 0.1, 0.5, 1 and 2 ppm for Deltamethrin. Groups of seven Tra catfish were exposed for 96h in aerated glass aquaria with 120L of test medium. During acute toxicity experiment. No food was 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 Tra catfish within 24 h (24-h LC_{50}), 48 h, 72 h, and 96 h was attained by probit analysis by Finney's 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 arithmically and also by graphical interpolation by taking logarithms of the diazinon and deltamethrin concentrations versus probit value of percentage mortality.

The 95% confidence limits for LC_{50} are estimated by using the formula LC_{50} (95% CL) = $LC_{50}\pm1.96$ [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

No fish died during the acclimation period before exposure, and there was no dead fish in control aquaria during acute toxicity tests. The mortality of Tra Catfish for diazinon doses 0, 0.05, 0.1, 0.5, 1, 2, 3, 5 and 10 ppm for diazinon and 0, 0.05, 0.06, 0.07, 0.08, 0.1, 0.5, 1 and 2 ppm for deltamethrin were examined during the exposure times at 24, 48, 72 and 96 h (Table 1,2). Significantly increased mortality of Tra Catfish was observed with increasing concentrations from 2 ppm to higher concentrations.

For diazinon there were 100% mortality at 3 and higher concentrations within the 96h after exposure, and 100% mortality at 10 ppm within the 30 h whereas 100% mortality for 20 ppm was 30h and for 40 ppm were 24h. For deltamethrin 100% mortality was observed at 0.10 ppm within few hours after exposure (Table 2).

Table 1: Cumulative mortality of Tra Catfish (n=7, each concentration) exposed to different concentrations of diazinon

Concentration (ppm)	No. of mortality 24h	48h	72h	96h
0.00	0	0	0	0
0.05	0	0	0	0
0.10	0	0	0	0
0.50	0	0	0	0
1.00	0	0	0	0
2.00	0	0	0	1
3.00	0	0	0	7
5.00	0	0	4	7
10.00	6	7	7	7
20.00	6	7	7	7
40.00	7	7	7	7

Table 2: Cumulative mortality of Tra Catfish (n=7, each concentration) exposed to different concentrations of Deltamethrin

Concentration (ppm)	No. of mortality 24h	48h	72h	96h
0.00	0	0	0	0
0.05	0	0	1	2
0.06	2	2	2	2
0.07	2	4	2	2
0.08	4	4	4	4
0.10	7	7	7	7
0.50	7	7	7	7
1.00	7	7	7	7
2.00	7	7	7	7

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

	Concentration (ppm) (95 % of confidence limits)			
Point	24h	48h	72h	96h
LC_1	0.66±0.04	5.40±0.66	3.20±1.02	1.32±0.77
LC_{10}	5.31±0.04	6.35±0.66	3.94±1.02	1.85±0.77
LC_{20}	7.27±0.04	6.75±0.66	4.25±1.02	2.08±0.77
LC_{30}	8.68±0.04	7.04±0.66	4.48±1.02	2.25±0.77
LC_{40}	9.88±0.04	7.29±0.66	4.67±1.02	2.39±0.77
LC_{50}	11.01±0.04	7.52±0.66	4.85±1.02	2.52±0.77
LC_{60}	12.14±0.04	7.75±0.66	5.03±1.02	2.65±0.77
LC_{70}	13.34±0.04	8.00±0.66	5.22±1.02	2.79±0.77
LC_{80}	14.76±0.04	8.28±0.66	5.45±1.02	2.95±0.77
LC_{90}	16.71±0.04	8.69±0.66	5.76±1.02	3.18±0.77
LC_{99}	21.36±0.04	9.64±0.66	6.50±1.02	3.72±0.77

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

	Concentration (ppm) (95 % of confidence limits)			
Point	24h	48h	72h	96h
LC_1	-	-	-	-
LC_{10}	-	-	-	-
LC_{20}	-	-	-	-
LC_{30}	0.03±2.49	0.03±2.49	0.02±2.60	0.01±2.71
LC_{40}	0.08±2.49	0.08±2.49	0.07±2.60	0.06±2.71
LC_{50}	0.13±2.49	0.13±2.49	0.11±2.60	0.10±2.71
LC_{60}	0.17±2.49	0.17±2.49	0.16±2.60	0.15±2.71
LC_{70}	0.22±2.49	0.22±2.49	0.21±2.60	0.20±2.71
LC_{80}	0.28±2.49	0.28±2.49	0.27±2.60	0.26±2.71
LC_{90}	0.36±2.49	0.36±2.49	0.35±2.60	0.34±2.71
LC_{99}	0.55±2.49	0.55±2.49	0.54±2.60	0.53±2.71

Median lethal concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% test presented in tables 3 and 4. 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.

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 and 2.

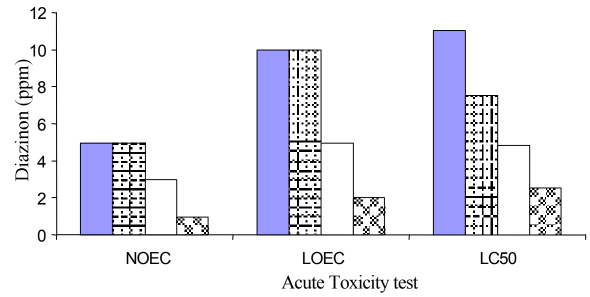

Fig. 1: Acute toxicity testing statistical endpoints in Tra Catfish exposed to crude Diazinon in different times (24h, 48h, 72 h and 96 h respectively)

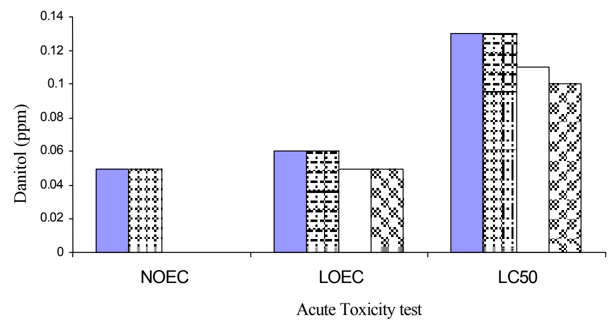

Fig. 2: Acute toxicity testing statistical endpoints in Tra Catfish exposed to crude Deltamethrin in different times (24h, 48h, 72 h and 96 h respectively).

DISCUSSION

The results of present study indicate that both chemicals diazinon and deltamethrin varied in their acute toxicity to Tra catfish (*Pangasius hypophthalmus*). The toxicity of deltamethrin and diazinon on young Tra catfish 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. hypophthalmus* .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] report 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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REFERENCES

1. Faruk, Md. A.R., 2008. Disease and health management of farmed exotic catfish *Panagasius hypopthalmus* in Mymensingh district of Bangladesh, pp. 193-204. In Bondad-Reantaso, M.G., C.V. Mohan, M. Crumlish and R.P. Subasinghe, (eds.). Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, Philippines.
2. Langston, W.J., 1990. Toxic effects of metals and the incidence of metal pollution in marine ecosystems. In: Firness, R.W. and P.S. Rainbow, (Eds.), Heavy Metals in the Marine Environment. CRC Press, Boca Raton, FL, pp: 101-122.
3. Miller, G.G., L.I. Sweet, J.V. Adams, G.M. Omann, D.R. Passino-Reader and P.G. Meier, 2002. 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., 13: 11-26.
4. Galloway, T. and R. Handy, 2003. Immunotoxicity of organophosphorous pesticides. Ecotoxicology, 12: 345-63.
5. Tinoco-Ojanguren, R. and D.C. Halperin, 1998. Poverty, production, and health: inhibition of erythrocyte cholinesterase via occupational exposure to organophosphate insecticides in Chiapas, Mexico. Arch. Environ. Health, 53: 29-35.
6. Capel, P.D., S.J. Larson and T.A. Winterstein, 2001. The behavior of thirty-nine pesticides in surface waters as a function of scale. Hydrol. Process, 15, 1251-1269.
7. Bazrafshan, E.S. Naseri, A.H. Mahvi and M. Shayedhi, 2007. Performance evaluation of electrocoagulation process for diazinon removal from aquaeous environments by using iron electrons, Iranian Journal of Environmental Health Science and Engineering, 4: 127-132.
8. Larkin, D.J. and R.S. Tjeerdema, 2000. Fate and effects of diazinon. Rev. Environ. Contam. Toxicol., 166: 49-82.
9. Smith, T.M. and G.W. Stratton, 1986. Effects of synthetic pyrethroid insecticides on nontarget organisms. Res. Rev., 97: 93-119.
10. Viran R., F.U. Erkoc, H. Polat and O. Kocak, 2003. Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). Ecotoxicology and Environmental Safety, 55: 82-85.
11. Bradbury, S.P. and J.R. Coats, 1989. Comparative toxicology of the pyrethroid insecticides. Rev. Environ. Contamin. Toxicol., 108: 133-177.
12. Srivastav, A.K., S.K. Srivastava and S.K. Srivastav, 1997. Impact of deltamethrin on serum calcium and inorganic phosphate of freshwater catfish, *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol., 59: 841-846.
13. Parrish, P.R. Acute toxicity tests, 1995. In Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment, 2nd ed. G. M. Rand. pp: 947±973. Taylor & Francis, Washington DC.

14. Pandey, S., R. Kumar, S. Sharma, N.S. Nagpure, S.K. Srivastava and M.S. Verma, 2005. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctatus* (Bloch). Ecotoxicology and Environmental Safety, 61: 114-120.
15. Gooley, G.J., F.M. Gavine, W. Dalton, S.S. De Silva, M. Bretherton and M. Samblebe, 2000. 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, pp: 84.
16. Hotos, G.N. and N. Vlahos, 1998. Salinity tolerance of *Mugil cephalus* and *Chelon labrosus*, Pisces: Mugilidae/fry in experimental conditions. Aquaculture, 167: 329-338.
17. Finney, D.J., 1971. Probit Analysis. Univ. Press, Cambridge, pp: 333.
18. Willis, G.H. and L.L. McDowell, 1982. Review: Pesticides in agricultural runoff and their effects on downstream water quality. Environ. Toxicol. Chem., 1: 267-279.
19. Tsuda, T., M. Kojima, H. Harada, A. Nakajima and S. Aoki, 1997. Acute toxicity, accumulation and excretion of organophosphorus insecticides and their oxidation products in killifish. Chemosphere, 35: 939-949.
20. Adedeji, O.B., A. Oadedeji, O.K. Adeyemo and S.A. Agbede, 2008. Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*). African Journal of Biotechnology 7(5): 651-654.
21. Keizer, J.D., G. Gostino and L. Vittozzi, 1991. The importance of biotransformation in the toxicity of xenobiotics to fish. 1. Toxicity and bioaccumulation of diazinon in guppy (*Poecilia reticulata*) and zebra fish (*Brachydanio rerio*). Aquat. Toxicol., 21: 239-254.
22. Oh, H.S., S.K. Lee, Y.H. Kim and J.K. Roh, 1991. Mechanism of selective toxicity of diazinon to killifish (*Oryzias latipes*) and loach (*Misgurnus anguillicaudatus*). Aquat. Toxicol. Risk Assess, 14: 343-353.
23. Boateng, J.O., F.K. Nunoo, E.H.R. Dankwa and M.H. Ocran, 2006. Acute toxic effects of deltamethrin on tilapia, *Oreochromis niloticus* (Linnaeus, 1758). West Africa Journal of Applied Ecology, 9: 1-5.
24. Mittal, P.K., T. Adak and V.P. Sharma, 1994. Comparative toxicity of certain mosquitocidal compounds to larvivorous fish. *Poecilia reticulata*. Ind. J. Malariol., 31(2): 43-47.
25. Mestres, R. and G., 1992. Deltamethrin: uses and environmental safety. Rev. Environ. Contamin. Toxicol., 124: 1-18.