

Acute toxicity of chlorpyrifos, cadusafos and diazinon to three Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) fingerlings

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Abstract

Fingerlings of three Indian major carps, viz. *Catla catla* (Hamilton-Buchanan), *Labeo rohita* (Hamilton-Buchanan) and *Cirrhinus mrigala* (Hamilton-Buchanan), were exposed to different concentrations of chlorpyrifos (lorsban 10 G), cadusafos (rugby 10 G) and diazinon (basudin 10 G) for a period of 96 h with a view to determine the median lethal concentrations (LC₅₀) values for each of chemicals. Of the tested concentrations, chlorpyrifos at a dose of 6.65 ppm, cadusafos at 2.0 ppm and diazinon at a dose of 8.40 ppm or above induced 100% mortalities within 96 h of exposure. The 96 h LC₅₀ values of chlorpyrifos, cadusafos and diazinon were 1.66, 0.72 and 2.10 ppm for *C. catla*, 2.35, 0.72 and 2.97 for *L. rohita* and 2.35, 0.72 and 2.10 ppm for *C. mrigala*, respectively. Pesticide induced behavioural abnormalities observed in the present study included erratic movements, rapid operculum activities, jumping of fish out of the test media, violent spasm and convulsion.

Key words: Toxicity, Chlorpyrifos, Cadusafos, Diazinon, Indian major carp

Introduction

Fish is the most accessible animal protein for majority of the population. Therefore, it is vital that the aquatic environment be used in a sustainable manner, and that the resource base is not destroyed. Unfortunately, presently, fish habitat of Bangladesh is jeopardized by pollution from different sources. Pesticides are one of the notorious causes of environmental pollution because the used pesticides drain off into open waterbodies through rainfall and floods and as a result the aquatic environment obviously gets polluted.

The organophosphorus pesticides are generally much more acutely toxic to vertebrates and are non-persistent. It is the latter quality that brought them onto the agricultural scene to gradually replace the persistent organochlorine, particularly the DDT. About 280 pesticides have so far been registered in Bangladesh for marketing and of them, 80% are organophosphorus.

Catla (*Catla catla*), rui (*Labeo rohita*) and (*Cirrhinus mrigala*) are the common fishes of south-east Asian countries. Carps use paddy fields as their nursing and feeding grounds.

Pesticides effects on fish mortality were observed by Kabir and Begum, 1978; Kabir and Ahmed, 1979; Ponce, 1984; El-Basyouni, *et al.* 1989; Hosny, *et al.* 1989; Samudra, *et al.* 1989; Medina, *et al.* 1991 and Mohd-Zulkifli, *et al.* 1993. Chlorpyrifos, cadusafos and diazinon are very important organophosphorus pesticide widely used in the paddy field. However, no work was carried out with these pesticides on the above mentioned fish species. Therefore, the purpose of the present study was to determine the LC₅₀ values of chlorpyrifos (lorsban 10 G), cadusafos (rugby 10 G) and diazinon (basudin 10 G) to three Indian major carps (*C. catla*, *L. rohita* and *C. mrigala*) fingerlings at different exposure times during a 96 h exposure period.

Materials and Methods

The insecticides: The empirical formula of chlorpyrifos is C₉H₁₁Cl₃NO₃PS. These are a product of O,O-diethyl O-(3,5,6-trichloro-2 pyridyl) phosphorothiate. Lorsban are emulsifiable concentrate and granular product containing 150 g/kg of chlorpyrifos as the active ingredient respectively. Chlorpyrifos has a very low solubility in water but is readily soluble in most common organic solvents. These two insecticides are effective for control of a wide range of important insects and certain other arthropod pests.

The empirical formula of cadusafos is C₁₀H₂₃O₂S₂P. It is a product of S, S-di-Sec-butyl O-ethyl Phosphorodithioate. It is a granular product containing 100 g/kg of cadusafos as the active ingredient. It is soluble in water and most organic solvents. It is effective to control nematodes and many soil insects.

The empirical formula of diazinon is C₁₂H₂₁N₂O₃PS. It is a product of O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl Phosphorodithioate. It is a granular product containing 100 g/kg of diazinon as the active ingredient. It is effective to control wide range of important insects of rice, sugarcane and vegetables. All the pesticides were procured from the local pesticide dealer.

Test animal: Fingerlings of *C. catla*, *L. rohita* and *C. mrigala* averaging 2.95 ± 0.09 g, 2.90 ± 0.08 g and 2.94 ± 0.10 g in body weight and 6.78 ± 0.13 cm, 7.59 ± 0.15 cm and 7.60 ± 0.14 cm in total length respectively were tested in the present study. The length and weight of the fingerlings were recorded by a measuring scale and a five figure digital electrical balance (model, JL-180, Japan). The test fingerlings were acclimatized without feeding in 500 L fibre glass tanks at the density of 0.03 g/l for 3 days at room temperature.

Experimental procedure: The experiments were conducted in a series of glass aquaria of size 30 cm x 60 cm x 28 cm having capacity of 50 litres of water. Aquaria were filled with 35 litres pond water which was free from insecticide contamination. For the test of lorsban 15 G, rugby 10 G and basudin 10 G 0.83, 1.66, 3.33, 6.65, 13.30 and 26.60 and, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 and 1.05, 2.1, 4.2, 8.4, 16.8, 33.6 and 67.2 ppm concentrations and a control were maintained for *C. catla*, *L. rohita* and *C. mrigala* each having three replicates. The required test materials (lorsban, rugby and basudin) were

weighed by a five figure digital electrical balance. Ten acclimatized fingerlings of uniform size were stocked in each aquarium as soon as after mix-up of test materials. Behaviour of test fish fingerling were observed and dead fish were recorded and removed as soon as they were seen. Temperature, dissolved oxygen, carbon-di-oxide, p^H, total alkalinity and hardness of test media were recorded everyday. Temperature and p^H were monitored with a model HI-8314 portable p^H meter. Dissolved oxygen were measured with a oxygen meter (model, HI 9142, England). Total alkalinity and hardness were determined with a Hach water quality test kit (model FF-2, USA). The LC₅₀ values of different concentrations and exposure times were calculated with the application of binomial formula of Ward and Parris (1982).

$$LC_{50} = (AB)^{1/2}$$

where, A= Highest toxin concentration in which none of the test organisms died.

B= Lowest toxin concentration in which all organisms died.

Results and discussion

Experiment with chlorpyrifos

The cumulative mortality percentage of *C. catla*, *L. rohita* and *C. mrigala* exposed to different concentrations of chlorpyrifos are presented in Table 1. Hundred percent mortalities occurred in all fish groups at the doses of 26.60, 13.30 and 6.65 ppm within the 96 h exposure period. While a dose of 0.83 ppm could not induce any mortality within the same period. Alam *et al.* (1995) reported that 100 % *C. mrigala* fry died at the doses of 1.75 and 1.50 ppm and, 80 %, 80 % and 50 % at the doses of 1.25, 1.00 and 0.75 ppm of diazinon, respectively. Haque (1989) also reported that 100 % *Oreochromis niloticus* died at the doses of 30.0, 27.5, 25.0, 22.5, 20.0, 17.5 and 15.0 ppm of sumithion. The results of the present experiment were very close with the findings of Haque (1989). In the present case, a 30% *C. mrigala* fingerlings died at the dose of 1.66 ppm, on the contrary Alam *et al.* (1995) reported that 100 % *C. mrigala* fry died at the doses of 1.75 and 1.50 ppm of diazinon. The LC₅₀ values of chlorpyrifos for *C. catla*, *L. rohita* and *C. mrigala* are shown in Table 2. The LC₅₀ values of phosphamidon on *Channa striatus* was 10.47 at 96 h (Choudhuri *et al.* 1984) and for *Puntius ticto* the LC₅₀ value of malathion was 4.0 ppm (Singh and Sahai, 1984). In the current experiment, the LC₅₀ for *C. catla*, and *L. rohita* was 9.41 ppm at 12 h and 4.7 ppm at 36 h and 48 h. The above mentioned results coincided with the findings of Choudheri *et al.*, 1984 and Singh and Sahai, 1984.

Table 1. Cumulative mortality percentage of *C. Catla*, *L. rohita* and *C. mrigala* fingerlings exposed to different concentrations of chlorpyrifos

Concentrations (ppm)	Cumulative mortality (%)								
	6 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
<u>Catla fingerlings</u>									
Control	00	00	00	00	00	00	00	00	00
0.83	00	00	00	00	00	00	00	00	00

1.66	00	00	00	00	10	23	30	40	40
3.33	00	00	20	40	60	67	70	87	100
6.65	00	27	40	67	87	90	100	100	100
13.30	20	50	80	100	100	100	100	100	100
26.60	100	100	100	100	100	100	100	100	100
<u>Rui fingerlings</u>									
Control	00	00	00	00	00	00	00	00	00
0.83	00	00	00	00	00	00	00	00	00
1.66	00	00	00	00	00	10	20	30	37
3.33	00	00	10	27	40	50	67	70	77
6.66	00	10	30	50	73	87	93	100	100
13.30	10	30	67	83	100	100	100	100	100
26.60	100	100	100	100	100	100	100	100	100
<u>Mrigal fingerlings</u>									
Control	00	00	00	00	00	00	00	00	00
0.83	00	00	00	00	00	00	00	00	00
1.66	00	00	00	00	00	20	23	27	30
3.33	00	00	00	10	37	47	60	67	70
6.65	00	00	13	30	63	80	90	100	100
13.30	10	20	40	67	90	100	100	100	100
26.60	70	100	100	100	100	100	100	100	100

Table 2. The median lethal concentration (LC₅₀) values of chlorpyrifos on *C. Catla*, *L. rohita* and *C. mrigala* fingerlings at different exposure times

Exposure time (hours)	LC ₅₀ values (ppm)		
	<i>C. catla</i>	<i>L. rohita</i>	<i>C. mrigala</i>
6	13.30	13.30	--
12	9.41	9.41	13.30
24	6.64	6.64	9.41
36	4.70	6.64	6.64
48	3.32	4.70	6.64
60	3.32	3.32	3.32
72	2.35	3.32	3.32
84	2.35	2.35	2.35
96	1.66	2.35	2.35

Experiments with cadusafos

The cumulative mortality percentages of *C. catla*, *L. rohita* and *C. mrigala* exposed to cadusafos are presented in Table 3. A dose 2 ppm cadusafos caused 100% mortality of catla fingerlings by 96 h of exposure. However, concentrations of 4 ppm cadusafos and diazinon killed 100% rui and mrigal fingerlings within 72 h of exposure. Alam *et al.* (1995) found 80 %, 50 %, 30 % and 10 % mortality of *C. mrigala* fry at the doses of 1.0, 0.75, 0.50 and 0.25 ppm diazinon respectively and thus agree favourably with the result of present study. The LC₅₀ values of cadusafos for *C. catla*, *L. rohita* and *C. mrigala* are shown in Table 4. The estimated LC₅₀ values for *C. mrigala* were 0.72 and 1.0 ppm at 96 and 84 hours respectively. The LC₅₀ value of sumithion for *O. niloticus* was 0.87 ppm at

24 hours (Haque, 1989). Alam *et al.* (1995) reported LC₅₀ values of 168 and 96 h as 0.739 and 1.002 ppm for the same fish.

Table 3. Cumulative mortality percentage of *C. Catla*, *L. rohita* and *C. mrigala* fingerlings exposed to different concentrations of cadusafos

Concentrations (ppm)	Cumulative mortality (%)								
	6 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
<u>Catla fingerlings</u>									
Control	00	00	00	00	00	00	00	00	00
0.125	00	00	00	00	00	00	00	00	00
0.25	00	00	00	00	00	00	00	07	13
0.50	00	00	00	00	00	10	27	37	50
1.00	00	00	00	00	10	20	40	60	73
2.00	00	00	10	27	30	60	80	90	100
4.00	10	30	40	60	77	100	100	100	100
8.00	50	100	100	100	100	100	100	100	100
<u>Rui fingerlings</u>									
Control	00	00	00	00	00	00	00	00	00
0.125	00	00	00	00	00	00	00	00	00
0.25	00	00	00	00	00	00	00	10	10
0.50	00	00	00	00	00	00	10	20	37
1.00	00	00	00	00	00	10	23	47	70
2.00	00	00	00	13	30	50	67	80	93
4.00	07	20	37	50	67	87	100	100	100
8.00	40	87	100	100	100	100	100	100	100
<u>Mrigal fingerlings</u>									
Control	00	00	00	00	00	00	00	00	00
0.125	00	00	00	00	00	00	00	00	00
0.25	00	00	00	00	00	00	00	10	07
0.50	00	00	00	00	00	00	00	13	30
1.00	00	00	00	00	00	10	20	40	60
2.00	00	00	00	10	27	47	60	80	87
4.00	00	10	27	40	60	80	100	100	100
8.00	30	60	100	100	100	100	100	100	100

Table 4. The median lethal concentration (LC₅₀) values of cadusafos for *C. Catla*, *L. rohita* and *C. mrigala* fingerlings at different exposure times

Exposure time (hours)	LC ₅₀ values (ppm)		
	<i>C. catla</i>	<i>L. rohita</i>	<i>C. mrigala</i>
6	--	--	--
12	4.0	--	--
24	2.83	4.00	4.00
36	2.83	2.83	2.83
48	2.00	2.83	2.83
60	1.00	2.00	2.00

72	1.00	1.00	1.41
84	0.72	1.00	1.00
96	0.72	0.72	0.72

Experiments with diazinon

The cumulative mortality percentages of *C. catla*, *L. rohita* and *C. mrigala* exposed to diazinon are shown in Table 5. Hundred percent *C. catla*, *L. rohita* and *C. mrigala* fingerlings died at 67.2, 33.6, 16.8, 8.4, ppm diazinon within 96 h of exposure. Diazinon was reported to cause 100 % mortality in *C. mrigala* at a concentration 1.5 ppm (Alam *et al.*, 1995). However, in the present study 100% mortalities were observed with 96 h of exposure in all three species of fish tested at a dose of 8.4 ppm diazinon. The median lethal concentrations (LC₅₀) values of diazinon for *C. catla*, *L. rohita* and *C. mrigala* at different exposure times are presented in Table 6. Bengeri *et al.* (1984) reported the LC₅₀ values of dimethyl parathion on *L. rohita* to be 6.34 ppm at 96 h and similarly, the LC₅₀ values of diazinon on *C. mrigala* fry were 1.399, 1.308, 1.218 and 1.002 ppm at 24, 48, 72 and 96 h respectively (Alam *et al.* 1995). In present experiments, the LC₅₀ values on *C. mrigala* were 16.80, 8.40, 5.94 and 2.10 ppm at 24, 48, 72 and 96 h respectively and, on *L. rohita* the LC₅₀ value was 2.97 ppm at 96 h. The results are somewhat lower than that of Bengeri *et al.* 1984 and Alam *et al.* 1995.

Table 5. Cumulative mortality percentage of *C. Catla*, *L. rohita* and *C. mrigala* fingerlings exposed to different concentrations of diazinon

Concentrations (ppm)	Cumulative mortality (%)								
	6 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
<u>Catla fingerlings</u>									
Control	00	00	00	00	00	00	00	00	00
1.05	00	00	00	00	00	00	00	00	00
2.10	00	00	00	00	10	20	30	40	57
4.20	00	00	00	10	30	40	70	80	100
8.40	00	00	20	37	70	90	100	100	100
16.80	10	40	67	70	90	100	100	100	100
33.60	30	50	80	90	100	100	100	100	100
67.20	100	100	100	100	100	100	100	100	100
<u>Rui fingerlings</u>									
Control	00	00	00	00	00	00	00	00	00
1.05	00	00	00	00	00	00	00	00	00
2.10	00	00	00	00	00	00	10	20	50
4.20	00	00	00	00	10	20	37	60	77
8.40	00	00	07	10	30	60	77	90	100
16.80	00	10	20	40	70	87	100	100	100
33.60	10	20	40	67	100	100	100	100	100
67.20	100	100	100	100	100	100	100	100	100
<u>Mrigal fingerlings</u>									
Control	00	00	00	00	00	00	00	00	00
1.05	00	00	00	00	00	00	00	00	00
2.10	00	00	00	00	00	00	00	10	40

4.20	00	00	00	00	00	10	37	60	87
8.40	00	00	7	10	20	40	77	90	100
16.80	00	10	20	40	60	87	100	100	100
33.60	10	20	40	67	97	100	100	100	100
67.20	100	100	10	100	100	100	100	100	100

Table 6. The median lethal concentration (LC₅₀) values of diazinon for *C. Catla*, *L. rohita* and *C. mrigala* fingerlings at different exposure times

Exposure time (hours)	LC ₅₀ values (ppm)		
	<i>C. catla</i>	<i>L. rohita</i>	<i>C. mrigala</i>
6	23.76	33.60	33.60
12	23.76	23.76	23.76
24	16.80	16.80	16.80
36	11.88	16.80	16.80
48	5.94	8.40	8.40
60	4.20	4.20	8.40
72	2.97	4.20	5.94
84	2.97	2.97	4.20
96	2.10	2.97	2.10

Several abnormal behaviour such as rapid swimming, loss of balance, increased operculum activities, jumping out of the test media, violent spasm, convulsion etc. were observed in all fish groups exposed to the pesticides. However, the extent of such abnormalities were dependent on the concentrations of the pesticides.

Water quality in aquaria was measured during the experiments. Temperature and p^H ranged from 24.7 to 29.8^oC and 6.4 to 7.8 respectively, monitored with a model HI-8314 portable p^H meter. Dissolved oxygen ranged from 5.8 to 7.6 mg l⁻¹, measured with a oxygen meter (model, HI 9142, England). In case of all experiments, dissolved oxygen was higher in the lower concentrated test media. Meletev *et al.* (1971) reported that fish kept in toxic concentration (pesticides) the oxygen requirement increases 2-3 times. Total alkalinity and hardness, determined with a Hach water quality test kit (model FF-2, USA) were 88.3 ± 5.3 and 80.3 ± 5.1 mg l⁻¹ respectively.

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