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Variable Sensitivity of *Cyprinus carpio* Eggs, Larvae, and Fry to Pesticides

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Pesticides used for controlling insect pests of crops like cotton, maize, sugarcane, oil seeds and pulses may run off to adjoining aquatic ecosystem. The hazards of these pesticides to aquatic organisms are well documented (Murty 1986). However, there is little information on acute toxicity of the pesticides to different life stages of fresh water culturable fishes (Kaur and Toor 1977; Kaur and Cheema 1985; Von Westernhagen 1988). The objectives of this study, therefore, were to evaluate the toxicity of four pesticides to eggs, larvae and fry stages of Cyprinus carpio, and to compare the sensitivity of these stages. These data will support environmental hazards assessment of pesticides.

MATERIALS AND METHODS

Three separate acute toxicity tests were conducted with eggs, larvae and fry of C. carpio which were obtained from Fish Seed Farm, Zoology Department, Punjab Agricultural University, Ludhiana. The pesticides (Table 1) used in this study were purchased from local market.

Table 1. Trade and technical name of the pesticides

Commercial name	Trade name	Technical name
Carbaryl	Sevin (50 WP)	1-nepthyl-N methyl carbamate
Carbofuran	Furadan (3 G)	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate
Malathion	Malathion (50 EC)	O,O-dimethyl-S-1-1,2 di(carboe thoxy) ethylphosphorodithioate
Phosphamidon	Dimecron (80 EC)	2-chloro-N,N-diethyl-3-hydroxy crotonamide dimethylphosphate

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Pesticide concentration range used in tests was based on preliminary range finding tests. Test solutions (having different concentration of pesticides) were prepared by diluting known volume of freshly prepared stock solution in tap water, since these pesticides are mixed with water for field application. The temperature, dissolved oxygen, pH and total hardness of water were $24\pm1^{o}C$, 5.5 ± 0.5 mgL^{-1}, 7.5 ± 0.2 and 272 ± 2 $mgL^{-1}CaCo_3$ respectively. In the egg-larval toxicity test about 50 eggs (attached to small pieces of vegetation), at early cleavage stages were placed in each cylindrical glass jar (15x10 cm) containing 1L of test solution. There were three replicates for each concentration and control. Eggs were examined every twelve hr until hatching was complete. Dead eggs were recorded and removed when observed. The time, when more than 50% of eggs at a given concentration had hatched, was recorded as mean time to hatch. Total number of larvae were recorded 2 d after the completion of hatch. Dead and deformed larvae were subtracted from the total to give viable hatch.

In the larval toxicity test, acclimatized 20 larvae (7 d old, 1.0 ± 0.2 cm in length) were released in each glass jar (15x10 cm) containing 1L of test solution. In the fry toxicity test, acclimatized 10 fry (30 d old, 2.5 ± 0.5 cm in length) were released in each plastic bucket of 15-L capacity containing 10 L of test solution. Mortality of larvae and fry was recorded at 24-hr intervals through 96-hr of exposure. Their physical responses were also recorded.

The 96-hr LC_{50} values with 95% confidence intervals and safe values were calculated by the method of Probit Analysis (Finney 1971) and by the formula of Hart et al (1945) respectively. To evaluate the differences in stage sensitivity, the data were analysed on computer by using Student's Newman Keul's Test (Zar 1984).

RESULTS AND DISCUSSION

Analysis of hatchability of eggs and viable hatch (Table 2) showed concentration related effects. 100% hatching success was observed at 0.05, 0.01, 0.1 and 100 mgL^{-1} concentration of carbaryl, carbofuran, malathion and phosphamidon, respectively. Higher concentrations of carbaryl(3-5 mgL^{-1}), carbofuran (2-4 mgL^{-1}), malathion (25-30 mgL^{-1}) and phospamidon (300-400 mgL^{-1}) arrested the development of eggs prior to the closure of the blastopore and heavy mortality (>50%) occurred during these stages, thus indicating the greater sensitivity of younger embryonic stages (before gastrulation) to pesticides.

Table 2. Effect of pesticides on hatchability and viable hatch of C. carpio

Concentration (mgL^{-1})	Hatchability (%)	Dead+Abnormal larvae (%)	Viable hatch(%)
	Carbaryl		
0.05	100	0 + 0	100
0.1	95	0 + 0	100
0.5	75	3 + 5	92
1.0	60	7 + 3	90
2.0	40	14 + 6	80
3.0	20	15 + 10	65
4.0	15	32 + 8	60
5.0	0	0 + 0	0
	Carbofuran		
0.01	100	0 + 0	100
0.1	85	0 + 0	100
0.5	70	12 + 8	80
1.0	50	18 + 6	76
2.0	40	22 + 8	70
3.0	20	30 + 10	60
4.0	0	0 + 0	0
	Malathion		
0.1	100	2 + 0	98
1.0	90	0 + 0	100
5.0	77	0 + 0	100
10.0	68	15 + 10	75
15.0	50	12 + 8	80
20.0	40	20 + 5	65
25.0	20	30 + 10	60
30.0	0	0 + 0	0
	Phosphamidon		
100	100	5 + 0	95
150	78	0 + 0	100
200	63	15 + 5	80
250	52	19 + 6	75
300	36	24 + 1	75
350	18	40 + 10	50
400	0	0 + 0	0

There was no mortality in the controls

Similar observations have also been made by Malone and Blaylock (1970), Kaur and Toor (1977), and Kaur and Cheema (1985) in the eggs of same species. Cyprinus carpio eggs incubated in different concentrations of all the four pesticides released many inactive and some abnormal (crippled and distorted) larvae which died within 2-3 days of hatching, thus reducing the viable hatch significantly (Table 2). Effects of chlorinated hydrocarbons on percent viable hatch are also known from the investigations of Hansen et al (1985) with herrings (Clupea harengus). The abnormal larvae of Cyprinus carpio exhibited vertebral column flexure, enlarged yolk and pericardial sacs and stunted tail. Similar teratogenic effects of malathion and carbaryl on Cyprinodon variegatus and Fundulus heteroclitus embryo (Weis and Weis 1974; 1976) and carbofuran on Cyprinus carpio (Kulshrestha and Arora 1985) have been reported earlier. These morphological deformities do not seem to be pollutant specific since the similar malformations have been produced in fish embryos by heavy metals, detergents, halogenated organic compounds, some petroleum fractions and naturally stressed conditions like low pH,temperature, salinity, and low DO (Von Westernhagen 1988;Rosenthal and Alderdice 1976). Little is known about toxic mechanism of pesticides during embryonic development. Probably these malformations and developmental abberations are ultimately caused by a blockage of the energy transfer system leading to an arrest of respiration and differentiation or to dedifferentiation (Von Westernhagen 1988).

In the acute toxicity test with larvae of C. carpio, no effects on survival were observed with phosphamidon at 10 mgL^{-1}. Although both carbofuran and malathion produced marked effect on the survival at 0.1 mgL^{-1} and carbaryl at 1.0 mgL^{-1}. In case of third acute toxicity test with fry of C. carpio, phosphamidon did not affect the survival upto 50 mgL^{-1} whereas malathion, carbofuran and carbaryl produced marked effects at 0.1, 0.5, and 1.5 mgL^{-1}, respectively.

Prior to death both larvae and fry exhibited signs of toxicity by swimming erratically near water surface and loss of equilibrium in higher concentrations of all the four pesticides. The fry also showed surface breathing and fast opercular movements. These symptoms clearly revealed that death was due to cholinesterase inhibition by carbaryl, carbofuran (carbamates) and malathion and phosphamidon (organophosphates)(Corbett 1974).

Analysis of 96-hr LC_{50}values (Table 3) revealed that the sensitivity of three life stages of C. carpio to carbamates (carbaryl and carbofuran) was in the order of eggs >larvae >fry and in case of organophosphates (malathion and phosphamidon) it was in the order of larvae >fry >eggs. Carbamate toxicity to different life stages of fresh water fish has not been compared previously. The high susceptibility of larvae to these

Table 3. 96-hr LC_{50} values(mgL^{-1}) with 95% confidence interval for eggs, larvae and fry of *C. carpio* exposed to different pesticides and their relative toxicities (RT) (compared with phosphamidon) and safe values(mgL^{-1})

Pesticides	Eggs		Larvae		Fry		SV*
	LC_{50}	RT	LC_{50}	RT	LC_{50}	RT	
Carbaryl	1.19^{a} (1.03-1.38)	201.36	2.86^{b} (2.56-3.20)	20.81	3.30^{c} (2.49-4.38)	31.47	0.012
Carbofuran	1.09^{a} (0.24-2.20)	219.83	1.29^{b} (1.02-1.70)	46.14	1.55^{c} (1.38-1.75)	67.01	0.003
Malathion	12.93^{c} (10.81-15.45)	18.53	0.71^{a} (0.24-1.24)	83.83	2.10^{b} (1.22-3.61)	49.46	0.043
Phosphamidon	239.62^{c} (225.1-255.1)	1.00	59.52^{a} (47.61-74.42)	1.00	103.87^{b} (90.55-118.9)	1.00	0.198

Values with different superscripts in same row differ significantly ($p=0.05$)
* SV (Safe Values) on the basis of most sensitive stage

organophosphate has also been reported by Peflitscheck (1979) in *Tilapia leucosticta* and *Heterotilapia multispinosa* exposed to Lebaycid and Baylusid and by Ansari and Kumar (1986) in *Brachydanio rerio* exposed to malathion. More resistance of eggs to both malathion and phosphamidon may be due to the protective effect of chorion preventing their free passage to embryo.

Present studies, therefore, emphasize that eggs and larvae being highly sensitive would be more crucial to the survival of population in water bodies receiving pesticides in concentration exceeding safe value (Table 3). Yet any recommendation made in this regard may depend on data such as chemical formulations of pesticides, their application and degradation rates, sorption estimates and residues in run off water and aquatic environment.

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