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Toxicity of Four Commonly Used Agrochemicals on *Oreochromis niloticus* (L.) Fry

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Abstract

The toxicity of organophosphate insecticides, monocrotophos and fenthion, a carbamate insecticide BPMC and a herbicide paraquat, to post-yolk fry and 3-week-old fry of *Oreochromis niloticus* (L.) was studied using static conditions and continuous aeration over a period of 48 hours. LT_{50} and LC_{50} levels were determined for all toxicants. LC_{50} levels for monocrotophos, paraquat, fenthion and BPMC were 16.5 ppm, 5.2 ppm, 0.6 ppm and 0.12 ppm, respectively, for post-yolk fry. Older fry were able to tolerate higher concentrations, corresponding to LC_{50}s of 45 ppm, 15 ppm, 5.2 ppm and 9 ppm for monocrotophos, paraquat, fenthion and BPMC, respectively, showing that the younger larvae were more sensitive to the toxicants and that toxicity changed with fish development. Measurements of oxygen consumption of the post-yolk fry at sublethal levels showed elevated levels to paraquat in pre-exposed fry but not under acute conditions. The sublethal levels studied showed no appreciable difference from that of the controls for the other toxicants.

Introduction

Use of pesticides in rice cultivation is common practice in Asia and Sri Lanka is no exception. Further, as water for cultivation in most of Asia is provided mainly from reservoirs which usually constitute parts of a system, water from the rice paddies also drains repeatedly into other reservoirs. This is very evident in Sri Lanka which has an intricate network of reservoirs for irrigation (De Silva 1985). The net effect is a distribution of pesticides over a very wide area. Since reservoir fish are now being increasingly used as a source of protein for the rural poor, the effects of pesticides and herbicides used in rice cultivation need to be monitored. The Nile tilapia, *Oreochromis niloticus* (Cichlidae), is widely used in fish culture programs in most of Asia and was chosen for toxicity testing of three insecticides and a herbicide commonly used in rice cultivation in Sri Lanka.

While many workers have investigated the effects of pesticides and herbicides on temperate species (see Hughes 1981), tropical species, particularly cichlids, have not been much studied. Rath and Misra (1979) studied the toxicity of dichlorovos to *O. mossambicus* of three different age groups while the effect of two agrochemicals on the hematology of *O. mossambicus* was studied by Koundinya and Ramamurthi (1979). Joshi and Desai (1984) and Chatterjee and Konar (1984) studied the effects of single pesticides, monocrotophos and diazinon, respectively, on tropical fish species. Cruz et al. (in press) studied the hematological and histopathological effects of the molluscicides aquatin and brestan on *O. mossambicus* and Matton and LaHam (1969) studied the effects of the organophosphate dylox on rainbow trout larvae. Kosoemadinata (1980) reviewed the effects of pesticides in agriculture-aquaculture integrated systems.

In this study the effects on *O. niloticus* fry of three common insecticides and a herbicide commonly used in rice cultivation were evaluated. The effects were quantified through LC_{50}. In addition, sublethal experiments were performed to determine the effects of the chemicals on the oxygen consumption of the fry.

Materials and Methods

Groups of 10-12 post-yolk fry of two different sizes, 1 to 7 mg (12-14 mm total length (TL)) and 20 to 80 mg (15-18 mm TL) were used. They were collected from the outdoor ponds in the Department of Zoology, University of Ruhuna, kept in 50-l recirculating tanks and fed a balanced diet used in growth experiments (De Silva and Perera 1984). Initially, the fry were tested over a period of 96 hours as recommended by Fry (1972). Varied concentrations of pesticides were tested under static conditions in 1-l glass beakers using distilled water with continuous aeration. The frequency of observations for mortality was based on pilot tests.

The pesticides tested were the organophosphate insecticides monocrotophos and fenthion, the carbamate insecticide butyl phenyl methyl carbamate (BPMC) and the carbamate herbicide paraquat (Table 1). At least six concentrations were tested for each chemical.

Table 1. List of toxicants used with common names, trade names, active ingredients and rate of application (r.a. - recommended rate of application in ml of toxicant diluted in liters of water).

Common name (Trade name)	Category	Chemical name (IUPAC)	% Active ingredient	r.a.	Practical use
Monocrotophos (Azodrin)	O.P.	dimethyl(E)-1-methyl-methyl carbamoylvinyl phosphate	60% W.S.C.	28 ml/14-23 l	Insecticide
Fenthion (Lebaycid)	O.P.	o,o-dimethyl o-13-methyl-4-methylthio-m-tolyl phosphorothioate	50% E.C.	28 ml/9.14 l	Insecticide
BPMC (Harcros)	Carbamate	2-sec-butyl phenyl methyl-carbamate	50% E.C.	28 ml/13.5-18 l	Insecticide
Paraquat (Paraquat)	Carbamate	1,1'-dimethyl-4,4'-bipyridyldiylium ion	Paraquat 24%	28 ml/4.5 l	Herbicide

The content of active ingredient is indicated by % figures following the chemical names; IUPAC - International Union of Pure and Applied Chemists; O.P. - organophosphate; W.S.C. - water soluble compound; E.C. - emulsifiable concentrate; BPMC - butyl phenyl methyl carbamate.

The test animals were starved for 24 hours prior to commencement of the experiments. Experiments were run in duplicate with two controls in each case in an air-conditioned room at 25 ± 1°C. The younger fry tended to attack the weaker or sickly ones during the experiment due to lack of food over the 96-hour period, thus increasing mortality. Therefore, the experiments were run only for 48 hours for both size groups. Concentrations of toxicants in the test solutions were not determined during the experiments. Stock solutions of the pesticide were made up using freshly distilled water. The pH of the test solutions was 7.0 for monocrotophos, 8.5 ± 0.5 for paraquat, 8.4 ± 0.1 for BPMC and 7.9 ± 0.1 for fenthion.

Fry were observed at 5-minute intervals for the higher concentrations of toxicants until complete mortality occurred. At the lower dosages they were observed every half hour over the 48-hour period. The post-yolk fry were considered to be dead when a gentle tap at the side of the beaker did not produce any movement in the organism. For older fry, complete cessation of opercular movement was taken as the time of death. In an earlier study on herring larvae, De Silva (1973) used the time when the brain turned white as the criterion for death even though slight movement was observed afterwards. However, it was not possible to use this criterion in the present study as the post-yolk *O. niloticus* fry are not transparent.

Oxygen consumption of the fry at sublethal levels of toxicants was measured using a Gilson differential respirometer in 25-ml vessels with 2 to 3 fry/flask at 25°C. Details of the experimental method are given in De Silva and Tytler (1973).

Results

At the higher concentrations of toxicant, the color of the fry became darker and the stripes along the body became more deeply etched. Death followed soon after. At the lower concentrations, such color changes were observed 1 to 2 hours before death. In addition, some of the fry tended to turn in a spiral motion in the same place with the mouth pointing downwards, with death occurring soon afterwards. A few swam around in this fashion in one place and then lay flat on the bottom, the process being repeated several times before death. In some fry, curvature of the body to either side was observed prior to death.

The results of the tests were expressed as LT_{50} and LC_{50} using the graphical methods described by Litchfield (1949). The typical pattern of mortality for the pesticide fenthion for post-yolk fry is shown in Fig. 1. The mortality patterns for the post-yolk fry followed

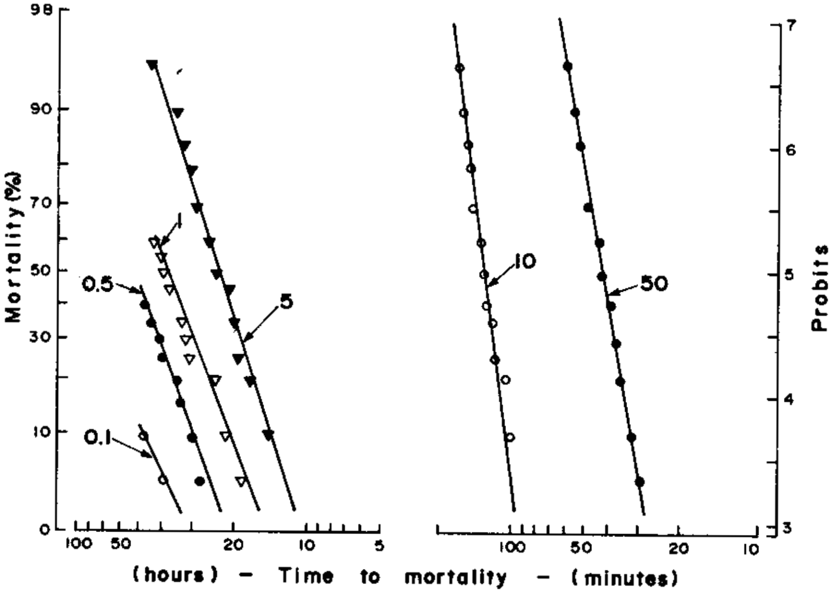

Fig. 1. Cumulative percentage mortality in post-yolk fry of *Oreochromis niloticus* exposed to various concentrations of fenthion expressed as ppm.

the normal mortality patterns common in dose-effect experiments (Shepard 1955; De Silva and Tytler 1973). The results of varying dosages of toxicants on time to 50% mortality (LT_{50}) on both groups of fry are summarized in Tables 2 and 3. At the higher dosages, mortality occurred very rapidly while at the lower dosages all the fry did not die over the 48-hour period. The LC_{50} values for all toxicants tested are given in Table 4. In order of decreasing toxicity to the younger stages, the chemicals are BPMC, fenthion, paraquat and monocrotophos. The sensitivity of 3-week-old fry was different; fenthion appeared to be the most toxic followed by BPMC and then paraquat and monocrotophos. Table 5 summarizes the toxicity of the chemicals to different fry stages. BPMC is 6 times more toxic than fenthion and 43 times more toxic than paraquat and 190 times more toxic than monocrotophos when used singly. However, in older fry not only does the sensitivity change slightly but the concentration required to produce 50% mortality of the population also increases greatly (Table 4).

Estimates of weight-specific oxygen consumption of fry at sublethal levels (Fig. 2) showed that monocrotophos and paraquat under acute conditions elevated oxygen consumption of post-yolk fry

Table 2. Time to 50% mortality (LT_{50}) over 48 hours in post-yolk fry exposed to several concentrations of pesticides and herbicides (c.d. - cumulative dead by 48 hours; S.D. - standard deviation equivalent to slope function; fLT_{50} - factor for LT_{50}; f_{se} - factor for slope).

Chemical	Nominal conc. (ppm)	LT_{50} (hours)	S.D.	fLT_{50}	f_{se}	c.d. (%)
Monocrotophos	5	>48	-	-	-	5
	10	>48	-	-	-	25
	25	46	1.20	1.095	1.085	60
	50	4.3	2.50	1.500	1.330	100
Paraquat	1	>48	-	-	-	20
	5	>48	-	-	-	40
	10	37.5	1.720	1.300	1.248	65
	50	20.5	1.700	1.260	1.180	95
	60	13.5	1.545	1.210	1.145	100
	100	0.72	1.663	1.248	1.172	100
Fenthion	0.1	>48	-	-	-	10
	0.5	>48	-	-	-	40
	1	40	1.600	24.1	122.2	60
	5	23.5	1.454	1.18	1.13	95
	10	2.17	1.175	1.075	1.05	100
	50	0.72	1.200	1.085	1.06	100
BPMC	0.1	42	1.590	1.280	1.26	50
	0.5	36	1.392	1.160	1.35	80
	1	31.5	1.353	1.140	1.15	90
	5	1.53	1.700	1.260	1.18	100
	10	0.60	1.700	1.260	1.18	100

Table 3. Time to 50% mortality (LT_{50}) over 48 hours in 3-week-old fry exposed to different concentrations of pesticides and herbicides.

Chemical	Nominal conc. (ppm)	LT_{50} (hours)	S.D.	fLT_{50}	f_{se}	c.d. (%)
Monocrotophos	15	>48	-	-	-	5
	30	>48	-	-	-	15
	50	>48	-	-	-	20
	100	12	1.50	1.900	1.135	100
	130	7.20	1.30	1.120	1.085	100
	150	2.42	1.50	1.900	1.135	100
	200	1.83	1.40	1.160	1.110	100
Paraquat	5	>48	-	-	-	20
	10	>48	-	-	-	40
	40	>48	-	-	-	40
	50	22	1.400	1.160	1.119	80
	70	16	1.500	1.900	1.135	100
	90	11	1.400	1.160	1.110	100
	120	1.00	1.500	1.900	1.135	100
	150	0.90	1.500	1.900	1.135	100
Fenthion	3	>48	-	-	-	10
	5	>48	-	-	-	20
	10	3.25	2.005	1.35	1.240	100
	20	1.13	1.300	1.12	1.085	100
BPMC	5	>48	-	-	-	20
	10	>48	-	-	-	45
	20	7.2	5.750	-	-	90
	25	1.92	2.400	1.450	1.31	100
	50	0.38	1.400	1.160	1.11	100

(c.d. - cumulative dead by 48 hours; S.D. - standard deviation equivalent to slope function; fLT_{50} - factor for LT_{50}; f_{se} - factor for slope)

Table 4. Median lethal dose (LC_{50}) over 48 hours in *O. niloticus* fry exposed to different concentrations of pesticides and herbicides.

Chemical	LC_{50} (ppm)	C.I.	S.D.	fLT_{50}	f_{se}
Post-yolk fry					
Monocrotophos	16.500	22.08-11.96	2.1	1.38	1.26
Paraquat	5.200	9.88- 2.74	4.5	1.90	1.57
Fenthion	0.600	1.06- 0.34	3.8	1.76	1.50
BPMC	0.115	0.21- 0.06	3.8	1.82	1.54
3-week-old fry					
Monocrotophos	45.00	85.50-23.68	1.5	1.90	1.14
Paraquat	15.00	19.80-11.36	1.9	1.32	1.22
Fenthion	5.20	6.03- 4.48	1.4	1.16	1.11
BPMC	9.00	11.34- 7.14	1.7	1.26	1.18

(S.D. - standard deviation; C.I. - is 95% confidence interval; fLT_{50} - factor for LT_{50}; f_{se} - factor for slope).

Table 5. Decreasing ratio of sensitivity of *O. niloticus* fry to pesticides and herbicides at two developmental stages.

Post-yolk fry

o	BPMC	Fen	Para	Mono
BPMC	-	6	43	190
Fenthion	0.18	-	8	35
Paraquat	0.02	0.12	-	4
Monocrotophos	0.06	0.03	0.23	-

Toxicity decreases (→ across columns; ↓ down rows)

8-week-old fry

	Fen	BPMC	Para	Mon
Fenthion	-	1.6	2.5	5.2
BPMC	0.6	-	1.59	3.3
Paraquat	0.4	0.6	-	2.0
Monocrotophos	0.04	0.3	0.5	-

Toxicity decreases (→ across columns; ↓ down rows)

(Fen - Fenthion; Para - Paraquat; Mono - Monocrotophos; numbers in table denote the rate of toxicity of each chemical in comparison to the others)

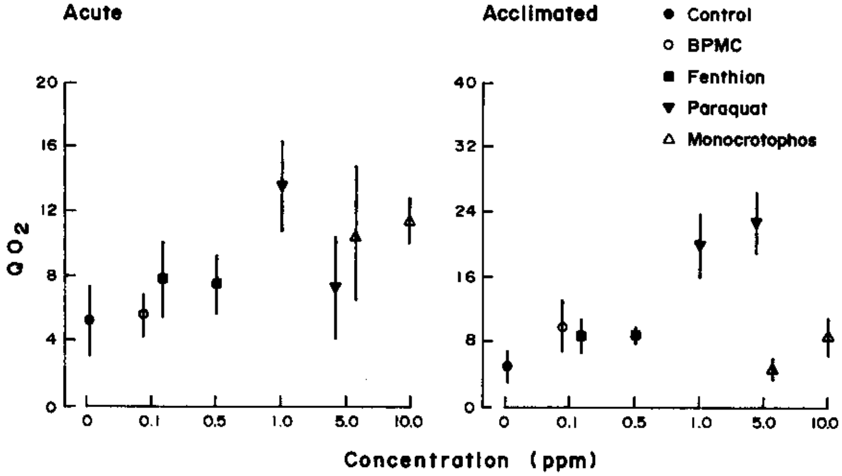

Fig. 2. Oxygen consumption of post-yolk fry of *Oreochromis niloticus* exposed to sublethal levels of toxicants and expressed as QO_2 ($\mu l.mg^{-1}$ dry wt $hour^{-1}$) under acute and pre-exposed conditions at 25°C.

over that of controls. Both size groups exposed to BPMC and fenthion showed no significant difference ($P > 0.01$) in oxygen consumption from that of the controls. Fry pre-exposed for 48 hours to the chemicals (Fig. 2) showed elevated oxygen levels only in the presence of paraquat.

Discussion

Cruz et al. (in press) have also observed the darkening of the body in 30-g *O. mossambicus* exposed to the organostannous molluscides aquatin and brestan. Matton and LaHam (1969) reported the curling of the body in rainbow trout larvae exposed to dylox. Decreasing sensitivity to the pesticide dichlorovos with age of fish was reported in *O. mossambicus* by Rath and Misra (1979). Hardjamulla and Koesoemadinata (1972) reported fish kills 5 to 10 days after application of emulsifiable concentrates of endrin 2G and thiodan 5G, respectively. On the basis of the Bathe et al. (1974) classification, the present study indicates that fenthion and BPMC are highly toxic to *O. niloticus* at the post-yolk stage while paraquat and monocrotophos are only slightly toxic. For the older fry fenthion is toxic while the other three substances are only slightly toxic. However, the test species used in the Bathe et al. (1974) study were the carp *Cyprinus carpio* and rainbow trout *Salmo gairdneri* (size 2-10 cm). Using the criteria of Koesoemadinata and Djajadireja (1976) for 48-hour LC_{50}s, fenthion and BPMC are extremely toxic (Rank A) while paraquat is highly toxic (Rank B) and monocrotophos is only moderately toxic (Rank C) to post-yolk *O. niloticus* fry. For the older fry, fenthion and BPMC fall into the Rank B category and paraquat and monocrotophos into Rank C. According to Koesoemadinata (1980) the recommended dosage of 0.5 to 10 $kg.ha^{-1}$ used in crop protection coresponds to a concentration of 0.5 to 10 ppm in irrigated rice fields with 10 cm of water and 0.5 ppm was taken as the boundary level for highly toxic pesticides.

From the present study it is evident that BPMC is too toxic to be used in aquaculture; fenthion is just within the boundary line; and the other two, especially monocrotophos, could be used in agriculture without apparent harm to fish fry. For the older fry the dosages applied are within the safety limits. Using the pesticide safety level for organophosphates and carbamates suggested by the FAO (1969) the safety range for monocrotophos , fenthion, BPMC and paraquat from this study corresponds to 1.65-0.165 ppm, 0.06-0.006 ppm, 0.012-0.0012 ppm and 0.52-0.052 ppm, respectively, for post-yolk fry and 4.5-0.45 ppm, 0.52-0.52 ppm, 0.9-0.09 ppm and 1.5-0.15 ppm for the older fry. However, it must be stressed that this is a slightly conservative estimate as the application factors quoted are for 96-hour LC_{50}s. It is also interesting that some authors (Macek and McAllister 1970; Koesoemadinata 1980) suggest that carbamates are less toxic than organophosphates to fish. However, this study shows that this does not apply to all fish species as BPMC is the most toxic of the four chemicals studied to both stages of fry of *O. niloticus*. It is also well known that most chemicals undergo 'biotransformation' to products that are more toxic than the parent chemical (see Buhler and Williams 1988). However, no assays were made for such breakdown products in this study.

There is little published information on the acute toxicity of pesticides to different developmental stages. Such changes have been observed with respect to other environmental parameters such as oxygen levels in larvae (De Silva and Tytler 1973; Blaxter and Hunter 1981). The changing sensitivity to decreasing oxygen levels in herring and plaice larvae was attributed to the development of gills and hemoglobin (De Silva and Tytler 1973; De Silva 1974). In *O. niloticus* the changing sensitivity could be due to the rapid gill development which occurs at this time (De Silva and Thabrew 1986). It is possible that pre-exposed post-yolk fry compensated for decreased blood oxygen transport by hyperventilation of the gills and increased perfusion rates. Increased operculation rates, heart rates and gill diffusion rates have been demonstrated in fish exposed to toxicants (Hughes 1981; Cairns et al. 1981). Compensation for decreased blood oxygen was observed in fathead minnows exposed to selenium (Watenpaugh and Beitinger 1985).

An important factor is that frequently more than one pesticide is used simultaneously for controlling pests in the rice fields and therefore it is important to test for synergistic and or antagonistic effects of two or more pesticides at the same time.

In conclusion, it is important for toxicity testing to be carried out on all developmental stages of fish and not merely on the adult. In addition, it is necessary to test the effects of breakdown products of these chemicals to the organisms concerned. Further, it is important that toxicity tests on fry be incorporated in the pesticide related laws of each country, especially those where rice-fish cultivation is being carried out.

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