MEASUREMENTS OF THE ACUTE TOXICITY OF CYPERMETHRIN TO NILE TILAPIA *(Oreochromis niloticus)*, USING A STATIC AND A CONTINUOUS-FLOW SYSTEM

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ABSTRACT

Experiments on the toxicity of the insecticide cypermethrin, a synthetic pyrethroid, to nile tilapia *(Oreochromis niloticus)* were conducted, using static and continuous flow systems. The acute toxicity of the insecticide, expressed in the values of the Median Lethal Concentrations (LC50s) were determined, to define its potential hazard to fish and fisheries. Cypermethrin was found to be highly toxic to the nile tilapia, with a 96-hour LC50 of less than 1 ppm of formulated insecticide material containing 100g cypermethrin per litre. The toxicity of cypermethrin was greatest in a continuous flow toxicity test, suggesting that substantial improvements in accuracy can be obtained in the measurement of lethal toxicity of synthetic pyrethroid insecticides by employing continuous flow tests rather than static tests. The variation in the results obtained using the two systems is discussed.

KEYWORDS: ***Insecticides, cypermethrin, acute toxicity test, nile tilapia.***

INTRODUCTION

Modern agriculture relies heavily on pesticides to control pests and to increase yields of many crops. Pesticides are also increasingly used to aid the control of many serious vector-borne diseases, such as bilharzia in Africa and malaria in Asia, as well as diseases in cattle. Unfortunately, despite the many benefits of pesticides there are considerable problems associated with their use (Willis and McDowell, 1982). For example many pesticides are now recognized as serious pollutants in aquatic environments, with deleterious effects on many aquatic organisms (Muirhead-Thomson, 1971).

The new synthetic pyrethroid insecticides have been found to be highly active against insects and larvae of several species of mosquitoes. Th`e chemical penetrates the cuticle of the insect and then transported in the haemolymph to the central nervous system, where toxic effects are manifested (Burt and Goodchild, 1974).

It has been reported, however, that at least some of the synthetic pyrethroids have a high level of toxicity to fish (Mulla *et al.*, 1978; Stephenson, 1982; Kumaraguru and Beamish, 1981; Shires, 1983). Decamethrin, for example, was found to be the most toxic pyrethroid compound to all the fish tested by Mulla *et al.* (1978), causing 90% mortality at concentration levels of 1-2 ppb (parts per billion).

The heavy usage of agricultural chemicals and their associated effects on fish represent considerable problems for fish culture in many parts of the world, particularly where agriculture and fish culture activities exist in the same agrosystem. The danger of pesticides is probably greatest where fish culture is practised in irrigated rice fields, a technique which is extensively conducted in Indonesia and many other Asian countries. Fish

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mortalities are commonly seen directly or shortly after insecticide application, depending on the toxicity of the chemical. Insecticides may persist in the rice field environment depending on their chemical structure (Edwards, 1977). High levels of insecticide usage can cause downstream contamination, associated with serious deleterious effects to fisheries (Gorbach *et al.*, 1971a, 1971b). The use of less toxic and less persistent insecticides in irrigated rice fields should be encouraged. Since many new insecticides that appear on the market pose a threat to the environment, there is a need for an assessment of the potential impact of insecticides, such as synthetic pyrethroids, on fish culture in rice fields.

This paper aimed to evaluate the acute toxicity of cypermethrin, a synthetic pyrethroid insecticide, to the nile tilapia, based on the results of static and continuous-flow toxicity tests.

MATERIAL AND METHODS

Insecticide Sample

Cypermethrin is the active ingredient of widely used formulated insecticides. The chemical name of cypermethrin is *(R,S)-α-cyano-3-pheno-xybenzyl (IR,IS,cis,trans)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane-carboxylate*. For the purpose of the experiments a commercial insecticide product containing 100g of cypermethrin per litre, was used. The material was a yellowish liquid, which was miscible in water, producing a whitish suspension.

A solution of cypermethrin in water was used for the experiments. This solution was prepared by mixing 1 mL of the formulated insecticide product in 100 mL of Analar acetone, to give 1 g cypermethrin per litre of acetone, as suggested by Stephenson (1982). The solution was then made up to 1 L with distilled water, producing a stock of 1000 ppm of the commercial insecticide product. Subsequent dilutions of this stock solution were prepared for the toxicity tests.

Test Fish

Fingerlings of nile tilapia, *Oreochromis niloticus*, with average weight of 1g, obtained from a local hatchery unit, were used in the tests. The fishes were reared in fibre glass tanks, supplied with water from a recycling water system which produced an average water flow of 0.72 L/sec., at a water temperature of 25°- 27°C. The fishes were fed with commercial fish pellets, containing 40% protein. Daily feeding rate of 5% body weight was given.

No mortality was recorded in the fish stock population, and no sign of any health problem was observed during the two weeks before the experiment. Two days before each experiment, fish were transferred to the test containers, and were starved to prevent fouling the test solutions.

Dilution Water

The average physical and chemical characteristics of the dilution water were as follows:

Temperature (°C)	: 23
pH	: 7
Total hardness (as ppm $CaCO_3$)	: 27
N-NH3 (ppm)	: 0.002
Dissolved oxygen (ppm)	: 7.5

The above parameters of water quality, except ammonia, were monitored and recorded daily during the course of the experiment.

Static Test

The main objective of the static toxicity test was to determine the acute or lethal toxicity of the insecticide to fish. The acute toxicity was defined as the Median Lethal Concentration or LC50, i.e the concentration which produces fifty percent mortality to test fish in a certain period of time (usually 24, 48 or 96 hours), under specified test conditions.

The methodology of fish toxicity testing has been described by many investigators and research institutions (Duodoroff *et al.*,1951;

Alabaster and Abrams, 1965; Sprague, 1969; APHA *et al.*, 1974; EIFAC/FAO, 1975 and Buikema *et al.*, 1982), and the basic procedures postulated in these standard methods were adopted in the tests.

Two tests were carried out in the static system. The tests were conducted in six test containers made of transparent plastic, each with dimensions of 40 x 18 x 20 cm (length, width and depth).

Ten litres of dilution water were added to each test container. Twenty fish were selected at random from the stock populations, and introduced into the test containers, allowing an average loading ratio of 2 g per 1 L of dilution water. In order to maintain the dissolved oxygen concentration in each test container, ventilation was provided by means of a compressor and air stones.

Aliquots of 1000 ppm cypermethin stock solution were added to each container to make certain concentration at the start of each experiment. To find out the lethal range of cypermethrin for the nile tilapia, an exploratory test was carried out using 0.01, 0.1 and 1.0 ppm of the commercial insecticide product, and five fishes per concentration. Full scale tests were conducted using five concentrations based on progressive bisection of intervals on a logarithmic scale (Duodoroff *et al.*, 1951 and APHA *et al.*, 1974).

These test concentrations were: 0.37ppm, 0.05ppm, 0.87ppm, 1.15ppm and 1.55ppm (formulated product of cypermethrin). The control fish were exposed to the highest concentrations of the solvent in which fish in the test solutions were exposed (i.e. 155ppm acetone which is non lethal to fish).

The test fish were observed and mortality recorded at the following time intervals: (a) 15, 30, 60 minutes, (b) 2, 4, 8, 16 and 24 hours, and (c) 2, 3, and 4 days. Test fish were considered dead when any movement of the operculum had ceased. Dead fish were immediately removed from the test containers to prevent fouling of the test solutions.

Cumulative fish mortality at 24, 48, 72 and 96 hours exposure periods, was recorded and plotted against concentration on logarithm-probability graph papers. The data were then analysed according to the statistical method described by Litchfield and Wilcoxon (1949), to determine the values of the Median Lethal Concentrations (LC50s), the slope functions of the dose-response lines, and their 95-percent confident limit intervals.

Testing for significant differences between LC50s (P = 0.05) was carried out by calculating the potency ratio of the values, as described by Litchfield & Wilcoxon (1949).

Continuous-Flow Test

The objective of this test was to determine the acute toxicity of the insecticide to fish by means of a continuous-flow system. This allows a continous supply of new test solution through the tank.

The system consisted of:

(a) a constant head water reservoir which supplied dilution water to the system;

(b) a peristaltic pump (Watson-Marlow HR Flow Inducer Type MHRE), which delivered insecticide solutions to the system by means of five tubings with different diameters and a pump speed of 20 rpm;

(c) mixing chambers which were attached to each test container, to receive the dilution water and the insecticide solution, respectively from the constant head water tank and the stock solution tank, and subsequently mix and deliver the new test solutions into the test containers.

The system allowed a rate of test solution flow of 1.2 L/g of fish/day, and a replacement time of test solution of 90% per 20 - 24 hours. More reasonable test solution rates of 2-3 L/g/day and replacement time of 90% in 8-12 hours, as recommended by Sprague (1969), could not be applied here due to limited supply of dilution water.

Except for the incorporation of the continuous-flow apparatus, the general procedures of the tests were the same as those described in the static test.

Three tests were carried out using water pH variations of 3.8-4.0, 4.6-5.3 and 8.7-9.6. High pH value was maintained by adding 20ppm of calcium hydroxide to the test solutions.

The test concentrations used in the above experiments were based on the formulated product of cypermethrin as indicated in Table 1.

RESULTS AND DISCUSSION

Static Tests

The two acute static tests showed that the insecticide is highly toxic to nile tilapia, with mean 24-hour LC50 concentrations of 0.90 ppm (0.090 mg/L active ingredient of cypermethrin) (Table 2).

Table 1. Concentration of cypermethrin formulation (100 g/L) used in the Continuous Flow System.

Test	Tube No	Flow rate of 100 ppm stock solution of test material (cc/hour)	Flow rate of dilution water from constant head tank (L/hour)	Final Concentration in the mixing chamber and test containers (ppm)
A and B	1	14.7	1	1.47
	2	10.6	1	1.06
	3	8.5	1	0.85
	4	6.9	1	0.69
	5	3.5	1	0.35
	6	Control	1	nil
C	1	17.0	1	1.70
	2	10.4	1	1.04
	3	6.2	1	0.62
	4	5.0	1	0.50
	5	1.4	1	0.14
	6	Control	1	nil

Table 2. The acute toxicity of cypermethrin formulation (100 g/L) to nile tilapia in test using a Static Test System.

Test	pH	Exposure time (hours)	Median Lethal Concentration (LC50) and 95% confidence limit interval values (ppm)	Slope function and 95% confidence limit interval values
1	6.7-7.4	24	0.90 (0.77-1.04)	1.19 (1.13-1.25)
		48	0.55 (0.41-0.74)	1.82 (1.21-2.73)
		72	0.48 (0.35-0.65)	1.64 (1.19-1.27)
		96	0.48 (0.35-0.65)	1.64 (1.19-2.27)
2	7.2-7.6	24	0.91 (0.73-1.13)	1.53 (1.26-1.85)
		48	0.80 (0.65-0.98)	1.50 (1.24-1.82)
		72	0.73 (0.57-0.94)	1.50 (1.19-1.90)
		96	0.73 (0.57-0.94)	1.50 (1.19-1.90)

The LC50s in both static tests were not signifcantly different (P <0.05), and there was no significant change in LC50 values after 24 hours (Table 3). This was indicated by the relative potency ratio of the action of the insecticide against nile tilapia between 24 hours and subsequent exposure periods, which is estimated after testing the parallelism of their dose-effect lines, as described by Litchfield and Wilcoxon (1949) (see Table 3).

Table 3. The potency ratio of cypermethrin formulation (100 g/L) to nile tilapia between 24, 48 and 72 hours exposure period in the Static Test 1 and 2.

Test No.	Potency Ratio (PR) and 95% Confidence Limit Intervals		
	24/48 hours	48/72 hours	72/24 hours
1	1.67 (1.18-2.37) P > 0.05	1.14 (0.75-1.73) P < 0.05	1.36 (0.96-1.93) P < 0.05
2	1.14 (0.86-1.50) P < 0.05	1.09 (0.93-1.27) P < 0.05	1.24 (0.92-1.47) P < 0.05

The physico-chemical characteristics of the test solutions measured were according to the recommended guidelines of APHA *et al.* (1974). No significant fluctuations was noted in the values of the parameter measured (Table 4).

Table 4. The physico-chemical characteristics of test solutions measured during the test in the Static System (Mean ± standard deviation, N = 5).

Parameter	Test	Day 1	Day 2	Day 3	Day 4
Temperature (°C)	1	21.50 (± 0.55)	22.30 (± 0.43)	22.70 (± 0.27)	22.20 (± 0.27)
	2	31.60 (± 0.40)	21.20 (± 0.26)	21.70 (± 0.29)	21.70 (± 0.27)
Dissolved Oxygen	1	8.25 (± 0.15)	7.47 (± 0.44)	6.80 (± 0.61)	8.10 (± 0.37)
(mg/L)	2	8.15 (± 0.54)	7.55 (± 0.83)	7.72 (± 0.32)	8.48 (± 0.28)
pH	1	7.36 (± 0.08)	7.24 (± 0.10)	6.68 (± 0.16)	7.28 (± 0.09)
	2	7.40 (± 0.11)	7.17 (± 0.03)	7.38 (± 0.24)	7.63 (± 0.26)
Total Hardness	1	25.85 (± 2.04)	25.02 (± 0.63)	25.51 (± 2.31)	25.08 (± 0.61)
(mg $CaC0_3$/L)	2	27.09 (± 2.01)	27.86 (± 2.04)	28.03 (± 1.79)	29.69 (± 1.51)
Total ammonia	1	-	-	-	0.051(± 0.018)
(mg/L)	2	-	-	-	-

Continuous-flow Tests

- **Acidic conditions**

A major feature of the results of the continuous-flow tests in comparison with the static toxicity tests, is that these tests showed continued mortality throughout the test period of 96 hours. The LC50 values in acidic conditions (pH: 3.6-3.9) are shown in Table 5. The data indicate a decrease in the median lethal concentrations through out the period of the test. There was more than 50% mortal-

Table 5. The acute toxicity of cypermethrin formulation (100 g/L) to nile tilapia in acidic conditions (pH: 3.6-3.9), using a Continuous-Flow Test System.

Exposure time (Hours)	Median Lethal Concentration (LC50), and 95% confidence limit intervals (ppm)	Slope Function and 95% confidence limit intervals
24	0.90 (0.69-1.17)	2.32 (1.76-3.69)
48	0.55 (0.44-0.69)	2.10 (1.44-3.07)
72	0.36 (0.27-0.48)	1.92 (1.28-2.88)
96	< 0.35	-

ity in the test fish after 96 hours in the lowest concentration, and thus the value of 96-hour LC50 could not be calculated, but was clearly less than 0.35 ppm. However, the potency ratios clearly showed that continuous exposure is more toxic over a longer period of time than a single dose of the insecticide under the present experimental conditions (Table 6).

Table 6. The potency ratio of cypermethrin formulation (100 g/L) to nile tilapia between 24, 48 and 72 hours exposure periods, in the Continuous-Flow Test System.

Test No.	Potency ratio and 95% confidence limit intervals		
	24/48 hours	48/72 hours	72/24 hours
1	1.64 (1.15-2.52) P > 0.05	1.53 (1.05-2.22) P > 0.05	2.50 (1.70-3.67) P > 0.05
2	1.57 (1.26-1.96) P > 0.05	1.39 (1.03-1.88) P > 0.05	2.19 (1.68-2.85) P > 0.05
3	1.30 (1.00-1.69) P > 0.05	1.64 (1.31-2.05) P > 0.05	2.94 (2.18-3.97) P > 0.05

Notes: Test No.1 : pH 3.8 - 3.9, Test No. 2 : pH 4.6 - 5.3, Test No.3 : pH 8.7 - 9.6

LC50 values obtained from tests in a slightly acidic water (pH: 4.6-5.3) are tabulated in Table 7. The results show, similar to those in the previous experiment, that there was an increasing mortality with longer exposure period. There was no significant difference (P>0.05) between the LC50s in both acidic experiments.

- **Alkaline conditions**

The values of LC50 obtained from the tests in alkaline water are presented in Table 8. The comparison of the results between the toxicity tests in acidic and alkaline media suggests that cypermethrin is more toxic in acidic conditions (P<0.05) (Table 9). There was no significant difference between the toxicity of the insecticide in static and continuous-flow test within 48 hours exposure time (P>0.05) (Table 10). However, the difference was significantly pronounced after 72 hours of exposure.

The physico-chemical characteristics of the water used in the tests did not show any significant fluctuations, except in pH. The hardness of the water was higher than those measured in the static test, as shown in Table 11.

Table 7. The acute toxicity of cypermethrin formulation (100 g/L) to nile tilapia in acidic condition (pH: 4.6-5.3), using the Continuous Flow System.

Exposure time (hours)	Median Lethal Concentration, and 95% confidence limit intervals (ppm)	Slope function and 95% confidence limit intervals
24	0.94 (0.82-1.08)	1.48 (1.32-1.65)
48	0.60 (0.49-0.75)	1.57 (1.29-1.91)
72	0.43 (0.34-0.54)	1.86 (1.38-2.51)
96	< 0.35	-

Table 8. The acute toxicity of cypermethrin formulation (100 g/L) to nile tilapia in alkaline condition, using the Continuous Flow Test System.

Exposure time (hours)	Median Lethal Concentration (LC50), and 95% confidence limit intervals (ppm)	Slope function and 95% confidence limit intervals
24	1.00 (0.83-1.21)	1.37 (1.17-1.60)
48	0.77 (0.65-0.92)	1.50 (1.25-1.78)
72	0.47 (0.41-0.53)	1.33 (1.17-1.51)
96	0.34 (0.27-0.43)	1.47 (1.78-1.89)

Table 9. The potency ratio of cypermethrin formulation (100 g/l) to nile tilapia between acidic, slightly acidic and alkaline condition, in the Continuous-Flow Test System.

Exposure time (hour)	Potency ratio and 95% confidence limit intervals		
	Test 1/Test 2	Test 2/Test 3	Test 1/Test 3
24	1.04 (0.77-1.40) P < 0.05	1.11 (0.81-1.52) P < 0.05	1.06 (0.85-1.32) P < 0.05
48	1.16 (0.66-1.57) P < 0.05	1.28 (0.98-1.66) P < 0.05	1.40 (1.04-1.89) P > 0.05

Notes: Test No.1 : pH 3.8 - 3.9, Test No. 2 : pH 4.6 - 5.3, Test No.3 : pH 8.7 - 9.6

Table 10. The potency ratio of cypermethrin formulation (100 g/L) to nile tilapia between the Static Test and the Continuous Flow Test conditions.

Exposure time (hour)	Potency Ratio and 95% confidence limit intervals Static Test / Continuous Flow Test
24	1.03 (0.76-1.39) $P < 0.05$
48	1.05 (0.77-1.44) $P < 0.05$

Note: The potency ratios were based on the average values of 24 and 48 hours LC50s obtained from two static tests and three continuous flow tests.

Table 11. The physico-chemical characteristics of test solutions measured during the test in the Continuous Flow System (Means ± standard deviation, N = 5).

Parameter	Test	Day 1	Day 2	Day 3	Day 4
Temperature (°C)	1	24.5 (± 0.45)	23.8 (± 0.41)	24.5 (± 0.45)	24.1 (± 0.20)
	2	23.8 (± 0.40)	23.0 (± 0.60)	23.0 (± 0.70)	22.5 (± 0.60)
	3	21.2 (± 0.26)	21.5 (± 0.45)	22.2 (± 0.50)	22.1 (± 0.55)
Dissolved Oxygen (mg/ℓ)	1	7.03 (± 0.60)	6.67 (± 0.58)	5.57 (± 0.79)	5.45 (± 1.35)
	2	6.35 (± 0.99)	6.75 (± 0.56)	7.03 (± 0.58)	6.67 (± 0.87)
	3	7.43 (± 0.23)	7.30 (± 0.75)	7.15 (± 0.63)	6.66 (± 1.07)
pH	1	3.95 (± 0.22)	3.91 (± 0.18)	3.50 (± 0.26)	3.82 (± 0.46)
	2	5.34 (± 0.81)	4.64 (± 0.72)	5.13 (± 1.12)	5.00 (± 1.12)
	3	8.75 (± 0.15)	8.80 (± 0.12)	9.41 (± 0.27)	9.55 (± 0.07)
Total Hardness (mg $CaCO_3$/L)	1	45.71 (± 3.56)	43.21 (± 1.47)	41.88 (± 4.67)	42.21 (± 6.56)
	2	41.04 (± 4.82)	43.71 (± 4.50)	44.71 (± 3.03)	41.04 (± 4.69)
	3	48.71 (± 0.36)	45.71 (± 5.32)	50.52 (± 5.11)	61.66 (± 9.80)
Total ammonia (mg/L)	1	-	-	-	0.040 (± 0.002)
	2	-	-	-	-
	3	-	-	-	-

The results of the above toxicity tests of cypermethrin strongly suggest the loss of the bioactivity of this material or the reduction of its initial concentration in the test solutions. It has been pointed by Stephenson (1982) that synthethic pyrethroids have a very low solubility in water (5×10^{-3} to 10×10^{-3} mg/L) and a strong tendency to be adsorbed onto surfaces. The cypermethrin may have been adsorbed onto the surfaces of the test containers or adsorbed onto organic matter during the course of the test, resulting in a reduction of its toxic effects on fish. The lower toxicity in static tests may also have been due to the removal and metabolism of the test material by the fish. However, specific

information about this effect with regard to cypermethrin is not available. It was also observed during the experiment that fish mortality was more rapid in the continuous-flow system relative to the static system in the same level of concentration, perhaps due to the constant availability of the insecticide in this system to enter the fish vascular system via the gills. This result is in accordance with similar experiments by Lincer *et al.* (1970) in determining the toxicity of DDT and Endrin. These investigators stated that toxicity of DDT and Endrin to fish are greater in a continuous-flow system than a the static system.

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Stephenson (1982) obtained a 96-hour value of 0.0022 ppm for technical cypermethrin containing more than 95% cypermethrin, using *Oreochromis niloticus* as a test fish in a continuous-flow system. This value is about 15 times lower than the value obtained from the present test, if calculated based on the active ingredient of cypermethrin in the test material. This large difference in results may be due to the different test material used or experimental method. According to Alabaster (1969) it is impossible in most instances to deduce LC50 values from concentrations of the active constituent, as the other components may themselves be toxic, or may reduce the toxicity of the pesticides. Variation of the results may also have been due to different testing conditions. One of the test conditions which was shown to influence toxicity was water pH, with toxicity being greater in acidic conditions. This result may be due to fish in acid water being stressed by the relatively low pH. It is well known that additional stress on a fish which is already stressed can cause mortality (Wedemeyer *et al.*, 1984). It is also possible that pH may influence the toxicity of pesticide itself (Muirhead-Thomson, 1972, and Alabaster, 1969). The results of the experiment suggest, however, that water quality in the field will play an important rule in the toxicity of cypermethrin to fish.

CONCLUSION

The results of these experiments showed that the insecticide cypermethrin is highly toxic to the nile tilapia. The experiments also confirmed that substantial improvements in determining more accurately the toxicity of cypermethrin could be made by employing a continuous-flow test system, relative to a static system. Accurate information from toxicity tests can be used in (a) prediction of environmental effects of pesticides, (b) comparison of potential hazard of pesticides to aquatic organisms, and (c) regulation of the usage of pesticides.

It is therefore advantageous to obtained toxicological informations of all new pesticides, before the materials are to be recommended for their use in agricultural pest control, especially in rice plants.

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