Research Paper

# Toxicity of cypermethrin to the juveniles of freshwater fish *Poecilia reticulata* (Peters) in relation to selected environmental variables

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**Abstract**

Toxicity of cypermethrin, a synthetic pyrethroid insecticide, was evaluated for the juveniles (8.5± 1.5 mm) of a freshwater fish, *Poecilia reticulata* (Peters) in relation to selected environmental variables such as temperature, hardness, *p*H and salinity. The $LC_{50}$'s were found to change significantly with the change in temperature, hardness, *p*H and salinity of water. The juveniles were found more susceptible to cypermethrin at low water hardness (270±1 mg/l) and low water *p*H (5.4) as compared to other variables. Based on $LC_{50}$'s the order of toxicity of cypermethrin to juveniles of *Poecilia* was observed as: *p*H <salinity <hardness < temperature. The range of safe dischargeable concentrations of cypermethrin (1.04-1.09 ppb) was too low as compared to harmless or safe concentrations (45.18-75.25 ppb) for the juveniles at selected environmental variables.

**Keywords:** Toxicity, Cypermethrin, Freshwater fish, *Poecilia reticulata*, Environmental variables.

**IPC code; Int. cl.[8]—** A01K 61/00, A01K 63/04, A01N 25/00

*Poecilia reticulata*

## Introduction

Cypermethrin (pyrethroid) is a widely used synthetic insecticide and has been found highly toxic for fish populations and aquatic invertebrates[1-4]. It is used in cotton, cereals, vegetables, fruits, food storage and in animal husbandry. Reports are also available on the toxicity of cypermethrin on the early stages of fish[5-7]. Further, activities of enzymes such as Glutamic oxaloacetic acid transaminase (GOT), Glutamic pyruvic acid transaminase (GPT) and Lactate dehydrogenase (LDH) have also changed marginally due to cumulative effects of cypermethrin in heart, liver and kidney of *Cyprinus carpio* Linn.[8] However, reports on cypermethrin toxicity in relation to environmental variable on early stages of fish are not available. In view of this, an attempt has been made to assess the effects of selected environmental variables, viz. temperature, hardness, *p*H and salinity on the short-term (96 h) toxicity of cypermethrin to the juveniles of a freshwater fish *Poecilia reticulata* (Peters) considering various parameters such as median lethal concentration ($LC_{50}$), safe or harmless concentration and safe dischargeable concentration.

## Materials and Methods

Short-term (96 h) static bioassays for cypermethrin to juveniles were conducted at selected environmental variables such as temperatures (16.5±1 and 25±1°C), hardness (270±1 and 660±5 mg/l), *p*H (5.4, 7.6 and 9.1) and water salinities (0.53, 1.18 and 1.54%) following standard methods of APHA[9]. The water having different hardness values, i.e. 270±1 and 660±5 mg/l were collected from public supply and bore well, respectively. The water of different *p*H, i.e. 5.4 and 9.1 were prepared by adding HCl and NaOH, respectively. Also the water of different salinity, i.e. 1.18 and 1.54% were prepared by adding common salt (NaCl). The *p*H and salinity of normal water were estimated as 7.6 and 0.53%, respectively. The experimental water were analysed for selected physico-chemical characteristics as per standard methods of APHA[9]. Glass jars of one litre capacity were used for conducting static bioassay tests. The juveniles (8.5± 1.5 mm) produced from healthy males and females of *Poecilia* were maintained in 100 litre capacity of plastic tank on artificial diet (rice bran and ground nut cake mixture - 1:1).

The pesticide cypermethrin marketed and manufactured by Crystal Phosphate Limited (jakpot 25 EC) was used in the present investigation. To commence with assays for cypermethrin a common stock solution was prepared by dissolving appropriate amount in one litre of deionized diluent water as per the following formula: $N_1V_1 = N_2V_2$ [where $N_1$-concentration of commercial formulation in per cent, $V_1$-volume or amount of commercial formulation required in ml, $N_2$-desired concentration of formulation required in ml, $V_2$- volume or amount of solution required in ml]. The series of different nominal concentrations of cypermethrin (as ppb) based on the progressive bisection of intervals on a logarithmic scale were prepared by adding stock solution into the measured diluent's water[9].

The experimental units, i.e. 1 litre l glass jars were filled with toxicant solutions and placed in three rows. Full scale experiments were conducted in triplicate by testing various concentrations in the range known by preliminary exploratory test. Each glass jar was labelled with the details such as concentration, water quality variables, replicate number, date and time of the experiment. Ten acclimatized healthy juveniles of size (8.5± 1.5 mm) were transferred to these jars after about 20 minutes of the preparation of test solution. Proper controls were run simultaneously. The experiments were continued for a period of 96h. The number of dead juveniles in each concentration of cypermethrin were observed carefully and recorded at the time intervals of 24, 48, 72 and 96h. The $LC_{50}$'s of cypermethrin for juveniles in relation to each environmental variable were estimated for the time intervals of 24, 48, 72 and 96h by probit analysis[10]. Presumable safe or harmless and safe dischargeable concentrations of cypermethrin were calculated by using the formula of Hart *et al*[11].

## Results and Discussion

The results revealed that cypermethrin is highly toxic to the juveniles of *Poecilia* as the $LC_{50}$ values were noticed in ppb. The $LC_{50}$'s were also found to change significantly with the change in temperature, hardness, *p*H and salinity (Table 1). The safe concentrations of cypermethrin for the juveniles were different for each selected environmental variables. The bioassays conducted at water of higher hardness (660±5 mg/l), moderate *p*H (7.6) and normal salinity (0.53%) at a temperature of 16.5±1°C showed slightly higher values of harmless concentrations (75.25 ppb) as compared to that of bioassays conducted at higher temperature (25±1°C; 56.10 ppb), hardness (270±1 mg/l; 45.62 ppb), both acidic and alkaline *p*H (5.4 and 9.1; 45.18 and 47.45ppb) and higher salinity levels (1.18 and 1.54%; 57.29 and 50.31 ppb). The calculated safe dischargeable concentrations of cypermethrin were recorded too low as compared to safe harmless concentrations (Table 1).

**Table 1 : Toxicity of cypermethrin for the juveniles of *Poecilia reticulata* (Peters) in relation to temperature, hardness, *p*H and salinity**

| Environmental variables | Durations (h) | $LC_{50,\,s}$ (ppb) | 95% confidence limit (UCL-LCL) | R = UCL-LCL | Heterogeneity factor | Safe or harmless concentration (as ppb) | Safe dischargeable concentrations (as ppb) |
|---|---|---|---|---|---|---|---|
| **Temperature** | | | | | | | |
| **a. 16.5±1°C** | 24 | 316.59 | 478.10-209.64 | 2.28 | 0.22 | | |
| | 48 | 281.71 | 351.13-226.01 | 1.55 | 0.25 | 75.25 | 1.06 |
| | 72 | 248.96 | 309.81-200.07 | 1.54 | 0.31 | | |
| | 96 | 221.96 | 264.03-186.60 | 1.41 | 0.30 | | |
| **b. 25±1°C** | 24 | 219.83 | 240.86-200.64 | 1.20 | 25.82 | | |
| | 48 | 202.72 | 228.37-179.95 | 1.26 | 0.75 | 56.10 | 1.04 |
| | 72 | 186.22 | 210.93-164.41 | 1.28 | 0.28 | | |
| | 96 | 168.07 | 191.94-147.16 | 1.30 | 0.44 | | |

| Environmental variables | Durations (h) | $LC_{50, s}$ (ppb) | 95% confidence limit (UCL-LCL) | R = UCL-LCL | Heterogeneity factor | Safe or harmless concentration (as ppb) | Safe dischargeable concentrations (as ppb) |
|---|---|---|---|---|---|---|---|
| **Hardness** | | | | | | | |
| **a. 270±1 mg/l** | 24 | 184.06 | 200.23-169.19 | 1.18 | 17.23 | | |
| | 48 | 167.28 | 189.45-147.71 | 1.28 | 0.05 | 45.62 | 1.05 |
| | 72 | 155.55 | 171.59-141.01 | 1.21 | 0.06 | | |
| | 96 | 148.80 | 161.91-136.75 | 1.18 | 0.66 | | |
| **b. 660±5 mg/l** | 24 | 316.59 | 478.10-209.64 | 2.28 | 0.22 | | |
| | 48 | 281.71 | 351.13-226.01 | 1.55 | 0.25 | 75.25 | 1.06 |
| | 72 | 248.96 | 309.81-200.07 | 1.54 | 0.31 | | |
| | 96 | 221.96 | 264.03-186.60 | 1.41 | 0.30 | | |
| ***p*H** | | | | | | | |
| **a. 5.4** | 24 | 179.65 | 200.18-161.23 | 1.24 | 0.82 | | |
| | 48 | 164.47 | 180.93-149.51 | 1.21 | 4.02 | 45.18 | 1.04 |
| | 72 | 160.64 | 181.02-142.55 | 1.26 | 0.78 | | |
| | 96 | 148.89 | 171.33-124.40 | 1.32 | 0.86 | | |
| **b. 7.6** | 24 | 316.59 | 478.10-209.69 | 2.28 | 0.22 | | |
| | 48 | 281.71 | 351.13-226.01 | 1.55 | 0.25 | 75.25 | 1.06 |
| | 72 | 248.96 | 309.81-200.07 | 1.54 | 0.31 | | |
| | 96 | 221.96 | 264.03-186.60 | 1.41 | 0.30 | | |
| **c. 9.1** | 24 | 190.47 | 215.47-168.37 | 1.27 | 0.29 | | |
| | 48 | 173.53 | 195.43-154.09 | 1.26 | 0.39 | 47.45 | 1.05 |
| | 72 | 160.63 | 179.13-144.03 | 1.24 | 5.71 | | |
| | 96 | 153.34 | 178.30-131.86 | 1.35 | 5.23 | | |
| **Salinity** | | | | | | | |
| **a. 0.53%** | 24 | 316.59 | 478.10-209.64 | 2.28 | 0.22 | | |
| | 48 | 281.71 | 351.13-226.01 | 1.55 | 0.25 | 75.25 | 1.06 |
| | 72 | 248.96 | 309.81-200.07 | 1.54 | 0.31 | | |
| | 96 | 221.96 | 264.03-186.60 | 1.41 | 0.30 | | |
| **b. 1.18%** | 24 | 241.78 | 338.29-172.80 | 1.95 | 0.08 | | |
| | 48 | 214.85 | 265.36-173.95 | 1.52 | 0.36 | 57.29 | 1.06 |
| | 72 | 188.59 | 235.91-150.77 | 1.56 | 0.12 | | |
| | 96 | 168.37 | 238.03-119.09 | 1.99 | 0.07 | | |
| **c. 1.54%** | 24 | 236.31 | 407.98-136.87 | 2.98 | 0.06 | | |
| | 48 | 199.06 | 249.02-159.12 | 1.56 | 0.17 | 50.31 | 1.09 |
| | 72 | 184.03 | 212.59-159.30 | 1.33 | 0.64 | | |
| | 96 | 161.61 | 179.32-145.65 | 1.23 | 5.15 | | |

$LC_{50s}$ - Median lethal concentrations; UCL - Upper confidence limit; LCL - Lower confidence limit; R- Ratio of confidence limits

Characteristics of the experimental water revealed that they were similar to the standard values under natural conditions as they did not contain any toxic substances. However, few changes have been observed in some of the characteristics of water such as hardness, total alkalinity, dissolved oxygen and electrical conductivity (Table 2).

Polat *et al*[6] have also investigated the acute toxicity of beta-cypermethrin and estimated 48h $LC_{50}$ value for guppy (*P. reticulata*) as 21.4 µg/l at a temperature of 22 ± 1°C. Whereas, Aguigwo[5] has recorded much higher 96h $LC_{50}$'s value of cypermethrin as 4.17 mg/l for *Clarias gariepinus* and also found that mortality increased with increase in concentrations. They however, observed that number of dead embryos of the common carp (*C. carpio*) significantly increased in response to even very low cypermethrin concentrations of 0.0001, 0.001, 0.01, 0.1, 1, 2, 4 and 8 µg/l and estimated the 48 h $LC_{50}$'s (with 95% confidence limits) and 96h $LC_{50}$'s (with 95% confidence limits) of cypermethrin for common carp embryos and larvae as 0.909 (0.256-5.074) and 0.809 (0.530-1.308) µg/l, respectively[7]. Wang *et al*[4] determined the $LC_{50}$ of cypermethrin at 96h for carp as 12.6µg/l. However, Collins and Cappello[3] have recorded the $LC_{50}$'s of cypermethrin to freshwater prawn *Palaemonetes argentinus* as 0.0031 and 0.0020µg/l for 24 and 96h, respectively. The present investigation is in agreement with the above findings as the developing stage, viz. juveniles of *Poecilia* showed highly susceptibility to even very low concentrations of cypermethrin at all selected environmental variables. The results of the present study also revealed that changes in the selected water quality parameters, viz. temperature, hardness, *p*H and salinity played significant role in intoxication of cypermethrin to the juveniles of *Poecilia* as considerable difference in $LC_{50}$'s was noticed for different durations. Vardia and Durve[12] also found 7-7.5 fold increase in toxicity of 2,4-D to *C. carpio* with the rise in temperature from 17ºC (Winter) to 39ºC (Summer). Whereas Baer *et al*[13] have noticed that the 96h $LC_{50}$ of profenophos to fathead minnows (*Pimephales promelas*) at higher temperature of 30 ± 2ºC was significantly lower as 21.5µg/l as compared to 333µg/l at a lower temperature of 20 ± 2ºC. More or less similar results have been observed in the present study as the higher 96h-$LC_{50}$ (221.96ppb) of cypermethrin to the juveniles was observed at a temperature of 16.5±1ºC as compared to lower 96h $LC_{50}$ (168.07 ppb) at a temperature of 25 ± 1°C. Cairns *et al*[14] also opined that temperature influences toxicity of chemicals to aquatic organisms. Whereas, Hashimoto and Nishiuchi[15] have reported that the majority of the pesticides are more toxic to carps and daphnids at higher temperatures, but some including folpet and DDT are less toxic at higher temperatures.

**Table 2 : Physico-chemical characteristics of the water used in static bioassays**

| Characteristics | Borewell water | | Public supply water | With change in *p*H of water | | With change in salinity of water | |
|---|---|---|---|---|---|---|---|
| | Winter | Summer | | A | B | A | B |
| Water temperature (ºC) | **16.5±1** | **25±1** | 16.5±1 | 16.5±1 | 16.5±1 | 16.5±1 | 16.5±1 |
| *p*H | **7.6** | 7.7 | 7.8 | **9.1** | **5.4** | 7.9 | 8.0 |
| Dissolved oxygen (mg/l) | 6.5 | 5.2 | 6.4 | 6.2 | 6.1 | 5.8 | 5.6 |
| Total alkalinity (mg/l) | 600 | 540 | 340 | 420 | 380 | 500 | 450 |
| Hardness (mg/l) | **660±5** | 655±5 | **270±1** | 665±5 | 657±5 | 662±5 | 666±5 |
| Salinity (%) | **0.53** | 0.54 | 0.4 | 0.54 | 0.51 | **1.18** | **1.54** |
| Electrical conductivity (µS) | 2600 | 2700 | 1600 | 4800 | 5000 | 5400 | 6300 |

Values are S.E.; n=10

The present results are in agreement with the above findings as low $LC_{50}$'s of cypermethrin for the juveniles were recorded at a higher temperature of 25 ±1°C. Further, results of the present study also revealed that hardness of water plays a significant role in determining the toxicity of cypermethrin to juveniles of *Poecilia*. According to McCann and Hitch[16] the toxicity of aquazine has been found to be highly significant in both soft and hard water to striped bass (*Morone saxatilis*) and reported a common 96h $LC_{50}$ as 180 mg/l. In the present study sensitivity of the juveniles of *Poecilia* to cypermethrin was decreased considerably with the increase in hardness of water. Datta *et al*[17] observed that the hardness of water significantly reduced the toxicity of the pesticide deltamethrin to scale carp fry (*C. carpio* var. *communis*). According to them the 96h $LC_{50}$ values of deltamethrin, which was observed as 0.102 mg/l in soft water, increased to 0.8 mg/l in hard water. Further, Donald *et al*[18] have observed that decrease in water hardness associated with decrease in alkalinity and *p*H also increased the toxicity of mixture of inorganic and organic contaminants. The same results have been obtained in the present study as the toxicity of cypermethrin to juveniles decreased subsequently with the increase in water hardness. Khillare and Wagh[19] have reported that $LC_{50}$ values of endosulfan 35 EC for 96h increases with *p*H and alkalinity. The present results are also in agreement with the findings of Wang *et al*[20], who suggested that salinity of water significantly enhanced aldicarb toxicity, cholinestarase inhibition and 14C aldicarb sulfoxide formation in rainbow trout (*Oncorhynchus mykiss*). Abir *et al*[21] have reported that environmental factors, such as salinity in addition to gender and development have significant impacts on the acute toxicity of aldicarb to Japanese medaka (*Oryzias latipas*). They also found that after 48h of aldicarb exposure, mortality of Japanese medaka was significantly increased ($P<0.05$) in males from 13 ± 5.7% at 1.5 % salinity to 56 ± 5.7% at 20% salinity. In females also the mortality significantly increased ($P<0.01$) from 17 ± 5.7 to 76 ± 5.6% at the same water salinity. Further, Evelyn *et al*[22] have also reported that the toxicity of organophosphorus insecticides increased with higher salinity.

In the present investigation, range of presumable safe or harmless concentrations and dischargeable concentrations of cypermethrin for the juveniles are interesting and significant since they are not constant with the succeeding experiments conducted for the selected environmental variables. More or less similar pattern of safe concentrations have been reported by other workers[7, 23-25].

## Conclusion

The result suggests that cypermethrin is highly toxic to the juveniles of *P. reticulata* at selected environmental variables (*p*H<salinity <hardness <temperature). It is also recommended that before using cypermethrin in any aquaculture processes, the estimated safe and dischargeable concentrations should be considered important to protect living organisms as well as fish.

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