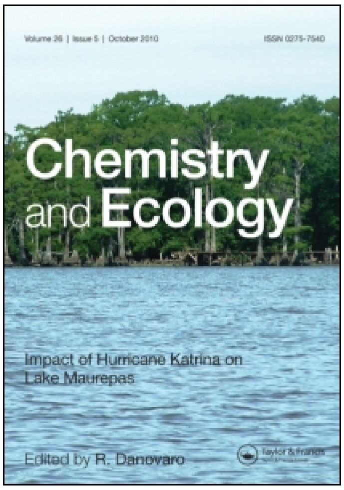

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Effect of cypermethrin on breeding performances of a freshwater fish, *Labeo rohita* (Hamilton)

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The effect of cypermethrin, a synthetic pyrethroid, on the breeding performances in the freshwater fish, *Labeo rohita* (Hamilton), has been investigated in the present study. Breeding of *Labeo rohita* was conducted after treating with three sublethal concentrations of cypermethrin, i.e. 0.16, 0.40, and 0.80 $\mu l\,l^{-1}$, respectively, for 96 h. The present investigation shows that the significant reduction ($P < 0.01$) of the total number of eggs, total amount of egg (litre), total amount of egg (litre per kilogram of body weight), fertilization percentage, and expected fertilized egg number at the concentrations of 0.40 and 0.80 $\mu l\,l^{-1}$ of cypermethrin. Also, the reduction in hatching percentage, expected number of hatchling, and expected number of hatched larvae were significantly different ($P < 0.01$) between the treatment and the control at all cypermethrin concentrations. No significant differences for the 96 h survivability of hatched larvae were reported at 0.16 and 0.40 $\mu l\,l^{-1}$ levels of cypermethrin, whereas significant differences ($P < 0.05$) were reported at 0.80 $\mu l\,l^{-1}$.

Keywords: Breeding; *Labeo rohita*; Cypermethrin

1. Introduction

Pesticides are a serious threat for non-target fauna, including fish which might be considered one of the major components of the aquatic habitats and a good source of protein for human. The pesticides sprayed on different crops are subsequently drained off to different water bodies, where the fishes live and breed. The indiscriminate use of pesticide in modern agriculture, forestry, and sanitation has caused different types of damage to non-target organisms [1].

In India, cypermethrin (cyanomethyl, 2.2-dimethyl, cyclopropane-carboxylate), a synthetic pyrethroid pesticide, has a wide range of applications in agriculture as a very effective systemic insecticide. The most common use is in controlling pests of cotton plants (*Gossipium borbedum*) and/or in controlling argulus (*Argulus* sp.) in fish. The toxic effects of sublethal concentrations of organochlorne and organophosphate pesticides to fish are

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well documented [2–5]. However, literature regarding the effect of pesticides on breeding performance, especially of Indian Major Carps (IMC), is practically non-existent and limited to catfishes and murrel [6–8]. In the present study, the sublethal effects of cypermethrin on the breeding performances of *Labeo rohita* (Hamilton), an important IMC, have been investigated.

2. Materials and methods

Breeding experiments were conducted taking *Labeo rohita* (Hamilton), rohu male and female brood stocks, collected from Krishi Vigyan Kendra (KVK) stocking ponds of the Institute. Average weight of the fishes ranged from 0.400 to 1.5 kg. The experimental process is discussed in the following subsections.

2.1 *Experimental design*

Only female rohu brood fishes were treated with pesticides, and one fish was maintained in each of 1000 l polyfibre tanks. The breeding experiments were conducted in the breeding season of 2001 using three sublethal concentrations of cypermethrin, i.e. 0.16, 0.40, and 0.80 $\mu l\,l^{-1}$. The sub-lethal concentrations were selected on the basis of 1/100, 1/40, and 1/20th of the 96 h LC_{50} value of *Labeo rohita* for cypermethrin, which was 16 $\mu l\,l^{-1}$ [9]. Fishes not treated with pesticides were considered as controls. All analyses were made using three replicates and were performed for 96 h.

2.2 *Breeding*

At the end of the treatments, fishes were transferred to KVK stocking ponds for breeding. Breeding was done in three separate breeding hapas and instituted in the morning of that day. The breeding programme started in the afternoon. Ovaprim was used as the inducing agent with a standard dose developed at the Institute (females: 0.5 ml kg^{-1} body wt.; males: 0.2 ml kg^{-1} body wt.). For each female, two males of equivalent weight were supplied. Males were harvested just before the injection. Weights of individual fish were measured at the same time. Fishes were brought to breeding hapa after injecting with proper doses of ovaprim. They spawned within 6–8 h. Then, all the fish were taken out and weighed again. Eggs were also collected from breeding hapa, and the quantity (litre) and number measured. Eggs were transferred to previously installed outer and inner hapa for further development, and some samples were carried to the laboratory to study the further development through binocular microscopy and constant monitoring.

2.3 *Post-breeding development and sampling*

Fertilization number and percentage were counted by placing a hundred eggs into each Petri dish. Three replications were maintained. Embryos were then kept for hatching, with the water being replaced every 1 h, and embryonic development was observed under a microscope. The hatching percentage was counted for all three replications. Hatched larvae were maintained properly up to 96 h. After 96 h, survivability number and percentage were calculated. Surviving larvae were transferred to pond conditions. The pH, alkalinity, hardness, conductivity, temperature, and calcium in the water were measured following standard methods [10].

2.4 *Statistical analysis*

Statistical analysis of data using ANOVA (Duncan's multiple range test) was performed to determine the level of significance among different concentrations of cypermethrin on the breeding performance of *Labeo rohita*.

3. Results

The effect of sublethal concentrations of cypermethrin on the breeding performance of *Labeo rohita* during 96 h of exposure is presented in table 1. The reduction in total egg number, total amount of egg (litre), total amount of egg ($l\,kg^{-1}$), fertilization percentage, and expected fertilized egg number at 0.40 and 0.80 $\mu l\,l^{-1}$ concentrations of cypermethrin were statistically significant ($P < 0.01$), while the reduction in total amount of egg (litres), total amount of egg ($l\,kg^{-1}$) and expected fertilized egg number at 0.16 $\mu l\,l^{-1}$ of cypermethrin showed no significant difference compared with the control.

The reduction in hatching percentage, expected number of hatching, and expected number of hatched larvae exhibited a significant statistical difference ($P < 0.01$) at all the tested concentrations of cypermethrin compared with the control (table 2). The 96 h survivability of hatched larvae showed no significant difference between the treatment and the control at 0.16 and 0.40 $\mu l\,l^{-1}$ levels of cypermethrin, whereas the result was found to be statistically significant ($P < 0.05$) at 0.80 $\mu l\,l^{-1}$ concentration of cypermethrin.

The water-quality parameters during breeding of *Labeo rohita* exposed to sublethal concentrations of cypermethrin are reported in table 3. The water-quality parameters, viz. hardness, total alkalinity, and calcium, were similar in all different breeding experiments of *Labeo rohita* exposed to sublethal concentrations of cypermethrin.

4. Discussion

The present investigation showed that the cypermethrin concentrations significantly decreased the egg production in terms of number and amount, and also the fertilization percentage. The reduction in fecundity and fertilization of eggs after cypermethrin treatment is in accordance with the results of inhibition in fertilization of eggs of *Heteropneustes fossilis* treated with malathion, an organophosphate pesticide [4]. The results of the present study are also highly corroborated with the investigation of brook trout treated with DDT [11], and sheepshead minnows treated with chlordecone [12]. Also, survival of developing eggs of carp (*Cyprinus carpio* Linn.) decreased with different pesticides [13]. Pesticide vitavax 200 EF greatly affected the reproductive endocrine functions of captive mallards, *Anas platyrhyachos*, and reduced their egg production [14]. Egg production was reduced with an orally administered mixture of selected PCBs in zebrafish (*Danio rerio*) [15].

The reduction in egg production may be due to cypermethrin-induced degenerative changes in the ovaries. The histopathological studies of ovaries of *Labeo rohita* at the spawning phase treated with 0.40 $\mu l\,l^{-1}$ of cypermethrin for 28 d showed an intense atresia of vitellogenic degeneration of follicular wall and ooplasm, clumped cytoplasm, mature ovaries in a stage of regression while at the pre-spawning stage, a reduction in size and deformity of oocytes, necrosis in ooplasm, disorganized nucleus, and degeneration of follicular wall [9]. The author also reported that medium atresia, increase in inter-follicular space, and the degeneration of the follicular wall were observed ooplasm of the ovary of *Labeo rohita* at the post-spawning phase treated with 0.40 $\mu l\,l^{-1}$ of cypermethrin for 28 d. The spawning phase showed maximum

Table 1. Effects of sublethal concentrations of cypermethrin on breeding performance of *Labeo rohita* after a 96 h exposure period.

Doses of cypermethrin	Body wt. (pre-breeding) (kg)	Ovaprim doses (ml)	Body wt. of female (post-breeding) kg	Total amount of egg (1)	Total egg number	Total amount of egg ($l\,kg^{-1}$ body wt.)	Percentage inhibition from control ($l\,kg^{-1}$ body wt.)	Fertilization percentage	Expected fertilized egg number
Control (without pesticide)	Female 0.450 ± 0.04	0.2	0.250 ± 0.04	1.50 ± 0.16	42 900 ± 1297	3.75 ± 0.14	0	92 ± 1.63	39 296 ± 2271
	Male 0.400 ± 0.03	0.1							
	Male 0.470 ± 0.06	0.1							
Cypermethrin at $0.16\,\mu l\,l^{-1}$	Female 0.600 ± 0.1936	0.3	0.400 ± 0.10	1.9 ± 0.28	47 500 ± 686	3.16 ± 0.51	15.73 ± 1.90	80 ± 3.87	38 000 ± 1745
	Male 0.400 ± 0.07	0.1							
	Male 0.600 ± 0.125	0.12							
Control (without pesticide)	Female 1.6 ± 0.208	0.8	1.20 ± 0.79	19 ± 2.08	450 680 ± 290	11.87 ± 0.74	0	96 ± 3.65	432 653 ± 886
	Male 1 ± 0.271	0.2							
	Male 1.10 ± 0.233	0.2							
Cypermethrin at $0.40\,\mu l\,l^{-1}$	Female 1 ± 0.24	0.5	0.80 ± 0.20	5.5 ± 1.20	170 720 ± 2141	5.5 ± 0.49	53.66 ± 3.13	79 ± 4.80	134 896 ± 1048
	Male 0.800 ± 0.12	0.16							
	Male 0.600 ± 0.06	0.12							
Control (without pesticide)	Female 1.5 ± 0.331	0.75	1.1 ± 0.21	23 ± 2.58	542 800 ± 1719	15.33 ± 1.71	0	89 ± 2.58	483 092 ± 2764
	Male 1.3 ± 0.369	0.26							
	Male 1.0 ± 0.351	0.20							
Cypermethrin at $0.80\,\mu l\,l^{-1}$	Female 1.5 ± 0.28	0.75	1.3 ± 0.24	8.50 ± 0.49	208 080 ± 1274	5.67 ± 1.16	63.01 ± 4.68	70 ± 3.69	145 656 ± 2866
	Male 1.0 ± 0.12	0.20							
	Male 0.7 ± 0.08	0.14							

Table 2. Effects of sub-lethal concentrations of cypermethrin on hatching performance of *Labeo rohita* after breeding.

Type of sampling	Hatching percentage	Expected number of hatchling	96 h survivability percentage of hatched larvae	Expected number of hatched larvae after 96 h	Expected number of hatched larvae per kilogram of body wt. of fish	Total inhibition percentage from control (from egg production to larvae)
Control (without pesticide)	90 ± 2	35 366 ± 247	88 ± 2	31 122 ± 911	77 805 ± 441	0
Cypermethrin at 0.16 $\mu l\ l^{-1}$	68 ± 5	25 840 ± 930	80 ± 5	20 672 ± 690	34 453 ± 451	55.71 ± 2.5
Control (without pesticide)	94 ± 2	406 694 ± 2591	92 ± 3	374 158 ± 3641	233 848 ± 1862	0
Cypermethrin at 0.40 $\mu l\ l^{-1}$	61 ± 5	82 270 ± 2695	85 ± 4	69 929 ± 1731	69 929 ± 1731	70.09 ± 3.2
Control (without pesticide)	88 ± 3	425 120 ± 3348	84 ± 4	357 100 ± 6113	238 066 ± 3532	0
Cypermethrin at 0.80 $\mu l\ l^{-1}$	58 ± 4	84 480 ± 2809	74 ± 4	62 515 ± 286	41 676 ± 1343	82.49 ± 3.44

Table 3. Variations in water-quality parameters during breeding of *Labeo rohita* exposed to sublethal concentrations of cypermethrin for 96 h.

Period of sampling	pH	Hardness (mg l^{-1}) as $CaCO_3$	Total alkalinity (mg l^{-1}) as $CaCO_3$	Conductivity (mmho cm^{-1})	Temperature (°C)	Ca^{2+} (mg l^{-1})
1. *During treatment*						
A. Cypermethrin at 0.16 µl l^{-1}	7.5 ± 0.47	192 ± 3.87	283 ± 4.93	0.60 ± 0.44	28.2 ± 0.50	76 ± 5.21
B. Control	7.8 ± 4.29	186 ± 4.39	280 ± 3.87	0.64 ± 0.38	28.1 ± 0.33	74 ± 4.08
2. *During breeding*						
A. Pre-breeding	7.4 ± 0.25	92 ± 3.36	136 ± 3.82	0.25 ± 0.38	28.3 ± 0.38	36 ± 5.16
B. Post-breeding	7.8 ± 0.36	96 ± 3.56	138 ± 3.92	0.26 ± 0.39	28.5 ± 0.397	38 ± 5.38
1. *During treatment*						
A. Cypermethrin at 0.40 µl l^{-1}	7.8 ± 0.36	198 ± 5.16	336 ± 6.24	0.63 ± 0.41	29.1 ± 0.45	79 ± 4.39
B. Control	7.4 ± 0.33	192 ± 5.01	310 ± 5.98	0.61 ± 0.40	29.2 ± 0.45	76 ± 4.21
2. *During breeding*						
A. Pre-breeding	7.5 ± 0.33	104 ± 6.7	134 ± 5.77	0.24 ± 0.071	29.1 ± 4.9	41 ± 3.82
B. Post-breeding	7.8 ± 0.34	104 ± 6.91	162 ± 6.13	0.24 ± 0.72	29.5 ± 5.2	41 ± 3.1
1. *During treatment*						
A. Cypermethrin at 0.80 µl l^{-1}	7.5 ± 0.34	176 ± 4.76	274 ± 7.74	0.59 ± 3.89	29.5 ± 0.65	70 ± 4.02
B. Control	7.9 ± 0.53	180 ± 4.93	288 ± 5.68	0.59 ± 0.046	29.1 ± 0.41	72 ± 4.65
2. *During breeding*						
A. Pre-breeding	7.5 ± 0.28	108 ± 3.10	142 ± 7.30	0.22 ± 0.041	28.5 ± 0.25	43 ± 3.109
B. Post-breeding	7.6 ± 0.35	112 ± 3.87	156 ± 6.58	0.21 ± 0.038	28.7 ± 0.402	44 ± 2.9

ovarian damage due to cypermethrin exposure, while the pre-spawning phase was the next impaired stage. It seems that a hormonal imbalance especially with the levels of gonadotropin and oestrogen used, plays an important role. Cypermethrin was capable of reducing fecundity and finally the fertility of fish by not allowing all oocytes to grow fully and also by causing atresia of growing follicles. It should also be mentioned that the alteration in ovarian activity due to insecticides may also be influenced by the physiological, metabolic, and cellular energy status of the ovary of the fish. The physiological mechanism of this reduced egg production appears to be a failure of synthesis of yolk proteins and their subsequent deposition in the egg. Thus, any such eggs produced may have a greatly reduced yolk content. Normally, vitellogenins from the liver are transported to the ovary as a complex of calcium, phosphoprotein, and lipid [16]. The process requires sufficient calcium in the blood, so that in fish forming eggs, a marked elevation in serum calcium is brought about by oestradiol [17]. Female rohu from insecticide-stressed populations which had produced fewer eggs could be due to a failure to show these normal increases in serum calcium. Therefore, this seems to be the primary cause of the lower level of egg production in insecticide-exposed fish.

The present study indicated that a decreasing trend in hatching percentage in *Labeo rohita* with increasing concentrations of cypermethrin than controls, which is in agreement with the findings of the hatchability of Coho salmon eggs exposed to PCBs, was much reduced, and exposure to PCB effectively reduced the embryonic development time [18]. A concentration of $0.1\,\mu g\,l^{-1}$ PCB could have adverse effects on survival and reproduction success for Everglades fish communities [19]. The hatchability among eggs decreased with increasing concentrations of malathion in the case of *Cyprinus carpio* [13], and the results showed that 84.5% eggs hatched at 1 ppm malathion, and no hatching took place at 5 ppm of malathion. In the same study, also, the eggs incubated at lower concentrations (0.01 ppm) exhibited a greater percentage of hatching (100%). A decrease in hatching percentage in *Puntius conchonius* at higher concentrations of malathion than controls were also reported; the results also showed that the hatching percentage was 52% at 0.0015 ppm, which gradually decreased as the concentration of malathion increased, and at 0.025 ppm concentration, total (100%) egg mortality occurred [20]. The decline in hatching success and failure of hatching with higher concentrations of insecticides, particularly organochlorine compounds, may be mediated through the inhibitory effects of pesticides on hatching enzymes [21], and the larvae that hatch from these eggs were reduced in number for insecticide-treated fish compared with the control. The reduction in expected number of larvae could be due to the hatching failure of many eggs, and these eggs could be deformed because of deposition of insecticides. Residual concentration of pesticide led to a profound alteration of the early developmental stages of fish [22, 23].

Physico-chemical parameters of water, especially temperature, pH, hardness, and total alkalinity, have a significant role in the successful breeding and hatching performance of fish [24]. In the present investigation, the environmental parameters did not change significantly during the experiment and were not influenced by the concentrations of cypermethrin.

5. Conclusion

Our results indicate that the reduction in total egg number, total amount of egg (litre), total amount of eggs (litre) per kilogram of body weight, fertilization percentage, and expected fertilized egg number were statistically significant ($P < 0.01$) at a cypermethrin concentration of 0.40 and $0.80\,\mu l\,l^{-1}$. Also, the hatching percentage, expected incidence of hatching, and expected number of hatched larvae decreased significantly ($P < 0.01$) at all concentrations of cypermethrin. Finally, the 96 h survivability of hatched larvae was not significantly different at

0.16 and 0.40 $\mu l l^{-1}$ of cypermethrin, whereas significant differences ($P < 0.05$) were reported at 0.80 $\mu l l^{-1}$ of cypermethrin.

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