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Cypermethrin induced respiratory and behavioural responses of the freshwater teleost, *Labeo rohita* (Hamilton)

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ABSTRACT

A short term definitive test by the static renewal bioassay method was conducted to determine the acute toxicity (LC_{50}) of technical grade pyrethroid insecticide, cypermethrin (92.25%) in freshwater indigenous carp, *Labeo rohita*. Carp fingerlings were exposed to different concentrations (2.0 to 6.0 µg/L) of cypermethrin for 96 h. The acute toxicity value was found to be 4.0 µg/L and one fifth of LC_{50} (0.57 µg/L) was selected for sub acute studies. Behavioural patterns and oxygen consumption were studied in lethal (1, 2, 3 and 4 d) and sub lethal concentrations (1, 5, 10 and 15 d). Carp in toxic media exhibited irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and sinking to the bottom, which might be due to inactivation of (AChE) acetyl cholinesterase activity which results in excess accumulation of acetylcholine in the cholinergic synapses leading to hyperstimulation. Variation in oxygen consumption (1.289 to 17.409 %; 20.580 to 109.77 %) was observed in both lethal and sub lethal concentrations of cypermethrin respectively. Alterations in oxygen consumption may be due to respiratory distress as a consequence of impairment in oxidative metabolism. Fish in the sublethal concentration were found under stress, but that was not fatal.

Key words: cypermethrin toxicity, oxygen consumption, behaviour, *Labeo rohita*

Introduction

The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems of the century. As a result of the pollutants' transport from industrial and agricultural areas into the environment and their chemical persistence, many freshwater ecosystems are faced with spatially or temporally alarming high levels of xenobiotic chemicals (BRACK et al., 2002; DIEZ et al., 2002). The recent development of biomarkers based on the study of the response of organisms to pollutants

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has provided essential tools for the implementation of programmes for contamination monitoring (KORAMI et al., 2000).

Due to the increasing regulatory restrictions on organophosphate pesticides, pyrethroids pesticides have replaced organophosphates for many residential and agricultural uses. Cypermethrin is a synthetic pyrethroid insecticide used to control many pests, such as moth pests attacking cotton, fruit and vegetable crops, including structural pest control, or landscape maintenance. This has resulted in its discharge into the aquatic environment and consequently several laboratory studies have been performed, which have shown that cypermethrin is extremely toxic to fish and aquatic invertebrates at very low concentrations. Fish sensitivity to pyrethroids may be explained by their relatively slow metabolism and elimination of these compounds (DAVID et al., 2003).

Any change in the behaviour and physiology of fish indicates the deterioration of water quality, since fish are the biological indicators of water quality. Hence, the present study was undertaken to evaluate the aquatic toxicity of cypermethrin-based pesticides, with special emphasis on behavioural and oxygen consumption of the freshwater teleost, *L. rohita* exposed to lethal and sublethal concentrations of technical grade cypermethrin.

Materials and methods

Healthy and active *L. rohita* (3 ± 0.5 g, 6 cm) fingerlings were procured from the State Fisheries Department, Dharwad, India. The carps were acclimatized to laboratory conditions for 15 d at 24 ± 1 ºC and held in large glass aquaria containing dechlorinated tap water of the quality used in the test. The physico-chemical characteristics of the water were analyzed following the methods mentioned in (ANONYM., 1998) and found to be as follows: Temperature: 24 ± 2 ºC, pH: 7.1 ± 0.2 at 24 ºC, Dissolved oxygen: 9.3 ± 0.8 mg/L, Carbon dioxide: 6.3 ± 0.4 mg/L, Total hardness: 23.4 ± 3.4 mg as $CaCO_3$/L, Phosphate: 0.39 ± 0.002 µg/L, Salinity-nil, Specific gravity: 1.0030 and conductivity less than 10 µS cm^{-1}. The water was renewed everyday and 12-12 h of photoperiod was maintained daily during acclimation and test periods. The fish were fed regularly with oil cake and rice bran during acclimation and feeding was stopped three days prior to exposure to the test medium.

The concentrations of the test compounds used in the short term definitive tests ranged from the highest concentration at which there was 0% mortality and the lowest concentration at which there was 100% mortality in the range finding tests. Replacement of the water medium was followed by the addition of the desired dose of the test compound. The fish were exposed in batches of ten to varying concentrations of cypermethrin with 20 L of water in six replicates for each concentration along with a control. Cypermethrin (92.95%) was procured from the Herbana Industries Limited, Borivli (W) Mumbai, India. The stock solution was prepared in acetone, which was found to be non-toxic to fish. The required quantity of cypermethrin was drawn directly from this emulsified concentrate using a variable micropipette.

For LC_{50} calculation, mortality was recorded every 24 h and dead fish were removed when observed, every time noting the number of fish deaths at each concentration up to 96 h. Duncan's multiple range test (DMRT) was employed for comparing mean mortality values after estimating the residual variance by repeated measures ANOVA (WINNER, 1971) for arc sine transformed mortality data (dead individuals/initial number of individuals). Time of exposure was the repeated measure factor while treatment (concentration and control) was the second factor. In addition, LC_{50} were compared by the method of ANONYM., 1998. The LC_{50} were determined/estimated with a 95% confidence limit for cypermethrin for 96 h by probit analysis (FINNEY, 1971).

One seventh of the LC_{50} (0.57 µg/L) was selected as a sub lethal concentration for the subacute study (1, 5, 10 and 15 d). The control and cypermethrin exposed fishes were kept under continuous observation during the experimental periods. The overall animal oxygen consumption was measured for lethal and sublethal concentrations as well as the controls by following the method of WELSH and SMITH (1953) as described by SAROJA (1959).

Each experiment was repeated six times and the mean value was calculated. The data obtained were analyzed statistically by following the DMRT (Duncan multiple range test).

Results

Acute toxicity. The acute toxicity of cypermethrin for the freshwater fish, *L. rohita* was found to be 4.0 µg/L. The upper and lower 95% confidence limits were found to be 4.231 µg/L and 3.668 µg/L, respectively (Table 1).

Table 1. 96 h LC_{50}, slope and 95% confidence limits of cypermethrin in the freshwater carp, *Labeo rohita*

Pesticide	96 h LC50 value (µg/L)	Slope	95% Confidence limits	
			Upper limit	Lower limit
Cypermethrin	4.0 ± 0.03	1.289	4.231	3.668

Oxygen consumption. Fish exposed to a lethal concentration depicted increased (Fig. 1) oxygen consumption on day 1 (8.597%) to day 2 (17.409%) and the increase was decreased (1.289%) on day 4 (Table 2). In the sublethal concentration oxygen consumption increased (Fig. 1) on days 1, 5, 10 and 15 as compared to the control (Table 2) in the order of 1 (20.580%) <5(39.44%) <10 (102.57%) <15 (109.77%).

Table 2. Oxygen consumption (mL of oxygen consumed/g wet wt of fish/h) of the fish, *L. rohita* following exposure to lethal (4.0 µg/L) and sub lethal (0.57 µg/L) concentrations of cypermethrin

		Exposure periods in days							
		Lethal				Sublethal			
Estimations	Control	1	2	3	4	1	5	10	15
Oxygen consumption	0.1861[I]	0.2021[F]	0.2185[E]	0.2005[G]	0.1885[H]	0.2244[D]	0.2595[C]	0.3770[B]	0.3904[A]
± SD	0.0003	0.0002	0.0003	0.0002	0.0004	0.0002	0.0003	0.0002	0.0004
% Change	-------	8.597	17.409	7.737	1.289	20.580	39.44	102.57	109.77

Values are means ± SD (n = 6) for oxygen consumption in a column followed by the same letters and are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test.

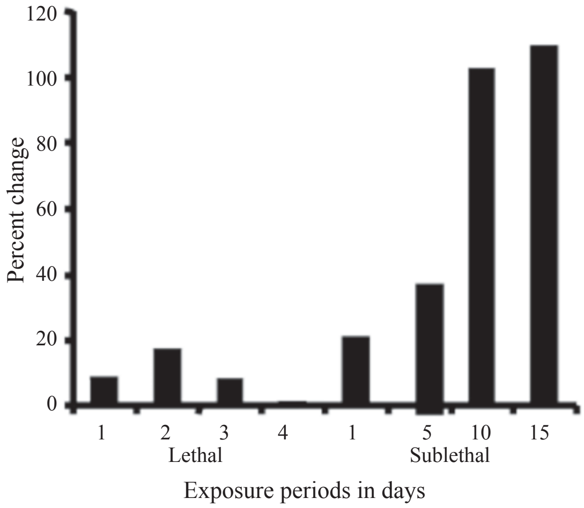

Fig 1. Oxygen consumption (mL of oxygen consumed/g wet wt of fish/h) of the fish, *L. rohita* following exposure to lethal (4.0 µg/L) and sub lethal (0.57 µg/L) concentrations of cypermethrin

Behavioural observations. The control fish behaved in a natural manner i.e., they were active with well-coordinated movements. They were alert to the slightest disturbance, but in the toxic environment fishes exhibited irregular, erratic and darting swimming movements and loss of equilibrium. They slowly became lethargic, hyper excited, restless and secreted excess mucus all over their bodies. Opercular movements increased initially in all exposure periods but decreased later steadily in the lethal as compared to the sub lethal exposure periods. Gulping air at the surface, swimming on the water surface, disrupted shoaling behaviour and easy predation was seen on the first day itself in the lethal and sub lethal exposure period and continued throughout the test tenures. Finally the fish sank to the bottom with their least opercular movements and died with their mouths open.

Discussion

Acute toxicity of cypermethrin for the freshwater fish, *L. rohita* was found to be 4.0 µg/L. The upper and lower 95% confidence limits were found to be 4.231 µg/L and 3.668 µg/L, respectively. It is evident from the results that cypermethrin can be rated as highly toxic to fish.

In the present study the control fish behaved in a natural manner i.e., they were active with well-coordinated movements. They were alert to the slightest disturbance, but in the toxic environment the fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium due to inhibition of AchE activity, leading to accumulation of acetylcholine in the cholinergic synapses, leading to hyperstimulation (MUSHIGERI and DAVID, 2005). They slowly became lethargic, hyper excited, restless and secreted excess mucus all over their bodies. Mucus secretion in fish forms a barrier between the body and toxic media thereby probably reduces contact with the toxicant so as to minimize its irritating effect, or to eliminate it through epidermal mucus. Similar observations were made by RAO et al. (2003) and PARMA DE CROUX et al. (2002) in *Prochilodus lineatus* under monocrotophos stress. Opercular movements increased initially in all exposure periods but decreased later, steadily in lethal compared to sub lethal exposure periods. The increased opercular gill movements observed initially may possibly compensate for increased physiological activity under stressful conditions (SHIVAKUMAR and DAVID, 2004).

Gulping air at the surface, swimming on the water surface, disrupted shoaling behaviour and easy predation was seen on the first day itself in lethal and sub lethal exposure periods and continued the same more intensely, which is in accordance with the observations made by URAL and SIMSEK (2006). Gulping of air may help to avoid contact with the toxic medium. Surfacing phenomenon i.e., significant preference of upper layers in the exposed group might be the result of the need for higher oxygen levels during the exposure period (KATJA et al., 2005). Finally fish sank to the bottom with the least opercular movements and died with their mouths open.

In sublethal exposure, the fish's bodies became lean towards the abdomen position compared to the control fish and they were found to be under stress, but this was not fatal. Leanness in fish indicates a reduced amount of dietary protein consumed by the fish under pesticide stress which is immediately utilized and not stored as body mass (KALAVATHY et al., 2001).

Variation in oxygen consumption is an indicator of stress, which is frequently used to evaluate the changes in metabolism under environmental deterioration. It is clearly evident from the studies that the cypermethrin affected oxygen consumption of *L. rohita* under lethal and sublethal concentrations. Fish exposed to lethal concentrations depicted increased oxygen consumption on day 1 to day 2 and the increase decreased on day 4. In sublethal exposure oxygen consumption increased on days 1, 5, 10 and 15 as compared to the control.

Since most fish breathe in the water in which they live, changes in the chemical properties in it may be reflected in the animal's ventilator activity, particularly if the environment factors affect respiratory gas exchange (MUSHIGERI, 2003). The fluctuated response in respiration may be attributed to respiratory distress as a consequence of the impairment of oxidative metabolism. Disturbance in oxidative metabolism has been reported earlier under cypermethrin toxicity in *Tilapia mossambica* (DAVID et al., 2003).

Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress (MAGARE and PATIL, 2000). Pronounced secretion of a mucus layer over the gill lamellae has been observed during cypermethrin stress. Secretion of mucus over the gill curtails the diffusion of oxygen (DAVID et al., 2002), which may ultimately reduce the oxygen uptake by the animal.

If the gills are destroyed due to xenobiotic chemicals (GRINWIS et al., 1998) or the membrane functions are disturbed by changed permeability (HARTL et al., 2001), oxygen uptake rate would rapidly decrease. On the other hand, the metabolic rate (in relation to respiration) of fish could be increased under chemical stress. KALAVATHY et al. (2001) reported that the dimethoate is efficiently absorbed across the gill and diffuses into the blood stream resulting in toxicity to the fish.

Conclusion

The analysis of data from the present investigation demonstrated that cypermethrin is highly toxic and had a profound impact on behaviour and respiration in *L. rohita* in both lethal and sublethal concentrations. Thus, it led to altered fish physiology. Variation in the oxygen consumption in cypermethrin exposed fish was probably due to impaired oxidative metabolism and pesticide induced stress.

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SAŽETAK

Brzi test statičke metode obnove rabljen je za određivanje akutne toksičnosti (LC50) piretroidnoga insekticida cipermetrina (92,25%) u autohtonoga slatkovodnoga indijskoga šarana, *Labeo rohita*. Šaranska mlađ bila je izložena različitim koncentracijama (2,0 do 6,0 µg/L) cipermetrina tijekom 96 sati. Akutna toksična doza iznosila je 4,0 µg/L, a jedna petina LC50 (0,57 µg/L) bila je uzeta za subakutno trovanje. Način ponašanja i potrošnja kisika bili su promatrani nakon izlaganja letalnim (1, 2, 3 i 4 d) i subletalnim koncentracijama (1, 5, 10 i 15 d). Šarani su u toksičnom mediju pokazivali nepravilno, lutajuće i nasrtajuće plivanje, razdražljivost, gubitak ravnoteže, potonuće na dno što se može pripisati oslabljenoj aktivnosti acetilkolinesteraze što dovodi do suvišnog nakupljanja acetilkolina na kolinergičnim sinapsama i time do prekomjerne stimulacije. Kolebanje u potrošnji kisika (1,289 do 17,409%; 20,580 do 109,77%) ustanovljeno je pri letalnim i subletalnim koncentracijama cipermetrina. Promjene u potrošnji kisika javljaju se zbog dišnoga poremećaja i nepravilne izmjene kisika. Ribe su pod subletalnim koncentracijama bile u stresu, koji nije bio fatalan.

Ključne riječi: cipermetrin, toksičnost, potrošnja kisika, ponašanje, *Labeo rohita*