World Journal of Zoology 4 (1): 19-23, 2009
ISSN 1817-3098

Impact of Cypermethrin on Behavioural Responses in the Freshwater Teleost, *Labeo rohita* (Hamilton)

[1]S.R. Marigoudar, [1]R. Nazeer Ahmed and [2]M. David

[1]Deparment of Post Graduate Studies and Research in Zoology,
Karnatak University, Dharwad-580 001, Karnataka, India
[2]Karnatak University's Research Laboratory, Environmental and Molecular Toxicology Division,
Department of Zoology, Karnatak Science College, Dharwad-580 001, Karnataka, India

Abstract: Static renewal bioassay test was conducted to determine the toxicity of technical grade (92.25% purity) insecticide cypermethrin on the freshwater teleost, *Labeo rohita*. Fishes were exposed to various concentrations of pyrithroid insecticide cypermethrin for 96 h and the percent mortality was recorded. The LC_{50} value was found to be 4.0 μg L^{-1} and one seventh of LC_{50} (0.57 μg L^{-1}) was selected as sub lethal concentration for sub acute studies. Behavioural patterns were observed in both lethal (1, 2, 3, 4 d) and sub lethal concentration (1, 5, 10, 15 d). *L. rohita* in toxic media exhibited erratic and darting movements with imbalanced swimming activity, which might be due to the malfunctioning of neurotransmitters, followed by hyper and hypo opercular activity, loss of equilibrium, and mucus secretion all over the body were observed.

Key words: Toxicity · Behaviour · Cypermethrin · *Labeo rohita*

INTRODUCTION

The pervasive use of pesticides in agriculture, public health and forestry ultimately leads to the contamination of aquatic biotopes posing a great threat to the environment [1]. Elucidation of the action of pesticides in non target organisms forms a thrust area in the field of toxicology due to the serious effects and persistent nature of these pesticides. Metabolic and pathologic derangements on exposure to pesticides are well documented.

Due to the increasing regulatory restrictions on organophosphate pesticides, pyrethroid pesticides have replaced the organophosphates for many residential and agricultural uses. Urban use of pyrethroid, in particular, has increased significantly due to professional pest control and retail sales for home usage [2]. Cypermethrin is a synthetic pyrethroid insecticide used to control many pests, such as moth pests of cotton, fruit and vegetable crops, including structural pest control, landscape maintenance, for residential and garden use. This has resulted in its discharge into the aquatic environment and consequently several laboratory studies have been performed, which evidenced that cypermethrin is extremely toxic to fish at very low concentrations and to aquatic invertebrates [3]. Fish sensitivity to pyrethroid may be explained by their relatively slow metabolism and elimination of these compounds through excretion. *Labeo rohita* is one of the prime cultured fresh water teleost in polyculture and of great economic importance. Hence, the present study is a contribution to the assessment of aquatic toxicity and behavioral impact of cypermethrin-based pesticide to freshwater fish; *L. rohita* exposed to lethal and sub lethal concentrations

MATERIALS AND METHODS

L. rohita fingerlings weighing 3±0.5 g and average length of 6 cm were collected from the Bhadra fish seed farm, Lakhalli, Shimoga, Karnataka and acclimatized to laboratory condition for 15 d in large cement tanks previously washed with potassium permanganate to free the walls from any microbial growth. Physico-chemical characters of water was maintained according to methods in APHA [4] and found as follows: Temperature: 26±1°C, pH: 7.8±0.2 at 26°C, Dissolved oxygen: 6.7 to 7.2 mg L^{-1}, Chloride 43.3 mg L^{-1}, Carbon dioxide 9.0 mg L^{-1}, Total hardness 105 mg L^{-1}, Total alkalinity 25.2 mg L^{-1}, Specific gravity 1.00361, Calcium 16 mg L^{-1} and Magnesium 0.8 mg L^{-1}. During acclimatization fish were fed with rice bran mixed with oil cake in the ratio 2:1 everyday.

For the present investigation, technical grade cypermethrin (92.95%) was obtained from Herbana Industries Limited, Borivli (W) Mumbai, India. The stock

Corresponding Author: Dr. R. Nazeer Ahmed, Deparment of Post Graduate Studies and Research in Zoology, Karnatak University, Dharwad-580 001, Karnataka, India.

solution was prepared in acetone, which was found to be non-toxic to fish. Required quantity of cypermethrin was drawn from this stock solution for the further experiment. Preliminary tests were carried out to find out the median tolerance limit (LC_{50}) of the fish to cypermethrin for 96 h by probit analysis method [5]. The concentration of cypermethrin at which 50% mortality occurred was taken as the median lethal concentration (LC_{50}) for 96 h, which was found to be 4.0 µg L^{-1}. One seventh of the LC_{50} value (0.57 µg) was selected for sub lethal concentration studies according to Sprague [6]. The control and the exposed fish were aerated frequently to prevent hypoxic condition of the medium. The control and cypermethrin exposed fish were kept under continuous observation during the experiment period, 24, 48, 72 and 96 h for lethal concentration and 1, 5, 10 and 15 days for sub lethal concentration. During this experiment the behavioural changes were also critically observed.

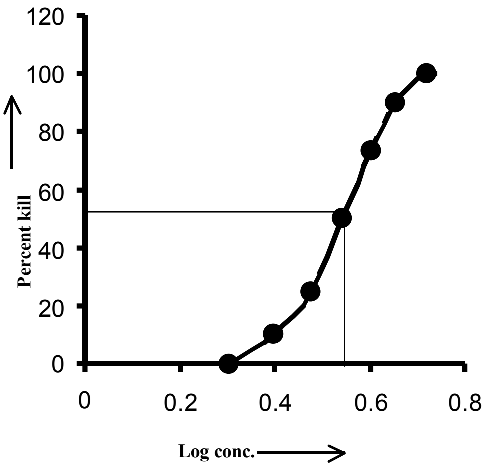

Fig. 1: The graph showing sigmoid curve between percent mortality of fish against log concentration in L.rohita on exposure to cypermethrin

RESULTS

The maximum concentration at which zero percent mortality and minimum concentration at which 100% mortality of *Labeo rohita* were observed at 2.5 and 5.0 µg L^{-1} respectively (Fig. 1). LC_{50} value obtained through sigmoid curve is 4.0 µg L^{-1} (Fig. 1) and linear curve is 4.0 µg L^{-1} (Fig. 2). The LC_{50} values were further verified by the Dragstedt and Behrens's equation and the LC_{50} value obtained was 3.97µg L^{-1}. Thus the average LC_{50} for 96 h is determined to be 4.0 µg L^{-1}.

Behavioural changes are physiological responses shown by the animal, which are often used as the sensitive measure of stress syndrome in the organism experiencing it, consequently the behavioural changes were observed in control and exposed fish.

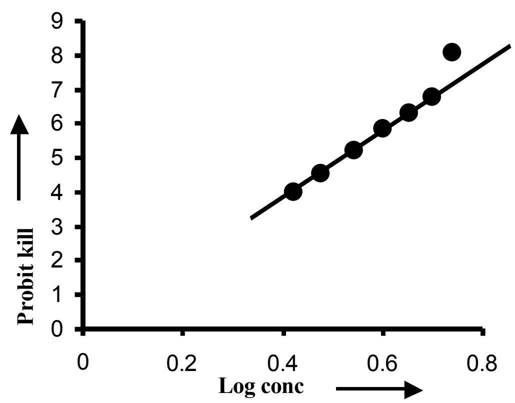

Fig. 2: The graph showing linear curve between probit mortality of fish against log concentration in *L. rohita* on exposure to cypermethrin

Control Fish: Control fishes maintained a fairly compact school, covering about one third of the bottom during the first five days of the 15 days experiment. By fifth day, the school became less compact covering up to two-third of

Table 1: Mortality of *Labeo rohita* in different concentration of cypermethrin at 96 h exposure period

Concentration of cypermethrin (µg L^{-1})	Log conc.	No. of fish alive out of ten	Percent kill (%)	Probit kill	Dragstedt- Behren's LC_{50} value (µg L^{-1})
2.5	0.3979	10	0	----	3.97
3.0	0.4771	9	10	3.72	
3.5	0.5440	8	20	4.16	
4.0	0.6020	5	50	5.00	
4.5	0.6532	4	60	5.25	
5.0	0.6989	1	90	6.28	
5.5	0.7403	0	100	8.09	

the tank area. Fishes were observed to scrap the bottom surface. When startled, they instantly formed a tight school that was maintained briefly. They were sensitive to light and moved to bottom of the tank when light was passed into the tank. Except a less response to form a dense school towards the end of the study, no other extraordinary behaviour was observed.

Exposed Fish: When the fish were exposed to the lethal concentration of cypermethrin, they migrated immediately to the bottom of the tank. The schooling behaviour was observed to be disrupted in the first day itself and the fish occupied twice the area than that of the control group. They were spread out and appeared to be swimming independent of one another. Irregular, erratic and darting movements followed this with imbalanced swimming activity. The fish exhibited peculiar behaviour of trying to leap out from the pesticide medium, which can be viewed as an escaping phenomenon. The frequency of surfacing phenomenon was greater on the second day of exposure wherein the fish frequently come to the water surface. Respiratory disruption was observed in the normal ventilating cycle (cough, yawn) with a more rapid, repeated opening and closing of the mouth and opercular coverings. Partially extended fins and singlewide opening of the mouth and opercula coverings accompanied by hyperextension of all fins were found and the fish was in a state of excitement on the third day. The swimming behaviour was in a corkscrew pattern rotating along horizontal axis and followed by \`s\` jerk, partial jerk, sudden, rapid, non-directed spurt of forward movement (burst swimming). The fish progressively showed signs of tiredness and lost positive rheotaxis characterized by weakness and apathy. On the 4^{th} day they lost their equilibrium and response, to external stimuli such as touch and light followed by drowning to the bottom. They often barrel-rolled or spiraled at intervals and engulfed the air through mouth before respiration ceased. The fish eventually died with their mouth and operculum wide opened. A change in colour of the gill lamellae from reddish to light brown with coagulation of mucus on gill lamellae was seen in dead fishes.

In sub lethal treatment, the schooling behaviour of the fish was slowly disrupted during the first day. The ventilation rate was increased, but hyperactivity, excitement, hyperventilation etc., were not influenced on exposure to the sub lethal concentration of cypermethrin at 5 and 10 days. Further, the fish at 15 days of exposure exhibited free, normal swimming and active feeding.

DISCUSSION

The acute test for a long time has been a major component in toxicity testing. In which acute chemical toxicity is determined as a 96hr LC_{50} value however the environmental significance of death of individuals after short term exposure to high concentration is questionable. In contrast to this our results shows cypermethrin is very toxic even at lower concentration (4 μg L^{-1}) for 96hr LC_{50}. Lethality in the present study is comparable to the few previously published studies that exist, but that LC_{50}'s for all species exceeded this concentration. This can be attributed to the inability of the *L.rohita* to withstand and metabolize the cypermethrin intoxication. The acute toxicity treatments showed strong negative effects on survival as pesticide concentration increased. This suggests dose-dependent survival and concentration graded lethality (Table 1). The varying degree of mortality reported in this study is consistent with the report of David *et al*. [7], who reported that differences in an organism's biological adjustment and behaviour response to change in water chemistry.

Behavioral characteristics are obviously sensitive indicators of toxicant's effect. It is necessary, however, to select behavioural indices of monitoring that relate to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural system.

The migration of the fish to the bottom of the tank following the addition of cypermethrin clearly indicates the avoidance behaviour of the fish, which was reported by Murthy [8] in trout. The opercular movement of the fish ceases immediately following exposure to cypermethrin. The decrease in opercular movement and corresponding increase in frequency of surfacing of fish clearly indicates that fish adaptively shifts towards aerial respiration (by obtaining atmospheric oxygen) and the fish tries to avoid contact with the pesticide through gill chamber [9]. The increased ventilation rate by rapid, repeated opening and closing of mouth and opercular coverings accompanied by partially extended fins was observed in the present study. This could be due to accumulation of mucus in the gill region for proper breathing [10].

The surfacing phenomenon of fish observed under cypermethrin exposure might be due to hypoxic condition of the fish as reported by Radhaiah and Jayantha Rao [11]. The increased surfacing during the initial periods of exposure to cypermethrin concentrations suggests an elevated rate of metabolism. Changes in ventilation rate and surfacing frequencies are the general symptoms noticed in the fish after exposure to the pesticide and these activities help the fish to avoid contact with poison and fight against stress. Chronic exposure of finfish to aroclor was found to induce surfacing phenomenon of fish as pointed out by Drummond [12]. Acute respiratory distress, degeneration of hepatocytes in perportal zones can imply the influence of toxic compounds in the digestive tract. The biochemical changes in liver profile can relate to hepatocytes damage [3].

Aggressive behaviour such as nudge and nip were increased following exposure to the toxic material. Orientation and locomotor patterns were found to be involved in most aspects of fish behaviour such as migration, mating, courtship and feeding, which were altered under cypermethrin stress and observations in the present investigation are consistent with the earlier reports [3, 8, 13 and 14] on exposure to environmental contaminants.

In sub lethal treatment, the schooling behaviour of the fish was slowly disrupted during the first day itself. The ventilation rate was increased, but hyperactivity, excitement, hyperventilation etc were not influenced on exposure to the sub lethal concentration of cypermethrin on day 1 and day 5. Further the fish exposed to 10 and 15 days exhibited balanced swimming and active feeding

The hyper excitability of the fish invariably in the lethal and sub lethal exposure to cypermethrin may be attributed to the hindrance in the functioning of the enzyme acetylcholine esterase enzyme (AChE) in relation to nervous system as suggested by many authors [15-18]. It leads to accumulation of acetylcholine, which is likely to cause prolonged excitatory postsynaptic potential. This may first lead to stimulation and later cause a block in the cholinergic system. In most cases changes were more pronounced during a lethal exposure compared to sublethal exposure period.

In the present study the abnormal changes in the fish exposed to lethal concentration of cypermethrin are time dependent. However, the normal behaviour of the fish at 10 and 15 days on exposure to sub lethal concentrations indicates its adaptability to the sub lethal concentration due to long term exposure of cypermethrin. The fish behaviour indicates that the fish has adapted to a compensatory mechanism to derive energy during pyrethroid toxicosis as suggested by Philip [19]. Hence this type of study can be useful to compare the sensitivity of the various species of aquatic animals and potency of chemicals using LC_{50} values and to derive safe environmental concentration, by which there is no lethality and stress to the animals.

ACKNOWLEDGEMENTS

The authors acknowledge grant of project fellow to first author and other financial assistance from SAP DRS-II of UGC, New Delhi and Department of Zoology Karnatak University, Dharwad to undertake the present work.

REFERENCES

1. Visweswaraiah, K., M. Jayaram and S.K. Majumdar, 1975. Environmental pollution with pesticides. J. Food. Sci. Tech., 2: 53-60.
2. Lydy, M., 2005. Relative contributions of agricultural or Pyrethroid usage to toxicity in California streams. Setac J. 380 (LYD-1117-740135).
3. Sarkar, B., A. Chatterjee, S. Adhikari and S. Ayyappan, 2005. Carbofuran and cypermethrin-induced histopathological alterations in the liver of *Labeo rohita* (Hamilton) and its recovery. J. Applied Ichthyol., 21: 131-135.
4. Arnold, E., R.R. Greenberg, and S. Lenore, 1985. Standard methods for the examination of water and waste water, 16th edn. American Public Health Association, Washington, DC.
5. Finney, D.J., 1971. Probit Analysis, 3rd Edn. Cambridge University Press, London.
6. Sprague, J.B., 1971. Measurement of pollutant toxicity to fish under Sub lethal effects and safe concentrations. Water Res., 5: 245-266.
7. David, M. and G.H. Philip, 2005. Accumulation of fenvalerate and induced alteration in Lactate and Succinate dehydrogenises activity in *Labeo rohita*. J. Ecophysiol Occup. Hlth., 5(3,4): 207-211.
8. Murthy, A.S., 1987. Sub lethal effect of pesticides on fish. Toxicity of pesticides to fish, 2: 55-100.
9. Santhakumar, M., M. Balaji, K.R. Saravanan, D. Soumady and K. Ramudu, 2000. Effect of monocrotophos on the optomotor behaviour of an air breathing fish, *Anabas testudinius* (Bloch). J. Environ. Biol., 21(1): 65-68.
10. David, M., S.B. Mushigeri and M.S. Prashanth, 2000. Toxicity of fenvalerate to the freshwater fish, *Labeo rohita* (Hamilton). Geobios., 29: 25-28.

11. Radhaiah, V. and R.K. Jayantha, 1988. Behavioural response of fish, *Tilapia mossambica* exposed to fenvalerate. Environ. Ecol,. 6(2): 2-23.
12. Drummond, R.A., D.G. Russom and L.D. David, 1986. Behavioural and morphological changes in Pathead minnow *Pimephles promelas* as diagnostic end point for screening chemicals according to mode of action. Aqua. Toxicol., 9: 415-434.
13. Sabita, B. and R.N.S. Yadav, 1995. Static Biossay and toxicity of two pesticides, rogor and endosulfan to the air-breathing fish, *Hetropneustes fossilis* with special references to behaviour. Poll. Res., 14(4): 435-438.
14. Madhab, P., Bandyopadhyay and Ajit Kumar Adithya, 2002. Xenobiotic impact on sensitivity in *Anabas testudineus* (both). J. Ecobiol., 14(2): 117-124.
15. Agarwal, S. and N.N. Balakrishanan, 1989. Physiological effect of malathion on the brain of *Sarotherodon mossambicus*. Curr. Sci., 58: 1046-1051.
16. Narasimha, M.B., 1983. Studies on the toxic potentiality of lindane on the fresh water teleost, *Tilapia mossambica*, with special emphasis on nitrogen metabolism, Ph.D Thesis, S.V. University, Tirupati, India.
17. David, M., 1995. Effect of fenvalerate on Behavioural, Physiological and Biochemical aspects of freshwater fish, *Labeo rohita* Ph.D. Thesis, S.K. University, Anantapur andhra Pradesh, India.
18. Deva, P.R., 2000. Fenvalerate induced changes in the Protein metabolism of freshwater fish, *Tilapia mossambica* (Peters). Ph.D. Thesis. S.K.University, Anantapur, A.P., India.
19. Philip, G.H., P.M. Reddy and Ramamurthi, 1988. Changes in protein metabolism in liver and kidney of *Mus boodurga* Gray after BHC feeding. Bull. Environ. Contam Toxicol., 41: 822-827.