Environment and Ecology 39 (2) : 414—419, April—June 2021
ISSN 0970-0420

Assessment of Acute Toxicity and Behavioral Responses in *Catla catla* Following Exposure to Pyrethroid Pesticide Cypermethrin

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Received 20 April 2021, Accepted 22 May 2021, Published on 9 June 2021

ABSTRACT

Pyrethroids are highly preferred for agriculture pest management owing to their high specificity and relative lower mammalian toxicity. However, these chemicals very often result in contamination of aquatic bodies through surface run-off, thereby causing detrimental effects on non-target organisms such as fish. In the present study, the acute toxicity of cypermethrin on freshwater teleost *Catla catla* was determined by probit analysis. The test was performed for 96 h period by exposing the fish to different concentrations of the pesticides and LC_{50} value was found to be 1.24 µg/L. In addition, morphological and behavioral alterations on chronic exposure of 45 days were also monitored. The observed endpoints included fin and opercular movements, swimming patterns and morphological alterations were also studied. Short term exposure to cypermethrin resulted in alterations like increased opercular and fin movements, hyper excitability loss of scales, hemorrhage and depigmentation in *Catla catla.*

Keywords Acute toxicity, Pyrethroid, *Catla catla*, Cypermethrin, LC_{50}.

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INTRODUCTION

Freshwater systems are adversely affected due to incessant release of pesticides from agricultural activities. Fish are exposed to these chemicals through runoff after rainfall or by spray drift. The lack of pyrethroid hydrolyzing enzymes in fish makes them highly sensitive to these chemicals (de Moraes *et al.* 2018). Cypermethrin (CYP) is a widely used pesticides used to control pests of wheat, cotton, soyabean and many other crops (Eni *et al.* 2019) and is reported to be highly toxic to fish (Dawar *et al.* 2016, Parvani *et al.* 2019).

Acute toxicity determines the harmful effects that occur after organisms are exposed to a single or multiple doses of a test substance within 24-96 hours by a known route (Saganuwa 2016). The results from acute toxicity test serve as a guide in dosage selection for long term toxicity studies as well as other studies that involve the use of animals (Maheshwari and Shaikh 2016). The test can help in determining the impending risks to the fish species, or to assess the regulatory requirement related to water contamination.

Behavior is a sensitive indicator of toxicity, serving as a link between physiology of an organism and its environment and therefore has been used to study the effects of environment stressors on organisms (Little and Brewer 2001). Fish are ideal organisms to study behavioral toxicity, since many ecologically relevant fish behaviors can be observed and quantified. Therefore, keeping in view the above, the present study was carried out to find the 96 h LC_{50} of CYP to Indian major carp *Catla catla.* Also, the alterations in morpho-behavioral patterns were observed during chronic exposure of the pesticide to the fish.

MATERIALS AND METHODS

Fish procurement and maintenance

The fingerlings of *Catla catla* (wt 15 ± 0.2 g, length 12.5±2 cm) were procured from local fish farm and brought to laboratory in oxygen filled polybags. The fish were treated with 0.1% $KMnO_4$ for 2-3 min to get rid of any dermal adherents. Prior to experimentation, the animals were acclimated in glass aquaria filled with dechlorinated tap water for 10 days. Continuous aeration was maintained with aerators. Commercial fish feed pellets were used to feed the fish (twice daily). During experimentation, the average values of physico-chemical parameters of test water analyzed were : Temperature 23 ± 1˚C, pH 7.2 and DO 8 ± 2 mg/l. All the tests were performed in triplicate.

Test chemical

Commercial formulation of cypermethrin (CYP), $C_{22}H_{19}Cl_2NO_3$ (10% EC), manufactured by Gujarat Agro Industries Corporation Ltd, Ahmedabad-14, India, was used. The stock solution was made which was further diluted get the desired concentrations.

Acute toxicity tests

Acute toxicity bioassay was conducted following standard methods (APHA 2012). Fish were starved for 24 h and feeding was withheld during the tests. 96 h LC_{50} of CYP to *Catla catla* was determined by Probit analysis (Finney 1980) following standard methods (APHA 2012). 10 fish in non-toxic plastic tanks (Sintax 60 cm×30 cm×30 cm) were exposed to varying concentrations of CYP (1.14-1.36 µg/L). The test water was renewed every 24 h to maintain the pesticide concentration in water. Fish mortality was observed at an interval of 24 h for a period of 96 h. Dead fish was removed immediately to avoid contamination.

Chronic toxicity tests

For chronic toxicity tests, sub lethal concentrations i.e., 1/3rd (0.413 µg/L) and 1/10th (0.124 µg/L) of 96 h LC_{50} of cypermethrin for *C. catla* were selected with parallel control. The sub-lethal concentrations selected are environmentally relevant. Three experimental groups were studied ; group I served as control, and was maintained in pesticide free dechlorinated water, group II and III were treated with lower (0.124 µg/L) and higher (0.413 µg/L) concentration of CYP respectively.

Morphological and behavioral studies

Observations were made to assess the influence of CYP exposure on behavioral and morphological responses in *C. catla,* such as swimming, hyper excitability, loss of equilibrium, restlessness, jerking, darting, surface movements, opercular movements, fin movements and air gulping. To measure the behavioral endpoints, the fish were observed for 20 min twice a day (morning and evening). Further, fish were monitored for any visible morphological alteration, such as body color, mucous secretion or deformities.

Statistical analysis

SPSS 21.0 software was employed for statistical analysis. Data obtained was tested for normal distribution using Kolmogorov-Smirnov test. Significance of differences between the control and treated groups were tested, using one way analysis of variance (ANOVA) followed by Tukey's post hoc test for all the parameters. The results were expressed as mean ± standard deviation. The differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Median lethal concentration (96 h LC_{50})

Of CYP to *C. catla* calculated using SPSS 21.0

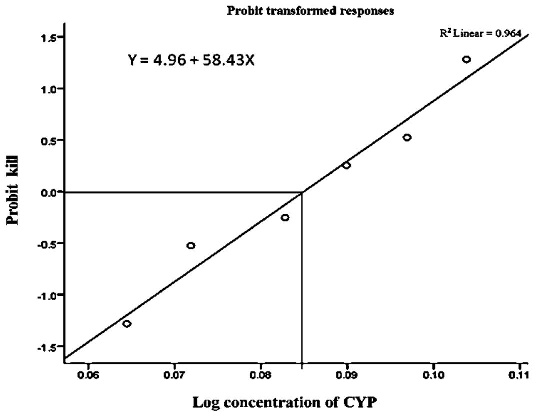

Fig. 1. Empirical probit values of the death rate of *C. catla* against log concentrations of CYP.

was found to be 1.24 µg/L (Y = 5.14 + 54.66 X, r^2 = 0.994) (Table 1, Fig. 1).

Behavioral and morphological studies

The behavioral changes observed as result of CYP exposure were represented by respiratory and neurological manifestations. In the present study, abnormal behavior was noticed in the fish on acute as well as chronic exposure to CYP. The monitored behavioral patterns and morphological features of the control fish were normal and the fish maintained a compact school. Normal reflexes were shown when startled. When exposed to the pesticide, the fish immediately moved to the bottom of the tank and exhibited noticeable reactions within first few hours of pesticide exposure. The schooling behavior was disrupted as fish spread out and swam independent of each other and also tried to leap out of the pesticide medium, thus exhibiting escaping phenomenon. Additionally, frequent surfacing, restlessness, erratic swimming and air gulping suggested avoidance behavior of the fish. The increase in surfacing frequency reflects the fish's response to hypoxic condition induced by the pesticide and also its effort to avoid contact with it (Kumari *et al.* 2017). Similar behavioral alterations have been recorded in *Labeo rohita* exposed to three pesticides ; malathion (Karmakar *et al.* 2016). The variations in fin and opercular movements in the fish treated with CYP are presented in Fig. 2.

During initial 15 days of treatment with CYP, fish displayed increase in fin as well as opercular movements at both the sub-lethal concentrations. Elevation in the opercular activity was observed after first few days of pesticide treatment. Specifically, there was 3.66% and 8.47% increase in opercular movements on exposure of the fish to CYP at 0.12µg/L and 0.41µg/L respectively (Fig. 2A). The dorsal fin movements increased by 8.68% and 17.39% at 0.12µg/L and 0.41µg/L of CYP respectively (Fig. 2B). Besides, fish displayed vertical hanging, loss of equilibrium, hyperexcitability and convulsions. Movements in caudal fin at lower concentration treatment remained unchanged, whereas 5.55% increase was registered at higher concentration (Fig. 2C). After 30 days of treatment, the opercular movements increased by 6.33% at lower concentration (0.12µg/L) and 11.38% at the higher concentration (0.41µg/L), while decreased significantly ($p < 0.05$) by 6.11% and 11.57% at lower and higher concentration respectively after 45 days. A declining trend in fin movements was noticed after

Table 1. Mortality and empirical probit values of *C. catla* exposed to different concentrations of CYP for 96 h.

CYP conc (µg/L)	Log conc	Total number of fish	Number of dead fish	Mortality (%)	Emperi-calprobit-value
1.16	0.05	10	1	10	3.72
1.18	0.07	10	2	20	4.16
1.21	0.08	10	3	30	4.48
1.23	0.08	10	4	40	4.75
1.25	0.09	10	6	60	5.25
1.27	0.10	10	7	70	5.52
1.29	0.11	10	8	80	5.84

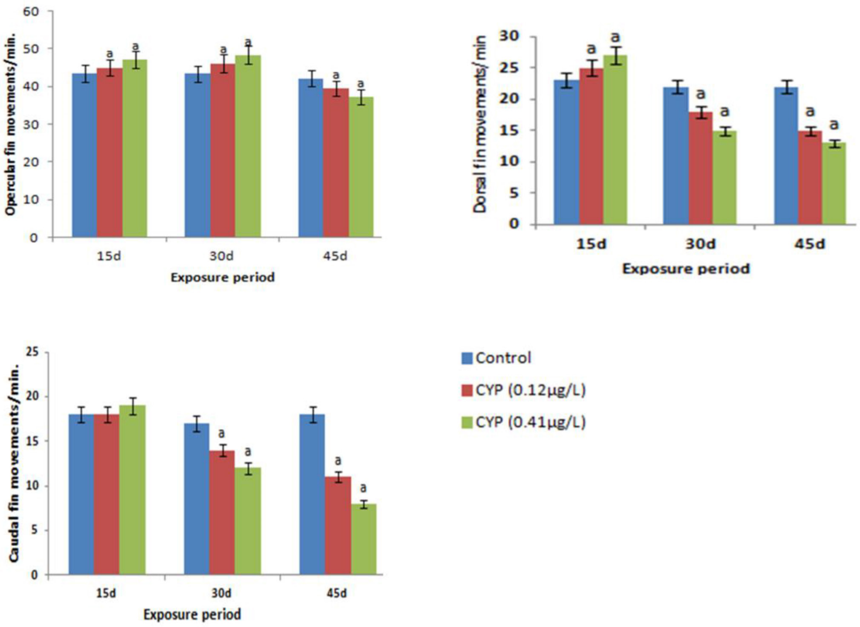

Fig. 2. Variation in (A) opercular, (B) dorsal and (C) caudal fin movements (per min) of *C. catla* exposed to cypermethrin (CYP) at different time intervals. Data are presented as mean ± SD (n = 6). $p < 0.05$ is considered to be statistically significant, determined by oneway ANOVA followed by Tukey's post hoc test.

15 days, showing a maximum decrease of 55.5% in caudal fin and 40.9% in dorsal fin after prolonged exposure of 45 days at higher toxicant concentration. The behavioral movements subsided after prolonged exposure and the fish progressively displayed signs of lethargy and tiredness and became unresponsive to external stimuli. There was decline in respiration rate and fish settled at the bottom tank. It remained motionless for longer intervals of time. Some fish suspended in vertical position with tail pointing downwards.

Elevated opercular movements as observed in the present study has also been reported in *Cirrhinusmrigala* in response to chlorpyrifos exposure (Cheema *et al.* 2018) and *Channapunctatus* exposed to cadmium chloride (Chilke and Bacher 2014). The increased opercular movements accompanied by labored breathing, repeated opening and closing of mouth indicated adaptive response of the fish in an effort to compensate the toxicant induced respiratory distress, or to meet out high energy requirement. It has also been postulated that it can be an extreme effort to clear the accumulated mucous in the gill region to restore proper breathing (Chilke and Bacher 2014).

The responses such as erratic swimming, hyperexcitability, loss of balance and convulsions could be the neurotoxic menifestations of cypermethrin, which directly bind to voltage gated sodium channels of nerve fibers and prevent its closing continuous nerve stimulation which consequently lead to uncoordinated movements (Singh *et al*. 2018) or could be attributable to alterations in the cholinergic system (Duarte *et al.* 2019).

Morphological alteration apparent during initial period of CYP treatment, at both the sub-lethal concentrations 0.12µg/L and 0.41µg/L was the deposition of thick mucus layer all over the body, particularly gills, a response for minimizing the irritating effect of the pesticide coming in contact with body and to prevent its absorption from body surface. Dutta and Chaudhary (1996), explained that heavy exudation of mucous is a defensive mechanism, according to them gills have the ability to metabolize and eliminate xenobiotic compounds and mucous

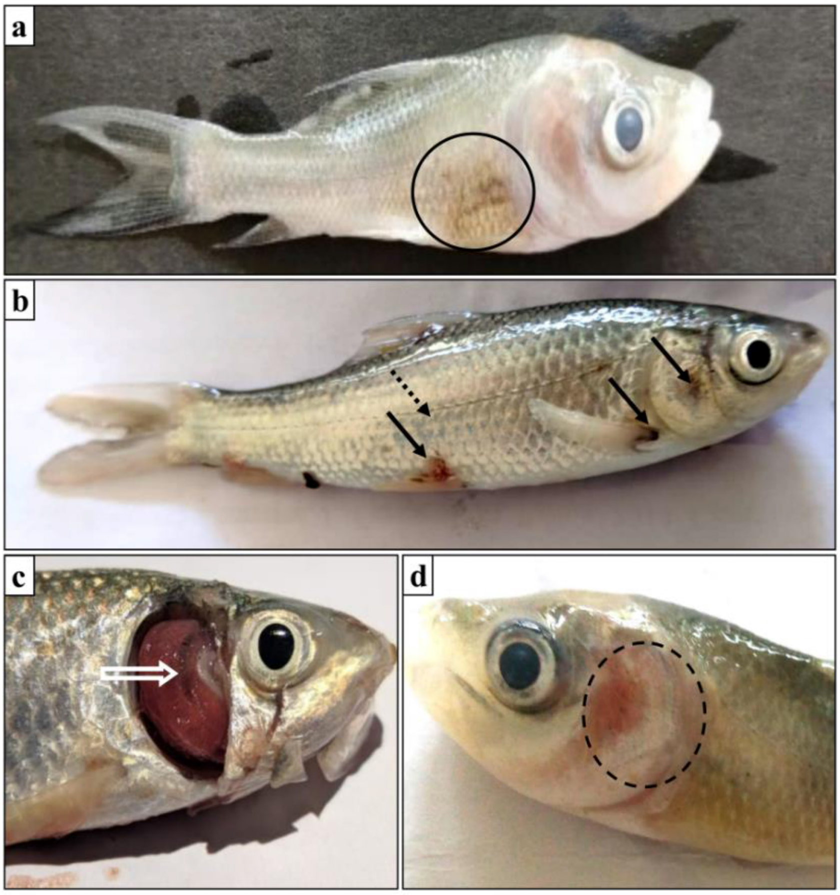

Fig. 3. Morphological alterations in *C. catla* exposed to CYP showing (a) depig mentation (circle), (b) loss of scales (broken arrow) and hemorrhage near fins (arrow), darkening of gills filaments (block arrow) and hemorrhage in cephalic region (dotted circle).

cells play key role in excretion to a considerable extent. Toxicants that enter permeable gill epithelium get efficiently excreted through mucous secretion (Al-Ghanim *et al.* 2008). Secretion of mucous and body depigmentation could be attributed to pituitary gland dysfunction under the toxic stress causing changes in number of mucous cells and chromatophores.

Extended treatment of the pesticide induced depigmentation, loss and loosening of scales and hemorrhage around fin and cephalic area at 0.12 µg/L of CYP (Fig. 3). Loosening of the scales could have been due to disruption of lepidonts of the scales (Jindal and Kaur 2015).

CONCLUSION

The recorded behavioral changes in *Catla catla* showed that on initial treatment with CYP, the fish tried to avoid the toxic environment by moving to the bottom of tank, jumping, frequent surfacing and air gulping. Simultaneously, efforts to adjust to the toxicant medium were manifested as increase in opercular and fin movements to compensate the respiratory distress and secretion of copious amount of mucous to minimize pesticide contact. But prolonged exposure to the toxicant caused physiological alterations, resulting in decreased opercular movements and displayed signs of lethargy in fish. Therefore, it is concluded that CYP is highly toxic to the fish and its use in farming practices needs to be strictly regulated.

ACKNOWLEDGEMENT

The authors are thankful to the Chairperson, Department of Zoology Panjab University, Chandigarh for providing research facilities and University Grants Commission for providing financial assistance to one of the authors (Ritu Sharma) (2061330615, 2013 EU- V).

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