Uttar Pradesh Journal of Zoology

Volume 44, Issue 23, Page 46-51, 2023; Article no.UPJOZ.3011
ISSN: 0256-971X (P)

Impact Assessment of Cypermethrin (A Pyrethroid) Induced Chronic Toxicity on Vitals of Adult *Cyprinus carpio* L.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2023/v44i233762

Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Israr Maqbool, Cholistan University of Vaterinary and Animal Sciences Bahwalpur Pakistan, Pakistan.
(2) Theeranat Suwanaruang, Kalasin University, Thailand.

Original Research Article

Received: 09/09/2023
Accepted: 14/11/2023
Published: 17/11/2023

ABSTRACT

The acute toxicity evaluation of cypermethrin, a pyrethroid to common carp i.e. *Cyprinus carpio* was done with an emphasis on histopathological effects. In this investigation, fish were exposed to different concentrations of Cypermethrin (25% EC) for 21 days. *C. carpio* were treated with different sublethal concentrations i.e. (5, 10, 15, and 20 percent of 96 hr LC50 value i.e. 2.924 µl/L) of cypermethrin for three different exposure periods, 7, 14, and 21 days. Control and exposed fish were sacrificed at an interval of 7 days and the vital tissues like brain, gills, and kidney were taken to analyze the histopathological alterations. During this experimental period, respiratory stress and erratic swimming activities, disrupted equilibrium, and response were observed which continuously varied with the increasing toxicant concentration. In gills, the proliferation of cells of filament, inflammation, hemorrhage, epithelial cell destruction, fusion of secondary lamellae, and necrosis were noticed. Hence it is concluded that cypermethrin poses a serious threat to aquatic fauna of the ecosystem which can be assessed via histological studies.

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Keywords: Histopathology; cypermethrin; Cyprinus carpio; sub-lethal concentration.

1. INTRODUCTION

Himachal Pradesh is one of the best farming states in India. In Himachal Pradesh, about 93% of the state population depends directly upon agriculture. Agriculture contributes nearly about 45% to the net state domestic product. With the passage of time in order to maintain crop yield, pesticides become more prevalent in farming practices especially synthetic ones [1]. Synthetic pyrethroids are insecticides that have been introduced over the past two decades for agricultural purposes and local use. Cypermethrin is a fourth-generation synthetic pyrethroid that is very effective against many insects of agriculture and households and is being used on a large scale [2]. The use of these pesticides gives rise to a number of environmental concerns [3]. Most of the sprayed insecticides reach a destination other than their target species, including non-target species, air, water, and soil. Thus, it is important to understand the relative toxicities of insecticides to predict their effects on the functioning of the ecosystem. Aquatic contamination due to pesticides causes acute and chronic poisoning in fish and other organisms. Histopathological studies are important in assessing the potency of pesticides to cause environmental pollution because even very low levels of pesticides are capable enough to induce noticeable cellular damage in spite of causing direct death of animals [4]. Therefore it is utilitarian to have a detailed analysis of the histology of gills to know the extent of damage cypermethrin can impose on *Cyprinus carpio*.

2. MATERIALS AND METHODS

2.1 Collection and Maintenance of Fish

The experimental fish *C. carpio* (weight 90-100gm; length up to 18±2cm) were collected from the Deoli fish farm Ghaghus, district Bilaspur, Himachal Pradesh in well-oxygenated polythene bags, and acclimatized in the aquarium of laboratory for 10 days at Himachal Pradesh University. Fish were kept in 0.2% potassium permanganate solution for 2-4 minutes to treat unwanted infection or injury. The experimental setup was done in the laboratory for 21 days. All the fish were kept in an aquarium of capacity approximately 180 liters. The water was dechlorinated and free from any other pollutants.

2.2 Analysis of Water Chemical Parameters

The average values for tap water used in both acclimation and experiments were pH 7.85 ± 0.510, dissolved oxygen 8.06 ± 0.014 mg/l, and water temperature was maintained at 24±2°C.

The aquariums were aerated with air stones attached to an air compressor to saturate with oxygen. The water quality parameters mentioned above were assessed in the experimental period. Throughout the acclimation period and experiment periods, fish were held under a photoperiod of 8 h of light and 16 h of darkness.

2.3 Feeding

During the acclimation period, the fish were fed on commercial pellet feed based on rice and wheat flour composition (minimum 29% protein, 3% fat, 8% fiber, 9% ash along with vitamin and mineral premix).

2.4 Test Chemical

The test chemical cypermethrin of technical grade (25%) was purchased from the local market of Shimla under the trade name Cyperkill. The stock solution of the test chemical was prepared by dissolving 100 µl of cypermethrin in 1 liter of distilled water. All experimental procedures were conducted after the approval of the Department of Fisheries, Himachal Pradesh.

2.5 Experimental Setup and Treatment

Experimental studies were carried out in a semi-static closed system. Uniform-sized healthy fish regardless of sex, were selected and evenly distributed in glass aquaria as one control and other treated groups. All aquaria were fitted with water-purifying filters, heaters, and air stones to ensure good aeration of water. Feeding was stopped 24 hours before starting the experiment. The control group was kept devoid of any chemical dosage while the treatment groups were exposed to different sublethal concentrations of 96 h LC50 (2.924 µl/L). After 24 hours water from each aquarium was changed and cypermethrin concentration was restored afresh. At an interval of 7 days fish from each aquarium were taken out and sacrificed for histological procedure.

2.6 Grouping of Animals

1. Group I fish were designated as the control.
2. Group II fish were treated with 0.1462µl/L concentration of cypermethrin for 21 days.
3. Group III fish were treated with 0.2924 µl/L concentration of cypermethrin for 21 days.
4. Group IV fish were treated with 0.439µl/L concentration of cypermethrin for 21 days.
5. Group V fish were treated with 0.584 µl/L concentration of cypermethrin for 21 days.

The fish of each group were dissected after 7, 14, and 21 days for each concentration. The gills and brain were dissected from normal and treated fish and further procedures were carried out.

2.7 Histopathological Procedure

Gills, of the fish (*Cyprinus carpio*) were excised immediately after sacrificing the fish. After dissection, tissues were immersed in a normal saline solution (0.9% Sodium chloride). Then tissues were fixed in Bouin's fixative for 24 hours and later washed under running tap water until the entire yellow color disappeared. Tissues were dehydrated serially in different grades of alcohol (30%, 50%, 70%, 90%,100%) and cleared in xylene. Tissues were then embedded in paraffin wax (58-60℃). Sections of about 5-6µm thickness were cut on the rotary microtome and subjected to hematoxylin-eosin staining.

3. RESULTS

Behavioral responses are obvious responses that can be assessed as sensitive indicators against oxidative stress and pesticide exposure or contaminated water. In order to derive authentic results, relevant behavioral aspects should be taken into observation to evaluate the exact potential hazard imposed by contaminants. During this whole experimental control group fish maintained a specific schooling behavior, proper opercular movements, and a good response and equilibrium. When disturbed by passing light or startled they immediately formed a tight school and moved to the bottom. In the control group, no extreme or unusual behavior was observed. While in treatment groups the absence or minimum schooling behavior was prevalent from the very beginning i.e. 7 days onward at every dose of cypermethrin. The onset of darting movements was seen on the 14th day at 0.294 µl/L concentration which became extreme on the 7th day at 0.584 µl/L (Table. 1). The ventilation rate was increased, at 14 and 21 days of all the concentrations. Equilibrium and response were distorted badly on the 21st day of 0.584 µl/L, fish lost the ability to balance during swimming and showed no response against light and touch stimulations. Tiredness, weakness, and apathy were reflected in fish. Bottom migration intensity was enhanced with an increase in concentration towards the end of the experiment. Opercular movements were drastically affected due to impairment caused by cypermethrin exposure. The surfacing phenomenon where the frequency to come towards the surface increased, was a prominent behavior during the mild concentrations and was continuously shown till the 21st day at 0.584µl/L concentration in order to enhance the oxygen availability by gulping air.

Table.1 Behavioural responses shown by *C. carpio* at different CYP concentrations

Doses→ Days↓		Control	0.1462 µl/L	0.2924 µl/L	0.439µl/L	0.584 µl/L
	SB	+ +	+	+	-	-
	DM	-	-	+	+ +	+ + +
7	SP	-	-	-	+	+ +
	ER	+ +	+ +	+	+	+
	BM	-	-	+	+ +	+ +
	OM	+ +	+ +	+ +	+	+
	SB	+ +	+	-	-	-
	DM	-	-	+	+ +	+ +
	SP	-	+	+ +	+ +	+ +
14	ER	+ +	+ +	+	+	-
	BM	-	+	+	+ +	+ + +
	OM	+ +	+ +	+	-	+
	SB	+ +	-	-	-	-
	DM	-	+	+ +	+ +	+ +
21	SP	-	+	+	+	+ +
	ER	+ +	+	+	-	-
	BM	-	+	+ +	+	-
	OM	+ +	+	+	+	+

Here SB-Schooling behavior, DM-Darting movement, SP-Surfacing Phenomena, ER-Equilibrium & response, BM-Bottom Migration, OM-Opercular movement. Frequency = (-) absent, (+) minimum, (++) average, (+++) maximum.

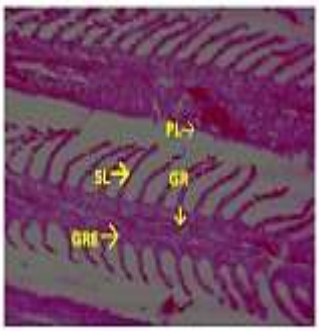


Fig. A

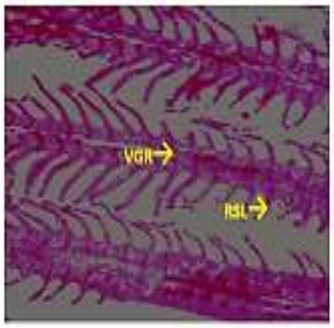
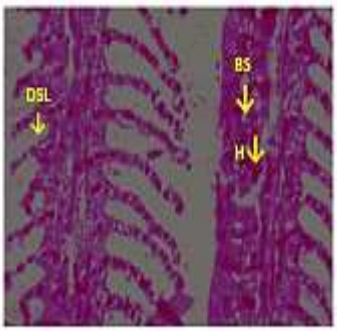


Fig. B



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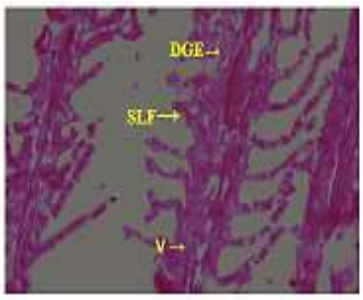

Fig. D

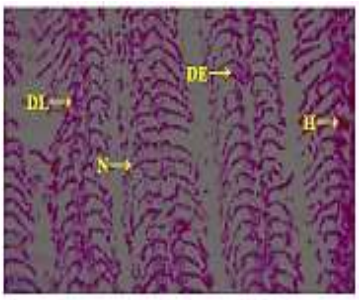

Fig. E

Fig. 1. A- Gills of *C.carpio* from the control group showing no pathological lesions showing laments (GF), gill arches (GA), primary lamellae (PL), secondary gill lamella (SGL), gill rakers (GR) and gill raker epithelium (GRE). Fig. B- Gills of fish at concentration 0.1462 µl/L (Day 21) showing ruptured secondary lamellae (SL), vacuolization of gill raker (GR), svasodilation of central sinus (CS). Fig. C- Gills of carp at concentration 0.2924 µl/L (Day 21) showing hyperplasia (H), disorganized secondary lamellae (SL), and blood spaces (BS). Fig. D- Gills of fish exposed to concentration 0.439µl/L on day 21 showing fusion of secondary gill lamellae (SLF), degenerated lamellae, epithelium (DLE), vacuolization (V). Fig. E- Gills of fish exposed to concentration 0.584 µl/L (21 Day) showing degenerated epithelium (DE), necrosis (N), disorganized gill lamellae (DL), and hyperplasia (H)

4. DISCUSSION

In the present investigation, certain behaviors of carp were situation-specific which justifies the potency of cypermethrin at very minute concentrations. There was a sudden disruption of the schooling behavior of fish after the onset of the experiment. Darting or erratic movements, surfacing phenomena, bottom migration, and loss of response and equilibrium were prominent with an increase in concentration and exposure time as supported by many previous investigations [5,6,7,8,9,10]. To avoid the presence of toxicant (cypermethrin), fish of treated groups showed the bottom migration as reported in trout fish [11]. Gills histology showed a negative shift in the structural organization characterized by some pathological lesions (fused or disorganized secondary lamellae, hyperplasia, curling of lamellae, vacuolization, increased blood spaces, edema, necrosis, etc.) after fish were treated with sub-lethal concentrations. Cypermethrin exposed *C.carpio* showed proliferation, edema, and vasodilation in the secondary lamellae along with lamellar lifting and proliferation in gill epithelium [12]. In *Oreochromis niloticus* during cypermethrin treatment epithelial hyperplasia and desquamation of epithelium with enhancement in interlamellar space as witnessed [13]. Cypermethrin treatment can induce hyperplasia of lamellar cells, and telangiectasia of gill lamellae and can cause thickening due to cellular infiltration in fish gills [14]. Shortening and curling of gill lamellae and gill epithelium showing hyperplasia was witnessed [15] accompanied by lamellar atrophy and fusion of secondary gill lamellae [16]. Damage to histopathology of gills in Lake Van fish *Alburnus tarichi* on cypermetnrin exposure can be seen as lamellar lifting,

aneurysm and expanded blood vessels [17]. Destruction of secondary gill lamellae lining and fusion with contiguous gill lamellae or adhesion and hemorrhage was seen on pyrethroid administration in fish like *Pangasius hypothalamus*, *Channa punctatus* and *Clarias gariepinus* [18,19,20].

5. CONCLUSION

Through this investigation, it can be concluded that cypermethrin (25%) is highly potent to induce disruptions at behavioral and cellular levels in common carp. Even very few quantities of cypermethrin can cause impairments due to its animal-unfriendly formulations that are commercialized widely in Himachal Pradesh and also in India as a whole. The results draw attention towards the potentiality of synthetic pyrethroid to vanish the aquatic fauna especially fish fauna of India.

ACKNOWLEDGMENT

The authors are thankful to the Department of Biosciences, Himachal Pradesh University, and institutional authorities for providing facilities to carry out the research work. The financial assistance to one of the authors (Jyoti Verma) from the Council of Scientific and Industrial Research (CSIR), India in the form of NET-JRF (File. No. 09/237 (0176)/2019 EMR-I) is gratefully acknowledged.

ETHICAL STANDARDS

It is declared that the experiments were conducted as per the guidelines of the institutional animal ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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