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EFFECT OF PYRETHROID CYPERMETRIN ON HEMATOLOGICAL PARAMETERS IN FRESHWATER FISH *Channa punctatus*

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Abstract

Pesticides are modern chemicals, which are used to kill the harmful insect, weed etc. of the crops, when they are applied on crop they also leached into soil and reach to water bodies. In water bodies they affect the aquatic life including fishes. The edible fishes are consumed by human and other livings. So they reached to next trophic level by biotransformation and causing adverse effects to living. Fishes are best bioindicator for these pesticides. Keeping these points in view, present study is designed to observe the effect of pyrethroid cypermethrin on hematological parameters viz. hemoglobin concentration, total leucocyte count, differential leucocyte count in freshwater fish *Channa punctatus*.

Keywords : Pyrethroid, Cypermethrin, Freshwater fish, *Channa punctatus*, Hematology.

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Introduction

The population of the world is increasing continuously since time immemorial and utilization of natural resources is also on increase. Scientists in the early 20th century had to resort to all means necessary to feed the world's rapidly expanding population, which had surpassed all previous records. Some of the produced compounds during this time period were beneficial to humanity but also contributed to his suffering. For a long time, pesticides and other chemical solutions were the norm, but they left behind several health and safety issues.

Cypermethrin is an effective contact insecticide and gastrointestinal toxin. It finds extensive application in such diverse fields as cotton, grains, vegetables, fruits, food storage, public health, and animal husbandry. Its molecular structure is similar to pyrethrum, a naturally occurring insecticide found in chrysanthemum flowers, however it is more potent biologically and more stable than its inspiration. It was first commercialized in 1977 after being synthesized in 1974. While cypermethrin is one of the most important insecticides for cereals and vegetables in the UK, pyrethroids accounted for 40% of global sales for insecticides for cotton treatment in 1988 (cypermethrin 8%). It is also widely used for pest control in the home and for impregnating mosquito nets to combat malaria.

The EPA may designate any or all products containing cypermethrin as Restricted Use Pesticides (RUP) due to the chemical's toxicity to fish. Only trained professionals can acquire and apply Restricted Use Pesticides. The signal word "Danger" or "Caution," depending on the specific formulation, must appear on the label of any pesticide containing cypermethrin. Synthetic pyrethroid pesticide cypermethrin is effective against numerous pests, including cotton, fruit, and vegetable crop moths. Stores, warehouses, factories, homes, apartments, greenhouses, labs, vehicles, trains, buses, trucks, and airplanes all use it to treat cracks, crevices, and problem areas to keep insects at bay. Also, it can be used as a barrier treatment and mosquito repellent for horses, as well as in non-food areas of educational institutions, medical facilities, dining establishments, hotels, and food processing factories. Cypermethrin can be purchased in wettable powder and emulsifiable concentrate forms. Eight distinct isomers make up cypermethrin, and it's possible that each one has its own unique chemical and biological features.

The snakehead fishes known as Channidae are classified under the genus Channa. There are roughly 29 species in this genus, although the northern snakehead (*Channa argus*) and the gigantic snakehead (*Lampropeltis*) are the most well-known (*Channa micropeltes*). The native range of channa extends from western Iran to eastern China and even some remote regions of Siberia. Thailand, Cambodia, Vietnam, and other South East Asian countries cultivate them extensively, making them a common staple meal fish. Snakeheads are not only prized for their culinary value, but also for their medicinal use in promoting wound healing and lowering post-operative pain and suffering, and for their value in the international aquarium pet trade. Fish, frogs, snakes, rodents, birds, and insects all make up part of the diets of several species of Channa. Some of them, like snakes, can walk on land and even breathe oxygen.

Material and Methods

For this study, we collected live Channa punctatus, often known as "soli," from ponds and the Agra fish market in and around Agra. The experimental fish Channa punctatus was chosen due to its accessibility, its resilience in the face of proposed pollutant treatments, and its potential to serve as a

useful indicator of the persistence of toxic effects in soft tissues. Fish also has a high monetary value as a food source.

Fishes of almost identical size and weight were utilized in the experiments so that a constant factor could be established, namely the same age range. In order to treat any potential skin infections, the fish were rinsed in a 0.1% $KMnO_4$ solution. They were then rinsed with regular water and spread into aquariums.

Experimental compound: Cypermethrin

CAS number : 52315-07-8
Trade name : Super killer
Chemical formula : $C_{22}H_{19}Cl_{12}NO_3$
IUPAC number : (R,S)-alpha-cyano-3-phenoxylbenzyl I(IRS)-cis, trans-3-(2,2-dichlorovinyl) -2,2-dimethylcyclopropane-carboxylate

Cypermethrin 25%EC is a synthetic pyrethroid insecticide used to control various pests.

Experimentation

For each the concentration duplicates were runs as control. The water was, however changed every alternate day to maintain the constant concentration of the cypermethrin during the period of exposure. The fishes were not provided any food for 24 hrs, prior to experiments in order to avoid any change in toxicity of the chemical likely to be caused by excretory materials.The sub-lethal concentrations were selected on the basis of LC_{50} value. The selected concentrations were- $1/10^{th}$ of LC_{50} for acute (4days) treatment, $1/20^{th}$ of acute for sub-lethal (20 days) treatment and $1/45^{th}$ of acute for chronic (45 days) treatment respectively. Recovery studies were also done at 45 days.

Haematological Methods

The haemoglobin concentration in the blood was calculated using Wintrobe's outlined Standard Sahli's method (1981).

Total leukocyte count was performed using a modified Standard Neubauer hemocytometer (Wintrobe, 1981) to determine the total number of white blood cells.

The creation of a smear, staining, and microscopic viewing are the three main stages of a differential leucocyte count (DLC).

Probability and Statistics: Online statistical tools were used to perform the calculations for this study.

Results and Discussion

Treatment with 8.124, 1.624, and 0.722 µg/l of cypermethrin for 4, 20, and 45 days correspondingly resulted in a significant decrease in hemoglobin concentration in *Channa punctatus*.

Table 1 : Haemoglobin Concentration (g/dl) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S.No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean ± S.Em.	Significance level
1.	Control	-	5	7.97-8.25	8.18±0.04	-
2.	Acute (4 days)	8.124	5	6.84-7.57	7.03±0.08	P>0.05
3.	Sub-acute (20 days)	1.624	5	6.10-6.48	6.15±0.09	P<0.05
4.	Chronic (45 days)	0.722	5	6.00-6.23	6.10±0.05	P<0.05

Table 2 : Total leucocytes count ($x10^9$/l) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S.No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean ± S.Em.	Significance level
1.	Control	-	5	3.71-3.97	3.85±0.05	-
2.	Acute (4 days)	8.124	5	2.52-2.96	2.82±0.07	P>0.05
3.	Sub-acute (20 days)	1.624	5	2.55-2.78	2.64±0.08	P<0.05
4.	Chronic (45 days)	0.722	5	2.48-2.66	2.55±0.07	P<0.05

Table 3 : Lymphocytes (%) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S.No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean ± S.Em.	Significance level
1.	Control	-	5	49.40-51.50	50.30±0.08	-
2.	Acute (4 days)	8.124	5	55.85-57.45	56.80±0.05	P>0.05
3.	Sub-acute (20 days)	1.624	5	58.20-59.92	58.41±0.07	P<0.05
4.	Chronic (45 days)	0.722	5	66.12-67.58	67.09±0.09	P<0.05

Table 4 : Monocytes (%) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S.No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean ± S.Em.	Significance level
1.	Control	-	5	5.10-5.49	5.29±0.12	-
2.	Acute (4 days)	8.124	5	3.12-3.72	3.52±0.10	P<0.05
3.	Sub-acute (20 days)	1.624	5	2.72-3.30	3.15±0.10	P<0.05
4.	Chronic (45 days)	0.722	5	2.15-2.58	2.35±0.09	P<0.01

Table 5 : Neutrophils (%) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S.No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean ± S.Em.	Significance level
1.	Control	-	5	1.85-1.99	1.90±0.02	-
2.	Acute (4 days)	8.124	5	0.65-0.79	0.70±0.04	P<0.05
3.	Sub-acute (20 days)	1.624	5	0.58-0.75	0.60±0.01	P<0.01
4.	Chronic (45 days)	0.722	5	0.30-0.50	0.40±0.02	P<0.001

Table 6: Eosinophils (%) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S.No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean ± S.Em.	Significance level
1.	Control	-	5	1.82-1.94	1.89±0.01	-
2.	Acute (4 days)	8.124	5	0.90-1.10	1.00±0.01	P<0.05
3.	Sub-acute (20 days)	1.624	5	0.85-1.00	0.94±0.04	P<0.01
4.	Chronic (45 days)	0.722	5	0.75-0.98	0.85±0.08	P<0.01

Table 7 : Basophils (%) in *Channa punctatus* after 4, 20 and 45 days treatment of cypermethrin

S.No.	Experimental Set	Dose (µg/l)	No. of fishes	Range	Mean ± S.Em.	Significance level
1.	Control	-	5	39.00-45.60	40.90±0.15	-
2.	Acute (4 days)	8.124	5	24.69-36.80	32.50±0.17	P>0.05
3.	Sub-acute (20 days)	1.624	5	19.00-33.10	22.10±0.12	P<0.05
4.	Chronic (45 days)	0.722	5	17.50-26.50	20.00±0.09	P<0.01

There is a lack of data on the hematological effects of oral exposure. Fifteen percent of 235 cases of pyrethroid poisoning in humans with blood tests revealed leukocytosis. No major changes were found in hematological end points in the majority of animal trials. However, Shakooriet al. (1992) found that after 7 days of daily oral treatment of 10 mg/kg of fenvalerate, there was a substantial drop in red blood cell count, hemoglobin content, and mean corpuscular hemoglobin, and an increase in white blood cell count in rabbits. Surviving dogs fed pyrethrins in the chow at a concentration resulting in a dose level of roughly 100 mg/kg/day for 8 weeks showed signs of anemia, as described by Shires (1985). Male and female mice given esfenvalerate in the diet at concentrations resulting in mean dosages of 106 and 113 mg/kg/day, respectively, for 90 days showed decreases in red blood cell counts, hemoglobin, and hematocrit.

Our results on the primary haematological responses to synthetic pyrethroid exposure in rainbow trout were slightly different from those published by other researchers (Dorucu and Girgin, 2001; Svobodova *et al*., 2003). There were no changes in the white blood cell profiles of carp after acute exposure to deltamethrin, despite reports of significantly decreased amounts of RBC, Hb, and PVC (P 0.01) (Svobodova *et al*., 2003). Whole leukocyte count and neutrophil granulocyte count were reduced in carp after acute poisoning with permethrin, and decreases in PVC, Hb, LEU, and RBC were recorded after cypermethrin poisoning (Dorucu and Girgin, 2001). (Sopinska and Guz, 1998). Clarias batrachus was tested for its reaction to acute exposure to arsenic in the form of sodium arsenite (Na_3AsO_5). Hemoglobin levels drop in this research. Hemolytic anemia is the outcome of intoxication-related disruptions in hematopoiesis, reduced red blood cell (RBC) iron uptake, and hemolysis.

Total leucocyte count (TLC) and differential leucocyte count (DLC) were shown to be significantly lower in Channa punctatus after exposure to cypermethrin at concentrations of 8.124, 1.624, and 0.722g/l for 4 days, 20 days, and 45 days, respectively. The drop in TLC after drinking may explain why the RBC/WBC ratio increased. Blood T-lymphocyte (TLC) counts were shown to be lower in fenvalerate-exposed C. idella (Shakoori *et al*., 1996). Physiological and chemical characteristics of fish blood may alter as a result of environmental changes. In the current study, DLC showed lymphopenia, decreased basophil, monocyte, and neutrophil counts, and finally increased neutrophil counts. Several fish species reported the same finding (Thakur and Sahai, 1987). These shifts in DLC abundance are further indication that fish exposed to toxins experience a reduction in their nonspecific immune response.

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