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Hematological Parameters of Freshwater Fish Anabas Testudineus after Sublethal Exposure to Cypermethrin

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Abstract. The experiment was conducted to evaluate the hematological effect of cypermethrin, a synthetic pyrethroid on *Anabas testudineus*. The effect was assessed based on the comparison results of control group and experimental groups exposed to sublethal concentrations (0.015, 0.030, 0.045 mg L^{-1}) of cypermethrin on 7^{th}, 14^{th} and 21^{st} days. It was observed that with the increase of cypermethrin concentration, Red Blood Cell (RBC) counts, hemoglobin (Hb) levels, hematocrit (Hct) levels and thrombocyte (platelet) counts decreased. White Blood Cell (WBC) counts increased on 7^{th} day of exposure but WBC counts decreased with the increase of cypermethrin concentrations after 14^{th} and 21^{st} days. The results are statistically significant at $p < 0.05$ level. These reports indicate that hematological parameters may be useful as a diagnostic test for cypermethrin exposure in *A. testudineus*.

Keywords: Cypermethrin, synthetic pyrethroids, *Anabas testudineus*, hematological parameters.

1 Introduction

Pyrethrins are insecticidal compounds obtained from flowers of the plant, *Tanecatum cinerariaefolium* also called *Chrsanthemum cinerariaefolium* or *Pyrethrum cinerariaefolium*. Pyrethroids are synthetic analogs of pyrethrin. An important characteristic of pyrethroids is their differential potency on insects and mammals [1].

Cypermethrin is a synthetic pyrethroid pesticide. Use of cypermethrin is rapidly increasing throughout the world because of its low toxicity to birds and mammals [2]. Cypermethrin is highly toxic to fish [3]. The 96-h LC50 values range between 0.7 and 350 µg L^{-1} [4]. Velmurugan et al. [5] have cited 96-h LC50 of cypermethrin as 0.3 µg L^{-1} for *Anabas testudineus*. Cypermethrin is metabolized and eliminated significantly more slowly by fish than mammals or birds [3] which may explain this compounds higher toxicity in fish compared to other vertebrates.

Hematological parameters are important for reflecting the pathophysiological status of a fish. These parameters have been widely used as indicators of disease or stress due to pollutants [6].

The selected fish *A. testudineus*, is a very hardy fish, which can tolerate extreme conditions. It is widely seen in canals, lakes, ponds, swamps and inland water bodies of India, hence selected as biological indicators of ecotoxicological studies. Several studies have been carried out on the impacts of cypermethrin on the hematology of different fish species [7-10]. But little is known about its tolerance to cypermethrin. Hematology of *A. testudineus* has not been much documented, so this paper would provide an important contribution to the knowledge of the specimen constitution variation.

The present paper is a contribution to the assessment of toxicity and effects of a cypermethrin-based pesticide to *A. testudineus*. Therefore, in the present investigation, it was decided to determine the hematological parameters as biomarker in fish *A. testudineus* exposed to sublethal concentrations of cypermethrin.

2 Materials and methods

2.1 Animals and experimental design

Experimental fish were collected from a freshwater source in the Puzhal Lake, Redhills, Chennai, Tamil Nadu, India. The specimens were transported to the laboratory in appropriately aerated plastic bags. Fish, *A. testudineus* with weight 72 ± 5 g and length 16 ± 1 cm mean ± SD was utilized as the model organisms in this study. The fish were acclimated to the laboratory conditions for at least 20 days prior to the experiment in a glass aquarium. The fresh water used had a pH of 7.5 ± 0.03, temperature of 27.5 ± 1.5 °C, dissolved oxygen 6.4 ± 0.2 mg L^{-1} alkalinity 250 ± 2.8 mg L^{-1} as CaCO3 total hardness 456 ± 3.5 mg L^{-1}. The fish were fed daily with commercially balanced fish food sticks. The fish were maintained on a photoperiod with 12 h light: 12 h dark.

The fish were divided into four groups and placed in separate glass aquaria. Present study was performed in three replicates in order to ensure the reproducibility of the results. Ten fish were used for each group per replicate. Groups I, II and III were exposed to sublethal concentration of cypermethrin. Group IV was maintained in pesticide-free water to serve as control. The sublethal concentrations of cypermethrin tested were 0.015, 0.030, 0.045 mg L^{-1} (5%, 10% and 15% respectively of LC50 value). The 96-hr LC50 for cypermethrin is 0.309 mg L^{-1} for *A. testudineus* [5].

2.2 Sampling and analysis

On 7^{th}, 14^{th} and 21^{st} days of exposure, both the experimental and control fish were anesthetized using 2-phenoxy ethanol. Anesthetized fish were used for collecting fish blood samples. Blood was obtained by severance of caudal peduncle and collected in Eppendorf tubes containing EDTA anticoagulant and mixed immediately [11]. These exposed and control anticoagulant blood samples were used for further haematological parameters.

The hematological parameters analysed were RBC counts, WBC counts, Hb levels, Hct levels and thrombocyte counts. The hematological parameters were measured using Sysmex KX-21N Fully Automatic Cell Counter (Sysmex Corporation, Japan) [12, 13].

2.3 Statistical analysis

One way ANOVA was used to evaluate measurements in this study. The Tukey HSD, Dunnet test was used for multiple comparisons. Normal distributions were evaluated using the Kolmogorov-Smirnow test and homogeneity was evaluated using Levene's test. All data were analyzed using the statistical package SPSS version 15.0 for Windows. The significance of test results was ascertained at $p < 0.05$.

3 Results

3.1 Red Blood Cell

In control, the RBC count was recorded as 4.08 ± 0.34 10^6 μL^{-1} on 7^{th} day. The RBC counts were observed as 3.74 ± 0.25 10^6 μL^{-1} at 0.015 mg L^{-1}, as 2.86 ± 0.18 10^6 μL^{-1} at 0.030 mg L^{-1} and as 2.47 ± 0.23 10^6 μL^{-1} at 0.045 mg L^{-1}. In control, the RBC count was recorded as 4.11 ± 0.27 10^6 μL^{-1} on 14^{th} day. The RBC counts were observed as 3.43 ± 0.22 10^6 μL^{-1} at 0.015 mg L^{-1}, as 3.02 ± 0.15 10^6 μL^{-1} at 0.030 mg L^{-1} and as 2.63 ± 0.14 10^6 μL^{-1} at 0.045 mg L^{-1}. In control, the RBC count was recorded as 4.19 ± 0.27 10^6 μL^{-1} on 21^{st} day. The RBC counts were observed as 1.75 ± 0.21 10^6 μL^{-1} at 0.015 mg L^{-1}, as 1.41 ± 0.21 10^6 μL^{-1} at 0.030 mg L^{-1} and as 1.11 ± 0.21 10^6 μL^{-1} at 0.045 mg L^{-1}. The RBC counts were in decreasing trend with increase in the concentration of cypermethrin on 7^{th}, 14^{th} and 21^{st} days. The overall decline was observed throughout the exposure period. The maximum decrease was found at concentration 0.045 mg L^{-1} as 1.11 ± 0.21 10^6 μL^{-1} on 21^{st} day. In the present study, significant changes of RBC were presented in Table and Figure 1.

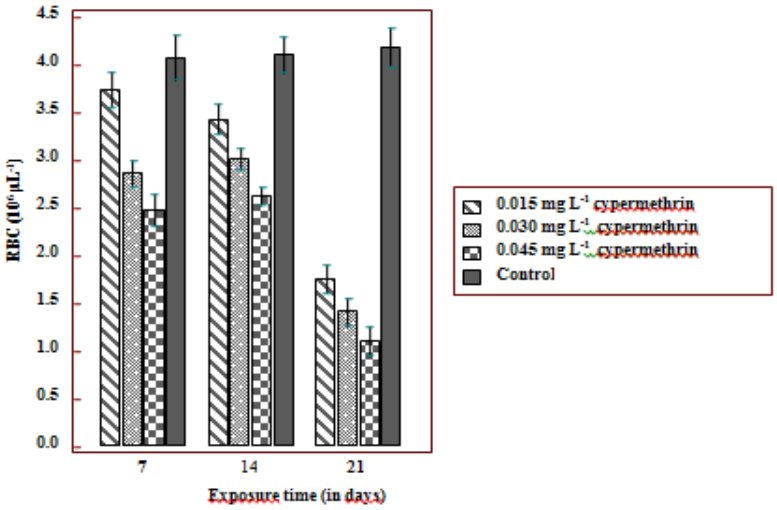

Figure 1. RBC counts in Anabas testudineus exposed to sublethal concentrations of cypermethrin on 7th, 14th and 21st days. Data are expressed as mean ± SD (N = 10). $p < 0.05$

Table. Hematological values of Anabas testudineus exposed to sublethal concentrations of cypermethrin.

Parameters	Cypermethrin (mg L^{-1})	7th day	14th day	21st day
RBC (10^6 µL^{-1})	Control	4.08 ± 0.34^{ax}	4.11 ± 0.27^{ax}	4.19 ± 0.27^{ax}
	0.015	3.74 ± 0.25^{bx}	3.43 ± 0.22^{by}	1.75 ± 0.21^{bz}
	0.030	2.86 ± 0.18^{cx}	3.02 ± 0.15^{cx}	1.41 ± 0.21^{cy}
	0.045	2.47 ± 0.23^{dx}	2.63 ± 0.14^{dx}	1.11 ± 0.21^{dy}
WBC (10^3 µL^{-1})	Control	195.00 ± 12.67^{ax}	194.10 ± 9.57^{ax}	198.40 ± 9.36^{ax}
	0.015	213.90 ± 8.97^{bx}	163.40 ± 11.30^{by}	62.60 ± 13.86^{bz}
	0.030	230.50 ± 10.54^{cx}	152.00 ± 12.79^{by}	53.40 ± 10.24^{bcz}
	0.045	245.90 ± 9.53^{dx}	112.10 ± 13.14^{cy}	40.90 ± 8.16^{cz}
Hgb (g dL^{-1})	Control	16.45 ± 0.93^{ax}	16.81 ± 1.08^{ax}	17.05 ± 1.01^{ax}
	0.015	14.73 ± 1.15^{bx}	12.95 ± 0.97^{by}	6.76 ± 0.77^{bz}
	0.030	11.21 ± 1.25^{cx}	11.13 ± 0.93^{cx}	5.92 ± 0.83^{bcy}
	0.045	10.69 ± 0.96^{cx}	9.43 ± 1.22^{dy}	4.95 ± 0.73^{cz}
Hct (%)	Control	49.67 ± 1.53^{ax}	50.50 ± 1.60^{ax}	51.00 ± 1.61^{ax}
	0.015	47.24 ± 1.29^{bx}	40.00 ± 1.74^{by}	19.14 ± 1.75^{bz}
	0.030	38.50 ± 1.01^{cx}	34.73 ± 1.94^{cy}	17.79 ± 1.89^{bz}
	0.045	30.81 ± 1.27^{dx}	28.85 ± 1.28^{dy}	15.03 ± 1.99^{cz}
Thrombocyte (10^4 µL^{-1})	Control	41.70 ± 5.44^{ax}	43.30 ± 5.12^{ax}	42.80 ± 4.94^{ax}
	0.015	36.40 ± 5.70^{ax}	12.00 ± 3.46^{by}	11.00 ± 2.21^{by}
	0.030	24.90 ± 5.00^{bx}	10.00 ± 3.16^{by}	8.60 ± 2.59^{bcy}
	0.045	18.10 ± 5.04^{cx}	9.30 ± 2.95^{by}	6.50 ± 2.42^{cy}

Values are expressed as mean ± SD (N = 10).

Letters "a," "b," "c"and "d"and indicate differences between groups at the same time, and letters "x", "y" and "z"and indicate differences between times for the same group. $p < 0.05$.

3.2 Hemoglobin

In control, the Hb level was recorded to be 16.45 ± 0.93 g dL^{-1} on 7th dav. The Hb levels were observed as 14.73 ± 1.15 g dL^{-1} at 0.015 mg L^{-1}, as 11.21 ± 1.25 g dL^{-1} at 0.030 mg L^{-1} and as 10.69 ± 0.96 g dL^{-1}

at 0.045 mg L^{-1}. In control, the Hb level was recorded to be 16.81 ± 1.08 g dL^{-1} on 14th day. The Hb levels were observed as 12.95 ± 0.97 g dL^{-1} at 0.015 mg L^{-1}, as 11.13 ± 0.93 g dL^{-1} at 0.030 mg L^{-1} and as 9.43 ± 1.22 g dL^{-1} at 0.045 mg L^{-1}. In control, the Hb level was recorded to be 17.05 ± 1.01 g dL^{-1} on 21st day. The Hb levels were observed as 6.76 ± 0.77 g dL^{-1} at 0.015, as 5.92 ± 0.83 g dL^{-1} at 0.030 mg L^{-1} and as 4.95 ± 0.73 g dL^{-1} at 0.045 mg L^{-1}. The Hb levels were in decreasing trend with increase in the concentration of cypermethrin on 7th, 14th and 21st days. The overall decline was observed throughout the exposure period. The maximum decrease was found at concentration 0.045 mg L^{-1} as 4.95 ± 0.73 g dL^{-1} on 21st day. In the present study, significant changes of Hb levels were presented in Table and Figure 2.

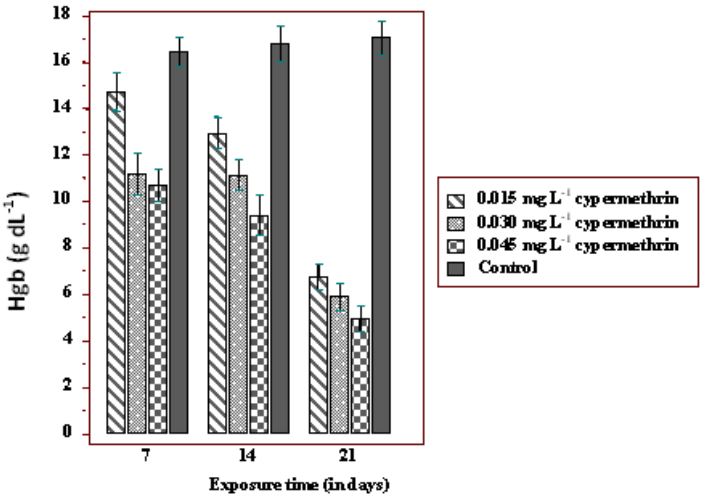

Figure 2. Hgb levels in Anabas testudineus exposed to sublethal concentrations of cypermethrin on 7th, 14th and 21st days. Data are expressed as mean ± SD (N = 10). $p < 0.05$.

3.3 Hematocrit

In control, the HCT level was recorded to be 49.67 ± 1.53 % on 7th day. The HCT levels were were observed as 47.24 ± 1.29 % at 0.015 mg L^{-1}, as 38.50 ± 1.01 % at 0.030 mg L^{-1} and as 30.81 ± 1.27 % at 0.045 mg L^{-1}. In control, the HCT level was recorded to be 50.50 ± 1.60 % on 14th day. The HCT levels were observed as 40.00 ± 1.74 % at 0.015 mg L^{-1}, as 34.73 ± 1.94 % at 0.030 mg L^{-1} and as 28.85 ± 1.28 % at 0.045 mg L^{-1}. In control, the HCT level was recorded to be 51.00 ± 1.61 % for 21 days. The HCT levels were observed as 19.14 ± 1.75 % at 0.015 mg L^{-1}, as 17.79 ± 1.89 % at 0.030 mg L^{-1} and as 15.03 ± 1.99 % at 0.045 mg L^{-1}. The HCT levels were in decreasing trend with increase in the concentration of cypermethrin on 7th, 14th and 21st days. The overall decline was observed throughout the exposure period. The maximum decrease was found at concentration 0.045 mg L^{-1} as 15.03 ± 1.99 % on 21st day. In the present study, significant changes of HCT levels were presented in Table and Figure 3.

3.4 White Blood Cell

In control, the WBC count was recorded to be 195.00 ± 12.67 10^3 μL^{-1} on 7th day. The WBC counts were observed as 213.90 ± 8.97 10^3 μL^{-1} at 0.015 mg L^{-1}, as 230.50 ± 10.54 10^3 μL^{-1} at 0.030 mg L^{-1} and as 245.90 ± 9.53 10^3 μL^{-1} at 0.045 mg L^{-1}. The WBC counts elevated with increase in the concentrations of cypermethrin on 7th day. In control, the WBC count was recorded to be 194.10 ± 9.57 10^3 μL^{-1} on 14th day. The WBC counts when exposed to 0.015, 0.030 and 0.045 mg L^{-1} were observed as 163.40 ± 11.30 10^3 μL^{-1}, 152.00 ± 12.79 10^3 μL^{-1} and 112.10 ± 13.14 10^3 μL^{-1} respectively. The WBC counts declined with increase in the concentrations of cypermethrin on 14th day. In control, the WBC count was recorded to be 198.40 ± 9.36 10^3 μL^{-1} on 21st day. The WBC counts were observed as 62.60 ± 13.86 10^3 μL^{-1} at 0.015 mg L^{-1}, as 53.40 ± 10.24 10^3 μL^{-1} at 0.030 mg L^{-1}, and as 40.90 ± 8.16 10^3 μL^{-1} at 0.045 mg L^{-1}. The WBC counts were in decreasing trend with increase in the concentrations of cypermethrin on

21st day. The WBC counts showed changes in *A. testudineus* exposed to sublethal concentrations of cypermethrin on 7th, 14th and 21st days. The increasing counts of WBC were observed on 7th day and the elevation was found to be dose dependant. The decreasing counts of WBC were observed on 14th and 21st days. The maximum decline was found as 40.90 ± 8.16 10^3 μL^{-1} at 0.045 mg L^{-1} on 21th day. In the present study, significant changes of WBC were presented in Table and Figure 4.

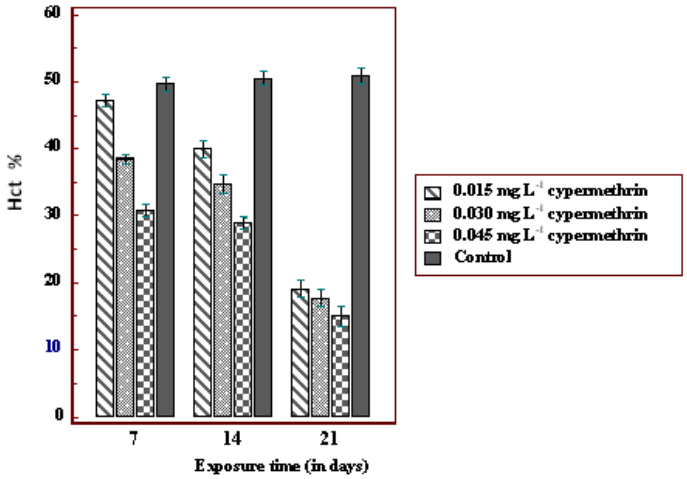

Figure 3. HCT levels in Anabas testudineus exposed to sublethal concentrations of cypermethrin on 7th, 14th and 21st days. Data are expressed as mean ± SD (N = 10). $p < 0.05$.

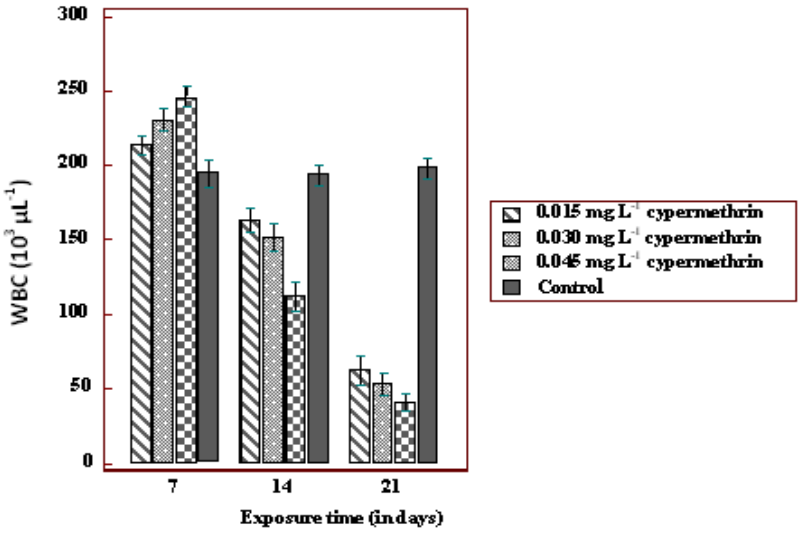

Figure 4. WBC counts in Anabas testudineus exposed to sublethal concentrations of cypermethrin on 7th, 14th and 21th days. Data are expressed as mean ± SD (N = 10). $p < 0.05$.

3.5 Thrombocyte

In control, the thrombocyte count was recorded to be 41.70 ± 5.44 10^4 μL^{-1} on 7th day. The thrombocyte counts were observed as 36.40 ± 5.70 10^4 μL^{-1} at 0.015 mg L^{-1}, as 24.90 ± 5.00 10^4 μL^{-1} at 0.030 mg L^{-1} and as 18.10 ± 5.04 10^4 μL^{-1} at 0.045 mg L^{-1}. In control, the thrombocyte count was recorded to be 43.30

± 5.12 10^4 μL^{-1} on 14th day. The thrombocyte counts were observed as 12.00 ± 3.46 10^4 μL^{-1} at 0.015 mg L^{-1} , as 10.00 ± 3.16 10^4 mg μL^{-1} at 0.030 mg L^{-1} and as 9.30 ± 2.95 10^4 μL^{-1} at 0.045 mg L^{-1}. In control, the thrombocyte count was recorded to be 42.80 ± 4.94 10^4 μL^{-1} on 21st day. The thrombocyte counts were observed as 11.00 ± 2.21 10^4 μL^{-1} at 0.015 mg L^{-1}, as 8.60 ± 2.59 10^4 μL^{-1} at 0.030 mg L^{-1} and as 6.50 ± 2.42 10^4 μL^{-1} at 0.045 mg L^{-1}. The thrombocyte counts decreased with increase in the concentration of cypermethrin on 7th, 14th and 21st days. The maximum decrease was found at concentration 0.045 mg L^{-1} as 6.50 ± 2.42 10^4 μL^{-1} on 21st day. In the present study, significant changes of thrombocyte counts were presented in Table and Figure 5.

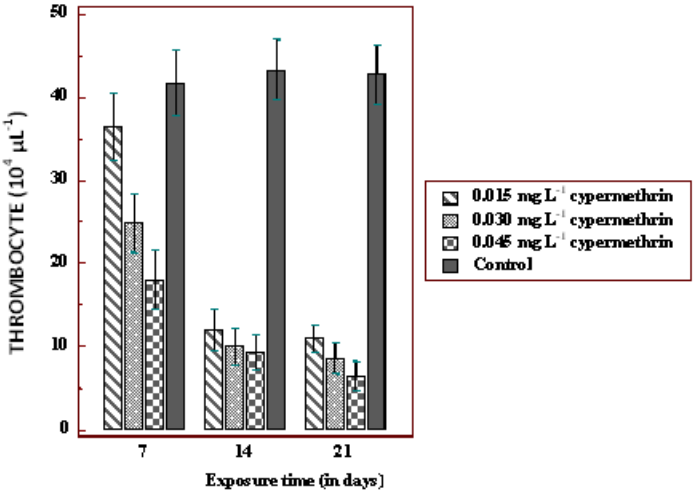

Figure 5. Thrombocyte counts in Anabas testudineus exposed to sublethal concentrations of cypermethrin on 7th, 14th and 21st days. Data are expressed as mean ± SD (N = 10). $p < 0.05$.

4 Discussion

The results obtained on the hematology parameters of *A. testudineus* exposed to sublethal concentrations of cypermethrin on 7th, 14th and 21st days showed a significant variation in the blood parameter compared with the control group of fish. Hematology parameters are important in the health status of any organism [14]. In fish, they are used for clinical diagnosis of fish physiology which is determined by the effect of the internal and external physical environment [15].

The results obtained in this study showed decreases in RBC counts, Hb and HCT levels of fish exposed to increased concentrations of cypermethrin. Similar reduction in RBC counts and Hb levels had been also reported in *Oncorhynchus mykiss* [16, 17), *Labeo rohita* [18, 19], *Sebastes schlegeli* [20], *Prochilodus lineatus* [21] exposed to cypermethrin. The significant reduction in RBC counts may be due to disruptive action of the cypermethrin on the erythropoietic tissue [22]. The significant decrease in the Hb levels may also be due to either an increase in the rate at which the Hb is destroyed or to a decrease in the rate of Hb synthesis [23]. Increased destruction of RBC can lead to decreased hemoglobin. The decrease in HCT in fish exposed to cypermethrin was due to decreased RBC count.

In the present study, WBC count increased on 7th day of exposure. On 14th and 21st days of exposure, WBC count decreased. The increases in WBC counts (leucocytosis) were reported in *Clarias gariepinus* [8], *L. rohita* [18, 19], *Cyprinus carpio* [9], *O. mykiss* [17] exposed to cypermethrin. But, the decreases in WBC counts (leucopenia) were also described in the *O. mykiss* [16, 24] exposed to cypermethrin. The increase in WBC counts can be related with an increase in antibody production which helps in survival and recovery of the fish exposed to cypermethrin. Reduced numbers of leucocytes in exposed fish can result in reduced disease resistance [25].

There was significant reduction in thrombocyte counts with the exposure of the fish to cypermethrin. Similar reduction in thrombocyte counts had been also reported in *O. mykiss* exposed to cypermethrin

[26]. The decrease of this parameter can be related with trapping of thrombocytes in the spleen, decreased thrombocyte production or increased destruction of thrombocytes.

In conclusion, our study clearly indicated that the physiology of the fish was disturbed by the cypermethrin. The cypermethrin caused hematological disturbances which could lead to impairment ability to combat diseases, reduce its chances for survival for growth of fish.

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