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Impact of Cypermethrin 25% EC and pH on Certain Haematological Parameters of Cyprinid Fish *Puntius javanicus*

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Abstract: In present study an attempt has been made to investigate the combined impact of sub lethal toxicity of Cypermethrin 25% EC and pH on certain blood parameters of *Puntius javanicus*. Adult fish were exposed to sub-lethal concentration (0.36 µg/L) of cypermethrin 25% EC in different pH water i.e. pH 5, pH 6, pH 7, pH 8.5 and pH 9.5 for 15 days. Significant changes in red blood cell (RBCs) count, total white blood cells (WBCs), haemoglobin % (Hb%), haematocrit value, total serum protein and blood glucose level were observed in fish exposed to sub-lethal concentration of cypermethrin. Significant decrease in red blood cell (RBCs) count, haemoglobin % (Hb %), haematocrit value and total serum protein content were found in fish exposed to sub-lethal concentration of cypermethrin in pH 5, pH 6 and pH 9.5, compared to fish exposed to sub-lethal concentration of cypermethrin in pH 7. A significant increase in these parameters were documented in fish exposed to sub-lethal concentration of cypermethrin in pH 8.5, compared to fish exposed to sub-lethal concentration of cypermethrin in pH 7. On the other hand a significant increase of total white blood cells (WBCs) and blood glucose level were recorded in fish subjected to sub-lethal concentration of cypermethrin in pH 5, pH 6 and pH 9.5, compared to fish exposed to sub-lethal concentration of cypermethrin in pH 7. Values of these parameters were decreased significantly in fish exposed to sub-lethal concentration of cypermethrin in pH 8.5, compared to fish exposed to sub-lethal concentration of cypermethrin in pH 7. The values of all studied blood parameters in fish subjected to sub-lethal concentration of cypermethrin in pH 8.5 were almost found to retain the values of control group (pH 7, without toxicant).

Keywords: Cypermethrin, Haemoglobin, Haematocrit, Blood glucose, Serum protein, Sub-lethal concentration,

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Introduction

There has been surging increase in the use of agricultural chemicals like pesticides to

preserve the standing crop from the attack of pests and to boost up crop production, in

order to meet the ever-increasing food demand of the increasing human population. (Aktar *et al.*, 2009; Chandola *et al.*, 2011; Gupta *et al.*, 2012). Due to injudicious and indiscriminate use of pesticides, the natural water resources such as lakes, reservoirs, wetlands, rivers, ponds, paddy-fields, streams, and other low-lying areas are getting polluted all over the world (Gilliom *et al.*, 2007; Ngidlo, 2013). Pesticides affect whole ecosystem, particularly the aquatic ones, leading to unwanted mortality of aquatic biota, in general and fish in particular as revealed by several workers (Kumari, 2005; Gupta *et al.*, 2013). Synthetic pyrethroids that have been introduced over the past two decades for agricultural and domestic use affect fish health adversely (Sanchez- Fortun *et al.*, 2005). The biochemical changes in different organs/tissues of fish due to toxicity stress of heavy metals and pesticides have been reported by number of workers (Khan, 1982; Balint *et al.*, 1995; Das *et al.*, 1999; Rao *et al.*, 2000; Khare *et al.*, 2002; Mishra *et al.*, 2011; Srivastav *et al.*, 2013, 2021). Pyrethroid is metabolized and eliminated comparatively more slowly by the fish than by mammals or birds (Bradbury *et al.*, 1989).

Blood is most important and abundant body fluid. Its composition often reflects the total physiological condition (Svoboda *et al.*, 2001; Adeyemo, 2008). Consequences of pesticides on haematological parameters have been investigated in number of carp species-- in *Oreochromis mosumbicus* (Ali and Rani, 2009; Deasi and Parikh, 2012); in *Cyprinus carpio* and *Puntius ticto* (Satyanarayan *et al.*, 2004; Salvo *et al.*, 2008); in *Puntius mesopotamicus* (Saxena and Seth, 2002; Carraschi *et al.*, 2012). The blood parameters have been used as diagnostic indices of

pathological condition and are very important tool for the assessment of systemic functions and overall health of animals. The alteration of haematology also help in diagnosis of structural and functional condition of fish exposed to toxicant (Talas and Gulhan, 2009; Atamanalp *et al.*, 2010; Prasad *et al.*, 2015). As fish live in aqueous environment the blood will illustrate conditions within the fish body long before visual manifestation of any disease (Okechukwu and Auta, 2007).

Many abiotic factors have been reported to affect the health of the fish. Abiotic factors such as temperature, pH, water column length, turbidity, salinity, alkalinity of water etc. are found to have profound effect on the reproductive success of the fish (Wilson, 1956; Herzig and Winkler, 1986; Ikuta and Kitamura, 1995; Ernst *et al.*, 2005; Tiffany *et al.*, 2009). Amongst all these abiotic factors, pH is one of the most important factor affecting aquatic life. Levels of pH 4 constitute acidic condition which is lethal to fish (Ikuta *et al.*, 1992).

Although ecotoxicological manifestations of pyrethroid intoxication have been documented by several workers in various organs of fish but despite our best efforts, we could not gather any information regarding the combined impact of pH and cypermethrin on *Puntius javanicus*.

Materials and Methods

Experimental design:

Healthy freshwater fish *Puntius javanicus* (100 ± 5; 17-19 cm) were obtained from a local fish reservoir of Sasan, near Taki Road, Barasat and treated with potassium permanganate solution (0.5% w/v) for 1 min to remove any dermal adherent. The fish were acclimatized

Table 1: Experimental groups

10 fish/ group Exposure period: 15 days	Group	Parameters
	1 (Control 1)	pH 7 with no Cypermethrin
	2 (Control 2)	pH 7 + Sublethal concentration Cypermethrin
	3	pH 5 + Sublethal concentration Cypermethrin
	4	pH 6 + Sublethal concentration Cypermethrin
	5	pH 8.5 + Sublethal concentration Cypermethrin
	6	pH 9.5 + Sublethal concentration Cypermethrin

for 2 weeks in a rectangular glass aquarium (500 L) containing de-chlorinated aerated tap water at room temperature (32 ± 1 C). Characteristics of water were tested according to the APHA. During this time fish were fed daily with commercial food pellet about 3% of their body weight. Dead fish were removed immediately to avoid fouling of water. 60 fish were divided into 6 groups (Table 1), 10 fish for each group and exposed to sub-lethal concentration of cypermethrin at different pH for a period of 15 days. Regulation of pH of water was done by using sodium hydroxide and hydrochloric acid. The water of aquaria was changed at an interval of 24 h and concentrations of cypermethrin as well as different pH were restored afresh as mentioned above. Equal volume of acetone was maintained in the control 1 aquarium (pH 7, no toxicant), as the pyrethroids used were solubilised in acetone.

Haematological estimation:

At the end of exposure period, both control and test fish were anaesthetized with mild dose of Benzocaine (Larid and Oswald, 1975). Blood was taken from caudal vein into (i) Eppendorf tube (no anticoagulant) and (ii) using heparinized disposable syringe containing 0.5 mg EDTA as anticoagulant. Blood collected in heparinized syringes with

anticoagulant was properly mixed and stored at -20 C for haematological analysis. RBC and WBC counts were performed using improved nebular haemocytometer and calculated (Blaxhall and Diasley, 1973). Replicated counts were made for each blood samples. Sahli's haemoglobinometer was used to estimate haemoglobin percentage (HB%). Haematocrit (HCT) was determined by the method of Wintrobe. Blood (anticoagulant free) was centrifuged (3000 rpm for 10 min) and serum was separated and stored at -80 C until the analysis. The glucose level in the blood was estimated by the method of Dubowski (1962). Total protein content was estimated by the method of Lowry *et al.* (1951).

Statistical analysis:

Data are presented as mean $\pm$ SE. Apart from descriptive statistics of the outcome variables, appropriate multivariate statistic such as One-way ANOVA, LSD was done with Minitab, version 17 Software to understand the causal relationship between parameters.

Results

Cypermethrin exposed fish in pH 7 exhibited significant decreases in RBC count, Haemoglobin %, Haematocrit and total serum protein compared to control group. A significant increase in total WBC count and

Table 2: Alteration of blood parameters of *P. javanicus* subjected to sub-lethal concentration of cypermethrin

Parameters	Control 1 (pH 7)	Control 2 (pH 7+toxicant)
Total RBC	5.638 ± 0.007	5.512 ± 0.004***
Total WBC	20.060 ± 0.093	20.520± 0.066***
Hb%	9.874 ± 0.029	8.786 ± 0.060***
HCT	39.272 ± 0.067	35.060 ± 0.198***
Serum Protein	4.680 ± 0.074	4.160 ± 0.040***
Serum Glucose	320 ± 2.381	342 ± 1.983**

** indicates P<0.01, *** indicates P<0.001, each value represents the mean ± SE

Table 3: Alteration of blood parameters of *Puntius javanicus* subjected to sub-lethal concentration of cypermethrin in different pH

Parameters	Control 2, pH 7+toxicant	pH 6+toxicant	pH 5+toxicant	pH 8.5+toxicant	pH 9.5+ toxicant
Total RBC	5.512 ± 0.004	5.374 ±0.007***	5.236±0.010***	5.574±0.007**	5.080±0.046***
Total WBC	20.520± 0.066	21.300±0.071***	21.820±0.086***	20.340±0.051*	21.400±0.100***
Hb%	8.786 ± 0.060	7.088±0.046***	6.536±0.088**	9.468±0.200***	6.612±0.032**
HCT	35.060 ± 0.198	33.254±0.200***	31.708±0.279***	38.072±0.202**	30.856±0.269***
Serum Protein	4.160 ± 0.040	3.880± 0.037***	3.260± 0.060***	4.560± 0.040**	3.240± 0.060***
Glucose	342 ± 1.983	376.5±3.063***	424.167±17.141***	326.333±1.687***	442.666±1.745*

The treated groups were compared with Control 2. ** indicates P<0.01, *** indicates P<0.001, each value represents the mean ± SE.

blood glucose in cypermethrin treated fish in pH 7 was recorded (Table 2). Present study showed considerable significant gradual decrease of RBC count, Haemoglobin %, Haematocrit and serum protein and significant gradual increase in total WBC count and blood glucose in fish exposed to pH 5 + toxicant, pH 6+ toxicant and pH 9.5+ toxicant compared to fish exposed to pH 7+

toxicant group (Table 3; Figure 1). A significant increase in RBC count, Haemoglobin %, Haematocrit and total serum protein was seen in pH 8.5+ toxicant group compared to pH 7+ toxicant group (Table 3; Figure 1). In this study there was significant gradual increase in total WBC count and blood glucose in fish exposed to pH 5 + toxicant, pH 6 + toxicant and pH 9.5+toxicant compared to

Table 4: One-way ANOVA for blood parameters in experimental fish, *Puntius javanicus*

		Sum of Squares	df	Mean Square	F	Sig.
Total RBC	Between Groups	1.147	5	0.229	117.342	0.000
	Within Groups	0.047	24	0.002		
	Total	1.194	29			
Total WBC	Between Groups	12.099	5	2.420	76.413	0.000
	Within Groups	0.760	24	0.032		
	Total	12.859	29			
Hb%	Between Groups	55.821	5	11.164	580.461	0.000
	Within Groups	0.462	24	0.019		
	Total	56.283	29			
Haematocrit	Between Groups	291.112	5	58.222	288.976	0.000
	Within Groups	4.835	24	0.201		
	Total	295.947	29			
Glucose	Between Groups	92382.222	5	18476.444	643.779	0.000
	Within Groups	861.000	30	28.700		
	Total	93243.222	35			
Total serum Protein	Between Groups	9.666	5	1.933	152.616	0.000
	Within Groups	0.304	24	0.013		
	Total	9.970	29			

fish exposed to pH 7+ toxicant group, whereas a significant decrease in total WBC count and blood glucose was seen in pH 8.5 + toxicant group compared to pH 7 + toxicant group. The difference of RBC count, WBC count, Haemoglobin %, Haematocrit, total serum protein and blood glucose is least in pH 8.5+ toxicant and control group (Figure 2).

A significant decrease in total WBC count and blood glucose was seen in pH 8.5 + toxicant group compared to pH 7 + toxicant group (Figure 1). The difference of RBC count, WBC count, Haemoglobin %, Haematocrit, total serum protein and blood glucose is least in pH 8.5 + toxicant and control group (Figure 2).

Fig. 1: Variation in different blood parameters in different experimental groups of *Puntius javanicus*. Data represented as Mean $\pm$ SE. (A) Total RBC, (B) Total WBC, (C) Haemoglobin %, (D) Haematocrit, (E) Blood glucose, (F) Total serum protein.

One-way ANOVA was done to determine the difference in total RBC count, Haemoglobin %, Haematocrit, total WBC

count, blood glucose and total serum protein between control and experimental groups. Results of the statistical analysis referred to

Fig. 2: Proximity in different blood parameters among experimental groups of *Puntius javanicus*. (A) Total RBC, (B) Total WBC, (C) Haemoglobin %, (D) Haematocrit, (E) Blood glucose, (F) Total serum protein.

significant differences ($P < 0.001$) between the control and different experimental groups for every parameter studied (Table 4).

Discussion

Haematological parameters are reported to be altered by number of factors such as size, age, physiological state, environmental condition and stress (Parma *et al.*, 2007; Ighwela *et al.*, 2012). The significant decrease in RBC count might be due to haemolysis and shrinkage of blood cell by the toxicity of the water. Reduction of RBC count indicates a reduced blood oxygen carrying capacity and anaemia

in fish due to stress (Tak *et al.*, 2014). The progressive reduction in Hb % in both acidic and alkaline conditions indicated that there was a possibility of respiratory stress in the carp. Decreased hemoglobin and haematocrit concentration indicate the RBC is being destroyed by the leukocytosis activity in an erythrocytic anaemia subsequent erythroblastosis. Haemodilution resulting from the impaired osmoregulation across the gill epithelium also might have contributed to such reduction in Hb%. Reduction of serum protein content in different pH medium also

supports the view of erythrocytic cell lysis and hemodilution (Wedemeyer *et al.*, 1984; Das *et al.*, 2006). A decrease in RBC and Hb content has been observed earlier in fish exposed to different stressor (John, 2007; Kori-Siakpere and Oghohene, 2008). An increased WBC count is considered to be an adaptive value for the tissue under chemical stress. Increase in WBC count can be correlated with an increase in the antibody production which help in survival and recovery of the fish exposed to toxicant (Joshi *et al.*, 2002). This also helps in removal of cellular debris of necrosed tissue at faster rate (John, 2007). In present study WBC count increased as a protective response of the body during stress imparted by both cypermethrin and pH. This is in agreement with earlier investigations (Svobodova *et al.*, 2003; Padash-Bramchi *et al.*, 2010; Desai and Parikh, 2012). Blood glucose is highly reliable secondary stress response parameters that are strongly influenced by handling and environmental stress, nutritional status and sexual maturity (Prasad *et al.*, 2010). Stress also induces elevation of plasma catecholamines in the fish within minutes (Mazeud *et al.*, 1981). These released catecholamines, adrenalin and nor-adrenalin increase the conversion of liver glycogen to blood glucose to satisfy the greater energy demands of the body to stress (Sriwastava and Srivastava, 1985). Increased blood glucose level in the present study indicated elevated stress level in carp which was most likely due to the disturbance in acid-base balance, respiratory homeostasis and ionic regulation during exposure to the changed water pH.

The serum protein reduction in fish in all cases may be attributed to protein catabolism, the process of converting blood and structural

protein to energy, to meet the higher demand during prevailing stress. Haemolysis and shrinkage of the erythrocyte also might cause dilution of plasma volume contributing to some extent to reduction of serum protein content (Das *et al.*, 2004, 2006).

Present study documented alteration of certain blood parameters due to exposure of sub-lethal dose of cypermethrin 25% EC. These alterations were very much influenced by alteration of pH of water. Fish exposed to the basic water (pH 8.5 and 9.5) can somehow able to minimize the alteration induced by the toxicant in comparison with the fish exposed to acidic water (pH 5 and 6). Fish maintained in pH 8.5 shows much less alteration of parameters retaining almost same values compared to control. Pesticide exposure is unavoidable fact in present day. Fish exposed to pH 8.5 were found to be able to minimize the alterations of certain blood parameters induced by cypermethrin 25% EC.

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