INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–0986/2018/20–3–601–608
DOI: 10.17957/IJAB/15.0527
http://www.fspublishers.org

Full Length Article

Effects of Sub-Lethal Concentration of Cypermethrin on Histopathological and Hematological Profile of Rohu (*Labeo rohita*) during Acute Toxicity

Nasir Khan[1], Munawar Saleem Ahmad[2], Sadia Tabassam[1], Faisal Nouroz[3], Ashfaq Ahmad[4] and Shehzad Ghayyur[4], Atta Ur Rehman[1] and Muhammad Fiaz Khan[1*]
[1]*Department of Zoology, Hazara University, Mansehra, Pakistan*
[2]*Department of Zoology, University of Swabi, Swabi, Pakistan*
[3]*Department of Botany, Hazara University, Mansehra, Pakistan*
[4]*Government Postgraduate College Haripur, KPK Pakistan*
[*]For correspondence: fiazkhanhu333@gmail.com

Abstract

The present investigation was designed to understand the lethal effects of Cypermethrin (CYP); an active pyrethroid, a widely used against an extensive range of pests in agriculture. In this study, Rohu (*Labeo rohita* Hamilton) was subjected to the treatment with three sub-lethal concentrations (0.50, 1.00 and 1.5 ppb) of CYP for a total period of 96 h. The results obtained indicated significant stress in the studied fish due to pesticide intoxication. Pronounced effects of CYP on fish hematology included significant increase ($p<0.05$) in white blood cells (WBCs), platelets and blood glucose level in treated group compared to control group. Conversely; however, substantial decrease in red blood cells (RBCs), hemoglobin (Hb) level, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was noticed among the treated group related to control group ($p<0.05$). Similarly, the histopathological lesions in the gills of exposed fish included lamellar disorder, disruption of cartilaginous core, epithelial lifting, blood mobbing, damage to secondary lamellae, fusion of secondary gills lamellae, twisting and shortening of secondary gills lamellae and degeneration and atrophy. While liver exhibited dissolution of cell membrane, Pyknosis, blood congestion, necrosis, hyperplasia and vacuolations; intestine was characterized by symptoms like necrosis, hemorrhages, over production of goblet cells in villi, disintegration, fusion and shortening of villi. In summary, we reported marked impacts of CYP on both hematological and histopathological traits of *L. rohita*. These, findings warrant that the indiscriminate use of CYP significantly increased WBCs, platelets and blood glucose level whereas it decreased RBCs, Hb, HCT, MCV, MCH and MCHC respectively. It also significantly affected the histopathology such as disruption of gills, liver and intestine architecture.

Keywords: Pesticides; Cypermethrin toxicity; *Labeo rohita*; Pakistan

Introduction

The use of pesticides is a common practice in agriculture in recent era owing to the increasing demand of food requirements. Nevertheless, the use of pesticide has resulted in contamination of the environment with far-reaching consequences for living organisms. For instance, only 0.1% of the total applied pesticides reach the pests while remaining 99.9% find their way to alter the components of environment (Marigoudar *et al.*, 2009). Organophosphorus compounds were commonly used in industries, medicines and agriculture (Amin and Hashem, 2012). However; until recently this pesticide is continuously substituted with pyrethroids owing to their greater photo stability, less toxicity in birds as well as mammals, greater efficiency even in low concentration, and easy to disintegrate (Bradbury and Coats, 1989). The pyrethroids, are decidedly toxic to fish with little capacity to neutralize and hydrolyze such compounds (Haya, 1989). Therefore, the core cause of elevated toxicity for fish aquaculture are the pesticides from farming fields brought with rain into coves, ponds and rivers. Another possible source of this contamination may be the phytoplanktons (Das and Mukherjee, 2003). Pyrethroids are proved to possess an elevated degree of gill absorption even at very low concentrations in the water and this has been contributed to their lipophilicity which in turn causes sensitivity in fish to aqueous pyrethroid exposure due to low rate of pyrethroids metabolism (Viran *et al.*, 2003).

Cypermethrin (CYP), a synthetic pyrethroid, is one of the most effective insecticide used in forestry, agriculture,

To cite this paper: Khan, N., S. Tabassam, M.S. Ahmad, F. Norouz, A. Ahmad, S. Ghayyur, A.U. Rehman and M.F. Khan, 2018. Effects of sub-lethal concentration of cypermethrin on histopathological and hematological profile of rohu (*Labeo rohita*) during acute toxicity. *Int. J. Agric. Biol*., 20: 601–608

buildings and farmyards (Casida *et al.*, 1983; Khan *et al.*, 2006). Commercially CYP is being used against cotton and soybean pests (Carriquiriborde *et al.*, 2007). The use of CYP is increasing day by day in the world for fishing. In addition, some aqua culturists also use CYP as a chemotherapeutic agent to eradicate different copepod infestations (Medina *et al.*, 2002; Athanassopoulou *et al.*, 2009; Nafees and Jan, 2009). Consequently, CYP due to its diffusion and surface runoff into natural water reservoirs, seemed as a main hazard to the aquatic fauna including fishes (John and Prakash, 2003).

Besides its well-developed nervous, immune, endocrine and osmoregulatory systems, fish species exhibit high sensitivity to fluctuations in external environment and is capable of bio-accumulating toxic materials in higher concentration relating to surrounding water. These attributes favored the fish to be used as an important eco-toxicological biomarker and suitable bio-indicator as compared to other vertebrates and invertebrates (Song *et al.*, 2012; Huang *et al.*, 2013; Eagles-Smith and Ackerman, 2014; Dhanakumar *et al.*, 2015; Zhao *et al.*, 2016). The pesticides accumulation in the important organs, including liver and muscles may leads to organ dysfunction resulting in demise of the fish. Similarly, efficiency of some enzymes indispensable for metabolic purposes of fish may be altered (Srivastava and Kaushik, 2001).

In Pakistan, CYP is being used extensively for a variety of purposes including, but not limited to, pest control of different crops, fruits and vegetables and illegitimate fishing (Khan *et al.*, 2006; Nafees and Jan, 2009). Around the world, it is being used in aquaculture as a chemotherapeutic agent against lice infiltration and preventing invasion by copepod parasites (Boxaspen and Holm, 2001; Barata *et al.*, 2002; Medina *et al.*, 2002). Thus, use of CYP on commercial level could cause ecological toxicity in water reservoirs, which in turn could have adverse implications on the aquatic biota generally and fish particularly (John and Prakash, 2003). The current investigation was undertaken to investigate the sub lethal effect of CYP on certain hematological and histopathological parameters of *Labeo rohita* Hamilton.

Materials and Methods

Physico-chemical Parameters of Water

The physico-chemical parameters of test water used in this experiment were: Temperature (21±1.19°C); PH (7.5±0.23); total dissolved solids (366±10.81 mg/L); dissolved oxygen (6.4±0.53 mg/L); total alkalinity (129±4.89 mg/L); calcium hardness (44±1.55 mg/L); chloride (18.5±2.1 mg/L) and conductivity (819±14.9 mS/cm).

Fish Collection and Acclimatization

A total of thirty Rohu fish (*Labeo rohita* Hamilton) with a body weight (336.25±28.53 g) were collected from Abdul Wahid fish farm Punjab and were transferred to the dechlorinated fresh-water reservoir under controlled temperature where they were kept for at least three days and were fasted for 24 h before embarking the experiments. The fish were then soaked for a few seconds in 0.2% K_2MnO_4 solution followed by washing with tap water. Fish were then left to adapt to laboratory environment for about a week preceding the onset of trials. They were kept in aerated glass aquarium filled with dechlorinated tap water having a temperature of 20–23°C, pH 7.6–7.8 and 100% dissolved oxygen. During this retro, fish were fed daily with commercial food. The residual food was eliminated after serving. Around 70% of the water was changed each day during acclimatization period.

Study Design

Cypermethrin ([S, R]-N-α-cyno-3-phenoxybenzyl-(IR, IS, cis, trans)-2, 2-dimethyl-3, (2, 2-dichlorovinyl, cyclopropane carboxylate), used in this study was purchased from a local market of Haripur, Khyber Pakhtunkhwa, Pakistan. So as to assess the impacts of CYP on *L. rohita*, test solutions of three varying concentrations i.e., 0.50 ppb, 1.00 ppb and 1.50 ppb of CYP were formulated from a commercial devising carrying 32% active ingredients. Randomly selected Five fishes were stocked in each of the four aquaria of 80 L water capacity which were half filled. Each of the tank was provided with different concentration of CYP and were labeled as, E0, E1, E2 and E3. E0 had zero concentration of CYP and considered as control group, E1 had 0.50 ppb, E2 had 1.00 ppb while E3 had 1.50 ppb CYP. Fishes were exposed to CYP for 96 h and tested solutions were renewed after 24 h throughout the experiment.

Hematological and Biochemical Effects of CYP

For studying hematological, blood samples were collected from each fish in EDTA containing tubes. The blood samples were analyzed using HEMA READER HRG 6300D (auto hematological analyzer Advanced Japanese Technology, China). The hematological parameters checked in this study included total red blood cell count, hemoglobin content, total white blood cell count and hematocrit level. Likewise, the corpuscular indices comprised mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Histopathological and Behavioral Effects of CYP

For biochemical analysis, the clotted tubes carrying blood samples were spun for separation of plasma from blood cells. Plasma was collected and glucose level was determined by using Chem Reader SBA-733 Plus (Semi-auto Chemistry analyzer, Advanced Japanese Technology).

For histopathological analysis, each fish was weighed on a digital balance. Moreover, for measurements of the total length, standard length and body depth measuring tape was used. The fish were then incised in order to dichotomize the particular biomarker organs including gills, intestine and liver. Each organ was separately weighed and preserved in 10% neutral buffered formalin (NBF) for 48 h. After fixation, tissues were allowed to dehydrate in ascending succession of alcoholic grades, cleared in xylene and entrenched in paraffin wax. Slices of 4–5 mm thickness were cut on a rotary microtome which were then stained with eosin and hematoxylin and were inspected under a camera fitted microscope, OPTICA TCB 3.0 Italy. Finally, the behavior of *L. rohita* subjected to treatment with different concentration of CYP was recorded during 96-h trial.

Statistical Analysis

Statistical data analysis was carried out using SPSS software (Version 20.0). Comparison was made among all of the four experimental assemblies using one-way ANOVA. It was then followed by comparison of hematological variables using independent t-test between control and treated groups. Variables were stated as means and standard deviations. $p<0.05$ was considered as statistically significant.

Results

Behavioral Effects of CYP

As a consequence of exposure to CYP, *L. rohita* showed severe symptoms in all the treated assemblies of fish (0.5, 1.00 and 1.50 ppb). Several changes in their behavior were observed during acute toxicity. For instance, the distinct changes seen in treated fishes included abnormalities like rapid swimming as fish was added to CYP solution. Furthermore, as the time passed, fishes lost body equilibrium, started swimming at lateral sides, showed high opercular movement, several fishes tried to jump out of aquariums indicating sign of stress, fishes secreted slime, directed their tails downwards and head upwards etc. Control fishes, on the other hand, did not deviated from their normal swimming behavior.

Hematological and Biochemical Effects of CYP

With CYP exposure, significant changes in the total blood cells count of treated fishes but not the control group were noticed. Overall, WBCs count increased significantly in the treated than control group. Conversely, total RBCs count declined markedly in the treated than control group. Likewise, hemoglobin level also decreased in all the treated groups of fish as compared to control group. While the hematocrit level was significantly decreased in all the three treated groups of fish. The MCV values were increased significantly in all three treated groups. A slight decline was observed in the MCH level in one treated group compared to control group (32.82 ± 0.70), the values decreased in two treated groups significantly $p<0.005$. In contrast with control group, the value of MCHC decreased slightly in two treated groups of fish, while a significant change was noticed in treated group III ($p<0.05$). Similarly, the number of platelets increased significantly in treated group compared to control group consistently with the increasing concentration of CYP. Blood glucose level was increased in all three treated groups of fish (0.50 ppb 68.2 ± 0.165, $p<0.05$; 1.00 ppb 84.96 ± 0.250, $p<0.05$; 1.50 ppb 103.16 ± 0.248) as compared to control group (60.02 ± 0.191). Thus, comparison of all the treated groups showed significant increase in WBCs, platelets and blood glucose level while a significant reduction in RBCs, Hb, HCT, MCV, MCH, and MCHC was noticed as the concentration of CYP increased (Table 1).

Histopathological Effects of CYP

Control gill tissues: Under ordinary light microscope, the gill tissues section of control group of *L. rohita* showed normal assembly of gill filament, primary and secondary gill lamellae having central cartilaginous core and a thin epithelial layer (Table 2). There is a thick lining of stratified epithelium between primary and secondary gill lamellae containing the mucous cells and chloride cells (Fig. 1A–B).

Treated gill tissues: As revealed by histopathological results, CYP primarily targeted gill tissue. For instance, group I treated with 0.50 ppb concentration of CYP showed disruption, fusion and shortening of secondary gills lamellae. In addition, mild blood congestion was also seen (Table 2; Fig. 1C and D). In treated group II and III exposed to 1.00 ppb and 1.50 ppb concentrations of CYP respectively, histopathological changes became more prominent in gills. The changes were shortening, fusion and disruption of secondary gills lamellae, epithelial lifting, and atrophy, disruption of cartilaginous core, blood congestion, lamellar disorganization and curling (Fig. 1E and H).

Control liver tissue: When examined with light microscope, liver tissue from control group of *L. rohita* exhibited normal structure of liver cells (hepatocytes) bearing foamy cytoplasm. Hepatocyte is a roundish polygonal cell body containing a clear spherical nucleus and nucleolus (Table 2; Fig. 2A and B).

Treated liver tissues: In group-I treated with 0.50 ppb of CYP hepatocytes exhibited slight vacuolations, blood congestion, mild pyknosis, dissolution of cell membrane, vacuolations in the hepatocytes (Fig. 2C–D). In group-II treated with 1.00 ppb concentration of CYP, these histopathological variations were moderate in liver (Fig. 2F). In group-III treated with 1.50 ppb of CYP, hepatocytes exhibited pulsating histopathological changes including vacuolations, dissolution of cell membrane, blood congestion, necrosis, pyknosis, and lymphocyte infiltration (Table 2; Fig. 2G–H).

Table 1: Summarized hematological and biochemical (glucose) parameters of both control and treated groups of rohu

Hematological and biochemical parameters	Control group	Treated groups		
	0.00 ppb	T1 (0.50 ppb)	T2 (1.00 ppb)	T3 (1.50 ppb)
Total WBCs	78.34 ± 0.49	79.46 ± 0.64$^{**\Delta}$	89.52 ± 1.38$^{**\Delta}$	97.40 ± 0.87$^{**\Delta}$
Lymphocytes	64.56 ± 0.55	64.52 ± 0.46$^{**\Delta}$	70.40 ± 0.61$^{**\Delta}$	74.76 ± 0.46$^{**\Delta}$
Monocytes	6.58 ± 0.56	6.86 ± 0.33$^{**\Delta}$	9.20 ± 1.12$^{**\Delta}$	9.78 ± 0.52$^{**\Delta}$
Neutrophils	7.20 ± 0.40	8.08 ± 0.27$^{**\Delta}$	9.92 ± 0.54$^{**\Delta}$	12.86 ± 0.50$^{**\Delta}$
RBCs	3.94 ± 0.14	2.92 ± 0.07$^{**\Delta}$	2.03 ± 0.06$^{**\Delta}$	1.34 ± 0.12$^{**\Delta}$
Hb	12.94 ± 0.39	10.40 ± 0.44$^{**\Delta}$	8.00 ± 0.22$^{**\Delta}$	5.90 ± 0.31$^{**\Delta}$
HCT	24.82 ± 0.34	19.52 ± 0.50$^{**\Delta}$	15.78 ± 0.31$^{**\Delta}$	12.54 ± 0.45$^{**\Delta}$
MCV	62.97 ± 1.85	66.87 ± 2.20$^{**\Delta}$	77.75 ± 3.98$^{**\Delta}$	93.94 ± 7.53$^{**\Delta}$
MCH	32.82 ± 0.70	28.77 ± 0.90	39.37 ± 0.49**	44.26 ± 4.86**
MCHC	52.15 ± 1.46	53.32 ± 3.06	50.73 ± 2.41	47.06 ± 2.44**
Platelets	48.40 ± 0.49	55.24 ± 0.49$^{**\Delta}$	61.34 ± 0.99$^{**\Delta}$	68.08 ± 0.58$^{**\Delta}$
Glucose Level	60.02 ± .191	68.2 ± 0.165$^{**\Delta}$	84.96 ± 0.250$^{**\Delta}$	103.16 ± 0.248$^{**\Delta}$

Unit of measurements: WBCs (10^9/L), RBCs (10^{12}/L), Hb (g/dL), HCT (%), MCV (fL), MCH (pg) MCHC (g/dL), Platelets (10^9/L) and Glucose Level (mg/dL). Values are expressed as Mean ± SD (n=5 fish per treatment). Mean with ** expresses significant difference ($p< 0.05$) while Δ shows significant intergroup difference

Control intestine tissues: The light microscopy of intestine section of control group of *L. rohita* presented normal structure of villi with no alteration (Table 2; Fig. 3A–B). The histological architecture of intestinal villi consists of mucosal epithelium, lamina properia, muscularis mucosa, stratum compactum and serous membrane.

Treated intestine tissues: In treated group-I exposed to 0.50 ppb of CYP, intestine showed mild alterations including goblet cells formation in villi and shortening of villi (Table 2; Fig. 3C–D). In treated group-II exposed to 1.00 ppb concentration of CYP these histopathological alterations were moderate along with fusion of villi, necrosis, hemorrhages and pyknosis (Fig. 3E–F). In treated group-III exposed to 1.50 ppb of CYP, the intestine showed severe histopathological changes (Table 2; Fig. 3G–H).

Discussion

The current investigation was conducted to understand the noxious level induced by commercial CYP pesticide on the hematological, histopathological and biochemical aspects of *L. rohita*. Overall, no mortality was witnessed during 96 h exposure. Because most of the pesticides influence the activity of acetylcholinesterase (AChE) consequently producing abnormal behavioral patterns in the fish. In our study, *L. rohita* was intoxicated with 0.50, 1.00 and 1.5 ppb concentrations of CYP and various signs of behavioral distress like hyper-excitability, erratic and darting swimming, increased mucous secretion, loss of equilibrium, opening of gills and sinking to the bottom was observed in the treated group of Rohu. Hematological parameters are one of the most important biomarkers to examine the effects of pesticides on health of an organism (Lavanya *et al.*, 2011). Pyrethroid pesticides causes significant alterations in hematological and histopathological parameters of fish (Velisek *et al.*, 2009; Mahboob *et al.*, 2017).

Hematological investigations have proved a valued tool for the fisheries experts to detect alterations in fish morphology and these alterations may lead to a change in behavior of fish and evident lacerations (Authman *et al.*, 2015). In this study, significant changes were noticed in blood components of treated fish. These changes included reduction in the red blood cells count, Hb level, Hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (HCH) and mean corpuscular hemoglobin concentration (MCHC) values. On the other hand, certain components of blood were increased as a result of intoxication with CYP. These components include white blood cells and platelets count. The reduction in Hb and several other blood components might be due to the inhibition of RBCs' and haem synthesis, osmoregulatory dysfunction and destruction of RBCs in hematopoietic organs as reported earlier in *Catla catla* (Vani *et al.*, 2011). Leukocytes play a significant role in regulation of immunological role and the variations in WBC counts after treatment with several toxicants may depict a decline in generic insusceptibility of the fish (Okomoda *et al.*, 2013).

The substantial increment in leukocyte count in the current investigation may be attributed to general immune response and a defensive response of CYP. According to (Ndimele *et al.*, 2015), hemoglobin, red blood cells, MCV and MCHC decreased significantly while WBC and Thrombocyte increased in *Clarias gariepinus* as a result of acute exposure to Endosulfan. Similarly, hematological examination reported by (Kavitha *et al.*, 2012) revealed a significant reduction in Hb, HCT, RBC and MCHC levels in fish exposed to *Moringa oleifera* seed extract. Generally, reduction in hemoglobin level, hematocri, and number of red blood cells might be owing to erythroblastosis causing anemia (Saleh and Marie, 2016). Similar observations were also previously reported in *C. gariepinus*, which was exposed to tobacco leaf extracts, and cassava effluents (Adamu, 2009). White blood cells are the main components of the blood that shield the organism in the time of injury, hemorrhage and entry of foreign antigen particle in the body (Velmurugan *et al.*, 2016). During stress, the number of leukocytes increases significantly to cope with conditions of stress and defend organism (Deshmukh, 2016).

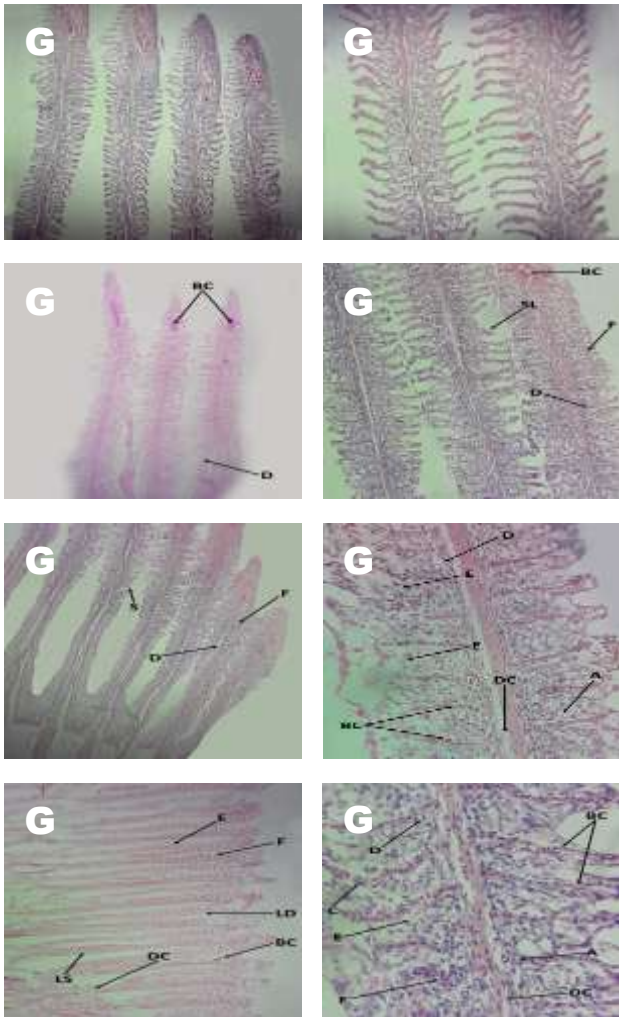

Fig. 1: A and B shows Gill structure of control fish while Gills tissues of treated fish C to H shows Epithelial lifting (E), Fusion of secondary gills lamellae (F), Lamellar disorganization (LD), Blood congestion (BC), Disruption of cartilaginous core (DC) and Shortening secondary gills lamellae (SL), Disruption of secondary gills lamellae (D), Curling (C) and Atrophy (A)

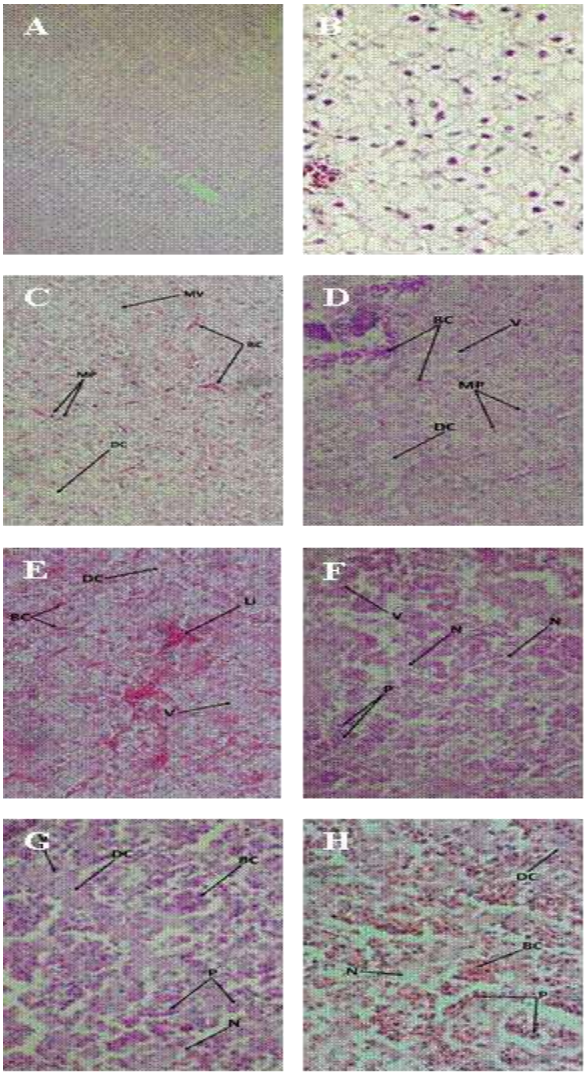

Fig. 2: A and B shows Liver structure of control fish while Liver tissues of treated fish C to H shows Dissolution of cell membrane (DC), Blood congestion (BC), Necrosis (N) and Pyknosis (P), Vacuolations in the hepatocytes (V), Lymphocyte infiltration (Li)

Thrombocytes are one of the indispensable component of blood playing major role in clotting of blood by absorbing various factors for blood clotting and delivering them to the site of injury of hemorrhage (Singh and Srivastava, 1981). Likewise, (Hasan *et al.*, 2015), reported a significant increase in platelet number during acute toxicity of Grass carp when exposed to endosulfan.

A momentous surge in the blood glucose level was noticed in all the treated groups of *L. rohita* as compared to the control group. This result is in accordance with that of (Das and Mukherjee, 2003) who had also reported hyperglycemia in *L. rohita* upon exposure to CYP. Hyperglycemia in fish is a general secondary stress response to acute toxic effect and reliable sign of ecological stress. It enables the animal to cope with the condition of stress by making more energy available to vital organ as per demand (Suvetha *et al.*, 2010). Same results was obtained by (Velisek *et al.*, 2006) in rainbow trout exposed to acute CYP, showed significant increased level of alkaline phosphatase, ammonia, aspartate aminotransferase, lactate dehydrogenase, creatine kinase and glucose in blood plasma. Contrarily (Agrahari *et al.*, 2007) who stated that Monocrotophos exposure induced hypoglycemia in *Channa puntatus*. Fish treated with lindane exhibited a substantial increase in plasma glucose level during the entire investigation related to the control groups (Saravanan *et al.*, 2011).

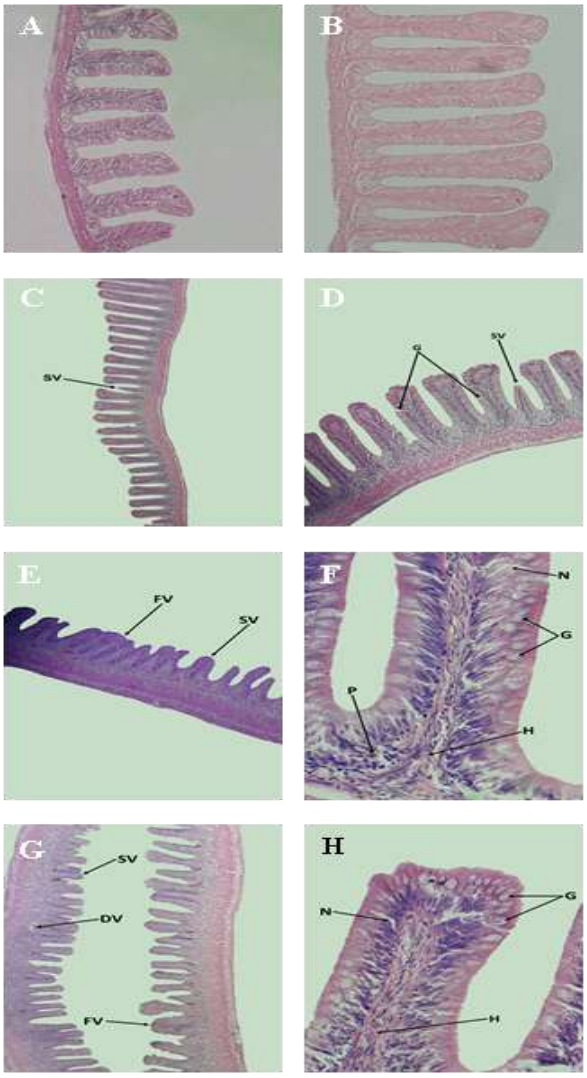

Fig. 3: A and B shows Intestine structure of control fish. Intestine tissues of treated fish C to H Shows Shortening of villi (SV), Goblet cells formation in villi (G) Fusion of villi (F), Necrosis (N), Hemorrhages (H) and Pyknosis (P), Detachment of villi (DV)

Various histopathological studies indicated that gills are the first target of fishes on exposure to pesticides and are prime indicators to show water quality (Qureshi *et al.*, 2016). Gills beside absorbing oxygen perform many important functions such as regulation of ions, acid base balance and elimination of nitrogenous wastes from body. So, the ecological toxicants affect these vital organs severely and indirectly impose significant effects on health of fish (Bantu *et al.*, 2017). In present study, various hematological alterations were noticed in gills of fish exposed to various concentrations of CYP. Among them prominent ones are lamellar disorganization, disruption of cartilage, epithelial lifting, loss, fusion, curling and shortening of secondary gills lamellae, atrophy and blood

Table 2: Summarized histopathological effects on gills, liver and intestine of both control group and groups exposed to various concentrations of cypermethrin

Body parts	Histopathological alterations	E-0	E-1	E-2	E-3
Gills	Lamellar disorganization	-	-	*	***
	Disruption of cartilaginous core	-	-	**	***
	Blood congestion	-	*	**	***
	Disruption of Sec gills lamellae	-	*	**	***
	Epithelial lifting	-	-	**	***
	Loss of secondary lamellae	-	-	*	***
	Fusion of Sec gills lamellae	-	*	**	***
	Shortening of Sec gills lamellae	-	*	**	***
	Atrophy	-	-	**	***
	Curling	-	-	*	***
Liver	Dissolution of cell membrane	-	*	**	***
	Blood congestion	-	*	**	***
	Vacuolations in hepatocytes	-	*	**	***
	Hyperplasia	-	-	**	***
	Pyknosis	-	*	**	***
	Necrosis	-	-	**	***
	Lymphocyte infiltration	-	*	**	***
Intestine	Disruption of villi	-	-	*	***
	Fusion of villi	-	-	**	***
	shortening of villi	-	*	**	***
	Goblet cells formation in villi	-	*	**	***
	Hemorrhages	-	-	**	***
	Necrosis	-	-	**	***

Absent (-), Rare (*), Frequent (**), Abundant (***)

congestion. The extent of tissue damage increased as a result of exposure to increasing concentration of CYP. Several other investigations have revealed parallel impacts of pesticides on fish gills. Studies conducted by (Ghanbahadur and Ghanbahadur, 2012) showed that deterioration of secondary gill lamellae, necrosis of respiratory epithelium and devastation of primary gill lamellae is also evident after exposure of *Rarbora daniconius* to endosulfan pesticide. According to (Hasan *et al.*, 2015) exposure of *Ctenopharyngodon idella* to endosulfan during acute toxicity results in blood mobbing, reduction in size and joining and fusion of both primary and secondary gills lamellae, epithelial lifting in the gills and hypertrophy. Histopathological alterations in gills of *L. rohita* during 96 h acute toxicity with CYP is same as the findings of toxicological impact of fungicide propiconazole on gill tissue including alterations such as desquamation of the epithelial lining, lamellar disorganization, hemorrhagic congestion and necrosis at the secondary lamellae (Tabassum *et al.*, 2016). Different pesticides showed similar results in different fishes. For instance *C. punctata* exposed to pendimethalin showed cellular hypertrophy with loss in the epithelial layer, fusion of secondary lamellae, cellular degeneration, necrosis of gill epithelial tissues, epithelial lifting and blood congestion in the vascular axis of primary filaments (Tabassum *et al.*, 2016), *Cyprinus carpio* exposure to buprofezin and fipronil (Qureshi *et al.*, 2016). *Arius thalassinus* exposed to heavy metals (Saleh and Marie, 2016), *Puntius ticto* exposure to 5.012 ppm of dimethoate (Marutirao, 2012),

common carp (Pal *et al.*, 2012) and *C. punctatus* (Devi and Mishra, 2013) exposed to Chlorpyrifos, resulting in ascending histopathological alterations with increasing various pesticides concentrations.

Liver is one of the vital organs in the body that plays a major role in carbohydrates, proteins and fats metabolism. It is also an organ for detoxifying harmful agents. Accumulation of most of the pesticides and their by-products in hepatocytes results in significant histopathological modification and variation in liver (Sharma *et al.*, 2012). In the current investigation, dissolution of cell membrane, blood mobbing and congestion, pyknosis, necrosis, hyperplasia and vacuolations of hepatocytes in *L. rohita* was noticed after exposure to CYP. (Murussi *et al.*, 2016; Saleh and Marie, 2016). The histological modifications reveled in the liver of *C. punctatus* during present trials are in accordance to the results in Nile tilapia i.e., *Oreochromis misniloticus* (Coimbra *et al.*, 2007) and rain bow trout i.e., *Oncorhynchus mykiss* (Altinok and Capkin, 2007) treated with varying concentrations of endosulfan, Common carp treated with chlorpyrifos (Pal *et al.*, 2012), *C. carpio* treated with buprofezin fipronil (Qureshi *et al.*, 2016). *C. catla* exposed to α-CYP (Muthuviveganandavel *et al.*, 2013).

Intestine is a prime part of fish digestive system, playing major role in digestion and assimilation of food materials. It is highly sensitive to any toxic material engulfed and can be used as an important biomarker organ for assessment of ecotoxicology. In this study, changes in intestinal tissues of *L. rohita* were predominantly necrosis, hemorrhages, overproduction of goblet cells in villi, fusion, detachment and shortening of villi as reported earlier by (Hasan *et al.*, 2015), for acute endosulfan toxicity exposure. The deterioration of villi, mucosal folds disintegration, vacuolations, hypertrophy, and necrosis in *C. carpio* and *Cirrhinus mrigala* treated with atrazine and fenvalerate, was observed (Velmurugan *et al.*, 2007). Severe mucosal secretion occurs due to distress enabling fish to cope with ecological stress (Samanta *et al.*, 2016). Leukocytes infiltration, necrosis in gut tissues in Mosquito fish, *Gambusia affinis* treated with deltamethrin (Cengiz and Unlu, 2006); Intestinal lesions, eosinophils invasion into the lamina properia and epithelial cells atrophy was observed in *C. mrigala* exposed to lambda-cyhalothrin for 60 days, which showed shortening of villi with inflammation, rupture of cells, degeneration changes in tips of villi, curved villi, hemorrhage, necrosis, numerous vacuoles, dilation in the blood vessels, completely damaged villi and loss of architecture in a number of fish species (Cengiz and Unlu, 2006; Velmurugan *et al.*, 2007; Vidhya and Nair, 2016).

Conclusion

This study unraveled the toxic effects of commercial CYP formulation on behavior, hematology, histopathology and biochemical (glucose) parameters of freshwater fish *L. rohita*. The present study could be useful to access the previously unanticipated possible environmental hazards of CYP to aquatic life. In summary, this study suggested that CYP significantly increased WBCs, Platelets and Blood Glucose Level whereas it resulted in decreased RBCs, Hb, HCT, MCV, MCH and MCHC values. It significantly affected the histopathology like lamellar disorder, disruption of cartilage, epithelial lifting, blood congestion, damage, fusion, twisting, shortening, degeneration of secondary gills lamellae and atrophy in gills, dissolution of cell membrane, pyknosis, blood congestion, necrosis, hyperplasia and vacuolations in liver and necrosis, hemorrhages, over production of goblet cells, disintegration, fusion and shortening of villi in intestine, respectively.

References

Adamu, K.M., 2009. Sublethal effects of tobacco (*Nicotiana tobaccum*) leaf dust on enzymatic activities of Heteroclarias (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). *Jord. J. Biol. Sci*., 2: 151–158

Agrahari, S., K.C. Pandey and K. Gopal, 2007. Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch). *Pest. Biochem. Physiol.*, 88: 268–272

Altinok, I. and E. Capkin, 2007. Histopathology of rainbow trout exposed to sublethal concentrations of methiocarb or endosulfan. *Toxicol. Pathol.*, 35: 405–410

Amin, K.A. and K.S. Hashem, 2012. Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (*Clarias gariepinus*): antioxidant defense and role of alpha-tocopherol. *BMC Vet. Res.*, 8: 45

Athanassopoulou, F., I. Pappas and K. Bitchava, 2009. An overview of the treatments for parasitic disease in Mediterranean aquaculture. *Options Méditerr. Ser A*, 86: 65–83

Authman, M.M., M.S. Zaki, E.A. Khallaf and H.H. Abbas, 2015. Use of fish as bio-indicator of the effects of heavy metals pollution. *J. Aquat. Res. Dev.*, 6: 1

Bantu, N., N. Kumari and R. Vakita, 2017. Histological Alteration in Different Tissues of Indian major carp, *Labeo rohita* (Hamilton) exposed to Profenofos 50% EC and Carbosulfan 25% EC formulations. *J. Biol. Today World*, 6: 38–45

Barata, C., M. Medina, T. Telfer and D. Baird, 2002. Determining demographic effects of cypermethrin in the marine copepod *Acartia tonsa*: stage-specific short tests versus life-table tests. *Arch. Environ. Contam. Toxicol.*, 43: 0373–0378

Boxaspen, K. and J.C. Holm, 2001. The development of pyrethrum- based treatments against the ectoparasitic salmon lice *Lepeophtheirus salmonis* in sea cage rearing of atlantic salmon *Salmo salar* L. *Aquacult. Res*., 32: 701–707

Bradbury, S.P. and J.R. Coats, 1989. Comparative toxicology of the pyrethroid insecticides. *Rev. Environ. Contam. Toxicol.*, Springer, The Netherlands

Carriquiriborde, P., J. Díaz, H. Mugni, C. Bonetto and A.E. Ronco, 2007. Impact of cypermethrin on stream fish populations under field-use in biotech-soybean production. *Chemosphere*, 68: 613–621

Casida, J.E., D.W. Gammon, A.H. Glickman and L.J. Lawrence, 1983. Mechanisms of selective action of pyrethroid insecticides. *Annu. Rev. Pharmacol. Toxicol.*, 23: 413–438

Cengiz, E.I. and E. Unlu, 2006. Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: a microscopic study. *Environ. Toxicol. Pharmacol.*, 21: 246–253

Coimbra, A.M., A. Figueiredo-Fernandes and M.A. Reis-Henriques, 2007. Nile tilapia (*Oreochromis niloticus*), liver morphology, CYP1A activity and thyroid hormones after endosulfan dietary exposure. *Pest. Biochem. Physiol.*, 89: 230–236

Das, B.K. and S.C. Mukherjee, 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences. *Comparative Biochem. Physiol. Part C: Pharmacol. Toxicol.*, 134: 109–121

Deshmukh, D., 2016. Hamatological response in a freshwater fish *Channa Striatus* exposed to endosulfan pesticide. *Biosci. Discov.*, 7: 67–69

Devi, Y. and A. Mishra, 2013. Histopathological alterations in gill and liver anatomy of fresh water, air breathing fish *Channa punctatus* after pesticide Hilban® (Chlorpyrifos) treatment. *Adv. Bioresour.*, 4: 57–62

Dhanakumar, S., G. Solaraj and R. Mohanraj, 2015. Heavy metal partitioning in sediments and bioaccumulation in commercial fish species of three major reservoirs of river Cauvery delta region, India. *Ecotoxicol. Environ. Saf.*, 113: 145–151

Eagles-Smith, C.A. and J.T. Ackerman, 2014. Mercury bioaccumulation in estuarine wetland fishes: Evaluating habitats and risk to coastal wildlife. *Environ. Pollut.*, 193: 147–155

Ghanbahadur, A. and G. Ghanbahadur, 2012. Histopathological effect of organochloride endosulphan on intestine and stomach of larvivorous fish *Rasbora daniconius*. *Res. Rev. J. Zool. Sci*., 4: 28–33

Hasan, Z., S. Ghayyur, Z.U. Hassan and S. Rafique, 2015. Histomorphometric and hematological profile of grass carp (*Ctenopharyngodon idella*) during acute endosulfan toxicity. *Pak. Vet. J.*, 35: 23–27

Haya, K., 1989. Toxicity of pyrethroid insecticides to fish. *Environ. Toxicol. Chem.*, 8: 381–391

Huang, B., B. Wang, D. Ren, W. Jin, J. Liu, J. Peng and X. Pan, 2013. Occurrence, removal and bioaccumulation of steroid estrogens in Dianchi Lake catchment, China. *Environ. Int.*, 59: 262–273

John, P. and A. Prakash, 2003. Bioaccumulation of pesticides on some organs of freshwater catfish Mystus vittatus. *Bull. Environ. Contam. Toxicol.*, 70: 1013–1016

Kavitha, C., M. Ramesh, S.S. Kumaran and S.A. Lakshmi, 2012. Toxicity of *Moringa oleifera* seed extract on some hematological and biochemical profiles in a freshwater fish, *Cyprinus carpio*. *Exp. Toxicol. Pathol.*, 64: 681–687

Khan, B.A., A. Farid, N. Khan, K. Rasul and K. Perveen, 2006. Survey of pesticide use on fruits and vegetables in district Peshawar. *Sarhad J. Agric.*, 22: 497

Lavanya, S., M. Ramesh, C. Kavitha and A. Malarvizhi, 2011. Hematological, biochemical and ionoregulatory responses of Indian major carp *Catla catla* during chronic sublethal exposure to inorganic arsenic. *Chemosphere*, 82: 977–985

Mahboob, S., S. Sultana, K.A. AlGhanim, F. Al-Misned, T. Sultana, B. Hussain and Z. Ahmed, 2017. Distribution and accumulation of polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAHs) and organo-chlorine residues in the muscle tissue of *Labeo rohita*. *Int. J. Agric. Biol.*, 19: 701–706

Marigoudar, S.R., R.N. Ahmed and M. David, 2009. Cypermethrin induced respiratory and behavioural responses of the freshwater teleost, *Labeo rohita* (Hamilton). *Vet. Arch.*, 79: 583–590

Marutirao, G.R., 2012. Histopathological changes in the gills of *Puntius ticto* (Ham) under Dimethoate toxicity. *Bioscan*, 7: 423–426

Medina, M., C. Barata, T. Telfer and D. Baird, 2002. Age-and sex-related variation in sensitivity to the pyrethroid cypermethrin in the marine copepod *Acartia tonsa* Dana. *Arch. Environ. Contam. Toxicol.*, 42: 17–22

Murussi, C.R., M.D. Costa, J.W. Leitemperger, L. Guerra, C.C. Rodrigues, C.C. Menezes, E.S. Severo, F. Flores-Lopes, J. Salbego and V.L. Loro, 2016. Exposure to different glyphosate formulations on the oxidative and histological status of *Rhamdia quelen*. *Fish Physiol. Biochem.*, 42: 445–455

Muthuviveganandavel, V., I. Hwang, V. Anita, P.S. Malarani, C. Selvam, M. Hemalatha and M. Pandurangan, 2013. Synthetic Pyrethroid effect on blood plasma biomarker Enzymes and histological changes in *Catla catla*. *Int. J. Exp. Pathol.*, 94: 104–108

Nafees, M. and M. Jan, 2009. Residues of cypermethrin and endosulfan in soils of Swat valley. *Soil Environ.*, 28: 113–118

Ndimele, P., A. Jenyo-Oni, C. Kumolu-Johnson, K. Chukwuka and S. Onuoha, 2015. Effects of Acute Exposure to Endosulfan (Organochlorine Pesticides) on Hematology of African Mud Catfish, *Clarias gariepinus*. *Bull. Environ. Contam. Toxicol.*, 95: 164–170

Okomoda, V., G. Ataguba and V. Ayuba, 2013. Hematological response of *Clarias gariepinus* fingerlings exposed to acute concentrations of Sunsate®. *J. Stress Physiol. Biochem.*, 9: 271–278

Pal, S., E. Kokushi, J. Koyama, S. Uno and A.R. Ghosh, 2012. Histopathological alterations in gill, liver and kidney of common carp exposed to chlorpyrifos. *J. Environ. Health Sci. Part B.*, 47: 180–195

Qureshi, I.Z., A. Bibi, S. Shahid and M. Ghazanfar, 2016. Exposure to sub-acute doses of fipronil and buprofezin in combination or alone induces biochemical, hematological, histopathological and genotoxic damage in common carp (*Cyprinus carpio* L.). *Aquat. Toxicol.*, 179: 103–114

Saleh, Y.S. and M.-A.S. Marie, 2016. Use of *Arius thalassinus* fish in a pollution biomonitoring study, applying combined oxidative stress, hematology, biochemical and histopathological biomarkers: A baseline field study. *Mar. Pollut. Bull.*, 106: 308–322

Samanta, P., S. Pal, A.K. Mukherjee, D. Kole and A.R. Ghosh, 2016. Histopathological study in stomach and intestine of *Anabas testudineus* (Bloch, 1792) under Almix exposure. *Fish Aqua J.*, 7: 1–7

Saravanan, M., K.P. Kumar and M. Ramesh, 2011. Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. *Pestic. Biochem. Physiol.*, 100: 206–211

Sharma, V., D. Anderson and A. Dhawan, 2012. Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria mediated apoptosis in human liver cells (HepG2). *Apoptosis*, 17: 852–870

Singh, N.N. and A.K. Srivastava, 1981. Effects of endosulfan on fish carbohydrate metabolism. *Ecotoxicol. Environ. Saf.*, 5: 412–417

Song, Y., B. Salbu, L.S. Heier, H.-C. Teien, O.-C. Lind, D. Oughton, K. Petersen, B.O. Rosseland, L. Skipperud and K.E. Tollefsen, 2012. Early stress responses in Atlantic salmon (*Salmo salar*) exposed to environmentally relevant concentrations of uranium. *Aquat. Toxicol.*, 112: 62–71

Srivastava, N. and N. Kaushik, 2001. Use of fish as bioindicator of aquatic pollution. *Proceedings Int. Congress Chem. Environ.*, pp: 23–27. Indore, India

Suvetha, L., M. Ramesh and M. Saravanan, 2010. Influence of cypermethrin toxicity on ionic regulation and gill Na^+/K^+-ATPase activity of a freshwater teleost fish *Cyprinus carpio*. *Environ. Toxicol. Pharmacol.*, 29: 44–49

Tabassum, H., A.Q. Dawood, P. Sharma, J. Khan, S. Raisuddin and S. Parvez, 2016. Multi-organ toxicological impact of fungicide propiconazole on biochemical and histological profile of freshwater fish *Channa punctata* Bloch. *Ecol. Indic.*, 63: 359–365

Vani, T., N. Saharan, S. Mukherjee, R. Ranjan, R. Kumar and R. Brahmchari, 2011. Deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of *Catla catla* (Ham.) and their amelioration by dietary supplement of vitamin C. *Pestic. Biochem. Physiol.*, 101: 16–20

Velisek, J., Z. Svobodova and V. Piackova, 2009. Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*). *Vet. Med.*, 54: 131–137

Velisek, J., T. Wlasow, P. Gomulka, Z. Svobodova, R. Dobsikova, L. Novotny and M. Dudzik, 2006. Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). *Vet. Med.*, 51: 469

Velmurugan, B., E.I. Cengiz, P. Senthilkumaar, E. Uysal and A. Satar, 2016. Hematological parameters of freshwater fish *Anabas testudineus* after sublethal exposure to cypermethrin. *Environ. Pollut. Prot.*, 1: 32–39

Velmurugan, B., M. Selvanayagam, E.I. Cengiz and E. Unlu, 2007. Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. *Environ. Toxicol. Pharmacol.*, 24: 286–291

Vidhya, V. and C.R. Nair, 2016. International Journal of Advanced Research in Biological Sciences. *Int. J. Adv. Res. Biol. Sci.*, 3: 43–47

Viran, R., F.Ü. Erkoç, H. Polat and O. Koçak, 2003. Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). *Ecotoxicol. Environ. Saf.*, 55: 82–85

Zhao, X., X. Ren, R. Zhu, Z. Luo and B. Ren, 2016. Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria-mediated apoptosis in zebrafish embryos. *Aquat. Toxicol.*, 180: 56–70

(Received 31 August 2017; Accepted 11 November 2017)