DOI: https://doi.org/10.54393/mjz.v4i02.71

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
Volume 4, Issue 2 (July-Dec 2023)

Original Article

Influence of Different Levels of Cyanazine on Blood Picture of *Labeo rohita*

Sidra Ayesha[1], Sikandar Hayat[1*], Sobia Alyas[1], Sajid Mahmood[2], Muhammad Asif Abbas Tahir[3] and Shahid Suleman[2]

[1]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan
[2]Department of Fisheries, Government of Punjab, Pakistan
[3]Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan

ARTICLE INFO

Key Words:
Fish, *Labeo rohita*, Hematological Effects, Cyanazine

How to cite:
Ayesha, S., Hayat , S., Alyas, S., Mahmood, S., Tahir, M. A. A., & Suleman, S. (2023). Influence of Different Levels of Cyanazine on Blood Picture of Labeo rohita : Cyanazine on Blood Picture of Labeo rohita. MARKHOR (The Journal of Zoology), 4(02). https://doi.org/10.54393/mjz.v4i02.71

***Corresponding Author:**
Muhammad Imran
Institute of Zoology, University of the Punjab, Lahore, Pakistan
imran.phd.zool@pu.edu.pk

Received Date: 3rd August, 2023
Acceptance Date: 28th November, 2023
Published Date: 31st December, 2023

ABSTRACT

The freshwater fish *Labeo rohita* is found in Asian countries and around the world. Cyanazine herbicide produce extensive threat to freshwater fishes and used to control annual grasses and broadleaf weeds. Subsequently, freshwater ecosystem was facing potential risk due to extensive application of this herbicide. **Objective:** To find the toxic effects of cyanazine on hematological parameters of *Labeo rohita*. **Methods:** For this purpose, fish was divided into four groups. Ten fish were introduced into each group. The group 1 was taken as control group. The group 2, 3 and 4 were treated groups and exposed to different concentration of cyanazine 3µgL^{-1}, 6µgL^{-1} and 8µgL^{-1} respectively. **Results:** The current study showed that WBC, RBC, HGB and HCT increased while the MCV, MCH, MCHC and PLT decreased significantly. **Conclusions:** Hematological parameters of *L. rohita* were significantly affected by exposure to Cyanazine. These alterations indicate that Cyanazine can potentially disrupt the fish's hematological balance, which could have implications for its overall health.

INTRODUCTION

Aquaculture has developed quickly in recent years and today it delivers major portion of food in the form of fish and shellfish. Aquaculture is the fundamental practices for getting more food from oceans [1]. The freshwater (*L. rohita*) is often known as Rohu and is found in Asian countries and around the world. It is a vegetarian column feeder and is a tropical and temperate fish that breeds in running water between June and July, and is used as a model fish/type specimen of bony fish due to its widespread eating [2]. Herbicides are frequently employed in agriculture to improve crop quality, but when they are discharged into the environment, they cause serious environmental concerns [3]. The problem in developing countries is more serious than in developed countries. Cyanazine is a harmful toxin and dangerous compound Cyanide toxicity, such as skin and eye irritation, has been found in several animals. This pesticide penetrates aquatic habitats mostly by runoff and it is hazardous to fish, and several aquatic invertebrates [4]. Pesticide pollution of water, whether direct or indirect, can result in fish fatalities, decreased fish production or high levels of undesired chemicals in fish tissue, all of which damage human[5]. Pesticides enter the different organs of fish; they can cause damage to certain physiological and When biochemical processes [6]. Herbicides are water soluble, mobile and they can be absorbed into the soil. It

DOI: https://doi.org/10.54393/mjz.v4i02.71

can also harm human health since herbicide contaminated water is taken as drinking water [7]. The possibility for herbicides to reach water systems varies greatly. Some herbicides are sufficiently water soluble to dissolve in rainwater or irrigation water. Their final destination is largely determined by the circumstances in which they are used. Both the soil and the herbicide have physical and chemical qualities that influence the extent to which each of these occurrences happens (Ahmed *et al.*, 2021). Bioassay methods, which are critical in the field of ecotoxicology, can determine the toxicity of any chemical. Fisher have model system as they have quick Behavioural response. Fish exhibit in contaminated water, blood measurements are critical for determining the functional and structural condition [8]. Because the blood in fish gills comes into close contact with the water medium, any changes in the aquatic environment can be detected in the circulatory system. As a result, fish blood has been widely employed as a possible indicator of physiological changes in fisheries in toxicological and environmental monitoring [9]. Pollutants cause fish to modify their hematopoietic properties rather quickly [10]. Blood parameters in fish are controlled by size, age, reproductive stage, health, sex and external factors such as water temperature, seasonal dynamic, environmental quality, stress and diet. Changes in hematological parameters indicate a fish's physiological reaction to environmental stress. The aims and objective of this study was to determine the effect of different sub-lethal concentration of cyanazine on blood parameters of fish. By calculating hematological parameters, the harmful effect of triclosan on the freshwater fish *Labeo rohita* was assessed in the current study. For 15 days, fish were exposed to a sub-lethal dose (0.09 mg/L). When compared to the control group, the TCS-exposed fish showed a significant ($p<0.05$) decrease in (Hb) content, (RBC), (HCT) and (MCV) which suggests that TCS has an adverse effect on erythropoitic tissues by causing RBC cells to shrink and making erythrocytes more brittle and porous. The findings revealed that the creation of inclusion complexes could improve the use of cyanazine in agricultural output while lowering environmental risk [11]. Rohu (*Labeo rohita*) was given three sub-lethal amounts of CYP (0.50, 1.00, and 1.5 ppb) for a total of 96 hours. The effects of CYP on fish haematological were well-defined, with a substantial increase ($p<0.05$). These findings support the hypothesis that indiscriminate use of CYP raised WBCs, platelets, and blood glucose levels while decreasing RBCs, Hb, HCT, MCV, MCH, and MCHC [12].

METHODS

Live samples of *L. rohita* were collected from fish seed hatchery Manawa Lahore and shifted to laboratory of The University of Lahore in polyethylene oxygen filled bag. The fish were initially acclimatized under laboratory conditions for one week in glass aquaria with proper oxygen system through aerators. During acclimatization protein-based diet was provided to fish twice a day. After acclimatization period 40 fish were divided into four groups. Each group contains 10 fish, group 1 was the control group while other three groups (2, 3 and 4) were treated group commonly named as T1, T2 and T3 respectively. A stock solution of cyanazine was prepared by dissolving 1mg of cyanazine. Then stock solution was stored in the cell line bottle for further use. After dose preparation, the fish groups were exposed to different doses of cyanazine. Control group fish were kept in simple tap water with proper feed. While T1 group was treated with 3µgL-1. T2 group was treated with 6µgL-1. T3 group was treated with 8µgL-1. A Chronic toxicity test 28 days. During conducted in triplicates in glass aquaria with 70% of clean water and fish were introduced in it. Fish were exposed to different concentration of cyanazine for the whole trial period, water, temperature, pH and dissolved oxygen was maintained. Fish behavioral changes having no breathing signs was picked up from aquarium immediately with the help of fry net. After the exposure to cyanazine fish were anesthetized, and blood sample were collected. CBC test was performed by using automatic hematological analyzer by following the standard procedures for the determination of WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT. A statistical analysis was applied to blood sample data to compare means for their statistical significance and to check variable.

RESULTS

Hematological parameters among the biological alterations that are thought to be possible biomarkers of chemical agent exposure since the latter can cause an increase or reduction in the various hematological components. The changes due to exposure of cyanazine in blood parameters of *L. rohita* are shown in table 1.

Table 1: Changes in the hematological parameters of *Labeo rohita* exposed to sub-lethal concentration of Cyanazine (Herbicide)

Blood Parameters	Exposure periods				
	Control	7days	14days	21days	28days
RBC 106/µl	1.95± (0.001)	1.70± (0.13)	1.25± (0.81)	1.34± (0.70)	2.3± (0.22)
t-value	1.614	0.946	0.151	2.258	1.40
%age change	0	0.08	0.47	0.40	0.12
WBC 103/µl	215.7± (0.001)	212.69± (0.85)	143.14± (41.813)	116.11± (101.11)	217.83± (10.53)
t-value	3.080	2.928	0.429	1.733	0.176
%age change	0	0.49	23.75	58.38	6.08
HGB g/l	8.30± (0.001)	8.12± (0.55)	8.27± (3.32)	5.23± (1.57)	9.62± (0.74)
t-value	0.281	0.074	1.431	4.737	1.539
%age change	0	0.32	1.92	0.91	0.43
HCT (%)	26.00± (0.001)	23.33± (1.15)	29.09± (20.78)	10.14± (5.47)	27.68± (9.34)
t-value	2.00	0.479	1.527	2.805	0.156
%age change	0	0.67	11.10	3.16	5.39

DOI: https://doi.org/10.54393/mjz.v4i02.71

In control group, the RBCs, WBCs, HGB and HCT are in normal range. While in the start of experiment when fish exposed to different doses of cyanazine, hematological study revealed that RBCs, WBCs decreased and then gradually increased after 14 days. At the end of experiment, the RBCs, WBCs reached at its maximum numbers when compared with control group.

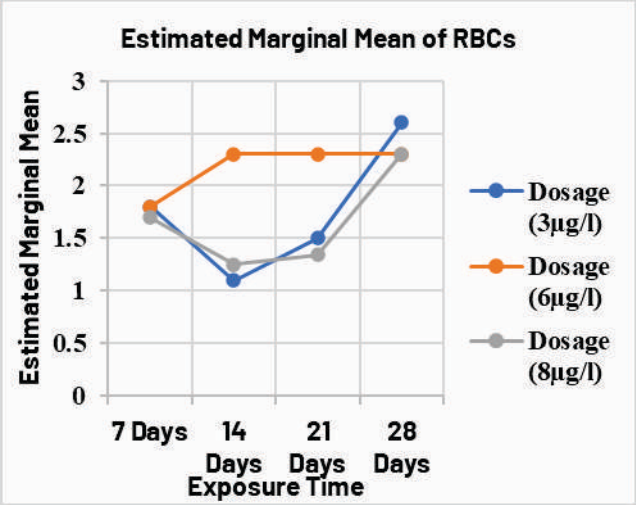

Figure 1: Dosage effect on RBC at different exposure time

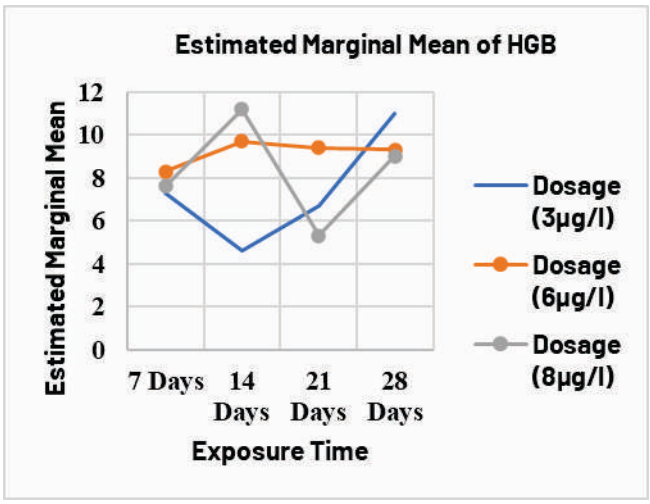

Figure 2: Dosage effect on HGB at different exposure time

In control group, the PLT, MCHC, MCH and MCV are in normal range. In the start of experiment when fish exposed to 3µg/L of cyanazine, hematological study revealed that PLT, MCHC, MCH and MCV decreased and then gradually increased after 14 days. When fish exposed to 8µg/L of cyanazine, study revealed that PLT, MCHC, MCH and MCV increased then decreased gradually after 14 days(Table 2)

Table 2: Changes in the hematological parameters of *Labeo rohita* in control and treated group

Blood Parameters	Exposure periods				
	Control	7days	14days	21days	28days
MCV (f/l)	133.30± (0.001)	128.39± (3.25)	101.89± (23.91)	79.81± (28.14)	119.31± (26.83)
t-value	1.307	1.902	1.035	1.760	0.452
%age change	0	1.88	13.81	16.25	15.49
M H (pg)	42.60± (0.001)	46.80± (7.56)	58.58± (26.36)	47.88± (27.34)	40.14± (1.61)
t-value	0.481	0.744	0.488	0.489	1.320
%age change	0	4.37	15.22	15.79	0.93
MCHC (g/dl)	31.90± (0.001)	36.98± (8.73)	53.02± (24.41)	42.34± (30.20)	33.39± (6.39)
t-value	0.503	1.072	0.476	0.502	0.202
%age change	0	5.04	14.09	17.44	3.69
PLT10^3/ul	36.00± (0.001)	33.55± (2.91)	49.89± (30.36)	35.11± (4.22)	16.66± (10.40)
t-value	0.727	0.927	0.835	2.847	1.610
%age change	0	1.68	17.53	2.44	6.00

DISCUSSION

In the present study, no hematological changes were observed in the control group. RBC, WBC, HGB and HCT values suddenly decreased till 14 days and then gradually increased at the end of experiment. While MCH, MCHC and PLT decreased in number in first week and then increased till 14 days and then again decreased at the end of experiment. MCV increased after 7 days and then gradually decrease till 21 days and then increased. In the present study, *Cirrhinus mrigala* treated with glyphosate showed many of these hematological alterations. The hematological parameters of control group show the normal values. The treated group showed that the WBC increased while the other parameters like MCV, MCH and PCV decreased [13]. The same results were shown in the *Cyprinus carpio* when treated with metribuzin. The RBCs and HCT increased in number when exposed to metribuzin, but other parameters were not changed significantly by any concentration of chemical [14]. The hematological study revealed that when *Cyprinus carpio* exposed to metribuzin showed significant decreased in numbers of MCV, PCV, WBC AND Hb. While no significant changes observed in MCHC, MCH and RBC [15]. This study found a significant decline in red blood cells, haemoglobin and hematocrit when tilapia was exposed to sub-lethal levels of pendimethalin. After exposure to pendimethalin, mean corpuscular volume increased but mean corpuscular haemoglobin concentrations fell [16]. In current study, when *P. hypophthalmus* exposed to sub-lethal dose of triclosan significant decrease in MCV, Hb and PCV was recorded. While the MCHC, blood glucose level and TLC were increased [17]. The current investigation was to determine the toxicity of propanil on the haematological parameters of juvenile *Oreochromis niloticus*. When compared to the control, fish subjected to propanil showed

DOI: https://doi.org/10.54393/mjz.v4i02.71

significant alterations and dose-dependent reductions in haemoglobin, pack cell volume and red blood cell values. Compared to the control, fish exposed to propanil had a progressive rise in their white blood cell count and leukocyte differentials (neutrophils and lymphocytes) [18]. By calculating haematological parameters, the harmful effect of triclosan on the freshwater fish *Labeo rohita* was assessed in the current study. When compared to the control group, the TCS-exposed fish showed a significant ($p<0.05$) decrease in haemoglobin (Hb) content, erythrocyte count (RBC), hemocrit (HCT) and mean corpuscular volume (MCV). While a substantial rise in leukocyte count (WBC), mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) suggests direct or indirect reactions of structural damage in RBC membranes leading to hemolysis [19]. The current investigation clarified the possible hazardous effects of fenitrothion on the blood biomarkers of the catfish (*Heteropneustes fossilis*). Blood glucose and white blood cell levels dramatically rose with rising fenitrothion concentrations, whereas haemoglobin, red blood cell and packed cell volume sharply dropped. However, throughout the exordial period, the mean corpuscular volume and mean corpuscular haemoglobin did not alter appreciably, while modifications were seen afterwards [20]. This investigation explored the haematological parameters of the freshwater fish *Labeo rohita* were affected by chlorpyrifos. Hematological markers such as total erythrocyte count (TEC), haemoglobin (Hb) and packed cell volume (PCV) declined over the study period, whereas total leukocyte count (TLC) rose [21]. Imidacloprid-exposed fish had considerably lower haemoglobin, RBC, PCV, MCV, and platelet levels, indicating severe anaemia. Insecticide treatments resulted in hyperglycemia, hypercalcemia, and hypoprotonemia, indicating a high energy demand by the fish to counteract the toxicant's effect [22]. In this experiment, Rohu (*Labeo rohita*) was given sub-lethal amount of CYP. The effects of CYP on fish haematological were well-defined, with a substantial increase ($p<0.05$). These findings support the hypothesis that indiscriminate use of CYP raised WBCs, platelets, and blood glucose levels while decreasing RBCs, Hb, HCT, MCV, MCH, and MCHC [23]. The goal of the current study was to evaluate the haematological alterations in major carp (*Catla catla*) subjected to various amounts of copper (Cu) and cadmium (Cd). Results demonstrated that total white blood cell count and neutrophil population dramatically increased in experimental groups when compared to the control group, red blood cells (RBCs), haemoglobin (Hb), hematocrit (Hct), lymphocyte, and monocyte declined significantly [24]. The effects different chlorpyrifos concentrations on the haematological parameters of *Oreochromis mossambicus* were investigated. RBCs, Hb, and HCT all decreased significantly ($p< 0.05$) in all treatment groups compared to the control group, indicating anaemia, whereas WBCs and platelet counts increased significantly ($p< 0.05$) in all treated groups [25].

CONCLUSIONS

Hematological parameters of *L. rohita* were significantly affected by exposure to Cyanazine. These alterations indicate that Cyanazine can potentially disrupt the fish's hematological balance, which could have implications for its overall health. However, further research is needed to elucidate the precise mechanisms and long-term consequences of these hematological changes.

Authors Contribution

Conceptualization: SA, MAAT, SS
Methodology: SH
Formal Analysis: SA
Writing-review and editing: SM, SS

All authors have read and agreed to the published version of the manuscript.

Acknowledgment

The authors extend their sincere gratitude to the University of Agriculture, Faisalabad, and University of the Punjab, Lahore, Pakistan for the provision of lab facilities to complete this study.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Munguti JM, Kim JD, Ogello EO. An overview of Kenyan aquaculture: Current status, challenges, and opportunities for future development. Fisheries and Aquatic Sciences. 2014 Jan; 7(1): 1-11. doi: 10.5657/FAS.2014.0001.

[2] Ullah S, Li Z, Hasan Z, Khan SU, Fahad S. Malathion induced oxidative stress leads to histopathological and biochemical toxicity in the liver of rohu (Labeo rohita, Hamilton) at acute concentration. Ecotoxicology and Environmental Safety. 2018 Oct; 161: 270-80. doi: 10.1016/j.ecoenv.2018.06.002.

[3] Rodríguez JA, Aguilar-Arteaga K, Díez C, Barrado E. Recent advances in the extraction of triazines from water samples. Herbicides advances in research. London: IntechOpen. 2013 May: 255-76.

[4] Ali I, Alharbi OM, Alothman ZA, Alwarthan A. Facile and eco-friendly synthesis of functionalized iron

DOI: https://doi.org/10.54393/mjz.v4i02.71

nanoparticles for cyanazine removal in water. Colloids and Surfaces B: Biointerfaces. 2018 Nov; 171: 606-13. doi: 10.1016/j.colsurfb.2018.07.071.

[5] Owolabi OD and Abdulkareem SI. Carica papaya and Mangifera indica modulate haematological, biochemical and histological alterations in atrazine-intoxicated fish, Clarias gariepinus (Burchell 1822). The Journal of Basic and Applied Zoology. 2021 Dec; 82: 1-8. doi: 10.1186/s41936-021-00241-y.

[6] Jasmin J, Rahman MM, Rahman MM. Haematological changes in Labeo rohita (H.) due to exposure of pesticides, difenoconazole and thiamethoxam. International Journal of Contemporary Research and Review. 2018; 9(1): 20199-205. doi: 10.15520/ijcrr/2018/9/01/390.

[7] Solomon KR, Dalhoff K, Volz D, Van Der Kraak G. Effects of herbicides on fish. InFish physiology. academic press. 2013 Jan; 33: 369-409. doi: 10.1016/B978-0-12-398254-4.00007-8.

[8] Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT, Ayyappan S. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, Labeo rohita (Hamilton). Ecotoxicology and Environmental Safety. 2004 Jun; 58(2): 220-6. doi: 10.1016/j.ecoenv.2003.12.003.

[9] Ismail M, Ali R, Shahid M, Khan MA, Zubair M, Ali T, *et al*. Genotoxic and hematological effects of chlorpyrifos exposure on freshwater fish *Labeo rohita.* Drug and Chemical Toxicology. 2018 Jan; 41(1): 22-6. doi: 10.1080/01480545.2017.1280047.

[10] Rezania S, Park J, Din MF, Taib SM, Talaiekhozani A, Yadav KK, Kamyab H. Microplastics pollution in different aquatic environments and biota: A review of recent studies. Marine Pollution Bulletin. 2018 Aug; 133: 191-208. doi: 10.1016/j.marpolbul.2018.05.022.

[11] Gao S, Bie C, Ji Q, Ling H, Li C, Fu Y, *et al*. Preparation and characterization of cyanazine–hydroxypropyl-beta-cyclodextrin inclusion complex. RSC Advances. 2019 Aug; 9(45): 26109-15. doi: 10.1039/C9RA04448E.

[12] Khan N, Tabassum S, Ahmad MS, Norouz F, Ahmad A, Ghayyur S, *et al*. Effects of sub-lethal concentration of cypermethrin on histopathological and hematological profile of rohu (Labeo rohita) during acute toxicity. International Journal of Agriculture Biology. 2018 Jan; 20(3): 601-8. doi: 10.17957/IJAB/15.0527.

[13] Pechiammal K and Vasanthi J. Studies on impact of glyphosate on selected haematological parameters of fresh water fish, cirrhinus mrigala. World Journal of Pharmaceutical Research. 2017 Jul; 6(8):1755-60. doi: 10.20959/wjpr20178-9074.

[14] Modra H, Haluzova I, Blahova J, Havelkova M, Kruzikova K, Mikula P, *et al*. Effects of subchronic metribuzin exposure on common carp (Cyprinus carpio). Neuroendocrinology Letters. 2008 Oct; 29(5): 669. doi:

[15] Velisek J, Stastna K, Sudova E, Turek J, Svobodova Z. Effects of subchronic simazine exposure on some biometric, biochemical, hematological and histopathological parameters of common carp (Cyprinus carpio L.). Neuroendocrinology Letters. 2009 Jan; 30(1): 236. doi: 10.1016/j.toxlet.2009.06.539.

[16] Kocamaz D. The Effect of Propolis On Hematological and Biochemical Parameters In Juvenile Tilapia Exposed To Pendimethalin. Fresenius Environmental Bulletin. 2019 Jan; 28(2 A): 1465-70.

[17] Paul T, Kumar S, Shukla SP, Pal P, Kumar K, Poojary N, *et al*. A multi-biomarker approach using integrated biomarker response to assess the effect of pH on triclosan toxicity in Pangasianodon hypophthalmus (Sauvage, 1878). Environmental Pollution. 2020 May; 260: 114001. doi: 10.1016/j.envpol.2020.114001.

[18] Abubakar YA, Iheanacho S, Ogueji E. Sublethal exposure and toxicity effect of propanil on hematology and serum biochemistry in Oreochromis niloticus in a static bioassay. Gazi University Journal of Science. 2018 Dec; 31(4): 1048-62.

[19] Geetha N and Priya N. Impact of triclosan an antimicrobial agent on haematological parameters of fresh water fish Labeo rohita. International Journal of Applied Research. 2020 May; 6: 118-23.

[20] Ritu RF, Islam SM, Rashid H, Haque SM, Zulfahmi I, Sumon KA. Application of fenitrothion on Heteropneustes fossilis causes alteration in morphology of erythrocytes via modifying hematological parameters. Toxicology Reports. 2022 Jan; 9: 895-904. doi: 10.1016/j.toxrep.2022.04.010.

[21] Ismail M, Ali R, Shahid M, Khan MA, Zubair M, Ali T, *et al*. Genotoxic and hematological effects of chlorpyrifos exposure on freshwater fish Labeo rohita. Drug and Chemical Toxicology. 2018 Jan; 41(1): 22-6. doi: 10.1080/01480545.2017.1280047.

[22] Qadir S, Latif A, Ali M, Iqbal F. Effects of imidacloprid on the hematological and serum biochemical profile of Labeo rohita. Pakistan Journal of Zoology. 2014 Aug 1;46(4): 1085-90.

[23] Khan N, Tabassum S, Ahmad MS, Norouz F, Ahmad A, Ghayyur S, Rehman AU, Khan MF. Effects of sub-lethal concentration of cypermethrin on histopathological and hematological profile of rohu (Labeo rohita) during acute toxicity. International Journal of Agriculture and Biology. 2018 Jan; 20(3):

DOI: https://doi.org/10.54393/mjz.v4i02.71

601-8. doi: 10.17957/IJAB/15.0527.

[24] Naz S, Hussain R, Ullah Q, Chatha AM, Shaheen A, Khan RU. Toxic effect of some heavy metals on hematology and histopathology of major carp (Catla catla). Environmental Science and Pollution Research. 2021 Feb; 28: 6533-9. doi: 10.1007/s11356-020-10980-0.

[25] Ghayyur S, Tabassum S, Ahmad MS, Akhtar N, Khan MF. Effect of chlorpyrifos on hematological and seral biochemical components of fish Oreochromis mossambicus. Pakistan Journal of Zoology. 2019 Jun; 51(3): 1047. doi: 10.17582/journal.pjz/2019.51.3.1047.1052.