JIPK. Volume 17 No 2 June 2025
Sinta 1 (Decree No: 158/E/KPT/2021)

e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Research Article

Bio-Monitoring of Aquatic Environment: Hematological and Biochemical Changes in Grass Carp due to Toxicity of Emamectin Benzoate, Acetochlor, and Topsin-m

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ARTICLE INFO

Received: January 20, 2025
Accepted: April 29, 2025
Published: May 02, 2025
Available online: May 25, 2025

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Keywords:
Grass carp
Emamectin Benzoate
Acetochlor and Topsin-M
Hematological changes
Biochemical markers
Aquatic biomonitoring

Abstract

This study investigates the novel combined effects of Emamectin Benzoate (pesticide), Acetochlor (herbicide), and Topsin-M (fungicide) on hematological and biochemical profiles in grass carp (Ctenopharyngodon idella), to fill critical gaps in understanding their ecotoxicological impacts on aquatic health and sustainable fisheries. Fishes from farm in Gujranwala were acclimatized in a freshwater aquarium laboratory for a week, then divided into four groups (E1, E2, E3, and E4) exposed to pesticide, herbicide, and fungicide concentrations for 5 and 14 days. Blood samples were collected for hematological and biochemical parameters. Exposure to Emamectin Benzoate induces significant increase in WBCs, neutrophils, MCV, MCH, and platelets, while a significant decrease was found in RBCs, lymphocytes, hemoglobin, PCV, and MCHC concentration. Acute toxicity of Acetochlor showed an increase in WBCs, neutrophils, MCV, MCH contents, and platelets, while a decrease in RBCs, lymphocytes, hemoglobin, PCV, and MCHC contents was noted. Effects of Topsin-M showed an increase in WBCs, neutrophils, MCV, MCH contents, and platelets. However, a significant decrease in RBCs, lymphocytes, hemoglobin, PCV, and MCHC contents has been observed. It reveals that Uric acid, serum lipase, Sodium, Phosphorous, Bilirubin, and Potassium increased significantly. Exposure to chemicals induced significant declines in the levels of Lactate dehydrogenase (LDH), Triglyceride, HDL Cholesterol, SGPT (ALT) Creatinine, and ALK Phosphatase which caused illness in fish. This work demonstrates the potential for biomonitoring aquatic ecosystems, providing early warning indications for aquatic biomonitoring, and contributing to safer aquaculture methods.

Cite this as: Badshah, K. D., Riaz, B., Ejiz, R., Khan, A. A., Mehdi, K., Khan, S., Riaz, S., Ahmad, K., & Ahmed, N. (2025) Bio-Monitoring of Aquatic Environment: Hematological and Biochemical Changes in Grass Carp due to Toxicity of Emamectin Benzoate, Acetochlor, and Topsin-m. *Jurnal Ilmiah Perikanan dan Kelautan,* 17(2):389–403. https://doi.org/10.20473/jipk.v17i2.68785

1. Introduction

One of the main causes of water contamination is pesticide use. Over 200 distinct kinds of organic pesticides are utilized in hundreds of different things, according to (Akram *et al.,* 2022). Many heavy metals, including Fe, Cr, Cd, Ni, Cu, Pb, Zn, and Mn, are included in these insecticides. These substances eventually find their way into water bodies, where they have a negative impact on fish and other non-target aquatic creatures' physiology, growth, reproduction, and even survival (Chaudhary *et al.,* 2023). Among these pesticides, synthetic organic chemicals such as Emamectin Benzoate, although promoted as environmentally benign continue to provide considerable hazards to aquatic organisms. Emamectin Benzoate, a semi-synthetic insecticide derived from the fermentation of avermectin B1, is characterized by its exceptional efficacy, broad-spectrum activity, and minimal toxicity to non-target organisms in comparison to conventional heavy metal-based pesticides (Okoboshi *et al*., 2023). However, its extensive application in aquaculture, particularly for addressing sea lice (*Lepeophtheirus salmonis*) infestations in cultivated Atlantic salmon (*Salmo salar*), has led to reservations of unwanted ecological implications (Perveen *et al*., 2019). Although emamectin benzoate is considered safer, its residues in aquatic environments have been linked to adverse hematological, immunological, and behavioral changes in fish, challenging its ecological safety profile (Shahjahan *et al*., 2022).

The most widely used active component in the herbicide compound chloroacetanilide is acetochlor. Acetochlor is taken by the roots and leaves of weeds, and it prevents the weeds from synthesizing proteins (Tomlin, 2001). Acetochlor's effects on human health at low ambient concentrations are unclear (Makled *et al.,* 2024). Acetochlor possesses a low acute toxic effect. Chronic animal trials revealed no developmental or fetal toxicity, but it did cause testicular shrinkage, renal damage, and neurologic movement problems (EPA, 2006). A systemic fungicide called Topsin-M is used to manage diseases including wheat rust, brown rust, downy mildew, smut, and powdery mildew (Das *et al*., 2023). According to studies, whatever physiological changes brought about by toxicants affecting the water quality would be seen in the results of one or more aquatic animals' hematological parameters (Opute and Oboh, 2021). The fish's physiological state and overall health are determined in large part by hematological indicators (Suchana *et al.,* 2021). Furthermore, these metrics accurately capture changes in an organism and are crucial for identifying illnesses and determining the metabolism of fish that inhabit various ecological niches (Giri *et al.*, 2021; Sharma and Behera, 2022).

Immunological and hematological parameters are key indicators of fish health, and aquaculture's hematological profile can help identify diseases and stressors, improving disease therapies' effectiveness(Hasan *et al.,* 2015). Hematocrit values are influenced by various factors, including species, temperature, age, stress, photoperiod, nutritional status, sexual maturity, health condition, water quality, fluctuations in dissolved oxygen, gender, lotic or lentic environments, handling stress, transportation, fish inflammation, size, feeding habits, stocking density, and microbial infection or parasitism (Adeyemo *et al.,* 2009). Precise assessment of these parameters requires appropriate reference values for each species. The assessment of hematologic analysis enhances aquaculture by enabling the early detection of stress and illnesses, hence preventing detrimental impacts on productivity (Jones *et al.,* 2013). Blood parameters are significant indications of physiological alterations in intensively farmed fish and provide essential insights for disease diagnosis and prognosis (Siwicki *et al.,* 1994). Recent years have seen a sharp increase in the application of hematological techniques in fish culture for toxicological research, monitoring of the environment, and fish medical conditions (Tahir *et al*., 2024). Rapid detection of hematological and clinical chemistry markers makes them useful for diagnosing and predicting pesticide damage. Variations in these measures reveal toxic stress on the blood and organs that generate blood in the treated animals in particular (Rahman and Siddiqui, 2006; Alammar, 2019). Many experimental investigations have shown that sub-lethal pesticide exposures, including glyphosate, endosulfan, cypermethrin, chlordane, dieldrin, aldrin, lambda-cyhalothrin (Karate), and sulfoxaflor cause negative hematological changes in fish. These alterations show up as major disturbances in packed cell volume (PCV), red and white blood cell counts (RBCs, WBCs), and hemoglobin levels across many fish species (Afzal *et al.,* 2024). The main objective of present research was to determine the effect of sub-lethal concentrations of Emamectin Benzoate, Acetochlor and Topsin-M on biochemical and hematological parameters including erythrocytes count, leucocytes count, hemoglobin and platelets and then comparison of the effects produced by these chemicals (Park *et al.,* 2021). The novelty of this research lies in its first comprehensive and detailed investigation of the effects of toxic agrochemicals Emamectin Benzoate, Acetochlor, and Topsin-M on hematological parameters in Grass carp (*Ctenopharyngodon idella*). These parameters include total leukocyte count (TLC), red blood cells (RBCs), neutrophils, lymphocytes, hemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (Satyanarayan *et al.,* 2004)

2. Materials and Methods

2.1 Materials

2.1.1 The equipments

The equipments that were used in this study, freshwater aquarium, where the fish were kept for an acclimatization period before exposure to the test chemicals. To maintain proper oxygen levels, air pumps (Hailea HX88 series water pump, UK) were used for continuous aeration in the aquariums. For blood sample collection, heparin syringes (Lovenox, LLC, USA) were utilized, ensuring that the samples were drawn effectively. For the collected blood samples which were transferred into EDTA tubes (BD, Vacutainer®, USA). Digital pH Meter (VIVOSUN®, USA) was used for measuring pH.

2.1.2 The materials

A systematic approach was applied for the sample collection and preparation. The experimental work of this study was conducted at Department of Chemistry, Government Postgraduate College Haripur, Khyber Pakhtunkhwa Pakistan. Forty healthy Grass carp fish were purchased from Atif farm, Gujranwala, Pakistan. Fish were collected within plastic bags containing water and transported to the laboratory for study. Three different types of commercial chemical/pesticides have been used for experimental work. These were Emamectin Benzoate, Acetochlor and Topsin-M of analytical grade all the chemicals purchased of Sigma-Aldrich©, Germany. During the sampling process, clove oil (Piping Rock® LLC, USA) was used to anesthetize the fish, ensuring minimal stress and discomfort.

2.1.3 Ethical approval

This research study was conducted in compliance with ethical guidelines and was approved by the Animal Ethics Committee of Government Postgraduate College Haripur, affiliated with the University of Haripur, Pakistan. The approval was granted under case number G/GE-24302 on March 14, 2024. All procedures involving the use of fish were carried out in accordance with the ethical standards and regulations established by the committee to ensure the humane treatment and welfare of the animals.

2.2 Methods

2.2.1 Acclimatization period

Fishes were allowed to adjust with laboratory conditions for a week before the start of experiment. Fishes were kept in 40 L oxygenated glass aquaria having de-chlorinated tap water having temperature 20–25°C, dissolved oxygen 100% saturation (bubbling) and pH 7.6–7.8 respectively. In this period fish were given pelleted commercial food. About 70 % of water was renewed on daily basis during the whole study.

2.2.2 Sample collection

The experiment was conducted for 14 days. Grass Carp fishes were captured with a hand net and were made unconscious using clove oil. Blood samples were taken from the veins with the help of heparin syringes containing ethylene diamine tetra acetate (EDTA) as anticoagulant and immediately transferred to EDTA tubes. The EDTA tubes were shaken very slowly and smoothly for anticoagulant.

2.2.3 Hematological parameters

The collected blood was used for biochemical and hematological tests; total red blood cells (RBCs) content, total white blood cells (WBCs) content, hemoglobin (Hb) content, neutrophils content, lymphocyte content, pack cell volume (PCV) content and platelets content. Similarly, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) constituted the corpuscular indices. All groups' biochemical and hematological parameters (aquaria) were analyzed.

2.2.4 Experimental design

Three different types of solutions (5ppb) for each of Emamectin Benzoate, Acetochlor, and Topsin-M were prepared. Four aquaria were taken and filled with 40 L of fresh tap water. Forty Grass Carp fish were divided randomly in 4 groups, 10 fish for each group in each aquarium. Group 1 served as control (without any treatment), Group 2 treated with pesticide, Emamectin Benzoate, Group 3 treated with herbicide, Acetochlor, Group 4 treated with fungicide, Topsin-M, Constant amount of chemicals and water were changed every 24 hours during this experiment. The water in each aquarium was aerated using air pumps at room temperature (20-25°C).

2.3 Analysis Data

After the experiment, statistical data from all four groups was compared using one-way analysis of variance (ANOVA) to see if at least one type of treatment is significantly different from the others and significant difference value is $p < 0.05$. This was done using Statistical Package for the Social Sciences (SPSS) version 20. The next section presents the analyzed results in the form of tables and graphs (Fiandini *et al.*, 2024).

For the assumptions of normality

$$H = 12/(N(N+1))\sum^{k}_{i=1}(Ri2-3)/ni(N+1) \text{.....................(i)}$$

For all four groups comparison

$$HSD = q\alpha, k, df_{within} \; _{\sqrt{((MS\ within)/n)}} \text{.......................(ii)}$$

Where :

q = critical value from Studentized range distribution

3. Results and Discussion

3.1 Results

3.1.1 Hematological parameters

Exposure to pesticides (Emamectin Benzoate), herbicides (Acetochlor), and fungicides (Topsin-M) by taking same concentration of 5 ppb has affected the hematology of Grass carp. Several changes in their hematological parameters; number of red blood cell, white blood cell and platelets, neutrophils content, lymphocytes content, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration during acute toxicity were observed.

3.1.1.1 Effects of emamectin benzoate on hematological parameters

Exposure of Grass carp to pesticides i.e., Emamectin Benzoate, number of WBCs significantly $p < 0.05$ increased 373.93 ± 10.12 /µL in this group than control group 132.53 ± 7.1.0 /µL. On the other hand, total RBCs significantly $p < 0.05$ decreased 1.58 ± 0.13×10^6 /µL as compared to control group 6.38 ± 0.55×10^6 /µL. Likewise, hemoglobin content also significantly $p < 0.05$ decreased 7.3 ± 0.4 g/dL in this group as compared to control group 13.9 ± 0.92 g/dL. Lymphocytes content slightly decreased 90.33 ± 1.53 % as compared to the control group 94.33 ± 0.58 % and Neutrophils content slightly increased 9.67 ± 1.53 % as compared to the control group 5.67 ± 0.58 %. The PCV contents, significantly $p < 0.05$ decreased 23.03 ± 1.40 % in treated group as compared to the control group 77.4 ± 3.45 %. While MCH content a significant $p < 0.05$ increased 46.57 ± 6.59 pg in treated group as compared to the control group 21.90 ± 3.06 pg and a significant $p < 0.05$ increase in MCV content 147 ± 21.79 fL as compared to control group 53.96 ± 9.62 fL was noted. The MCHC contents were significantly $p < 0.05$ decreased 31.7 ± 0.53 g/dL in treated group than control group 40.85 ± 2.85 g/dL. Similarly, the number of platelets significantly $p < 0.05$ increased 250.49 ± 10.81×10^3 /µL in treated group as compared to control group 82.5 ± 7.00×10^3 /µL due to exposure to 5 ppb concentration of Emamectin Benzoate. The results of the effects of Emamectin Benzoate are shown in Table 1.

3.1.1.2 Effects of acetochlor on hematological parameters

Exposure of Grass carp to Acetochlor has shown several changes in the total blood cells count of treated fishes. Overall, WBCs count significantly $p < 0.05$ increased 202.91 ± 2.61 /µL in the treated group than control group 132.53 ± 7.10/µL. Otherwise, total RBCs count significantly $p < 0.05$ decreased 3.41 ± 0.51×10^6 /µL in the treated than control group 6.38 ± 0.55×10^6 /µL. Likewise, hemoglobin content also significantly $p < 0.05$ decreased 9.54 ± 0.73 g/dL as compared to control group 13.9 ± 0.92 g/dL. While both Lymphocytes 93.67 ± 1.53 % and Neutrophils 6.33 ± 1.53 % content of treated group is just like the control group. The MCV contents were increased non-significantly $p > 0.05$ in this group 84.08 ± 17.56 fL than control group 53.96 ± 9.62 fL and MCH contents showed non-significant $p > 0.05$ increased 28.19 ± 2.64 pg than control group 21.90 ± 3.06 pg. The PCV content showed non-significant $p > 0.05$ decrease 28.10 ± 2.23 % in treated group as compared to the control group 77.4 ± 3.45 %. The value of MCHC significantly $p < 0.05$ decreased 34.18 ± 5.14 g/dL as compared to the control group 40.85 ± 2.83 g/dL. Similarly, the number of platelets increased significantly 179.76 ± 2.04×10^3/µL in treated group as compared to the control group 82.5 ± 7.00×10^3 /µL during the exposure to 5 ppb of Acetochlor. The results of the effects of acetochlor are shown in Table 1.

3.1.1.3 Effects of topsin-M on hematological parameters

Exposure of Grass carp to fungicides i.e., Topsin-M has affected the hematological parameters. Overall, number of WBCs significantly $p < 0.05$ increased 141.77 ± 2.86 /µL in treated group than control group 132.53 ± 7.10 /µL. On the other hand, total RBCs significantly $p < 0.05$ decreased 5.17 ± 0.30×10^6 /µL than control group 6.38 ± 0.55×10^6 /µL. Likewise, hemoglobin content non-significantly $p > 0.05$ decreased 12.65 ± 1.93 g/dL in treated group as compared to control group 13.9 ± 0.92 g/dL. Lymphocytes and Neutrophils contents were slightly affected i.e., less than 1 % decrease noted as compared to control group 94.33 ± 0.58 % and less than 1 % increase noted as compared to control group 5.67 ± 0.58 % respectively. In case of PCV content, a non-significant $p > 0.05$ decreased 32.39 ± 0.81 % in treated group occurred as compared to control group 77.4 ± 3.45 %. While MCH content was slightly increased 24.5 ± 4.82 pg in treated group as compared to control group 21.90 ± 3.06 pg and an increase in MCV content 62.74 ± 4.22 fL as compared to control group 53.96 ± 9.62 fL has occurred. In case of MCHC content a decreased 38.97 ± 5.10 g/dL in treated group than control group 40.85 ± 2.83 g/dL was noted. Similarly, the number of platelets significantly $p < 0.05$ increased 132.13 ± 2.20×10^3/µL in treated group as compared to the control group 82.5 ± 7.00×10^3 /µL with the exposure of 5 ppb of Topsin-M. The results of the effects of Topsin-M are shown in Table 1.

Table 1. Summarized Hematological parameters of both control and treated groups on grass carp.

S. No.	Hematological Parameters	Control Groups	Emamectin Benzoate (5ppb)	Acetochlor (5ppb)	Topsin-M (5ppb)
1.	T.L.C	132.53±7.10	373.93±10.12*	202.91±2.61*	141.77±2.86*
2.	RBCs	6.38±0.55	1.58±0.13*	3.41±0.51*	5.17±0.30*
3.	Neutrophils	5.67±0.58	9.67±1.53*	6.33±1.53	6.33±0.58
4.	Lymphocytes	94.33±0.58	90.33±1.53*	93.67±1.53	93.67±0.58
5.	Hemoglobin	13.9±0.92	7.3±0.4*	9.54±0.73*	12.65±1.93
6.	PCV	77.4±3.45	23.03±1.40*	28.10±2.23	32.39±0.81
7.	MCV	53.96±9.62	147±21.79*	84.08±17.56	62.74±4.22
8.	MCH	21.90±3.06	46.57±6.59*	28.19±2.64	24.5±4.82
9.	MCHC	40.85±2.83	31.7±0.53*	34.18±5.14	38.97±5.10
10.	Platelets	82.5±7.00	250.49±10.81*	179.76±2.04*	132.13±2.20*

Units of measurement: T.L.C (/µL), RBCs (×10^2//µL), Neutrophils (%), Lymphocytes (%), Hemoglobin (g/dL), PCV (%), MCV (fL), MCH (Pg), MCHC (g/dL) and Platelets (×10^3//µL). Values are expressed as Mean ± S.D (n=3 fish per treatment). Mean with * expresses differences statistically significant in comparison to the control group at $p < 0.05$.

3.1.1.4 Comparison of the results of toxic effects of emamectin benzoate, acetochlor and topsin-M on hematological parameters of grass carp

Comparison of the results of toxic effects of Emamectin Benzoate, Acetochlor and Topsin-M in case of all the treated groups of Grass carp fish have shown several changes in their hematological parameters i.e., number of red blood cell, white blood cell and platelets, neutrophils content, lymphocytes content, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration as shown in Table 1. In case of Emamectin Benzoate, a significant $p < 0.05$ increase in WBCs, neutrophils, MCV, MCH contents and platelets, while a significant $p < 0.05$ decrease in RBCs, lymphocytes, hemoglobin, PCV and MCHC contents have been observed. An exposure to Acetochlor showed an increase in WBCs, neutrophils, MCV, MCH contents and platelets, while a decrease in RBCs, lymphocytes, hemoglobin, PCV, and MCHC contents was noted. However, the observed effects of Acetochlor were lesser then Emamectin Benzoate. In case of Topsin-M, an increase in WBCs, neutrophils, MCV, MCH contents and platelets have been shown, while a decrease in RBCs, lymphocytes, hemoglobin, PCV and MCHC contents were noted. However, the observed effects Topsin-M were lesser than Acetochlor.

3.1.2 Biochemical parameters

3.1.2.1 Effects of emamectin benzoate on biochemical parameters

Exposure to pesticides Emamectin Benzoate, number of total Bilirubin significantly increased 4.00 ± 0.65 than control group 0.07 ± 0.17. Uric Acid also increased 7.43 ± 0.50 than control group 0.80 ± 0.55. Likewise, Sodium content also increased 164 ± 5.56 as compared to control group 75.00 ± 5.29. Potassium content showed huge increase 15.66 ± 3.05 as compared to control group 2.63 ± 0.40 and Phosphorous increased 22.63 ± 5.84 than control group 2.70 ± 0.300. In case of Serum Lipase, it increased 10.66 ± 4.04 as compared to control group 1.33 ± 0.35. In case of HDL Cholesterol, it decreased 15.33 ± 6.50 than control group 58.33 ± 4.16. LDH content decreased 569.3 ± 5.50 in treated group as compared to the control group 940 ± 9.45. Similarly, the number of Creatinine decreased 0.45 ± 0.47 as compared to the control group 1.33 ± 0.07. Triglycerides also decreased 16.00 ± 4.58 than control group 62.00 ± 6.55 and same SGPT (ALT) also decreased 31.66 ± 4.16 as compared to the control group 53.66 ± 6.02 and decreased in alkyl phosphatase content 5.66 ± 3.05 than control group 16.00 ± 3.00 with the 5-ppb concentration of Emamectin Benzoate Table 2.

3.1.2.2 Effects of acetochlor on biochemical parameters

Exposure to herbicide acetochlor, number of total bilirubin significantly increased 2.46 ± 0.83 as compared control group 0.07 ± 0.17. Uric Acid also increased 3.70 ± 0.72 than control group 0.80 ± 0.55. Likewise, Sodium content also increased 133.6 ± 4.04 as compared to control group 75.00 ± 5.29. Potassium content show huge increase 7.50 ± 0.30 as compared to control group 2.63 ± 0.40 and Phosphorous increased 13.66 ± 4.36 than control group 2.70 ± 0.300. In case of Serum Lipase, it increased 5.66 ± 3.51 as compared to control group 1.33 ± 0.35 and increased in alkyl phosphatase content 6.33 ± 2.08 than control group 16.00 ± 3.00. In case of HDL Cholesterol, it decreases 39.33 ± 4.50 than control group 8.33 ± 4.16. LDH content decreased 842 ± 4.58 in treated group as compared to control group 940 ± 9.45. Similarly, the number of Creatinine decrease 0.85 ± 0.30 as compared to control group 1.33 ± 0.07. Triglycerides also decreased 24.66 ± 5.50 than control group 62.00 ± 6.55 and similarly SGPT (ALT) also decreased 43.00 ± 6.245 as compared to control group 53.66 ± 6.02 with exposure of 5 ppb concentration of Acetochlor as shown in Table 2 and in Figure 1.

3.1.2.3 Comparison of the results of toxic effects of emamectin benzoate and acetochlor on biochemical parameters of grass carp

Comparison of all the treated groups which showed several changes in their biochemical parameters that are number of total bilirubin, uric Acid, sodium, potassium, phosphorous, serum Lipase increased while number of creatinine, triglyceride, HDL cholesterol, SGPT (ALT), LDH and alkyl phosphatase decreased as compared to control group during chronic toxicity shown in Table 2. In case of Emamectin Benzoate (5ppb), which showed increase of total Bilirubin, uric acid, sodium, potassium, phosphorous, serum lipase in number, while a significant decrease in creatinine, triglyceride, HDL cholesterol, SGPT (ALT), LDH and alkyl Phosphatase was noted as compared to untreated (control) group. In case of acetochlor (5ppb), which shows increase of total bilirubin, uric acid, sodium, potassium, phosphorous, serum lipase in number, while a significant decrease in creatinine, triglyceride, HDL cholesterol, SGPT (ALT), LDH and alkyl phosphatase was noted as compared to untreated (control) group.

Different types of chemicals; like insecticides, herbicides and fungicides, are in use to produce high yield of crops. However, their excessive use cause harmful effect to the human health and contaminate the environment. The current study aimed to determine the effects of sub-lethal concentrations (5 ppb) of insecticide (Emamectin Benzoate), herbicide (Acetochlor), and fungicide (Topsin-M) on hematological parameters including erythrocytes count, leucocytes count, hemoglobin and platelets of Grass carp fish during acute toxicity (120 hours).

3.2 Discussion

The Grass carp was first described by Valenciennes in 1844, they are commercially available for food in Pakistan. According to Gabriel *et al.* (2007a), an aquatic animal, fish are directly exposed to the aquatic environment, which may influence their hematological traits. Blood is an excellent bio-indicator of an organism's health and is one of the hematological indicators used to assess the health state of fish (Joshp *et al.*, 2002). Since fish blood has been demonstrated to be susceptible to stress caused by pesticides, analyzing hematological features is crucial to comprehending both healthy and unhealthy processes as well as the effects of toxins (Erlista *et al.,* 2023; Fazio *et al.*, 2014).

Hematological profile analysis and histology analysis are the standard techniques used to investigate the negative impacts of various toxicants. The changes in blood parameters show how stressed out

Table 2. Summarized Biochemical parameters of both control and treated groups on grass carp.

S. No.	Biochemical Parameters	Control Groups	Emamectin Benzoate (5ppb)	Acetochlor (5ppb)
		Metabolites		
1.	Total Bilirubin (mg/dL)	0.07±0.17	2.46±0.83*	4.00±0.65*
2.	Creatinine (mg/dL)	1.33±0.07	0.85±0.30	0.45±0.47*
3.	Uric acid (mg/dL)	0.80±0.55	3.70±0.72*	7.43±0.50*
4.	Triglycerides (mg/dL)	62.00±6.55	24.66±5.50*	16.00±4.58*
5.	HDL Cholesterol (mg/dL)	58.33±4.16	39.33±4.50*	15.33±6.50*
6.	SPGT(ALT) (U/L)	53.66±6.02	43.00±6.245	31.66±4.16*
		Enzymes		
7.	Serum Lipase	1.33±0.35	5.66±3.51	10.66 ±4.04*
8.	ALK Phosphatase (μ/L)	16.00±3.00	6.33±2.081*	5.66 ±3.055*
9.	LDH (U/L)	940±9.45	842±4.58*	569.3±5.50*
		Electrolytes		
10.	Phosphorous	2.70±0.300	13.66±4.36*	22.63 ±5.84*
11.	Sodium (mmol/L)	75.00±5.29	133.6±4.04*	164±5.56*
12.	Potassium	2.63±0.40	7.50 ±0.30*	15.66 ±3.05*

fish are when they are subjected to various toxicants, including pesticides, rodenticides, heavy metals, and biocides (Singh *et al.*, 2008). So, the hematological parameters of blood can provide the information about the body of the fish and can indicate the symptoms of disease.

3.2.1 Impact of environmental toxicants on fish hematology

The results of this study demonstrate that grass carp exposed to the same concentration of 5 ppb of Acetochlor, Topsin-M, and Emamectin Benzoate alters hematological parameters, such as platelets, neutrophils, and white blood cells (WBCs), all of which significantly increase. In contrast to the control group, the treated groups showed a substantial drop in red blood cells (RBCs), pack cell volume (PCV), lymphocytes, hemoglobin (Hb) level, and mean cor puscular hemoglobin concentration (MCHC) values. According to David *et al.* (2015), the decrease in RBCs may be due to decrease iron uptake by intestine or due to the damage of intestinal villi and mucosal lining. In the present work, the significant $p < 0.05$ decrease in the RBCs were observed, which showed the breakdown of RBCs of Grass carp fish on exposure to Emamectin Benzoate, Acetochlor and Topsin-M. It was proposed that the drop in RBC counts during exposures may be brought on by the emergence of hypoxia, which might result in an increase in RBC destruction or a decrease in RBC genesis since the Hb levels in the cellular medium are unavailable (Akinrotimi and Gabriel, 2012). Marked reduction in the

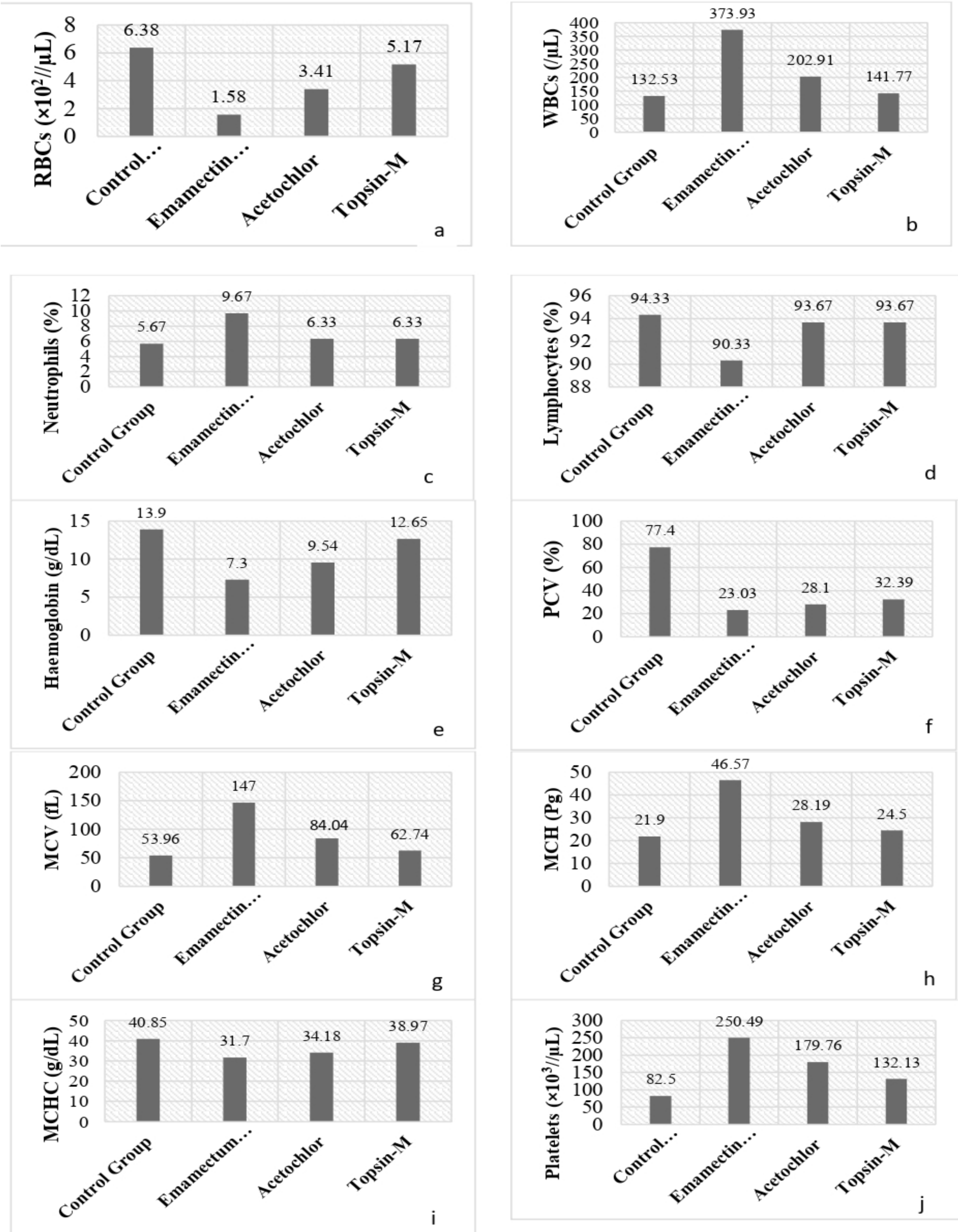

Figure 1. (a) Showing comparison between RBCs of control and treated group of fish (b) WBCs of control and treated group of fish (c) Neutrophils of control and treated group of fish (d) Lymphocytes of control and treated group of fish (e) Hemoglobin of control and treated group of fish (f) PCV of control and treated group of fish (g) MCV of control and treated group of fish (h) MCH of control and treated group of fish (i) MCHC of control and treated group of fish and (j) Platelets of control and treated group of fish.

RBC, Hb and PCV in fishes infected by hemiparasites had been reported (Shahi *et al.*, 2013). However, it may be concluded that pesticides caused harmful effects on erythrocytes during the acute toxicity.

3.2.2 Immunomodulatory effects of pesticides on fish leukocytes

In the present study, the white blood cell was significantly $p < 0.05$ increased in all treated group as compared to the control group. A significant $p < 0.05$ increased in the number of WBCs was noted due to immunological response of fish exposed to sub-lethal concentration of Emamectin Benzoate, Acetochlor and Topsin-M. The rise in white blood cell count was a pathological reaction since WBCs are important throughout infestation because they stimulate the immune system and hemopoietic tissues to produce chemicals that act as defense against infection and antibodies (Lebelo *et al*., 2001). In the present investigation significant $p < 0.05$ increase in WBCs indicate the effect of Emamectin Benzoate, Acetochlor and Topsin-M on the immune system of fish.

The primary blood components that protect the body against damage, bleeding, and the admission of foreign antigen particles into the body are white blood cells (Velmurugan *et al*., 2016). During stress, the number of leukocytes increases significantly to cope with conditions of stress and defend organism (Deshmukh, 2016). In this study, significant $p < 0.05$ increase in WBCs during acute toxicity showed protective response and recovery of the fish on exposure to Emamectin Benzoate, Acetochlor and Topsin-M.

Experimental results show that across treatment groups, exposure to Emamectin Benzoate, Acetochlor, and Topsin-M each given at an identical dose of 5 ppb markedly raised neutrophil level. However, Lymphocytes got decreased by exposure to these three pesticides. Lymphocytes make up around 85% of all leukocyte cells and are the most prevalent type of leukocyte detected in the blood of several fish (Groff and Zinkl, 1999). Emamectin Benzoate caused the increase and decrease in number of neutrophils and lymphocytes respectively as compared to Acetochlor and Topsin-M. However, later two chemicals, both numbers of neutrophils and lymphocytes were noted to be same.

3.2.3 Agrochemical effects on RBC indices

The results of exposure of Grass carp to Emamectin Benzoate, Acetochlor and Topsin-M noted to cause alteration in Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Pack Cell Volume (PCV). Potential erythrocyte shrinkage and physiological disturbance of red blood cells (RBCs) owing to agrochemical interference are suggested by the observed decrease in packed cell volume (PCV) and hemoglobin (Hb) levels. The inverse link between MCV (mean corpuscular volume) and MCH (mean corpuscular hemoglobin) levels served to confirm these phenomena even further (Atamanalp *et al*., 2002). Consistent with macrocytic anaemia a disease marked by fewer but bigger, haemoglobin-enriched erythrocytes this research shows a significant decrease in RBC count across treated groups compared to controls in PCV amid increased MCV and MCH levels. According to Dzenda *et al*. (2004), MCHC is a good indicator of red blood cell swelling. Hence it reduced as concentration of toxicant increased. Our experimental study showed a significant decrease in the MCHC, which is the indication of red blood cell swelling and hence a decrease in hemoglobin of fish (Zhou *et al.*, 2016).

Decreased in Hemoglobin (Hb) have been observed in the treated groups of fishes on exposure to three different chemicals of this study compared to the control group. Numerous researchers examined how toxicants affected the hematology of diverse fish species, reporting varying degrees of hematological alterations and speculating that a decrease in hemoglobin, red blood cells, and platelet count was connected to the blood's ability to transport oxygen (Adhikari *et al*., 2004; Gabriel *et al*., 2007b) It has been discovered that hemoglobin is degraded when fish are exposed to pesticides.

The latest study exhibited a significant increase in platelet count $p < 0.05$ across all treatment groups exposed to 5 ppb of Emamectin Benzoate, Acetochlor, and Topsin-M, in all cases. According to Singh and Srivastava, (2010), platelets play a crucial role in blood coagulation by absorbing numerous substances essential for clotting and transporting them to the site of hematological changes. The findings indicate that a considerable increase in platelet count $p < 0.05$ enhances blood coagulation in Grass carp exposed to pesticides.

3.2.4 Hepatic and plasma triglyceride alterations in Grass carp from pesticide exposure

Grass Carp subjected to a 5-ppb dosage of Emamectin Benzoate and Acetochlor exhibited significant changes in biochemical markers. The objective was to investigate the biochemical changes in Grass Carp subjected to equivalent concentrations of Emamectin Benzoate and Acetochlor for a duration of 14 days. After statistical analysis of triglyceride shows a regular decreasing trend in the result as compared to control group. According to the finding of Abdali *et al.* (2011), which are compatible to our result. They exposed the Grass carp against different diluted concentration of atrazine for 30 days and found the triglyceride to be decreased compared to control group. Studies have been done on the effects of increased toxin concentration on the liver, digestive tract, and associated enzymes, as well as on fish hormone levels and natural metabolic imbalances, and on the decrease of triglyceride volume in blood plasma (Robinson, 1990). According to the results concluded by Rajini *et al.* (2014), the elevation is reported in the value of triglyceride when 50 % of alachlor was introduced for biochemical alteration in *Calarias batrachus* during the cessation exposure. Triglycerides were shown to be significantly elevated in the liver tissue of fish subjected to 0.32 mg/L and 0.65 mg/L following the exposure, as compared to the control group. Following the reversal phase, fish exposed to 0.32 mg/L and 0.65 mg/L showed a substantial reduction in triglycerides in their liver tissues and a rise in their gill tissues; the elevation in gill tissue was not significant in either of the exposed fish.

Exposure of Grass Carp to 5 ppb of Emamectin Benzoate and Acetochlor resulted in significant modifications of the biochemical parameters. The current investigation found a reduction in SGPT levels when exposed to equivalent concentrations of Emamectin Benzoate and Acetochlor compared to the control group. According to the finding of (Singh and Zahra, 2019), which was not compatible to present results showed an increasing order of SGPT, for fresh water fishes *Heteropneustes fossilis* exposed against Cypermethrin for 15 days. This study found that the CYP treated group had higher concentrations of SGPT enzyme activity in their serum during both acute and long-term exposure periods. This finding may be related to the harmful effects of nitroso compounds, which are formed in the stomach's acidic environment and cause enzymatic changes because of cellular damage. Many enzymes that are typically found in the cytosol are released into the bloodstream when the liver cell membrane is disrupted. An increase in SGPT signifies the use of amino acids in oxidation processes to release energy (Fazlolahzadeh *et al.,* 2011).

To investigate the biochemical changes in Grass carp subjected to equivalent concentrations of Emamectin Benzoate and Acetochlor over a 14-day period. The statistical analysis of uric acid demonstrated a consistent upward trend in comparison to the control group. According to the finding of Qadir *et al.* (2014), which was not compatible to our result showed decreasing trend *Labeo rohita* for sub lethal dose of 120 mgL^{-1} of Imidacloprid for 16 days. According to (Alwan *et al.,* 2009), which was compatible to present result showed an increasing trend of uric acid in For ninety-six hours, freshwater fish (*Tilapia zillii*) were exposed to aluminum. The blood analysis in this investigation revealed a considerably higher level of uric acid than in the control. Increased muscle tissue catabolism, impaired glucose metabolism, or glomerular insufficiency might all be contributing factors to this elevation in uric acid (Murray *et al.,* 1990).

3.2.5 Emamectin and acetochlor effects, bilirubin rise, creatinine/ALP drop in Grass carp

Exposure of Grass Carp to 5 ppb of Emamectin Benzoate and Acetochlor resulted in significant changes in biochemical parameters. The current investigation found an elevation in total bilirubin levels upon exposure to Emamectin Benzoate and Acetochlor compared to the control group. A significant elevation in total bilirubin levels was found in all treatment groups. The result reported by (Inyang *et al.,* 2016), were same when common African catfish (*Clarias gariepinus*) was exposed to different concentration of fluazifop-p-butyl to determine the biochemical parameter. The bilirubin was showing increased value which was compatible with result of present study. The results indicated a reduction in Creatinine levels in the treatment groups relative to the control group. A significant reduction in creatinine levels was found in all treatment groups. The result reported by (Inyang *et al.,* 2016) were different when common African catfish (*Clarias gariepinus*) was exposed to different concentration of fluazifop-p-butyl to determine the creatinine and was showing an increase, which was not compatible with present result.

Grass Carp exposed to comparable concentrations of Emamectin Benzoate and Acetochlor for 14 days exhibited a consistent decline in ALP levels, as shown by statistical analysis, relative to the control group. According to the findings of Adedeji *et al.* (2009), acute effect of diazinon on plasma biochemical values of African catfish (*Clarias gariepinus*) a decrease in ALP was observed after exposure to alachlor for 21 days during which reduction as well as elevation was reported. Changes in alkaline phosphatase may be caused by the effects of pesticides, as multiple researchers have observed. Alkaline phosphatase is involved in the maintenance of the orthophosphate pool transport of phosphoryl groups as well as the hydrolysis and esterification of the metabolites through the membrane transport (Rana *et al.,* 2002). The organism's damage may be the cause of the alkaline phosphatase enzyme's activity. In a freshwater edible fish species called *Sarotherodon mossambicus* (Peters), the impact of a sub-lethal dosage of a carbamate pesticide was investigated on the activity of the enzyme's acid and alkaline phosphatase in the liver and muscle following 24, 48, 72, and 96 hours of exposure. The drastic decrease in tissue alkaline phosphatase enzyme may have been an adaptive response by the fish to satisfy energy requirements, facilitating the anaerobic breakdown of glycogen (Shaikila *et al.,* 1993).

To find out the biochemical alteration in Grass Carp, it was exposed to similar concentration of Emamectin Benzoate and Acetochlor for 14 days after the statistical analysis of LDH showed a regular decreasing trend as compared to control group. Findings of (Christo Queensly *et al.,* 2015), were compatible to the outcome of this investigation for *Cyprinus carpio* (L) treated to 0.129 mg/L of cypermethrin and organ phosphorus insecticide for 96 hours. Heart, liver, kidney, pancreas, spleen, lung (gill), red blood cells, and brain tissue all contain LDH. These tissues are impacted by illnesses or injuries, and the cells are killed, particularly in the liver. According to Yaji *et al.* (2011), the substantial drop in LDH activity in the blood serum of Oreochromis niloticus indicates liver dysfunction, which might have impacted the liver's ability to synthesize triglycerides or caused a slowdown in metabolic rate because of exposure to cypermethrin. Also observed in similar findings by Naveed *et al.* (2011), that substantial drop in LDH activity in *Channa punctatus* blood plasma moreover indicates that exposure to triazophos has caused a reduction in the glycolytic process because of the lowered metabolic rate.

To determine the biochemical alteration in Grass carp, they were exposed to similar concentration of Emamectin Benzoate and Acetochlor for 14 days. After the statistical analysis of serum lipase showed a regular increasing trend as compared to control group. The result reported by Samanta *et al.* (2014), was compatible with our result and an increasing trend was shown when teleostean fish exposed to Almix herbicide for 30 days. Lipase activity was significantly elevated from control value. Stomach, liver and intestine showed increased activity of lipase. The result reported by Rani *et al.* (2017), was not compatible with result of this study and decreasing trend was shown when *L. rohita* exposed to organophosphates for 30 days. Fish treated to all three herbicides separately or in combination have shown a significant dose-dependent decrease in lipase activity levels. When *L. rohita* is exposed to malathion together with dimethoate and chlorpyrifos at 0.001 ppm and 0.0001 ppm concentrations, respectively, lipase activity in blood serum is reduced by up to 23.1 % and 15.1%, respectively.

To determine the biochemical alteration in Grass Carp, they were exposed to similar concentration of Emamectin Benzoate and Acetochlor for 14 days. After the statistical analysis of data for potassium showed a regular increasing trend in the result as compared to control group. The result reported by (Adedeji *et al.,* 2009), was compatible with our result and increasing trend was shown when African catfish (*Clarias gariepinus*) was exposed to different concentration of diazinon for 96 hours. To examine the biochemical alteration in Grass Carp for 168 hours, when it was exposed to similar concentration of Emamectin Benzoate and Acetochlor the phosphorous being electrolyte was increased in results. Electrolytes play a fundamental role in the body by regulating fluid distribution, preserving intra- and extracellular acid-basic balance, preserving bodily fluid osmotic pressure, and promoting appropriate neuro-muscular irritability (Harper, 1997).

Emamectin Benzoate, Acetochlor, and Topsin-M are hematotoxin agents that induce oxidative stress, dysfunction of the organs, and disruption of hematopoiesis in fish. Emamectin Benzoate changes the flow of ions and the activity of neurons and muscles. It also changes how the body regulates osmoregulation and absorbs oxygen, which causes low oxygen levels and damage through reactive oxygen species (ROS). Acetochlor, Emamectin Benzoate, and Topsin-M are hematotoxic chemicals that damage organs, cause oxidative stress, and mess up the process of hematopoiesis in fish. Emamectin Benzoate affects the transport of ions and neuromuscular activity, affecting osmoregulation and intake of oxygen, resulting in systemic hypoxia or damage from reactive oxygen species (ROS). Acetochlor, a chloroacetanilide herbicide, stimulates an increase in metabolism in the liver, which results in peroxidation of lipids and methylation of DNA, hence affecting hematopoiesis in the kidneys and spleen. Topsin-M, which has thiophanate-methyl in it, stops hematopoietic stem cells from putting together microtubules. This stops mitosis and erythropoiesis, which causes microcytic anemia and lower hemoglobin levels. Its estrogen-mimicking characteristics enhance immune system dysfunction and chronic inflammation. The level of toxicity is based on how quickly Emamectin affects important ion channels and makes reactive oxygen substances. Acetochlor, similarly, requires an accelerated metabolism, while Topsin-M provides a reduced influence on mitosis. Further study on oxidative indicators and transcriptome analyses is necessary to identify dysfunctional hematopoiesis pathways. The herbicide acetanilide enhances metabolic activity.

4. Conclusion

In this study, it has been concluded that by exposing the Grass carp (*Ctenopharyngodon idella*) at room temperature to concentration (5 ppb) of Emamectin Benzoate (an insecticide), Acetochlor (a herbicide) and Topsin-M (a fungicide), the Biochemical and Hematological parameters like RBCs, WBCs, Platelets count, Neutrophils, Lymphocytes, Hemoglobin, Pack Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) get altered in different extents in treated groups of fish. This has been also concluded that Emamectin Benzoate is more toxic than Acetochlor and Acetochlor is more toxic than Topsin-M. They noted to cause harmful effects on hematological profile of Grass carp fish and hence are deleterious to aquatic life and environment. This study recommend that great care must be done to control aquatic pollution and to present aquatic life by the minimum or controlled usage of investigated three pesticides because the use or presence of such pesticides in aquatic environment is the cause of ailment in fish. In this study only single species were used and presumably evaluated acute effects a short-term exposure, but chronic, low-dose exposure is more pertinent for analyzing long-term consequences on fish populations and ecosystems. Future studies can examine long-term ecotoxicological assessments of agrochemical combinations, new remediation methods, stronger regulations, and pesticide toxicity using omics technologies. Aquatic biodiversity protection requires community-based awareness campaigns and a coordinated science, policy, and public engagement strategy. The findings emphasize the need for stricter environmental monitoring policies and suggest further investigations using molecular and chronic exposure endpoints to assess long-term ecosystem risks.

Acknowledgement

We would like to extend our heartfelt appreciation to the entire team at GPGC Haripur for their invaluable contributions to this research. Your dedication and hard work made this achievement possible. Thank you for your commitment and collaboration.

Authors' Contributions

Khan Dil Badshah: Experimental work, Writing, Supervision, Data analysis, Bilal Riaz: Experimental work, Writing, Editing, Data analysis, Rameen Ejiz: Experimental work, Writing, Editing, Data analysis, Ashfaq A. Khan: Experimental work, Writing, Data editing, Supervision, Data analysis, Kamran Mehdi: Writing, Editing, Data analysis, Saqib Khan: Writing, Editing, Data analysis, Curation, Sania Riaz: Writing, Editing, Data analysis, Naseer Ahmed: Writing, Editing, Data analysis, curation, Graphing.

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

The author(s) affirm that no artificial intelligence (AI) tools, services, or technologies were employed in the creation, editing, or refinement of this manuscript. All content presented is the result of the independent intellectual efforts of the author(s), ensuring originality and integrity.

Funding Information

There was no source of funding.

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