

EFFECTS OF LOW DOSES OF LAMBDA CYHALOTHRIN ON HEMATOBIOCHEMICAL REACTIONS OF NILE TILAPIA

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Abstract

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Lambda Cyhalothrin is a synthetic pesticide that is used to kill nematodes and insects in agricultural crops. It is a major contaminant polluting aquatic ecosystems, having a harmful effecting health of fish; therefore, the current experiment was performed to assess the effects of low doses of Lambda Cyhalothrin on the hematobiochemical reactions of Nile Tilapia. In order to do this, fish were obtained from a fish farm in the area and then separated into three separate groups (A, B, and C). While group A served as a control, groups B as well as C were given two doses of Lambda Cyhalothrin (0.01 mg/L and 0.04 mg/L) that were below the threshold of being deadly over a period of 28 days. The LC50 value determined by probit analysis was 0.103 mg/L. Fish were provided a normal feed each day based on their body weight. The findings of the current study showed that concentrations of triglycerides, VLDL, ALT, AST, cholesterol, blood glucose, serum creatinine, WBCs, PLT, MPV, neutrophils, lymphocytes, and monocytes increased while the results of total protein, albumin, globulin, urea, HGB, RBCs, HCT, LDL, HDL, MCHC, PDW, MCH, PCT, serum BUN (blood urea nitrogen), and MCV, T3 (triiodothyronine hormones), and T4 (thyroxin hormone) levels significantly decreased when comparing the experimental groups to the pesticide-free group. According to current research, Nile Tilapia fingerlings exposed to lambda experience changes in their biochemical and haematology parameters.

Keywords: *pesticide, Nile Tilapia, toxicity, biochemistry, haematology.*

1. Introduction

Pesticides, insecticides, fungicides, herbicides, and other biocides are used to control crop pests in agricultural production (Alam, 2002). Pesticides are still thought to be the most effective way to decrease pests and promote crop growth in agriculture; hence, their use has increased (Annastesia et al., 2023). The use of pesticide combinations with various mechanisms of action to combat resistance to a single ingredient has grown significantly (Sewell et al., 2016). The consequences of pesticide effects on the ecosystem have therefore attracted attention from all over the world (Levine & Borgert, 2018). However, these pesticides are introduced into the environment through various means, such as food chains, soil, water, and air (Naveed et al., 2010).

Synthetic pyrethroid insecticide Lambda Cyhalothrin (LCT) is used to eradicate a variety of insects from homes and fields (Hasan, 2019). Furthermore, it has the potential to be used in public health for insect control and structural pest management (Kidd, 1991). The Lambda Cyhalothrin generally enters the soil through the materials used for packing and storage and discharge during spraying, then via runoff from its use in fields for agriculture into the aquatic environment. (de Moraes et al., 2013). The main risk of synthetic pesticides is environmental pollution, especially in water systems, where they induce a variety of negative effects in aquatic resources (fish) and, ultimately, humans. Research has revealed that freshwater fish variety is threatened by a range of environmental stresses such as pollutants and nutrient loading, habitat deterioration, and change of climate (Jelks et al., 2008; Bilala,b, 2021). On the other hand, most ecotoxicological studies conducted in aquatic environments and regulatory risk assessment of pesticides concentrate primarily on the toxicity of specific compounds in controlled environments. (Hassold & Backhaus, 2014). Fish have been used as bio indicators of the effects of pollutants as they are sensitive to environmental changes (Bharti & Rasool, 2021; Bilala,b et al., 2024).

Because they are very sensitive to toxic substances, fish can serve as bioindicators in a variety of aquatic habitats. Pesticides are used widely and carelessly in farming, causing pollution in the environment and contaminating water bodies. The bioaccumulation of pesticides in non-target aquatic organisms, especially fish or animals that eat fish, like humans, poses a serious ecological threat to aquatic environments (Nicolopoulou-Stamati et al., 2016). When pesticides penetrate fish organs, they can harm their biochemical and biological processes (BANAEI et al., 2008). The Nile Tilapia (Oreochromis niloticus) is a tropical omnivore fish that is widely bred globally. O. niloticus has commercial value, but it's also important to research the impacts of aquatic contaminants in field research and lab bioassays, where it can serve as a risk indicator for human intoxication. Validated methods for evaluating the long and short-term impacts of agrochemicals on fish include blood biomarkers (hemoglobin, white blood cells, and red blood cells) and biochemical indicators (blood glucose, lipid profile, total protein) (Bharti & Rasool, 2021). Previously, Yekeen et al. (2013) worked on the effects of Lambda Cyhalothrin on hematobiochemical of fish. A similar study of hematology conducted by Dhanya and Sushama (2006) reported the long and short-term exposure of Lambda Cyhalothrin to fish. The present study was aimed to assess the toxicity of Lambda Cyhalothrin on certain hematological and biochemical parameters of a freshwater teleost Nile Tilapia. Moreover, the selected parameters can serve as excellent biomarkers in environmental monitoring of Lambda Cyhalothrin contamination in aquatic ecosystems.

2. Methods

2.1 Chemicals

A commercial lambda-cyhalothrin pesticide was purchased from Distributor Company Al Rehman agrochemical Pvt. Ltd., Renala, Pakistan. A suitable amount of lambda-cyhalothrin was dissolved in distilled water to prepare the stock solution.

2.2 Specimen Collection and acclimatization

A total of 15 randomly selected fingerlings of the freshwater fish Nile Tilapia (Oreochromis niloticus) of body length 12-17cm and body weight 19-30g were purchased from the "Fish seed nursery unit" in Manga, Lahore and shifted to the fisheries laboratory at the zoology department, University of Okara, Renala khurd. Prior to transferring to aquariums all the fish were screened for any pathogenic infection. The fish were transferred to aquariums made of glass, containing tap water. The fish were acclimated to the laboratory environment for a period of 10 days before the experiment. This was done using a recirculation-aerated system (RAS) with a water renewal system that replaced the water daily to eliminate any leftover food and fish waste. Oxygen concentration of 7.25 ± 0.23 mg/L, temperature 24.5 ± 2.7 oC and pH of 7.46 ± 0.28 of water were upheld throughout the experimental duration of 28 days.

2.3 Experimental Design

The experiment was designed for 30 days, and fish were kept in aquariums with 40-liter water capacity. The fish were fed with commercial feed once in 24 hours during the experimental duration. It was preferred that fish of similar weight should be placed in the same group. Three treatments were performed depending on the concentration of Lambda Cyhalothrin. The first group was control with no exposure to Lambda Cyhalothrin; group II was given 0.01 mg/L Lambda Cyhalothrin; group III was given 0.04 mg/L Lambda Cyhalothrin. These sublethal doses were chosen by earlier research, as stated by Oluah and Chineke (2014). All the protocols for handling of fish were implied after having approval from ethical committee of department of zoology at University of Okara, Renala Khurd.

2.4 Hematobiochemical analysis

Three fish were taken out of each group after the experimental trial. Using a BD syringe, blood was taken from the caudal vein and kept in EDTA vials for hematological analysis and in gel vials for serology analysis. These vials were obtained from Hunan YBK Medical Technology Co. Ltd. A hematological analyzer examined all hematological parameters. The RBC and WBC counts were performed using a hemocytometer. The amount of HGB was determined by a laboratory reagent kit and the method of cyanmethemoglobin (Bihari et al., 2016). To compute HCT, the microhematocrit technique was used (Nwani et al., 2014). Using standard formulas, the RBC, HGB, and HCT were used to determine the fish erythrocyte indicates (MCHC, MCH, and MCV) (Ramesh et al., 2015). A chemistry analyzer assessed the biochemical indicators. Plasma was isolated from other samples of blood via a cooling centrifuge at 9400×g for 20 minutes and stored at 4°C. The amounts of protein were calculated by the Waterborg (2009) method. Glucose was assessed using the Khan et al. (2019) technique. Reitman and Frankel (1957) describe the method to calculate the AST and ALT. Kits from Biomerieux (France) were used to assess serum urea and creatinine. Cholesterol, triglycerides, and HDL were assessed using standard kits using the methodology of (Hassan et al. (1995). We calculated the levels of VLDL and LDL using the basic formula provided by Zaahkouk et al. (1996). Using standard kits, Hadie et al. (2013)'s methodology was used to evaluate T3, and T4.

2.5 Statistical Analysis

The mean and standard variations of all the values are shown. One-way analysis of variance (ANOVA) was used to compare all the factors that were studied between the three experimental conditions. This was done with Mini Tab statistical program Graph pad (version 9.1).

3. Results

3.1 Hematological analysis:

Hematological findings revealed that red blood cells (WBCs, PLT, MPV, neutrophils, monocytes, and lymphocytes) were increased, while HGB, RBC, HCT, MCV, MCHC, MCH, and PDW decreased in comparison to the lambda-free group, as shown in Table 1. The results are shown as mean \pm SD. Asterisk (*) values indicate significant differences (P < 0.05) when compared with the control group. NS = not significant.

3.2 Lipid profile

The statistical values of triglycerides, VLDL, HDL, LDL, and cholesterol are presented in Figure 1. The result of the one-way ANOVA statistic showed a significant ($p \le 0.05$) elevated in VLDL, triglycerides, and cholesterol levels in the experimental groups compared to the control group. A significant decrease in LDL and HDL was observed.

3.3 Effects on protein and liver enzymes

The statistical (mean and SD) values of total protein, albumin, globulin, AST and ALT are shown in Figure 2. ANOVA statistics revealed a significant ($p \le 0.05$) elevated in ALT and AST after exposure to lambda-cyhalothrin in the experimental group. While a decrease in globulin, total proteins, and albumin was observed.

3.4 Blood glucose and renal effects

The mean values of creatinine, blood sugar, urea, and BUN are presented in Figure 3. ANOVA statistics revealed a significant ($p \le 0.05$) rise in blood sugar, creatinine, and BUN after exposure to lambda cyhalothrin in the experimental group. While a decrease in urea was observed.

3.5 Effects on thyroid hormones

The mean values of T3 and T4 are presented in Figure 4. ANOVA statistics revealed a significant ($p \le 0.05$) decrease in T3 and T4 after exposure to lambda cyhalothrin in the experimental group.

Parameters	Control	Low (Mean ±SD)	High (Mean ±SD)
HGB (g/dl)	6.83±1.007	3.90±0.700*	2.00±0.45*
WBC (×10 ³ /µL)	22.33±2.22	32.83±2.75*	43.03±0.95*
RBC (×10 ⁶ / µL)	2.00±0.46	1.500±0.200 ^{NS}	1.08±0.21
HCT (%)	16.77±1.59	9.83±1.07*	7.73±0.76*
MCV (FL)	160.5±9.87	85.47±4.91*	59.30±2.56*
MCH (pg)	50.43±2.50	37.63±2.55*	32.90±2.21*
MCHC (g/dl)	91.60±4.06	67.93±2.91*	33.17±3.35*
PLT (×10 ³ / μ L)	241.5±62.09	351.2±4.81*	519.3±7.48*
MPV (FL)	11.13±1.48	14.77±2.41	18.13±0.81*

Table 1. Showing the hematological profile of Nile Tilapia exposed to different concentrations ofLambda Cyhalothrin.

PDW (%)	16.87±1.96	10.37±0.78*	5.87±0.45*
PCT (%)	0.560±0.954	0.240±0.300*	0.110±0.010*
Neutrophils (%)	60.43±3.50	74.93±1.68*	81.83±3.00*
Lymphocytes (%)	32.90±2.59	39.90±1.85*	49.30±3.25*
Monocytes (%)	2.77±0.32	4.53±0.47*	5.93±0.152*

4. Discussion

Many toxicological and environmental problems arise from pollution spilled into neighboring streams from agricultural areas (Akpomie & Dawodu, 2015). Organisms that are exposed to certain quantities and durations of hazardous compounds, both in acute and sublethal concentrations, might give valuable insights into the detrimental consequences of these chemicals in toxicological evaluations (Lemly, 2002). Lambda-cyhalothrin is classified as a pyrethroid insecticide. Farmers in the agricultural industry choose synthetic pyrethroids due to their superior efficacy in comparison to other pesticides (Shah, 2010). The increasing usage of synthetic pyrethroids as pesticides in agricultural and pest management activities has led to a rise in pyrethroid residues found in both freshwater and marine sediments. Lambda-cyhalothrin infiltrates the aquatic environment via rivers, direct application, spray, drift, aerial spraying, and washing. It also enters from the atmosphere by precipitation, erosion, and runoff from agricultural land, manufacturing effluents, and sewage (Edwards, 2013). Lambda-cyhalothrin poses a serious risk to fish and other aquatic life (Kidd & James, 1991; Sattar et al., 2024). Our toxicity experiments employed Nile Tilapia to examine the toxicological effects of lambda-cyhalothrin on fish lipid profile, renal function, thyroid function, liver enzymes, blood glucose, and hematology. The physiological state of an organism and its blood properties can be better understood and studied with the help of hematological measurements. The number of red blood cells has enormous physio pathological significance. Lambda Cyhalothrin directly caused the fish Nile Tilapia to have a reduced RBC count. The current study's anemic condition could be the result of mature RBC destruction or inhibition of erythrocyte formation. Such reductions in RBC and the state of anemia were observed by Adhikari et al. (2004) and Sajjad et al. (2024) following exposure to various submission concentrations in Sarotherodon mossambicus. Adhikari et al. (2004) revealed a drop in Hb and RBC, which caused anemia. Anemia may result from the attraction of the cell membrane or the impact of pesticides on hemopoiesis. Anemia may result from the attraction of the cell membrane or the effect of pesticides on hemopoiesis. It was discovered that fish with stressful conditions had a lower erythrocyte count. The decreased count could result from erythropoiesis suppression or from the breakdown of red blood cells. The observed rise in white blood cells (WBC) during treatment in this study may have been due to Lambda Cyhalothrin stimulating the immune system and protecting the fish from toxicity. The impact of lindane and malathion exposure on specific blood parameters in a freshwater teleost fish Clarias batrachus was investigated by Joshi et al. (2002) found that the rise in the WBC count could be the result of an increase in antibody synthesis, which aids in the survival and recovery of fish exposed to pesticide. Yekeen et al. (2013) and Dhanya and Sushama (2006) discovered that Oreochromis niloticus subjected to an acute dose of lambda cyhalothrin showed similar results. In the present study, sign cant elevated PLT, MPV, neutrophils, lymphocytes, monocytes, and eosinophil levels were reported after exposure to lambda. The value of HCT (hematocrit) decreased due to the harmful effect of Lambda Cyhalothrin on the liver and kidneys of exposed fish. The value of mean cell hemoglobin (MCH) and mean corpuscular volume (MCV) decreased by exposure to pesticide due to the changes in RBC's and HB values (Ramesh & Saravanan, 2008). It also leads to hypochromic microcytic anemia (Ucar et al., 2019; Basharat et al., 2024), but opposite results were reported by Dhanya and Sushama (2006).

Biochemical parameters are helpful to understand the alteration in body physiology of fish (Sharafeldin et al., 2015). The pesticide lambda caused significant changes in the serum lipid profile (Oluah & Chineke, 2014). The acute lambda poisoning was found to cause changes in the lipid profile and related components (Fig. 1). Levels of cholesterol, VLDL, and triglycerides were noticeably higher, which is corroborated by the findings of (Oluah & Chineke, 2014). When compared to the insecticide-free group, the levels of HDL and LDL were decreased. The rise in cholesterol, triglycerides and VLDL could be due to catecholamine stimulation, which causes lipolysis and increased fatty acid production. One reason for the aforementioned modifications could be enhanced tissue lipogenesis brought on by an accelerated acetyl CoA pathway (Rai et al., 2009). HDL level in blood was significantly decreased (Patrick, 2009). Blood proteins provide essential information about biochemical, physiological and ecological state of fish. They are necessary for the transportation of many different exogenous substances and metabolites that keep fish healthy (Kovyrshina & Rudneva, 2012). The most significant blood protein that are able to utilized as biochemical indicators for liver health are albumin and globulin (Sunmonu & Oloyede, 2007). The blood of Nile Tilapia treated with pesticides showed a significant decrease in total proteins, globulin and albumin (Fig. 2). Sunmonu and Oloyede (2007) stated that a low amount of globulin could be a sign of weakened immunity in the body because the liver produces less of it for immunological purposes. Serum ALT and AST were significantly elevated (Fig. 2). Our results are corroborated by Harabawy and Ibrahim (2014). Increased ALT and AST levels were associated with severe necrosis and hepatocyte degeneration, indicating injury to several organ tissues, particularly the liver (Palanivelu et al., 2005). Elevated liver enzyme levels (ALT, AST) indicate hepatocellular damage and are among the blood parameters affected by liver damage. Animals utilize blood glucose as their primary energy source, and elevated levels of glucose are commonly used as a stress indicator in reaction to environmental exposure (Authman et al., 2013). The results of this experiment demonstrated that fish exposed to lambda had increased blood glucose levels. Increases in amino acids, glucocorticoids, and plasma catecholamines may activate the gluconeogenesis process, resulting in hyperglycemia (Mekkawy et al., 2013). In vertebrates, the preservation of metabolic balance depends on the thyroid hormones T3 and T4 (Fig 4). Thyroid hormones appear to promote almost every stage of the synthesis, mobilization, and breakdown of lipids and proteins in teleost fish (Sheridan, 1994). The results of this investigation also demonstrated a comparable decline in T3 and T4 levels in the experimental fish, which was directly correlated with the Lambda Cyhalothrin concentration and exposure time. Our findings are corroborated by Saravanan et al. (2009). The production of hormones and their release into the bloodstream may have been directly affected by Lambda Cyhalothrin. Thyroid function may have been affected by environmental pollutants (Brown et al., 1989). Thyroid hormone secretion reduction may result in physiological changes related to their possible functions in controlling development and metabolism (Waring & Brown, 1997).

Present study suggested that Lambda Cyhalothrin; an organophosphate is the primary factor that causes significant changes in blood and tissue of fish in aquatic ecosystem. Harmful toxicological alterations in hematological and biochemical parameters after 28 days of exposure in Nile Tilapia. The current observation indicates that this pesticide poses a significant thread to aquatic life. To minimize the toxic effects of pesticide, their use should be reduced and more environmentally friendly organophosphate pesticide should be developed with faster degradation ability.

Statement of Significance:

Three main factors make this study on the consequences of low-dose Lambda Cyhalothrin exposure on Nile Tilapia noteworthy:

Novelty and Importance: It closes a significant gap in the literature by offering vital insights into the sublethal effects of a commonly used pesticide on a globally significant freshwater fish species.

Impact on Science: Beyond Lambda Cyhalothrin toxicity, the results provide useful markers of environmental stress that might guide future biomonitoring initiatives and improve our capacity to evaluate the general well-being of freshwater ecosystems.

International Relevance: Policymakers, environmental managers, and aquaculture experts around the world will find this study extremely interesting, especially considering the global significance of Nile Tilapia and the extensive use of synthetic pesticides.

Author Contributions

I.J. and A.I. collected data, conducted data analysis. A.I. and A.B. wrote and edited the manuscript. F.T. supervised it. A.D. and S.J. helped in writing. All authors approved of the final publication.

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N/A

Conflicts of Interest

The authors declare no conflicts of interest.

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