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RESEARCH PAPER

Physiological changes in common carp exposed to pyrethroid pesticides using well water

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A B S T R A C T:

The current research was carried out to assess the negative impacts of water cypermethrin concentrations on certain hematological and biochemical parameters and stress hormones in the juvenile common carp *Cyprinus carpio* L. for 96 hours. The experiment was arranged in completely randomized design (CRD) and data were analyzed through one-way ANOVA.

The percentage of mortality was increased significantly following the exposure of common carp to cypermethrin and increasing the exposure level. Cypermethrin has a detrimental impact on fish hematological and biochemical parameters. Hematological profiles (LYM, MON, GRA, Hb, RBC, MCV, and Hct) decreased significantly with exposure of common carp to cypermethrin and increasing the level of exposure. Exposing fish to cypermethrin led to a significant rise in the level of triglycerides, AST, ALT, and ALP and a significant decline in the level of cholesterol. The levels of cortisol and T3 were significantly increased following the exposure of common carp to cypermethrin. While, significant reduction in AChE level was found by exposing common carp to cypermethrin.

This research concluded that hematological, biochemical, and stress hormone parameters were adversely affected by exposing juvenile common carp to cypermethrin.

KEY WORDS: Pesticides, cypermethrin, common carp *Cyprinus carpio*, haematological parameters, biochemical parameters, stress hormone
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1.INTRODUCTION

Using pesticides is the main concern for polluting freshwater ecosystems due to their wide range of hazardous compounds, which adversely affect freshwater fish health (Kumar *et al*., 2021). Insects, water weeds, and plant diseases are controlled by utilizing pesticides. Fish health is negatively impacted by pesticide use in agricultural areas since they are particularly toxic to non-target species like fish and impede their metabolism, which can occasionally result in fish mortality (Sabra and Mehana, 2015).
Pesticides are arranged according to their intended purpose.

Herbicides (for weed control), insect sprays (for controlling creepy crawlies), and fungicides (for controlling mycotics) are the three main pesticides; nevertheless, bug sprays are the most dangerous (Perwaiz, 2020).

Several researchers have examined the consequences of pesticide toxicity in fish, finding that it has a range of long-term impacts, including oxidative damage, the suppression of ACHE function, and histological alterations (Aktar et al., 2009). Pesticides' biological availability (also known as bioavailability), bioconcentration, biomagnifications, and environmental persistence all have a role in how readily fish and other aquatic species are exposed to them. The quantity of pesticide in the environment that is accessible

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to fish and wildlife is referred to as bioavailability (Sabra and Mehana, 2015)

These harmful compounds can get into the water sources through a number of various channels, including spills, industrial effluent, surface runoff, or soils that have been exposed to pesticides (Picó *et al*., 2020). Depending on the exposure duration, which may be short or lengthy, and exposure type, which can be lethal or sub-lethal, the toxic impacts brought on by exposure to these hazardous chemicals can be divided into several categories. Short-term exposure is defined as lasting less than 96 hours, whereas long-term exposure is defined as lasting longer than 96 hours (Kumar *et al.*, 2021)

Aquatic species are typically exposed to pesticides in one of three ways: (i) through the skin; (ii) through breathing because they breathe through gills. Because aquatic species are in direct contact with water, they quickly absorb pesticides through their skin pores while eating and breathing. Aquatic animals that eat food polluted with pesticides may die as a result of secondary poisoning. For instance, if fish eat pesticide-exposed insects, the fish can die if the insects take in a lot of the toxin (Kumar *et al*., 2021).

The most common and frequently used synthetic insecticide is cypermethrin. Even at very low doses, it has been known to be hazardous to a variety of fish and aquatic invertebrates (Prusty *et al*., 2015). Cypermethrin is the most widely used synthetic insecticides though it is reported that cypermethrin is toxic to many fishes and aquatic invertebrates even at low concentration. Cypermethrin is active compound of many insecticide viz. killer ammo, barricade, kafil super, polytrin, and stockade. The chemical formula of cypermethrin is $C_{22}H_{19}Cl_2NO_3$ (Biswas et al., 2019)

The aim of the current study was to evaluate the effects of pesticides on haematological, biochemical parameters, cortisol, and thyroid hormones in common carp.

2. MATERIALS AND METHODS

2.1 Experimental diets

The experimental diets were prepared according to the NRC (2011) guiding principle on the dietary necessities for carp. An experimental diet was formulated to be isonitrogenous (38%) and isolipidic (8%).

Diet was created to begin with weighting and mixing dry ingredients before homogenizing via a food mixer. Diet was extruded using a cold press extruder (SUNRRY, model: SYMM12, China) with a 2mm aperture die. Pellets were then dried for a period of 24 hours using a dehumidifying oven at 40 °C. Experimental diet formulation and approximate composition are shown in table 1

2.2 Experimental fish

Common carp (*Cyprinus carpio*) juveniles were obtained from the Ankawa hatchery station, Erbil, Kurdistan Region, Iraq. Fish were transported to the closed system in the aquaculture unit at the Salahaddin University- Erbil, Kurdistan Region, Iraq. Fish adapted to the experimental system for two weeks prior to the commencement of the experiment. Fish were fed on a maintenance diet 2% during that time (34 percent protein and 7 percent lipid). A total of 180 healthy common carp *Cypriuns carpio* juveniles (mean weight =15±1.5.) were divided into 12 tanks (15 each).

2.3 Experimental conditions

The experiment was undertaken in indoor system in the aquaculture unit, College of Agriculture Engineering Sciences, Salahaddin University- Erbil, Kurdistan Region-Iraq. The trial was carried out in the experimental closed system. Fish were randomly distributed into 74L^{-1} (each measuring 30×38×65 cm^3) polyethylene tanks, each of these tanks was equipped with one inlet pipe (to form dissolved oxygen in tanks) all pipes connected with low noise air pump (RESUN, Model: LP-60). Fish were exposed to 0ug/L, 75 ug/L, 150 ug/L and 250 ug/L respectively. Four days was the duration of the trial. Fish were fed three times daily at a share level of ~3% of the fish body weight. The level of mortality were closely tracked and reported at 24, 48h, 72h and 96h, respectively, from the beginning of the experiment. The level of dissolved oxygen (6.82 ± 0.19 mg L^{-1}), temperature (24 ± 1.20 °C) and pH (7.02 ± 0.6) being measured daily (TRANS instruments, HD3030 8403,HANNA instruments, HI98129, CE; Made in Romania).

2.4 Haematological and biochemical analysis

The fish were anaesthetised by buffered tricaine methane sulphate (MS222, Phamaq, Norway) at 200 mgL^{-1} at the end of the toxic trail. Blood was collected in the caudal vein via 25-

gauge heparinized needle and 1-ml syringe (Campbell, 2015).

Blood samples were separated into two sec tions, with the first half of each sample put for hae matological examination in 2 heparinized vials.
The other halves of the blood samples were put in clot activator and sun-val and then placed in ice then placed immediately in centrifuge at 3,500 rpm for 15 minutes and the supernatant serum collected and located in labeled in eppendrof tubes stored at -80˚C for biochemical tests and stress hormones. For haematological analysis white blood cells (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRA), mean cellular hemoglobin (MCH), mean cellular hemoglobin concentration (MCHC), red blood cells (RBC), mean cellular volume (MCV), hemoglobin (HGB), hematocrit (Hct), platelet (PLT) and mean platelet volume (MPV) were measured using fully-auto hematology analyzer (MCL-3800 made in China). Biochemical tests such as cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride and alkaline phosphate (ALP) were measured using Cobas c111.

2.5. Acetylcholinesterase Assay:

Acetylcholinesterase (AchE) activity was determined by the method of (Dembele *et al*., 2000). 100 µL supernatant was added to a test tube (1.5 mL) containing 880 µL of phosphate buffer (0.1M, pH 7.5), 10 µL of 100 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), and 10 µL of 0.1M acetylthiocholine chloride. Then, the contents were mixed and the absorbance was read spectrophotometry for 1 min using a StatFax 100 spectrophotometer, USA. Three samples were assayed for each tissue. Enzyme activity was reported as millimoles of product formed per mg protein per minute.

2.6. Stress Hormone determinations (ng/ml):

Serum corticosterone (cortisol) levels were determined by ELISA Kit (MBS2700193) for cortisol. Thyroxin (T4) and triiodothyronin (T3) hormones concentration in plasma was determined using ELISA according to the instructions of the kit included in the Cusabio Technology LLC, according to the manufacturer's instruction.

2.7 Statistical analysis

All data are stated as mean values ± standard error (±SE). Statistical analyses were performed by SPSS statistics version 26 for windows (SPSS Inc., an IBM company, copyright 1989-2019). One-way ANOVA was used to analyze data. To determine where significant differences occurred at the 95% confidence level (associated probability ≤0.05), Duncan's multiple ranging ad-hoc LSD test was applied.

Table 1: Formulation and proximate composition of the experimental diet (dry weight).

Ingredient g kg-[l]	Ratio per 1kg
Soybean[a]	530
Corn [b]	150
Fishmeal[c]	100
Premix	25
Soya oil	50
Wheat flour[d]	100
Wheat bran[e]	13
Vitamin Premix [f]	11
Enzyme	1
Mineral premix [g]	20
Proximate composition (%)	
Moisture (%) Protein (%) Lipid (%) Ash (%)	7.8 38.8 6.7 6.9

[a] Soybean obtained from Kosar local Company and originally sourced in BAF in Turkey and consists of (Dry mater =89%, MEn=2230 kcal/kg, protein=44%, crude lipid= 0.8% crude fiber=7%, total phosphorus 0.65).
[b] Corn: (Dry mater=92%, MEn=1525 kcal/kg, protein=19.2%, crude lipid= 2.1%, crude fiber=14.4%, total phosphorus %0.65)
[c] Fish meal: (Dry mater 90%, protein=65%).
[d] Wheat flour: (Dry mater =87%, MEn=2900 kcal/kg, protein=14.1%, crude lipid= 2.5%, crude fiber=3%, total phosphorus %0.37).
[e] Wheat bran: (Dry mater =89%, MEn=1300 kcal/kg, protein=15.7%, crude lipid= 3% crude fiber=11%, total phosphorus %1.15).
[f] Vitamin Premix sourced in Kosar Company and originally sourced in BAF in Turkey and consists of: Vitamin D3 (300000 IU per kg), Vitamin A (2000000 IU per kg), Vitamin K3 (1600 MG per kg), Vitamin E (40000 MG per kg), Vitamin C (150000 MG per kg), Vitamin B6 (2000 MG per kg), Vitamin B2 (3000 MG per kg), Vitamin B1 (2000 MG per kg), Pantothenic acid B5 (20000 MG per kg), Niacin B3 (8000 MG per kg), Folic acid (800 MG per kg), Cholin (45000 MG per kg), Biotin (2000 MG per kg).
[g] Mineral premix consists of: 1-trace minerals consist of selenium (60 MG per kg), manganese (3000 MG per kg), Cobalt (20 MG per kg), Iodine (200 MG per kg), Zinc (6000 MG per kg), Copper (30000 MG per kg)2- calcium carbonate 41% 3- salt 1g per kg limestone 14g per kg.

3. Results

3.1 Mortality

Figure 1 shows the effect of exposing common carp to different levels of cypermethrine on mortality. The level of mortality was significantly increased by exposing fish to cypermethrin. The highest level of mortality was recorded in groups of fish exposed to 250 ug per L, which was significantly higher than the non-exposed and groups exposed to 75 ug per L.

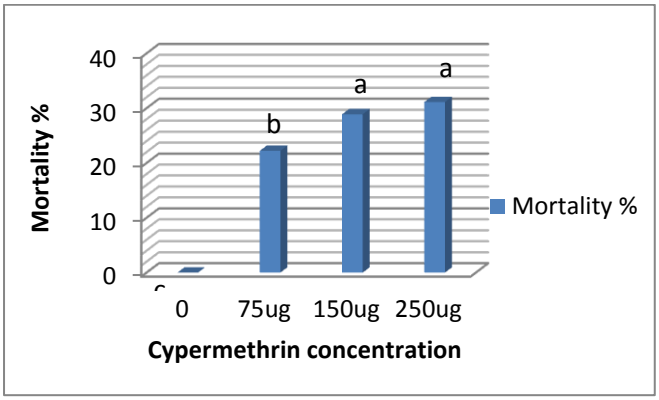

Figure 1: Percentage of mortality of juvenile common carp *Cypriuns carpio* exposed to cypermethrine for 96 hours

3.2 Haematology

Cypermethrin exposure alters the hematology of common carp. The haematological parameters in experimented fish are shown in Table 2. There were no significant ($P \geq 0.05$) variations in the levels of WBC and monocytes among experimental fish. The level of lymphocytes was significantly ($P \leq 0.05$) decreased by exposing fish to cypermethrin and increasing the exposure concentration. The level of granulocytes was generally reduced by exposing fish to cypermethrin, while the trend was only significant by exposing fish to 250 ug per L.

The RBC level was reduced ($P \leq 0.05$) progressively, in response to exposing fish to cypermethrin. The HGB, HCT, and MCV values were significantly ($P \leq 0.05$) declined in response to exposure doses of cypermethrin and decreased with increasing exposure concentration.

Table 2: Haematological parameters juvenile common carp (*Cyprinus carpio*) exposed to cypermethrin (n=3).

Parameters	C	75ug	150ug	250ug
WBC ($\times10^9$ /L)	96.56± 0. 72^a	98.63± 0. 29^a	97.46± 1.28^a	95.83± 1.43^a
LYM# ($\times10^9$ /L)	66.41± 0.67^a	39.32± 1.63^b	32.74± 0.85^c	31.91± 0.35^c
MON# ($\times10^9$ /L)	4.25± 0.1^a	4.23± 0.13^a	4.33± 0.28^a	4.48± 0.17^a
GRA#	19.85± 0.15^a	19.55± 0.26^a	19.18± 0.25^a	17.89± 0.35^b
RBC ($\times10^6$ /L)	2.52± 0.21^a	1.72± 0.16^b	1.56± 0.27^b	1.27± 0.12^b
HGB g\dL	7.42± 0.06^a	6.06± 0.44^b	4.62± 0.2^c	4.57± 0.27^c
MCHC g\dL	23.10± 0.39^b	29.79± 2.77^a	25.82± 1.83^{ab}	25.40± 1.08^{ab}
MCH pg	29.83± 2.45^a	35.89± 4.22^a	32.53± 8.12^a	36.26± 1.42^a
HCT %	32.13± 0.28^a	20.43± 0.42^b	17.97± 0.48^c	17.96± 0.33^c
MCV fL	122.16± 6.00^a	110.83± 3.26^{ab}	101.36± 0.6^c	105.33± 1.45^c

Data are presented as mean ± S.E.
Data in the same row with different superscript are significantly different ($P<0.05$).

3.3 Biochemical

Biochemical parameters in the serum of juvenile common carp (*Cyprinus carpio)* exposed to cypermethrin is presented in Table 3. Fish exposed to the cypermethrin showed a significant ($P \leq 0.05$) decrease in the value of cholesterol in comparison with the unexposed group, and this trend gradually continued with increasing the exposure concentration. The value of TG was significantly ($P \leq 0.05$) increased by exposing fish to 150ug/L and 250 ug/L compared with unexposed fish. The levels of AST, ALT, and ALP gradually and significantly ($P \leq 0.05$) increased with increasing exposure concentration.

Table 3: Biochemical parameters in serum of juvenile common carp (*Cyprinus carpio*) blood exposed to cypermethrin (n=3).

Parameters	C	75ug	150ug	250ug
Cholesterol mg/d	102.36 ±1.57^{a}	97.02± 0.85^{b}	91.02± 1.19^{c}	89.81± 1.06^{c}
TG mg/d	155.63 ±0.9^{c}	157.60± 0.36^{c}	161.90± 0.90^{b}	167.23 ±1.58^{a}
AST U\L	118.82 ±1.51^{d}	128.69± 0.70^{c}	148.69± 0.70^{b}	162.03 ±3.84^{a}
ALT U\L	19.41± 0.57^{d}	21.18± 0.16^{c}	24.93± 0.33^{b}	28.90± 0.7^{a}
ALP U\L	22.96± 0.63^{d}	25.86± 0.16^{c}	28.66± 0.79^{b}	32.50± 0.75^{a}

Data are presented as mean ± S.E.
Data in the same row with different superscript are significantly different (P<0.05).

3.4 Serum hormones

The levels of stress hormones in the serum of common carp exposed to cypermethrin are presented in Table 4. The AChE levels were significantly ($P \leq 0.05$) reduced in response to exposing fish to cypermethrin. The levels of cortisol and T3 were significantly increased by exposing fish to cypermethrin and increasing exposure concentrations. The level of T4 was slightly increased by exposing fish to cypermethrin, but the trend was not significant ($P>0.05$).

Table 4: Serum hormones in common carp exposed to cypermethrin (n=3)

Parameters	C	75ug	150ug	250ug
(AChE) 10^{-3}	3.47± 0.25^{a}	2.50± 0.14^{b}	2.14± 0.01^{bc}	2.00± 0.03^{c}
Cortisol	73.83± 0.70^{d}	81.53± 0.6^{c}	88.53± 0.69^{b}	93.13± 0.52^{a}
T3	0.57± 0.01^{d}	0.65± 0.00^{c}	0.77± 0.01^{b}	0.84± 0.01^{a}
T4	0.92± 0.00^{a}	0.96± 0.01^{a}	1.02± 0.01^{a}	1.36± 0.26^{a}

AChE: acetylcholinesterase (10^{-3}); T3: triiodothyronine (ng mL^{-1}); T4: thyroxine (ng mL^{-1}).
Data were represented as means ± SD. Different letters in each column indicate significant differences ($P < 0.05$).

4. Discussion

The results revealed that cypermethrin is highly toxic to the juvenile common carp. The findings of current research demonstrated that the level of mortality was significantly increased by exposing fish to cypermethrin. This is agreed with Shaluei *et al.* (2012) who found that the level of mortality was significantly increased in Caspian roach (*Rutilus rutilus caspicus*) and silver carp (*Hypophthalmicthys molitrix*) exposed to cypermethrin. According to Ayoola and Ajani's (2008) study on African catfish (Clarias gariepinus), cypermethrin is extremely toxic to juvenile fish because it causes respiratory distress and instant death in exposed fish. These findings varied with the toxicant's concentration and showed increased mortality with increasing concentration.

The results of the current study demonstrated that exposing juvenile common carp (*Cyprinus carpio)* to cypermethrin caused a reduction in the absolute LYM, MON, GRA, RBC, HGB, RBC, MCV, and Hct. Cypermethrin exposure causes severe disfunction in the hematopoietic system (Biswas *et al*., 2019). This is in agreement with (Masud and Singh, 2013) study, which found that the levels of RBC and HGB were significantly reduced by exposing common carp (0.01 µl/L) and (0.05 µl/L) of cypermethrin. The development of hypoxic conditions during treatment is the main cause of the reduction in RBC, which causes an increase in RBC destruction or a decrease in the rate of its formation due to the lack of Hb content in the cellular medium (Chen *et al*., 2004). Nevertheless, (Velisek *et al*., 2011) who observed that cypermethrin exposure in common carp resulted in substantially increased levels of RBC, MCV, MCH, and lymphocyte count compared to controls, disagreed with the findings of the current study. Haematological results indicated decrease in nonspecific immunity.Cypermethrin may have caused changes in hematological parameters as an anemic state because of reduced Hb and RBC generation in hemopoietic organs (Masud and Singh, 2013).

On the contrary, the level of MCHC was significantly higher in exposed fish compared with unexposed fish. This is agreed with (Velisek *et al.*, 2011) study, which found that mean corpuscular haemoglobin concentrations increased significantly in response to exposing common carp to 0.2 and 2 µg/L terbutryn. Also, the level of MCH was increased, but the trend was not significant.

Fish exposed to the cypermethrin showed a significant reduction in cholesterol values in comparison with the unexposed group, and this trend was gradually continued by increasing the exposure concentration. The level of TG was significantly increased by exposing fish to

150ug/L and 250 ug/L compared with unexposed fish. The elevation in the triglycerides may be attributed to enhanced lipid synthesis and/or reduced lipid catabolism (Mohamed et al., 2019).

ALT and AST are categorized among stress enzymes, their rise in the common carp when the salinity concentrations rise is an indicator that the fish were under stress; in addition to the process of the protein breaking down, especially in the liver, that would lead to a rise in both enzymes (Al-Khshali and Al Hilali, 2019). The levels of AST, ALT, and ALP gradually and significantly increased with increasing exposure concentration. According to Loteste *et al*. (2013), exposure to cypemethrin significantly elevated asparate and alanine amino transaferase levels as well as alkaline phosphatase levels in Prochilodus lineatus fish. Furthermore, Kumar *et al*., (2011) found obvious alterations in enzymes of nitrogen metabolism in fish such as AST, ALT, glutamate dehydrogenase and gutamine synthetase in fish *Channa striatus* and *C. batrachus.* These results agree with (Majumder and Kaviraj, 2017) who showed a significant rise in ALT and AST levels as a response to exposing *Oreochromis niloticus* to Cypermethrin. Elevated the activities of hepatic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as recorded in the current research point out active catabolism of amino acids to meet the immediate energy request under cypermethrin stress (Kumar *et al*., 2011). Furthermore, Velisek *et al*. (2006) investigated a significant rise in the level of ALP in response to exposure rainbow trout to cypermethrin. In other study, Sabra and Mehana (2015) recorded a significant increase in the level of ALP and ALT in carp exposed to 4 µg/L simazine.

The main enzyme for hydrolytic metabolism and a crucial indicator of neurotoxicity in fishes is acetylcholinesterase (AChE) (Kumar *et al*., 2017; Sahu *et al*., 2018). Choline acetylase produces acetylcholine in neural tissue, while cholinesterase further degrades it (Singh *et al*., 2019). The synaptic area is region to the enzyme acetylcholinesterase (AChE), which converts acetylcholine into acetic acid and choline to cause the propagation of nerve impulses. Hyperexcitability may occur as a consequence of inhibiting acetylcholinesterase (Majumder and Kaviraj, 2017). This response was noticeable in the current study's experiment on the acute toxicity of cypermethrin. In reaction to exposure to technical grade cypermethrin, Reddy and Philip (1994) found a considerable inhibition of AChE and an increase in the amount of acetylcholine in all tissues, including the gill, brain, liver, and muscle of *Cyprinus carpio.* The AChE levels were significantly decreased in response to exposing fish to cypermethrin. Our findings support those of (Majumder and Kaviraj, 2017) who found a significant decrease in level of AChE in response to exposing *Oreochromis niloticus* to Cypermethrin.

Cortisol is among the most frequently measured indicators of the fish stress response (Baker *et al*., 2013); increased plasma cortisol indicates significant physiological stress, with no signs of acclimation. In the current study, exposed fish had considerably higher serum cortisol levels than the control group.

Thyroid hormones in the gill and liver are involved in the control of osmoregulation, metabolism, and growth performance in fish. However, many chemicals found in the environment may affect the thyroid system (Singh, 2014). Thyroid hormones are involved in many physiological processes, including growth, development, behavior, and stress (Scott, 2007). The significant increase in thyroid hormone levels in juvenile common carp following exposure to Cypermethrin demonstrates the thyroid endocrine disruption potential of the Cypermethrin.

5. Conclusion

Based on the results of this research, cypermethrin is very toxic to juvenile common carp (*Cyprinus carpio*). Short-term exposure to various amounts of cypermethrin can cause alterations in the fish's biochemical and hematological markers. These alterations show that the fish is stressed. It also affects stress hormones. A further study could assess the long-term influences of cypermethrin and use various doses of cypermethrin for different ages of carp and for other economically important fish species.

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Conflict of Interest

The authors state that there is no conflict of interests.

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