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ORIGINAL RESEARCH

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Effects of dietary black cumin (*Nigella sativa* L.) oil on growth performance, hemato-biochemical and histopathology of cypermethrin-intoxicated Nile tilapia (*Oreochromis niloticus*)

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Abstract

This study was conducted to investigate the effects of dietary black cumin (*Nigella sativa* L.) oil on growth performance, hemato-biochemical, and histopathology of cypermethrin-intoxicated Nile tilapia. After determining the LC_{50} (96 h) value of cypermethrin, cypermethrin was added to the water at a ratio of 1:20 of this concentration, and the fish were fed for 42 days. To reduce the effects of cypermethrin, 1% black cumin oil was added to the fish feed as a potential protectant. Growth parameters, hematology, blood biochemistry, and histopathological changes of Nile tilapia were examined after the feeding period. In this study, the best growth was observed in the group fed with feed containing 1% black cumin oil, while the worst growth performance was observed in the group fed with water containing cypermethrin and without black cumin oil in the feed. As a result of the study, it was observed that black cumin oil added to the fish diet reduced the negative effects of water-borne cypermethrin on growth, hematology, blood biochemistry, and histopathological parameters of Nile tilapia.

KEYWORDS
black cumin oil, blood parameters, cypermethrin, growth performance, histopathology, Nile tilapia

1 | INTRODUCTION

The rapid increase in the world population continues to raise the need for quality food, and the demand for agricultural products is increasing daily (Farag et al., 2021). In the process of agricultural development, pesticides became a vital tool for plant protection and for enhancing crop yield (Abhilash & ve Singh, 2009). In addition to their use in agriculture, pesticides have started to be used in urban and industrial activities, which has led to a continuous increase in pesticide accumulation in aquatic ecosystems (Syafrudin et al., 2021). Rivers, lakes, and ponds are constantly exposed to pesticides through runoff as pesticides dissolve, making fish and aquatic organisms susceptible to the toxic effects of these pesticides (Majumder & Kaviraj, 2022). Cypermethrin is one of the most widely used pesticides worldwide (Ullah et al., 2018). Cypermethrin, a synthetic pyrethroid, is a toxic substance that can change from light yellow to dark yellow. It is widely used in many areas, such as agricultural control, flea and lice control in shelters, public health, and ectoparasite control of animals (Issi et al., 2014). Most pyrethroids have a short life span because they are easily metabolized in animals. Fish is an exception among other animals as they lack the enzyme system that hydrolyzes pyrethroids (Korkmaz et al., 2009). Black cumin (*Nigella sativa*) belongs to the Ranunculaceae family and is cultivated in most African and Asian countries and used as a medicinal plant. For 1000 of years, black cumin oil has been used worldwide as a spice, carminative, feed additive, food preservative, and treatment of various diseases (Nada et al., 2015; Nair et al., 2005). In addition, antimicrobial, antioxidant, anticancer, and immunostimulation effects are observed (Salem & Hossain, 2000). Black cumin oil is known to increase growth performance in fish, improve meat quality (Öz, Dikel, & Durmus, 2018), support the immune system (Dorucu et al., 2009; Öz et al., 2017) and extend shelf life after harvest (Öz, 2017; Öz et al., 2015; Öz, Dikel, & Durmus, 2018).

With the increase in consumer awareness in recent years, the demand for aquatic products has increased, and aquaculture activities are gradually increasing to meet this demand. The aquaculture sector is one of the fastest-growing food sectors (Keskin and Erdem, 2005; Öz, 2016; Öz et al., 2021; Öz & Üstüner, 2021). According to FAO data, Nile tilapia (*O. niloticus*) ranks 3rd among the cultured fish species worldwide. While world production of Nile tilapia was nearly 1 million tons/year in 2000, it increased to almost 4.5 tons in 2020 (FAO, 2022a, 2022b).

Nile tilapia is one of the most important farmed fish in many countries throughout the world due to its high growth performance, easy adaptation to commercial diets, and high tolerance to disease and environmental stress (El-sayed, 2020). Nile tilapia is important in production and consumption in the world. According to FAO (2022a, 2022b), it ranks third among inland aquaculture fish species and thirteenth among marine and coastal aquaculture fish species worldwide. Fish is one of the most frequently used experimental models for the general condition of the aquatic ecosystem, toxicology, and pathology studies.

This research investigated the effects of pesticides on Nile tilapia, which has an important place in the aquaculture sector, and the protective effects of black cumin oil, which may be potentially used as a feed additive, and have a protective effect against pesticides.

2 | MATERIALS AND METHODS

2.1 | Fish material and experimental design

This study was conducted within the framework of ethical approval obtained from "Aksaray University Animal Experiments Local Ethics Committee" on January 8, 2021, in accordance with the principles of the Local Ethics Committee. The study used 264 Nile tilapia with a live weight of 10.61 ± 0.87 g as material. The fish used in the study were obtained by sizing, and care was taken to distribute the groups homogeneously. To determine the amount of cypermethrin to be put into the water in the study, the LC_{50} value was first determined by the probit analysis method (Finney, 1971), and the second experiment was planned by using cypermethrin at the rate of 1:20 of the calculated LC_{50} (96 h) value (Acar et al., 2018). To determine the LC_{50} value, 120 fish were used, and six different ratios

(0.00, 4.00, 8.00, 16.00, 32.00, and 64.00 mg/L) of cypermethrin were used. The first part of this research, in which the LC_{50} value was determined, and the second part, which included the feeding part, were carried out in aquariums in the laboratory of experimental animals. When determining the LC_{50} value, the fish were checked thrice daily for 96 h, and the dead fish were quickly removed from the environment. During the LC_{50} experiment, the aquariums (50 L) were continuously aerated, and the water was changed every 24 h from the prepared stocks. In addition, the fish were not fed during the experiment.

The research was carried out in three replicates with 12 fish in 80-L aquariums, and 36 fish were used for each group (G1: Control, G2: 1:00% black cumin oil in the feed, G3: $LC_{50}/20$ cypermethrin in the water, G4: 1.00% black cumin oil in the feed and $LC_{50}/20$ cypermethrin in the water). To keep the water temperature constant in all groups during the research period, an Eheim brand 100 W thermostat heater was used, and the water temperature was kept constant at 25°C. The research groups and fish numbers are shown in detail in Table 1.

2.2 | Preparation of experimental feeds and feeding

The fish in the experiment were fed with tilapia feed produced by a commercial brand (Hem yem, Gaziantep, Turkey) with a crude protein content of 39%, crude fat content of 6.7%, crude cellulose content of 4.30% and crude ash content of 6.79%.

Black cumin oil used in the experiment was obtained from a commercial company and produced by cold press. In the study, only 1% black seed oil was supplemented to G2 and G4 feeds and the rate of black seed oil to be added to the feed was determined according to Öz, Dikel, & Durmus, 2018.

The feeds were prepared in 100 g batches. Black cumin oil was sprayed into the feed and diluted with 2 mL sunflower oil for a homogeneous distribution. In the other two groups, 3 mL of sunflower oil was added to the feed to ensure that the feed contents of the groups were the same.

The research experiment started 1 day after the initial measurement and lasted 42 days. Fish were fed two meals daily at 09:00 in the morning, and 4:00 p.m. Fish were fed 2% of their body weight throughout the experiment.

2.3 | Measurement of fish growth parameters

Initial (IW), intermediate, and final weight (FW) measurements were made individually with a KERR brand precision balance sensitive to 0.1 g. The following calculations were made to evaluate growth performance and feed data in the experiment.

$$\text{Specific Growth Rate (SGR)} = [(\ln \text{FW} - \ln \text{IW})/\text{number of days}] \times 100, \text{(Company et al., 1999)}.$$

$$\text{Live weight gain (LWG, g)} = \text{Final Weight (FW)} - \text{Starting Weight (IW)}.$$

TABLE 1 Research groups, black cumin seed oil ratios, cypermethrin amount, and fish numbers.

Groups	The amount of black cumin oil in the feed (%)	The amount of cypermethrin in the water (mg/L)	Numbers of fish
G1	0.00	0.00	36
G2	1.00	0.00	36
G3	0.00	LC_{50}:20 (0.3193)	36
G4	1.00	LC_{50}:20 (0.3193)	36
Total			144

Daily feed intake (DFI) = Amount of feed consumed/time.

Feed conversion ratio (FCR) = Amount of feed consumed/weight gain (Santinha et al., 1999).

Protein efficiency ratio (PER) = Live weight gain (g)/protein intake (g), (Skalli & Robin, 2004).

2.4 | Blood sampling and analysis

At the end of the feeding trial, fish were anesthetized with 300 ppm of 2-phenoxyethanol, immediately wiped with 70% ethanol, and then blood samples were drawn from the *vena caudalis* using heparinized syringes. A blood sample was allocated into a standard lavender-top blood collection tubes containing anticoagulant (EDTA) for hematological analysis and a standard red-top (SST™ II) advance serum separator tubes for serum biochemical parameters. The latter samples were centrifuged for 10 min at 13,000 × g at 4°C to obtain serum. Hematological parameters were measured immediately while serum biochemical parameters were stored at −80°C until analysis.

White blood cell (WBC) were counted using a counting chamber. Red blood cell (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), hematocrit (Hct), hemoglobin (Hb) were analyzed by a hematology auto analyzer MS4-S (Melet Schloesing Laboratories, Osny, France). Serum alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), glucose (GLU), albumin (ALB), cholesterol (CHOL), creatinine (CRE), total bilirubin (TBL), total protein (TPR), globulin (GLO), uric acid (URA) were measured by a biochemical analyzer (MScan II, Melet Schloesing, Osny, France).

2.5 | Histopathological method

Gill, liver, and brain tissue samples taken from fish were fixed in phosphate-buffered formol solution (0.1 M, pH: 7.4) and blocked in paraffin following washing, dehydration, and polishing with known histological methods. After trimming, the tissues were transferred to cassettes and washed overnight under tap water. After washing, the tissues were kept in 70% alcohol for 1 h, 80% alcohol for 1 h, 90% alcohol for 1 h, 96% alcohol for 1 h each, 100% alcohol for 30 min, xylol for 30 min, xylol-paraffin for 30 min, soft paraffin (46–48°C) for 15 min and hard paraffin (56–58°C) for 30 min in Leica TP 1020 tissue tracking device and then embedded in paraffin blocks. The 6 µm thick sections taken from the paraffin blocks were stained with the Hematoxylin–eosin (Culling et al., 2014) method. The stained sections were passed through a graded alcohol and xylene series and coverslipped with synthetic glue (Entellan, Merck). The preparations were examined with a light microscope with a digital camera (Leica DM-2500), and digital images of the required regions were recorded. The histopathology scoring method was performed according to Capkin et al., 2009 and Gibson-Corley et al., 2013.

2.6 | Statistical analysis

Data from each treatment were subjected to one-way analysis of variance (ANOVA) followed by post hoc Tukey's HSD tests. All statistical analyses were performed using (SPSS 18.0 software Illinois, USA). It is significantly different when $p < 0.05$.

3 | RESULTS

At the end of the study, the effects of black cumin (*N. sativa* L.) oil on growth parameters, blood parameters, and histopathology of Nile tilapia (*O. niloticus*) under cypermethrin exposure were investigated.

3.1 | Determination of LC_{50} value

The 96-h LC_{50} value of cypermethrin for Nile tilapia was calculated as 6.386 mg/L cypermethrin. The LC_{50} value of cypermethrin was calculated as $y = 2.9996x + 2.5853$, $R^2 = 0.9745$ using the regression function found by the probit analysis method (Figure 1).

3.2 | Growth parameters

In the study, the effects of black cumin oil added to Nile tilapia (*O. niloticus*) feed at the rate of 1%, cypermethrin (6386/20 mg/L) in water and the simultaneous presence of both were calculated on the growth parameters of fish. The results are shown in Table 2.

3.3 | Blood parameters

The hematology findings of the study are shown in Table 3. The blood biochemistry of the fish fed within the scope of the research was examined, and the results are shown in Table 4.

3.4 | Histopathologic findings

Light microscopic examination revealed histopathologic lesions in the gills, liver, and brain. No histopathologic lesions were observed in the gills of group 1 (Control). In this study, gills were the most affected organ due to direct exposure to cypermethrin compared to the brain and liver.

The most common lesions in the gills of Nile tilapia in the cypermethrin group were lamellar edema, vascular hyperemia, and inflammatory cell infiltrations. Histopathologic findings are given in Table 5. Histopathologic evaluations of the gills revealed no histopathologic changes in group 1. In group 2, minimal inflammatory cell infiltrations

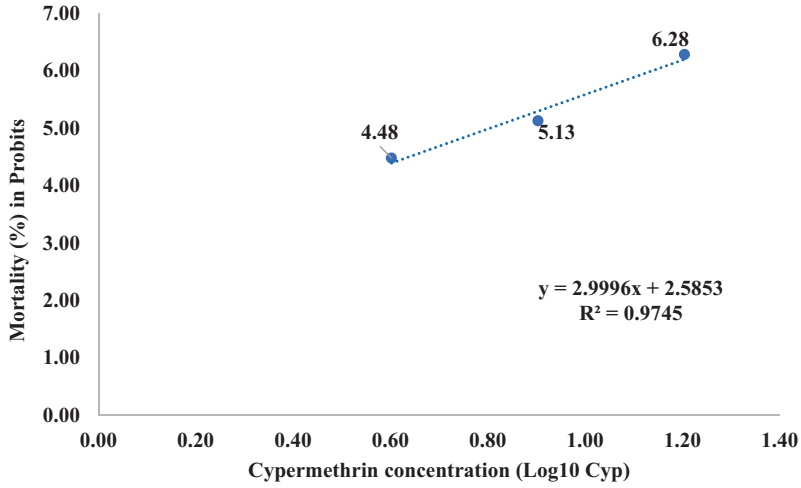

FIGURE 1 Calculation of 96-h LC_{50} values of cypermethrin for Nile tilapia (*Oreochromis niloticus)* and regression function with probit.

TABLE 2 Effects of black cumin (*Nigella sativa* L.) oil on growth parameters of Nile tilapia (*Oreochromis niloticus*) under cypermethrin exposure.

	Control (G1)	G2	G3	G4
IFW	10.61 ± 0.87	10.61 ± 0.87	10.61 ± 0.87	10.61 ± 0.87
FW	14.90 ± 0.05^{c}	18.25 ± 0.05^{a}	13.54 ± 0.31^{d}	16.55 ± 0.06^{b}
LWG	4.29 ± 0.05^{c}	7.64 ± 0.05^{a}	2.93 ± 0.31^{d}	5.94 ± 0.07^{b}
FI	5.83 ± 0.01^{c}	7.85 ± 0.04^{a}	5.54 ± 0.18^{d}	6.76 ± 0.00^{b}
FCR	1.36 ± 0.02^{b}	1.02 ± 0.01^{c}	1.90 ± 0.13^{a}	1.13 ± 0.01^{c}
SGR	0.81 ± 0.01^{c}	1.29 ± 0.01^{a}	0.58 ± 0.05^{d}	1.05 ± 0.01^{b}
PER	1.89 ± 0.02^{c}	2.49 ± 0.02^{a}	1.35 ± 0.09^{d}	2.25 ± 0.03^{b}
CF	1.66 ± 0.12^{b}	1.55 ± 0.07^{c}	1.76 ± 0.03^{a}	1.61 ± 0.01^{cb}
HSI	6.65 ± 0.70^{a}	5.33 ± 0.56^{b}	4.24 ± 0.21^{c}	4.93 ± 0.44^{b}
SUR (%)	100	100	100	100

Note: Statistical difference ($p < 0.05$) is indicated by different letters (a–d) within each row.
Abbreviation: CF, Condition factor; FCR, Feed conversion rates; FI, Feed intake; FW, Final weight; HIS, Hepatosomatic index; IFW, Initial fish weight; LWG, live weight gain; PER, Protein efficiency ratio; SGR, Specific growth rate; SUR, Survival rate.

TABLE 3 Hematology findings of Nile tilapia.

	Control (G1)	G2	G3	G4
WBC (m/mm^3)	3.90 ± 0.09^{b}	2.91 ± 0.06^{a}	4.48 ± 0.20^{d}	3.50 ± 0.07^{c}
RBC (m/mm^3)	2.75 ± 0.017^{c}	3.23 ± 0.10^{a}	1.89 ± 0.09^{d}	2.88 ± 0.05^{b}
MCV (fl)	123.66 ± 1.58^{b}	119.26 ± 4.55^{b}	167.24 ± 15.32^{a}	124.99 ± 1.58^{b}
MCH (pg)	37.39 ± 0.20^{b}	35.15 ± 1.12^{c}	50.17 ± 2.48^{a}	34.56 ± 1.39^{c}
MCHC (g/dL)	30.24 ± 0.26^{b}	29.48 ± 0.38^{b}	34.34 ± 0.75^{a}	27.64 ± 0.94^{c}
Hct (%)	34.00 ± 0.30^{c}	38.50 ± 0.28^{a}	27.66 ± 0.53^{d}	36.05 ± 0.58^{b}
Hb (g/dL)	10.28 ± 0.04^{b}	11.35 ± 0.10^{a}	9.50 ± 0.17^{d}	9.96 ± 0.28^{c}
RDW (%)	11.35 ± 0.05^{b}	9.16 ± 0.15^{c}	12.61 ± 0.45^{a}	11.30 ± 0.07^{b}
MPV (fl)	6.70 ± 0.08^{b}	7.21 ± 0.11^{a}	6.25 ± 0.10^{c}	6.08 ± 0.10^{d}
Pct (%)	0.07 ± 0.00^{c}	0.09 ± 0.00^{b}	0.11 ± 0.01^{a}	0.09 ± 0.00^{b}
PDW (%)	12.80 ± 0.06^{a}	10.63 ± 0.10^{d}	11.83 ± 0.15^{b}	11.05 ± 0.12^{c}

Note: There is a statistical difference between the data shown with different letters (a–d) in the same row ($p < 0.05$).
Abbreviations: Hb, Hemoglobin; Hct, Hematocrit; MCH, Mean corpuscular hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; MPV, Mean Platelet Volume; Pct, Plateletcrit; PDW, Platelet Distribution Width; RBC, Red Blood Cell; RDW, Red Cell Distribution Width; WBC: White Blood Cell.

were observed in the venous sinuses between the primary lamellae. In group 3, edema in the lamellae, hyperemia in the vessels, and inflammatory cell infiltrations were observed more severely and frequently in the gills. When inflammatory cell infiltrations were analyzed, severe lesions were observed in three cases in group 3. In group 4, the gills showed normal histologic structure except for mild inflammatory cell infiltrations in fish fed with cypermethrin + black cumin oil diets. Minimal pathologic changes were observed in group 4 (Figure 2a–d).

The most common lesions in the liver tissues of Nile tilapia in the cypermethrin group were hydropic and vacuolar degeneration and necrotic changes in hepatocytes. Histopathologic findings are given in Table 1. In the histopathologic evaluations of the liver tissue, no histopathologic changes were observed in group 1 and group 2. It was

TABLE 4 Blood serum biochemical values.

	Control (G1)	G2	G3	G4
ALK (U/l)	15.33 ± 1.15^{c}	12.52 ± 0.50^{c}	49.16 ± 1.61^{a}	37.33 ± 1.53^{b}
GOT (U/l)	28.55 ± 0.83^{c}	28.41 ± 0.52^{c}	64.66 ± 0.58^{a}	58.66 ± 0.57^{b}
GPT (U/l)	8,87 ± 0.15^{c}	8.03 ± 0.21^{c}	24.33 ± 0.58^{a}	20.33 ± 1.15^{b}
P (mg/dL)	15.60 ± 0.65^{b}	19.01 ± 0.72^{a}	5.00 ± 0.60^{d}	11.8 ± 0.34^{c}
GLU (mg/dL)	38.00 ± 1.00^{c}	32.86 ± 1.02^{d}	62.33 ± 1.52^{a}	45.67 ± 0.57^{b}
ALB (g/dL)	0.19 ± 0.00^{c}	0.40 ± 0.01^{a}	0.12 ± 0.01^{d}	0.22 ± 0.01^{b}
CHO (mg/dL)	115.34 ± 1.52^{c}	102.33 ± 1.53^{c}	470 ± 25.94^{a}	248.33 ± 1.15^{b}
CRE (mg/dL)	0.33 ± 0.03^{c}	0.23 ± 0.02^{d}	0.82 ± 0.04^{a}	0.44 ± 0.03^{b}
Ca (mg/dL)	8.77 ± 0.40^{a}	10.39 ± 0.40^{a}	6.03 ± 0.32^{b}	6.54 ± 1.67^{b}
TP (g/dL)	6.63 ± 0.21^{b}	7.13 ± 0.06^{a}	4.67 ± 0.21^{d}	5.28 ± 0.03^{c}
GLO (g/dL)	2.86 ± 0.03^{a}	2.83 ± 0.06^{a}	1.72 ± 0.13^{c}	2.47 ± 0.4^{b}

Note: There is a statistical difference between the data shown with different letters in the same row ($p < 0.05$).
Abbreviations: ALB, Albumin; ALK, Alkaline Phosphatase; Ca, Calcium; CHO, Cholesterol; CRE, Creatine; GLO, Globulin; GLU, Glucose; GOT, Glutamic Oxaloacetic Transaminase; GPT, Glutamate Pyruvate Transaminase; P, Phosphorus; TP, Total Protein.

observed that the liver showed normal histologic structure. In addition, exocrine pancreatic acini were observed in the liver of Nil tilapia. In group 3, hydropic and vacuolar degeneration in the liver was severe and frequent. Hydropic and vacuolar degenerations were observed moderately in two cases and severely in 4 in group 3. In addition, hepatocyte necrosis was observed mildly in three cases and moderately in 1 in group 3. In group 4, minimal histopathologic lesions were observed in the gills of fish fed with cypermethrin + black cumin oil diets (Figure 3a–d).

The most common lesions in Nile tilapia brain tissues in the cypermethrin group were hyperemia, intramyelinic edema, and degeneration of neurons. Histopathologic findings are given in Table 5. In histopathologic evaluations of the brain tissue, hyperemia in the brain was mild in one case and moderate in one case in group 1. In groups 2 and 4, it was mild and moderate. However, it was severely present in group 3. Intramyelinic edema was observed in all groups except group 1, the most severe case was seen in three cases in group 3 (Figure 4a–d).

4 | DISCUSSION

Body weight gain is one of the best growth parameters showing whether the feed given to the fish for a certain period is used effectively or not. When the experimental groups with an average initial weight of 10.61 g were examined at the end of the experiment, it was observed that the groups fed with black cumin oil-supplemented feed (G2 and G4) showed a better growth performance than the control group. In addition, the best growth was determined in group 2 fed with 1% black cumin oil-supplemented feed and without cypermethrin in the living environment, and the growth between all groups was found to be statistically different ($p < 0.05$). Similar to the results of this study, Öz, Dikel, and Durmus (2018), Öz, Inanan, and Dikel (2018), reported that black cumin oil increased the growth performance of rainbow trout. Dikel et al. (2010) fed rainbow trout with feed containing L-carnitine and reported better growth performance than the control group. In another study on rainbow trout, boric acid was added to the feed at different rates, and it was reported that the growth parameters of the experimental groups were different (Öz, Inanan, & Dikel, 2018). In this study, it was concluded that cypermethrin in water slowed down the growth of Nile tilapia. The decrease in growth may be attributed to the physiological stress of cypermethrin toxicity, which is caused by the insufficient accumulation of building blocks such as protein and lipids in the fish muscles

TABLE 5 Number and scores of histopathological findings observed in organs.

		Gill			Liver		Brain		
Groups (n:32)	Lesion scores	Edema in the lamellae	Hyperemia	Infiltrates of inflammatory cells	Hydropic and vacuolar degeneration	Necrosis in hepatocytes	Hyperemia	Intramyelinal edema	Degeneration in neurons
G1	-	8	7	8	8	8	7	8	7
	+1	-	1	-	-	-	1	-	1
	+2	-	-	-	-	-	-	-	-
	+3	-	-	-	-	-	-	-	-
	Toplam	-	1	-	-	-	1	-	1
G2	-	8	7	6	8	7	6	7	7
	+1	-	1	1	-	1	1	1	1
	+2	-	-	1	-	-	1	-	-
	+3	-	-	-	-	-	-	-	-
	Toplam	-	1	2	-	1	2	1	1
G3	-	1	3	-	-	4	-	-	5
	+1	4	2	1	2	3	1	2	2
	+2	3	2	4	2	1	2	3	1
	+3	-	1	3	4	-	5	3	-
	Toplam	7	5	8	8	4	8	8	3
G4	-	6	7	5	5	7	5	5	8
	+1	2	1	1	2	1	1	1	-
	+2	-	-	2	1	-	2	1	-
	+3	-	-	-	-	-	-	1	-
	Toplam	2	1	3	3	1	3	3	-

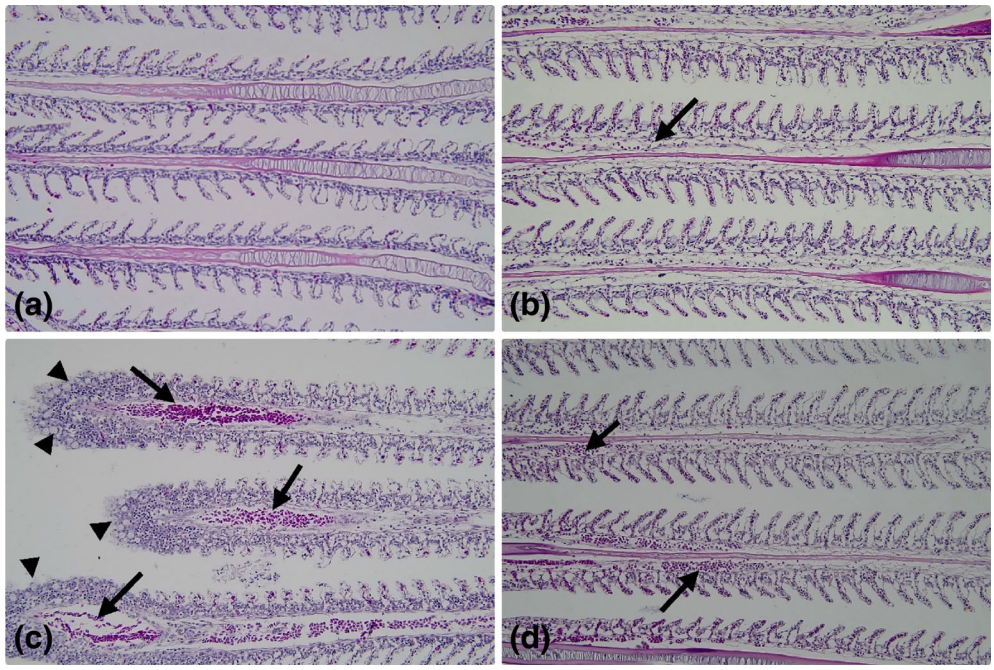

FIGURE 2 (a) Normal histologic appearance of gills, Group 1. Hemotoxylin–Eosin staining (H-E) Bar: 100 µm. (b) +1 Slight changes in the gills. Infiltration of inflammatory cells in the venous sinuses between the primary lamellae of the gill (arrow). Group 2. Hemotoxylin–Eosin staining (H-E) Bar: 100 µm. (c) +3 Severe lesions in the gills. Intense Inflammatory cell infiltrations (arrows) and lamellar edema (arrowhead). Group 3. Hematoxylin–Eosin staining (H-E) Bar: 100 µm. (d) +1 Mild changes in the gills. Infiltration of inflammatory cells in the venous sinuses between the primary lamellae in the gill (arrows). Group 4. Hemotoxylin–Eosin staining (H-E) Bar: 100 µm.

(Majumder & Kaviraj, 2017). The toxic effect of bisphenol-A on Nile tilapia was examined, and similar results were found that it slowed down growth (Abdel-Tawwab & Hamed, 2018; Hamed & Abdel-Tawwab, 2017). Nile tilapia exposed to imidacloprid showed decreased growth performance (Abdel-Tawwab et al., 2021). Hossain et al. (2022), found that chlorpyrifos, one of the organophosphate pesticides, slowed down the growth of Nile tilapia. Abdel-Tawwab and Hamed (2020), similar to this study, found that cypermethrin decreased the growth performance of Nile tilapia. When we look at the results of this research, it is seen that black cumin oil added to fish feed reduces the negative effects of cypermethrin on growth. Black cumin oil contains essential fatty acids such as linoleic acid and oleic acid. Additionally, it contains vitamins A, B1, B6, C, biotin, folic acid, niacin, and minerals such as zinc, magnesium, calcium, selenium, and iron.

In addition to this rich content, pharmacological properties are also quite high in black cumin oil. Its most known properties are antibacterial, antifungal, antiviral, antiprotozoan, antihistamine, antioxidant, non-inflammatory, and immunostimulating properties (Altinterim, 2010; Öz, Dikel, & Durmus, 2018; Öz, Inanan, & Dikel, 2018). The content and pharmacological properties of black cumin oil stimulate growth in fish, reduce resistance to diseases and increase the appetite of the fish. It is also thought that black cumin oil reduces stress due to cypermethrin, increases feed intake by stimulating digestive enzymes, and thus contributes to growth. Abdel-Tawwab and Hamed (2020), reported that *Psidium guajava* reduced the negative effects of cypermethrin. Kiran Kumar et al. (2021), found that *Moringa oleifera* had a protective effect against nitrate intoxication in Nile tilapia. Similar to this study, it has been shown that herbal and chitosan-derived applications will provide a protective effect and survival of fish and protection against pollutants such as heavy metals and carbofuran (Hamed & Abdel-Tawwab, 2021; Hamed & Osman, 2017).

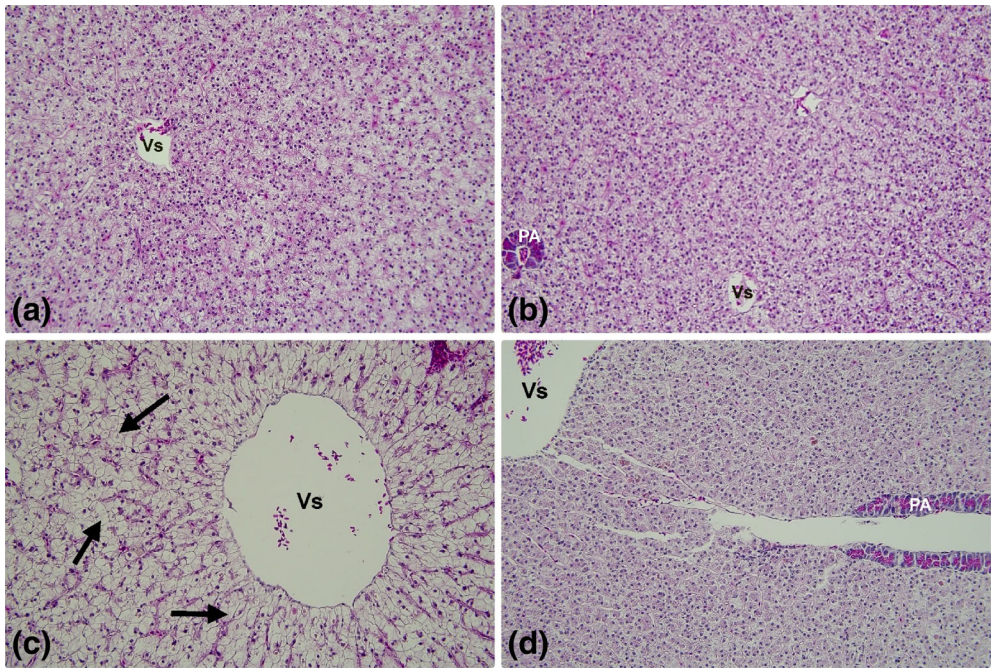

FIGURE 3 (a) Normal histologic appearance of the liver, Group 1. Hemotoxyl–Eosin staining (H-E) Bar: 100 μm. (b) No lesion was seen in the liver, Group 2. Hemotoxylene–Eosin staining (H-E) Bar: 100 μm. Exocrine pancreatic acini (PA) (c) +3 Severe changes in liver, Hydropic, and vacuolar degenerations in hepatocytes (arrows) Group 3. Hemotoxylene–Eosin staining (H-E) Bar: 50 μm. (d) +1 minimal histopathologic changes in the liver, Group 4. Exocrine pancreatic acinus (PA), Vena centralis (Vs), Hemotoxylene–Eosin staining (H-E) Bar: 50 μm.

An excessive increase in the condition factor is considered obese, and a decrease is considered underweight (Timur, 2006). In this research, the condition factor increased in the groups fed with cypermethrin, while it decreased in those fed with a black cumin oil diet. Yılmaz et al. (2019), investigated the effects of rosemary and aloe vera extract on growth performance and feed utilization of Nile tilapia (*O. niloticus*) and concluded that plant extracts decreased the condition factor. In another study, it was reported that the condition factor decreased when green tea by-product and 2% black cumin were used, similar to this study (Cho et al., 2007; Diab et al., 2008).

HSI is an index measured by calculating the ratio between the liver and body weight of fish. Regardless of the size of the fish, enlarged livers can be seen in fish, and one of the most important reasons for this situation is that there is too much fat in the ration, which is stored in the liver (Storebakken & Austreng, 1987; Yılmaz et al., 2019). In this study, the highest HSI index value was calculated in the fish in the control group. Black cumin oil slightly decreased the HSI value due to its positive effects on fish health, but the toxic effect of cypermethrin caused severe damage to the liver and shrunk liver. Öz et al. (2020), added boric acid to rainbow trout feed at different ratios and reported that HSI values of groups with high boric acid feed decreased due to toxication at the end of the feeding period.

In fish, WBCs increase their immunologic functions and numbers in stress factors and diseases (Gonca et al., 2018). The increase in WBC count may be associated with increased antibody production that helps survival and recovery in Nile tilapia exposed to cypermethrin (Joshi et al., 2002). Leukocyte cells are important cells of the immune system and play an important role in the defense mechanism of fish. Therefore, an increased or decreased number of leukocyte cells is a normal reaction to the chemical, as found in other studies. Similar results were reported in *Clarias gariepinus* (Ojutiku et al., 2013), *Cyprinus carpio* (Masud & Singh, 2013), and *Channa orientalis* (Shinde et al., 2014). There are also studies on cypermethrin exposure in *Oncorhynchus mykiss* with WBC reduction

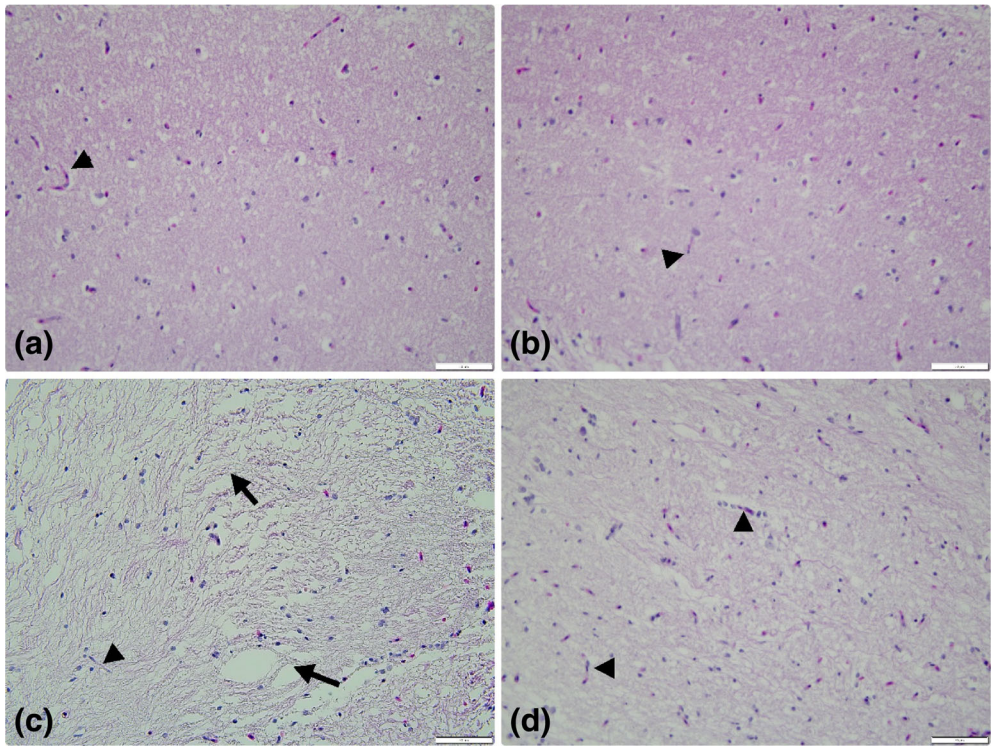

FIGURE 4 (a, b) Group 1,2. +1 mild changes in the brain. Hyperemia in vessels (arrowhead). Hemotoxylin–Eosin staining (H-E) Bar: 100 µm. (c) +3 Severe lesions in the brain, diffusely distributed intramyelinic edema in myelin sheaths of neuronal axons (arrows), hyperemia in vessels (arrowhead). Group 3. Hematoxylin–Eosin staining (H-E) Bar: 100 µm (d) +1 Mild changes in the brain. Hyperemia in vessels (arrowhead). Group 4. Hemotoxylene–Eosin staining (H-E) Bar: 100 µm.

(leukopenia) (Atamanalp et al., 2002). It can be said that black cumin oil decreased the effect of cypermethrin in the G4 group.

Hb, which shows the erythrocyte count, RBC, and Hb value, generally gives information about hypoxia and anoxia. The decreases in RBC and Hb values may be due to the destructive effect of cypermethrin on erythrocyte production in erythropoietic tissues (Chen et al., 2004). The increase in the number of erythrocytes in the G2 group and the increase in RBC count in cypermethrin-exposed fish in the G4 group may indicate that black cumin oil may have a protective effect. The decrease in the amount of RBC may cause a decrease in the amount of Hb. Decreases in RBC and Hb values were reported in *Labeo rohita* (Das & Mukherjee, 2003) and *O. mykiss* (Çakmak & Gorgon, 2003). Htc (%) gives the value of RBCs (erythrocytes) in percentage. Since the number of RBCs decreases will decrease the Htc percentage, Htc decreased in percentage. The mean corpuscular volume (MCV) increase may be due to osmotic stress, RBC swelling due to hypoxia, or macrocytic anemia. This may increase the dissolved oxygen level in the blood (Ramasamy et al., 2009). Adedeji et al. (2009) reported increased MCV after diazinon treatment in African catfish. The higher Hb count per erythrocyte (MCHC) and erythrocyte quantity Hb (MCH) is due to the larger size of RBC with less Hb content (Kumar & Banerjee, 2016). We think that the increase in MCV and MCH levels in this research caused an anemic condition due to the effect of cypermethrin on the gills.

The presence of ALP, GOT, and GPT enzymes in the blood gives information about liver damage (Abhijith et al., 2016). The increase in these enzyme activities is due to leaching from the liver cytosol into the bloodstream due to liver damage by pesticides, which shows its hepatotoxic effect on the liver (Fırat et al., 2011). In the study,

ALP, GOT, and GPT enzymes increased only in the G3 group exposed to cypermethrin. On the other hand, the G4 group supplemented with black cumin oil and cypermethrin decreased compared to the group exposed to purely cypermethrin. It can be concluded that the liver damage caused by cypermethrin was reduced by the protective effect of black cumin oil on the liver. Similar findings to our study were reported in *O. mykiss* (Imani et al., 2015), and *Cyprinus carpio* (Lee et al., 2014). According to Olusola et al. (2023) investigated the effects of thorn apple (*Datura stramonium*) extract on growth, histopathology, and blood profiles in Nile tilapia and reported that the experimental groups showed an increase in TPR and ALB values, and a decrease was observed in ALT and AST values when compared to the control group. An increase in the amount of GLU (blood glucose) in fish indicates stress (Pacheco & Santos, 2001). The increase in GLU in this study may be thought to be due to the breakdown of glycogen to GLU to meet the increased energy demand in fish under stressful conditions of cypermethrin exposure. Glucocorticoids and catecholamine hormones cause hyperglycemia in animals and cause rapid secretion from the adrenal tissues of fish in stressful situations (Pickering, 1981). Similar to this study, increased cortisol and GLU levels were reported in *Prochidolus lineatus* (Martinez et al., 2004), and *O. niloticus* (Monteiro et al., 2005). Thiamine in black cumin oil is effective on growth, development, and cell functions, niacin on blood fat level, and pyridoxine on protein metabolism. Substances such as folic acid and selenium in black cumin are effective in the production of RBCs, and due to their antioxidant properties, they have protective properties against tissue damage. Thus, these substances in black seed oil might have a positive effect by participating in the production of liver hepatocytes blood cells.

Cypermethrin is highly toxic to fish (Bradbury & Coats, 1989). Therefore, it is necessary to investigate the harmful effects of this insecticide on fish and their ecosystems. Histopathological studies on the effects of cypermethrin on fish have been conducted (Khafaga et al., 2020; Korkmaz et al., 2009; Ullah & Zorriehzahra, 2015; Velmurugan et al., 2009). Korkmaz et al. (2009) reported the ameliorative effect of dietary Vitamin C against histopathological lesions caused by cypermethrin in Nile tilapia. In this study, histopathologically examining the brain, gill, and liver tissues of Nile tilapia exposed to cypermethrin showed that the liver and gills were the most affected ones compared to other organs. Ullah and Zorriehzahra (2015) reported that cypermethrin caused histopathological changes such as vacuolation, hemorrhage, fatty infiltration, and hepatic necrosis in fish livers. This study observed that cypermethrin caused hydropic and vacuolar degeneration in the liver and necrotic changes in hepatocytes.

While no significant changes were observed in the gill tissues of the control group fish, cellular infiltration, congestion, swelling at the tip of gill lamellae, and gill damage were reported in fish exposed to cypermethrin for 96 h. It was reported that cypermethrin caused necrosis, hyperplasia of primary epithelial cells, edema, epithelial hypertrophy, epithelial elevation, and fusion of secondary lamellae in the gills of African catfish (*C. gariepinus*) (Velmurugan et al., 2009). Korkmaz et al. (2009) reported that cypermethrin caused obstruction and dilation in the capillaries of primary and secondary lamellae, hyperplasia and fusion of epithelial cells, and epithelial separation and separation and necrosis in secondary lamellae in Nile tilapia. This study observed edema in the lamellae, vessel hyperemia, and gills' inflammatory cell infiltrations. Ullah and Zorriehzahra (2015) reported that cypermethrin affects the brain structure and causes discoloration, neuronal degeneration, and mononuclear infiltration besides the liver and gills. Akhtar et al. (2021) reported that pyknosis, degenerative changes, necrosis, and occlusion of blood vessels were observed in *Cyprinus carpio* brain exposed to different concentrations of cypermethrin. Sayim et al. (2005) reported that some deformation areas were observed in the cytoplasm of neurons due to ischemia and pyknosis in the brain tissues of rats given cypermethrin. It was also reported that cypermethrin induced neurotoxicity, histopathological changes, and activation of apoptotic changes in the brain of *C. catla* (Jindal & Sharma, 2019). In this study, hyperemic vessels were especially noticed. Intramyelinic edema and degenerative changes in neurons are thought to be caused by the toxic effect of cypermethrin.

5 | CONCLUSIONS

This study investigated the protective effect of dietary black cumin oil on Nile tilapia exposed to cypermethrin. Waterborne cypermethrin was toxic to Nile tilapia, decreased growth performance, and negatively affected blood

parameters. Waterborne cypermethrin caused histopathological lesions in the fish's gills, liver, and brain. Black cumin oil added at 1% in the diet significantly improved fish growth, hemato-biochemical parameters, and histopathologic tissues. In conclusion, black cumin oil in the diet minimized the toxic effects of CYP on Nile tilapia. Based on the results of this research, it can be suggested that black cumin oil can be a good feed additive and can be used to reduce toxicity.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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