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Effects of Diazinon on the Survival, Blood Parameters, Gills, and Liver of Grass Carp (*Ctenopharyngodon idella* Valenciennes, 1844; Teleostei: Cyprinidae)

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Abstract: Diazinon (DZN) is a widely used pesticide that can affect the vital organs of non-target aquatic animals—mainly fish. This study evaluated the acute toxicity (LC_{50}) of six DZN concentrations (0.5, 0.75, 1.5, 2, 2.5, and 3 $mg{\cdot}L^{-1}$) and, based on its evaluation after 24 h, 48 h, 72 h, and 96 h, two sublethal concentrations for chronic toxicity testing (0.01 and 0.05 $mg{\cdot}L^{-1}$) for 21 days of exposure to DZN on grass carp fingerlings (*Ctenopharyngodon idella* Valenciennes, 1844). The median lethal concentrations of DZN at 24, 48, 72, and 96 h were 1.83, 1.57, 1.35, and 1.12 $mg{\cdot}L^{-1}$, respectively. Next, histological observations after 96 h LC_{50} showed oedema of the primary lamellae of the gills at low pesticide concentrations (0.5 to 1 $mg{\cdot}L^{-1}$) and extensive necrosis of primary lamellae of the gills at higher concentrations (1.5 to 3 $mg{\cdot}L^{-1}$). Moreover, cytoplasmic vacuolation and extensive necrosis were observed in liver tissue. Increased skin mucus, unbalanced swimming on the water surface, and increased gill opercula movements were noted during chronic exposure. Haematological parameters such as haematocrit, red blood cell count, white blood cell count, haemoglobin, and mean corpuscular volume were significantly reduced after 21 days of exposure to 0.05 $mg{\cdot}L^{-1}$ of DNZ ($p < 0.05$). The present study shows that DZN has various toxic effects on grass carp, which may pose a potential risk for other fish species.

Keywords: Cyprinidae; haematology; histopathology; insecticide; LC_{50}

1. Introduction

Today, residual concentrations of pesticides are ubiquitously found in all environmental compartments worldwide [1–6]. Due to the increasing usage of pesticides, these environmental pollutants directly or indirectly threaten aquatic ecosystems the most [1,7–9]. On the other hand, pesticides are needed for maintaining increasing efficiency of agricultural production due to growing populations [10–13]. The importance of aquatic ecosystems is priceless for humans, organisms, and even for the function of other compartments of nature [1,7–9].

The provinces of Guilan, Mazandaran, and Golestan, with a combined surface area of 1.5 million ha in the north of Iran, are dedicated to cultivating various standard and rain-fed crops [14–17]. According to the FAO database [18], more than 6.8 tons of pesticides were used in agricultural areas in 2019, with 1.7 tons of insecticides in Iran. There is growing global consumption of one of the most frequently used organophosphorus insecticides—diazinon (DZN), which is primarily used in agriculture, households, and recreational areas [19–22]. Generally, the highest concentration of DZN in catchments usually occurs after leaching from agricultural areas, followed by heavy rainfall and runoff into areas where DZN is not used [23]. Moreover, depending on their manufacturer or classification, commercial pesticides usually have a different percentage of the active ingredient. Commercial pesticides usually contain different solvents, which increase their dissolution, half-life, and toxicity compared with initial compositions [4]. The next issue may be the bioaccumulation index, which in the case of DZN varies in different fish species [24]. The most important abnormality recorded due to DZN toxicity is vertical and horizontal deformation of the spine [25]. The effects of DZN on blood indices in grass carp include decreased red blood cell (RBC) counts and drops in haematocrit (Hct) and haemoglobin (Hb) values [26].

All of these reasons together may lead to the problematic detection of source pollution of DZN and its monitoring. Despite these difficulties, Dahmardeh Behrooz et al. [27] detected diazinon in three major rivers of the Caspian Sea at levels of 41 to 145 ng·L^{-1}. Notably, residual pesticide concentrations have more destructive effects on non-target aquatic organisms than on target organisms (pests) in several cases, resulting in higher susceptibility and faster and higher mortality [28–32]. DZN at a concentration of more than 10 mg·L^{-1} in water can cause acute toxic effects on freshwater ecosystems [33]. The toxicity of DZN is based on its ability to block acetylcholinesterase (AChE) activity, which influences the nervous systems of fish and, consequently, disturbs the secretion of the hormones [19,33]. Generally, organophosphorus pesticides in the fish lead to loss of physical strength, stiffness, and deformity of the spine in larvae and juveniles—possibly due to impaired muscle control [34].

Fish populations are one of the essential links in the food network, with humans at the top [19,33]. Considering the linking of trophic levels, pesticides are closely related to fish protein when absorbed through the gills, as the primary target organ, and pose a health risk when contaminated fish is eaten [35]. Therefore, fish may be considered good indicators of contamination, because of their biochemical responses [15,24,35–39]. Pesticides strongly influence fish's vital organs in various ways. Environmental stress caused by xenobiotics usually affects fish and other aquatic organisms through reactive oxygen species mechanisms, leading to tissue damage (e.g., hyperplasia, cytoplasmic vacuolation, and necrosis), as well as changes in behavioural responses (e.g., changes in swimming patterns) and haematological parameters (e.g., haematocrit, red blood cell counts, and haemoglobin) [35,40–46].

The objective of this study was to evaluate the acute and chronic toxicity of the commercial insecticide diazinon (DZN, 60% active agent) to grass carp fingerlings (*Ctenopharyngodon idella*, Valenciennes 1844), which belong to the family Cyprinidae (Teleostei). The euryhaline grass carp is mainly native to East Asia's freshwater rivers and lakes, tolerating a wide temperature range from 7 to 33 °C [47]. It is one of the most highly steamed fish species in the northern part of Iran. Histological changes in gills and livers were observed after an acute toxicity test (96 h LC_{50}). Changes in red blood cell (RBC) and white blood cell (WBC) counts, haemoglobin concentration (Hb), haematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were detected after 21 days of chronic exposure.

2. Materials and Methods

2.1. Ethical Standard

The used procedures for rearing, toxicity, and sampling agreed with protocol 27140 recommended by the ethical committee of the Gorgan University of Agricultural Science and Natural Resources in 2019. Fish were acclimated with methods for experimental tests as suggested for fish, macroinvertebrates, and amphibians [41–43].

2.2. Testing Chemical

The commercial insecticide diazinon, with 60% active agent, was purchased from the company Alborz Behsam Co. in Tehran (Iran).

2.3. Experimental Animals

Fingerling grass carp (*Ctenopharyngodon idella*; N = 217, weight 14.4 ± 1.3 g) were purchased from a local commercial hatchery (Iran). Adaptation for laboratory conditions was maintained in 10 tanks, each with 250 L of tap water. During one week of acclimatisation, juveniles were fed twice per day at a rate of 2% body weight by commercial feed (GC-705, Faradane Co., Shahrekord, Iran) until 24 h before the initiation test [48]. The experimental room was maintained with a 12 h light and 12 h dark photoperiod and a temperature of 23 ± 2 °C. Water physicochemical parameters were measured daily (pH 7.4–7.9, NH3 < 0.02 mg·L^{-1}, BOD 865 ± 45 mg·L^{-1}, $CaCO_3$ 210 mg).

2.4. Acute Toxicity Test (LC_{50})

A preliminary acute toxicity test was conducted according to the procedure described in OECD guideline 203 [49]. In total, 147 grass carp fingerlings were randomly selected and divided into one control group and six treatment groups (21 fingerlings per group). Diazinon (DZN) concentrations were 0, 0.5, 0.75, 1.5, 2, 2.5, and 3 mg·L^{-1} during the acute toxicity test (LC_{50} 96 h). The chemical was used as described by Chorehi et al. [16] in the laboratory facility. Each test was performed in triplicate with four time intervals (24, 48, 72, and 96 h). Experiments were carried out in 21 fiberglass tanks (dehydration volume: 50 L). Fish were not fed during the acute toxicity tests, and water was not replaced. The survival under different DZN concentrations was evaluated and counted over the exposure period, and the dead fish were removed immediately. Median lethal concentrations of DZN were calculated by probit analysis using SPSS software (version 19.0) (Armonk, NY, USA).

Histopathological Observation

Samples were taken from live fish after 96 h. They were dissected from the gills to the anus and the viscera. The gills and liver were fixed in Bouin's fluid for 48 h and kept in a buffer solution for the next 12 h. After dehydration, the organs were submerged in paraffin. Slides at a thickness of 5–7 μm were produced using a microtome. After staining with hematoxylin and eosin (H&E), the slides were examined under a light microscope and photographed [23].

2.5. Chronic Toxicity Test

The chronic toxicity of diazinon (DZN) was determined according to acute toxicity test evaluation on grass carp fingerlings (N = 63). Fish were randomly divided into three groups (21 fish per group). Diazinon concentrations were 0, 0.01, and 0.05 mL·L^{-1}. Each group of tests was performed in triplicate, and sublethal concentrations were tested for 21 days [2]. The nominal concentration of DZN was restored by computing daily water exchange in each tank (dehydration volume: 50 L) and adding the DZN. During the test, fish were fed twice a day at a rate of 2% body weight with commercial feed (GC-705, Faradane Co., Shahrekord, Iran). The photoperiod and water physicochemical parameters responded to the adaptation period.

Haematological Parameters

After 21 days, seven fish were randomly sampled from each treatment and killed quickly with a blow to the head. Blood was taken immediately from the caudal vessels using heparinised syringes and transferred to heparinised 1.5 mL Eppendorf tubes, kept on ice. The whole blood was suspended in the diluent for red and white blood cell levels using a haemocytometer [50]. Haemoglobin concentration (Hb) was measured using a commercial kit (Pars Azmun Co., Tehran, Iran) by photometric assay of cyanmethemoglobin. Furthermore, the chemical solutions were centrifuged before measuring the absorbance. Haematocrit (Hct) was determined by centrifuging whole blood in heparinised micro-haematocrit capillary tubes at 3500× *g* for 10 min (Osterode, Germany). Mean corpuscular volume (MCV), mean red blood cell count (RBC), mean white blood cell count (WBC), and mean haemoglobin concentration (Hb) were calculated as described by Houston [51].

2.6. Data Analysis

Data analyses were performed using SPSS 19.0 software (IBM, SPSS Inc., Chicago, IL, USA). Before conducting statistical analyses, all data were tested for normality (Kolmogorov–Smirnov test). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to assess the significant effects of DZN concentrations on mortality rate. All of the data were expressed as the mean ± standard deviation (SD). Median lethal concentrations of DZN for these fish were calculated by probit analysis. A value of $p < 0.05$ was considered statistically significant for all tests. The levels of tissue damage in the samples were scored as described by Banaee et al. [20] and Yalsuyi et al. [15].

3. Results

3.1. Acute Toxicity Test (LC_{50})

Mortality was not observed during the adaptation period in the control group (without DZN) or in any of the treatments during the LC_{50} 96 h test. Fish's physical symptoms due to DZN's toxicity to grass carp species were clinically observed, including body tremor; haemorrhage in the caudal fin and head; irregular, rapid, and sudden circular swimming; and lethargy and discolouration of the studied fish. Results concerning the effects of DZN on grass carp fingerlings were observed at concentrations of 1.5 and 2 mg·L^{-1}, with 48 and 72 h mortality rates over 50%, while at 2.5 and 3 mg·L^{-1}, almost 100% loss was always observed. There was a significant correlation between DZN concentration and fish mortality rate ($p < 0.01$; Figure 1).

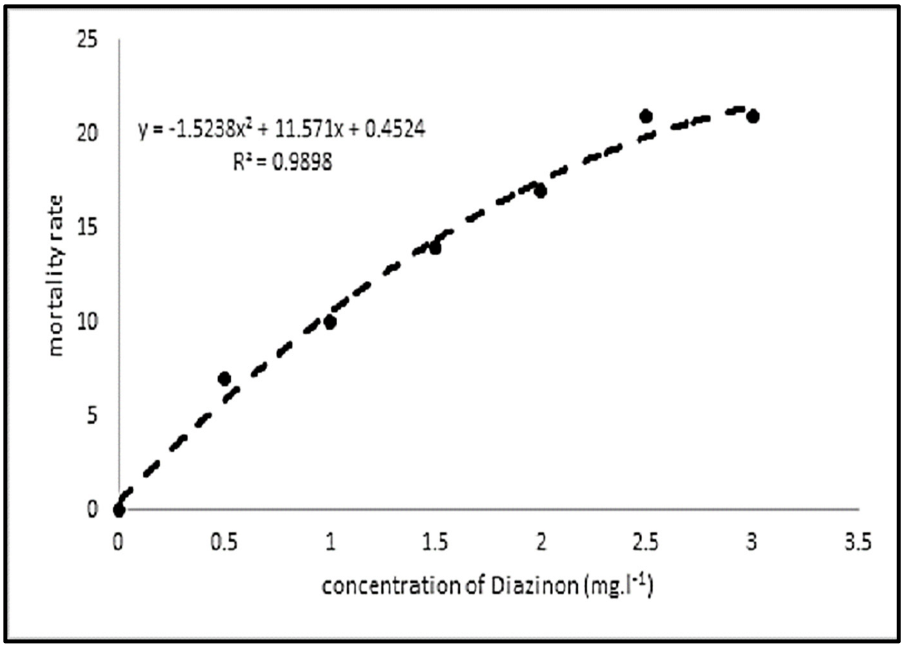

Figure 1. Correlation between mortality rates of grass carp (*Ctenopharyngodon idella*) and diazinon (DZN) concentrations after 96 h.

After 96 h, results showed that nominal DNZ concentrations higher than 0.099 mg·L^{-1} can lead to fish mortality; moreover, the 96 h LC_{50} of DZN was 1.124 mg·L^{-1} (Table 1).

Table 1. Lethal concentration (LC_{50}) of diazinon (DZN) for grass carp fingerlings (*Ctenopharyngodon idella*).

Point	Concentration (mg·L^{-1})			
	24 h	**48 h**	**72 h**	**96 h**
LC_{10}	0.885	0.521	0.347	0.099
LC_{20}	1.211	0.882	0.694	0.451
LC_{30}	1.446	1.142	0.943	0.704
LC_{40}	1.647	1.365	1.157	0.921
LC_{50}	1.835	1.573	1.356	1.124
LC_{60}	2.022	1.780	1.556	1.326
LC_{70}	2.223	2.003	1.769	1.543
LC_{80}	2.458	2.263	2.019	1.796
LC_{90}	2.784	2.624	2.365	2.148
LC_{95}	3.053	2.922	2.651	2.438

Note: all concentrations of DZN were nominal concentrations.

Histopathological Observation

Tissue damage was not observed in the control group samples. There was a significant correlation between tissue damage and concentrations of DZN ($p < 0.01$). Several forms of liver damage—such as cytoplasmic vacuolation of the hepatocytes, necrosis of liver tissue, dilation of sinusoids, and vascular dilation—were observed in all treatments (Table 2). Damage to the liver at different doses of DZN is shown in Figure 2.

Table 2. Liver lesions in premature grass carp (*Ctenopharyngodon idella*) after exposure to six different concentrations of diazinon (DZN).

Liver Tissue Damage	Concentration of DZN (mg·L^{-1})						
	0	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**
Cytoplasmic vacuolation of the hepatocytes (CV)	–	++	++	+++	+++	+++	++++
Necrosis (N)	–	–	++	+++	+++	++++	++++
Dilation of sinusoids (DSS)	–	–	++	+++	++++	++++	++++
Vascular dilation (D)	–	++	+++	+++	++++	++++	+++

Note: (–) no damage could be seen; (++) damage from 1 to 5; (+++) damage from 5 to 10; (++++) damage of more than 10; these scores were determined as described by Banaee et al. [20].

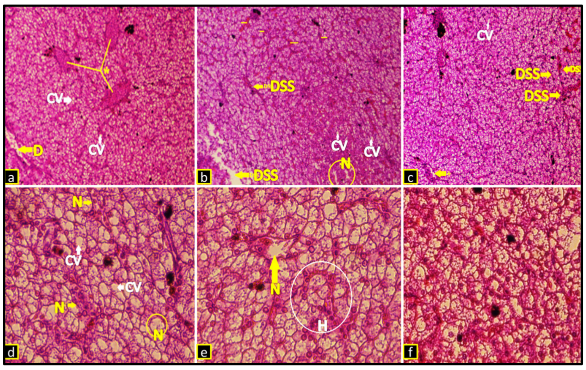

Figure 2. The tissue damage in grass carp (*Ctenopharyngodon Idella*) fingerlings exposed to DZN: (**a**) Liver exposed to 1 mg·L^{-1} after 96 h (H&E, 10×), displaying cytoplasmic vacuolation (CV), vascular dilation (D), and the aggregation of erythrocytes within hepatic blood vessels (*). (**b**) Exposure to 1.5 mg·L^{-1} after 96 h (H&E, 10×), revealing cytoplasmic vacuolation (CV), necrosis (N), congestion of erythrocytes (yellow line), and dilation of sinusoids (DSS). (**c**) Exposure to 1.5 mg·L^{-1} after 96 h (H&E, 10×), indicating cytoplasmic vacuolation (CV), dilation of sinusoids (DSS), and the aggregation of erythrocytes within hepatic blood vessels (*). (**d**) Exposure to 2 mg L^{-1} after 96 h (H&E, 40×), exhibiting cytoplasmic vacuolation (CV) and necrosis (N). (**e**) Exposure to 2.5 mg·L^{-1} after 96 h (H&E, 40×), illustrating necrosis (N) and hyperaemia (H). (**f**) Exposure to 3 mg·L^{-1} after 96 h (H&E, 40×), depicting the extensive necrosis of the liver.

Gill lesions were observed in all treatments. There was a significant correlation between gill lesions and DZN concentration ($p < 0.01$). Lethal concentrations of DZN led to primary lamellar oedema, hyperplasia, and hypertrophy of squamous cells of the secondary lamellae. Secondary lamellar sloughing, haemorrhage, and necrosis of gill cells and tissue were also observed (Figure 3). However, primary lamellar oedema, hyperplasia, and hypertrophy of the gills were more commonly seen than other lesions (Table 3).

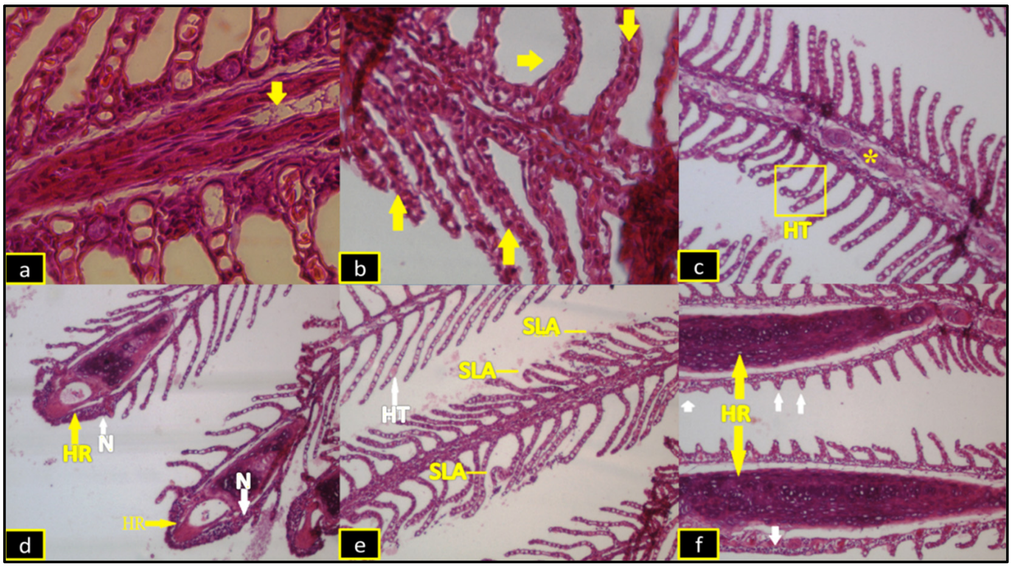

Figure 3. The different tissue damage to the gills of fish exposed to different concentrations of DZN: (**a**) Fish gill lamellae exposed to 1 mg·L^{-1} DZN after 96 h (H&E, 40×), exhibiting primary lamellar oedema (yellow arrow). (**b**) Exposure to 1.5 mg·L^{-1} DZN after 96 h (H&E, 40×), displaying hyperplasia of secondary lamellae (yellow arrow). (**c**) Exposure to 1.5 mg·L^{-1} DZN after 96 h (H&E, 10×), indicating hypertrophy (HT) and primary lamellar oedema (*). (**d**) Exposure to 2 mg·L^{-1} DZN after 96 h (H&E, 10×), revealing haemorrhage (HR) and secondary lamellar shortening (N). (**e**) Exposure to 2.5 mg·L^{-1} DZN after 96 h (H&E, 10×), depicting secondary lamellar sloughing (SLA) and hypertrophy (HT). (**f**) Exposure to 3 mg·L^{-1} DZN after 96 h (H&E, 10×), illustrating necrosis (white arrow) and haemorrhage (HR).

Table 3. Gill histopathology in grass carp (*Ctenopharyngodon idella*) after exposure to different concentrations of diazinon (DZN).

Tissue Damages	**Concentration (mg·L^{-1})**						
	0	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**
Primary lamellar oedema	−	++	++	+++	+++	++++	+++
Hyperplasia of epithelial cells	−	+	++	+++	+++	++++	+++
Hypertrophy of epithelial cells	−	++	++	+++	+++	+++	+++
Secondary lamellar sloughing	−	−	++	+++	+++	+++	+++
Haemorrhage	−	−	++	+++	++	+++	++++
Necrosis	−	−	−	++	+++	+++	++++

(−) No gill tissue damage could be seen; (+) gill tissue damage from 1 to 3; (++) gill tissue damage from 3 to 5; (+++) gill tissue damage from 5 to 9; (++++) gill tissue damage from 9 to 15; these scores were determined as described by Yalsuyi et al. [15].

3.2. Chronic Toxicity Test

Some clinical signs—such as behavioural alterations, increased skin mucus, unbalanced swimming on the water surface, slow swimming near the water surface, increased opercular movements, and alterations in the fish's colour patterns—were observed in fish exposed to sublethal concentrations of DZN.

Haematological Parameters

Haematocrit, haemoglobin, red blood cells, and white blood cells were significantly reduced after 21 days of exposure (Table 4). However, there were no significant differences between blood parameters in the control group and those treated with 0.01 mg·L^{-1} of DZN ($p > 0.05$). Finally, the mortality rate at 0.05 mg·L^{-1} of DNZ was less than 10% of the fish population.

Table 4. Blood parameters of grass carp (*Ctenopharyngodon idella)* after 21 days of exposure to sublethal concentrations of diazinon (DZN).

Blood Parameters	Treatments		
	Control	**0.01 ***	**0.05 ***
Haematocrit (%)	26.73 ± 0.36 ^{a}	26.66 ± 0.39 ^{a}	19.73 ± 0.14 ^{b}
Haemoglobin (g/dL)	8.04 ± 0.31 ^{a}	7.91 ± 0.76 ^{a}	5.25 ± 0.46 ^{b}
Red blood cells (10^6/µL)	1.87 ± 0.07 ^{a}	1.85 ± 0.11 ^{a}	1.34 ± 0.4 ^{b}
White blood cells (10^3/µL)	14,833.33 ± 828 ^{a}	13,837 ± 782 ^{a}	6000 ± 569 ^{b}
Mean corpuscular volume (fL)	149.25 ± 1.23 ^{a}	147.91 ± 1.63 ^{a}	124.17 ± 0.87 ^{b}

* Nominal concentration of diazinon (DZN) mL·L^{-1}. Different letters (^{a},^{b},^{c}) indicate significant differences between values on the same row ($p < 0.05$).

As shown in Table 5, the coefficient of determination did not differ significantly during the experiments. The minimum exposure time was considered to be 24 h. Based on LC_{50}, DZN can be classified as a toxic pesticide.

Table 5. Toxicity ratings of pesticides in living organisms according to the U.S. EPA [52].

Toxicity Rating of Pesticides for Living Organisms	LC_{50} (mg·L^{-1})
Relatively non-toxic	0 > 500
Less toxic	100–500
Moderate toxicity	10–100
Toxic	1–10
Very toxic	0.1–1
Highly toxic	<0.1

4. Discussion

Environmental pollution—especially with industrial wastewater containing various pollutants, toxic metals, and agricultural pesticides—is the most crucial factor in the morbidity and mortality of aquatic organisms—especially fish [53–56]. There are several methods for evaluating the toxicity of pollutants, such as blood assessments, physiological studies, or histopathological studies. However, each of these methods has several limitations. For example, the 96 h LC_{50} test can measure only lethal concentrations of contaminants in laboratory conditions. Hence, using two or three methods together can be a valuable tool for assessing toxicity to organisms. [57]. This assessment was proven by this study on the toxicity of DZN to grass carp (*Ctenopharyngodon idella*) fingerlings. The 96 h LC_{50} test was performed with an evaluation of gill and liver histology, followed by a chronic toxicity test of complementary changes in haematological parameters.

This study's haematological assessments showed that sublethal concentrations of DZN can cause liver and gill lesions, and reduce haematocrit and blood cell counts in fish, as was made evident during the tests. A fish's physiological response to the contaminated environment is reflected in haematological indices, such as haemoglobin, haematocrit, and blood cell counts, inducing alterations in fish's oxygen-carrying capacity via reducing RBC counts and osmotic disturbances via tissue lesions. Significant changes occur in the liver structure due to DZN exposure, including necrosis and hypertrophy, decreasing the fish's Hb, RBCs, WBCs, and Hct values. According to Table 2, a decrease was observed in RBC counts, haemoglobin, and haematocrit levels in the fish exposed to different concentrations of DZN compared to the control group. Fish exposed to pesticides are likely to be overactive so as to get out of a stressful environment, and they need more oxygen to increase their energy requirements [6]. Alternatively, fish may secrete mucus in large quantities to cover the body—especially the gills—to eliminate the toxin's irritating effects. Undoubtedly, this coating reduces gas exchange through the gills. Due to the decrease in supply and elevated oxygen demand, oxygen deficiency is expected to occur in the fish [58]. The reduction in RBC counts depends on the damage to membrane structure and the accumulation of RBCs in the gills, stress resulting from malnutrition, the disintegration of RBCs, and the biological concentration of toxins in the kidneys and liver [51,59]. WBCs play an essential role in the body's immune system. The reduced number of WBCs and, consequently, weakened immune system observed in the present study may reflect leucopoiesis degradation and direct damage to the cell membrane [53,60,61]. Al-Otaibi et al. [62] evaluated the chronic effects of DZN on the blood, liver, and gills of catfish (*Clarias gariepinus*), reporting that increased levels of DZN reduced the number of WBCs, similar to the present study. One of the reasons for the decrease in RBCs is the decrease in haemoglobin levels, and iron is one of the main components of haemoglobin in the blood stored in the liver [58]. One disadvantage of DZN we observed was the reduction in iron content due to the effect of this toxin on the liver, which reduces the levels of haemoglobin in the blood. With the decrease in haemoglobin levels, tissues are exposed to low oxygen levels, leading to decreased metabolism and physical activity [20]. DZN, as an organophosphate, is an acetylcholinesterase inhibitor, causing continuous stimulation of muscle and nerve fibres and, consequently, fatigue and tetanus [63]. This nerve stimulation can induce immune system alterations by affecting the transmission of signals from nerve terminals to cells [64,65].

Fish's increasing or decreasing motility and behavioural patterns mainly interfere with nervous systems and sensory receptors, resulting from the fish's responses to environmental stimuli, such as pesticides [20,66]. In the present study, several clinical signs of behavioural changes were recorded in fish, such as unbalanced swimming on the water surface, slow swimming near the water surface, and anxiety, which can constitute a direct effect of DZN. Al-Asgah et al. [67] linked behavioural changes and blood cell responses. They reported that alterations in these indices reveal toxic stress in the treated animals. Therefore, it is crucial to understand the destructive effects of pesticides on the fish as an essential link in the food chain, because changes in the food chain may lead to an imbalance in the whole aquatic ecosystem [67]. The present study's results were similar to those of Pirbeigi et al. [11] and Kavitha et al. [1].

Insecticides may influence acetylcholinesterase activity, leading to decreased motility in the fish [54,55,59]. DZN has also shown to be metabolised into toxic derivatives in the liver via cytochrome P450 monooxygenase [68].

The effect of certain insecticides on acetylcholinesterase activity may also lead to decreasing motility in the fish. DZN was reported to be metabolised into toxic derivatives in the liver by cytochrome P450 monooxygenases, hydrolysed in microsomes, and then excreted from the body. In a study on the effects of DZN on the Persian sturgeon, (*Acipenser persicus*), the 96 h LC_{50} was reported as 4.38 $mg \cdot L^{-1}$ [69]. In the case of *A. nudiventris* and *Rutilus kutum*, it was 3.6 and 1.9 $mg \cdot L^{-1}$, respectively [70]. Measuring haematological indices is a good tool for evaluating the effects of pollutants on fish. Al-Asgah et al. [67] reported alterations in several haematological indices as indicators of exposure to pollutants. In the present study, the liver was exposed to 1, 1.5, 2, 2.5, and 3 $mg \cdot L^{-1}$ for 96 h. When elevating the DZN concentration, the damage to the liver increased. These lesions started through the aggregation of erythrocytes within hepatic blood vessels at 1 $mg \cdot L^{-1}$, and continued to extensive necrosis at 3 $mg \cdot L^{-1}$. In this study, the gills were also exposed to 1, 1.5, 2, 2.5, and 3 $mg \cdot L^{-1}$ for 96 h. The lesions started with hyperaemia at 1 $mg \cdot L^{-1}$, and continued to extensive necrosis of gill cells and haemorrhage at 3 $mg \cdot L^{-1}$ (Table 3).

Hedayati et al. [47] reported that deltamethrin causes more serious gill damage than DZN during chronic toxicity tests on the iridescent shark (*Pangasius hypophthalmus*). Other studies have reported changes in the morphology of the gills when exposed to toxins [21,71].

Gill damage leads to impaired gas exchange capacity and respiratory distress. Similar results concerning gill damage in fish after exposure to some toxins were reported by Omitoyin et al. [72], Rahman et al. [73], and Ezemony and Ogbomida [74]. Svoboda et al. [75] reported that DZN significantly affects blood indices in grass carp by reducing the number of red blood cells, as well as levels of haemoglobin and haematocrit. They suggested that these alterations after exposure to DZN could be due to the destruction of haematopoietic tissues in the fish's kidneys. Although haematopoietic organs were not examined in the present study, the results related to blood parameters were similar to the findings of their study. These results reinforce the possibility that diazinon may have a toxic effect on hematopoietic tissues.

5. Conclusions

This study showed that diazinon (DZN) is classified as poisonous to grass carp (*C. idella*) and, even at low concentrations, can alter their haematological indices and histopathology. Histopathological parameters are sensitive and significant indicators for the evaluation of the effects of DZN on fish. Moreover, tissues such as the gills and liver can be used to monitor the effects of these toxins in fish over a short time. Further investigations are needed to better understand the harmful effects of DZN—especially with respect to the recovery of blood and other tissues over time. In general, the findings of this study provide information about the white blood cell counts and red blood cell counts in fish, and the effects of DZN on these indices, as well as on liver and gill tissues.

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