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Research article

Ecotoxicological effects of carbendazim on the freshwater food fish *Channa striata*: An approach to safeguard fish and its consumers' health

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Abstract

Carbendazim, a systemic benzimidazole fungicide widely used in agriculture, poses environmental risks due to its persistence and toxicity to non-target aquatic organisms. This study examines the impact of carbendazim on hematological, cytotoxic, genotoxic, and histopathological parameters in *Channa striata*, a commercially important food fish species. It hypothesizes that carbendazim at concentrations above the permissible limit (1.0 µg/L) affects fish health. Fish were exposed to carbendazim at concentrations of 1.05 µg/L (Group II) and 1.10 µg/L (Group III), representing 5% and 10% above the permissible limit, respectively, for 96 hours. A control group (Group I) was maintained without exposure. Hematological analysis showed a dose-dependent reduction in red blood cell count, hemoglobin level, and packed cell volume percentage, along with an increase in white blood cell count and poikilocytosis. The micronucleus assay revealed a significant increase in micronuclei frequency, indicating genotoxicity. Histopathological examination showed damage to the liver, kidney, and muscle tissues. The observed behavioral changes included hyperactivity, gulping, abnormal skin pigmentation, and mucus secretion. These findings indicate that exposure to carbendazim above permissible levels induces hematological disorders, cytotoxic damage, genetic damage, and tissue degeneration in Channa striata, posing ecological risks. The study emphasizes the necessity for stricter regulations on the use of carbendazim to protect aquatic ecosystems and the consumers who rely on fish.

Keywords: *Channa striata*, Carbendazim, Hematology, Genotoxicity, Histopathology, Poikilocytosis

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Introduction

Environmental pollution, an unavoidable consequence of modernization, poses a significant global challenge. Among the most urgent concerns is the contamination of water resources, driven by various pollutants, including pesticide residues (Yamuna et al., 2022). Pesticides, widely used to enhance agricultural productivity and food quality, significantly contribute to environmental pollution. While these chemicals play a crucial role in modern farming, their extensive use leads to their accumulation in ecosystems, particularly in water bodies, posing serious risks to food safety, human health, and aquatic biodiversity. Carbendazim, a systemic benzimidazole carbamate fungicide, is widely used for preventing and treating fungal infections caused by *Ascomycetes, Basidiomycetes*, and *Deuteromycetes* (Nazir et al., 2024) in crops like wheat, rice, citrus, bananas, grapes, and soybean according to Central Insecticides Board Registration Committee (CIBRC, 2021). However, beyond its intended application, carbendazim has been reported to exert toxic effects on non-

target organisms (Abdel-Rahman et al., 2022; Hassanen et al., 2022; Salem et al., 2021). Carbendazim exerts its toxicity by targeting β-tubulin in cells, disrupting microtubule polymerization, and interfering with spindle formation during mitosis, which ultimately leads to impaired nuclear division and cell cycle arrest (Merel et al., 2018). Although effective against fungal pathogens, this mechanism also leads to harmful effects on aquatic organisms.

Despite its widespread use, research on the toxicological impact of carbendazim on fish remains scarce. Carbendazim exhibits prolonged environmental persistence due to the stability of its benzimidazole ring and slow degradation rate, leading to its accumulation in aquatic ecosystems (Yamuna et al., 2022). Recognizing its potential risks, the European Commission's Health and Food Safety Directorate-General and its Standing Committee on Pesticides enforced a ban on carbendazim (Götte et al., 2020). Nevertheless, its use continues in several regions worldwide. It has been detected in the Yangtze River in China (Liu et al., 2015), in northern Vietnam's drinking and surface water (Wan et al., 2021), and even in rainfall-runoff in South China (Liu et al., 2018).

The maximum permissible concentration of carbendazim in freshwater is 1.0 µg/L according to the United Kingdom Technical Advisory Group on the Water Framework Directive (WFD-UKTAG, 2009). However, its extensive agricultural use increases the risk of exceeding this threshold through pathways such as agricultural runoff, leaching, drainage, and atmospheric deposition, leading to contamination of aquatic ecosystems. As fish are sensitive to waterborne pollutants, they serve as key bioindicators of environmental health. *Channa striata* (*C. striata*), a commercially valuable species with high nutritional demand in India, was chosen as the model organism for this study (Phoonaploy et al., 2019). This study hypothesizes that exposure to carbendazim above permissible limits induces hematological alterations, cytotoxicity, genotoxicity, and histopathological damage in *C. striata*, posing significant ecological and physiological risks.

Fish is a highly nutritious protein source, often termed a "rich food for poor people" due to its high biological value, proteins, and beneficial fats (Sujatha et al., 2013). Fish contains 15-20% protein relative to its total live weight and supplies essential amino acids that enhance diet quality (Balami et al., 2019). Additionally, fish is rich in easily absorbable micronutrients and omega-3 fatty acids, contributing to disease prevention and overall health. However, if fish become contaminated with carbendazim, their nutritional value is compromised, posing potential health risks to humans as direct consumers.

Pesticides have been shown to interact with erythrocytes, inhibiting delta-aminolevulinic acid dehydrogenase, altering plasma membrane integrity, and leading to poikilocytosis and alteration in red blood cell (RBC) count (Sharma and Langer, 2014). These alterations can further alter white blood cells (WBC). Genotoxicity assessment, an essential tool in ecotoxicology, evaluates a substance's propensity to damage genetic material. The micronucleus (MN) assay is a well-established genotoxic marker for identifying DNA damage induced by xenobiotics (Jain et al., 2024; Kumar et al., 2022). Histological alterations serve as reliable biomarkers for evaluating tissue morphology changes in fish, which may compromise organ structure and function under toxicant exposure (Badroo et al., 2020). This study aimed to assess the impact of fungicide carbendazim on *C. striata* when present above the permissible limit, providing insights into its potential effects on fish health and the associated risks to human consumers.

Materials and methods

Ethical approval

Experimentation was permitted by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Ministry of Environment and Forests, Government of India, under registration no.1861/GO/Re/S/16/ CCSEA, and approved by the institutional ethical committee of the University of Lucknow, Lucknow, India. All methods were performed according to the relevant ARRIVE guidelines and regulations.

Test chemical

Carbendazim 50% WP (Wettable powder) manufactured by Crystal Crop Protection Ltd., Factory Unit Jammu-181133 (J&K), was procured from a local dealer in Lucknow.

Animal model and acclimatization

C. striata (122±2.0 g, 21±3.0 cm) were procured from a local hatchery in Lucknow and immediately transported to the laboratory. To eliminate external contaminants, fish were subjected to a 0.05% $KMnO_4$ treatment for two minutes, followed by rinses with fresh water. Fish were acclimatized for 10 days in 40 L of aged tap water within a 100 L aquarium, maintained under standardized physicochemical conditions: hardness 77.38±2.2 mg/L as $CaCO_3$, temperature 23.1±2.6°C, dissolved oxygen 6.3±0.9 mg/L, and pH 7.2±0.6 (Baird et al., 2017). During this period, fish were fed with commercial pellets (Perfect Companion Group Company Limited, Bangkok, Thailand) twice daily. A fasting period of 24h was implemented before the commencement of the experiment (OECD, 2019).

Experimental set-up

After acclimatization, 45 fish were randomly assigned to three groups (15 fish each), maintained in triplicate. The control group (T1) was unexposed, while T2 and T3 were subjected to carbendazim concentrations exceeding permissible limits by 5% (1.05 µg/L) and 10% (1.10 µg/L), respectively, for a 96h exposure period. No mortality was observed during the experiment. Water quality parameters were monitored at the start of the experiment and after the sampling (Table 1). After 96h, three fish per replicate were euthanized with Tricaine Methanesulfonate (MS-222, Sigma-Aldrich, USA) (Jain et al., 2025) for blood and tissue sampling. Blood was collected in EDTA-coated vials for poikilocytosis, MN, and hematological analysis. The whole fish, liver, and kidney were weighed to calculate the condition factor (CF), hepato-somatic index (HSI), and reno-somatic index (RSI). Tissue samples were collected for histopathology, and behavioral changes were monitored.

Behavioral study

Behavioral changes, including hyperactivity, gulping, mucus secretion, and abnormal skin pigmentation, were monitored during the exposure period.

Hematological analysis

RBC and WBC counts were performed using a Neubauer haemocytometer following the procedure described by Shah and Altindag (2004). For RBC estimation, blood samples were diluted at a ratio of 1:200 using Hayem's fluid (Qualigen, Mumbai, India) (Mishra et al., 1977), while a 1:20 dilution with Turk's fluid (Qualigen, Mumbai, India)was used for WBC counts. The prepared samples were loaded onto the haemocytometer, and cells were counted manually. Results were expressed as $10^6/mm^3$ for RBCs and $10^3/mm^3$ for WBCs, as per the method of Wintrobe (1967).

Hemoglobin concentration (Hb%) was determined using the Sahli method and reported in g/dL (Godkar and Godkar, 2003). Packed cell volume (PCV) was determined using the microhematocrit method and expressed as a percentage (Dacie and Lewis, 2001). Genotoxicity (MN assay) and cytotoxicity (poikilocytosis). The MN assay was conducted to evaluate genotoxicity in fish following the protocol of Trivedi et al. (2022). Blood smears were prepared on clean slides, air-dried, fixed with methanol, and stained using May-Grünwald's (Himedia, India) and 5% Giemsa stain (Sigma-Aldrich, USA). The slides were mounted in DPX and examined under a microscope (Nikon ECLIPSE Ci) at 100× magnification. MN and erythrocyte morphological abnormalities (Poikilocytosis) were assessed by analyzing 2000 erythrocytes per slide, and their frequencies were calculated by the formula as follows: MN frequency = (Number of cells containing micronucleus/Total number of cells counted) ×100. Poikilocytosis frequency=(Number of cells with altered morphology/Total number of cells counted)×100.

Biometric assay

The length (cm) and weight (g) of the fish were measured to assess the general health condition using Fulton's condition factor (Fulton et al., 1902), calculated by the following equation:

$CF=(W/L^3) \times 100$, W=weight of the fish in grams, L=length of the fish in cm. Hepatosomatic and reno-somatic indices were determined using the following formula (Maddock and Burton, 1999): Organosomatic index = Weight of organ (g)/ Total body weight (g)×100.

Histopathological analysis

The dissected tissues were fixed in 9% formaldehyde, dehydrated through a graded alcohol series, and embedded in paraffin wax. Thin sections (3 µm) were prepared using a rotary microtome (YSI062 Yorco Precision Rotary Microtome, India). Then, the sections

were stained with hematoxylin and eosin (HiMedia, Mumbai, India) and mounted with Distyrene Plasticizer Xylene (DPX, Qualigen, Mumbai, India) (Jia et al., 2019). Following drying, slides were examined under a Nikon ECLIPSE Ci microscope (Nikon Instruments Inc., Tokyo, Japan).

Statistical analysis

All values (mean±standard error of the mean) were analyzed at a significance level of $p<0.05$ using one-way ANOVA followed by Tukey's post hoc test. Data was analyzed using Statistical Package for the Social Sciences (SPSS) software (version 20.0, Chicago, IL, USA). Additionally, Pearson correlation analysis was performed to assess the strength of relationships among the tested parameters related to carbendazim toxicity.

Results

Physico-chemical parameters

The physicochemical parameters of the test medium were measured before and after the 96hexposure period in both control and treated groups (Table 1). The pH values recorded ranged from 7.04 ± 0.13 to 7.20 ± 0.08. The temperature values ranged from 23.86 ± 0.38°C to 25.08 ± 0.13°C. Dissolved oxygen concentrations were between 6.48 ± 0.21 mg/L and 7.39 ± 0.32 mg/L. Water hardness varied from 72.31 ± 0.27 mg/L to 79.24 ± 0.12 mg/L, and alkalinity values ranged from 71.15 ± 0.10 mg/L to 78.13 ± 1.12 mg/L.

Behavioral Study

The observed behavioral changes,including activity levels, gulping, mucus secretion, and abnormal skin pigmentation, are shown in Table 2.

Hematological analysis

In fish exposed to carbendazim, a significant ($p<0.05$) dose-dependent decrease in RBC count, hemoglobin (Hb), and packed cell volume (PCV) was observed, with the lowest values recorded in T3, followed by T2, compared to the control group (T1). In contrast, the WBC count increased significantly ($p<0.05$), with the highest levels in T3, followed by T2, compared to T1 (Table 3).

Genotoxicity (MN-assay)

A significant dose-dependent increase ($p<0.05$) in MN frequency was observed in the erythrocytes of carbendazim-exposed fish, with T3 exhibiting the highest frequency (1.723±0.007), followed by T2 (1.243±0.009), in comparison to the untreated group T1 (0.414±0.0032) (Figure 1).

Table1: Descriptive statistics of physicochemical parameters of the test medium.

Physicochemical Parameters	Group	Exposure period in hours	
		0h	96h
pH	T1	7.04 ± 0.13	7.18 ± 0.15
	T2	7.11 ± 0.09	7.15 ± 0.23
	T3	7.07 ± 0.16	7.20 ± 0.08
Temperature (°C)	T1	23.86 ± 0.38	24.49 ± 0.35
	T2	24.03 ± 0.66	24.83 ± 0.18
	T3	24.26 ± 0.25	25.08 ± 0.13
Dissolved Oxygen (mg/L)	T1	6.48 ± 0.21	7.09 ± 0.17
	T2	7.12 ± 0.13	6.99 ± 0.28
	T3	6.87 ± 0.11	7.39 ± 0.32
Hardness (mg/L)	T1	78.18 ± 0.15	72.31 ± 0.27
	T2	74.15 ± 0.21	77.79 ± 0.19
	T3	76.27± 0.23	79.24 ± 0.12
Alkalinity (mg/L)	T1	77.56 ± 0.23	74.11 ± 0.29
	T2	71.15 ± 0.10	78.13 ± 1.12
	T3	73.94 ± 0.28	72.37 ± 0.16

T1 is the control group, T2 and T3 were subjected to carbendazim concentrations exceeding permissible limits by 5% (1.05 µg/L) and 10% (1.10 µg/L), respectively, for a 96h exposure period.

Table 2: Behavioral abnormalities of *C. striata* due to the exposure to carbendazim within the 96h exposure period.

Group	Hyperactivity	Gulping	Mucus secretion	Abnormal skin pigmentation
T1	-	-	-	-
T2	-	+	+	+
T3	++	+	++	++

'-' indicates no behavioral abnormality; '+' = Mild; '++' = Moderate. T1 is the control group, T2 and T3 were subjected to carbendazim concentrations exceeding permissible limits by 5% (1.05 µg/L) and 10% (1.10 µg/L), respectively, for a 96h exposure period.

Table 3: Hematological parameters of *C. striata* post 96h exposure period. Data are presented as mean ± standard error of the mean. Statistically significant differences ($p < 0.05$) are indicated by different superscript letters within the same row.

Parameters	T1	T2	T3
RBC (10^6 mm^{-3})	2.117 ± 0.233^a	1.8167 ± 0.035^b	1.610 ± 0.0062^c
WBC ($10^3 mm^{-3}$)	26.716 ± 0.516^c	30.231 ± 0.142^b	33.813 ± 0.160^a
HB (g/dL)	7.82 ± 0.032^a	6.920 ± 0.674^b	6.231 ± 0.030^c
PCV (%)	36.173 ± 0.040^a	32.160 ± 0.015^b	29.490 ± 0.317^c

T1 is the control group, T2 and T3 were subjected to carbendazim concentrations exceeding permissible limits by 5% (1.05 µg/L) and 10% (1.10 µg/L), respectively, for a 96h exposure period.

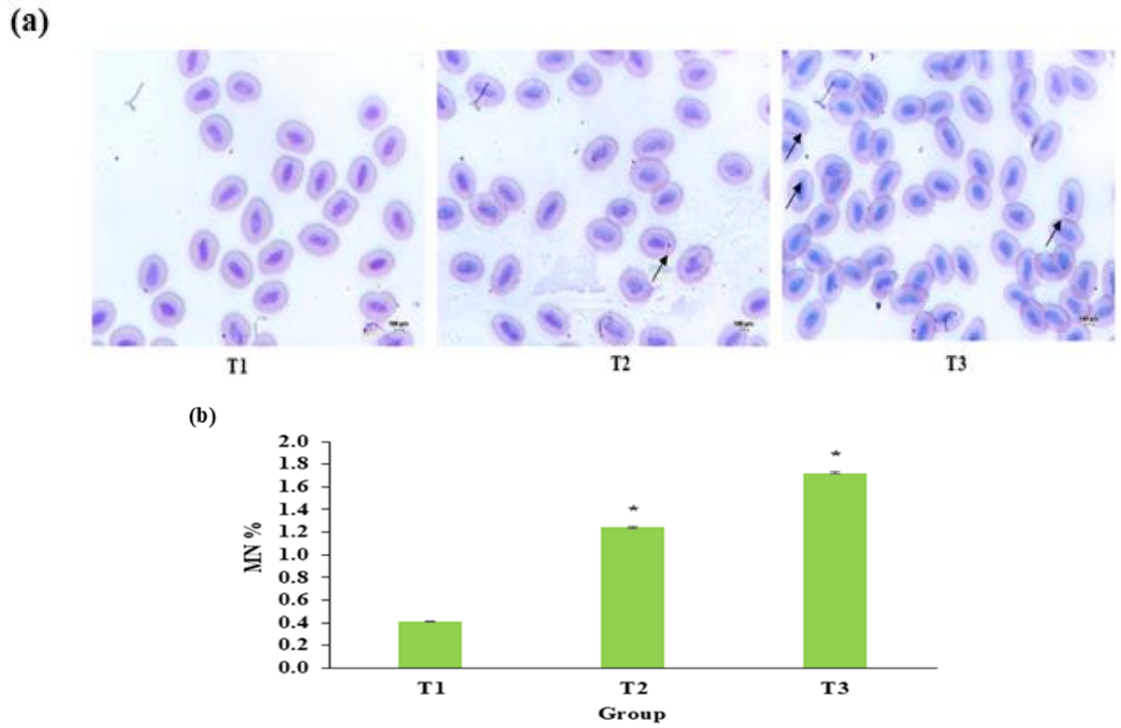

Figure 1: (a) Microphotograph showing micronuclei (MN) in *C. striata* erythrocytes at 100X magnification.Scale bar – 100 µm (b) MN frequency induced by carbendazim in groups [T2=5% above the permissible limit (1.05µg/L) and T3=10 % above the permissible limit (1.10µg/L) as compared to the control group (T1) for 96h of exposure periods] (mean±SD., n=3 fishes of three replicates of each group). (* represents the significant ($p<0.05$) difference from the control).

Cytotoxicity (poikilocytosis)

Carbendazim exposure induced a dose-dependent increase in the percentage of poikilocytotic RBCs in *C. striata*, with significantly higher ($p<0.05$) values observed in T2 (1.05 µg/L) and T3 (1.10µg/L) compared to the control (T1) (Table 4). The erythrocytes in the blood smears of the control fish had an ellipsoidal form with a centrally placed ellipsoidal nucleus. The blood smear of fish treated with carbendazim (T2 and T3) revealed poikilocytosis, including teardrop (TD), keratocyte (K), boat-shaped (BS), elliptocyte (E), and vacuolated cytoplasm (VC) (Figure 2).

Biometric assay

A progressive decline in CF values was observed with increasing concentrations of carbendazim when compared to the control. The HSI and RSI for the control group of fish were recorded as 1.122 ± 0.015 and 0.869 ± 0.011, respectively (Table 5). After 96h of carbendazim exposure, the HSI of *C. striata* significantly decreased ($p<0.05$) by 39.96 % (T2) and 47.14 % (T3), respectively, when compared to the control group (Table 5). The RSI decreased significantly ($p<0.05$) by 28.07% (T2) and 43.84% (T3) in comparison to the control group (T1).

Histopathological analysis

Histopathological analysis revealed significant alterations in the liver, kidney, and muscle tissues of carbendazim-exposed fish compared to

the control group (T1). Liver sections from T2 exhibited cytoplasmic degeneration, pyknotic nuclei, and vacuolization, while T3 showed cytoplasmic disintegration, necrosis, and vacuolization, indicating hepatocellular damage (Figure 3). Renal histology demonstrated hypertrophy and reduced tubular lumen in T2, whereas T3 presented necrosis, hypertrophy, and further tubule cavity reduction, suggesting compromised renal function (Figure 4). Muscle tissue in T2 displayed degenerative changes, including muscle fiber splitting, whereas T3 exhibited fiber breakage, degeneration, and fragmentation (Figure 5).

Correlation analysis

The correlation matrix illustrates a close relationship among the evaluated parameters in carbendazim-exposed fish *C. striata* after 96h (Table 6).

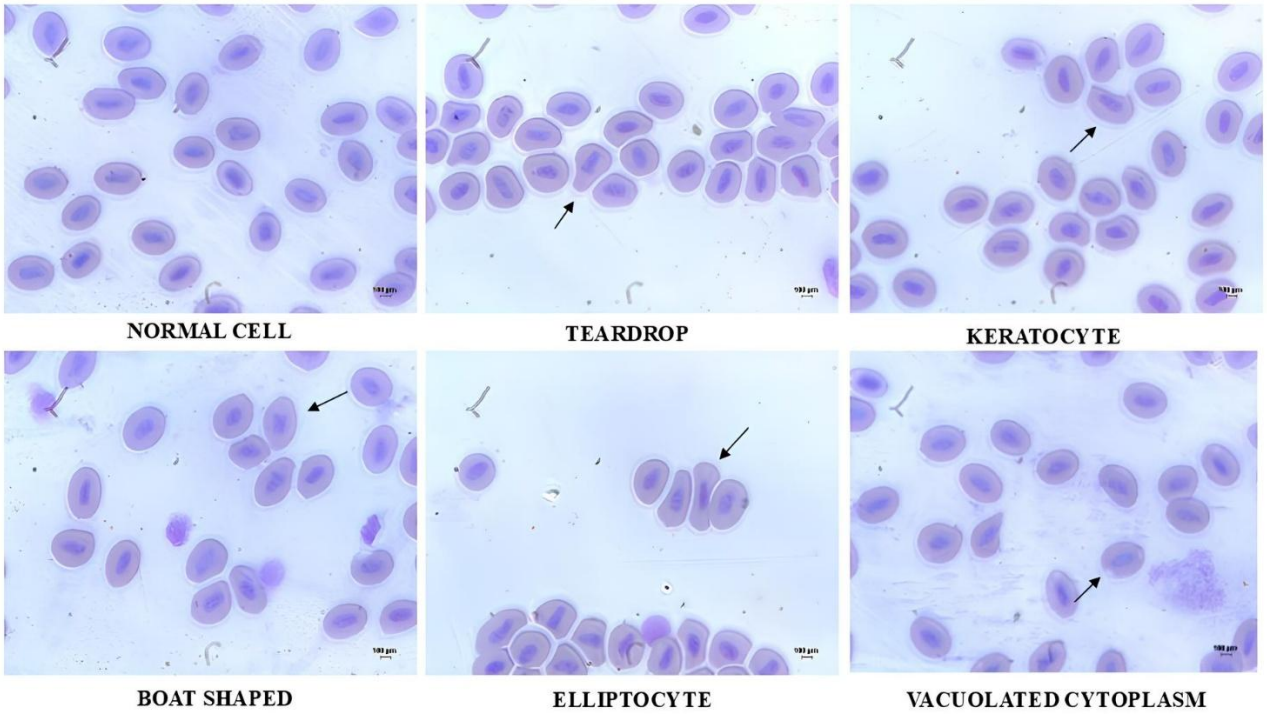

Figure 2: Stained blood smears from *C. striata* in group T3 show normal erythrocytes and various abnormalities. Scale bar – 100 µm.

Table 4: Percentage of poikilocytotic cells in *C. striata* following 96h carbendazim exposure. Data are presented as mean ± standard error of the mean. Statistically significant differences ($p < 0.05$) are indicated by different superscript letters within the same row.

Cellular deformity	T1	T2	T3
TD	0.98 ±0.32^{a}	1.21 ± 0.11^{b}	1.43 ± 0.26^{c}
K	0.51 ± 0.27^{a}	0.78 ± 0.22^{b}	0.93 ± 0.21^{c}
VC	0.42 ±0.19^{a}	0.71 ± 0.17^{b}	1.02 ± 0.13^{c}
E	0.34 ± 0.21^{a}	0.61 ±0.12^{b}	0.84 ± 0.15^{c}
BS	0.68 ± 0.23^{a}	0.88 ± 0.19^{b}	1.18 ± 0.17^{c}

T1=control group, T2 and T3 were subjected to carbendazim concentrations exceeding permissible limits by 5% (1.05 µg/L) and 10% (1.10 µg/L), respectively, for a 96h exposure period.

Table 5: Changes in the condition factor (CF), hepato-somatic index (HSI), and reno-somatic index (RSI)of *C. striata* fish exposed to carbendazim after 96h of exposure. Data are presented as mean ± standard error of the mean. Statistically significant differences ($p < 0.05$) are indicated by different superscript letters within the same column.

Group	CF	HSI	RSI
T1	1.241 ± 0.041^{a}	1.122 ± 0.015^{a}	0.869 ± 0.011^{a}
T2	0.984 ± 0.012^{b}	0.741 ± 0.021^{b}	0.625 ± 0.017^{b}
T3	0.772 ± 0.015^{c}	0.593 ± 0.014^{c}	0.488 ± 0.026^{c}

T1=control group, T2 and T3 were subjected to carbendazim concentrations exceeding permissible limits by 5% (1.05 µg/L) and 10% (1.10 µg/L), respectively, for a 96h exposure period.

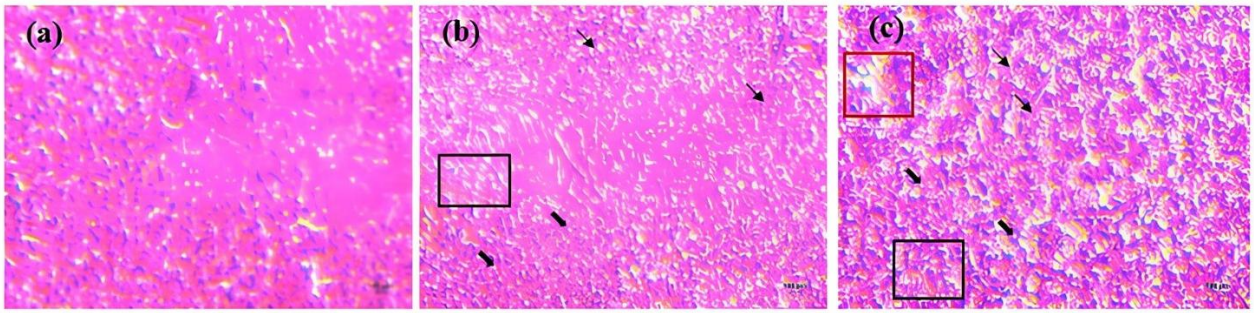

Figure 3: Microphotographs of liver sections of *C. striata* subjected to carbendazim exposure. (a) The control group (T1) showed normal hepatocytes. (b) The liver section from group T2 exhibited cytoplasmic degeneration (rectangle), pyknotic nuclei (arrow), and vacuolization (thick arrow). (c) Liver section from group T3 displaying cytoplasmic degeneration (black rectangle), necrosis (red rectangle), vacuolization (thick arrow), and pyknotic nuclei (arrow). Scale bar – 100 µm.

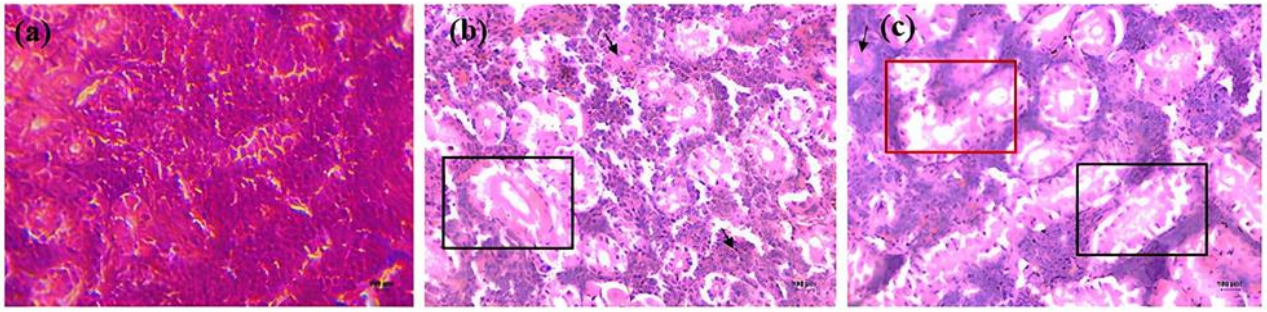

Figure 4: Microphotographs of the kidney sections of *C. striata* subjected to carbendazim exposure. (a) The control group showed a normal structure. (b) The kidney section from group T2 exhibited hypertrophy (rectangle), and cavity reduction in the renal tubule (arrow). (c) The kidney section from group T3 displays hypertrophy (black rectangle), necrosis (red rectangle), and cavity reduction in the renal tubule (arrow). Scale bar – 100 µm

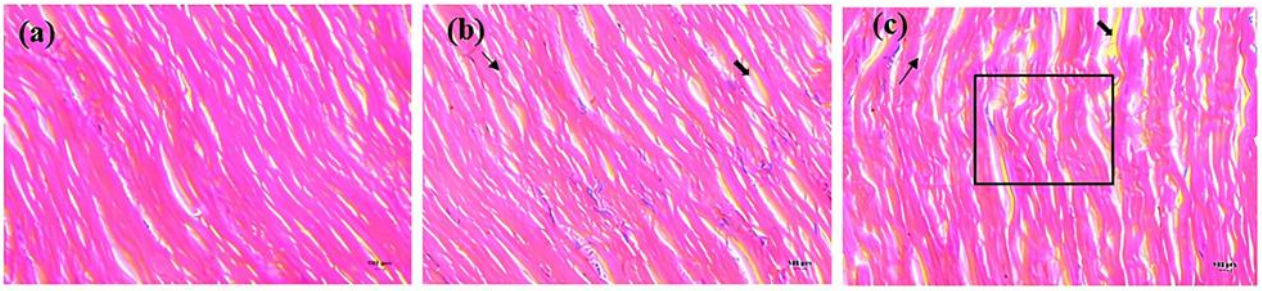

Figure 5: Microphotographs of the muscle sections of *C. striata* subjected to carbendazim exposure. (a) The control group showed a normal muscle structure. (b) The muscle section from group T2 exhibited muscle fiber degeneration (thick arrow) and splitting of muscle fiber (arrow). (c) The muscle section from group T3 displayed breakage of muscle fibers (black rectangle), muscle fiber degeneration (thick arrow), and splitting of muscle fiber (arrow). Scale bar – 100 µm.

Table 6: The correlation between MN, RBC, WBC, Hb, PCV, CF, HSI, and RSIin carbendazim-exposed fish, *C. striata* post 96h exposure period (* p<0.05).

	MN%	RBC	WBC	Hb	PCV	CF	HSI	RSI
MN	1.000*							
RBC	-0.999	1.000*						
WBC	0.988*	-0.994	1.000*					
Hb	-0.997	1.000*	-0.997	1.000*				
PCV	-0.781	0.808*	-0.870	0.826*	1.000*			
CF	-0.995	0.999*	-0.998	1.000*	0.838*	1.000*		
HSI	-0.995	0.990*	-0.968	0.985*	0.717*	0.981*	1.000*	
RSI	-1.000	0.999*	-0.986	0.996*	0.776*	0.994*	0.996*	1.000*

MN: Micronuclei; RBC: Red blood cell; WBC: White blood cell; Hb: Hemoglobin; PCV: Packed cell volume; CF: Condition factor; HSI: Hepatosomatic index; RSI: Reno-somatic index.

Discussion

Assessing the potential harm of pesticides to non-target organisms, especially fish, is of utmost importance to ensure the sustainability of aquatic life. This evaluation is necessary to prevent inadvertent exposure to contaminants and to protect human health from the negative effects of consuming contaminated fish. Hence, this experiment was conducted to study the deleterious effects of the fungicide carbendazim on the economically important fish *C. striata* at concentrations 5% (1.05 µg/L) and 10% (1.10 µg/L) above the permissible limit in freshwater. The physico-chemical parameters of the test medium were assessed throughout the study, with no notable alterations detected. Blood serves as a pathophysiological reflector of the entire body. Hematological parameters can be used as reliable indicators to detect physiological changes following exposure to toxicants and to reflect the overall health status of fish (Trivedi et al., 2022). The results obtained from this study reflect a significant decrease in RBC count, Hb level, and PCV percentages, which may confirm that the fish have suffered from anemic conditions. Anemia may arise from the destruction of mature erythrocytes, leading to a decrease in RBC count, suppression of erythropoiesis, or disturbance of hemosynthesis (Burgos-Aceves et al., 2019). However, a significant rise in WBC count was observed. This elevation in WBC count represents a protective immunological response to counteract the toxic effects of the fungicide, facilitate tissue repair, and maintain homeostasis. The hematopoietic system of fish is primarily located in the renal interstitium, distinguishing it from humans. Any structural changes in this kidney can significantly affect the efficiency of hematopoiesis, leading to alterations in hematological parameters (Smorodinskaya et al., 2023). Results similar to the present study were recorded earlier by Sharma et al. (2021) in *Channapunctatus* exposed to carbendazim, and Ibrahim et al. (2023) in *Oreochromis niloticus* exposed to fungicide mancozeb. Acar et al. (2023) reported a similar result in *Cyprinus carpio* exposed to the fungicide folpet, and Crupkin et al. (2021) in *Australoheros facetus* exposed to the fungicide azoxystrobin.

This hematotoxicity coincided with a significant rise in MN frequency after 96h, indicating genotoxic stress.MN primarily arises from acentric chromosomes or chromatid fragments (clastogenicity) or whole chromosomes (aneugenesis) that fail to integrate into daughter nuclei during mitosis due to improper spindle attachment in anaphase (Götte et al., 2020). The exposed fish exhibited a notable induction in micronuclei frequency compared to the control, aligning with findings from previous studies on different fungicides. Crupkin et al. (2021)observed MN formation in *Australoheros facetus* exposed to the fungicide azoxystrobin. Similar results were found by Ray et al. (2024) in *Pethiaconchonius* exposed to azoxystrobin, by Nataraj et al. (2023) in *Labeorohita* exposed to difenoconazole, and by Khatun et al. (2021) in *Danio rerio* exposed to fenitrothion.

This genotoxic impact on fish erythrocytes is often accompanied by morphological alterations before external symptoms appear, making poikilocytosis a valuable early toxicity biomarker (Bardhan et al., 2024). Our findings indicate that carbendazim interfered with erythrocyte function by inhibiting delta-aminolevulinic acid dehydrogenase activity, resulting in plasma membrane disruption and the formation of

poikilocytes (Hamed et al., 2021). In our results, the morphological abnormalities of RBCs (poikilocytosis) included teardrop-like shapes, keratocytes, erythrocytes with cytoplasmic vacuolization, boat-shaped cells, and elliptocytes. These structural changes, collectively termed shape-shifting erythrocytes, may result from internal damage, environmental stressors, or genetic abnormalities. Alterations in erythrocyte morphology have been documented for various pollutants, such as pyrogallol-induced poikilocytosis in *Clarias gariepinus* (Hamed et al., 2024) and in emamectin benzoate-exposed *Lates calcarifer* (Ananda Raja et al., 2020). The concurrent increase in MN frequency and poikilocytosis suggests a direct relationship between genotoxic stress and erythrocyte deformation.

Histopathological studies are a sensitive endpoint for detecting organ toxicity during exposure to toxicants (Jain et al., 2024). As the liver is the primary organ for detoxification and metabolic processes, hepatic abnormalities provide valuable insights into the extent of environmental contamination. The liver's histopathological alterations following carbendazim exposure indicate a progressive toxic response. In the T2 group, vacuolization, cytoplasmic degeneration, and pyknotic nuclei appeared, indicating increasing cellular damage due to metabolic stress. In the T3 group, this progressed to mild necrosis, signifying structural disruption and loss of cellular integrity. This highlights the escalating impact of carbendazim on hepatic tissue, consistent with liver toxicity patterns observed in fish and mammals exposed to fungicides. Similar liver damages were seen in *Danio rerio* exposed to fungicide tebuconazole (Macirella et al., 2022), dimoxystrobin (Macirella et al., 2024) and difenoconazole (Nataraj et al., 2023), and in rats exposed to carbendazim (Abdel-Rahman et al., 2022), epoxiconazole (Hamdi et al., 2019). These highlight carbendazim's hepatotoxicity and the liver's vulnerability.

The kidney's role in xenobiotic excretion makes it vital for assessing carbendazim toxicity in fish. After 96h of carbendazim exposure, significant histopathological changes, including cavity reduction in renal tubules, hypertrophy, and necrosis, were observed—cavity reduction from tubular cell proliferation or swelling limits toxicant infiltration (Kumari et al., 2020). With progression, hypertrophy counters cellular damage (Lavecchia et al., 2022), but increased stress leads to necrosis. These findings align with previous studies on pesticide-induced renal toxicity in fish, such as *Danio rerio* exposed to hexaconazole and epoxiconazole (Jia et al., 2019), bisphenol A (Smorodinskaya et al., 2023), and difenoconazole (Nataraj et al., 2023). Similar alterations have been observed in *Channa punctata* exposed to propiconazole (Tabassum et al., 2016), in *Cyrinuscarpio* exposed to difenoconazole (Li et al., 2024) and *Labeorohita* exposed to difenoconazole (Nataraj et al., 2023).

Similarly, muscle tissue exhibited structural impairments due to carbendazim exposure. The observed alterations in the muscle were muscle fiber degeneration and splitting of muscle fibers in group T2. Additionally, breakage of muscle fiber was seen in group T3. These findings are consistent with the observations of Bhuvaneshwari and Rajendran (2015) in *Danio rerio* exposed to organochlorine pesticides, Loganathan et al. (2024) in *Heteropneustes fossilis* exposed to the fungicide triazophos, and Singh et al. (2021) in *Channa punctata* exposed to fenvalerate. Since muscle integrity is crucial for locomotion and its nutritional value, histopathological changes in this tissue reflect the toxic effects on fish health and raise concerns about food safety.

The Pearson correlation matrix for *C. striata* exposed to carbendazim demonstrated pronounced negative correlations between MN% and RBC, Hb, PCV, CF, HSI, and RSI, indicating genotoxic effects, impaired erythropoiesis, hemolytic activity, and organ dysfunction. Positive correlations among RBC, Hb, CF, HSI, and RSI suggest coordinated physiological adjustments to maintain metabolic homeostasis under toxic stress. Conversely, negative correlations between WBC and these parameters reflect immunological responses to cytotoxic exposure. These correlation patterns elucidate the hematotoxic, cytotoxic, genotoxic, and organ-specific impacts of carbendazim, underscoring its detrimental effects on fish health.

Carbendazim exposure disrupted key hematological parameters in *C. striata*, including reductions in RBC count, WBC count, Hb level, and PCV, along with morphological abnormalities and increased micronuclei frequency, compromising circulatory function and affecting organs responsible for detoxification, excretion, and locomotion.

Moreover, the muscle tissue, a determinant of the fish's nutritional and commercial value, exhibited histopathological alterations. These

findings indicate that the fungicide not only impairs the physiological health of the fish but also affects food safety and its marketability, underscoring the broader ecological and economic consequences of chemical exposure in aquatic environments.

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