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Azoxystrobin-Induced Oxidative Stress and Reduced Oxygen Consumption in Grass Carp (*Ctenopharyngodon idella*): A Multi-Biomarker Toxicity Evaluation

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Abstract

The widespread application of Azoxystrobin, a strobilurin fungicide, has raised ecological concerns because of its persistence and potential toxicity in freshwater environments. Building upon previously published findings[1] on haematological alterations in *Ctenopharyngodon idella*, the present study comprehensively evaluates the toxic effects of azoxystrobin on oxygen consumption, oxidative stress biomarkers, haematological parameters, and neurobehavioral responses in this species. Juvenile grass carp were exposed to 10% LC_{50} (1.1 mg/L) and 20% LC_{50} (2.2 mg/L) in a semistatic bioassay for 96 hours. The oxygen consumption rates initially increased during the first 6 hours but declined significantly thereafter, with a 43.5% reduction observed in the high-dose group at 96 hours ($p < 0.01$), indicating metabolic suppression and respiratory distress. Haematological assessments revealed a 40% reduction in Red Blood Cell (RBC) count and a 34.4% decrease in Haemoglobin (Hb) level, accompanied by a 52% increase in White Blood Cell (WBC) count ($p < 0.01$), suggesting hypoxia-induced anaemia and immune activation. Biochemical assays revealed significantly elevated reactive oxygen species (ROS) and lipid peroxidation (LPO) levels ($p < 0.001$), as well as decreased Superoxide Dismutase (SOD) and Catalase (CAT) activity, confirming oxidative damage and depletion of antioxidants. Behavioural alterations, such as surface gasping, erratic swimming, and loss of equilibrium, correlated with a 49% inhibition of Acetylcholinesterase (AChE) activity ($p < 0.001$), indicating neurotoxicity. These findings underscore the multifaceted toxicity of azoxystrobin and highlight oxygen consumption as a sensitive early biomarker of pesticide exposure.

Major Findings: This study provides novel insights into aquatic toxicology by demonstrating integrated physiological, haematological, and neurobehavioral responses in *C. idella*, highlighting the need for stricter environmental regulations and sustainable pesticide management strategies.

Keywords: Aquatic Toxicology, Azoxystrobin Toxicity, *Ctenopharyngodon idella*, Haematological Biomarkers, Neurotoxicity, Oxidative Stress, Oxygen Consumption.

1. Introduction

Aquatic ecosystems are increasingly threatened by complex mixtures of environmental contaminants, including pesticides, heavy metals, pharmaceuticals, and industrial effluents, which pose severe risks to aquatic biodiversity and ecosystem functioning. In our earlier study, we reported the toxicological and haematological

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effects of azoxystrobin in *Ctenopharyngodon idella*, which provides a foundation for the present investigation[1]. Among these, synthetic pesticides have emerged as a major cause of ecotoxicological concern because of their widespread agricultural usage and potential to accumulate in freshwater bodies via surface runoff and leaching[2,3]. Azoxystrobin, a strobilurin-based fungicide, is one of the most commonly applied fungicides in agriculture worldwide and is known for its systemic action and persistence in aquatic systems[4]. Residual levels of azoxystrobin have been reported in rivers, lakes, and estuarine waters in many regions, raising concerns about its sublethal effects on nontarget aquatic organisms such as fish[5,6].

Fish, which are continuously exposed to waterborne pollutants through their gills, skin, and alimentary canal, are excellent bioindicators of aquatic toxicity[7]. As aerobic ectotherms, fish depend on dissolved oxygen for metabolism and homeostasis. However, exposure to environmental toxicants often disrupts normal oxygen uptake, gill function, and energy metabolism[8]. Oxygen consumption is therefore widely used as a sensitive biomarker in toxicological studies, reflecting the physiological stress, metabolic alterations, and respiratory efficiency of fish under pollutant exposure[9,10]. Pollutants can impair oxygen uptake by damaging gill structures, increasing mucus secretion, and triggering mitochondrial dysfunction, collectively compromising respiratory efficiency[11,12].

Ctenopharyngodon idella (grass carp), an ecologically and commercially important freshwater fish, is widely cultured in Asia and has been recognised for its high sensitivity to aquatic pollutants. Its feeding behaviour, fast growth, and habitat adaptability make it a model species for ecotoxicological assessments. Our previous study[1] demonstrated significant haematological and biochemical alterations in *C. idella* upon exposure to azoxystrobin. However, the effects of this fungicide on oxygen consumption, oxidative stress biomarkers, and neurobehavioral endpoints in *C. idella* have not been comprehensively studied, particularly at sublethal concentrations that reflect realistic field conditions.

The presence of azoxystrobin in aquatic environments has been linked to mitochondrial toxicity, oxidative stress, neurotoxicity, and respiratory dysfunction in various fish species[13,4]. Azoxystrobin disrupts mitochondrial respiration by inhibiting complex III in the electron transport chain, leading to reduced Adenosine Triphosphate (ATP) synthesis and increased Reactive Oxygen Species (ROS) generation[14]. This oxidative imbalance adversely affects vital enzymes such as Superoxide Dismutase (SOD) and Catalase (CAT), compromises cellular integrity, and induces lipid peroxidation[2,15]. Behavioural impairments, such as surface gasping, erratic swimming, and loss of equilibrium, often linked to Acetylcholinesterase (AChE) inhibition, have also been reported in pesticide-exposed fish, reflecting neurotoxicity[16,17].

Furthermore, histopathological studies have revealed that pesticides cause severe gill injuries, including lamellar fusion, epithelial hyperplasia, and necrosis, thereby limiting oxygen diffusion and exacerbating metabolic suppression[6]. Under such conditions, fish may initially exhibit compensatory hyperventilation, followed by a decline in oxygen uptake as damage progresses[18]. Measuring oxygen consumption under toxicant exposure thus serves as a powerful physiological endpoint to assess early stress responses and energy imbalance before overt signs of toxicity manifest[19].

Despite the known toxicity of azoxystrobin in aquatic organisms, limited information exists on its integrated impact on respiratory, haematological, oxidative, and neurobehavioral functions in freshwater fish. To bridge this gap, the present study investigated the sublethal toxicity of azoxystrobin in *C. idella* by assessing oxygen consumption, haematological indices, oxidative stress markers, and behavioural anomalies.

The novelty of this study lies in the finding that oxygen consumption is an early and sensitive biomarker of azoxystrobin-induced stress in *C. idella*, which is supported by mechanistic evidence from haematological and neurobehavioral endpoints. This integrated biomarker approach enhances the understanding of sublethal toxicity mechanisms and supports ecotoxicological risk assessments for aquatic pesticide exposure.

1.1 Oxygen Consumption as a Biomarker of Toxicity

Oxygen consumption is a key physiological indicator that reflects the metabolic rate and respiratory efficiency of aquatic organisms. In fish, pollutants such as pesticides are known to interfere with this process through several mechanisms, including gill impairment leading to inefficient gas exchange, excessive mucus secretion obstructing respiratory pathways, and mitochondrial

dysfunction caused by oxidative stress, which reduces cellular energy production[8,11].

In pesticide-exposed fish, oxygen uptake often shows a biphasic trend: an initial increase due to stress-induced hyperventilation followed by a significant decline due to respiratory failure and metabolic suppression[18]. Despite growing evidence of respiratory toxicity caused by pesticides, no comprehensive study has examined the effects of azoxystrobin on oxygen consumption in *Ctenopharyngodon idella*. This study addresses that gap by evaluating oxygen consumption under sublethal azoxystrobin exposure and proposes that it is a sensitive early biomarker of pesticide-induced physiological stress in grass carp.

1.2 Mechanisms of Azoxystrobin Toxicity

Azoxystrobin inhibits mitochondrial respiration in fungi by blocking complex III of the electron transport chain. However, this mechanism can also adversely affect nontarget aquatic organisms. In fish, azoxystrobin exposure has been linked to the overproduction of ROS, resulting in lipid peroxidation, cellular membrane damage, and oxidative stress[2]. Studies have also reported mitochondrial fragmentation and ATP depletion in exposed fish[4], as well as the inhibition of AChE, which causes neuromuscular dysfunction, erratic swimming, and impaired equilibrium[13].

These pathophysiological responses justify the integration of multiple toxicity biomarkers, such as oxygen consumption, haematological changes, oxidative stress markers, and behavioural symptoms, in the assessment of the impact of azoxystrobin on aquatic organisms.

1.3 Research Objectives and Hypotheses

This study builds upon our previous findings[1] to expand the understanding of azoxystrobin toxicity in *Ctenopharyngodon idella*. The objectives of this study included evaluating the impact of azoxystrobin on oxygen consumption, with respiratory metabolism assessed as a biomarker of toxicity. Haematological parameters such as RBC count, Hb concentration, and WBC count were analysed to assess stress and immune responses. This study also examined oxidative stress through the levels of ROS, lipid peroxidation, and the activities of antioxidant enzymes such as SOD and CAT. Neurobehavioral outcomes, including surface gasping, erratic movement, and AChE inhibition, were observed to assess neurotoxicity. Finally, the results were compared with existing pesticide toxicity studies to derive broader ecological implications.

Hypothesis: Azoxystrobin exposure significantly impairs oxygen uptake, disrupts haematological and antioxidant balance, and induces behavioural and neurotoxic effects in *C. idella*.

1.4 Expected Contributions

This study offers comprehensive insight into the sublethal toxicity of azoxystrobin in *Ctenopharyngodon idella*, addressing critical gaps in respiratory, oxidative, and neurobehavioral toxicity profiling. One of the key contributions of this study is the establishment of oxygen consumption as an early and sensitive biomarker of azoxystrobin-induced stress. This research also provides mechanistic evidence of oxidative damage and AChE inhibition, reinforcing the potential of this compound to disrupt metabolic and neural functions in fish. Furthermore, the findings enrich ecotoxicological data essential for refining environmental risk assessments and informing regulatory frameworks on pesticide use in aquatic ecosystems. By highlighting the potential hazards of strobilurin pesticide contamination, this study supports sustainable aquaculture practices and contributes to the development of targeted conservation policies and Integrated Pest Management (IPM) strategies, which are particularly important in the context of increasing concerns over pesticide runoff and aquatic ecosystem health.

2. Materials and Methods

This study was conducted to evaluate the acute toxicity and physiological effects of azoxystrobin on *C. idella*. The experimental design included oxygen consumption analysis, haematological assessments, oxidative stress biomarkers, and behavioural observations to establish a comprehensive toxicological profile. All procedures were conducted following ethical guidelines and institutional animal care regulations.

2.1 Experimental Fish and Acclimatisation

Juvenile *C. idella* (average length: 12.5 ± 1.2 cm, weight: 25.6 ± 2.3 g) were obtained from a certified fish hatchery and acclimatised under controlled laboratory conditions for 14 days. The fish were housed in fibreglass tanks (100

L capacity) with aerated dechlorinated water under the following environmental conditions:

Temperature: 24.5 ± 1.2°C

Dissolved oxygen (DO): 6.5–7.3 mg/L

pH: 7.0 ± 0.2

Photoperiod: 12 hours light:12 hours dark cycle

Feeding: Fish were fed a formulated floating pellet diet containing 35% crude protein at 5% body weight per day. The feed was manufactured by Growel Feeds Pvt. Ltd. (Andhra Pradesh, India) and procured from a certified aquaculture supplier.

Water quality was monitored daily to ensure stable conditions, and no mortality or abnormal behaviour was observed during the acclimatisation period.

2.2 Toxicant and Experimental Design

Azoxystrobin (98% purity, Sigma–Aldrich) was used for toxicity evaluation. The 96-hour LC_{50} value (11 mg/L) was determined via probit analysis before the bioassay was conducted.

The fish were randomly assigned to three groups (n = 15 per group, triplicate per treatment):

Control Group: Exposed to pesticide-free water.

Low-dose group: Exposed to 10% LC_{50} (1.1 mg/L) azoxystrobin.

High-dose group: Exposed to 20% LC_{50} (2.2 mg/L) azoxystrobin.

The bioassay was conducted for 96 hours in a semistatic system with 50% water renewal every 24 hours to maintain the pesticide concentration and minimise degradation.

2.3 Oxygen Consumption Analysis

Oxygen consumption was measured via a closed respirometry system (YSI ProODO optical oxygen meter). Each fish was individually placed in a sealed metabolic chamber (1 L capacity), and dissolved oxygen levels were recorded at 0, 6, 12, 24, 48, and 96 hours. The oxygen depletion rates were calculated via the following formula:

$$Oxygen\ Consumption = \frac{(Initial\ DO - Final\ DO)}{Time(h)}$$

Control experiments without fish were conducted to correct for background oxygen depletion due to bacterial activity.

2.4 Haematological Analysis

At the end of the experiment, blood samples were collected from the caudal vein via heparinised syringes and analysed as follows:

RBC count ($\times 10^6/\mu L$) – Haemocytometer method

Hb concentration (g/dL) – Cyanmethemoglobin method

Hematocrit (HCT) percentage – Microhematocrit method

WBC count ($\times 10^3/\mu L$) – Neubauer chamber method

All measurements were performed in triplicate to ensure accuracy and reproducibility.

2.5 Oxidative Stress Biomarkers

Liver and gill tissues were homogenised (10% w/v) in phosphate-buffered saline (pH 7.4) and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatants were used for the following biochemical assays:

ROS levels – Fluorometric assay

LPO levels – Malondialdehyde (MDA) quantification

SOD activity – Nitroblue Tetrazolium (NBT) assay

CAT activity–Hydrogen peroxide (H_2O_2) decomposition assay

Enzyme activities were normalised to total protein content (Bradford assay) to account for variations in tissue mass.

2.6 Behavioural Observations

The behaviour of the fish was continuously monitored for signs of neurotoxicity and physiological stress, including the following:

Erratic swimming patterns

Surface gasping

Loss of equilibrium

Lethargy and hypoactivity

A behavioural scoring system (0–4 scale) was used, where 0 = normal behaviour and 4 = severe impairment.

2.7 AChE Activity Assay

Brain tissues were carefully dissected from euthanised fish and homogenised in 0.1M phosphate buffer (pH 7.4) via a glass-Teflon homogeniser. The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C, and the supernatant was collected for AChE analysis. Acetylcholinesterase activity was measured following the

method of Ellman *et al.* (1961)[20], using acetylthiocholine iodide as a substrate and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) as the chromogenic reagent. The change in absorbance was monitored at 412nm via an Ultraviolet (UV)–Vis spectrophotometer. AChE activity was expressed as nmol of acetylthiocholine hydrolysed per minute per mg of protein (nmol/min/mg protein). The protein content was determined via the Bradford method.

2.8 Statistical Analysis

All the data were analysed via SPSS version 26.0. One-way analysis of variance (ANOVA) was performed to determine significant differences among the treatment groups. Tukey's post hoc test was applied for pairwise comparisons between groups. The results are presented as the mean ± Standard Error (SE), and statistical significance was considered at $p < 0.05$.

Table 1. LC_{50} values at different exposure durations

Exposure Duration (Hours)	LC_{50} Value (mg/L)	Mortality Rate (%)
24	17.0	20
48	15.0	45
72	13.0	70
96	11.0	90

3. Results

3.1 Acute Toxicity (LC_{50}) Determination

The 96-hour LC_{50} of azoxystrobin for *Ctenopharyngodon idella* was determined to be 11 mg/L via probit analysis. The mortality response followed a dose-dependent pattern, with increased mortality at higher concentrations and longer exposure durations (Table 1 and Figure 1).

The progressive decrease in the LC_{50} values suggests cumulative toxicity, indicating bioaccumulation and prolonged physiological stress in *C. idella*. These findings align with previous studies that reported time-dependent increases in pesticide toxicity among freshwater fish, including *Labeo rohita* and *Danio rerio,* exposed to azoxystrobin and other strobilurin fungicides[2,21&14].

3.2 Oxygen Consumption Trends

The oxygen consumption rates of fish exposed to azoxystrobin exhibited a biphasic response. During the

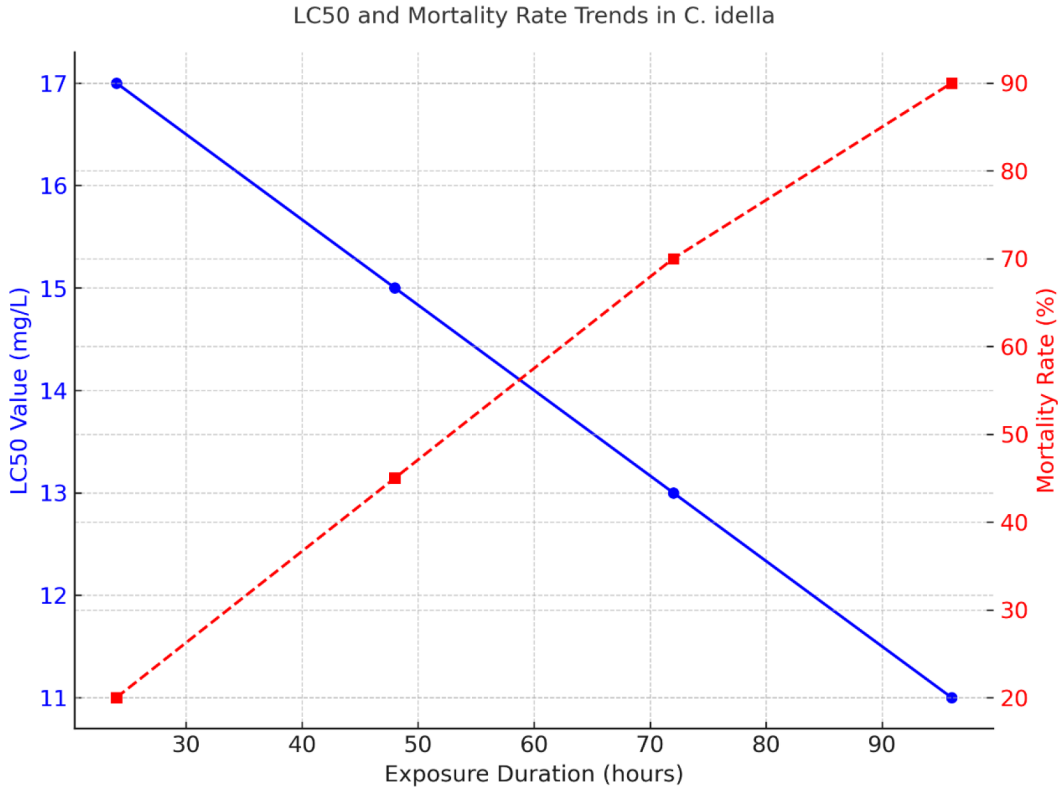


Figure 1. Trends in the LC_{50} and mortality rate.

initial exposure phase (0–6 hours), a slight increase in oxygen consumption was observed, likely reflecting stress-induced hyperventilation caused by gill irritation. This was followed by a progressive decline from 12 to 96 hours, indicating respiratory impairment and metabolic suppression. By the end of the 96-hour exposure period, oxygen consumption in the high-dose groups was 43.5% lower than that in the control groups, a statistically significant difference ($p < 0.01$), as shown in Table 2 and Figure 2.

These findings align with previous studies reporting respiratory suppression in freshwater fish, such as *Labeo rohita* exposed to cypermethrin[8] and *Danio rerio* subjected to chlorpyrifos[14], where significant reductions in oxygen consumption and gill function were observed.

Table 2. Oxygen consumption rates (mg/g/h) of *C. idella*

Exposure Time (hours)	Control	Low Dose (10% LC_{50})	High Dose (20% LC_{50})
0	0.82 ± 0.03	0.79 ± 0.04	0.76 ± 0.03
6	0.80 ± 0.02	0.92 ± 0.03	1.10 ± 0.05
12	0.78 ± 0.02	0.85 ± 0.02	0.92 ± 0.04
24	0.76 ± 0.03	0.69 ± 0.02	0.55 ± 0.03
48	0.75 ± 0.02	0.61 ± 0.03	0.40 ± 0.02
96	0.72 ± 0.02	0.50 ± 0.03	0.41 ± 0.02

3.3 Haematological and Oxidative Stress Biomarkers

Dose-dependent decreases in RBC count, Hb, and HCT were observed, indicating hypoxia-induced anaemia. In contrast, WBC counts increased, suggesting an immune response to oxidative stress (Table 3 and Figure 3). Although haematological parameters were also examined in our previous study[1], their inclusion in the present investigation serves to validate their utility as consistent biomarkers in the context of azoxystrobin-induced respiratory and oxidative stress. Unlike earlier studies, which focused solely on haematology and biochemistry, the current study integrated respiratory metabolism, oxidative damage, and neurotoxicity endpoints to present a comprehensive biomarker-based toxicity profile.

In the high-dose groups, the RBC count decreased by approximately 40% ($p < 0.01$), indicating a significant reduction in the oxygen-carrying capacity. Conversely, the WBC count increased by 52%, suggesting an

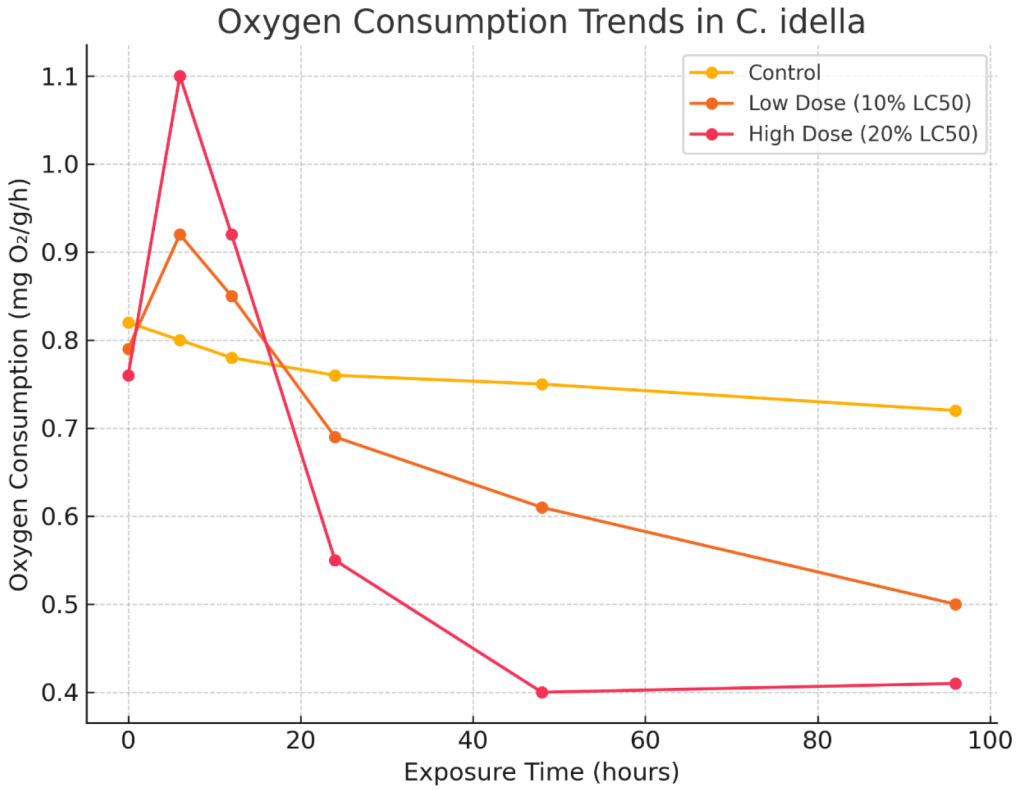


Figure 2. Trends in oxygen consumption.

active inflammatory or immune response triggered by azoxystrobin-induced stress.

Table 3. Haematological and oxidative stress biomarkers in *C. idella*

Parameter	Control (Mean ± SD)	Of Test Animals	High Dose (20% LC_{50})
RBC (×10^6/μL)	2.5 ± 0.3	1.9 ± 0.2	1.5 ± 0.2
Hb (g/dL)	9.6 ± 0.5	7.8 ± 0.4	6.3 ± 0.3
HCT (%)	27 ± 2	20.5 ± 1.5	17.7 ± 1.3
WBC (×10^3/μL)	15 ± 1.8	19.2 ± 2.1	22.8 ± 2.3
ROS (μmol/mg protein)	1.5 ± 0.2	2.8 ± 0.3	4.2 ± 0.4
LPO (MDA, nmol/mg protein)	0.8 ± 0.1	1.6 ± 0.2	2.9 ± 0.3
SOD (U/mg protein)	3.2 ± 0.4	2.5 ± 0.3	1.8 ± 0.2
CAT (U/mg protein)	5.0 ± 0.5	3.7 ± 0.4	2.2 ± 0.3

These biomarkers validate the potential of azoxystrobin to induce oxidative toxicity, necessitating further research on its long-term effects. The haematological parameters observed in this study were similar to those previously reported for larger *C. idella*[1]. This consistency likely stems from standardised husbandry practices, species-specific physiological stability, and controlled environmental factors across studies. Haematological indices in teleost fish often remain within a narrow physiological range, even across moderate size differences under similar rearing conditions.

3.4 Behavioural Responses and Neurotoxicity

Fish exposed to azoxystrobin exhibited clear, dose-dependent behavioural impairments. During the early exposure phase, erratic swimming and hyperactivity are frequently observed, indicating acute neurological excitation. As exposure progressed, surface gasping and increased opercular movement became prominent, indicating that respiratory distress was likely linked to

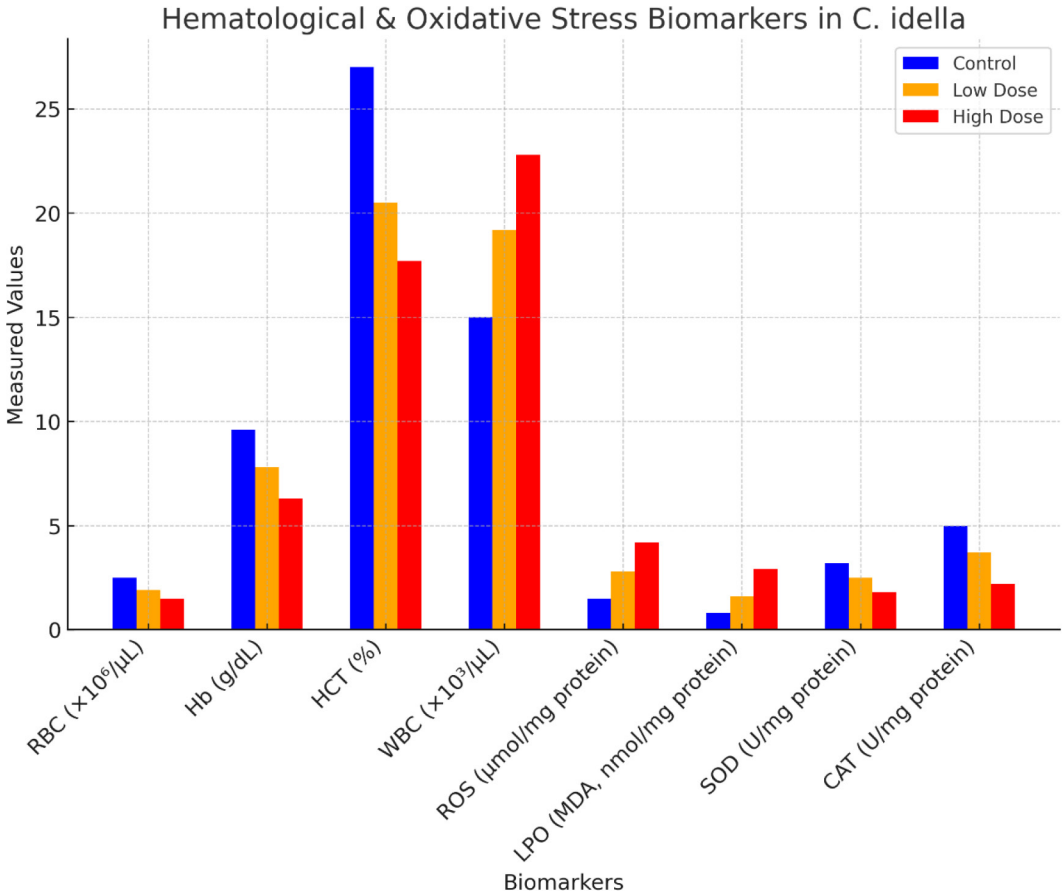


Figure 3. Haematological and oxidative stress biomarkers.

gill irritation and hypoxia (Table 5). In the later phases, behavioural symptoms included loss of equilibrium, diminished responsiveness to stimuli, and general lethargy, which coincided with significant inhibition of AChE activity, suggesting neuromuscular dysfunction (Table 4 and Figure 4). These neurobehavioral alterations provide strong evidence of azoxystrobin-induced neurotoxicity in *C. idella*. AChE activity was significantly inhibited in exposed fish, and this inhibition closely correlated with the observed neuromuscular dysfunction and behavioural abnormalities, including loss of equilibrium and reduced responsiveness.

Table 4. Ache activity in *C. idella*

Group	AChE Activity (nmol/min/mg Protein)
Control	12.4 ± 0.5
Low Dose	9.8 ± 0.4 ($p < 0.05$)
High Dose	6.3 ± 0.3 ($p < 0.001$)

These behavioural alterations align with previous studies on pesticide-induced AChE inhibition in freshwater fish, including those by Brander *et al.*[13] and

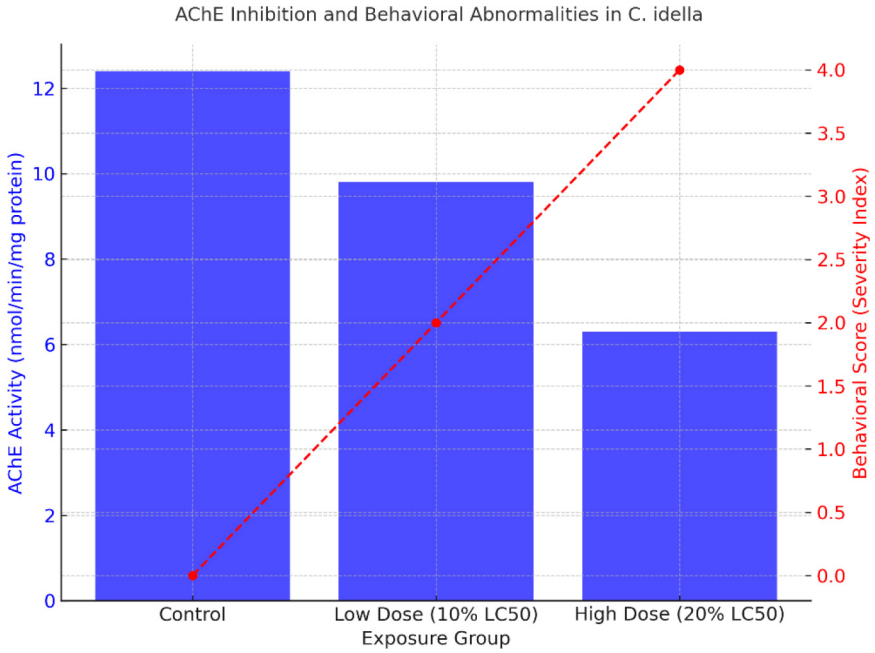


Figure 4. Ache inhibition and behavioural abnormalities.

Table 5. Qualitative observations of the ocular movement and behavioural responses of *C. idella* exposed to azoxystrobin

Exposure Phase	Treatment Group	Opercular Movement Trend	Behavioral Observation	Interpretation
0–6 hours	Control	Normal (baseline)	Normal swimming, no distress	Healthy state
0–6 hours	Low Dose (1.1 mg/L)	Increased (visibly rapid opercular beats)	Erratic swimming, surface gasping	Compensatory hyperventilation due to early gill irritation
0–6 hours	High Dose (2.2 mg/L)	Strongly increased (frequent, forceful movement)	Hyperactivity, frequent surfacing	Acute stress response; respiratory overcompensation
12–96 hours	Control	Normal (baseline)	Normal behavior maintained	No physiological disruption
12–96 hours	Low Dose (1.1 mg/L)	Decreased (less movement than the initial phase)	Lethargy, reduced responsiveness	Onset of metabolic suppression
12–96 hours	High Dose (2.2 mg/L)	Significantly decreased (slow, weak movement)	Loss of equilibrium, hypoactivity	Respiratory failure, neurotoxicity onset

Mohapatra *et al.*[16], where significant reductions in AChE activity were linked to neurobehavioral impairments in species such as *D. rerio* and *Oreochromis mossambicus*.

Qualitative summary of opercular movement and behaviour in *C. idella* during different phases of exposure to azoxystrobin. Although opercular movements were not recorded quantitatively, consistent patterns were observed across all replicates. The early increase (0–6 hours) reflects a stress-induced hyperventilation phase, whereas the subsequent decline (12–96 hours) aligns with reduced oxygen uptake and systemic impairment.

3.5 Comparative Analysis with Other Studies

The haematological, oxidative stress, and neurobehavioral responses observed in *C. idella* in this study are consistent with findings in other freshwater fish species exposed to pesticides, particularly azoxystrobin and organophosphates.

Similarly, reduced RBC, Hb, and HCT levels were reported in *L. rohita* exposed to azoxystrobin, indicating anaemia and hypoxia-induced haematological stress[21]. In *Oreochromis niloticus*, exposure to azoxystrobin resulted in significantly elevated levels of ROS and LPO and suppressed SOD and CAT activities, which aligns with the oxidative biomarker trends observed in the present study[2,12]. Neurotoxic effects, including erratic swimming, hyperactivity, and AChE inhibition, were also observed in *D. rerio* exposed to chlorpyrifos and azoxystrobin, reflecting a common mode of action across different fish models[13,16]. Surface gasping and opercular hyperactivity have been reported in *Tilapia mossambica* exposed to synthetic pyrethroids, further supporting the link between behavioural stress and respiratory distress in pesticide-exposed fish[10]. Qiu *et al.*[4] reported that mitochondrial dysfunction and oxidative damage in fish liver cells upon azoxystrobin exposure correlated well with the decline in antioxidant enzymes observed in this study. Similar patterns of early hyperventilation followed by suppressed oxygen uptake have also been described in *Cyprinus carpio* and *Channa punctatus* exposed to sublethal pesticide levels[18].

These findings collectively reinforce that azoxystrobin exerts its toxic effects through a combination of oxidative stress, haematological disruption, and neurotoxicity. The consistency across species highlights the ecological relevance and environmental risks posed by azoxystrobin residues in freshwater ecosystems.

4. Discussion

The present study demonstrated that azoxystrobin has moderate acute toxicity to *C. idella*, with LC_{50} values decreasing from 17 mg/L at 24 hours to 11 mg/L at 96 hours. This time-dependent increase in toxicity suggests bioaccumulation and delayed physiological stress. Similar findings have been reported in *L. rohita* and *D. rerio*, where exposure to strobilurin fungicides led to cumulative toxicity and increased mortality over time[2,21]. Waheed *et al.*[18] reported that the toxic impacts of pesticides intensify over time, even at sublethal concentrations, due to their persistent interaction with vital organs. One of the primary mechanisms of azoxystrobin toxicity appears to involve gill impairment, as gills serve as the first site of contact with waterborne toxicants. Histopathological alterations such as epithelial lifting, lamellar fusion, and mucus hypersecretion have been associated with pesticide exposure and are known to disrupt gas exchange and trigger respiratory stress[6].

The consumption of oxygen, an important indicator of metabolic health in fish, significantly decreased in a dose-dependent manner, with a 43.5% reduction in the high-dose group at 96 hours. This trend reflects reduced respiratory efficiency and increased hypoxia, similar to findings in *Channa punctatus* and *Cyprinus carpio* exposed to synthetic pesticides[8,11]. During the early exposure phase (0–6 hours), the fish exhibited increased opercular movement and surface gasping, which are typical hyperventilatory responses to early gill irritation and hypoxic conditions. Sharma and Singh[10] reported a comparable hyperventilation phase in *T. mossambica* exposed to pesticides. However, this was followed by a significant decline in both opercular activity and oxygen consumption during the later exposure phase (12–96 hours), likely due to cumulative mitochondrial dysfunction and metabolic depression. This biphasic respiratory pattern has been widely observed in fish and reflects an initial attempt to compensate for oxygen stress, followed by eventual physiological suppression[12,4]. These findings affirm the potential of oxygen consumption as a sensitive early biomarker for pesticide-induced metabolic stress in aquatic organisms.

Haematological responses in the present study revealed that azoxystrobin exposure led to anaemia, as evidenced by significant reductions in RBC count and Hb and haematocrit levels. These disruptions are likely the result of oxidative injury to erythrocytes or inhibited

erythropoiesis and are consistent with previous reports in *L. rohita* and *Oreochromis niloticus* following exposure to azoxystrobin or chlorpyrifos[21,16]. The observed leucocytosis, an increase of 52% in the WBC count, further suggests an immune response to chemical stress. Similar immune activation under pesticide exposure has been reported by Zhang *et al.*[2] in azoxystrobin-exposed fish. In support of these trends, oxidative stress biomarkers presented significantly elevated levels of ROS and LPO, whereas antioxidant enzymes such as SOD and CAT were suppressed. This pattern of oxidative imbalance has been observed in several freshwater species, including *D. rerio* and *O. mossambicus*, under fungicidal and insecticidal exposure[12,9]. Suppressed antioxidant enzyme activities indicate that the antioxidant defence system was overwhelmed, increasing susceptibility to cellular injury.

Neurotoxicity was another significant outcome observed in this study, with behavioural impairments including erratic swimming, lethargy, and loss of equilibrium. These alterations were closely associated with 49% inhibition of AChE activity in the high-dose group, confirming the disruption of cholinergic neurotransmission. Brander *et al.*[13] and Sharma and Singh[10] reported similar behavioural and enzymatic impairments in freshwater fish exposed to neurotoxic pesticides. The early respiratory hyperactivity observed at 0–6 hours may be linked to overstimulation of cholinergic pathways, whereas the subsequent decline (12–96 hours) could result from sustained neuroinhibition and energy depletion. This biphasic neuro-respiratory effect aligns with previous studies on fish exposed to deltamethrin and chlorpyrifos[16]. The correlation between behavioural symptoms and biochemical markers such as AChE inhibition supports the use of integrated endpoints in ecological risk assessment.

Although this experiment was conducted in a freshwater environment, the findings carry broader ecological implications. Azoxystrobin, which is widely used in agriculture, can enter aquatic ecosystems through runoff and reach estuarine and marine habitats, where it may bioaccumulate in aquatic food chains. Such contamination can affect biodiversity and pose risks to human health via the consumption of contaminated seafood. Bhuiya *et al.*[3] reported significant levels of pesticide residues in river systems, highlighting the risk of chronic toxicity to both benthic and pelagic organisms. The present study highlights the need for more stringent pesticide regulations, regular environmental monitoring, and the promotion of IPM and biopesticide use to protect aquatic life and ecosystem sustainability.

5. Future Research Directions

Future research should focus on the chronic effects of azoxystrobin exposure, particularly on fish reproduction, growth, and survival under environmentally relevant concentrations. Long-term studies examining bioaccumulation and trophic transfer in freshwater and marine ecosystems are essential for understanding their persistence and potential ecological consequences. Investigating detoxification mechanisms, such as the upregulation of antioxidant enzymes and liver biotransformation pathways, may help identify adaptive responses and inform mitigation strategies. Additionally, expanding toxicological evaluations to include a wider range of ecologically significant fish species and marine invertebrates will provide a more comprehensive understanding of the environmental impact of azoxystrobin and support more effective regulatory frameworks for aquatic ecosystem protection.

6. Conclusion

The present study clearly establishes that azoxystrobin exerts significant sublethal toxicity on *C. idella*, impacting respiratory, haematological, oxidative, and neurological functions. A marked reduction in oxygen consumption of 43.5% in the high-dose group over 96 hours confirmed impaired respiratory metabolism. Haematological alterations, including a 40% decrease in RBC count and a 34% reduction in haemoglobin levels, indicate anaemia and reduced oxygen-carrying capacity. Oxidative stress was evidenced by elevated ROS and lipid peroxidation levels, coupled with significant suppression of the antioxidant enzymes SOD and CAT ($p < 0.001$). Neurobehavioral changes, such as erratic swimming and loss of equilibrium, were directly linked to 49% inhibition of AChE activity, confirming neuromuscular toxicity.

These integrated findings demonstrate that oxygen consumption is a sensitive and early biomarker of pesticide-induced stress. This study underscores the ecological risks associated with azoxystrobin runoff and highlights the urgent need for stricter pesticide regulations, regular monitoring of aquatic contaminants, and adoption of environmentally sustainable pest control practices.

7. Acknowledgements

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9. Ethical Statement

This study was conducted in strict accordance with the ethical standards for animal research outlined by the Institutional Animal Ethics Committee (IAEC), Acharya Nagarjuna University. All experimental procedures involving fish handling, exposure, and sampling followed the National Guidelines for the Care and Use of Laboratory Animals and adhered to international ethical standards for animal welfare in scientific research. Efforts were made to minimise stress and discomfort to the experimental animals throughout the study.

10. Author Contributions

Gopal Anapana – Conceptualisation, methodology, data collection, formal analysis, writing – original draft. Venkata Rathnamma V – Supervision, Project Administration, Resources, Writing – Review and Editing.

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