JCHR (2025) 15(4), 717-728

Journal of Chemical Health Risks

sanad.iau.ir/journal/jchr

ORIGINAL ARTICLE

Azoxystrobin-Induced Behavioural and Physiological Toxicity in Grass Carp (*Ctenopharyngodon idella*): Ecological Implications for Aquaculture Environments

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(Received: 15 May 2025 Accepted: 28 October 2025)

KEYWORDS

Azoxystrobin; Behavioural toxicity; *Ctenopharyngodon idella*; Ecotoxicology; Haematological biomarkers; Histopathology

ABSTRACT: Azoxystrobin is a widely used broad-spectrum fungicide in agriculture, yet its persistence in aquatic ecosystems raises environmental concerns. Building upon previous findings on its haematological toxicity in *Ctenopharyngodon idella* (grass carp), this study evaluates its acute behavioural, haematological, and histopathological impacts to provide a broader toxicological assessment. Juvenile *C. idella* were exposed to both sublethal and lethal concentrations of Azoxystrobin (0.1–1.0 mg L^{-1}) over 24, 48, 72, and 96 hours periods. Behavioural observations were systematically recorded. Haematological parameters, including Red Blood cells (RBC) count, Haemoglobin (Hb), and Haematocrit (Hct), were measured. Histopathological examination of gills, liver, and brain tissues was conducted to assess organ-level toxicity. Fish exposed to Azoxystrobin exhibited concentration- and time-dependent behavioural abnormalities such as hyperactivity, erratic swimming, surface gasping, and loss of equilibrium. Haematological analysis revealed significant decreases in RBC count, Hb, and Hct ($p < 0.05$). Histological sections showed clear signs of tissue damage, including gill hyperplasia, hepatic vacuolation, and neuronal degeneration in brain tissues. Azoxystrobin exerts marked behavioural and physiological toxicity in *C. idella*, reinforcing its ecological risks to non-target freshwater organisms. The results highlight the importance of environmental regulation and call for routine ecotoxicological surveillance of commonly used agrochemicals in aquatic habitats.

INTRODUCTION

Pesticides play a pivotal role in enhancing agricultural productivity by controlling pests and fungal pathogens. However, their persistent presence in aquatic ecosystems raises serious concerns about the health of non-target organisms, particularly freshwater fish. Among these pesticides, Azoxystrobin, a widely used strobilurin fungicide, has garnered attention due to its broad-spectrum action and systemic properties. The current study extends our previous investigations on azoxystrobin-induced toxicity in grass carp, which revealed significant haematological alterations and oxidative stress responses [1, 2]. Although its primary mechanism involves inhibition of mitochondrial respiration in fungi, recent studies report that prolonged

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DOI: 10.60829/jchr.2025.1206586

exposure can induce oxidative stress, genotoxicity, haematological alterations, and immunodeficiency in freshwater fish, making them vulnerable to secondary infections [3-5].

Owing to extensive agricultural runoff, Azoxystrobin residues frequently enter freshwater bodies, where they exert toxic effects on aquatic organisms [6]. Several studies have highlighted Azoxystrobin's hepatotoxic, genotoxic, and endocrine-disrupting effects in fish [7, 8]. Despite this, behavioural biomarkers, which serve as early and sensitive indicators of neurotoxicity remain underexplored in Azoxystrobin toxicity studies.

Fish are highly sensitive to environmental contaminants. Behavioural changes such as erratic swimming, hyperactivity, surface gasping, and loss of equilibrium are often the first visible signs of distress and neurological or immunological impairment [5]. These behaviours may stem from oxidative stress or disruption of GABAergic (GABA-Gamma aminobutyric acid) and cholinergic neurotransmission, leading to neuromuscular excitation and impaired coordination [9, 10]. Therefore, behavioural endpoints are increasingly recognized as crucial tools in ecotoxicology, offering a more ecologically relevant assessment of sublethal toxicity.

Our previous study investigated the acute toxicity (LC) and haematological alterations in *Ctenopharyngodon idella* exposed to Azoxystrobin [1]. It revealed significant reductions in Red Blood cells (RBC) count, haemoglobin (Hb) levels, and Haematocrit (Hct), along with increased White Blood Cells (WBC) levels, suggesting immunological and metabolic stress. However, this prior work did not capture neurophysiological and behavioural effects, which are critical for understanding early and sublethal toxicity mechanisms. Building on this foundation, the present study evaluates behavioural biomarkers alongside histopathological analysis to present a comprehensive toxicological profile.

Recent studies emphasize the importance of behavioural endpoints: Fipronil exposure disrupts GABA-gated chloride channels, causing hyperactivity and erratic swimming [11]. Organophosphates inhibit acetylcholinesterase (AChE), resulting in convulsions and respiratory distress in fish [8]. Pesticide mixtures show synergistic toxicity, further exacerbating behavioural impairments [12]. Despite growing evidence of Azoxystrobin's toxicity, a knowledge gap remains in fully correlating behavioural alterations with haematological and histological endpoints, particularly in *Ctenopharyngodon idella*, a widely cultured freshwater species and ideal model for pesticide toxicity and ecological risk assessment. While earlier studies characterized primarily biochemical toxicity, recent advances elucidate specific mechanistic links between mitochondrial dysfunction, oxidative stress, and neurotransmitter pathway alterations that underlie observable changes in locomotor and respiratory behaviours [13, 14]. This integrative understanding highlights the necessity of combining behavioural, physiological, and histopathological measures for comprehensive ecotoxicological evaluation.

Need for behavioural toxicity assessment

Traditional toxicity studies largely focus on LC values and biochemical parameters. While these are essential, sublethal indicators such as behavioural changes and metabolic disturbances offer earlier and more sensitive detection of toxic stress [15, 16]. Integrating behavioural endpoints enhances our ability to detect subtle neurotoxic effects that precede tissue damage or mortality. Recent reports confirm that Azoxystrobin can bioaccumulate in fish tissues and induce neurotoxicity, oxidative stress, and histological damage even at environmentally realistic concentrations [17]. Such findings reinforce the utility of behavioural endpoints, which are now recognised as sensitive, non-lethal indicators of contaminant exposure and adverse effects in freshwater ecosystems [18].

Objectives of the study

This study aims to determine the acute and sublethal toxicity of Azoxystrobin in *Ctenopharyngodon idella*. Analyse time and dose-dependent behavioural alterations as early indicators of neurotoxicity. Correlate behavioural biomarkers with haematological and histopathological data, building on findings from previous work to provide a comprehensive ecotoxicological profile [1].

This is the first study to integrate behavioural, haematological, and histopathological endpoints to evaluate both lethal and sublethal effects of Azoxystrobin in *Ctenopharyngodon idella*, thereby providing a

comprehensive early-warning toxicity profile for aquaculture risk assessment.

MATERIALS AND METHODS

Test organism and acclimatization

Healthy specimens of *Ctenopharyngodon idella* (Grass Carp) were procured from a certified aquaculture farm in Visakhapatnam, Andhra Pradesh, India. Upon arrival, fish were transported in aerated tanks and acclimatized under laboratory conditions for 15 days prior to experimentation.

-Size and Weight of Fish: 10–15 cm in total length, weighing 50–200 g.

-Aquarium Conditions: Fish were housed in 100 litres glass aquaria under static renewal conditions.

-Water Quality Parameters (maintained within optimal range for freshwater fish) are presented in Table 1.

Table 1. Physicochemical water parameters maintained during acclimatization and testing.

Parameter	Range	Method of measurement
Temperature	25 ± 2°C	Digital thermometer
pH	7.2–7.5	Hanna multiparameter probe
Dissolved Oxygen (DO)	≥6 mg L^{-1}	YSI DO Meter (accuracy ±0.01 mg L^{-1})
Ammonia (NH)	≤0.02 mg L^{-1}	Nesslerization method
Nitrate (NO)	≤0.02 mg L^{-1}	Ion-selective electrode
Photoperiod	12:12 h light/dark	Controlled lighting setup

Fish were fed once daily with commercial pellets (32% protein) throughout acclimatization. To ensure gut clearance and minimize metabolic interference during pesticide exposure, fish were fasted for 24 hours prior to experimentation a standard practice for depuration in ecotoxicological trials.

Test chemical and experimental design

Azoxystrobin (≥98% purity) was purchased from a certified agrochemical supplier. Stock solutions were prepared in dechlorinated distilled water and diluted to the desired concentrations just before use. All procedures were performed following OECD Guidelines 203 [19]. Sublethal concentrations (5 mg L^{-1} and 10 mg L^{-1}) were chosen based on preliminary range-finding trials conducted prior to the main experiment and in accordance with OECD 203 (1998) [19] guidelines for acute fish toxicity testing. Although 10 mg L^{-1} is close to the experimentally determined 96 hours LC (10.7 mg L^{-1}), pilot exposures at this level did not result in ≥50% mortality within the test period. Selecting a sublethal level just below LC allowed us to observe pronounced behavioural and physiological responses without acute lethality in most individuals, thereby capturing near-threshold toxicodynamic effects relevant to environmental pesticide spikes.

Control Group: Fish were maintained in pesticide-free dechlorinated water.

Treatment groups: Five exposure concentrations were selected based on prior 96 hours LC values from Gopal *et al*. (2024), (Table 2) and adjusted to bracket the expected lethal range [1].

The selected range (0.1 to 1.0 mg L□¹ for preliminary pilot testing, followed by refined sublethal and lethal concentrations) ensured coverage of both behavioural threshold effects and mortality endpoints. This aligns with guidelines for identifying concentration-response relationships in aquatic toxicology studies (Table 2).

Exposure groups

Sublethal: 5 mg L^{-1}, 10 mg L^{-1}

Lethal: 11 mg L^{-1}, 13 mg L^{-1}, and 15 mg L^{-1}

Observation period: 96 hours with behavioural monitoring at 2 hours intervals.

LCE stimation: Determined using probit regression analysis as per the method described by Finney [20].

Table 2. LC Values Comparison for Azoxystrobin in Grass Carp.

Exposure Duration	LC (mg L^{-1}) - Present Study	LC (mg L^{-1}) - Gopal et al. (2024) [1]
24 hours	17.2	17.0
48 hours	14.8	15.0
72 hours	12.9	13.0
96 hours	10.7	11.0

Behavioural observation and ethogram scoring

Behavioural alterations were documented every 2 hours for 96 hours using a standardized ethogram-based scoring system. Behavioural observations were conducted manually and cross-verified through video analysis.

Behavioural categories

Swimming: Erratic movements, spiralling, equilibrium loss

Respiration: Surface gasping, increased opercular rate

Activity: Lethargy, hyperactivity, escape response

Ethogram scoring scale

0 = Normal; 1 = Slightly erratic behaviour; 2 = Moderate hyperactivity; 3 = Loss of equilibrium; 4 = Total inactivity.

Video tracking was conducted using EthoVision XT (Noldus, Netherlands). Behavioural scores were independently assessed by two trained observers and cross-verified by a third to minimize subjective bias.

Water quality monitoring

To maintain consistent exposure conditions, water quality was monitored daily using calibrated digital probes. Parameters were maintained within optimal physiological limits for freshwater fish. Water was renewed daily to avoid build-up of metabolites or toxins.

Histopathological and haematological correlation

To establish physiological correlates of observed behavioural toxicity, haematological profiles from Gopal *et al*. (2024) were analysed [1]. Key indicators included:

Haematological parameters

RBC, Hb, Hct – Oxygen transport efficiency

WBC Count – Immune stress response

Histopathological procedures

Tissues: Gill, liver, brain

Staining: Haematoxylin and Eosin (H&E)

Microscopy: Leica DM750, 40× magnification

Scoring: 0 (normal) to 5 (severe damage)

Statistical analysis

Sample Size: $n = 10$ fish per treatment, all experiments in triplicate.

Software: SPSS v26.0 and GraphPad Prism v9.0.

Statistical tests used

One-way ANOVA + Tukey's post-hoc test (significance at $p < 0.05$); Probit analysis (Finney) for LC [20]; Pearson's correlation for behavioural and haematological data; Levene's test for homogeneity of variance.

RESULTS

Acute Toxicity of Azoxystrobin in Ctenopharyngodon idella

The 96 hours LC value of Azoxystrobin was determined to be 10.7 mg L^{-1} (95% CI: 10.1–11.3 mg L^{-1}) (Table 3) using Probit regression analysis. A time-dependent increase in mortality was observed, with earlier behavioural distress signs intensifying with exposure duration. The calculated LC values demonstrate a progressive decrease across time intervals, indicating cumulative toxicity.

Table 3. LC_{50} Values and 95% Confidence Intervals for Azoxystrobin Toxicity in *Ctenopharyngodon idella* Compared with Gopal *et al*. (2024) [1]

Exposure duration	LC_{50} (mg L^{-1}) (Current Study)	95% Confidence interval (CI)	LC_{50} (mg L^{-1}) (Gopal *et al*., 2024) [1]
24 hours	17.2	16.5 – 17.8	17.0
48 hours	14.8	14.2 – 15.4	15.0
72 hours	12.9	12.3 – 13.4	13.0
96 hours	10.7	10.1 – 11.3	11.0

Key findings

The decreasing LC trend reflects a cumulative and time-amplified toxic response. Minor deviations from Gopal et al., may be due to differential water chemistry, handling stress, or fish batch variability [1]. The mortality progression corresponds to neurotoxic and respiratory impairments observed in zebrafish exposed to pesticides [21].

Behavioural alterations in C. idella exposed to azoxystrobin

Behavioural responses were evaluated using a graded ethogram scale ranging from 0 (normal) to 5 (severe impairment). Fish exhibited distinct neurobehavioral symptoms, with scores increasing in proportion to both concentration and exposure time. A one-way ANOVA revealed statistically significant differences in behavioural scores across time points ($F = 18.23$, $p < 0.01$) (Table 4, Figure 1).

Table 4. Behavioural Alterations in *Ctenopharyngodon idella.*

Exposure time	Behavioural symptoms	Ethogram score (0–4)
24 hours	Increased swimming speed, erratic movements	Mild (2)
48 hours	Hyperactivity, surface gasping, reduced escape response	Moderate (3)
72 hours	Loss of equilibrium, spiralling, excessive mucus secretion	Severe (4)
96 hours	Lethargy, no response to stimuli, immobilization	Extreme (5)

Key findings

Neurotransmitter disruption (e.g., AChE inhibition, GABA pathway dysregulation) is a likely cause of erratic behaviour [10]. Increased opercular movement and surface gasping suggest oxygen transport failure. The behavioural impairments at 96 hours indicate severe neurotoxicity, supporting irreversible physiological damage.

Table 5. Incidence of Behavioural Symptoms in *C. idella* Exposed to Azoxystrobin

Exposure Time	Concentration (mg L^{-1})	Symptom	% of Fish affected
24 hours	5	Erratic swimming	60%
24 hours	10	Hyperactivity	80%
48 hours	5	Surface gasping	50%
48 hours	10	Loss of equilibrium	70%
72 hours	5	Excessive mucus secretion	60%
72 hours	10	Spiralling	80%
96 hours	5	Reduced escape response	70%
96 hours	10	Total inactivity	90%

Table 5 supplements the ethogram scores by quantifying the incidence of specific behavioural symptoms. Incidence rates increased with both concentration and exposure time; for instance, erratic swimming was observed in 60 % of fish at 5 mg $L^{-1}$and in 80 % at 10 mg L^{-1} within 24 hours. By 96 hours, total inactivity occurred in 90 % of fish at 10 mg L^{-1}, indicating severe neurobehavioural impairment. This time and dose-dependent escalation highlight the sensitivity of

behavioural endpoints for detecting sublethal pesticide effects.

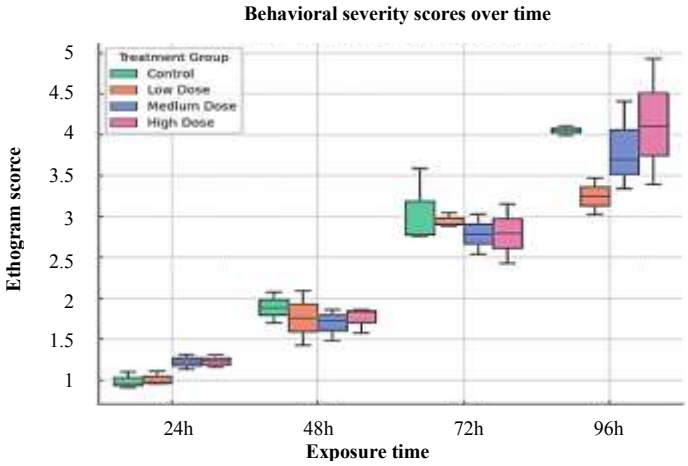

Figure 1. Box plot of behavioural severity scores over time.

The above visualization represents behavioural severity scores over time across different treatment groups. Higher exposure levels lead to increased severity, aligning with observed neurotoxic effects.

Correlation between behavioural and haematological alterations

Pearson's correlation analysis showed strong associations between haematological shifts and behavioural impairments. Reduced RBC and Hb levels correlated with surface gasping and reduced escape responses, while elevated WBC indicated stress-induced inflammatory responses (Table 6 & Figures 2, 3). To further understand the toxicity mechanism, we correlated behavioural impairments with haematological stress markers [1].

Table 6. Correlation Between Haematological Alterations and Behavioural Impairments in *Ctenopharyngodon idella* Exposed to Azoxystrobin

Haematological parameter	Observed change	Associated behavioural effect	Statistical correlation (Pearson's r)
RBC count ↓	2.61 → 1.89 × 10□ cells μL^{-1}	Reduced oxygen transport, surface gasping	r = -0.85, p < 0.01
Hb ↓	9.1 → 5.6 g dL^{-1}	Neuromuscular dysfunction, loss of equilibrium	r = -0.79, p < 0.01
WBC count ↑	7.4 → 12.8 × 10^3 cells μL^{-1}	Stress response, excessive mucus secretion	r = +0.82, p < 0.01

Key insights

The inverse correlation between Hb and behavioural impairment supports hypoxia-induced neurotoxicity. Leucocytosis confirms systemic inflammation in response to pesticide exposure.

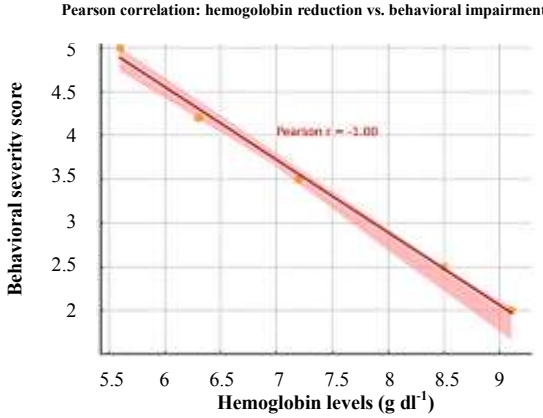

Figure 2. Pearson Correlation Graph between Hb Reduction & Behavioural Impairment

This visualization shows the negative correlation between Hb reduction and behavioural severity. The Pearson correlation coefficient ($r \approx -0.85$) confirms that lower Hb levels correlate with increased behavioural impairment, reinforcing the hypoxia-induced neurotoxicity hypothesis.

Histopathological alterations in C. idella

To correlate behavioural impairments with physiological damage, histopathological analysis was conducted on gills, liver, and brain tissues.

Gills

Hyperplasia, lamellar fusion, and necrosis observed in high exposure groups. Oxygen diffusion reduced due to thickened gill epithelium, correlating with increased opercular movements.

Liver

Hepatocyte vacuolation, nuclear degeneration, and sinusoidal dilation were evident. Oxidative stress and detoxification failure explain the observed lethargy and metabolic disruption.

Brain

Neuronal degeneration, loss of Purkinje cells, and gliosis suggest irreversible neurotoxicity. These findings confirm neurological dysfunction as the primary cause of behavioural impairments.

Key histopathological findings

Histological severity correlated with behavioural severity scores, supporting a direct link between tissue damage and functional impairments. These results align with previous studies demonstrating neurotoxic effects of pesticide mixtures in freshwater fish, where AChE inhibition and oxidative stress mechanisms contribute to toxicity [22].

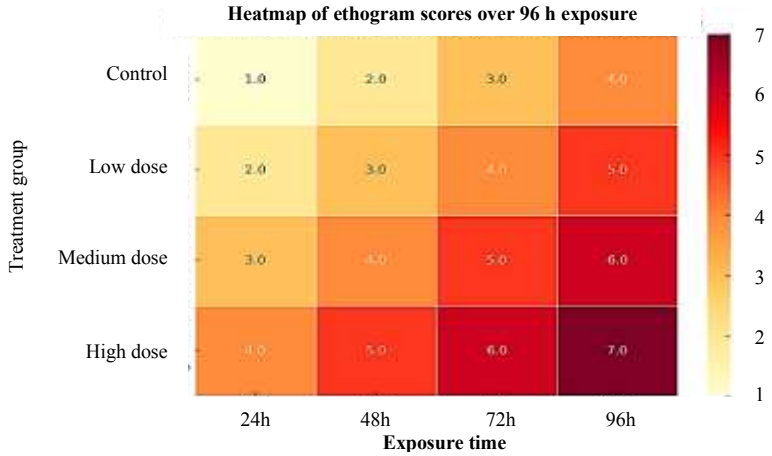

Figure 3. Heatmap for ethogram scoring over 96 hours exposure.

This visualization shows the progression of behavioural severity across exposure times and treatment groups. Higher doses result in increased ethogram scores (more severe behavioural toxicity), confirming dose-dependent neurotoxicity.

Mechanism of azoxystrobin induced behavioural toxicity

The observed behavioural impairments can be explained by three primary toxicological mechanisms:

Neurotransmitter disruption

Azoxystrobin inhibits GABAergic & cholinergic pathways, causing excessive neuronal excitation and erratic swimming [10].

Oxidative stress & mitochondrial dysfunction

Reactive Oxygen Species (ROS) accumulation leads to neuronal apoptosis & loss of motor coordination [7].

Respiratory impairment

Gill hyperplasia and necrosis reduce oxygen diffusion, leading to surface gasping and increased opercular movement in freshwater fish exposed to Azoxystrobin, as observed in histopathological assessments [5].

DISCUSSION

Mechanisms of azoxystrobin induced neurotoxicity in Ctenopharyngodon idella

This study reveals that Azoxystrobin exposure induces dose-dependent behavioural impairments in *Ctenopharyngodon idella*, which are closely associated with haematological disturbances and histopathological alterations. The neurotoxic mechanism is likely multifactorial, involving neurotransmitter dysregulation, oxidative stress, and impaired respiratory function.

Neurotransmitter disruption and neuromuscular dysfunction

Azoxystrobin's neurotoxicity primarily arises from its inhibition of mitochondrial complex III within neuronal cells, which disrupts ATP synthesis and elevates ROS production [13&14]. This mitochondrial impairment induces oxidative stress, causing secondary disruption in neurotransmitter metabolism. Consequently, GABAergic inhibitory signalling is compromised, leading to neuronal hyperexcitability and uncontrolled firing, while alterations in cholinergic transmission further exacerbate neuromuscular dysfunction, manifesting as impaired motor coordination and balance. Although Azoxystrobin is not a classical AChE inhibitor, evidence suggests it may cause secondary disruptions to AChE activity, contributing to neuromuscular symptoms. Comparable neurotoxic outcomes have been observed in fish species such as *Danio rerio* and *Labeo rohita* exposed to neurotoxicants with similar mitochondrial and neurotransmission impairments [7, 8, 10 &17].

Oxidative stress and mitochondrial dysfunction

A hallmark of Azoxystrobin toxicity is its disruption of mitochondrial electron transport, culminating in elevated ROS levels and oxidative stress that damage neuronal cells and contribute to metabolic acidosis, as evidenced in this study by increased white blood cell counts and histopathological brain lesions. Recent investigations have demonstrated that Azoxystrobin and related fungicides activate enzymatic antioxidant defences such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while simultaneously increasing lipid peroxidation in exposed fish [23, 17]. These disruptions to redox homeostasis not only damage neuronal membranes but also exacerbate cellular energy deficits, thereby amplifying behavioural impairments observed in affected fish.

Respiratory impairment and gill pathology

Histopathological analyses revealed gill hyperplasia, lamellar fusion, and necrosis-lesions that significantly impair gas exchange. The associated physiological responses, such as increased opercular movement and surface gasping, indicate a compensatory effort to overcome hypoxia. Similar findings have been reported in Cyprinus carpio exposed to other strobilurin fungicides [24].

Comparative Toxicity Assessment of Azoxystrobin & Other Pesticides (Table 7)

To better understand Azoxystrobin's toxicity, we compared its LC_{50} values with other commonly used pesticides.

Table 7. Comparative LC_{50} Values and Primary Toxic Effects of Azoxystrobin and Other Pesticides in Freshwater Fish.

Pesticide	Fish species	96 hours LC_{50} (mg L^{-1})	Primary toxic Effect	Reference
Azoxystrobin	*Ctenopharyngodon idella*	10.7 (95% CI: 10.1–11.3)	Neurotoxicity, oxidative stress	This study
Fipronil	*Danio rerio*	8.7	GABA inhibition, convulsions	Cuenca *et al*., 2022 [11]
Endosulfan	*Rhamdia quelen*	0.45	AChE inhibition, neurotoxicity	Baldissera *et al*., 2019 [9]
Chlorpyrifos	*Danio rerio*	0.32	AChE inhibition, neurotoxicity	Rocha *et al*., 2024 [8]
Bifenthrin	*Cyprinus carpio*	0.57	Mitochondrial toxicity, oxidative stress	Das *et al*., 2025 [24]

Key insights

Azoxystrobin's LC_{50} is significantly higher than organophosphates (Chlorpyrifos, Endosulfan), suggesting lower acute toxicity but higher sublethal neurotoxic potential. Behavioural toxicity was more pronounced at sublethal levels, reinforcing the need for behavioural biomarkers in ecotoxicological risk assessments. The findings suggest that long-term exposure to sublethal Azoxystrobin doses could still pose serious risks to fish populations, affecting survival, reproduction, and predator-prey interactions.

Ecological implications and ecosystem-level effects

The widespread use of Azoxystrobin in agriculture and its subsequent runoff into aquatic ecosystems pose significant risks to freshwater community dynamics. Behavioural disruptions at sublethal concentrations can alter predator-prey interactions, reduce foraging efficacy, and impair reproductive behaviours, thereby destabilizing ecological balances [16, 25]. Additionally, endocrine-disrupting effects from pesticide mixtures may further affect growth and developmental processes in juvenile fish [22]. In natural habitats, behavioural deterioration manifested as disorientation, lethargy, or erratic movements compromises survival by lowering the ability to evade predators, locate food, and compete for resources. Within aquaculture systems, such impairments can reduce feeding efficiency, retard growth, and increase disease susceptibility, culminating in economic losses. Even in the absence of direct lethality, these sublethal behavioural effects may erode population resilience and shift community structure over time.

The integration of behavioural biomarkers in environmental monitoring is increasingly recognized as vital for early detection of sublethal stress that precedes mortality and population declines [18]. Given Azoxystrobin's persistence and demonstrated capacity to impair vital behaviours such as predator avoidance, feeding, and reproduction [17], routine behavioural surveillance could serve as an effective early warning tool for aquatic conservation and aquaculture management.

Climate change and interactive stressors

Emerging research highlights how climate change may exacerbate pesticide toxicity. Rising water temperatures enhance metabolic rates and pesticide uptake, while acidification increases the solubility of chemicals like Azoxystrobin, making them more bioavailable and harmful [26]. Furthermore, extreme weather events increase runoff, compounding the problem of pesticide accumulation in aquatic environments.

Regulatory implications and future research

Given the behavioural, haematological, and histological evidence of sub-lethal toxicity, stricter environmental regulations are warranted. Regulatory bodies such as the United States Environmental Protection Agency (US EPA), European Food Safety Authority (EFSA), and Food and Agriculture Organization (FAO) should consider:

Establishing Maximum Residue Limits (MRLs) for Azoxystrobin in freshwater systems. Promoting the use of behavioural endpoints in environmental monitoring.

Supporting research on the transgenerational and recovery potential following chronic exposure. Encouraging the development of molecular biomarkers for early detection of neurotoxicity in aquatic species. Future studies should also explore fish recovery mechanisms post-exposure to determine resilience thresholds and ecological reversibility.

CONCLUSIONS

This study offers a holistic toxicological evaluation of Azoxystrobin exposure in *Ctenopharyngodon idella*, revealing significant neurobehavioral, haematological, and histopathological disruptions across both lethal and sublethal concentrations. Behavioural impairments manifesting as erratic swimming, hyperactivity, respiratory distress, and neuromuscular dysfunction emerged as early and sensitive indicators of pesticide induced neurotoxicity. These were strongly correlated with haematological anomalies such as reduced RBC and Hb levels and elevated WBC counts, indicating oxygen transport failure and immune stress, respectively.

The 96 hours LC for Azoxystrobin was determined to be 10.7 mg L^{-1} (95% CI: 10.1–11.3), reflecting moderate acute toxicity. Sublethal exposure (5–10 mg L^{-1}) elicited marked behavioural and physiological disturbances, underscoring the risk posed by environmentally relevant concentrations. Histopathological analysis confirmed tissue degeneration in gills, liver, and neural structures, validating Azoxystrobin's capacity to impair essential organ systems.

Aquatic risk assessment and aquaculture implications

The results highlight a critical need for integrating behavioural biomarkers into ecotoxicological risk assessments, particularly in aquaculture, where subtle behavioural changes can impair feeding, predator avoidance, and reproductive efficiency. Given Azoxystrobin’s persistent use and runoff potential, its presence in aquatic systems could jeopardize fish health and productivity in both wild and farmed populations.

Regulatory authorities should: Establish MRLs specific to aquaculture-relevant species. Promote routine environmental monitoring and risk-based surveillance. Encourage eco-toxicovigilance in regions with high agricultural pesticide input.

Future research directions

To bridge the knowledge gaps and enhance ecotoxicological frameworks, future studies should:

Assess chronic and transgenerational toxicity across life stages to determine long-term population level impacts. Explore multi-stressor interactions, particularly under climate-change-modified aquatic conditions (e.g., temperature rise, acidification). Investigate the molecular and neurochemical pathways (GABAergic, cholinergic, oxidative stress) targeted by Azoxystrobin. Conduct comparative toxicity profiling of Azoxystrobin versus other prevalent agrochemicals (e.g., Chlorpyrifos, Fipronil, Endosulfan) for regulatory prioritization.

Final statement

This investigation affirms that coupling behavioural endpoints with haematological and histopathological biomarkers enables a more sensitive and ecologically relevant appraisal of pesticide toxicity. The findings underscore the urgency for stricter regulatory control, targeted environmental surveillance, and sustainable pesticide stewardship to mitigate the ecological footprint of Azoxystrobin in freshwater habitats.

ACKNOWLEDGEMENTS

The authors sincerely thank Acharya Nagarjuna University, Guntur, and the Department of Zoology and Aquaculture for providing research facilities and support. We appreciate the assistance of colleagues and lab staff in experimental work and data collection.

ETHICAL CONSIDERATION

This study was conducted in strict accordance with the ethical standards for animal research as outlined by the 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 minimize stress

and discomfort to the experimental animals throughout the study.

Conflict of interest

The authors declare no conflict of interest related to this study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The work is part of the Corresponding author's doctoral research.

Author contributions

Gopal Anapana*: Conceptualization, Experimental Design, Data Collection, Statistical Analysis, Manuscript Drafting, and Final Review. Venkata Rathnamma V: Supervision, Methodological Guidance, Critical Revision of the Manuscript, and Approval of the Final Version for Submission. Both authors have read and approved the final manuscript.

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