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Behavioral biomarkers of isoproturon toxicity in freshwater carp *Cyprinus carpio* (L.)

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Abstract
The present study aimed to investigate the toxic effects of the herbicide Isoproturon on *Cyprinus carpio* (L.) with a particular focus on behavioral alterations that serve as early and sensitive biomarkers of stress. Fish were exposed acutely for 96 hours to a sub-lethal concentration of 1.65mg/L, and their behavior was carefully monitored at 24-hour intervals (24, 48, 72, and 96 hours). Throughout the exposure period, the fish exhibited a range of noticeable disturbances, including darkening of body pigmentation, excessive mucus secretion, erratic swimming, hyperactivity, and occasional loss of equilibrium. These symptoms reflect the fish's physiological attempts to cope with the toxicant pigmentation changes indicating stress, mucus secretion acting as a defensive response, and disorientation suggesting impaired neuromuscular coordination. Importantly, the severity and frequency of these behavioral anomalies increased steadily with prolonged exposure, demonstrating a clear time-dependent relationship. Such progressive deterioration highlights the disruptive impact of Isoproturon on the nervous and physiological systems of *C. carpio*. Overall, the results reinforce the value of behavioral biomarkers in environmental toxicology.

Keywords: Behavioral alterations, Toxicity, Herbicide, Isoproturon, *Cyprinus carpio* (L.), Swimming patterns

Introduction
Herbicides often spread beyond their intended application zones through environmental pathways such as wind drift and rainfall runoff, ultimately entering aquatic systems like lakes and rivers. A substantial portion of the herbicides used in agriculture ends up contributing to environmental pollution, where they adversely affect non-target species. Exposure to herbicide residues has been associated with changes in fish behavior, inhibited growth, and impaired reproduction, which can alter population structure and reduce biodiversity (van der Oost *et al.,* 2003; Velmurugan *et al.,* 2009) [18, 20]. Numerous studies have documented the harmful impacts of herbicides on aquatic ecosystems, showing that rising levels of chemical contaminants cause significant toxicological stress in aquatic organisms (Livingstone, 2001; Matsumoto *et al.,* 2006) [11, 13].
Isoproturon is a widely used phenyl-urea herbicide belonging to the substituted urea class of chemicals (Devika Rani and Parimala, 2025) [3]. India has emerged as one of the top five global markets for agrochemicals, with production increasing at an estimated rate of 14% per year, partly due to excise-duty-free zones established by central and state governments that encourage large-scale manufacturing (Sorensen *et al.,* 2003; Bhatt *et al.,* 2022) [1, 16]. The unintentional release of non-biodegradable herbicide residues into aquatic environments further aggravates pollution problems and can disrupt the natural structure and functioning of ecosystems (Grollier *et al.,* 1997; Knauert *et al.,* 2010) [7, 9, 10].
Isoproturon exhibits high water solubility, low volatilization, and considerable persistence in aquatic systems, making it a potential environmental contaminant. Behavioral bioassays in fish provide a rapid, sensitive, and ecologically relevant tool for assessing toxic effects, offering advantages over growth or reproduction-based assays that require longer durations. These behavioral changes often serve as early warning signals of environmental stress that may not be detected by traditional lethality tests (Madhu Sharma, 2019) [12, 15].
Fish are extremely responsive to even minimal levels of contaminants, making them valuable sentinel organisms for assessing environmental pollution. Their capacity to accumulate toxic substances, their central role in aquatic food chains, and their importance as a human food source further strengthen their significance in ecotoxicological research, as highlighted by (Madhu Sharma, 2019) [12, 15]. In the present study, *Cyprinus carpio* was chosen to assess behavioral alterations as indicators of sub-lethal Isoproturon exposure. According to (Devika Rani and Parimala, 2025) [3] this work aims to examine how Isoproturon affects fish behavior and to offer a clearer understanding of its possible ecological consequences.

Materials and Methods
Healthy and active *Cyprinus carpio* (L.) fingerlings were obtained from the Turvekere Fisheries Farm in Turvekere, Tumakuru. After being brought to the laboratory in well-aerated containers, the fish were placed in 25-L tubs and allowed to acclimatize for 30 days. Throughout this period, they were fed commercial dry pellet feed and maintained under standard husbandry conditions.
The control group followed the same water-change routine but received no Isoproturon. The experimental fish were exposed to Isoproturon for a duration of 96 hours, and their behavior was closely monitored at 24-hour intervals specifically at 24, 48, 72, and 96 hours.
During these observations, a wide range of behavioral changes was assessed in both control and treated fish. These included signs such as increased activity, altered swimming patterns, loss of balance, changes in opercular movement, pigmentation shifts, faster breathing, frequent gulping at the surface, sudden jumping, excess mucus secretion, heightened aggression, periods of immobility, vertical swimming, and repeated surfacing. Together, these

behavioral markers helped reveal the sub-lethal impacts of Isoproturon on *C. carpio* (Devika Rani and Parimala, 2025; Sharma, 2019) [3, 12, 15].

Result

Control fish displayed normal behavior throughout the experiment, including regular swimming, moderate opercular movement, and appropriate responses to mild external stimuli. In contrast, fish exposed to Isoproturon exhibited notable behavioral disturbances. Initially, upon introduction to the herbicide-contaminated water, the fish tended to move toward the corners of the tank, indicating stress. Within a few hours, they became mildly hyperactive and began swimming individually rather than in groups.

After 24 hours of exposure, slight increases in pigmentation and the formation of a thin mucus layer on the body surface were observed. As the duration of exposure increased, more severe behavioral changes developed, including frequent surfacing, hyperventilation, loss of equilibrium, pronounced pigmentation, thick mucus secretion, reduced swimming activity, and diminished responsiveness to external stimuli (Table 1). By the final observation period (96 hours), the effects were most severe, with intense pigmentation, complete loss of equilibrium, excessive mucus production, and frequent surface gulping, indicating strong sub-lethal stress induced by Isoproturon in *C carpio*.

Table 1: Behavioral responses of *C carpio* exposed to 1.65 mg/L Isoproturon

SL. No.	Parameter	Control	24 hrs.	48 hrs.	72 hrs.	96 hrs.
1	Hyperactivity	–	+	+	++	+
2	Operculum movement	++	++	++	++	+
3	Rate of swimming	—	++	++	++	+
4	Corner behavior	—	+	++	++	–
5	Pigmentation	+++	–	–	–	–
6	Mucus secretion	+	+++	+++	+++	–
7	Loss of balance	+	++	++	++	–
8	Surface gulping	–	–	–	–	+

Legend: (–) None, (+) Mild, (++) Moderate, (+++) Strong

Discussion

Behavioral assessment is widely recognized as a sensitive and reliable method for detecting acute chemical exposure in fish (Scott and Sloman, 2004) [14]. Such behavioral shifts may serve as temporary protective strategies that help fish avoid unfavorable environments and fishes may reflect direct toxic impacts on the nervous system. In the present study, *Cyprinus carpio* displayed clear behavioral disturbances when exposed to Isoproturon, particularly noticeable in altered swimming patterns. These locomotor disruptions are often associated with neurological impairment, and phenyl-urea herbicides like Isoproturon are known to interfere with neuromuscular coordination and neurotransmission (Knauert *et al.,* 2010) [9, 10].

Respiratory disturbances observed in the exposed fish may indicate an increased metabolic demand caused by toxic stress. The progressive reduction in swimming activity over time may be linked to higher energy requirements and the depletion of glycogen reserves as the fish attempt to maintain physiological balance (Tripathi and Singh, 2004) [17]. The appearance of aquatic surface respiration at later exposure stages suggests gill impairment and the development of herbicide-induced hypoxic conditions, a common response seen in fish exposed to herbicide (Velisek *et al.,* 2009) [19].

The heavy mucus secretion recorded during exposure likely represents a protective strategy. The mucus layer helps reduce direct contact with toxicants and minimizes irritation caused by chemicals in the surrounding water (Fujii, 2000) [5]. Increased pigmentation observed in the exposed fish indicates possible endocrine disruption, particularly involving the pituitary gland, which regulates chromatophore activity. Similar pigmentation changes have been reported in fish exposed to various herbicides (Fanta *et al.,* 2003) [4].

Previous research has shown that many agricultural chemicals, including herbicides such as atrazine, diuron, and Isoproturon, can trigger abnormal behavioral responses in fish due to their neurotoxic and oxidative effects (Gao *et al.,* 2011; Blais *et al.,* 2016) [2, 6]. Studies across different fish species consistently report comparable behavioral alterations in response to chemical contaminants, reinforcing the importance of behavioral endpoints as early-warning indicators in ecotoxicological studies (Hellou, 2011) [8].

Conclusion

The results of this study clearly show that Isoproturon has a noticeable impact on the behavior of *Cyprinus carpio*, even at sub-lethal levels. The fish displayed several unusual behaviors, such as erratic swimming, reduced movement, imbalance, and changes in feeding activity. These early signs of stress suggest that the fish are highly sensitive to this herbicide, and even low exposure can disrupt their normal functioning. Over time, such behavioral changes can affect how well the fish find food, escape predators, or maintain their overall health, which may eventually influence their survival. Altogether, these findings highlight the potential ecological risks linked to Isoproturon pollution and emphasize the importance of better monitoring and responsible use of herbicides to protect freshwater ecosystems.

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