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Acute toxicity and 96-hour LC_{50} determination of Isoproturon in the freshwater fish *Cyprinus carpio* (L.)

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Abstract
The present study was carried out to determine the 96-hour LC_{50} value of the herbicide Isoproturon in the freshwater fish *Cyprinus carpio*. Acute toxicity experiments were conducted following standard APHA procedures, and the LC_{50} value was calculated using Probit analysis. Healthy fish were acclimatized to laboratory conditions for a period of 30 days before the commencement of the experiment. During acclimatization, suitable water quality parameters such as temperature, pH, and dissolved oxygen were maintained under controlled conditions. A stock solution of Isoproturon was prepared, and different groups of fish were exposed to varying concentrations of the herbicide for 96 hours under semi-static conditions. Throughout the exposure period, several behavioral changes were noticed in the treated fish, including erratic swimming movements, increased mucus secretion, loss of equilibrium, frequent surfacing activity, and reduced feeding behavior. These abnormalities became more prominent with increasing concentration and duration of exposure.

The results revealed that the 96-hour LC_{50} value of Isoproturon for *Cyprinus carpio* was found to be 1.65 mg/L, indicating the toxic nature of the herbicide to freshwater fish. Mortality of the fish increased progressively with higher concentrations of the toxicant, showing a clear dose-dependent response. The observed toxic effects suggest that Isoproturon may cause physiological stress and metabolic disturbances in aquatic organisms even at relatively lower concentrations. The findings of the present investigation highlight the potential ecological risk of herbicide contamination in freshwater ecosystems. The study also provides baseline toxicological information that may be useful for future research related to biochemical, physiological, neurotoxic, and genotoxic alterations induced by Isoproturon exposure in aquatic organisms.

Keywords: 96-hour LC_{50}, *Cyprinus carpio*, Isoproturon, acute toxicity, aquatic pollution, ecotoxicology

Introduction

Freshwater ecosystems are among the most valuable natural resources, supporting biodiversity, fisheries, agriculture, and human livelihoods. However, these ecosystems are increasingly threatened by various pollutants originating from industrial, agricultural, and domestic activities (Smith *et al,* 2019) [25]. Among these contaminants, agrochemicals such as herbicides have gained major environmental concern because of their extensive use in modern agriculture. Large quantities of herbicides enter aquatic environments through agricultural runoff, leaching, spray drift, and atmospheric deposition, where they adversely affect non-target aquatic organisms (Brown and Taylor, 2018; Khatib *et al,* 2022) [4, 14]. Continuous exposure to these chemicals can disturb the ecological balance of aquatic ecosystems by affecting the physiology, metabolism, growth, reproduction, and survival of aquatic organisms (Johnson *et al,* 2020) [13].

Isoproturon, a selective phenyl urea herbicide widely used in cereal crop cultivation for weed control, has been frequently detected in surface water bodies and sediments due to its persistence and moderate solubility in water (Kumar and Singh, 2017; Rao *et al,* 2016) [15, 23]. The persistence of Isoproturon in aquatic environments increases its bioavailability and enhances the possibility of bioaccumulation in aquatic organisms. Prolonged exposure to this herbicide has been reported to induce oxidative stress, disrupt carbohydrate and protein metabolism, alter enzymatic activities, and cause histopathological and behavioral abnormalities in fish and other aquatic organisms (Patil *et al,* 2021; Hernandez and Lopez, 2019) [11, 19]. In addition, Isoproturon exposure may impair cellular respiration and energy metabolism, leading to physiological stress and reduced adaptive capacity in fish.

Fish are considered important bioindicators for assessing aquatic pollution because they respond sensitively to environmental contaminants. Among freshwater fishes, *Cyprinus carpio* (common carp) is extensively used in Ecotoxicological investigations due to its wide distribution, ecological importance, adaptability to laboratory conditions, and sensitivity to toxic substances (Rahman *et al,* 2014) [21]. Since fish occupy an important position in the aquatic food chain, toxicants accumulated in fish tissues may also pose risks to higher trophic levels, including humans. Therefore, studying herbicide toxicity in fish is essential for evaluating the ecological and public health consequences of aquatic contamination. Several studies have highlighted the importance of fish bioaccumulation and biomarker responses in environmental risk assessment, emphasizing their usefulness in monitoring aquatic pollution and toxicant-induced physiological alterations (Van der Oost *et al,* 2003) [26]. In addition, fish have been widely recognized as reliable bioindicators for assessing the effects of environmental contaminants due to their ability to accumulate pollutants and exhibit measurable biochemical, physiological, and behavioral changes following toxic exposure (Authman *et al,* 2015) [3].

Acute toxicity studies play a significant role in determining the harmful effects of pollutants on aquatic organisms. The 96-hour LC_{50} value is one of the most widely accepted parameters for assessing toxicity, representing the concentration of a toxicant capable of causing mortality in 50% of the exposed organisms within a specified period (Anderson and Miller, 2015; Garcia *et al,* 2018) [2, 7]. Such studies provide baseline information for environmental monitoring, pollution control strategies, and safe usage guidelines for agrochemicals.

Exposure to Isoproturon has been associated with several organ-specific toxic effects, particularly in metabolically active organs such as the liver, kidney, and gills, which are primary sites for detoxification and bioaccumulation. Sediment-associated Isoproturon can be absorbed by rooted aquatic macrophytes such as *Elodea densa* and subsequently released into the surrounding water during plant decomposition, resulting in localized contamination pulses (Grollier *et al,* 1997) [9]. Fish exposed to contaminated environments may accumulate the herbicide in muscle and other tissues, exhibiting delayed elimination and prolonged toxic effects (Hernandez *et al*, 2013) [10]. Moreover, sublethal exposure to Isoproturon can induce behavioral and morphological abnormalities such as erratic swimming, hyperactivity, loss of equilibrium, increased mucus secretion, respiratory distress, and body deformities, indicating neurophysiological stress and impaired normal functioning (Devika Rani and Parimala, 2025) [6].

Recent studies have also suggested that herbicides can generate reactive oxygen species (ROS), leading to oxidative damage in cellular components such as lipids, proteins, and nucleic acids. Alterations in antioxidant defense enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) have been observed in herbicide-exposed fish, reflecting biochemical stress responses. Such changes may ultimately affect fish health, growth performance, immune function, and reproductive success.

Although several studies have reported the acute and sublethal toxicity of Isoproturon in aquatic organisms, information regarding the combined influence of sediment accumulation, aquatic macrophytes, and subsequent transfer to fish remains limited. Therefore, the present study was undertaken to determine the 96-hour LC_{50} value of Isoproturon in *Cyprinus carpio* and to evaluate associated behavioral and organ-specific toxic responses. The findings of this investigation may contribute to a better understanding of the ecological risks posed by herbicide contamination in freshwater ecosystems and help in developing sustainable management strategies for herbicide usage and aquatic environmental protection.

Materials and Methods

Healthy fingerlings of *Cyprinus carpio* (L.) were procured from the Turvekere Fisheries Farm, Tumakuru, Karnataka. The fish were carefully transported to the laboratory in large aerated plastic containers in order to minimize handling stress and maintain sufficient oxygen supply during transportation. On arrival at the laboratory, the fish were gently transferred into 25-L capacity tubs containing dechlorinated tap water and acclimatized to laboratory conditions for 30 days. During acclimation, dead or injured fish, if any, were removed immediately to maintain healthy stock conditions. The fish were regularly fed with commercially available dry pellet feed to ensure proper nutrition, growth, and adaptation to the laboratory environment.

The experimental fish measured approximately 5–6 cm in length and weighed around 3.5–4.5 g. To stabilize physiological conditions before experimentation, the fingerlings were subjected to an additional acclimation period of 20 days under controlled laboratory conditions. The acclimation tubs were continuously aerated using air pumps to maintain adequate dissolved oxygen levels throughout the study period.

Physicochemical characteristics of the test water were analyzed according to standard APHA (2005) [1] procedures. The recorded water quality parameters included temperature 24 ± 2 ºC, pH 6.9 ± 0.2, dissolved oxygen 9.4 ± 0.8 mg/L, free carbon dioxide 6.3 ± 0.4 mg/L, total hardness 23.4 ± 3.4 mg as $CaCO_3$/L, phosphate 0.39 ± 0.002 µg/L, salinity nil, specific gravity 1.001, and conductivity below 10 µS/cm. These parameters were maintained within permissible limits throughout the acclimation and experimental periods to avoid additional environmental stress on the fish. Fresh dechlorinated water was renewed daily in order to maintain water quality and reduce the accumulation of metabolic wastes. A photoperiod of 12 h light and 12 h dark was maintained consistently throughout the study.

During acclimation and experimentation, the fish were fed once daily with commercial pellet feed. However, feeding was stopped 48 hours before the initiation of acute toxicity tests to minimize contamination of the test medium from fecal matter and to maintain stable experimental conditions. Care was taken not to disturb the fish unnecessarily during the exposure period.

The herbicide Isoproturon (IPU), used as the test toxicant in the present investigation, was procured from a local agrochemical supplier in Tumakuru, Karnataka, India, under the trade name "Srirama." The chemical was selected due to its extensive agricultural use and possible contamination of nearby freshwater ecosystems through agricultural runoff. Prior to use, the expiry date and purity of the herbicide were carefully checked. A stock solution of Isoproturon was prepared freshly by dissolving the required quantity of the herbicide in deionized water to obtain a concentration of 1000 ppm. Accurate measurements were carried out using a micropipette to ensure precision and uniformity in the preparation of test concentrations.

For acute toxicity assessment, healthy fish were randomly divided into different experimental groups, with each group exposed to varying concentrations of Isoproturon for 96 hours under semi-static bioassay conditions. A control group without herbicide exposure was also maintained simultaneously under identical laboratory conditions. The experimental media were renewed periodically to maintain constant toxicant concentration and water quality throughout the exposure duration.

During the exposure period, the fish were continuously monitored for mortality and abnormal behavioral responses. Behavioral changes such as erratic swimming, hyperactivity, rapid opercular movement, excessive mucus secretion, surfacing behavior, loss of equilibrium, reduced feeding activity, and sluggish movement were carefully observed and recorded. Mortality was noted at regular intervals of 24, 48, 72, and 96 hours, and dead fish were removed immediately from the test containers to prevent deterioration of water quality.

The mortality data obtained from the acute toxicity tests were subjected to statistical analysis using the Probit

Statistical Bioassay method for the determination of the 96-hour LC_{50} value and corresponding lethal concentrations. The 95% confidence intervals were also calculated to determine the reliability of the toxicity estimates. Further statistical evaluation and graphical interpretation of the data were performed using NCSS software. The obtained LC_{50} value served as the basis for selecting sublethal concentrations for subsequent biochemical, physiological, and genotoxic studies.

Results and Discussion

The acute toxicity study conducted on *Cyprinus carpio* exposed to different concentrations of the herbicide Isoproturon revealed significant toxic effects under controlled laboratory conditions (30 °C and pH 7.5). Mortality data obtained during the 96-hour exposure period were analyzed using Probit analysis, and the 96-hour LC_{50} value of Isoproturon for *C. carpio* was determined to be 1.65 mg/L (Table 1). The obtained LC_{50} value indicates that Isoproturon possesses considerable toxicity toward freshwater fish even at relatively low concentrations.

The study clearly demonstrated that the toxic effects of Isoproturon on *C. carpio* were both concentration- and time-dependent. Mortality increased progressively with increasing herbicide concentrations and longer exposure durations, indicating a clear dose response relationship. Fish exposed to lower concentrations survived for longer periods, whereas fish exposed to higher concentrations exhibited rapid toxic responses and increased mortality within a shorter duration of exposure. Similar observations have been reported in previous studies on herbicide-induced toxicity in freshwater fishes, where mortality increased proportionally with concentration and exposure period (Rana *et al,* 2024; Ghelichpour *et al,* 2019) [8, 22]. Comparable findings were also reported by Kumar and Singh, 2017 [15], who observed increased mortality and physiological stress in herbicide-exposed freshwater fish under laboratory conditions.

During the exposure period, several abnormal behavioral responses were observed in the treated fish. The exposed fish showed erratic and rapid swimming movements, hyperactivity, frequent surfacing behavior, excessive mucus secretion, rapid opercular movement, reduced feeding activity, loss of equilibrium, and sluggishness before death. These behavioral abnormalities became more pronounced with increasing concentration and exposure duration. Such changes are considered sensitive indicators of toxic stress and may result from disturbances in respiratory, nervous, and metabolic functions caused by herbicide exposure. Similar behavioral changes have been documented in fish exposed to agricultural contaminants by Patil *et al,* 2021 and Hernandez and Lopez, 2019 [11, 19], who reported that pesticide exposure altered swimming performance and respiratory activity in freshwater fishes.

The observed increase in mortality and behavioral alterations may be associated with impairment of vital physiological processes such as respiration, osmoregulation, and energy metabolism. Herbicides like Isoproturon are known to induce oxidative stress through the generation of reactive oxygen species (ROS), leading to cellular damage and physiological imbalance in aquatic organisms. Excessive mucus secretion and rapid opercular movements observed in the treated fish may indicate irritation and damage to gill tissues, resulting in reduced oxygen uptake efficiency and respiratory stress. Similarly, reduced feeding activity and sluggish behavior may reflect metabolic disturbances and reduced energy utilization under toxic conditions. Ribas *et al,* 2024 [24] reported that exposure to glyphosate-based herbicides significantly altered antioxidant defense systems and induced oxidative damage in zebrafish tissues, supporting the present observations of physiological stress caused by herbicide exposure.

Prolonged exposure to toxicants may also disrupt enzymatic activities and alter biochemical pathways associated with carbohydrate, protein, and lipid metabolism. Such physiological disturbances can eventually lead to tissue damage, weakened immune responses, impaired growth, and reduced survival of aquatic organisms. Burch *et al,*2025 [5] reported that pesticide contamination in aquatic systems can significantly interfere with metabolic enzymes and protein turnover, thereby affecting fish health and survival. Similarly, Nagaraju *et al,*2016 [18] observed reductions in protein band intensity in the tissues of *Labeo rohita* exposed to pesticides, suggesting protein degradation and metabolic impairment under toxic stress.

Sensitivity to toxic substances may vary among different fish species and even within the same species depending on factors such as age, body size, metabolic activity, nutritional condition, sex, and environmental parameters including temperature, dissolved oxygen, and pH (Devika Rani and Parimala, 2025; Hernandez *et al,* 2013) [6, 10]. Younger and smaller fish are generally more susceptible to toxicants because of their higher metabolic rate and lower detoxification capacity. Environmental conditions may further influence the absorption, accumulation, and toxicity of herbicides in aquatic organisms. According to Rahman *et al,* 2014 [21], environmental stressors can significantly modify the toxic response of fish by altering physiological adaptation mechanisms.

No mortality or abnormal behavioral responses were observed in the control group during the experimental period, confirming that the toxic effects observed in the treated fish were specifically due to Isoproturon exposure. The findings of the present investigation agree with earlier reports indicating that herbicides can adversely affect aquatic organisms even at low concentrations by causing physiological stress, behavioral disturbances, tissue damage, and increased mortality. Ivantsova *et al,* 2024 and Martyniuk and Ivantsova 2024 [12, 17] also emphasized that herbicides may trigger biochemical and cellular stress responses that contribute to long-term ecological disturbances in aquatic organisms.

Determination of LC_{50} values plays an important role in Ecotoxicological investigations as it provides baseline information regarding the toxic potential of pollutants in aquatic ecosystems. The present study highlights the ecological significance of herbicide contamination in freshwater habitats and indicates that uncontrolled discharge of Isoproturon into aquatic environments may pose serious threats to non-target organisms, particularly fish populations. Continuous contamination of water bodies through agricultural runoff and leaching may disturb ecological balance and reduce aquatic biodiversity.

Therefore, careful monitoring and regulated use of herbicides are essential to minimize environmental pollution and protect freshwater ecosystems. Adoption of eco-friendly agricultural practices, integrated weed management strategies, and regular monitoring of pesticide residues in water bodies may help reduce ecological risks associated with herbicide contamination. Furthermore, the present findings may serve as baseline information for future studies focusing on biochemical, histopathological, neurotoxic, oxidative stress, and genotoxic effects of Isoproturon in freshwater fish and other aquatic organisms.

Table 1: Mortality of *Cyprinus carpio* by varying Isoproturon concentrations during 96h exposure

Concentration (mg L^{-1})	% Actual	% Mortality (Observed)	No. of Fish	% Mortality (Expected)	Error % Mortality	Difference	χ^2
1.0	5.2	10.0	10	1.0	0.52	0.48	0.23
1.2	12.45	20.0	10	2.0	1.25	0.75	0.28
1.4	26.72	30.0	10	3.0	2.67	0.33	0.04
1.6	41.18	50.0	10	5.0	4.12	0.88	0.18
1.8	61.33	70.0	10	7.0	6.13	0.87	0.12
2.0	82.9	90.0	10	9.0	8.29	0.71	0.06

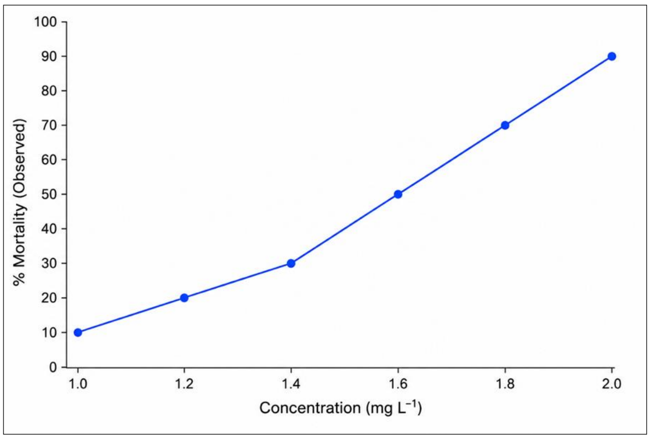

Fig 1: Graph showing percentage of Mortality v/s Concentration of Isoproturon exposed to *C. carpio*

Conclusion

The present investigation clearly demonstrates that freshwater organisms, particularly *Cyprinus carpio*, are highly sensitive to exposure to the herbicide Isoproturon. The determined 96-hour LC_{50} value indicates that even relatively low concentrations of the herbicide can produce significant toxic effects in fish. Mortality and behavioral abnormalities observed during the study confirmed that the toxic response of *C. carpio* was concentration- and time-dependent. Increasing concentrations of Isoproturon resulted in progressive physiological stress, abnormal swimming behavior, respiratory distress, loss of equilibrium, and increased mortality, reflecting the harmful impact of the herbicide on aquatic organisms.

The study provides important baseline toxicological information that may be useful for establishing permissible exposure limits and maintaining water quality standards in freshwater ecosystems. Acute toxicity assessment serves as an essential tool for evaluating the ecological safety of agrochemicals before their extensive use in agricultural practices. Since fish are important components of aquatic food chains and valuable bioindicators of environmental contamination, toxic effects observed in fish populations may indirectly indicate the overall health status of aquatic ecosystems.

The environmental implications of Isoproturon contamination are highly significant because of its persistence, mobility, and bioaccumulation potential in aquatic environments. The herbicide can accumulate in sediments and aquatic macrophytes, which may act as secondary sources of contamination over long periods. Decomposition of contaminated plants and sediment resuspension can release the herbicide back into the water column, exposing aquatic organisms repeatedly even after the initial application period. Such prolonged contamination may adversely affect non-target organisms, including fish, zooplankton, benthic fauna, amphibians, and aquatic microorganisms.

In addition to acute toxicity, long-term exposure to sublethal concentrations of Isoproturon may lead to biochemical, physiological, neurotoxic, and genotoxic alterations in fish. Disturbances in carbohydrate, lipid, and protein metabolism, oxidative stress generation, enzymatic dysfunction, and tissue damage may ultimately impair fish growth, reproduction, immune response, and survival. Continuous exposure may also reduce biodiversity and alter the ecological balance of freshwater habitats.

The findings of the present study emphasize the need for careful and regulated use of herbicides in agricultural practices. Improper application and uncontrolled disposal of agrochemicals can severely affect nearby water bodies through agricultural runoff and leaching. Therefore, regular monitoring of pesticide residues in aquatic ecosystems is necessary to minimize ecological risks and protect aquatic biodiversity. Adoption of eco-friendly agricultural practices, integrated weed management strategies, and public awareness regarding pesticide contamination may help reduce the harmful effects of herbicides on the environment.

Furthermore, the present study may serve as a foundation for future research focusing on chronic toxicity, oxidative stress biomarkers, histopathological changes, enzymatic alterations, reproductive toxicity, and molecular responses induced by Isoproturon in aquatic organisms. Detailed investigations at biochemical and genetic levels are essential for understanding the long-term ecological consequences of herbicide contamination and for developing effective environmental protection and management strategies.

Overall, the study highlights that uncontrolled use of Isoproturon poses a serious threat to freshwater ecosystems. Proper environmental regulations, sustainable agricultural practices, and continuous ecological monitoring are essential to safeguard aquatic life and maintain the ecological integrity of freshwater environments.

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