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DETERMINATION OF 96-HOUR LC_{50} VALUE OF ISOPROTURON FOR THE FRESHWATER FISH *CYPRINUS CARPIO (L.)*

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ABSTRACT

The present study focuses on estimating the 96-hour LC_{50} value of the herbicide Isoproturon in the freshwater fish *Cyprinus carpio*. Acute toxicity tests were conducted according to APHA standard procedures, and the LC_{50} was determined using Probit analysis. Before experimentation, the fish were acclimated to laboratory conditions for 30 days. A stock solution of Isoproturon was prepared, and groups of fish were exposed to different concentrations of the herbicide for 96 hours. Findings indicated that the 96-hour LC_{50} value for *Cyprinus carpio* was 1.56 mg/L. The sensitivity of the fish to Isoproturon increased with both higher concentrations and longer exposure times, with mortality rising proportionally to the herbicide concentration.

Keywords: 96-hour LC_{50}, *Cyprinus carpio,* Isoproturon, Herbicide, Acute toxicity

INTRODUCTION

Freshwater ecosystems are increasingly threatened by pollutants from industrial, agricultural, and domestic sources (Smith *et al.,* 2019). Agrochemicals, particularly herbicides, frequently enter aquatic environments via surface runoff, leaching, and atmospheric deposition, posing risks to non-target organisms (Brown and Taylor, 2018; Khatib *et al.,* 2022). These compounds can disrupt physiological and biochemical processes, impairing growth, reproduction, metabolism, and survival (Johnson *et al.,* 2020).

Isoproturon, a phenylurea herbicide commonly used in cereal cultivation, is persistent in water and sediments and can accumulate in aquatic plants and fish (Kumar and Singh, 2017; Rao *et al.,* 2016). Its prolonged bioavailability increases the likelihood of toxic effects, including oxidative stress, alterations in carbohydrate and protein metabolism, enzymatic dysfunction, and behavioral and histopathological abnormalities (Patil *et al.,* 2021; Hernandez and Lopez, 2019).

Cyprinus carpio (common carp) is widely used in Ecotoxicological research due to its ecological relevance, broad distribution, and sensitivity to pollutants (Rahman *et al.,* 2014). Acute toxicity testing, using a 96-hour LC_{50} determination, provides critical data for environmental risk

assessment by identifying concentrations that cause mortality in 50% of exposed fish (Anderson and Miller, 2015; Garcia *et al.,* 2018).

Isoproturon exposure can induce organ-specific changes, particularly in the liver and kidneys, which are key sites of bioaccumulation. Sediment-bound Isoproturon may be absorbed by rooted macrophytes such as *Elodea densa* and released into water following plant decay, creating localized contamination pulses (Grollier *et al.,* 1997). Fish can bioaccumulate the herbicide in muscle tissue, exhibiting long elimination half-lives and potential trophic transfer (Hernandez *et al.,* 2013). Sublethal exposures also cause behavioral and morphological effects, including erratic swimming, loss of equilibrium, and body deformities, reflecting persistent neurotoxic stress (Devika Rani H. K. and Parimala B., 2025). Although LC_{50} values and sublethal effects of Isoproturon are documented, studies on the interplay between sediment and macrophytes accumulation and subsequent fish exposure remain limited. This study aims to determine the 96-hour LC_{50} of Isoproturon for C. *carpio* and assess associated behavioral and organ-specific responses, contributing to a better understanding of ecological risks in freshwater ecosystems and informing sustainable herbicide management.

MATERIALS AND METHODS

Healthy *Cyprinus carpio* (L.) fingerlings were sourced from Turvekere Fisheries Farm, Tumakuru. Upon arrival at the laboratory, the fish were carefully transferred into large aerated containers for transport. They were subsequently acclimated for 30 days in 25-L tubs under controlled laboratory conditions, during which they were fed commercial dry feed pellets to ensure proper nutrition and adaptation.

The fingerlings, with an average weight of 3.5-4.5 g and an average length of 5-6 cm, underwent an additional 20-day acclimation period in 25-L tubs maintained at 24 ± 1 °C. The tubs contained dechlorinated tap water, whose physicochemical parameters were assessed according to APHA (2005) guidelines. The measured water quality parameters were Temperature 24 ± 2ºC, pH 6.9 ± 0.2 at 24ºC, Dissolved oxygen 9.4 ± 0.8 mg/L, 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. Water was replenished daily, and a 12-hour light/12-hour dark photoperiod was maintained throughout both acclimation and experimental periods.

During acclimation and experimental phases, the fish were fed commercial pellets, with feeding withheld for two days before acute toxicity testing. The herbicide Isoproturon (IPU), obtained locally in Tumakuru, Karnataka, India, under the trade name “Srirama,” was used as the test chemical. Before use, the expiry date was confirmed, and precise volumes were measured by dissolving the substance in deionized water to prepare a stock solution of 1000 ppm using a micropipette.

Mortality data were statistically analyzed using the Probit Statistical Bioassay system to calculate 96-hour LC_{50} and lethal concentrations along with 95% confidence intervals. All data were further analyzed using the NCSS software.

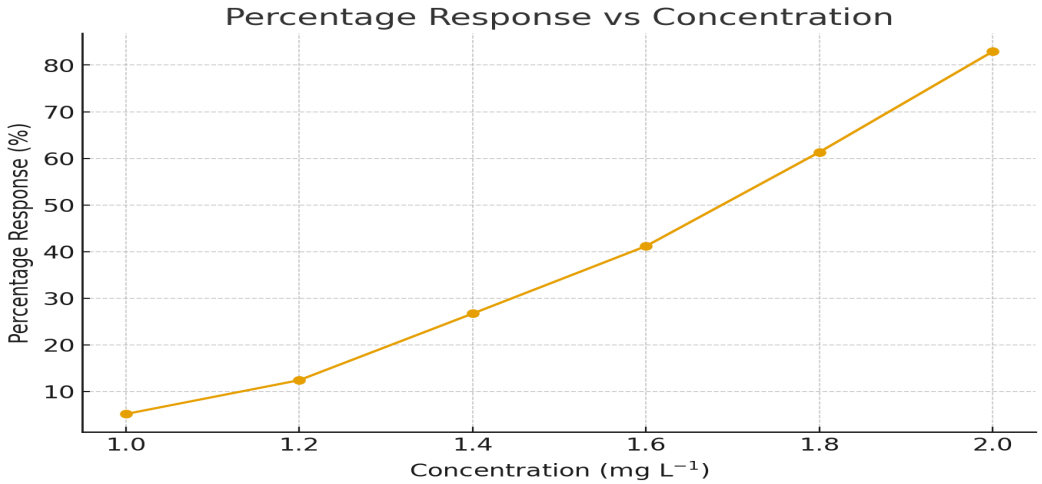
RESULTS

A total of ten C. *carpio* were exposed to different concentrations of the herbicide Isoproturon to determine the 96-hour LC_{50} and lethal concentrations under controlled conditions (30 °C and pH 7.5). The 96-hour LC_{50} of Isoproturon for C. *carpio* was estimated to be 1.56 mg/L (Table 1). The study demonstrated the toxic effects of Isoproturon on C. *carpio* were dose- and time-dependent. As the concentration of the herbicide increased, the mortality rate of the fish also rose, indicating a clear concentration–response relationship. Sensitivity to toxicants can vary between species, and even within the same species, responses may differ depending on factors such as age, size, and experimental conditions (Devika Rani and Parimala, 2025; Hernandez *et al.*, 2013). These findings align with previous research on herbicide-induced toxicity in freshwater fish (Rana *et al.*, 2024; Ghelichpour *et al.*, 2019).

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: Percentage response and concentration of Isoproturon



CONCLUSION

Freshwater organisms, particularly C. *carpio*, are highly sensitive to elevated concentrations of **Isoproturon**, which can cause lethal and sublethal effects. The study provides data for establishing permissible exposure limits and maintaining water quality standards. Environmental implications are significant persistence and bioaccumulation in sediments and

macrophytes can create secondary contamination sources, affecting non-target organisms over extended periods. Careful herbicide management and regular monitoring of aquatic ecosystems are essential to mitigate these ecological risks.

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