J. Sci. Innov. Nat. Earth: Vol. 5, Issue 4, 2025, Page 64-65 ISSN (Online) 2583-2093 OPEN ACCESS

Journal of Science Innovations and Nature of Earth

Journal homepage : https://jsiane.com/index.php/files/index

International, Double-Blind, Quarterly, Peer-Reviewed, Refereed Journal, Edited and Open Access Research Journal

Assessment of Malathion-Induced Toxicity in *Mystus seenghala* Using Biochemical Biomarkers

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DOI: https://doi.org/10.59436/jsiane.471.2583-2093

Abstract

The massive use of organophosphate pesticides in agriculture has caused the contamination of freshwater ecosystems, which is a serious threat to non, target aquatic organisms. Malathion, an organophosphate insecticide that is commonly used, is classified as moderately safe for mammals but highly toxic to fish even at very low concentrations. The present investigation is aimed at determining the biochemical changes induced by sublethal exposure of malathion in the freshwater catfish *Mystus seenghala* by using selected biochemical biomarkers. Healthy adult fish were exposed to malathion at the concentration of 1/10 of the 96, h LC for different time intervals (1-60 days). Serum lipid profile parameters (total cholesterol, triglycerides, HDL, LDL) and renal biomarkers (urea and creatinine) were analyzed. Outcomes showed that with the increase in duration of exposure, the levels of total cholesterol, triglycerides, LDL, urea, and creatinine increased while the level of HDL decreased significantly which means that lipid metabolism was severely disturbed and there was renal dysfunction. All in all, these results confirm that biochemical parameters are very sensitive, early indicators of malathion poisoning and thus, may be utilized as an effective means of pesticide pollution surveillance in freshwater habitats.

Keywords*:* Malathion, Organophosphate toxicity, Biochemical biomarkers, *Mystus seenghala*

Received 06.08.2025 Revised 16.09.2025 Accepted 18.11.2025 Online Available 01.12.2025

Introduction

The extensive use of pesticides in today's agriculture has resulted in growing contamination of our aquatic ecosystems, which poses serious risks to non-target organisms. Among these chemicals, organophosphate pesticides are widely used because they are highly effective at killing insects and break down more quickly than the more persistent organochlorines. However, their ongoing entry into freshwater systems through agricultural runoff, spray drift, and improper disposal has raised significant ecological concerns (Ullah & Zorriehzahra, 2015). Fish are especially at risk from these contaminants since they easily absorb harmful substances through their gills, skin, and digestive systems. Malathion is an organophosphate insecticide that's commonly used in agriculture, for household pest control, and in public health efforts. While it's considered to have low toxicity for mammals, it can be highly toxic to aquatic organisms, particularly fish, even at low concentrations (WHO, 2010). The primary way malathion causes toxicity is by inhibiting the activity of acetylcholinesterase, which leads to neurotoxicity, oxidative stress, and a disruption of normal metabolic functions (Ahmad *et al*., 2012). Prolonged exposure to malathion can adversely affect fish growth, reproduction, and survival, ultimately threatening aquatic biodiversity and the stability of ecosystems. Biochemical biomarkers are widely recognized as effective indicators for assessing the initial effects of environmental pollutants on aquatic organisms. They provide significant insights into physiological and metabolic disturbances that can occur long before any visible signs of disease or mortality appear (Van der Oost *et al*., 2003). Changes in serum biochemical parameters, such as lipid profile components, kidney markers, and metabolic enzymes, can reveal functional damage to crucial organs, especially the liver and kidneys, which are essential for detoxification and metabolism. Lipids are essential components in our bodies, playing key roles in energy storage, forming membranes, and synthesizing hormones. Changes in serum lipid levels like cholesterol, triglycerides, HDL, LDL, and VLDL can indicate problems with lipid metabolism and liver function, especially when under toxic stress (Ramesh *et al*., 2017). Similarly, markers related to kidney function, such as urea and creatinine, are valuable for spotting renal issues caused by pesticide exposure. Research has shown that fish exposed to organophosphate pesticides exhibit significant biochemical changes, highlighting their relevance in assessing toxicity (Chitra & Abdu, 2013). *Mystus seenghala* is a important freshwater catfish that holds significant economic value and is commonly found in Indian rivers and reservoirs, making it a crucial part of inland fisheries. Despite its importance both commercially and ecologically, there's a surprising lack of information regarding how *M. seenghala* responds biochemically to malathion exposure. This study aims to investigate the toxicity of malathion on *Mystus seenghala* by examining selected biochemical markers. The outcomes are expected to shed light on pesticide-induced metabolic issues and advocate for the use of biochemical parameters as effective measures of aquatic pollution.

Materials and Methods

Experimental Fish-We collected healthy adult specimens of the freshwater catfish *Mystus seenghala* from local freshwater bodies and transported them to the lab in aerated containers. We chose fish that were about 40–60 cm long and weighed between 350–550 g to keep size differences to a minimum. Before starting the experiments, we carefully checked each fish to make sure they showed no visible signs of disease or injury.

Acclimatization-The fish were acclimatized to laboratory conditions for a period of 15 days in large glass aquaria containing dechlorinated tap water. During acclimatization, fish were fed a standard commercial pellet diet twice daily, and feeding was stopped 24 hours before the start of the experiment. Water in the aquaria was renewed every alternate day to maintain optimal water quality. Physicochemical parameters such as temperature (25–30 °C), pH (7.0–7.5), and dissolved oxygen (6.0–7.5 mg/L) were regularly monitored.

Test Chemical-We used malathion, specifically a 5% dust powder formulation, as our test pesticide. To prepare stock solutions, we dissolved the necessary amount of malathion in distilled water. Each time we conducted an exposure experiment, we made sure to freshly prepare the working concentrations.

Determination of LC_{50} Value-We determined the 96-hour median lethal concentration (LC_{50}) of malathion for *Mystus seenghala* through static bioassay tests, sticking to standard procedures. The fish were exposed to various concentrations of malathion, and we kept track of mortality at regular intervals. To find the LC_{50} value, we used probit analysis.

Selection of Sublethal Concentration

Using the LC_{50} value we obtained, we decided on a sublethal concentration that's $1/10^{th}$ of the 96-hour LC_{50} for our chronic exposure studies. This level was selected to investigate the biochemical impacts of malathion without causing mortality.

Experimental Design-In this study, we randomly assigned fish into two groups: one control group and one treated group, each consisting of ten fish. The control group lived in water that was free from pesticides, while the treated group was exposed to a sublethal concentration of malathion for different time frames: 1, 7, 15, 30, 45, and 60 days. Throughout the experiment, we renewed the water daily and ensured that the pesticide concentration remained constant.

Blood Collection and Serum Separation-At the end of each exposure period, the fish were anesthetized and then sacrificed. Blood samples were taken from the caudal vein using sterilized syringes. After collection, the blood was allowed to clot at room temperature and then centrifuged at 2000 rpm for 30 minutes to isolate the serum. The serum samples were stored at 4 °C until they were analyzed biochemically.

Biochemical Biomarkers

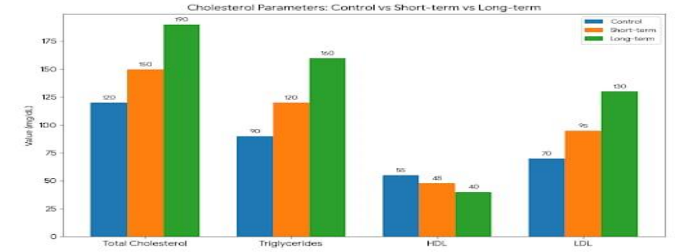
•**Serum Lipid Profile-**We measured serum total cholesterol, triglycerides, and high-density lipoprotein (HDL) levels using standard enzymatic diagnostic kits, carefully following the manufacturer's guidelines. For low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels, we applied Friedewald's formula.

•**Renal Function Markers**-We measured serum urea and creatinine levels using standard colorimetric methods with diagnostic kits that are commercially available.

Result

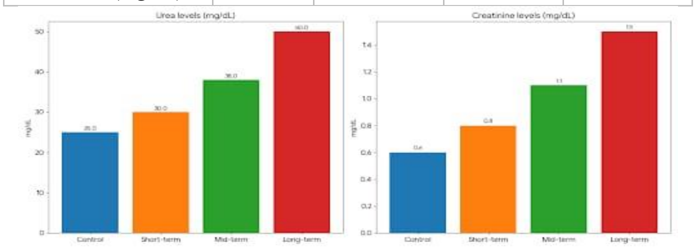
Changes in Serum Lipid Profile- Exposure of *Mystus seenghala* to malathion significantly altered serum lipid profile. Total cholesterol, triglycerides, and LDL levels increased progressively with exposure duration, while HDL levels declined. Control fish maintained normal lipid levels, indicating that malathion induced duration-dependent dyslipidemia and disrupted lipid metabolism.

Parameter	Control	Short-term	Long-term
Total Cholesterol	120	150	190
Triglycerides	90	120	160
HDL	55	48	40
LDL	70	95	130



Alterations in Renal Biomarkers-After short-term exposure, a slight increase in urea and creatinine levels was recorded. With extended exposure periods, these increases became more pronounced, reaching maximum values in the long-term exposure group. The elevation of renal biomarkers followed a clear exposure-duration-dependent pattern.

Parameters	Control	Short-term	Mid-term	Long-term
Urea (mg/dL)	25	30	38	50
Creatinine (mg/dL)	0.6	0.8	1.1	1.5



Discussion

Based on the present research, there is strong evidence to support the hypothesis that exposure to sublethal quantities of malathion leads to significant alteration of biochemical profiles in freshwater catfish (i.e., *Mystus seenghala*) indicating the potential for significant, chronic toxicity of organophosphate pesticides, even in very low concentrations. Agricultural runoff and spray drift are two common ways in which malathion is introduced into aquatic ecosystems, resulting in long-term exposure of non-target species such as fish to sublethal doses of malathion on a regular basis (Ullah & Zorriehzahra, 2015; Rani *et al*., 2021). While malathion is considered to be of moderate toxicity to mammals, it is known to have a very high level of toxic effect on fish because of fish's greater ability to absorb pesticides through the gills and the skin (WHO, 2010). An increase in serum levels of total cholesterol, triglycerides, Low-Density-Lipoproteins (LDL) and a decrease in High-Density-Lipoproteins (HDL) due to malathion exposure were observed in this current study; therefore, indicating altered lipid metabolism. Organophosphate pesticide results in similar dyslipidemia in aquatic animals (i.e. fish), indicating the amount of lipid synthesis, lipid transport, and lipid clearance due to toxicants (Ramesh *et al*., 2017; Kumar *et al*., 2020). The decrease in HDL levels also indicates the decrease in the ability to scavenge free radicals and, therefore, enhance lipid peroxidation, which may lead to the degradation of cellular membranes (Ahmad *et al*., 2012). Prolonged exposure to urea and creatinine increases the concentration of renal biomarkers. The increased marker concentrations indicate less efficient removal of blood from the kidney (reduced glomerular filtration) and possible structural damage due to the accumulation of pesticides in renal tissues (Van der Oost *et al*., 2003; Chitra & Abdu, 2013). Other freshwater fish exposed to malathion and other organophosphate pesticides have been observed to show similar renal dysfunction as well (Singh *et al*., 2019).

Conclusion

The results show that malathion causes a biochemical stress response in the freshwater catfish (*Mystus seenghala*) at sublethal concentrations. Even though 1/10 of the 96-hour LC_{50} concentration caused significant changes in serum lipid and renal parameters, it indicates the potential for chronic toxicity from malathion. The increase in total cholesterol, triglycerides, and low-density lipoprotein (LDL) and the decrease in high-density lipoprotein (HDL) indicate that lipid metabolism has been disrupted. The increased levels of urea and creatinine also indicate that renal filtration and excretion have been compromised. These changes, which are dependent on the duration of exposure to pesticides, support the idea that there is a cumulative effect from the extended time that aquatic organisms are exposed to pesticides. Biochemical disturbances may negatively affect growth, reproduction and overall physiological function, leading to decreased sustainability of economically significant fish stocks. This research demonstrates that renal biomarkers and serum lipid profiles can be used as sensitive indicators of organophosphate toxicity, and therefore could be included in monitoring programs. The results emphasize the urgent need for strict regulation, controlled application, and continuous surveillance of organophosphate pesticides to safeguard freshwater ecosystems and maintain ecological balance.

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