J. Sci. Innov. Nat. Earth: Vol. 5, Issue 3, 2025, Page No-51-53 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

Toxicological Evaluation of Lipid Profile Changes in *Mystus seenghala* Under Malathion Stress

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DOI: https://doi.org/10.59436/jsiane.HG.2583-2093

Abstract

The use of organophosphate pesticides in agricultural production has led to increasing levels of contamination in freshwater sources and are a serious hazard for aquatic life. The current research focuses on examining the effects of exposure to non-lethal levels of malathion, through modified experimental studies, on the serum lipids from an important freshwater catfish species, *Mystus seenghala*. Using a non-lethal dose of malathion, fish were kept in a controlled laboratory environment for 1, 7, 15, 30, 45, and 60 days, respectively. The results demonstrated an overall increase of total cholesterol, triglycerides, LDL, and VLDL, and a decrease of serum HDL levels which was related to the length of exposure to malathion. Total cholesterol, triglycerides, LDL, and VLDL exhibited their highest levels at the end of 60 days, and this provides strong evidence of accumulated toxic effects of malathion.. In our case, these findings reflect on changes in lipid metabolism and dysfunction of the liver due to exposure to pesticides. The findings of this research identify serum lipids as being an appropriate biochemical marker for measuring toxicity of malathion to freshwater fish species. Disruption of the lipid function due to continuous pesticide presence may hinder the metabolic homeostasis of aquatic organisms, thus emphasising the need for controlled pesticide use and monitoring of aquatic environments.
Keywords: Malathion toxicity; Lipid profile; *Mystus seenghala*; Organophosphate pesticide; Dyslipidemia; Biochemical biomarkers

Received 12.07.2025 Revised 18.07.2025 Accepted 03.09.2025 Online Available 20.09.2025

Introduction

A growing threat to aquatic ecosystems is the application of agrochemicals, particularly organophosphate pesticides, onto agricultural land. Agrochemical application occurs via agricultural run-off (as well as drainage, atmospheric deposition), impacting nearby aquatic ecosystems. Of all organophosphate insecticides, malathion is one of the most extensively used due to its ability to control a broad range of pest species in agriculture. Malathion is regarded as moderately toxic to mammals; however, it has been shown to have a high degree of toxicity to non-target aquatic organisms (particularly fish) because fish are able to absorb dissolved xenobiotics (pesticides and other chemicals) readily through the use of their gills, skin, and gastrointestinal tract (Ullah & Zorriehzahra, 2015) Malathion is responsible for chronic and sublethal toxicity to freshwater fish and has been associated with biochemical, hematological and histopathological changes in freshwater fish (Ahmad *et al*., 2021; Sivanandan & Binukumari, 2021). The biological indicators of water pollution are suggested to be fish due to their place in the food chain and their ability to respond physiologically to stress. Organophosphate exposure inhibits acetylcholinesterase and creates oxidative stress, resulting in problems with your body's metabolism (Lal *et al*., 2013). In addition to its neurotoxic effect, malathion affects how the liver works and how lipids are metabolized, since the liver plays an important role in producing, transporting and storing fat (Muthulingam *et al*., 2010). Thus, examining lipid profile parameters (total cholesterol, triglycerides, HDL, LDL and VLDL) is necessary to assess the extent of metabolic disturbances caused by toxins. Changes in the lipid profile have been accepted as valid biochemical indicators of environmental stress; for example, cholesterol has a critical structural role in the membranes of cells and is necessary for the production of steroid hormones. Triglycerides, on the other hand, provide a source of energy; therefore, both cholesterol and triglyceride levels can affect the ability of an organism to perform essential functions. Organisms transport lipids in their bloodstream using lipoproteins as transport proteins. When lipoproteins are out of balance, this is an indication that there is metabolic disruption in the liver (Mir 2014). Previous studies have documented that exposure to pesticides can disrupt lipid homeostasis in freshwater fishes (e.g. increased cholesterol and triglyceride concentrations, and reduced levels of high-density lipoprotein (HDL) (Abdel-Ghany *et al*. 2016; Narra 2016). Disruption in lipid homeostasis can result in impaired function of the cell membrane, impairment of the endocrine system, and impairment of reproductive function. The commercial and ecological significance of the freshwater catfish *Mystus seenghala* (family Bagridae) extends across the Indian subcontinent. This fish's abundance, adaptability, and economic importance make it a suitable species for conducting ecotoxicological studies. There is limited research published on the effects of malathion on the lipid profiles of this species, particularly after being subjected to extended periods of exposure. Past research has demonstrated that the hematological and biochemical parameters of multiple teleost fish species, including Clarias gariepinus and Oreochromis niloticus (Ahmad, 2012; Al-Ghanim, 2012), alter significantly following exposure to the pesticide malathion. Examining malathion-related alterations to lipid metabolism is vital because changes in lipid metabolism not only indicate damage to the liver, but also affect energy allocation, physiological stress responses, and reproductive performance. High concentrations of plasma lipids may be reflective of the body's response to stress by releasing stored lipids into the blood stream; however, reduced concentrations of high-density lipoprotein (HDL) represent a potential risk to reverse cholesterol transport and the body's ability to protect its cells (Gordon *et al*., 1977). Thus, systematically analyzing alterations to lipid profiles of *Mystus seenghala* that have been exposed to malathion will serve as useful toxicological indicators for environmental monitoring purposes and assessing risks associated with aquatic ecosystems. This study will create a baseline for establishing toxicological changes in the lipid profile of *Mystus seenghala* as a result of malathion exposure and generate valuable information about how pesticides disrupt metabolic processes within freshwater ecosystems. Findings from this study could promote development of Sustainable Agriculture Programs and enhance existing biological criteria for environmental monitoring and management.

Materials and Methods

•**Experimental Fish and Acclimatization**- Healthy *Mystus seenghala* (freshwater catfish) were collected from nearby freshwater bodies and transport to the lab in aerated containers. The fish were of uniform size (90 to 110 grams); this reduced variability among the fish used in the study. The fish were acclimated to laboratory conditions for 10 to 14 days prior to testing by placing them in glass aquaria containing controlled water conditions (temp: 26 ± 2°C; pH 7.2 – 7.6; DO = 6.0 to 7.0mg/L). They were fed commercial feed pellets on a continuous basis ad libitum and fresh water was added periodically to help maintain water quality.

•**Determination of LC_{50} and Experimental Design**- Using probit analysis, the median lethal concentration (LC_{50}) of malathion was found according to standard procedures for toxicity testing. A potential sublethal concentration was chosen for a long-term exposure study based on the LC_{50} value of 5.11 mg/l. Fish (10 in each group) were then split into two separate groups: a control group and an experimental group. The experimental group will be exposed to the selected sublethal dose of malathion for 1 day, 7 days, 15 days, 30 days, 45 days, or 60 days while the control will remain in clean, non-pesticide water under identical conditions.

•**Blood Collection and Lipid Profile Analysis**- Every interval of exposure all fished will be put to sleep then have blood taken from their tail on a sterile syringe for later analysis. The blood was then spun in a centrifuge at 3,000 rpm for 10 min. The following lipid profile parameters will also be measured; Total cholesterol, triglycerides, HDL, LDL and VLDL lipid profiles were measured using standard enzymatic methods. The LDL and VLDL levels calculated by the Friedewald formula were also converted to mg/dL.

Results

In the current study, there were notable changes seen with respect to lipids after malathion was given to M. seenghala at sub-lethal concentrations of malathion for 60 days (i.e. 30%) exposed to varying levels of malathion for 1 to 60 days, had increasing amounts of total cholesterol, triglycerides, LDL, & VLDL in their lipid profile on day 1 at 1mg/L compared to the day previous to that. Conversely, HDL exhibited a considerable decrease (p<).The degree of alteration increased with exposure time, with maximum deviation recorded on the 60th day. These findings indicate the development of pesticide-induced dyslipidemia under prolonged malathion stress.

Table 1. Effect of Malathion on Lipid Profile of *Mystus seenghala*

Duration (Days)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	120 ± 4	95 ± 3	52 ± 2	48 ± 3	19 ± 1
1 Day	130 ± 5	105 ± 4	49 ± 2	55 ± 3	21 ± 1
7 Days	142 ± 6	118 ± 5	46 ± 3	65 ± 4	23 ± 2
15 Days	155 ± 7	130 ± 6	42 ± 3	78 ± 5	26 ± 2
30 Days	168 ± 8	148 ± 7	38 ± 3	92 ± 6	29 ± 2
45 Days	182 ± 9	162 ± 8	34 ± 3	110 ± 7	32 ± 3
60 Days	198 ± 10	178 ± 9	29 ± 2	130 ± 8	35 ± 3

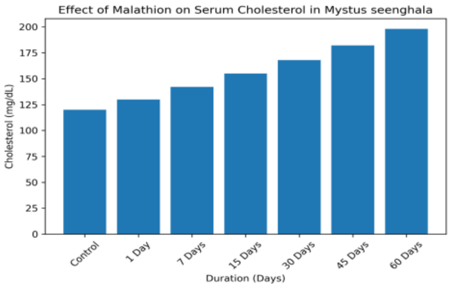
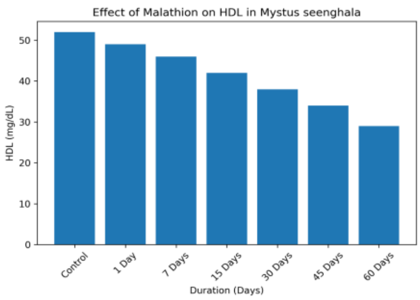


Figure.1



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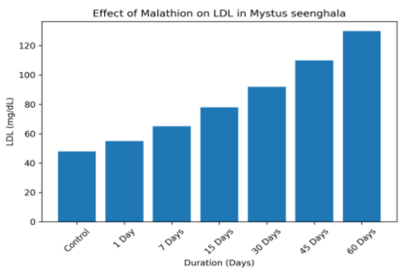

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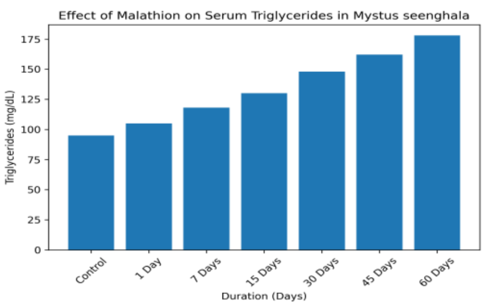

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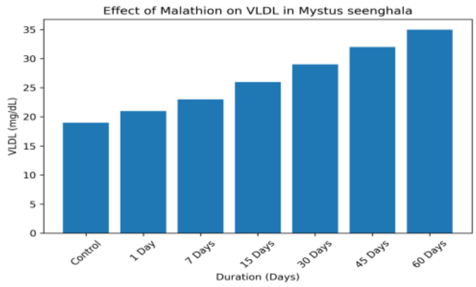

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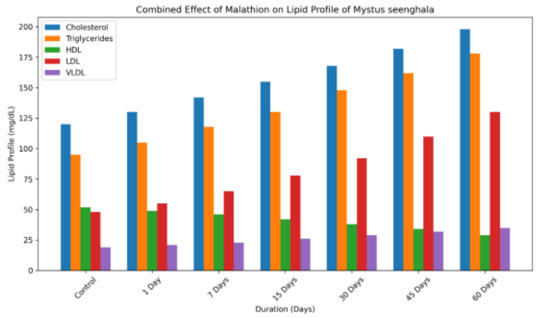

Figure.6

Discussion

The current study shows that exposure to malathion at sub-lethal concentrations significantly alters the lipid profile of *Mystus seenghala*. Experimental results demonstrated increases in total cholesterols, triglycerides, LDL/VLDL, and progressively decreasing concentrations of HDL, as the exposure progressed from day one to day sixty. These results are strong evidence for chronic exposure to malathion inducing pesticide-related dyslipidemia. The liver is primarily responsible for regulating lipids in fish. If the liver is not working correctly, the levels of various lipids in the blood will be affected. Some organophosphate pesticides (i.e., malathion) lead to the production of free radicals (oxidative stress) and damage to liver cells (hepatocytes). This can result in impaired fatty acid metabolism, lipid transport, and removal of lipids. The increase in total cholesterol and triglycerides indicates disturbance to lipid homeostasis and the release of lipid reserves when fish are exposed to some form of stress. Other studies (Ahmad *et al*., 2021; Sivanandan & Binukumari, 2021) have reported similar increases in the cholesterol concentration of freshwater fish exposed to the same organophosphate pesticides, indicative of disruption of liver function and metabolic processes. In fish, the primary factor regulating lipid metabolism is the liver. A disturbance in the physiology of this organ can directly affect the lipid fractions found in the fish's bloodstream. Organophosphate pesticides (malathion) are known to produce oxidative stress and damage to liver cells, which can negatively impact the fish's synthesis of lipids, transport of lipids, and clearance of lipids from the blood. The increased concentrations of cholesterol and triglycerides (as indicated by the elevations of total cholesterol and triglycerides) suggest that the fish do not have a stable level of lipids (lipid homeostasis) and that the fish are mobilizing stored lipids in response to stressful conditions. Other studies have reported the same increase in cholesterol levels in freshwater teleosts (fish) exposed to organophosphate pesticides, indicating that the fish are experiencing liver dysfunction and metabolic distress (Ahmad *et al*., 2021; Sivanandan & Binukumari, 2021). The noticeable rise in concentrations of both VLDLs and LDLs in this study is evidence of this altered metabolism. LDL is the primary carrier of cholesterol to the tissues, while VLDL carries most of the body's endogenous triglycerides. Thus, when LDL and VLDL rise, this indicates either impaired lipid transport or increased lipogenesis from toxic stress. Elevated levels of all lipoprotein fractions of Clarias gariepinus and Oreochromis niloticus exposed to organophosphate pesticides have been identified along with damage occurring to the liver and disruption of the endocrine system (Ahmad, 2012; Al-Ghanim, 2012).The significant drop in HDL levels during all exposure periods suggests that reverse transport of cholesterol is diminished, and that protective lipid fractions (HDLs) have been decreased. The HDL is essential for stabilizing membranes and preventing lipid peroxidation; therefore, a decrease in HDL may indicate compromised cellular defence mechanisms and increased susceptibility to oxidative damage. Previous studies of metabolic imbalance and physiological dysfunction have also reported decreased HDL under pesticide stress (Narra, 2016). Cumulative toxicity of Malathion was demonstrated by the progression of changes in lipids over the 60-day study period. The toxicological effects of organophosphates have been well documented, particularly with regard to interference with Enzymatic

Activity, Hormonal Regulation, Energy Metabolism (Lal *et al*., 2013); disruption of the endocrine system could further affect lipid metabolism, as cholesterol metabolism and steroid hormone synthesis are very closely related. The present results are consistent with previous studies showing that biochemical markers are sensitive enough to detect sub-lethal levels of pesticide exposure (Ullah & Zorriehzahra, 2015). Data supports that the lipid profile parameters can be used as useful biochemical markers of metabolic stress caused by pesticides applied to freshwater fish. The duration-dependent dyslipidemia observed in the fish species *Mystus seenghala* highlights the potential ecological hazard of long-term exposure to malathion in aquatic environments.

Conclusion

The current research indicates that a significant increase in the levels of total cholesterol, triglycerides, low density lipoprotein (LDL), and very low density lipoprotein (VLDL) occurred in the serum lipid profile of *Mystus seenghala* as a result of prolonged, sublethal exposure to malathion. In addition, a significant decrease in high density lipoprotein (HDL) levels occurred as an effect of malathion. These data suggest pesticide induced dyslipidaemia and metabolic imbalance occur as a result of chronic exposure to malathion. Elevated levels of cholesterol and triglycerides indicates that the liver is not functioning properly, and that the body is mobilizing its fat stores as a response to stress. The increased levels of HDL and LDL confirm that there are disruptions in lipid transport mechanisms. Additionally, the decreased level of HDL demonstrates impaired protective and reverse cholesterol transport capability. The above biochemical aberrations all point to both hepatocellular dysfunction and oxidative stress, both of which are associated with organophosphorus compound toxicity. Day 1 to Day 60 progressive changes demonstrate the cumulative nature of toxicity following long-term exposure to malathion. Freshwater fish already experience significant disruption of metabolism at sub-lethal malathion concentrations. Lipid profile measurements represent an effective indicator of physiological stress, and they are therefore a good tool to measure contamination by pesticides in aquatic ecosystems.The ongoing release of organophosphate pesticides (such as malathion) into freshwater ecosystems is creating significant risks to non-target species such as *Mystus seenghala*. These findings support the need for better regulation of the use of pesticides, the establishment of regularly scheduled monitoring of the environment and the move to more sustainable agricultural practices, thus reducing the damage to the ecosystem. Future work incorporating markers of oxidative stress and conducting histopathological assessments would provide insight into the long-term impacts of pesticide use to the ecosystem.

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