



Research Article

Inquisition of Malathion Induced Histopathology, Nuclear Abnormalities in Erythrocytes and DNA damage in *Hypophthalmichthys molitrix*

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Abstract | This study investigates the impact of a newly introduced insecticide, Malathion on the health of *Hypophthalmichthys molitrix* fish. The fish serve as valuable biomarkers for ecological harm caused by toxins, especially as they are a significant source of protein and easily accessible. The current study focuses on histopathological alterations, nuclear changes in erythrocytes, and DNA damage in fish brain after exposure to Malathion at environmentally relevant concentrations. The research aimed to assess the potential toxicity of Malathion on fish health, utilizing histological examinations and genetic toxicity assessments through micronuclei and Comet assays. Freshwater fish were exposed to Malathion for 18 days under constant pH, temperature and hardness conditions. The study involved sacrificing fish on the 6th, 12th, and 18th days of exposure for tissue and blood sample collection. Histological examinations revealed significant alterations in the fish brain, including necrosis of neurons, cytoplasmic vacuolization, edema, and congestion of neural cells. The severity of these changes increased with higher Malathion concentrations and longer exposure periods. The micronuclei and Comet assays were employed to evaluate genetic toxicities, providing insights into the potential harm posed by Malathion to fish health. The findings underscore the importance of understanding the impact of environmental pollutants on aquatic ecosystems and emphasize the necessity for effective monitoring and mitigation strategies. The observed histopathological alterations and genetic toxicities highlight potential risks associated with Malathion exposure, emphasizing the need for careful consideration of the environmental consequences of new insecticides. The results showed significant morphological changes in red blood cells, severe histological changes and DNA damage in brain tissues of exposed fish. Thus, malathion in water have toxic effects on fish therefore its seepage into aquatic bodies should be carefully monitored.

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Keywords | Malathion, Histopathology, Nuclear abnormalities, DNA damage, *Hypophthalmichthys molitrix*



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Introduction

Water bodies quality is impacted by the introduction of undesired substances, causing water pollution (Alrumman *et al.*, 2016). Which can endanger both human well-being and the environment. Water plays a crucial role in recycling nutrients and serves as a vital natural asset for drinking and other developmental needs (Kaur and Dua, 2015). Malathion (O, O- dimethyl -S-1,2-bisethoxy carbonyl ethyl- phosphorodithioate) is organophosphate pesticide with wide range expansion which is mostly preferred for its high selectivity to target pests (Moore *et al.*, 2011). Malathion, even at low concentrations are harmful for fish. And, caused a reduction of glycose, proteins and lipids, which reported by Huculeci *et al.* (2009) as malathion is lipophilic in nature. Its accumulation in membranes generating free radicals' production causing increase in Lactoperoxidase and hepatocytes damage. Lactoperoxidase is the highly resultant harmful attacks by free hydroxide radical through Fenton reaction (Ullah *et al.*, 2015), resulting of oxidative damage to different tissues (Dabas *et al.*, 2014). The study demonstrated that Malathion is toxic to Tilapia, *Oreochromis mossambicus* and the histological observations showed that exposure to the toxic concentration of Malathion caused the alterations in the gill tissues of *Oreochromis mossambicus* to a greater extent. These degenerative changes are expected to adversely affect the physiological process of respiration and cause many abnormal effects in the fishes in due course (Subburaj *et al.*, 2018). In a study entitled "Malathion induced histological modifications in gills and kidney of *Carassius auratus gibelio* gills and kidney tissues were exposed to malathion to see the histological changes for 24 hours exposure period. These changes included vacuolization of cytoplasm, changes in cell and nuclear volumes, detached cubic epithelial cells lining and renal tubuli from the basal membrane (Deka and Mahanta, 2016). After fish exposure to Malathion Extended exposure of fish to pesticides or insecticides poses a heightened risk, particularly through the consumption of contaminated fish, which serve as valuable and easily accessible sources of protein. The frequency and excessive utilization of pesticides or insecticides stand as key factors contributing to the deterioration of aquatic ecosystem quality (Sabra and Mehana, 2015). Fish offer a rich source of nutrients, proteins, and minerals not readily found in other foods, making them a major global protein resource (Lynch *et al.*, 2016). As alternatives

to fishmeal gain attention, research is underway on the utilization of carbohydrates in aquafeeds (Ngugi *et al.*, 2017). Declines in fish populations and fisheries can adversely affect population growth and economic factors (Limburg *et al.*, 2011). Ensuring the safety and quality of fish remains crucial, given that consuming contaminated fish can lead to adverse health outcomes. Therefore, extensive global research has been conducted on heavy metal contamination in fish (You *et al.*, 2018). Fish offer a comprehensive nutritional package encompassing energy, protein, amino acids, vitamins, and minerals, including omega-3 fatty acids Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA) (Rahman *et al.*, 2012). These nutritional advantages contribute to positive health impacts, such as protection against diabetes and cardiovascular diseases (Hossain *et al.*, 2023). However, fish contamination undermines these health benefits and introduces risks to consumers (Varol *et al.*, 2017). The Indian major carp, scientifically known as *Cirrhinus mrigala* and commonly referred to as Mrigal fish that holds significant economic importance within Southeast Asian countries, there it is cultivated for its role as a food source (Rahman *et al.*, 2008; Joardar *et al.*, 2015).

The main objective of present research was to assess the potential toxicity of Malathion on fish health. Therefore, the present study aimed to investigate the effect of Malathion on histopathological alterations, nuclear changes in erythrocytes, and DNA damage in *Hypophthalmichthys molitrix* brain after exposure at environmentally relevant concentrations.

Materials and Methods

A research initiative was launched to assess the potential toxicity of malathion on *Hypophthalmichthys molitrix* (body weight: 140-160g) at the Department of Zoology, Government Sadiq College Women University. Specimens were obtained from a public fish hatchery in Bahawalpur. Fish harvesting involved cleaning, acclimating, and testing in a 12:12 light-dark cycle with a 25% protein diet. Water quality was monitored, and 150 fish samples were collected from Hasilpur Fish Seed hatchery. After transportation, tanks were prepared with pH-adjusted tap water, and fish acclimatized for 14 days with daily checks, feeding, and measurements. Analytical-grade malathion was used to create stock solutions (T_0 , T_1 , T_2 , T_3 at concentrations: 0.066g/L, 0.132g/L, and 0.199g/L). The concentrations of malathion were determined

based on prior research (Rathnamma and Nagaraju, 2013). Over 18 days, fish were exposed to these solutions. Post-trial, fish were anesthetized, and DNA damage was examined in brain tissues. Histopathological studies involved brain sample collection from control and experimental fish (exposed for 40 days). Organ samples were dissected, labeled, preserved in 10% formalin, and fixed in Bouin's solution. Subsequent procedures included tissue dehydration, clearing, embedding in paraffin wax, section cutting (4 to 6 μ m thickness), and staining with hematoxylin and eosin. Observation at 100X and 400X magnification, along with photographic documentation using a digital camera, facilitated comparative histopathological analysis. Genetic toxicity was assessed through micronuclei and comet assay techniques (Hussain *et al.*, 2020).

Statistical analysis of collected data utilized ANOVA, and means were compared using Tukey's test through SPSS software (Waheed *et al.*, 2014).

Results and Discussion

A research study assessed the toxicity of malathion in *H. molitrix* fish. The study observed various clinical and behavioral signs in fish exposed to different malathion concentrations, including loss of balance, mucus secretion, gasping, increased swimming, rapid gill movement, protruding eyes, lack of coordination, erratic swimming, and solitary swimming. These signs were quantitatively scored. Control treatment T_0 showed no symptoms, while treatments T_1 to T_3 were exposed to different malathion concentrations for 18 days. Detailed data is available.

Table 1: Absolute weight of visceral organ weights of *hypophthalmichthys molitrix* exposed to malathion.

Absolute weight of brain (g)	T_0 (0.0)	T_1 (0.066 g/L)	T_2 (0.132g/L)	T_3 (0.199g/L)
6d	0.72 ± 0.26	0.723 ± 0.25	0.76 ± 0.20	0.99 ± 0.19
12d	0.75 ± 0.267	0.74 ± 0.01	0.77 ± 0.25	0.98 ± 0.28
18d	0.72 ± 0.26	0.75 ± 0.24	0.78 ± 0.26	0.99 ± 0.31

Absolute and relative weight of brain of *Hypophthalmichthys molitrix*

This study evaluated the impact of malathion treatments on the body and brain weight of *Hypophthalmichthys molitrix* over 18 days. The data suggests potential correlations between malathion concentration

and changes in weight. Further, analysis is required to understand the physiological implications of malathion on these aspects (Table 1 and Figure1).

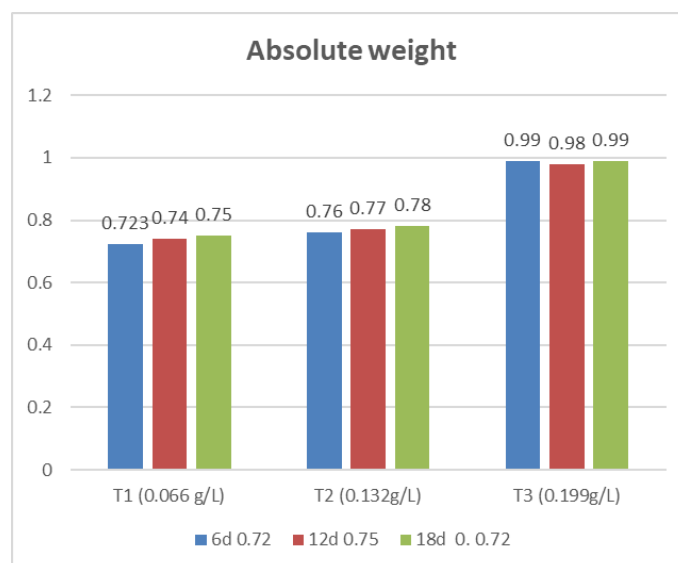


Figure 1: Absolute weight of visceral organ weights of *hypophthalmichthys molitrix* exposed to malathion.

DNA damage

To assess DNA damage, you can observe the mean values for each group at different time points. In general, higher mean values indicate more DNA damage. Smaller standard errors (SE) suggest more consistent or reliable measurements based on the data, you can see that DNA damage tends to increase over time for all groups, with the most significant increase observed in treatment T_4 . However, it's important to consider that the interpretation of DNA damage may require additional context and statistical analysis to draw meaningful conclusions about the significance of these changes (Table 2).

Table 2: Percentage of DNA damage in brain.

Parameters/ days	T_0 (0.0)	T_1 (0.066 g/L)	T_2 (0.132 g/L)	T_3 (0.199 g/L)
6	1.05 ± 0.02	1.38 ± 0.02	1.49 ± 0.03	2.11 ± 0.05
12	1.02 ± 0.02	1.43 ± 0.03	1.48 ± 0.04	2.36 ± 0.05
18	1.8 ± 0.02	1.45 ± 0.03	2.47 ± 0.04	2.58 ± 0.05

Morphological alterations in erythrocytes

In the experimental trial, significant morphological changes were observed in red blood cells of treatment T_3 fish on the 18th day, compared to the control group. Specifically, there was an increase in the percentage of leptocytes, spherocytes, microcytes, erythrocytes with broken nuclei, erythrocytes with lobed nuclei, blebbed nuclei, vacuolated nuclei, and nuclear

remnants in treatment T_3 on the 18th day. However, no substantial distinctions were noted in pear-shaped red blood cell ratios and leptocyte percentages among all treated and untreated fish on days 6 and 12.

Table 3: Morphological alterations in erythrocytes.

Parameters/ days	T_0	T_1	T_2	T_3
Leptocytes				
14	0.32±0.08	0.33±0.05	0.34±0.04	0.42±0.04*
28	0.35±0.098	0.39±0.03	0.42±0.04	0.47±0.03*
40	0.36±0.11	0.38±0.05	0.45±0.06*	0.46±0.07*
Spherocytes				
14	0.36±0.03	0.38±0.02	0.40±0.01	0.42±0.02*
28	0.39±0.02	0.41±0.02	0.44±0.02*	0.46±0.02*
40	0.41±0.02	0.52±0.03*	0.58±0.04*	0.78±0.04*
Microcytes				
14	0.66±0.01	0.68±0.02	0.68±0.03	0.69±0.03*
28	0.67±0.01	0.68±0.01	0.72±0.01*	0.76±0.03*
40	0.69±0.03	0.78±0.03	1.88±0.11	2.93±0.10*
Erythrocyte with broken nucleus				
14	0.32±0.02	0.34±0.02	0.35±0.01*	0.37±0.01*
28	0.33±0.02	0.34±0.02	0.37±0.03*	0.39±0.03*
40	0.35±0.06	0.39±0.06*	0.40±0.05*	0.93±0.05*
Erythrocyte with lobed nucleus				
14	0.44±0.02	0.47±0.02	0.48±0.01	0.49±0.01
28	0.45±0.05	0.54±0.04	0.56±0.02*	0.57±0.02*
40	0.47±0.04	0.63±0.04*	0.57±0.03	0.90±0.08*
Erythrocyte with micronucleus				
14	0.36±0.02	0.39±0.03	0.42±0.03	0.44±0.03*
28	0.38±0.03	0.41±0.04	0.44±0.03	0.46±0.04*
40	0.39±0.04	0.47±0.05	0.50±0.04	0.52±0.05*
Erythrocyte with blabbed nucleus				
14	0.28±0.01	0.30±0.01	0.32±0.03	0.33±0.03
12	0.29±0.02	0.31±0.03	0.33±0.02	0.35±0.02
18	0.30±0.03	0.39±0.04	0.41±0.04*	0.44±0.06*
Erythrocyte with vacuolated nucleus				
14	1.19±0.02	1.21±0.03	1.22±0.03	1.24±0.03
28	1.21±0.02	1.23±0.04	1.25±0.03	1.26±0.03*
40	1.23±0.05	1.33±0.04	1.35±0.02*	1.89±0.09*
Erythrocyte with nuclear remnants				
14	0.26±0.03	0.27±0.04	0.29±0.04	0.29±0.04
28	0.27±0.03	0.29±0.05	0.30±0.06	1.38±0.08*
40	0.29±0.05	0.39±0.06	0.99±0.09	2.58±0.09*

Additionally, there were no significant differences in the values of erythrocytes with micronuclei among

the groups on all observation days (Table 3 and Figure 2).

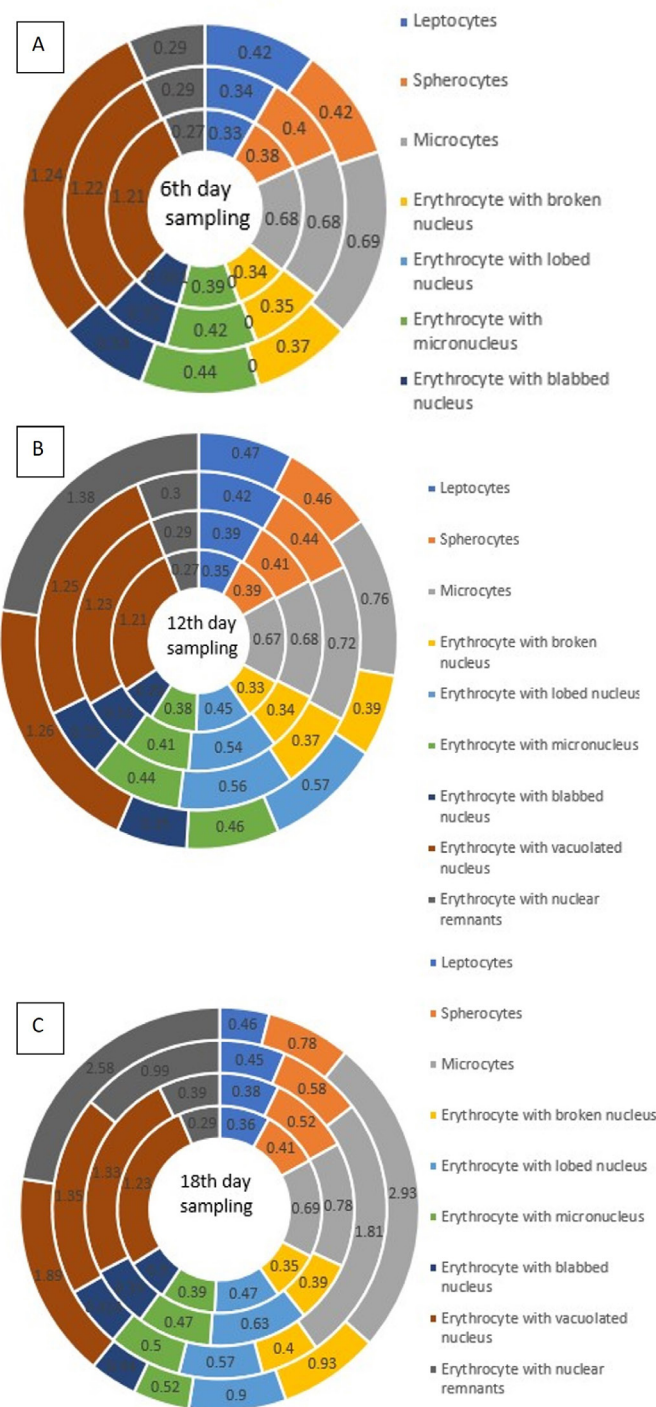


Figure 2: Morphological alterations in blood of fish exposed to malathion on sampling days (a= 6th sample collection day, b=12th sample collection day, c=18th sample collection day)

Histopathology

Histological examination revealed significant alterations in the fish brain of T_3 fish including necrosis of neurons, cytoplasmic vacuolization, edema, and congestion of neural cells when comparison was made with control group. Other treatment groups including

T_1 and T_2 also caused structural changes in brain cells including necrosis and vacuolization but these changes were not significant than control group.

Investigation was done on different species of fish encounter physiological, biochemical, histopathological ailments and DNA damage due to exposure to several toxic insecticides. Hence, this study investigated effects of insecticides on status of genotoxicity and histopathological ailments of silver carp fish (Naz *et al.*, 2022). In our trial, the results on genotoxic investigation exhibited significantly increased DNA damage potential of ferterra in different tissues of silver carp fish. The higher values of DNA damage in visceral tissues of silver carp fish (Raza *et al.*, 2022). In the experimental trial, a comprehensive analysis of red blood cell (RBC) morphology in fish from treatment T_3 revealed noteworthy changes on the 18th day of the study. Specifically, there was a marked increase in pear-shaped RBCs and leptocytes, indicating an alteration in the overall shape and size of these blood cells. These changes, however, were not observed on days 6 and 12, suggesting that they manifested after prolonged exposure to the experimental conditions. Moreover, significant increases in spherocytes and microcytes were noted in both treatments T_2 and T_3 on the 18th day. This elevation in these particular RBC types serves as a potential indicator of physiological and health changes in the fish. Yet again, these alterations were absent on days 6 and 12, underlining the importance of extended exposure in manifesting these effects. Micronucleus induction frequencies in erythrocytes were scored at 100x magnification, and the presence of nuclear abnormalities followed established protocols (Obiakor *et al.*, 2010).

Intriguingly, treatment T_3 exhibited a distinct pattern of change in erythrocytes, with notable differences in cells displaying broken and lobed nuclei on the 18th day. This suggests a potential detrimental impact on fish health within this group, albeit not evident on the earlier time points of the study. Surprisingly, micronuclei within erythrocytes displayed no significant variations across all treatments when compared to the control group on days 6, 12, and 18, suggesting that this particular measure of genetic damage remained relatively stable (Alink *et al.*, 2007). Furthermore, treatment T_3 demonstrated an increase in erythrocytes with lobed nuclei and vacuolated nuclei on the 18th day. These findings are indicative of potential oxidative stress and the presence of toxins. Importantly,

these effects seemed to require prolonged exposure to the experimental conditions and were not observable on the shorter time intervals of days 6 and 12. Likewise, treatment T_3 displayed an increase in erythrocytes with nuclear remnants, which could imply cellular damage, yet this was only evident after the extended exposure duration.

Shifting focus to DNA damage in the brain, the treatments administered in this study did not induce significant differences when compared to the control group on days 6, 12, and 18. This suggests that the treatments had a limited impact on the genetic material in fish brain cells and potentially lower risk to the integrity of these cells. In summary, this study unveiled substantial alterations in RBC morphology and provided some indicators of potential changes in fish health, particularly in treatment T_3 after 18 days of treatment. It is crucial to note that many of these effects were not discernible during the shorter exposure durations of days 6 and 12, underscoring the importance of prolonged exposure in assessing these physiological changes. Additionally, the treatments did not significantly affect DNA damage in fish brain cells, indicating a relatively lower risk to the genetic integrity of these cells from the treatments employed in the study. Nonetheless, further research is warranted to delve into the underlying mechanisms and long-term consequences of the observed changes, which could have implications for understanding the impact of these treatments on aquatic ecosystems and fish populations. For DNA damage of assessments, we used a novel, reliable and most sensitive technique comet assay. This technique was applied on fish erythrocytes. Recent research has confirmed these genotoxic modifications occur in response to the exposure to toxic agents in the environment. Such findings verify the genotoxicity in column fish *Hypophthalmichthys molitrix* and proved that DNA damage along with nuclear abnormalities could be used as biomarkers in response to insecticides. It could also be used for early monitoring of freshwater bodies by using simple and trustworthy techniques comet and a micronucleus assay (Pavlica *et al.*, 2011; Sharma and Chadha, 2023).

Conclusions and Recommendations

The research on malathion in fish *Hypophthalmichthys molitrix* reveals its complex impact on various physiological aspects, including brain histopathology, erythrocyte nuclear changes, and DNA changes in visceral

organ. These findings underscore the need for further investigation into malathion mechanisms and the importance of monitoring and managing its presence in aquatic environments to protect aquatic life and potential human interactions with affected ecosystems. Further research is crucial for a comprehensive understanding and mitigation of adverse consequences from malathion exposure.

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Novelty Statement

The current study highlighting the insecticide contamination as major freshwater environmental issue which poses a significant threat to aquatic organisms.

Author's Contribution

Saima Naz: Planned research, supervision of the study and arrangement of supplies, manuscript write-up and reviewed the final version of manuscript.

Moazama Batool: Execution of study, formatting, data analysis and reviewed the final version of manuscript.

Qurat Ul Ain: Helped in analyze the experimental data and writing research paper.

Ahmad Manan Mustafa Chatha: Helped in conducting research and helped in data analysis.

Sheeza Bano and Sadia Nazir: Helped in data compilation and manuscript writing.

Ghulam Abbas: Reviewed the manuscript and data analysis.

Unab Zahra: Performed the experiment in laboratory.

Conflict of interest

The authors have declared no conflict of interest.

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