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Effects of Pesticide Contamination on Fish Health and Aquatic Biodiversity

Bharat Singh*[1], Manoj Kumar[1], Sheetal Rajput[1] and Harendra Nath Sharma[2]

[1]*Department of Zoology, Shri Varshney College, Aligarh Affiliated to Raja Mahendra Pratap Singh University, Aligarh, Uttar Pradesh, India*
[2]*Department of Zoology, Agra College, Agra, Affiliated to Dr. Bhimrao Ambedkar University, Agra, Uttar Pradesh, India*

* Corresponding author. E-mail address: bharatsinghggc@gmail.com

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ABSTRACT

Remote Sensing Increasing utilization of agricultural pesticides is leading to severe contamination of aquatic animals particularly fish. The present study is aimed at inducing toxic action of organophosphate pesticide Malathion on fish *Channa Punctatus* specifically in the context of hematological, biochemical, oxidative stress and histological aspect. Fishes were treated with Malathion the sub lethal fraction and were studied at different intervals (7, 14, 21, and 28 days). Physiological response was determined. The fish showed anemia and low transport of oxygen is ascertained by the decrease in Hb and RBC's count Leucocytosis, the increased WBC's count is seemingly an immune reaction to the toxic stress. A significant increase was observed in liver enzymes, ALT, AST and ALP indicating hepatocellular damage and disturbance in metabolism. Detection of oxidative damage was inferred from an increased ROS and LPO (MDA) with a highly significant decreased SOD, CAT and GPx. Histopathological study revealed damage in the tissues of liver, gills and kidney shown by necrosis, lamellar fusion and tubular degeneration.Thus *Channa Punctatus* was a victim of toxic action from Malathion by various physiological pathways

Introduction

The agricultural intensification is a worldwide increase in the use of pesticides, helping to improve crop yields and protect against pests and diseases. While these chemicals are essential tools in modern agriculture, improper or overuse can cause widespread environmental contamination, particularly in aquatic environments. Pesticides can enter water bodies through surface water runoff, leaching, drift from spray applications, and returns to surface water from irrigation. Thus, they present a particularly serious risk to aquatic animals, particularly fish due to their continual exposure to contaminated water as well as being key components of aquatic food webs. Fish are widely recognized as effective bioindicators of environmental pollution because of their sensitivity to toxic materials as well as their ability to bioaccumulate contaminants within their bodies. Exposure to pesticides can affect the normal physiological and biochemical functions of fish, including their normal growth, reproduction and survival even at sub-lethal levels of exposure (Kumar *et al*., 2010). Organophosphate insecticides, e.g., chlorpyrifos and malathion, are two of the most commonly used agrochemicals and pose high risks to non-target aquatic organisms due to their toxicity. Organophosphates mainly exert their toxicity by inhibiting acetylcholinesterase activity, resulting in the accumulation of acetylcholine at nerve synaptic junctions, causing neurotoxicity (Fulton & Key, 2001). Pesticides can cause neurotoxicity as well as oxidative stress in aquatic organisms. The lack of balance between producing reactive oxygen species (ROS) and the production of an organism's own antioxidants is known as oxidative stress, and can result in excess ROS leading to cell membrane damage, damaging proteins, and damaging DNA which all lead to cells compromising their own integrity (Livingstone 2001). Research has shown pesticide exposure can significantly alter the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These antioxidant enzymes help detoxify organic compounds by removing ROS (Banerjee *et al*., 2016). Fish hematological and biochemical parameters have also been suggested to be a sensitive indicator of toxic stress-based responses as physiological changes in fish degree of hemoglobin, RBC counts, and WBC counts may demonstrate sign of anemia, immune responses or stress. Biochemical responses in fish include changes in hepatic alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), all being able to identify disruptions to hepatic function and distress to metabolic processes due to toxicant exposure (Adhikari *et al*., 2004). The liver being the organ primarily responsible for detoxification is an area commonly targeted with pesticides. Histopathological changes may take place due to chronic exposure to pesticides through the gills, liver, and eventual at the kidneys. The gill tissue being directly exposed to the external world undergoes disruptions on its structure (lamellar fusion and epithelial damage) with consequences in respiration and the regulation of ions. Likewise the liver tissue can show through cellular degeneration and necrosis, vacuolation and decreased metabolic activity as evidenced by histological staining processes (Hinton & Lauren 1990). The identification of such structural changes prove an extra indication of pesticide toxicity within any tissue. As agricultural practise is making more and more aquatic environments becoming permeated there is need to assess the adverse effects such pesticides have on the fish health. By appreciating in general the biochemistry of the oxidative stress, hormonal and cellular responses fish undergo to such toxic exposures we are not only able to appreciate how pesticides are toxic, but also assist in environmental monitoring as well as creating sustainable agriculture systems. The need for this study was to assess aquatic toxicity of agricultural pesticides and its effects on fish health using hematological parameters, oxidative stress and antioxidant enzymes activity.

Materials and Methods

2.1-Experimental Fish- Healthy freshwater fish, *Channa Punctatus*, were procured from local fish markets/fish farms in the Aligarh

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region, Uttar Pradesh, India. Fish of uniform size (average length: 10–12 cm; weight: 20–30 g) were selected to minimize variability. The fish were acclimatized to laboratory conditions for 10–15 days in aerated glass aquaria (capacity 50–100 L) prior to experimentation. During acclimatization, fish were fed with commercial fish feed and water was renewed regularly to maintain optimal conditions. Feeding was stopped 24 hours before the start of the experiment.

2.2Test Chemical- The organophosphate pesticide Malathion was used as the test toxicant. Analytical-grade malathion was procured from a certified supplier. A stock solution was prepared in distilled water and required concentrations were obtained by appropriate dilution.

2.3Determination of LC_{50}- The median lethal (LC_{50}) concentration of malathion for *Channa Punctatus* determined by standard methods (APHA, 2017). The fishes were exposed to different concentrations of malathion for 96 hours in separate tanks and mortality was recorded periodically. The LC_{50}value was calculated by probit analysis. Based on the LC_{50} value, a sub-lethal concentration was chosen for further studies (generally 1/10th LC_{50}).

2.4Experimental Design- The experimental fish were randomly divided into two groups:

•Control Group: Maintained in pesticide-free water

•Treatment Group: Exposed to sub-lethal concentration of malathion

Each group consisted of 10–15 fish, maintained in separate aquaria under controlled conditions. The exposure durations were:

7 days , 14 days , 21 days ,28 days

Water was renewed regularly, and pesticide concentration was maintained throughout the experimental period. Fish were observed daily for behavioral changes.

2.5 Physico-Chemical Parameters of Water

Water quality parameters were maintained within permissible limits:

•Temperature: 24–28°C

•pH: 7.0–7.5

•Dissolved Oxygen: 5–7 mg/L

•Total Hardness: 100–150 mg/L

These parameters were monitored regularly using standard methods (APHA, 2017).

2.6 Hematological Analysis

Blood samples were collected from the caudal vein using sterilized syringes. The following parameters were analyzed:

•Hemoglobin (Hb) – Cyanmethemoglobin method

•Red Blood Cell (RBC) count – Hemocytometer method

•White Blood Cell (WBC) count – Hemocytometer method

Standard procedures described by Dacie and Lewis (1991) were followed.

2.7 Biochemical Analysis

Serum was separated by centrifugation of blood samples at 3000 rpm for 10 minutes. The following biochemical parameters were estimated:

•Alanine aminotransferase (ALT)

•Aspartate aminotransferase (AST)

•Alkaline phosphatase (ALP)

These enzymes were measured using standard diagnostic kits.

2.8 Oxidative Stress Markers

Oxidative stress in fish tissues (liver and gills) was assessed by measuring:

•Reactive Oxygen Species (ROS) levels

•Lipid peroxidation (Malondialdehyde, MDA) using thiobarbituric acid reactive substances (TBARS) method

2.9 Antioxidant Enzyme Assay

The antioxidant defense system was evaluated by estimating:

•Superoxide dismutase (SOD)

•Catalase (CAT)

•Glutathione peroxidase (GPx)

Enzyme activities were determined using standard spectrophotometric methods.

2.10 Histopathological Examination

Liver, gill, and kidney tissues were dissected and fixed in 10% formalin. The tissues were dehydrated, embedded in paraffin, sectioned (4–6 µm), and stained with hematoxylin and eosin (H&E). Microscopic examination was carried out to observe structural alterations.

2.11 Statistical Analysis- All data were expressed as Mean ± Standard Deviation (SD). Statistical analysis was performed using one-way ANOVA followed by post hoc tests to determine significant differences between control and treated groups. A significance level of $p < 0.05$ was considered statistically significant.

Results

The present study revealed significant alterations in hematological, biochemical, oxidative stress, and behavioral parameters in *Channa Punctatus* exposed to the organophosphate pesticide Malathion. The magnitude of changes increased progressively with exposure duration, indicating time-dependent toxicity.

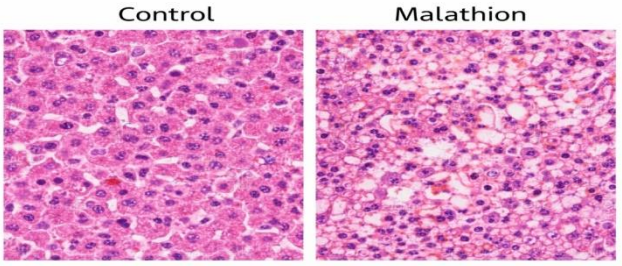

Fig: 1 Control liver normal; malathion shows necrosis, vacuolation, cellular degeneration

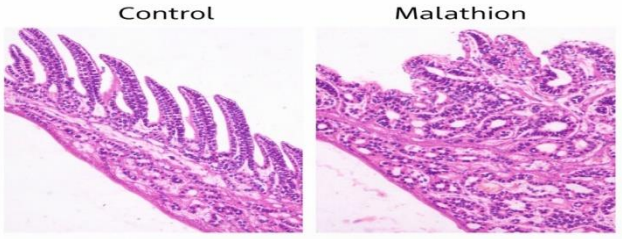

Fig2: Control gills normal; malathion shows lamellar fusion, epithelial lifting, damage

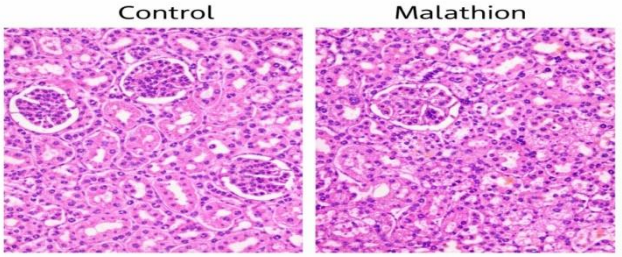

Fig3: Control kidney normal; malathion shows tubular damage, glomerular degeneration, infiltration

3.1 Hematological Changes- A significant decline ($p < 0.05$) in hemoglobin (Hb) concentration and red blood cell (RBC) count was observed in treated fish compared to the control group. The reduction in Hb levels became more pronounced with increasing exposure duration, indicating the development of anemia and impaired oxygen transport capacity. Conversely, white blood cell (WBC) count showed a significant increase ($p < 0.05$) in pesticide-exposed fish. This elevation suggests activation of the immune system in response to toxic stress. The progressive rise in WBC count over time reflects a physiological defense mechanism against pesticide-induced damage.

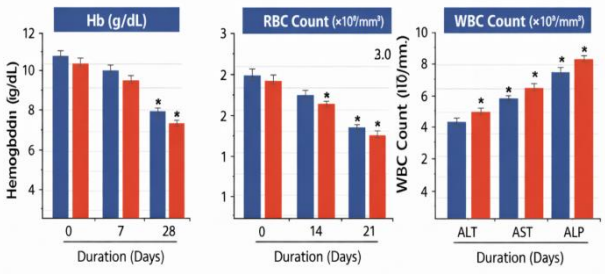

Fig:4 Control (Blue) stable: Malathion (Red) decreases Hb, RBC, increases WBC significantly

3.2 Biochemical Alterations- Biochemical analysis revealed a significant increase ($p < 0.05$) in liver enzyme activities, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), in treated fish. The elevation of these enzymes indicates hepatocellular damage and increased membrane permeability, resulting in leakage of intracellular enzymes into the bloodstream. The increase in enzyme levels was directly

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proportional to the duration of exposure, suggesting cumulative toxic effects of malathion on liver function. These findings confirm that the liver is a primary target organ for pesticide toxicity.

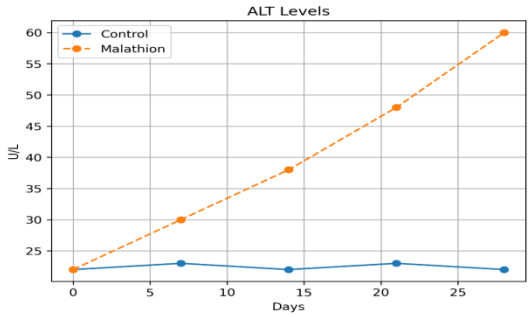

Fig:5 ALT increases in malathion; control remains stable over exposure duration

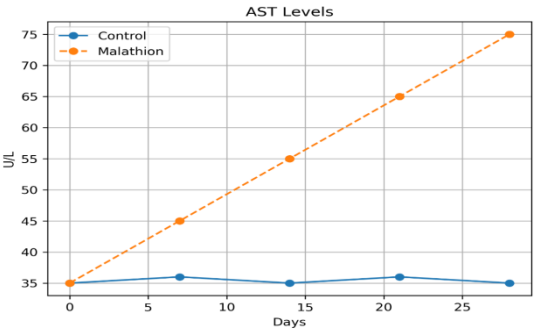

Fig:6 AST increases with malathion; control levels remain stable over time

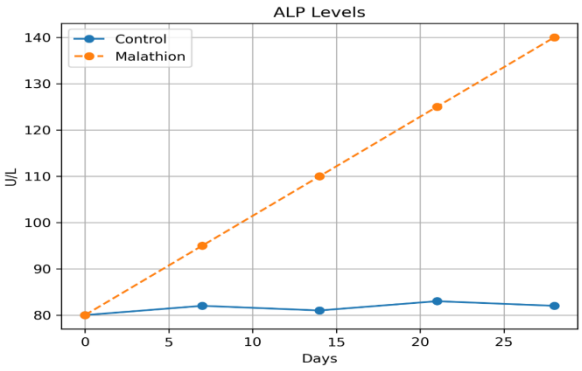

Fig: 7 ALP increases with malathion; control shows minimal change over time

3.3 Oxidative Stress- Exposure to malathion resulted in a significant increase ($p < 0.05$) in reactive oxygen species (ROS) levels in fish tissues. The generation of ROS showed a progressive rise with increasing exposure periods, indicating enhanced oxidative stress. Additionally, lipid peroxidation levels, measured as malondialdehyde (MDA), were significantly elevated in treated fish. The increase in MDA levels confirms oxidative damage to cellular membranes, leading to loss of structural integrity and cellular dysfunction. These results clearly demonstrate that oxidative stress is a key mechanism of malathion-induced toxicity.

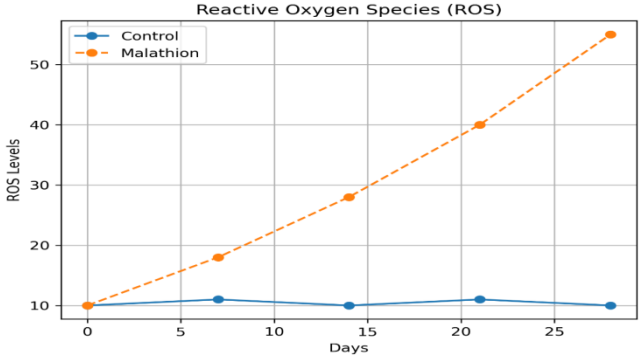

Fig:8 ROS increases significantly with malathion indicating enhanced oxidative stress

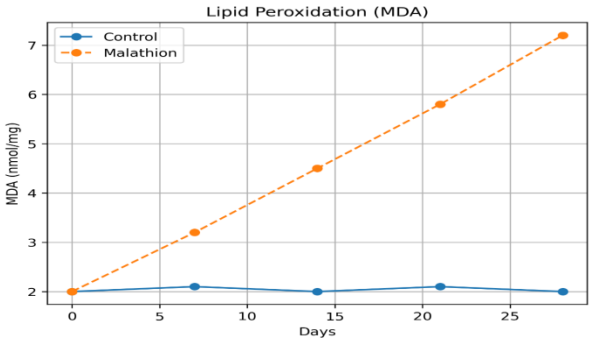

Fig: 9 MDA increases with malathion confirming lipid peroxidation and cell damage

3.4 Antioxidant Enzyme Activity- A significant decrease ($p < 0.05$) in antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), was observed in pesticide-exposed fish. The decline in these enzymes suggests exhaustion of the antioxidant defense system under continuous oxidative stress. The reduction in antioxidant enzyme activity became more severe with prolonged exposure, indicating an imbalance between pro-oxidant generation and antioxidant defense. This imbalance ultimately contributes to cellular and tissue damage.

Discussion

The present study reveals that exposure to organophosphate pesticide Malathion causes respective hematological, biochemical, oxidative and histopathological changes in organs of *Channa Punctatus* indicating toxicity. Our results corroborate findings that even sub lethal concentrations of the pesticide may evoke significant physiological stress on the physiological thermometry of fishes. The decrease in levels of Hb and RBC indicates the probable development of anaemia due to destruction of erythrocytes or inhibition of hematopoiesis. Such declines in Hb and RBC levels have been reported for the fish exposed to pesticides due to lack of oxygen transport and metabolic dysfunctions (Adhikari *et al*., 2004). Increase in counts of WBC may be due immune response activated against toxic stress, which was observed earlier (Kumar *et al*., 2010) as leukocytosis associated with pollutant exposure. The rise in liver enzymes levels (ALT, AST, ALP) is indicative of hepatocellular damage and increase in permeability of cell membrane. The liver is primary organ for detoxification and its association makes it highly susceptible to the toxicity of pesticide. The leakage of these enzymes indicates damaged cells and disturbance of metabolic activity. Increase in transaminases were reported earlier when fish were exposed to organophosphate pesticides (Fulton & Key, 2001). Oxidative stress appears to be a noteworthy mechanism of toxicity involved with malathion exposure. The increasing levels of ROS and MDA progressively indicates over active lipid peroxidation leading to oxidative damage to cell membrane. Such finding have been reported in earlier studies that the excess generation of ROS causes cellular damage and induces tissue injury (Livingstone, 2001). The increased level of MDA reflects the breakdown of cell membrane polyunsaturated fatty acid confirming the structural integrity. Decrease in levels of antioxidant enzymes (SOD, CAT, GPx) indicates that antioxidant system protective is evaded by excess of free radicals. Such observations are documented in the literature concerning decline of antioxidant activity (Banerjee *et al*., 2016). Effect of Malathion on oxidative stress and their significance in Fish and Animal Health indicating a major finding. Fish were exposed to various pesticides. A significant correlate in the study with 0.5& to 0.7 % decrease in SOD levels. Histopathology of liver, gills, kidneys shows that structures such as necrosis, vacuolization lamellar fusion and tubular degeneration establish the organ specific nature of toxicity corroborating (Hinton & Lauren, 1990) that pesticide exposure give rise to tissue damage in fish.

Conclusion

The present findings represent that the organophosphate pesticide malathion is a potential toxin to fish *Channa Punctatus* and detrimental even at a sub-lethal concentrations on the physiological, biochemical and cellular levels of the fish. There was a significant decrease in Hb and RBC, indicating a condition of anaemia and reduced oxygen transport. The compensatory increase in the WBC indicated the organism was under duress, an adaptive response to the toxic agent. The recovery of these blood parameters strongly implicate them as biomarkers of possible early warning of

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intoxication coming from sublethal exposure to the pesticide. The biochemical parameters reflected a disturbance in liver function, with the increase in ALT, AST and ALP indicating cellular damage and some dysfunction of metabolic processes. The liver is well known to be the target organ for detoxification and the effects were exacerbated by an increase of oxidative stress as indicated by increased levels of ROS and peroxides (MDA) coupled with a decrease of antioxidant status (SOD, CAT, GPx) indicating that the homeostasis of various antioxidant activity had been disturbed by the denaturing effect of the pesticide. The hepatocytes could be impaired by ROS perturbing the physiology of the liver, affecting both metabolic as well as cellular information as evidenced by histological findings of which the parameters of note were necrosis in the derivatives of the hepatic parenchyma, lamellar fusion of gill epithelium, degenerative tubules in the kidney, and other lesions. The results, which show positive correlation, lend credence to a probable complex and cumulative mechanism at work in the function of fish under malathion toxicity on the one hand, and the more intensive the contact, the greater the noxious manifestations from all systems of the organism. Extrapolating, the practical import is clear; namely, that significant remedy be formulated to stroke the possible deleterious effects of malathion upon aquatic organisms and their connection with the targeted beneficial pedigree of man. Regulation of pesticides use through strict public policy to screen people and impact studies and eco-friendly alternatives adopted, and monitoring aquatic organisms in random batches to check for pollution outburst./ Fish based biomarker studies such as this work can fill in a important utility zone within the sustainable enviromnent and ecology programme apparatus.

References

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C. T., & Ayyappan, S. (2004). Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, Labeo rohita. Ecotoxicology and Environmental Safety, 58(2), 220–226. https://doi.org/10.1016/j.ecoenv.2003.12.003

Aktar, M. W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: Their benefits and hazards. Interdisciplinary Toxicology, 2(1), 1–12. https://doi.org/10.2478/v10102-009-0001-7

Banerjee, S., Chattopadhyay, P., & Roy, S. (2016). Oxidative stress and antioxidant responses in fish exposed to pesticides. Environmental Toxicology and Pharmacology, 45, 135–144.

Fulton, M. H., & Key, P. B. (2001). Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphate and carbamate insecticide exposure. Environmental Toxicology and Chemistry, 20(1), 37–45. https://doi.org/10.1002/etc.5620200104

Hinton, D. E., & Lauren, D. J. (1990). Integrative histopathological approaches to detecting effects of environmental stressors on fishes. American Fisheries Society Symposium, 8, 51–66.

Kumar, A., Prasad, M. R., Srivastava, K., Tripathi, S., & Srivastav, A. K. (2010). Branchial histopathological study of catfish exposed to pesticides. Bulletin of Environmental Contamination and Toxicology, 84(3), 321–324. https://doi.org/10.1007/s00128-010-9950-7

Livingstone, D. R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollution Bulletin, 42(8), 656–666. https://doi.org/10.1016/S0025-326X(01)00060-1

Rajput, Sharma and Khan, 2024. ‘A review on Toxic Effect of Triazine on Hematological Parameters of *Channa Punctatus* (Bloch)’, Journal of Science Innovations and Nature of Earth, 4(4), 58–62. https://doi:10.59436/jsiane.288.2583-2093

Singh, B., Maheshwari, M., & Sharma, H. (2024). Nutritional studies on fish Catla catla found in Ganga River with hydrobiological studies. Journal of Science Innovations and Nature of Earth, 4, 17–22. https://doi.org/10.59436/ht5mmg32

Singh, B., Sharma, H. N., Prveen, & Singh, S. (2024). A review on effect of cypermethrin on hematology of fresh water fish *Channa Punctatus* (Bloch.). Journal of Science Innovations and Nature of Earth, 4. https://doi.org/10.59436/jsiane.271.2583-2093

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