International Journal of Bioinformatics and Biological Sciences
Citation: IJBBS: **11**(1&2): 23-31, June & December 2023
DOI: 10.30954/2319-5169.1.2023.3
Peer Reviewed Journal

RESEARCH PAPER

Chemical Pesticide vs. Bio-pesticides: Impact on Climbing Perch- Special Reference to Biochemical Parameters, Histology and DNA Integrity

Arijit Chaudhuri[1], Swagata Parui[1], Pratyusha Adhikary[1], Amit Chattopadhyay[1*], Chiranjeeb Dey[2], Sandip Mukherjee[3] and Tiyesh Paul[3]

[1]Vector Molecular Biology Unit, Department of Zoology for UG and PG Studies, Serampore College, Serampore, Hooghly, West Bengal, India
[2]Parasitology and Immunology Unit, Department of Zoology for UG and PG Studies, Serampore College, Serampore, Hooghly, West Bengal, India
[3]Endocrinology & Reproductive Physiology Unit, Department of Physiology, Serampore College, Serampore, Hooghly, West Bengal, India

*Corresponding author: chattopadhyayamit5@gmail.com

Received: 21-09-2023 **Revised:** 29-11-2023 **Accepted:** 06-12-2023

ABSTRACT

This study investigates the comparative effects of the chemical pesticide deltamethrin and the biopesticide neem extract on the climbing perch (*Anabas testudineus*) by assessing biochemical parameters, histological changes, and DNA damage using the comet assay. Deltamethrin, a widely used synthetic pyrethroid, is known for its high efficacy against pests but also poses significant risks to aquatic life. Conversely, neem extract, derived from the neem tree (*Azadirachta indica*), is considered a safer alternative due to its biodegradability and lower toxicity to non-target species. The results of this study indicated that deltamethrin exposure led to significant increases in stress protein levels, liver enzyme activities, along with pronounced histological damage in liver tissue. In contrast, neem extract exposure resulted in comparatively lower biochemical and histological alterations. The comet assay revealed higher DNA damage in fish exposed to deltamethrin compared to those treated with neem extract. These findings suggest that while both pesticides impact the health of climbing perch, neem extract poses a lower risk of biochemical and genetic damage. This study underscores the need for adopting biopesticides like neem extract to mitigate the adverse effects on non-target organisms associated with chemical pesticides.

Keywords: Aquatic Toxicology, Pesticide Impact, Biochemical Analysis, Genotoxicity, Environmental Safety

Pesticides are indispensable in modern agriculture, playing a crucial role in protecting crops from pests and diseases, thereby ensuring food security and economic stability. However, their extensive use has raised significant environmental concerns, and environmental toxicology has become a critical global issue, particularly regarding their impact on non-target organisms in aquatic ecosystems (Chandran *et al.* 2005). Over recent decades, the extensive application of pesticides in industrial, chemical, and

How to cite this article: Chaudhuri, A., Parui, S., Adhikary, P., Chattopadhyay, A., Dey, C., Mukherjee, S. and Paul, T. (2023). Chemical pesticide vs. Bio-pesticides: Impact on Climbing Perch- Special Reference to Biochemical parameters, Histology and DNA Integrity. *International Journal of Bioinformatics and Biological Sciences (IJBBS)*, **11**(1&2): 23-31.

Source of Support: None; **Conflict of Interest:** None

agricultural activities has significantly contributed to environmental pollution, posing severe risks to living organisms. Aquatic environments are especially vulnerable, as pollutants invariably accumulate in water bodies, leading to increased exposure of aquatic organisms, particularly fish, to toxic substances. Fish, due to their habitat, accumulate pesticide residues at levels much higher than the surrounding water, resulting in acute and chronic toxicity. This contamination adversely affects their skeletal systems, vital organs, and biochemical processes (Sathyamoorthi *et al*. 2018; Ravichandran *et al*. 2018). Additionally, the biomagnification of synthetic pesticides within aquatic food webs exacerbates the risk to aquatic life and, subsequently, to humans consuming contaminated fish, raising significant food safety concerns (Caulibaly *et al*. 1994).

Fish are a crucial dietary component, providing essential amino acids and high nutritional value. They significantly contribute to the supply of macro and micronutrients in human diets, particularly in coastal areas where fish is a primary protein source (Wahengbam *et al*. 2013). Proteins in fish are vital for structural integrity, biocatalysis, hormone function, and innate immunity. However, exposure to pesticides like deltamethrin can cause oxidative stress, genotoxicity, and histopathological alterations in fish (Anadón *et al*. 2013). Among the various pesticide classes, pyrethroids, such as deltamethrin, are extensively used in India for their efficacy against numerous pests in the field of agriculture (Devi *et al*. 2014; Mueller-Beilschmidt, 1990; Velisek *et al*. 2007; Marques *et al*. 2014). It functions by disrupting the normal function of the nervous system in insects, leading to paralysis and death. Despite their rapid degradation and minimal bioaccumulation, the excessive use of pyrethroids has led to significant contamination of aquatic environments through runoff and leaching. Pyrethroids exhibit properties like low water solubility, strong adsorption to soil and sediments, and rapid metabolism, yet fish are particularly susceptible due to the absence of specific hydrolytic enzymes and the high absorption rate through gills (Srivastava *et al.* 1997; Sayeed *et al*. 2003). The primary mechanism of pyrethroid toxicity involves the disruption of voltage-dependent sodium channels in nerve cell membranes, leading to neurotoxicity and oxidative stress, which significantly impairs fish physiology (Oliveira *et al*. 2012; Yonar *et al*. 2011). Numerous studies have been conducted in India to evaluate the effects of commonly used pesticides on non-target organisms. Chlorpyrifos, an organophosphate pesticide, is widely used in agriculture, while bifenthrin is employed not only in agricultural practices but also in public health programs, including mosquito control. Research examining the genotoxic effects of a mixture of bifenthrin and chlorpyrifos on *Labeo rohita* at sub-lethal concentrations reported a time-dependent increase in DNA damage over a 56-day exposure period, with a slight decrease observed after 14 more days (Bano *et al.* 2021). Comparative studies on the chronic exposure of *Cirrhinus mrigala* to chlorfenapyr, dimethoate, and acetamiprid revealed significant alterations in the blood and thyroid profiles, elevated liver biomarker enzyme levels, and pronounced histological damage to gills and liver tissues indicating that the indiscriminate use of such chemicals poses severe risks to non-target organisms, ecosystems, and human health (Ghayyur *et al.* 2021, Tarbah *et al.* 2007). Experiments on *Channa punctatus* exposed to malathion demonstrated a progressive decline in morphometric indices, biochemical parameters, and antioxidant enzyme activity with increased exposure time (Bharti & Rasool, 2021). These findings emphasize the need for stricter control over pesticide use to mitigate environmental and ecological damage.

Given the detrimental effects of synthetic pesticides, biopesticides offer a promising, environmentally friendly alternative. In this study, the ethanolic extract of neem leaves (*Azadirachta indica*) was employed as a biopesticide. Neem-based products are noted for their low toxicity towards non-target aquatic life and their potential to reduce bacterial populations and subsequent infections in fish (Gupta *et al*. 1993). Previous research has indicated that neem extract may induce less severe biochemical and physiological stress in aquatic organisms (Boeke *et al*. 2004; Raut & Swarup, 2019).

Stress proteins, particularly C-reactive protein (CRP), are part of the acute phase response to stress and inflammation. CRP is a well-known biomarker for physiological stress and is used to assess the impact of various stressors, including chemical exposure, on fish health (Cray *et al*. 2009). Elevated levels of

CRP in fish blood serum indicate an increased stress response, which can result from exposure to harmful substances such as pesticides (Thakur & Barman, 2017). These alterations not only impact fish health but also pose significant risks to human health, as the consumption of the fish with reduced protein content and increased pesticide residues can compromise dietary nutrition. The inclusion of contaminated fish in the human diet can lead to the accumulation of harmful chemicals in the body, potentially causing adverse health effects (Aktar *et al.* 2009).

Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) are crucial enzymes used as biomarkers to assess fish health, particularly in response to environmental stressors such as chemical exposure. AST is an enzyme found in various tissues but most common in liver, and is involved in amino acid metabolism. Elevated levels of AST in fish blood serum indicate tissue damage and are commonly associated with exposure to toxic substances, including pesticides. Similarly, ALP is an enzyme linked to the liver and bone health, playing a vital role in dephosphorylation processes. Increased ALP activity in fish can signal hepatobiliary damage and is often observed following exposure to environmental contaminants (Pelgrom *et al*. 1995). The presence of elevated AST and ALP levels in fish not only reflects compromised health but also poses significant risks to human consumers. Fish with high enzyme levels may indicate underlying tissue damage and contamination, leading to the accumulation of harmful substances in the fish. Consuming such fish can result in the ingestion of these contaminants, potentially causing adverse health effects in humans (Abdel-Tawwab *et al*., 2007). Therefore, monitoring AST and ALP levels in fish is essential for ensuring both aquatic and human health, highlighting the need for safer pest management practices in aquaculture.

The study of liver histology provides critical insights into the structural and functional integrity of this vital organ, particularly in response to environmental stressors such as pesticides. The liver is composed of numerous microscopic units called hepatic lobules, each centred around a central vein and bordered by portal triads consisting of a portal vein, hepatic artery, and bile duct. Hepatocytes, the primary liver cells, are arranged in cords radiating from the central vein, interspersed with sinusoids that facilitate blood flow. Histological examination of liver tissue can reveal various alterations, including cellular degeneration, necrosis, and fibrosis, which are indicative of toxic exposure and liver damage.

In the context of pesticide exposure, histological studies can identify specific changes in liver architecture, such as hepatocyte swelling, vacuolation, and bile duct proliferation. These alterations can compromise liver function, leading to impaired detoxification processes and overall health deterioration in fish. By comparing the histological effects of chemical pesticides like deltamethrin with biopesticides, researchers can assess the relative safety and impact of these substances on liver tissue. This information is crucial for developing safer pest management practices in aquaculture, ensuring the health of aquatic organisms and the safety of human consumers.

Chronic exposure to harmful chemicals can lead to DNA damage in liver cells, heightening the risk of liver diseases. The integrity of liver function is compromised by DNA damage, which also poses significant health risks to humans consuming contaminated fish. The ingestion of fish with DNA damage can lead to the bioaccumulation of harmful substances in the human body, potentially causing adverse health effects. Therefore, monitoring DNA damage in liver tissue is essential for assessing the genotoxic impact of environmental contaminants and ensuring the safety of both aquatic organisms and human consumers (Lodovici *et al.* 1996).

The freshwater tropical fish *Anabas testudineus* was selected as the model organism due to its economic importance, high survival rate even in polluted water, and rapid development under laboratory conditions. This study involved treating climbing perch with both deltamethrin and neem extract for 96 hours, followed by an analysis of enzymatic activity compared to control group. The findings from this study underscore the importance of adopting safer pest control methods to mitigate the ecological and health impacts of pesticide contamination, ensuring the sustainability of both aquatic ecosystems and human food sources.

MATERIALS AND METHODS

Study Design and Subjects

This study was conducted to compare the effects of the chemical pesticide deltamethrin and the biopesticide neem extract on climbing perch. A total of 30 healthy climbing perch, with an average weight of 30 ± 5 grams, were obtained from a local aquaculture farm. The fish were acclimatized in laboratory conditions for 72 hours before the experiment. The study was designed with three groups: a control group, a deltamethrin-treated group, and a neem extract-treated group, each consisting of 10 fish.

Chemicals and dosage regimen

Deltamethrin (commercial grade, 2.8 EC) was procured from Bayer Decis, and ethanolic-neem extract was prepared by percolation method with a 2:5 ratio of neem to ethanol. The fishes in both the deltamethrin-treated group and neem extract treated group were exposed to a sub-lethal concentration of 2 µg/L. The control group was maintained in pesticide-free water. The exposure period for both treatments was 30 days, with water parameters (temperature, pH, and dissolved oxygen) monitored and maintained consistently.

Biochemical analysis

Blood samples were collected from the caudal vein and heart of the fish at the end of the exposure period. The collected blood samples were incubated at 37°C for 30 minutes and centrifugation at 3000 rpm for 15 minutes to separate the components. The resulting supernatant, known as serum, was carefully extracted and subsequently stored at -20°C until analysis. Stress protein, C-reactive protein (CRP), was measured using a CRP agglutination test kit (Aspen Laboratories). CRP is a well-known biomarker for physiological stress and is used to assess the impact of various stressors, including chemical exposure, on fish health. Elevated levels of CRP in fish blood serum indicate an increased stress response, which can result from exposure to harmful substances such as pesticides. The CRP latex test involves mixing a latex reagent, which consists of polystyrene latex particles coated with anti-CRP, with a test serum. If the CRP concentration exceeds 0.6 mg/dl, visible agglutination occurs; otherwise, no agglutination is observed. The CRP latex reagent is a suspension of uniform polystyrene particles coated with monospecific anti-CRP in a glycine buffer at pH 8.8±0.5. The procedure began with the preparation of serial dilutions of the test sample (1:2, 1:4, 1:8) using 0.9% NaCl solution in labelled microcentrifuge tubes. Each diluted sample was then pipetted onto designated circles on a test slide using a sample dropper. One drop of CRP latex reagent was added to each circle containing the diluted sample and mixed thoroughly with a mixing stick. The slide was then slowly rotated for exactly 2 minutes and observed for agglutination under a high-intensity light source. Liver enzyme activities, including Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP), were determined using the 2,4-DNPH assay and Kind & Kings assay, respectively (Arkray) following the manufacturer's instructions.

Histological study

Liver tissue was excised and fixed in Carnoy's fixative for 24 hours. The tissues were then dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a microtome and stained with Haematoxylin and Eosin (H&E) for histological examination under a light microscope. Histopathological changes were documented and compared across the control and treated groups.

Assessment of DNA Damage

DNA damage in liver cells was assessed using the comet assay (single-cell gel electrophoresis). Liver tissues were minced into small cubes and digested with trypsin, followed by incubation at 37°C for 2.5 hours with intermittent vortexing. The resulting suspension was triturated and centrifuged at 5000 rpm to pellet the cells. The cell pellet was then washed with phosphate-buffered saline (PBS) and resuspended to obtain a single-cell suspension, which was subsequently stained with 4',6-diamidino-2-phenylindole (DAPI). For the comet assay preparation, 1% low-gelling-temperature agarose was prepared. Pre-coated slides were made by dipping them in molten agarose and allowing them to air dry. The single-cell suspension was mixed

with warm agarose and layered onto the pre-coated slides. After gelling, the slides were lysed overnight at 4°C in an alkaline buffer. The slides were then rinsed and subjected to electrophoresis at 0.6 V/cm for 60 minutes. Finally, the slides were stained with Ethidium Bromide to assess DNA damage and examined under a fluorescence microscope. DNA damage was quantified by measuring the tail length and tail moment using image analysis software (Comet Assay IV, Perceptive Instruments).

RESULTS AND DISCUSSION

Biochemical Parameters

The levels of C-reactive protein (CRP), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) were assessed in the serum of climbing perch across different treatment groups, including control, deltamethrin-treated, and neem extract-treated groups. The data is summarized in Table 1. The results, presented in the table, demonstrate a significant elevation in the levels of all three parameters in the deltamethrin-treated group, indicating a strong physiological stress response and potential liver damage due to pesticide exposure, while the neem extract may offer some mitigation of these harmful effects.

Histological Changes

Histological study of liver tissue showed normal liver architecture with no significant histological changes *(Fig. 1 A)*. However, the study revealed pronounced damage in the deltamethrin-treated group, including hepatocyte swelling, vacuolation, and bile duct proliferation (Fig. 1 B). In contrast, the neem extract-treated group exhibited comparatively minor alterations, with only slight hepatocyte swelling and minimal vacuolation (Fig. 1 C).

DNA Damage

The comet assay results indicated significantly higher DNA damage in liver cells of the deltamethrin-treated group compared to the control and neem extract-treated groups (Fig. 2 A, B, C). The tail length and tail moment were markedly increased in the deltamethrin group, indicating greater genotoxic stress. The neem extract-treated group showed moderate DNA damage, while the control group exhibited minimal DNA damage.

This study provides a comprehensive analysis of the differential impacts of chemical and biopesticides on climbing perch (*Anabas testudineus*), focusing on histological changes, DNA damage, and stress protein expression. The findings reveal significant insights into the relative safety and toxicity of these pesticides, contributing to the broader understanding of their effects on aquatic organisms. The liver tissue of the control group exhibited normal histological conditions, characterized by homogeneous, parenchymatous, polygonal cells with vesicular nuclei and a clear central vein. This observation aligns with the expected healthy liver structure, indicating that the control conditions did not induce any adverse effects. The absence of histological changes in the control group serves as a baseline for comparing the effects of the biopesticide and chemical pesticide (Isman, 2006). In the biopesticide (neem extract) group, the liver tissue showed mild lymphocyte infiltration but otherwise maintained normal histological conditions. The hepatocytes remained compact with centrally placed nuclei, and the sinusoids were clearly visible. This suggests that neem extract has a minimal impact on liver histology, with only slight immune response activation. Previous studies have also reported the safety of neem-based biopesticides, highlighting their potential as environmentally friendly alternatives to chemical pesticides (Schmutterer, 1990). Conversely,

Table 1: Serum Levels of CRP, AST, and ALP in Climbing Perch Across Different Treatment Groups

Parameter	Control group	Deltamethrin-treated group	Neem extract-treated group
CRP (mg/dL)	1.2	3.2	1.6
AST (IU/L)	18.375	185.75	33.25
ALP (KA/100 ml)	5.8	36.95	8.3

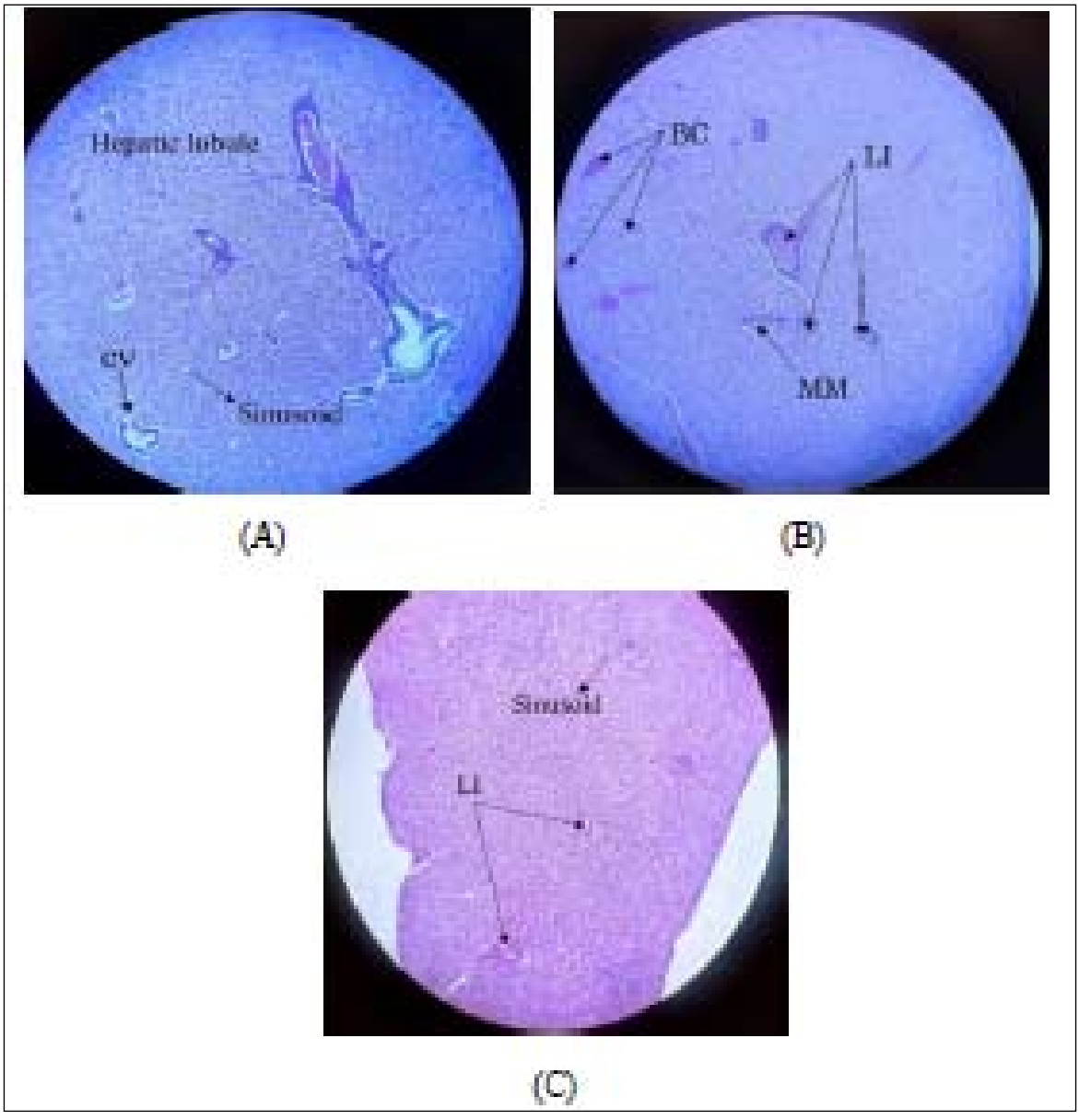

Fig. 1*:* **(A)** The photomicrograph of liver tissue from the control group reveals homogeneous, parenchymatous, polygonal cells formed from double layers separated by blood capillaries. Large polyhedral cells are visible within a network of minute canaliculi between the liver cells. The nuclei appear vesicular with a large nucleolus, indicating normal histological conditions. Irregular distribution of bile ducts, blood capillaries, and sinusoids filled with erythrocytes is observed. Hepatocytes surrounding the central vessels are compact with centrally placed nuclei, and the central vein (CV) is clearly visible. No histological changes are noted in the control group. **(B)** The photomicrograph of liver tissue exposed to deltamethrin shows homogeneous, parenchymatous, polygonal cells formed from double layers separated by blood capillaries. Sinusoids are clearly visible. Melano macrophage centres (MM) and blood congestion (BC) are observed, indicating excessive accumulation of blood within vessels. Lymphocyte infiltration (LI) is also noted. Numerous histological changes are evident in the liver tissue of fish exposed to the chemical pesticide. **(C)** In the photomicrograph of liver tissue exposed to the biopesticide, the tissue consists of homogeneous, parenchymatous, polygonal cells formed from double layers separated by blood capillaries. The nuclei are vesicular with a large nucleolus, indicating normal histological conditions. Mild lymphocyte infiltration (LI) is observed. Hepatocytes surrounding the central vessels are compact with centrally placed nuclei, and sinusoids are clearly visible. Apart from lymphocyte infiltration, no significant histological changes are observed in the liver tissue of biopesticide-exposed fish.

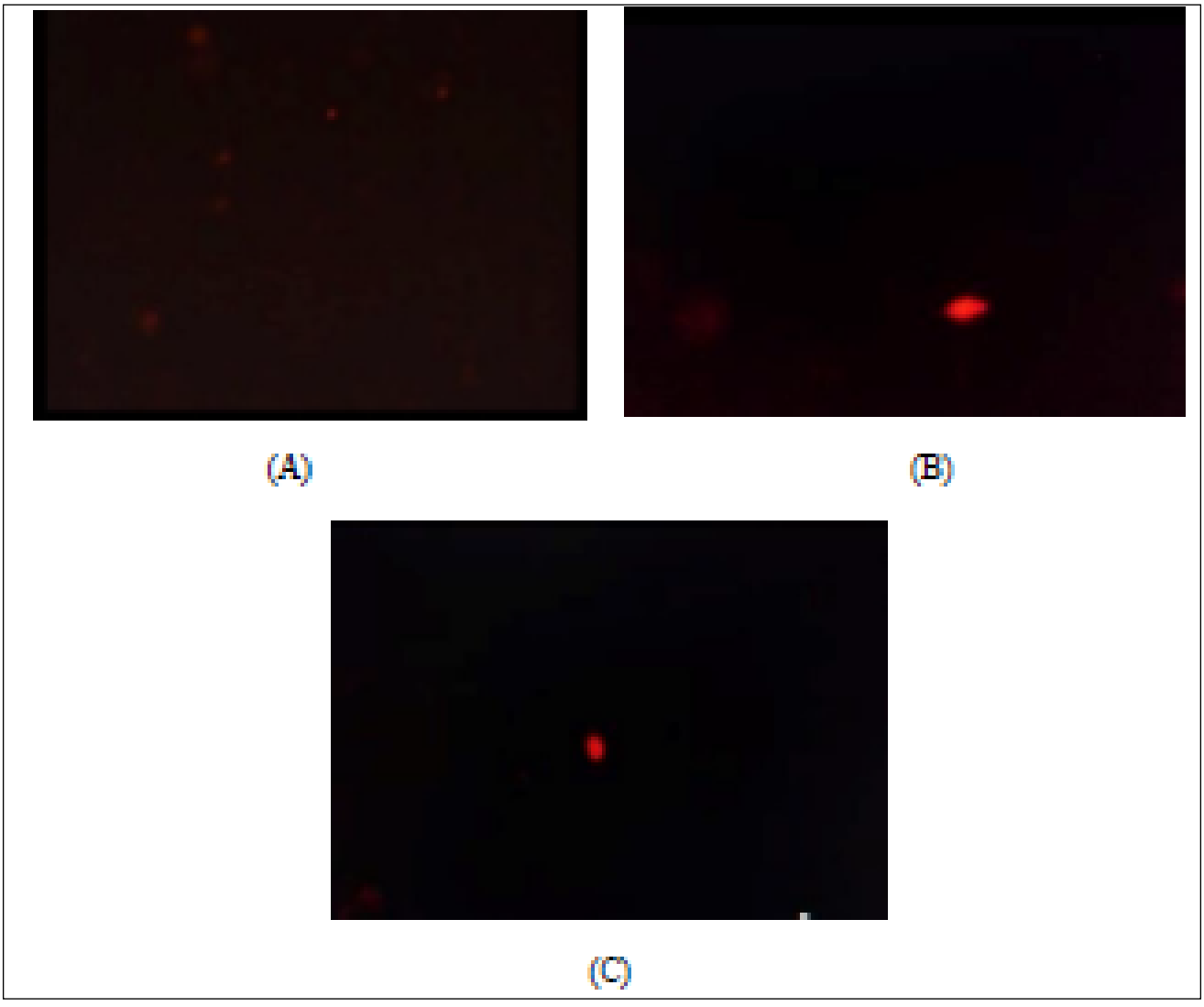

Fig. 2: (A) In the liver tissue of fish exposed to the control group, no DNA comet tail or nuclear disintegration was found, indicating normal histological conditions. **(B)** Conversely, in the liver tissue of fish exposed to the chemical pesticide (deltamethrin), a prominent DNA comet tail was evident, along with clear nuclear disintegration. **(C)** Fish exposed to the biopesticide, no prominent DNA comet tail was observed, although slight nuclear disintegration was noted.

the liver tissue of fish exposed to the chemical pesticide (deltamethrin) displayed significant histological changes. These included melano-macrophage centres (MM), blood congestion (BC), and lymphocyte infiltration (LI). The presence of these features indicates a pronounced inflammatory response and potential liver damage due to the chemical pesticide. These findings are consistent with earlier research demonstrating the deleterious effects of deltamethrin on aquatic organisms, underscoring its high toxicity (Bradbury *et al.* 2005).

The DNA damage was assessed using the comet assay, which revealed distinct differences between the control, biopesticide, and chemical pesticide groups. In the control group, no DNA comet tail or nuclear disintegration was observed, indicating intact DNA and normal cellular conditions. This serves as a reference point for evaluating the extent of DNA damage in the other groups. The slight increase in stress protein expression further supports the notion that neem extract induces minimal physiological stress. The chemical pesticide-exposed group displayed a prominent DNA comet tail and clear nuclear disintegration, indicating significant

DNA damage and cellular stress. In contrast, the biopesticide-exposed group, no DNA comet tail was observed, although slight nuclear disintegration was noted. This suggests minimal DNA damage, which is consistent with the mild histological changes observed.

The expression of stress protein (CRP) was analysed to assess the cellular response to pesticide exposure. In the control group, baseline levels of stress proteins were observed, reflecting normal physiological conditions. This serves as a reference point for evaluating the stress response in the other groups. The biopesticide-exposed group showed a slight increase in stress protein expression, indicating a mild stress response. This is consistent with the minimal histological changes and slight DNA damage observed, suggesting that neem extract induces minimal physiological stress. These findings align with previous studies that have highlighted the safety and efficacy of neem-based biopesticides in various aquatic environments (Schmutterer, 1990).

One of the strengths of this study is the comprehensive approach to assessing the impacts of pesticides, including histological analysis, DNA damage assessment, and stress protein expression. However, there are some limitations to consider. The study was conducted under controlled laboratory conditions, which may not fully replicate the complex interactions and environmental variables present in natural aquatic ecosystems. Additionally, the long-term effects of pesticide exposure were not assessed, which could provide further insights into the chronic impacts of these substances. Investigating the potential for bioaccumulation and the impacts on reproductive and developmental processes would also be valuable. Moreover, exploring the mechanisms underlying the differential toxicity of these pesticides could inform the development of safer and more effective pest management strategies.

CONCLUSION

In conclusion, this study underscores the importance of using biopesticides as a safer alternative to chemical pesticides. The significant toxic effects of deltamethrin call for cautious use and stringent regulations to protect aquatic ecosystems. In contrast, the minimal adverse effects of neem extract on climbing perch highlight its potential for sustainable pest management practices.

ACKNOWLEDGEMENTS

The authors would like to express sincere gratitude to Dr. Subhro Sekhar Sircar, Principal of Serampore College and Dr. Soumyajit Banerjee, HOD of Department of zoology for their encouragement in this work. They also thankful to Dr. Pranab Kumar Banerjee and Dr. Subarna Ghosh for their continuous help and support during this period.

REFERENCES

1. Abdel-Tawwab M. and Ahmad M.H. 2009. Effect of dietary protein regime during the growing period on growing performance, feed utilization and whole-body chemical composition of Nile Tilapia, *Oreochromis niloticus*. *Aquaculture Research,* **40**: 1532-1537.
2. Aktar, M.W., Sengupta, D. and Chowdhury, A. 2009. Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology,* **2**(1): 1-12.
3. Anadón, A., Martínez, M.A., Martínez, M., Castellano, V. and Martínez-Larrañaga, M.R. 2013. Pyrethroids: Advances in exposure, toxicity, and detection. *Journal of Toxicology and Environmental Health,* **15**(7): 444-467.
4. Bano, N., Nadeem, A., Maalik, S., Mushtaq, S., Iqbal, N. and Khan, A.K. 2021. Effect of pesticides on erythrocytes of indigenous fish *Labeo rohita. Journal of King Saud University-Science,* **33**(7): 101586.
5. Bharti, S. and Rasool, F. 2021. Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on *Channa punctatus* (Bloch) fish. *Toxicology Reports,* **8**: 443-455.
6. Boeke, S.J., Baumgart, I.R., Van Loon, J.J.A., Van Huis, A., Dicke, M. and Kossou, D.K. 2004. Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against *Callosobruchus maculatus*. *Journal of Stored Products Research,* **40**(4): 423-438
7. Bradberry, S.M., Cage, S.A., Proudfoot, A.T. and Vale, J.A. 2005. Poisoning due to pyrethroids. *Toxicological Reviews,* **24**(2): 93-106.
8. Caulibaly, R.T., Ouattara, A., Gourene, G., Koné, T. and Teugels, G.G, 1994. Fishes of the Comoé National Park (Ivory Coast). *Cybium,* **18**(4): 343-367.
9. Chandran, R. and Sivakumar, A.A. 2005. Biochemical effects of monocrotophos on tissue enzymes of the freshwater fish, *Labeo rohita*. *Journal of Environmental Biology,* **26**(1): 123-128.
10. Cray, C., Zaias, J. and Altman, N.H. 2009. Acute phase response in animals: A review. *Comparative Medicine,* **59**(6): 517-526.

11. Devi, Y.B. and Gupta, A. 2014. Effects of neem extract on the haematology of freshwater fish *Channa punctatus*. *International Journal of Advanced Research,* **2**(3): 431-435.
12. Ghayyur, S., Khan, M.F., Tabassum, S., Ahmad, M.S., Sajid, M. and Badshah, K. 2021. A comparative study on the effects of selected pesticides on hemato-biochemistry and tissue histology of freshwater fish *Cirrhinus mrigala* (Hamilton, 1822). *Saudi Journal of Biological Sciences,* **28**(1): 603-611.
13. Gupta, P.K., Mathur, N. and Singh, Y.D. 1993. Histopathological changes induced by cypermethrin in kidney of *Cirrhinus mrigala*. *Bulletin of Environmental Contamination and Toxicology,* **51**(6): 900-904.
14. Isman, M.B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology,* **51**: 45-66.
15. Lodovici, M., Casalini, C., Briani, C. and Dolara, P. 1996. Oxidative DNA damage after chronic exposure to a common air pollutant mixture in rats. *Environmental Health Perspectives,* **104**(11): 1350-1354.
16. Marques, S.M., Antunes, S.C., Nunes, B., Gonçalves, F. and Pereira, R. 2014. Toxicological effects of environmental contaminants in freshwater fish species. *Environmental Research,* **135**: 379-388.
17. Mueller-Beilschmidt, D. 1990. Toxicology and environmental fate of synthetic pyrethroids. *Journal of Pesticide Science,* **15**(3): 29-35.
18. Oliveira, R., Domingues, I., Grisolia, C.K. and Soares, A.M.V.M. 2012. Effects of pyrethroid pesticide cypermethrin on growth and biochemical biomarkers in the mosquito fish (*Gambusia holbrooki*). *Environmental Toxicology and Chemistry,* **31**(9): 2039-2045.
19. Raut, P.K. and Swarup, D. 2019. Protective effect of ascorbic acid on lipid peroxidation and oxidative stress in fish exposed to deltamethrin. *Fish Physiology and Biochemistry*, **45**(2): 603-611.
20. Ravichandran, P., Selvanayagam, M. and Kumar, K, 2018. Impact of malathion on haematological and biochemical parameters of *Labeo rohita*. *International Journal of Fisheries and Aquatic Studies,* **6**(4): 17-23.
21. Pelgrom, S.M.G.J., Lamers, L.P.M., Lock, R.A.C., Balm, P.H.M., and Wendelaar Bonga, S.E. 1995. Interactions between copper and cadmium modify metal organ distribution in mature tilapia, *Oreochromis mossambicus*. *Environmental Pollution,* **90**(3): 415-423.
22. Sathyamoorthi, A., Manogaran, M., Jothivel, S. and Vinothkumar, P. 2018. Toxicity effect of cypermethrin on the histological changes in the gill, liver and kidney of freshwater fish *Catla catla. Journal of Entomology and Zoology Studies,* **6**(2): 2035-2040.
23. Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R. and Raisuddin, S. 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicol. Environ. Saf.,* **56**(2): 295-301.
24. Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annu. Rev. Entomol.,* **35**: 271-97.
25. Tarbah, F.A., Shaheen, A.M., Benomran, F.A., Hassan, A.I. and Daldrup, T. 2007. Distribution of dimethoate in the body after a fatal organphosphate intoxication. *Forensic Science International,* **170**(2-3): 129-132.
26. Thakur, P. and Barman, P. 2017. Stress responses in fish due to environmental pollutants. *International Journal of Fisheries and Aquatic Studies,* **5**(2): 169-172.
27. Velisek, J., Svobodova, Z. and Piackova, V. 2007. Effects of acute exposure to deltamethrin on some hematological and biochemical indices of rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Pharmacology,* **23**(3): 430-436.
28. Wahengbam, P.D. and Reddy, P.R. 2013. Effect of sublethal concentrations of chlorpyrifos on haematological parameters of the fish *Clarias batrachus*. *The Bioscan.,* **8**(1): 105-10.
29. Yonar, M.E., Sakin, F. and Yonar, S.M. 2011. The protective effect of propolis on some biochemical parameters in *Cyprinus carpio* exposed to pyrethroid deltamethrin. *Fish Physiology and Biochemistry,* **37**(4): 959-963.