

Assessment of the Micronucleus Assay and Analysis of Fish RBCs Exposed to Pyridaben in *Hypophthalmichthys Molitrix* and *Labeo Rohita*: A Public Health Concern

Original Article

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Citation

Rauf, S., Khan, M.Z.U., Rehman, A., Khan, I., & Shams, A. (2025). Assessment of the micronucleus assay and analysis of fish RBCs exposed to pyridaben in *Hypophthalmichthys Molitrix* and *Labeo Rohita*: A public health concern. *Open Access Public Health and Health Administration Review*, S-1(1), 16-23.

WEBSITE: www.mdpi.com

ISSN: Print: 2959-619X

ISSN: Online: 2959-6203

PUBLISHER: MDPIP

Abstract

The present study was conducted to examine how exposure to pyributicarb affects the shape of red blood cells (RBCs) and the production of micronuclei in two commercially significant fish species: *Labeo rohita* (Rohu) and *Hypophthalmichthys molitrix* (silver carp). Pyribaden is a chemical that is commonly used in agriculture and has been linked to several harmful impacts on aquatic life. In the present study, 33 specimens were taken and exposed to Pyribaden. Blood then blood sample were taken and a micro assay on them. The results show that micronuclei were formed, and production was increased by increasing the chemical concentration. These results point to the possibility of genotoxic and hematotoxic effects of pyridabin on fish, underscoring the need for more investigation to clarify its modes of action and any possible ecological ramifications. To evaluate pyridabin overall toxicity and implement practical mitigation techniques to protect aquatic ecosystems, it is imperative to understand the effects of pyribaden on fish red blood cells. We need to act regarding the control of pesticides, such as pyridabin and others like it, to control aquatic pollution.

Keywords: Pyridaben, Micronuclei, Chemicals, *Labeo Rohita*, *Hypophthalmichthys Molitrix*.



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Introduction

Pesticide toxicity adversely affects the growth, physiology, reproduction, immunity, hemato-biochemical profile, and induces serious histopathological alterations in several tissues of aquatic organisms, including fish (Arani, Kermani, Kalantary, Jaafarzadeh, & Arani, 2023). Water-based ecosystems exhibit greater biodiversity compared to terrestrial environments. While pesticides play a crucial role in modern agriculture by protecting crops from pests and diseases, their extensive usage poses significant risks to both the environment and human health. Prolonged exposure to pesticides has been linked to various health issues, including respiratory problems, neurological disorders, and cancer (Alengebawy, Abdelkhalek, Qureshi, & Wang, 2021). Additionally, the widespread application of pesticides can result in environmental contamination, adversely affecting soil quality, water sources, and non-target organisms, thereby posing threats to biodiversity and ecosystem health (Zhang *et al.*, 2021).

To tackle these challenges, it is essential to adopt sustainable pest management strategies. Integrated Pest Management (IPM) practices offer a promising approach by combining chemical controls with biological and cultural methods. This approach aims to reduce reliance on pesticides while minimizing environmental and health risks (Koul, Yakooob, & Shah, 2022). By embracing such strategies, agriculture can effectively address pest control needs while safeguarding human health and ecological balance. The utilization of pesticides dates back millennia, as ancient civilizations employed natural substances to safeguard their crops against pests. Early societies like the Sumerians, Egyptians, and Chinese utilized botanical extracts and minerals to deter insects and rodents from damaging agricultural harvests.

The history of pesticides traces back thousands of years, with early civilizations using natural substances to protect crops from pests. The ancient Sumerians, Egyptians, and Chinese employed various botanical extracts and minerals to deter insects and rodents from devouring their agricultural yields (Khursheed *et al.*, 2022). However, it was not until the 19th century that synthetic pesticides emerged with the discovery of chemicals such as arsenic and sulfur compounds, which were widely used to combat pests.

The introduction of DDT (dichloro-diphenyl-trichloroethane) in the mid-20th century marked a significant milestone in pesticide history, hailed for its effectiveness against insects and disease vectors. However, the indiscriminate use of DDT led to environmental degradation and health hazards, prompting its ban in many countries due to its persistence and bioaccumulation in ecosystems (Carson, 1962). Subsequent generations of pesticides, including organophosphates, carbamates, and pyrethroids, have continued to evolve, with a focus on enhancing efficacy while minimizing environmental and health risks through regulatory frameworks and technological advancements. Despite ongoing efforts to develop safer and more sustainable pest management practices, the history of pesticides underscores the complex interplay between agricultural productivity, human health, and environmental conservation (Ahmed, Thompson, & Turchini, 2020).

Pyridaben is powerful and dangerous acaricide and insecticide, widely utilized in agriculture. Its primary mechanism of action involves disrupting complex I of the mitochondrial electron transport chain, thereby hindering adenosine triphosphate (ATP) production and triggering cellular respiration failure in targeted pests (Fish-Trotter *et al.*, 2020). This disruption results in paralysis and eventual mortality among the affected organisms, underscoring pyridaben's effectiveness against diverse pests such as mites, aphids, and whiteflies (Kumar, Singh, Dwivedi, Dubey, & Trivedi, 2022). Given its broad-spectrum activity and relatively low toxicity to mammals, pyridaben is deemed an asset in integrated pest management strategies for agriculture. However, persistent use raises concerns regarding its environmental impact and the potential development of resistance in target populations. (Helfrich, Neves, & Chapman, 2019).

Figure 1
Labeo rohita (Rohu)

Figure 2
Silver Carp


The present study aims to conduct a micronucleus assay on silver carp (*Hypophthalmichthys molitrix*) and *Labeo rohita* exposed to varying concentrations of Pyribaden to evaluate its genotoxic potential. It further seeks to examine the histological effects of Pyribaden on fish tissues, providing insights into cellular and tissue-level alterations induced by chemical exposure. Additionally, the study aims to assess the resistance levels of different fish species to Pyribaden and to analyze micronuclei formation as a biomarker of genotoxic stress in both species, thereby contributing to a better understanding of the toxicological impact of Pyribaden on aquatic organisms.

Literature Review

According to [Khursheed et al. \(2022\)](#), the utilization of pesticides dates back millennia, as ancient civilizations employed natural substances to safeguard their crops against pests. Early societies like the Sumerians, Egyptians, and Chinese utilized botanical extracts and minerals to deter insects and rodents from damaging agricultural harvests. The history of pesticides traces back thousands of years, with early civilizations using natural substances to protect crops from pests. The ancient Sumerians, Egyptians, and Chinese employed various botanical extracts and minerals to deter insects and rodents from devouring their agricultural yields ([Khursheed et al., 2022](#)). [Shankhu et al. \(2015\)](#) stated that Pyridaben is powerful and dangerous acaricides and insecticide, widely utilized in agriculture. Its primary mechanism of action involves disrupting complex I of the mitochondrial electron transport chain, thereby hindering adenosine triphosphate (ATP) production and triggering cellular respiration failure in targeted pests. This disruption results in paralysis and eventual mortality among the affected organisms, underscoring pyridaben's effectiveness against diverse pests such as mites, aphids, and whiteflies ([Shankhu et al., 2020](#)). As a result of the growth of the human population, the consumption of fish and fish products by fisheries forms a lucrative supply of proteins in a diet to be well. In 2009, 6.5% of protein consumed was that of fish and 16.6% of the animal protein was also fish that constituted 16.6 of the total protein intake by the world population ([Food, 2008](#)). The role of socio-economic development of the country is recognized. Fisheries plays an important role in the economy of the country and create a precious asset to a massive percentage of the population ([Aich, Goswami, Roy, & Mukhopadhyay, 2015](#)).

Methods and Materials

The present study was conducted to determine the effects of Pyribaden on the histology of silver carp (*Hypophthalmichthys molitrix*) and *Labeo rohita*. The research involved a series of carefully designed experimental steps using specific materials and procedures. Essential materials included gloves, syringes, distilled water, a dissection box, Petri dishes, methanol, EDTA tubes, permanent markers for labeling, and blood slides. Healthy specimens of both fish species were collected from a local hatchery in February 2024 using a casting net, with approximately 33 individuals of each species obtained and safely transported alive to the university laboratory in water tanks. The fishes were identified to the species level using identification keys such as *Fresh Water Fishes of the Indian Region* by [Jayaram \(1999\)](#) and *Pakistan Mein Taaza Pani Ki Machlian* by [Mirza \(2004\)](#). Silver carp were recognized by their small scales, terminal mouth, and elongated body with a rounded snout, while *Labeo rohita* were identified by their upturned mouth, laterally compressed body, large scales, and absence of barbels. In the laboratory, the fish

were maintained in water tanks with continuous electricity supply to ensure proper aeration and temperature regulation, creating optimal conditions for survival.

After a seven-day acclimatization period, Pyribaden was administered using a pipette in controlled doses due to its high toxicity. Four tanks were prepared—one as a control group and three as experimental groups. The control group contained seven fish maintained under natural conditions without pesticide exposure. Experimental Group 1 included seven fish exposed to 10 μL of Pyribaden, Experimental Group 2 contained eleven fish exposed to 20 μL , and Experimental Group 3 included eleven fish exposed to 30 μL of Pyribaden under identical artificial conditions. After seven days of exposure, blood samples were collected from both control and experimental groups using sterile syringes. The collected blood was immediately transferred into EDTA tubes to prevent clotting. For slide preparation, blood smears were made on clean glass slides and left to air dry for 30 minutes. The dried smears were then fixed with ethanol to preserve the cell structure. A total of eight slides were prepared—two for each group—for subsequent microscopic examination and histological analysis to assess Pyribaden-induced changes in blood cell morphology and tissue structure.

Results and Findings

After performing experimental work which was done to investigate the effect of pyribaden (pesticides) on the RBCs of Silver carp and Labeo rohita to look where the micronuclei are formed or not. The pesticides were added to water and allowed the fish to take it through their gills. After the exposure time, we took blood samples from the fish and made blood slides. The slides were studied under a compound fluorescent microscope in the Biotechnology Department University of Peshawar, which gave the following results.

Table 1

14th day MN analysis of Silver Carp

Concentration	MN	P
Control	00 $\pm$ 00	0.05
G1	11.06 $\pm$ 6.45	0.041
G2	13.66 $\pm$ 9.10	0.017
G3	23.060 $\pm$ 11.70	0.006

Figure 3

14th Day MN analysis

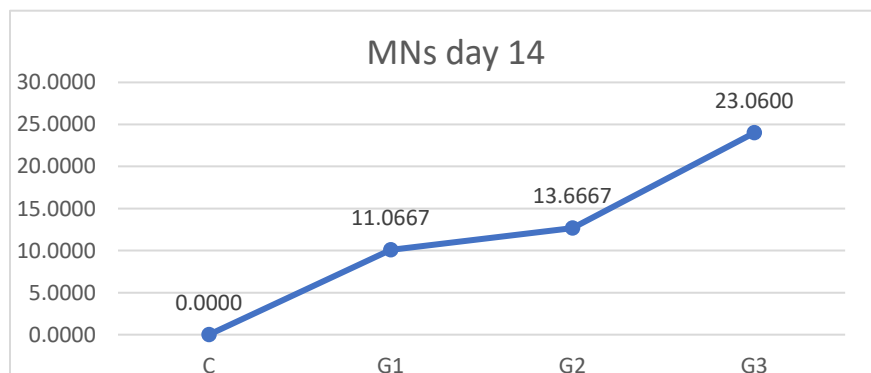


Table 2

Micro nucleus Analysis *Labeo rohita*

Concentration	MN	P
Control	00±00	0.05
G1	28.13±4.73	0.01
G2	32.33±5.81	0.00
G3	42.13±6.40	0.00

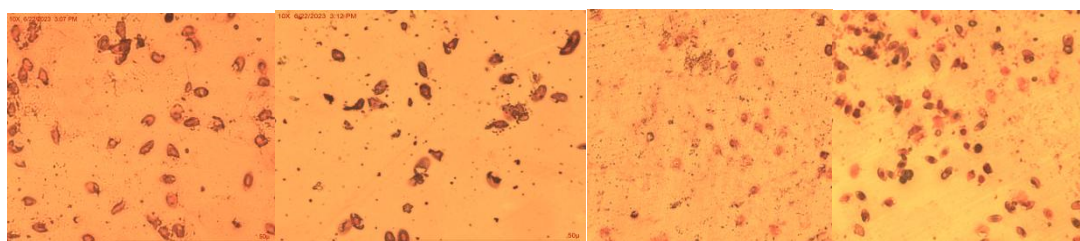
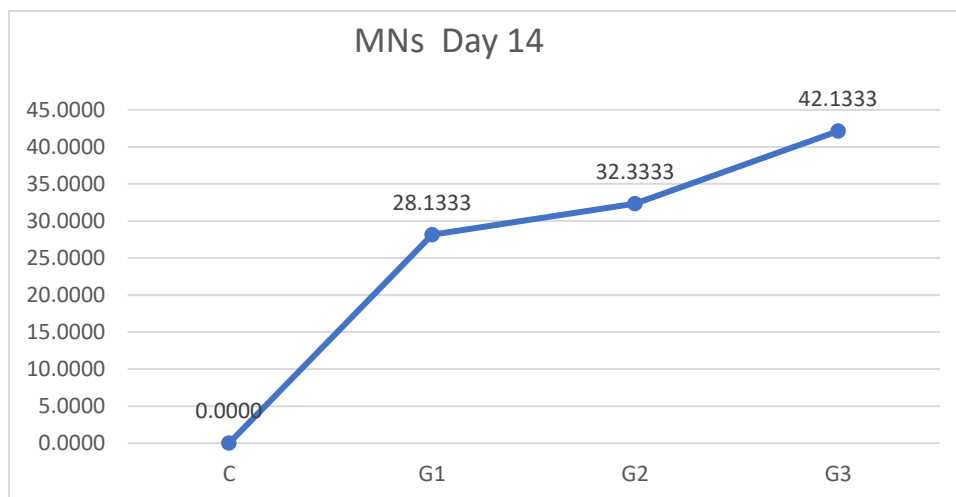
Figure 4 14th day MN analysis of *Labeo rohita*


Figure 5

Control, G1, G2, and G3 analysis of *Labeo rohita*



Discussion

Pesticides are potent chemical contaminants in the aquatic environment. These chemicals are routed into the aquatic environment through several natural and anthropogenic activities. Pesticide toxicity adversely affects growth, physiology, reproduction, immunity, hemato-biochemical profile, and induces serious histopathological alterations in several tissues of aquatic organisms, including fish (Zannat, Rohani, Jeba, & Shahjahan, 2024). The fish blood gives a lot about its histopathology, and we can obtain different kinds of information by studying it. It is also research regarding the effect of pyribaden, a pesticide that decreases the number of RBCs and develops micronuclei which would be a topic of global concern since it is reported that chlorpyrifos is the biggest selling organophosphate agrochemical that has been commonly detected in the surface waters of India. The investigations of long-term

genotoxic biomarkers are not much, thus the current experiment was conducted, to examine the rate of nuclear abnormalities in the blood cells of freshwater fish, *Cirrhinus mrigala* using micronucleus (MN) assays as a possible genotoxicity assessment method. The probity was used in determining the LC50 of chlorpyrifos by exposing fingerlings to various dosages of chlorpyrifos (1/20, 1/10, and 1/5 of LC50) and LC50 was calculated as 0.44 mg L. Overall, it was found that both concentration and duration of exposure had a significant impact on treated fish. The findings indicated that the maximum day 14 was 0.08mg/l concentration of chlorpyrifos which induced the most significant MN. It was determined that chlorpyrifos is a nuclear anomaly causing genotoxic pesticide in *Cirrhinus mrigala*. The current paper indicates the formation of the micronuclei in the silver and *Labeo rohita* fish, too.

Conclusion

The present study was conducted to examine the effect of pyribaden on the hematology of silver carp and *Labeo rohita*. In this research experiment there was one control group and two experimental groups. When we increase the amount of chemicals, they become responsible for the formation of micronuclei. After adding the chemical to the water, we observe that the fish's behavior changes. The present study suggests that when we increase the amount of chemicals, they directly related to the formation of micronuclei. The most affected group was 3rd one, where the amount of chemicals was too high. We conclude that fish are too sensitive to pyribaden and have effect on their morphology, behavior, and genotoxicity. Therefore, we should use another chemical besides pyribaden as a pesticide. We need to act against the use of pesticides because they have a direct effect on fish and other aquatic animals, also responsible for the extinction of different kinds of local species.

Recommendations

Fishes is largely eaten for its flavor, with little awareness of its nutritional value. The current study is an important step in considering the implications of future research on the effects of different pesticides on fish and what kind of disturbances they produce in them. The goal of this research is to assess the effect and resistance of pesticides on fish. According to different research and scientists, environmental pollution, specifically aquatic pollution, is very dangerous for humans as well as for aquatic life, and pesticides badly affect them. Fish are only exposed to pesticides, so they are more vulnerable to them. Some recommendations are as follows.

Future Directions

Future studies need to investigate longitudinal impacts of the pharmacist-led AMS programmes in different health care environments, such as community pharmacies and primary-care clinics. It would also be worth examining how newer technologies, including AI-based prescription auditing, electronic health records, and telepharmacy, may contribute to pharmacists managing and overseeing antimicrobial prescriptions. Comparison of different countries with varying healthcare setups would shed some light on the best practices and implementation barriers. Also, further qualitative research, attention on the views of physicians as well as patients on the role of pharmacists in AMS may aid in the process of customization of further policies and interventions. Overall, by increasing research scope to technological implementation, behavioral change, and international partnerships, sustainable combat against antibiotic resistance will be developed further.

Proper Usage of Pesticides

If we use any kind of pesticides, we should know about their harmful effects and non-targeted species. If we use them in a proper way, they cannot affect the non-targeted species.

Farmer Knowledge

Most of the farmers in Pakistan are uneducated and are unaware of the usage of the pesticides we should educate them about the uses and hazardous effects of pesticides.

Role of Fisheries Department

The fisheries department should take some serious steps towards reducing the usage of pesticides. Pesticides have adverse effects on fish physiology and development. The play takes some steps to control water pollution, which has harmful effects on aquatic animals, specifically on fishes.

Declarations

Ethical Approval and Consent to Participate: This study strictly adhered to the Declaration of Helsinki and relevant national and institutional ethical guidelines. Informed consent was not required, as secondary data available on websites was obtained for analysis. All procedures performed in this study were by the ethical standards of the Helsinki Declaration.

Consent for Publication: Here, we, the authors, give our consent for publication.

Availability of Data and Materials: Data will be provided upon written request from the corresponding author.

Competing Interest: There is no conflict of interest among the authors.

Funding: Not applicable.

Authors' Contribution: The research work is a collective effort of all co-authors, and all have reviewed and approved the final version of the manuscript.

Acknowledgement: We are thankful to all the learned personalities who helped us in conducting the study in hand.

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