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RESEARCH ARTICLE

Toxicological Impact of Profenofos and Azadirachtin on Vital Organs and Biochemical Indices in Fish (*Labeo Rohita*)

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ABSTRACT

The present study investigated the histopathological effects of a synthetic organophosphate pesticide, profenofos, and a neem-derived biopesticide, azadirachtin, on the freshwater fish *Labeo rohita*. The fish were exposed to sub-lethal concentrations of 40 µL/L profenofos and 25 mg/L azadirachtin for 96 hours and upto 28 days under controlled conditions. Histological observations revealed notable toxicological effects with profenofos causing more severe damage. The gills, being primary sites of pesticide absorption, exhibited progressive deterioration, including lamellar fusion, blood congestion, and epithelial cell degradation. Muscle tissues showed mild atrophy after 96 hours, progressing to significant necrosis by the end of 28 days. In contrast, azadirachtin exposure induced milder effects, including fibrosis and vacuolation in the gills and moderate muscle atrophy during the same exposure periods. This study highlights the differential toxicity between synthetic and natural pesticides, underlining the need for stringent regulation of profenofos due to its pronounced ecological risks. The relatively lower toxicity of azadirachtin suggests its potential as an eco-friendlier pest control alternative.

KEYWORDS: Ecosystem, Pesticide, Necrosis, Fibrosis, Toxicity

INTRODUCTION

Pesticides are widely used in agriculture to manage various pests; however, they often find their way into aquatic ecosystems, posing risks to non-target organisms such as fish (Pandey *et al.*, 2024). Profenofos, a commonly used pesticide, has significant adverse effects on living organisms. These insecticides are favored in agriculture due to their rapid biodegradation and non-persistent nature. Nonetheless, in developing countries, unregulated and excessive use of pesticides to prevent crop damage is a prevalent (Kumar *et al.*, 2021). Through surface runoff, pesticides infiltrate aquatic environments such as ponds and rivers, altering water's physio-chemical properties and negatively impacting aquatic organisms (Santhakumar & Balaji, 2000; Kamble & Muley, 2000).

Organophosphate pesticides, due to their inability to break down efficiently in natural conditions, contribute significantly to environmental damage. The overuse of fertilizers, pesticides, and herbicides leads to contamination of water sources, serving as a clear indicator of pollution. Fish absorb these pollutants through various pathways, making vulnerable. Concerns about freshwater pollution by toxic substances have grown over the past few decades (Sindhe *et al*., 2007; Vutukuru, 2005). Due to their sensitivity to the environmental changes, fishes are valuable bioindicators for assessing the risks of chemical contamination in aquatic systems (Dirilgen, 2021).

Tandon & Dubey (1983) noted that, while herbicides, insecticides, and other chemicals used in agriculture and public health efforts might not seem to disrupt aquatic or terrestrial ecosystems directly, they can have harmful effects on non-target organisms, including fish. These pesticides can alter habitats, influence behavior, hinder growth, and reduce reproductive capacity, posing an ongoing threat to aquatic life (Taha, 2022).

Profenofos, a widely used organophosphorus insecticide, is primarily employed in agriculture to control pests on crops such as cotton, tobacco, and paddy. This chemical is highly toxic to fish (El-Houseiny, 2022). The acute toxicity of profenofos is primarily due to its inhibition of acetylcholinesterase, which leads to neurotoxicity in both humans and aquatic organisms. Pesticides like

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profenofos are a significant concern because of their extensive use and potential toxicity to mammals (Kushwaha *et al*., 2016). Hydrolysis is the key detoxification mechanism for organophosphate compounds, as it makes them more prone to further degradation. Several studies have documented the toxic effects of organophosphorus pesticides on fish (Raj *et al*., 2024; Kaliwal & Ksheerasagar, 2006).

Neem (*Azadirachta indica*), a highly versatile plant, is well-known for its insecticidal, medicinal, and pharmacological properties (Biswas *et al*., 2002). Traditionally, it has been widely used in the treatment of various ailments (Van Der Nat *et al*., 1991). Synthetic pesticides, while effective, are associated with disruptions to reproductive functions and significant biochemical changes in fish (Sharma & Ansari, 2010; Ahmad *et al*., 2012). In contrast, natural pesticides derived from plant extracts, such as azadirachtin, are gaining traction as safer alternatives for pest management (Schmutterer, 1990).

Azadirachtin, an active compound extracted from neem, is increasingly regarded as an environmentally friendly alternative to synthetic pesticides (Isman, 2006). Recent studies suggest that neem leaf powder can serve as an effective medium for delivering pesticides (Singh *et al*., 2010). Neem extracts have also been successfully employed in aquaculture to control fish predators (Dunkel & Ricilards, 1998). Azadirachtin (AZA), a secondary metabolite in neem, has been recognized for its antiviral, antibacterial, and antifungal properties for over 2,000 years. This compound is biodegradable, breaking down within 100 hours when exposed to light and water, and is commonly applied as a pesticide at a concentration of 7.8 mL/L of water (Isman *et al.*, 1990; Harikrishnan *et al.*, 2003).

The continuous exposure of aquatic organisms, particularly vital organs like gills, to water makes them highly susceptible to toxicants. Fish absorb pesticides primarily through their gills, leading to noticeable respiratory effects. As toxic exposure intensifies, oxygen consumption rates in fish increase, indicating physiological stress (Premdas & Anderson, 1963).

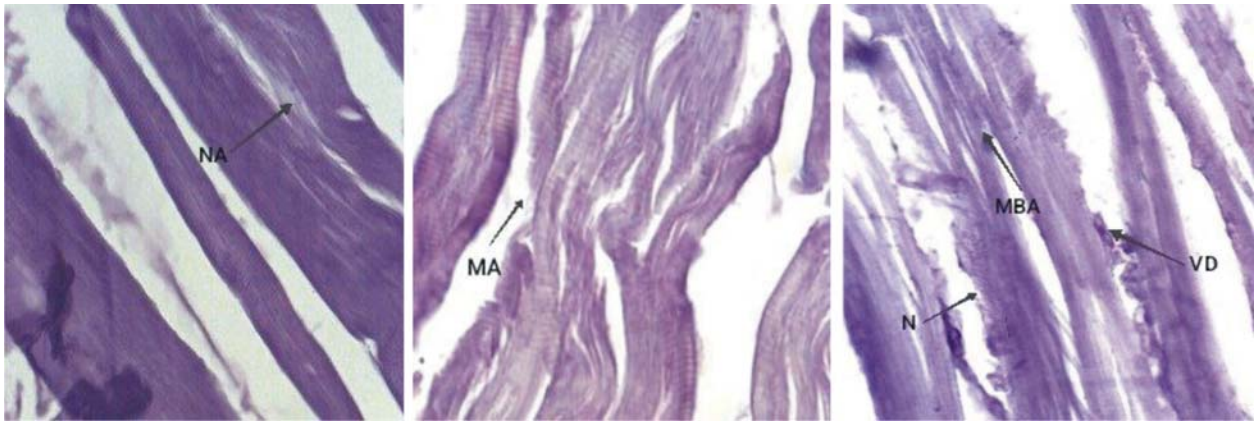
Jawale *et al.* (2010) reported that freshwater fish *Cyprinus carpio* exposed to sub-lethal levels of piscicidal chemicals exhibited significant hematological changes. Similarly, Shaikh *et al.* (2012) observed behavioral changes in *Lamellidens marginalis* following acute exposure to cadmium. The present study aims to evaluate the histo-pathological effects of profenofos on the gills and muscle tissues of a freshwater air-breathing fish.

MATERIALS AND METHODS

Experiment Design

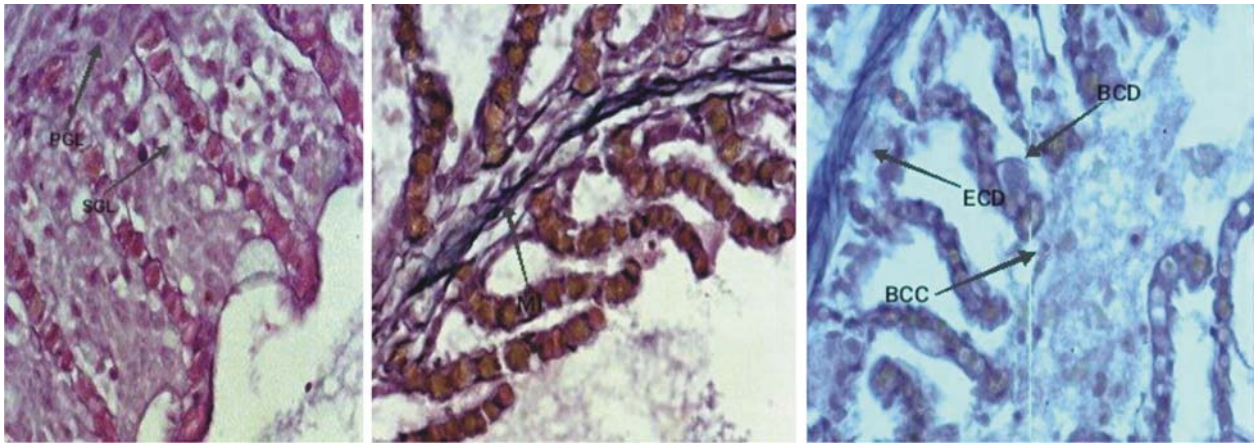
This study examines the toxic effects of profenofos on *Labeo rohita*, a freshwater Indian major carp widely found in ponds, swamps, and ditches. This species, part of the family Cyprinidae and order Cypriniformes, is an important food fish. Specimens were sourced from local fish farms in Sultanpur, Uttar Pradesh, India, and transported to the laboratory under careful conditions.

To eliminate potential external infections, the fish were treated with a 0.05% potassium permanganate ($KMnO_4$) solution for two minutes. They were then housed in 100-liter plastic tanks and acclimatized under controlled laboratory conditions for 14–15 days. During this period, the fish were fed a diet of wheat flour and mustard cake every other day. Tank water and any leftover food were



(A) (B) (C)

Fig.1. Muscles tissue (A) Control, Profenofos exposed tissue: 96 hours (B), 28 days (C). (NA, MA, MBA, N, VD must be abbreviated in the bracket)



(A) (B) (C)

Fig.2. Gills: Control (A), Profenofos exposed Gills: 96 hours (B), 28 days (C). (PGL, SGL, MI, ECD,BCD,BCC must be abbreviated in the bracket).

replaced daily with fresh water to maintain optimal conditions. The tanks were covered with transparent nylon nets to prevent contamination and ensure safety.

Feeding was ceased 24 hours before the toxicity tests began. The fish were maintained at room temperature with exposure to natural light cycles. The water's physicochemical parameters, including temperature, dissolved oxygen (DO), and pH, were monitored regularly. Toxicity tests were conducted under static conditions in 10-liter glass aquariums.

Fish *L. rohita* were exposed to 40 µg/L concentration of profenofos for durations of 96 hours and 28 days. The pesticide consistently impacted the gills and muscles, which are vital organs for the fish. Histopathological analyses were conducted on these tissues to assess the effects. Thin tissue sections were prepared using the microtomy technique and stained with Hematoxylin and Eosin for microscopic examination to highlight cellular and tissue structures.

Based on prior LC50 assessments (Mrinalini *et al.,* 2025), stock solutions of analytical-grade profenofos and azadirachtin were prepared and diluted to achieve sub-lethal concentrations equal to 50% of the LC50 value. Experimental groups, with replicates, were exposed to these concentrations, while a control group remained unexposed. Water quality was consistently monitored to ensure stable conditions throughout the 96-hour acute toxicity tests and the 28-days extended exposure period.

SAMPLING AND PROCESSING OF SAMPLES

- **Organ Collection:** After exposure, dissect the fish and gather its important organs, such as the gills and muscles. For histological examination, organs fixed in 10% neutral buffered formalin.
- **Tissue Processing:** A sequence of graded ethanol to dehydrate tissues was used. Wrap tissues in paraffin wax after clearing them with xylene. Using a microtome, cut tissues into thin slices that are 4-5 µm.
- **Staining:** To perform a general histological assessment, Hematoxylin and Eosin used.
- **Microscopic Examination:** Examine stained sections for histopathological alterations (such as necrosis, inflammation, and degeneration) using a Olympus compound microscope.

RESULT

Histological effect of on gills of *Labeo rohita*

A. Profenofos

Muscles: The arrangement of muscle tissue is normally arranged in single layer and straight fibres (Fig. 1A). Exposure to 40 µg/L of profenofos caused noticeable changes in the muscle tissue after 96 hours, mild atrophy was observed (Fig1. B). Prolonged exposure for 28 days led to significant atrophy of muscle bundles, vacuolar degeneration, and focal areas of necrosis (Fig. 1C).

Gills: The gills of a fish displayed a normal structure, consisting of four branchial arches beneath the operculum. Each arch contained two hemi-branches, composed of rows of primary gill lamellae (PGL), which were flattened and tapered. The hemi-branches were arranged perpendicular to the arch and parallel to each other. Secondary gill lamellae (SGL), flattened leaf-like structures, formed the respiratory surface on the upper and lower

sides of each PGL (Fig. 2A). Gills show mild to moderate alterations after 96 hours of exposure to 40 µg/L profenofos (Fig. 2B). However, after 28 days, the gill tissues exhibited pronounced histopathological changes, including blood congestion, curved lamellae, fusion of lamellae, bone cell deformities, and epithelial cell damage (Fig. 2C).

B. Azadirachtin

Muscles : The normal muscle tissue and cells have been described and shown earlier (Fig.1A). Exposure to 25 mg/L of azadirachtin resulted in moderate atrophy in the muscle tissue after 96 hours (Fig. 3A). Prolonged exposure for 28 days led to bundle atrophy, vacuolar degeneration, and focal necrotic areas in the muscle tissue (Fig. 3B).

Gills : In control fish, the gills displayed a normal structure as described and shown earlier (Fig. 2a). Exposure to 25 mg/L of azadirachtin for 96 hours resulted sign of gill fibrosis in the primary gill lamellae, expansion of the interstitium (the space between the secondary gill lamellae), and narrowed, shrunken secondary gill lamellae. Vacuolation was noted in the epithelial cells of the SGL, with minimal shrinkage in the nuclei (Fig.4A). About 28 days of exposure to the same sub-lethal concentration of azadirachtin, the gills displayed further pathological changes. These included infiltration of inflammatory cells into the PGL, moderate to severe dilatation of the central part of the PGL, vacuolar degeneration, and distortion of the SGL. The SGL also showed dilatation and infiltration of inflammatory cells (Fig. 4B).

DISCUSSION

This study assessed the histopathological effects of profenofos and azadirachtin on the gills and muscle tissues of *L. rohita*. Exposure to 40 µg/L of profenofos resulted in progressive damage to both gills and muscles. After 96 hours, the gills exhibited mild to moderate alterations, while prolonged exposure for 28 days led to significant damage, including blood congestion, lamellar fusion, epithelial cell damage, and necrosis. Muscle tissues initially showed mild atrophy at 96 hours, which progressed to marked degeneration and necrotic areas by the 28-day mark. Similarly, exposure to 25 mg/L of azadirachtin induced histological changes, though these were less severe compared to profenofos. After 96 hours, gills showed fibrosis, expansion of interstitial spaces, and epithelial vacuolation. Prolonged exposure for 28 days resulted in increased inflammatory cell infiltration, vacuolar degeneration, and dilation of lamellae. In muscle tissues, azadirachtin exposure caused atrophy and vacuolar degeneration, with focal necrosis becoming evident after extended exposure.

The histopathological findings revealed that the gills were the primary target tissue affected by profenofos exposure. As the main entry point for pesticides, gills are widely recognized as reliable indicators of water quality (Rankin *et al.*, 1982). These structures play crucial roles in the respiratory, osmoregulatory, and excretory systems of the fish. Histological alterations in the gills of various fish species exposed to pesticides have been extensively documented.

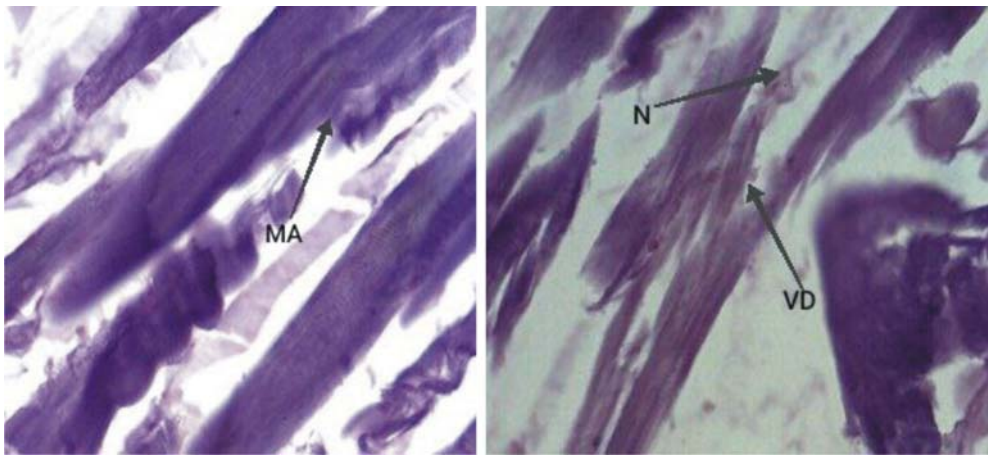


Fig. 3. Muscle: *Azadirachtin* exposure: 96 hours (3A), 28 days (3B)

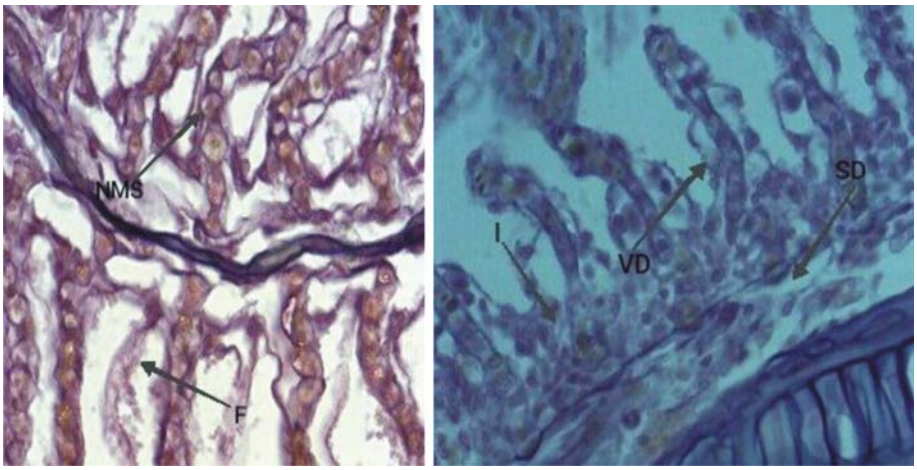


Fig. 4. Gills: *Azadirachtin* exposure: 96 hours (A), 28 days (B)

Santhakumar *et al.* (2001) observed that Anabas testudineus exposed to sub-lethal concentrations of monocrotophos exhibited mucus secretion, lamellar swelling, and microridge reduction, along with lamellar fusion. Kumaraguru *et al*. (1982) reported that synthetic pyrethroids primarily affect the gills of fish, with both technical and commercial formulations penetrating the gills and disrupting movements essential for respiration, ultimately impacting oxygen uptake. Ramamurthy (1988) also noted that pesticide exposure caused gill damage and reduced respiratory metabolism in fish. Additionally, the 96-hour LC50 value for common carp was recorded as 62.4 µg/L, while for zebrafish, it was 0.057 mg/L (Ismail *et al*., 2021). The grass carp (*Ctenopharyngodon idella*), a commercially significant freshwater fish from the Cyprinidae family, is noted for its rapid growth and ability to manage aquatic vegetation effectively (Qu *et al.*, 2016; Cudmore *et al*., 2017).

The spread of contamination in water bodies has raised concerns regarding the effects of toxins on both human health and the survival and well-being of fish. Similar findings were observed in studies on Nile tilapia (*Oreochromis niloticus*) and Labeo rohita exposed to both lethal and sublethal doses of profenofos (Khan, 2019). According to Joshi *et al.* (2002), the damage caused by the toxicant to the intestine may have contributed to reduced iron absorption, leading to deficiencies. In a similar study by Mamatha *et al*. (2014), it was found that azadirachtin could penetrate fish tissues within four days, aligning with the tissue damage observed in the gills of *Glossogobius giuris*, a species commonly found in the rice fields of the Cauvery belt region in Mysore.

Kapinga *et al*. (2018) studied the effects of neem leaf powder supplementation at varying doses (1.0, 2.0, 4.0, and 8.0 g per kg of feed) over a three-month period in *Oreochromis niloticus*. Their results revealed that even the lowest dose significantly reduced the absolute fecundity and gonadosomatic index of the fish. While extracts from *Azadirachta indica* are generally considered to have low toxicity to non-target aquatic organisms, studies have shown some effects on *Tilapia zilli*. Osmoregie & Okpanhchi (1997) reported respiratory issues in this species when exposed to water-based neem bark extracts. Furthermore, prolonged exposure to low concentrations of crude *Azadirachta indica* extract was found to hinder the growth of this cichlid fish (Omoregie & Okpanhchi, 1992). Tiwari and Singh (2006) suggested that, the reduction in protein levels in the muscles in fishes was due to exposure to *A. indica* extract could be due to increased protein hydrolytic activity within the tissues.

CONCLUSION

In the present study, histopathological effects of profenofos and azadirachtin on the gills and muscle tissues of *Labeo rohita*. Exposure to 40 µL/L profenofos caused the gills showing progressive deterioration, from mild changes at 96 hours to severe alterations, including blood congestion, lamellar fusion, and epithelial damage after 28 days. Similarly, the muscles exhibited increasing degeneration, transitioning from mild atrophy to severe vacuolar changes and necrotic regions over the extended exposure period. In contrast, azadirachtin at 25 mg/L induced relatively less severe changes. The gills showed structural modifications such as fibrosis and vacuolation within 96 hours, which progressed to inflammation, lamellar

dilation, and degeneration after 28 days. The muscles displayed progressive atrophy, vacuolar degeneration, and necrosis over time. These findings highlight the toxic effects of both pesticides, with profenofos proving more harmful. The study emphasizes the need for careful regulation of these chemicals to minimize ecological and environmental risks, advocating for the use of safer, alternative pest control strategies.

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