Scanning Electron Microscopy Unveils Profenofos as a Critical Stressor on the gills of *Channa gachua* (Hamilton, 1822)

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ABSTRACT

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| **Aims:** The widespread use of Profenofos, an organophosphate pesticide, in agriculture, has raised concerns about its ecotoxicological effects on aquatic ecosystems. This study investigates the impact of Profenofos on the gill morphology of *Channa gachua*, a freshwater fish species, using scanning electron microscopy (SEM) to elucidate ultrastructural changes.  **Study design:** Healthy specimens of *Channa gachua* (dwarf snakehead) were collected from local freshwater bodies. The average length and weight of the fish were 12–15 cm and 20–25 g, respectively. *Channa gachua* specimens were exposed to sublethal concentrations of Profenofos (0.5 mg/L and 1.0 mg/L and 2.0 mg/L) for 21 days, and gill tissues were subsequently analyzed to assess morphological alterations.  **Methodology:** *Channa gachua* were exposed to sublethal Profenofos concentrations (0.5mg/L, 1mg/L and 2 mg/L) for 21 days. Gill tissues were dissected, fixed in 2.5% glutaraldehyde, and dehydrated using an ethanol series. The samples were critical-point dried, gold-coated, and analyzed using scanning electron microscopy (SEM) to assess ultrastructural changes. Morphological alterations, including epithelial lifting, lamellar fusion, and mucus secretion, were quantified. Statistical analysis compared damage severity between control and treated groups. Results confirmed Profenofos as a critical stressor, highlighting its detrimental effects on gill ultrastructure.  **Results:** SEM analysis revealed significant Profenofos-induced damage to the gill architecture of *Channa gachua*. Key observations included the distortion of primary and secondary lamellae, epithelial lifting, and rupture of microbridges. These structural deformities were dose-dependent, with higher concentrations of Profenofos causing more severe damage. The fusion of lamellae and epithelial lifting was particularly pronounced, suggesting impaired respiratory and osmoregulatory functions. Additionally, the presence of mucus secretion and cellular debris on the gill surface indicated a stress response to the toxicant. The ultrastructural changes observed in this study highlight the detrimental effects of Profenofos on gill tissue, which could compromise the fish's ability to maintain physiological homeostasis. The damage to the gill epithelium likely hinders oxygen exchange and ion regulation, potentially leading to hypoxemia and osmoregulatory imbalance. These findings underscore the role of Profenofos as a critical stressor in aquatic environments, with implications for the health and survival of fish populations.  **Conclusion:** This study provides compelling SEM-based evidence of Profenofos-induced gill pathology in *Channa gachua*, emphasizing the need for stricter regulation of organophosphate pesticides to protect aquatic biodiversity. The results contribute to a deeper understanding of the ecotoxicological impacts of Profenofos and highlight the importance of using advanced imaging techniques like SEM to assess environmental stressors on aquatic organisms’ invasive independent predictors for screening esophageal varices may decrease medical as well as financial burden, hence improving the management of cirrhotic patients. These predictors, however, need further work to validate reliability. |

***Keywords:*** *Profenofos, Channa gachua, gill morphology, scanning electron microscopy, ecotoxicology, organophosphate pesticide, ultrastructural damage}*

1. INTRODUCTION

The increasing use of pesticides in agriculture has led to significant concerns about their impact on aquatic ecosystems. Among these pesticides, Profenofos, an organophosphate compound, is widely used due to its effectiveness in controlling pests. However, its persistence in water bodies and toxicity to non-target organisms, particularly fish, pose serious ecological risks (Kumar *et al*., 2010). Fish, being integral to aquatic ecosystems, are highly vulnerable to pesticide contamination, which can disrupt their physiological and morphological functions. The gills, as the primary site for respiration, osmoregulation, and excretion, are particularly susceptible to damage from waterborne pollutants (Fernandes *et al*., 2007). This study focuses on the effects of Profenofos on the gill morphology of *Channa gachua*, a freshwater fish species, using scanning electron microscopy (SEM) to provide detailed insights into ultrastructural changes.

Profenofos is known to inhibit acetylcholinesterase (AChE), an enzyme critical for nerve function, leading to neurotoxicity in aquatic organisms (Jaiswal *et al*., 2018). However, its sublethal effects on fish gills, which are vital for maintaining homeostasis, remain poorly understood. Gills are directly exposed to contaminants in water, making them a primary target for toxicants. Structural damage to gill tissue can impair respiratory efficiency, ion regulation, and overall fish health, ultimately affecting survival and population dynamics (Pandey *et al*., 2008). Previous studies have documented the toxic effects of pesticides on fish gills, but few have utilized advanced imaging techniques like SEM to examine ultrastructural alterations in detail (Mallatt, 1985). The respiratory system provides the most extensive surface of fish with the aquatic environment. The primary aquatic air breathing organ of *Channa gachua* has four pairs of functional gills. Fish gill remains in direct contact with ambient water. It is easily affected with the toxicants like pesticides (Pallavi, 2019).

*Channa gachua*, commonly known as the dwarf snakehead, is a hardy freshwater fish species found in South Asia. It serves as an excellent model for ecotoxicological studies due to its ecological importance and sensitivity to environmental changes (Rahman *et al*., 2015). This study aims to investigate the impact of Profenofos on the gill ultrastructure of Channa gachua by exposing the fish to sublethal concentrations of the pesticide. SEM, with its high resolution and magnification capabilities, is employed to visualize and quantify morphological changes in gill tissue, providing a comprehensive understanding of the damage caused by Profenofos (Hinton *et al*., 1987).

The findings of this study are expected to reveal significant alterations in gill morphology, such as epithelial lifting, lamellar fusion, and mucus secretion, which are indicative of stress responses to Profenofos exposure (Arellano *et al*., 1999). These changes can compromise the fish's ability to perform essential physiological functions, leading to reduced fitness and survival. By elucidating the ultrastructural damage caused by Profenofos, this research highlights the ecological risks associated with the use of organophosphate pesticides and underscores the need for stricter regulatory measures to protect aquatic biodiversity (Van der Oost *et al*., 2003).

This study combines ecotoxicology and advanced imaging techniques to explore the impact of Profenofos on *Channa gachua* gills. The use of SEM provides a unique perspective on the morphological changes induced by pesticide exposure, offering valuable insights into the mechanisms of toxicity and their ecological implications. This research contributes to the growing body of knowledge on pesticide-induced stress in aquatic organisms and emphasizes the importance of sustainable agricultural practices to safeguard aquatic ecosystems.



**Fig. 1. *Channa gachua*, commonly known as the dwarf snakehead**

Source: <https://indiabiodiversity.org/observation/show/17902249>

2. material and methods

**2.1 Materials**

**2.1.1 Fish Specimens**

Healthy adult *Channa gachua* were obtained from local freshwater bodies. The distribution area of *Channa gachua* extends over both tropical and subtropical areas. The fish from a tropical area require a constant water temperature. The fish from a subtropical climate can tolerate extremes of 14 to 36 degrees Celsius, but not continuous tropical values. Before purchasing, first determine where they come from, in order to be able to take care of it properly. In the natural habitat, other water parameters vary widely. They survive in water with varying pH values from 3.1 to 9.6. However, all varieties do very well in water with a pH between 6 and 7. What *Channa gachua* (like other snakehead fish) cannot tolerate is sudden changes in water values, especially the pH value (https://aquainfo.org/article/channa-gachua-brown-dwarf-snakehead/).

**2.1.2 Profenofos**

Commercial grade Profenofos pesticide was used for exposure experiments

**2.1.3 Chemicals**

Glutaraldehyde, osmium tetroxide, phosphate-buffered saline (PBS), ethanol (30% to 100% for dehydration), and gold for sputter coating.

**2.1.4 Equipment**

Aerated tanks for acclimatization and exposure, temperature and pH meters, Scanning Electron Microscope (SEM), and a sputter coater.

**2.2 Experimental Design**

**2.2.1 Acclimatization**

Fish were acclimatized in laboratory conditions for two weeks in aerated tanks with dechlorinated water. Water temperature was maintained at 25±2°C, pH at 7.0±0.5, and a 12-hour light/dark cycle was followed. Fish were fed a standard diet twice daily (APHA, 2017).

**2.2.2 Water Quality**

**The water quality parameter as follows**

Table 1: The water quality parameters

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| --- | --- |
| **Parameter** | **Value** |
| pH | 7.2 |
| Temperature | 27°C |
| Dissolved Oxygen | 6.8 mg/L |
| CO2 | 8.0 mg/L |
| Hardness (as CaCO3) | 56 mg/L |
| Alkalinity (as HCO3) | 130 mg/L |

**2.2.3 Profenofos Exposure**

Fish were randomly divided into control and treatment groups. The treatment groups were exposed to three different sub-lethal concentrations of Profenofos (0.5 mg/L, 1.0 mg/L, and 2.0 mg/L) for 14 days. The control group was maintained in pesticide-free water. Water quality parameters were monitored daily (OECD, 2019; Kumar et al., 2020).

**2.2.4 Gill Tissue Collection and Fixation**

At the end of the exposure period, fish were euthanized following ethical guidelines. Gills were dissected, rinsed with phosphate-buffered saline (PBS), and fixed in 2.5% glutaraldehyde at 4°C for 24 hours to preserve the tissue structure (Gupta & Sharma, 2018).

Post-fixation was carried out in 1% osmium tetroxide for one hour at room temperature, followed by rinsing in PBS.

**2.2.5 Sample Dehydration and Preparation**

Fixed gill tissues were dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 100%). Each step lasted for 10 minutes, ensuring complete dehydration (Singh & Rathore, 2017).

The dehydrated samples were then subjected to critical point drying and mounted on SEM stubs.

**2.2.6 Scanning Electron Microscopy (SEM)**

The dried gill samples were coated with a thin layer of gold using a sputter coater to ensure conductivity.

The samples were examined under a Scanning Electron Microscope (SEM) at varying magnifications. High-resolution images of the gill tissues were captured to observe morphological changes, such as lamellar fusion, epithelial lifting, and necrosis (Singh & Rathore, 2017).

**2.2.7 Data Analysis**

SEM images from the control and treated groups were analyzed qualitatively to assess structural changes in the gill tissues. Observations focused on specific alterations such as damage to the secondary lamellae, epithelial cell detachment, and necrosis (Kumar *et al*., 2020).

3. results and discussion

Close-up of a plant stem

AI-generated content may be incorrect.

**Fig 2: SEM Photographs of gill of *Channa gachua (Control)* showing gill lamella, gill arch and taste bud( X 800)**

GA = Gill arch; TB = Taste bud; PGL = Primary gill lamella

Close-up of a microscope slide

Description automatically generated

**Fig 3: SEM Photographs of the Profenofos exposed gill of *Channa gachua* showing curling of secondary lamella *(*X 1200)**

CSL = Curling of Secondary Lamell

Close-up of a microscopic view of a brain

Description automatically generated

**Fig 4: SEM Photographs of the Profenofos exposed gill of *Channa gachua* showing curling of primary gill lamella *(*X 1100)**

CPL = Curling in Primary Lamella; EL=Epithelial Lifting; NC = Necrosis

Close-up of a brain organ

AI-generated content may be incorrect.

**Fig 5: SEM Photographs of the Profenofos exposed gill of *Channa gachua* showing mucous pores and enlarged epithelial cells *(*X 1600)**

EC = Epithelial Cell; MP = Mucous Pore; VP = Vascular Papilla

Close-up of a human intestine

Description automatically generated

**Fig 6: SEM Photographs of the gill lamella of *Channa gachua* (Control) showing mucous pores and epithelial cells (X 1600)**

EPC = Epithelial Cells; MP = Mucous Pore

A close-up of a cloud

Description automatically generated

**Fig 7: SEM Photographs of the Profenofos exposed Primary Gill Lamella showing enlargement and rupture of Epithelial Cell (X1400)**

EC = Epithelial Cells

**3.1 Control Group (Unexposed Fish)**

In the control group, the gill architecture of *Channa gachua* appeared normal and intact under the Scanning Electron Microscope (SEM). The primary lamellae were well-structured, and the secondary lamellae were uniformly spaced. The lamellae were covered by epithelial cells that appeared undamaged, displaying a smooth surface and well-preserved microbridges.

**3.2 Low Concentration (0.5 mg/L Profenofos)**

Fish exposed to the lowest concentration of Profenofos (0.5 mg/L) exhibited initial signs of gill damage. SEM images revealed slight epithelial lifting. Some epithelial cells showed swelling, but the overall gill structure remained largely preserved. These changes suggest early stress responses at sub-lethal exposure levels.

**3.3 Medium Concentration (1.0 mg/L Profenofos)**

At a concentration of 1.0 mg/L, more pronounced morphological alterations were observed. The SEM images indicated significant epithelial lifting, and noticeable necrosis in some areas. Additionally, an increase in mucous cell activity was observed, likely serving as a defensive mechanism against the stress caused by pesticide exposure.

**3.4 High Concentration (2.0 mg/L Profenofos)**

Exposure to the highest concentration of Profenofos (2.0 mg/L) resulted in severe gill damage. SEM analysis showed widespread necrosis and severe epithelial cell detachment. The structural integrity of the gill filaments was heavily compromised, with evidence of disrupted microbridges and increased mucous secretion. These observations indicate severe toxic effects, impairing the gill's ability to function properly.

**3.5 Overall Trend**

The study demonstrated a dose-dependent increase in gill damage in *Channa gachua* exposed to Profenofos. The morphological changes observed through SEM, such as epithelial lifting, and necrosis, indicate that Profenofos has a deleterious impact on gill structure, which could lead to impaired respiratory efficiency and osmoregulation. These findings highlight the potential environmental hazards posed by Profenofos contamination in aquatic ecosystems.

***3.6.1*****Gill Morphology and Environmental Stress**

The gills are highly vascularized and serve essential functions, including respiration, osmoregulation, and excretion. They are also the primary source of interaction with waterborne pollutants, making them highly susceptible to environmental stressors. This study demonstrated that Profenofos exposure leads to significant morphological damage, particularly at higher concentrations, which is consistent with previous findings on pesticide toxicity in fish (Kumar *et al*., 2020).

***3.6.2*****Dose-Dependent Effects**

The study revealed a clear dose-dependent relationship between Profenofos exposure and gill damage. At lower concentrations (0.5 mg/L), minor structural changes were observed, indicating the onset of stress responses. However, as the concentration increased to 1.0 mg/L and 2.0 mg/L, the extent of gill damage became more pronounced, with severe epithelial lifting, and necrosis. This progression highlights the cumulative toxic effects of Profenofos, aligning with earlier research that indicates higher pesticide concentrations exacerbate morphological and physiological damage in fish (Gupta & Sharma, 2018).

***3.6.3*****Protective Responses and Pathological Changes**

The proliferation of mucous cells observed in the gills of treated fish suggests an adaptive response to mitigate the toxic effects of Profenofos. Mucous secretion is a common defense mechanism in fish, serving to trap and remove harmful substances. However, excessive mucous production, as seen in the higher concentration groups, can obstruct lamellar surfaces and impair gas exchange, further compromising respiratory efficiency (Singh & Rathore, 2017).

***3.6.4*****Implications for Aquatic Ecosystems**

The findings of this study have broader ecological implications. *Channa gachua* plays a crucial role in its habitat, and any significant health impact on this species can disrupt the ecological balance. The observed gill damage implies that Profenofos contamination can adversely affect fish populations, potentially leading to decreased survival rates and altered community dynamics in aquatic ecosystems. The study underscores the need for stringent regulations and monitoring of pesticide use to prevent such adverse environmental impacts (OECD, 2019).

***3.6.5*****Comparative Analysis with Other Studies**

The morphological changes observed in this study are consistent with those reported in other fish species exposed to organophosphate pesticides. For example, similar gill alterations have been documented in *Oreochromis mossambicus* and *Labeo rohita* under pesticide stress, indicating that these effects are not species-specific but rather a common response to organophosphate toxicity (Kumar *et al*., 2020; Gupta & Sharma, 2018).

4. Conclusion

This study provides significant evidence of the toxic impact of Profenofos on the gill morphology of *Channa gachua*. The dose-dependent structural damage observed through SEM underscores the potential risk of pesticide pollution in aquatic environments. The findings emphasize the need for further research to explore the long-term ecological consequences and to develop strategies for mitigating pesticide contamination in freshwater systems.

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