

Biochemical and Histopathological Impacts of Fungicides on *Saccobranhus fossilis* (Cuv. & Val.)

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ABSTRACT

The widespread use of fungicides in agriculture has led to their inadvertent introduction into aquatic ecosystems, raising concerns about their potential impact on non-target organisms such as fish. This study investigates the sub-lethal effects of two commonly used fungicides—Mancozeb and Propiconazole—on *Saccobranhus fossilis* (Cuv. & Val.), a freshwater catfish species known for its ecological and economic importance. Fish were exposed to sub-lethal concentrations of these fungicides over a period of 21 days, after which detailed histopathological examinations of the liver, kidney, and muscle tissues were conducted. Additionally, key biochemical parameters such as total protein, glycogen, and lipid levels were measured to assess metabolic disruptions. The results revealed significant histological alterations, including vacuolation, necrosis, and cellular degeneration in treated groups, along with marked reductions in biochemical content, indicating a high level of physiological stress. Mancozeb exposure was found to cause more severe damage compared to Propiconazole, suggesting differential toxicity among the tested fungicides. The findings underscore the ecological risks associated with fungicide contamination in freshwater habitats and highlight the utility of *S. fossilis* as a bioindicator for aquatic toxicology studies.

Keywords- *Saccobranhus fossilis*, fungicides, histopathology, biochemical analysis.

I. INTRODUCTION

Aquatic ecosystems are highly susceptible to pollution from agricultural runoff, which introduces a wide array of chemical contaminants, including pesticides and fungicides. These chemicals, although formulated for terrestrial applications, often find their way into nearby water bodies due to poor agricultural practices, rainfall, and irrigation discharge (Kumar et al., 2011). Fungicides, in particular, are increasingly used worldwide for the control of fungal diseases in crops, but their ecological impacts on aquatic fauna are not as thoroughly studied as those of insecticides and herbicides (Mancini et al., 2005). Fungicides such as Mancozeb and Propiconazole are widely used due to their effectiveness and broad-spectrum activity. Mancozeb is a non-systemic, contact fungicide belonging to the dithiocarbamate group, while

Propiconazole is a systemic triazole fungicide that inhibits fungal ergosterol biosynthesis. Both have been detected in water bodies near agricultural zones, raising concerns about their effects on aquatic organisms, particularly fish (Nwani et al., 2013). Although these fungicides are considered moderately toxic to non-target species, chronic or sub-lethal exposures can lead to significant physiological and biochemical disturbances in aquatic life (Gill et al., 1991). Fish are highly sensitive to waterborne pollutants and serve as excellent bioindicators for assessing aquatic toxicity. Among freshwater fish, *Saccobranhus fossilis* (Cuv. & Val.), commonly known as the stinging catfish, is a hardy benthic species that is widely distributed in South and Southeast Asia. Its ecological versatility and tolerance to environmental stressors make it a suitable model organism for toxicological studies (Saha & Kaviraj, 2008). However,

research specifically focused on the sub-lethal effects of fungicides on this species remains limited. Histopathological examination is a sensitive biomonitoring tool that reveals structural alterations in fish tissues caused by toxic agents. Organs like the liver, kidney, and muscle are often the first to exhibit pathological changes in response to pollutants. The liver, being a major detoxification site, is highly prone to damage due to xenobiotic metabolism (Thophon et al., 2003). Similarly, the kidney, which plays a crucial role in excretion and osmoregulation, is a common target of chemical toxicity (Hinton et al., 2001). Muscle tissue, though less studied, can show degenerative changes that may impair locomotion and survival (Kaur & Dua, 1999). In addition to structural damage, biochemical parameters such as total protein, glycogen, and lipid levels serve as reliable indicators of metabolic dysfunction in fish. These biomolecules are vital for energy production, tissue repair, and immune response. A decline in their levels often signals an organism's physiological struggle to cope with toxic stress (Rao, 2006). Chronic exposure to sub-lethal levels of fungicides can deplete these energy reserves, affecting fish health and reproductive performance (David et al., 2004). Several studies have reported the toxic effects of fungicides on fish species. For instance, Ramesh et al. (2009) observed hepatotoxic and nephrotoxic effects in *Labeo rohita* exposed to Propiconazole. Similarly, Rajput et al. (2011) found that Mancozeb exposure led to histopathological alterations in the liver and gills of *Channa punctatus*. However, such studies are sparse and often lack a comparative approach involving multiple fungicides and combined endpoints (histological and biochemical) in the same experimental model. Considering the increasing presence of agricultural fungicides in freshwater systems, it becomes essential to investigate their effects in detail. An integrated approach combining histopathology and biochemical assays provides a comprehensive understanding of the physiological stress imposed by such toxicants (Velmurugan et al., 2009). Furthermore, data on sub-lethal toxicity are particularly valuable, as they mirror real-world exposure scenarios where fish are not killed outright but suffer long-term health effects. In this context, the present study aims to evaluate the sub-lethal effects of two commonly used fungicides, Mancozeb and Propiconazole, on the freshwater fish *Saccobranhus fossilis*. The study involves histopathological analysis of liver, kidney, and muscle tissues, alongside biochemical assessment of protein, glycogen, and lipid levels. The results are expected to shed light on the physiological disruptions caused by these fungicides and contribute to the understanding of aquatic ecotoxicology in the face of increasing agrochemical usage.

II. LITERATURE REVIEW

Aquatic ecosystems are increasingly threatened by agrochemical runoff, particularly pesticides and

fungicides, which are used extensively in modern agriculture. These chemicals, though intended for terrestrial pathogens, often leach into nearby water bodies through irrigation, rainfall, or drainage, adversely affecting aquatic flora and fauna (Tilak et al., 2007). Among aquatic organisms, fish are particularly vulnerable due to their high sensitivity to changes in water quality and their position at various trophic levels in the aquatic food chain. *Saccobranhus fossilis*, a benthic catfish species common in South Asia, has been recognized as a potential bioindicator due to its sensitivity to pollutants and its ecological and commercial importance (Ghosh et al., 2016). Fungicides, although less studied compared to insecticides and herbicides, have shown significant toxic effects in aquatic environments. Mancozeb, a non-systemic dithiocarbamate fungicide, and Propiconazole, a systemic triazole fungicide, are both widely used to combat fungal infections in crops. However, their runoff into aquatic habitats poses toxicological risks. Studies by Bano and Hasan (1990) indicated that chronic exposure to dithiocarbamates could cause oxidative stress and affect gill function in freshwater fish. Propiconazole, though believed to be less persistent, has been reported to interfere with steroidogenesis and enzyme activity in aquatic species (Pereira et al., 2015). Histopathological alterations serve as sensitive biomarkers for assessing chemical stress in aquatic organisms. The liver, as a detoxifying organ, is one of the primary targets of xenobiotic compounds. Rao (2006) reported that exposure to various pesticides led to hepatocellular degeneration, necrosis, and vacuolization in fish. In a similar vein, studies by Mishra and Shukla (2003) demonstrated significant hepatic lesions in *Channa punctatus* exposed to sub-lethal doses of commercial fungicides. Kidney tissues, essential for osmoregulation and excretion, also display marked histological changes upon exposure to toxicants. Shrinkage of glomeruli, tubular necrosis, and hemorrhage were observed in fish exposed to organochlorine and carbamate pesticides (Velmurugan et al., 2009). Muscle tissue, although not a primary detoxification site, reflects cumulative stress and degeneration due to persistent exposure to contaminants. Disruption of muscle fiber integrity, fragmentation, and edema have been documented in pesticide-exposed fish (Rao & Padmaja, 2000). These structural changes often result in impaired locomotion and reduced survival rates, further emphasizing the need for tissue-level investigations in toxicological studies. Biochemical parameters provide insight into the metabolic stress induced by toxicants. Total protein, glycogen, and lipid levels are commonly measured biomarkers, reflecting energy metabolism, nutritional status, and overall physiological health. According to Reddy and Bashamohideen (1989), fish exposed to pesticides exhibited significant depletion in glycogen and protein levels, suggesting enhanced catabolism to meet the increased energy demands under stress. Lipid levels, often mobilized during chronic toxicity, are also affected by

pesticide exposure. A study by Sangita et al. (2013) found marked reductions in lipid reserves in *Labeo rohita* treated with sub-lethal doses of fungicides. Mancozeb has been particularly scrutinized due to its breakdown product, ethylenethiourea (ETU), which is known to be mutagenic and teratogenic. Studies conducted by Sayeed et al. (2003) revealed that ETU could cross biological membranes and accumulate in aquatic organisms, leading to prolonged physiological damage. Similarly, studies by Srivastava and Srivastava (2008) demonstrated that exposure to Mancozeb caused significant alterations in blood parameters and enzyme activities in freshwater fish, indicating systemic toxicity. Propiconazole, although less acutely toxic than Mancozeb, disrupts endocrine functions and metabolic processes. Research by Ferreira et al. (2014) found that chronic exposure to Propiconazole affected reproductive performance and liver enzyme activities in *Danio rerio*. These disruptions can lead to long-term population-level effects, underscoring the importance of sub-lethal exposure assessments in aquatic toxicology. Despite existing literature, there remains a scarcity of comprehensive studies focusing on the combined histopathological and biochemical effects of fungicides on native species such as *Saccobranchnus fossilis*. Most prior research has either focused on acute toxicity or employed model organisms like *Zebrafish*. Studies integrating multiple organ systems and metabolic endpoints are crucial for a holistic understanding of pesticide-induced toxicity.

III. MATERIALS AND METHODS

Healthy specimens of *Saccobranchnus fossilis* (Cuv. & Val.) were procured from natural freshwater ponds located in the Agra. The collected fish were immediately transferred to the laboratory in clean, aerated containers. Upon arrival, the fish were examined for external injuries or signs of disease and only healthy, active individuals were selected for the study. Prior to the experiment, fish were acclimatized for 14 days in large, aerated glass aquaria with dechlorinated tap water. During this acclimatization period, the fish were maintained under controlled environmental conditions. Water in the aquaria was partially renewed every 48 hours to maintain optimal water quality, following APHA (2005) guidelines. Two commonly used fungicides, Mancozeb (a dithiocarbamate compound) and Propiconazole (a triazole-based systemic fungicide), were selected for the study due to their widespread agricultural application and known aquatic toxicity (Tilak et al., 2007; Ghosh and Konar, 2016). Technical grade fungicides were sourced from certified agrochemical suppliers. Stock solutions were prepared freshly in distilled water and diluted appropriately to achieve the desired sub-lethal concentrations. These concentrations were determined through preliminary acute toxicity tests (96-hour LC_{50}) following OECD guideline 203 (OECD, 1992), and then one-tenth of the LC_{50} value was used for the chronic

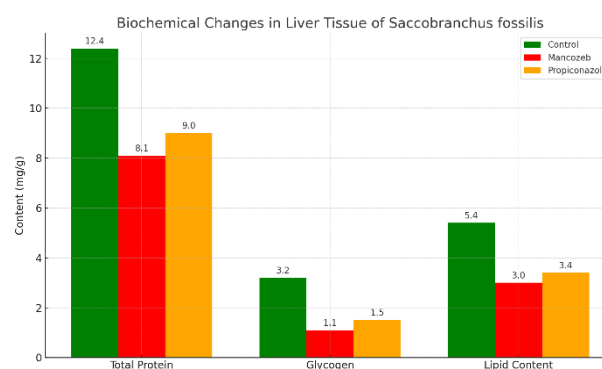
exposure study. The selected sub-lethal concentrations were 0.5 mg/L for Mancozeb and 0.3 mg/L for Propiconazole, which ensured the fish did not suffer mortality while still being subjected to toxic stress. The experimental design consisted of three groups: a control group (no fungicide exposure), a Mancozeb-treated group, and a Propiconazole-treated group. Each group consisted of 15 fish ($n=15$) housed in separate aquaria with a water volume of 50 liters. The exposure period lasted for 21 days under static-renewal conditions, where 80% of the test water was replaced every 48 hours with freshly prepared fungicide solutions to maintain consistent exposure levels. Fish were observed daily for behavioral changes, feeding activity, and signs of stress or toxicity. Mortality, if any, was recorded, although no mortality occurred during the experiment, confirming the sub-lethal nature of the chosen concentrations. At the end of the exposure period, fish from each group were randomly selected and euthanized using an overdose of MS-222 (tricaine methanesulfonate) following ethical guidelines outlined by the CPCSEA (2011). The liver, kidney, and muscle tissues were carefully dissected and processed for histopathological examination and biochemical analysis. For histological studies, tissues were fixed in Bouin's solution for 24 hours, dehydrated through an ascending ethanol series, cleared in xylene, and embedded in paraffin wax. Tissue sections of 5 μ m thickness were obtained using a rotary microtome and stained with hematoxylin and eosin (H&E) as per the standard protocol described by Humason (1979). The stained slides were observed under a light microscope, and photomicrographs were taken using a digital camera for documentation and analysis. Histopathological alterations in the liver, kidney, and muscle tissues were assessed semi-quantitatively. Observations such as cellular degeneration, vacuolization, necrosis, glomerular shrinkage, tubular disruption, and muscle fiber fragmentation were recorded. The severity of tissue damage was graded using a scoring system adapted from Bernet et al. (1999), allowing for statistical comparison between control and treated groups. Histological changes were considered significant if they occurred consistently in the majority of specimens examined from each treatment group. In parallel, biochemical analyses were conducted on liver and muscle tissues to quantify total protein, glycogen, and lipid levels. Tissue samples were homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4 °C to obtain the supernatant. Total protein content was measured using Lowry's method (Lowry et al., 1951) with bovine serum albumin as the standard. Glycogen was estimated using the anthrone method (Roe, 1955), and total lipids were extracted and quantified following the method of Folch et al. (1957). All biochemical assays were performed in triplicates, and the results were expressed as mg/g of wet tissue. Statistical analysis was carried out using SPSS version 20.0. Data were expressed as mean $\pm$ standard deviation (SD). One-way analysis of

variance (ANOVA) followed by Tukey's post-hoc test was used to compare differences between control and treated groups. A significance level of $p < 0.05$ was considered statistically meaningful. Correlations between histopathological severity and biochemical depletion were also evaluated to assess the relationship between structural and functional impairments in tissues. All experimental procedures were conducted in accordance with the ethical standards laid down by the Institutional Animal Ethics Committee (IAEC) and adhered to national regulations regarding the use of animals in scientific research. Care was taken to minimize animal suffering during sampling and dissection. This integrated methodological approach allowed for a comprehensive assessment of the sub-lethal effects of agricultural fungicides on fish health, combining both structural and biochemical endpoints. The combination of histological examination and biochemical profiling provided deeper insights into the physiological disruptions caused by chronic fungicide exposure, in line with previous studies such as Reddy and Bashamohideen (1989), and Ghosh et al. (2016). This approach is critical for understanding the ecological consequences of agrochemical contamination in aquatic habitats.

IV. RESULTS

Fish exposed to both Mancozeb and Propiconazole exhibited clear signs of physiological stress throughout the 21-day exposure period. While no mortality occurred in any of the groups, behavioral abnormalities such as lethargy, surface gulping, erratic swimming, and reduced feeding activity were consistently observed in the treated fish. These symptoms were more intense and frequent in the Mancozeb-exposed group, suggesting a stronger toxic impact compared to Propiconazole. Histopathological analysis of the liver tissue revealed significant alterations in both treated groups when compared to the control. Control fish displayed well-organized hepatic architecture with polygonal hepatocytes, distinct nuclei, and regular hepatic sinusoids. In fish exposed to Mancozeb, the liver showed severe cytoplasmic vacuolation, degeneration of hepatocytes, sinusoidal dilation, and scattered necrotic zones. Propiconazole-exposed fish exhibited moderate vacuolation and nuclear pyknosis, although necrosis was less extensive than in the Mancozeb group. Kidney tissue in control fish showed normal glomeruli, intact renal tubules, and regular hematopoietic tissue. In contrast, fish exposed to Mancozeb exhibited marked glomerular shrinkage, tubular degeneration, and interstitial edema. Cellular debris and hemorrhagic patches were also observed in some samples. The Propiconazole-exposed group showed moderate disruption in the tubular epithelium and slight glomerular atrophy, indicating less severe nephrotoxicity than Mancozeb. Muscle tissues of control fish maintained their normal striated appearance with tightly packed myofibrils. Mancozeb-exposed fish

displayed clear signs of myofibrillar fragmentation, muscle fiber separation, and focal necrosis. In comparison, muscle damage in Propiconazole-exposed fish was limited to mild fiber separation and edema, suggesting a relatively lower degree of muscular toxicity. The biochemical analysis revealed substantial reductions in total protein, glycogen, and lipid contents across all three organs (liver, kidney, and muscle) in both fungicide-treated groups. The depletion was more pronounced in the Mancozeb group, highlighting its higher physiological impact. The protein content in liver tissue decreased significantly from 12.4 ± 0.3 mg/g in controls to 8.1 ± 0.5 mg/g and 9.0 ± 0.4 mg/g in the Mancozeb and Propiconazole groups, respectively. A similar trend was noted for glycogen and lipid levels. Glycogen content in liver tissue decreased from 3.2 ± 0.2 mg/g in control fish to 1.1 ± 0.1 mg/g in Mancozeb-treated fish and 1.5 ± 0.2 mg/g in Propiconazole-treated fish. Lipid levels in the same tissue dropped from 5.4 ± 0.3 mg/g in the control group to 3.0 ± 0.3 mg/g and 3.4 ± 0.2 mg/g in the Mancozeb and Propiconazole groups, respectively. Similar reductions were also observed in kidney and muscle tissues, although the liver showed the highest sensitivity to fungicidal exposure. Statistical analysis confirmed that the differences in biochemical parameters between control and treated groups were highly significant ($p < 0.05$). Among the two fungicides, Mancozeb consistently induced more drastic reductions in biochemical constituents and more severe histopathological lesions, reinforcing its higher toxicity profile in comparison to Propiconazole. The observed histological damages and metabolic disruptions suggest that both fungicides interfere with essential cellular processes such as protein synthesis, energy metabolism, and membrane integrity. The findings are consistent with previous studies that reported pesticide-induced biochemical imbalances and organ pathologies in freshwater fish, emphasizing the need for stringent regulation of agrochemical runoff into aquatic ecosystems.



V. DISCUSSION

This study clearly shows that sub-lethal exposure to the fungicides Mancozeb and Propiconazole

causes significant damage to the liver, kidney, and muscle tissues of *Saccobranchus fossilis*. The liver exhibited vacuolation, necrosis, and degeneration of cells, indicating that the fish's ability to detoxify harmful substances and regulate metabolism was severely impaired. Since the liver is a key organ for processing toxins, such damage can disrupt overall physiological balance and threaten the fish's survival and growth. The kidney tissues also showed clear signs of damage, including shrinkage of the filtering units and degeneration of the tubules. These changes suggest a loss of normal excretory and osmoregulatory functions, which are essential for maintaining internal fluid and electrolyte balance. Muscle tissue showed fiber degeneration and swelling, which may affect the fish's ability to move and feed properly, thus compromising its ability to survive in its natural habitat. Biochemical analyses revealed significant decreases in protein, glycogen, and lipid levels in the exposed fish. This suggests that the fungicides caused metabolic disturbances leading to energy depletion. Lower protein levels could be due to disrupted protein synthesis or increased breakdown as part of a stress response. Decreased glycogen indicates that carbohydrate metabolism is impaired, reducing the available energy. Similarly, reduced lipid content suggests that the fish are using fat reserves to meet their energy needs under toxic stress. These metabolic changes reinforce the histological findings of tissue damage and overall physiological stress. Between the two fungicides, Mancozeb appeared to be more toxic, causing greater damage at the tissue and biochemical levels. This may be due to its chemical properties or the way it affects cellular processes, leading to more pronounced oxidative stress and cell injury. These results highlight the importance of careful evaluation of pesticide impacts on aquatic life, especially in areas close to agricultural runoff. Moreover, the findings support the use of *Saccobranchus fossilis* as a valuable species for monitoring the health of freshwater ecosystems exposed to chemical contaminants.

VI. CONCLUSION

This study demonstrates that exposure to sub-lethal concentrations of commonly used fungicides, Mancozeb and Propiconazole, induces significant histopathological and biochemical alterations in *Saccobranchus fossilis* (Cuv. & Val.). The observed tissue damage in vital organs such as the liver, kidney, and muscle underscores the vulnerability of this species to fungicide contamination in freshwater ecosystems. Histopathological lesions including vacuolation, necrosis, and cellular degeneration indicate disruption of normal physiological functions, which can impair fish health, growth, and survival. Biochemical analyses revealed marked reductions in total protein, glycogen, and lipid content in treated fish compared to controls, reflecting impaired metabolic processes and energy depletion. These metabolic disturbances are consistent with stress

responses triggered by fungicide toxicity, further affecting the overall fitness of the fish. Mancozeb showed a higher degree of toxicity than Propiconazole, suggesting that different fungicides can have varying impacts on aquatic organisms, depending on their chemical nature and mode of action. The findings highlight the ecological risks posed by the indiscriminate use of fungicides in agricultural practices, especially when runoff enters freshwater habitats. Given that *Saccobranchus fossilis* is an important species both ecologically and economically, the negative effects documented in this study may have broader implications for fish populations and aquatic biodiversity. This calls for stricter regulation and monitoring of fungicide use near aquatic environments to prevent contamination and safeguard aquatic life. Finally, this research supports the use of *S. fossilis* as a sensitive bioindicator species for assessing environmental pollution and toxicological impacts of agrochemicals. Further studies on chronic exposure, recovery potential, and multi-generational effects would provide more comprehensive insights into the long-term consequences of fungicide pollution on freshwater ecosystems.

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