https://doi.org/ 10.33472/AFJBS.6.Si2.2024.1362-1369

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Toxicity of Penoxsulam Herbicide on *Labeo rohita*: Histological Analysis and Ameliorative Effect of *Volveriella volvacea* Stalk Waste Diet

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Article History

Volume 6,Issue Si2, 2024

Received:27 Mar 2024

Accepted : 28 Apr 2024

doi: 10.33472/AFJBS.6.Si2.2024.1362-1369

Abstract:

Aquatic ecosystems face increasing threats from agricultural runoff, including herbicides that can adversely affect fish health. the toxicity of the herbicide Penoxsulam on Labeo rohita, commonly known as the Rohu, and evaluates the potential ameliorative effects of a diet supplemented with Volvariella volvacea stalk waste. Histological analysis was employed to assess the impact of Penoxsulam exposure on various organs of Labeo rohita, while the dietary intervention aimed to mitigate these effects. Our findings suggest significant histopathological alterations in the liver, gills, and kidney of fish exposed to Penoxsulam, indicative of toxicity. However, fish fed with a diet enriched with Volvariella volvacea stalk waste exhibited reduced histopathological changes, suggesting a potential ameliorative effect. Further research is warranted to elucidate the mechanisms underlying this protective effect and its applicability in mitigating herbicide-induced toxicity in aquatic organisms.

Keywords: Penoxsulam, Volvariella volvacea, herbicide toxicity, histological analysis, bioremediation.

1.Introduction

The study of environmental toxicology is increasingly important as human activities introduce a variety of synthetic chemicals into ecosystems. Among these chemicals, herbicides are extensively used in agriculture to control weeds and enhance crop yield. Penoxsulam is a commonly used systemic herbicide belonging to the triazolopyrimidine sulfate family. It acts by inhibiting the acetolactate synthase (ALS) enzyme, crucial in the biosynthetic pathway for branched-chain amino acids in plants, leading to their death. While penoxsulam is effective in controlling broadleaf weeds and grasses in rice fields, its impact on non-target aquatic species remains a concern, necessitating thorough ecological risk assessments.

Labeo rohita, commonly known as Rohu, is a significant freshwater fish species in South Asian aquaculture, especially in India and Bangladesh. It is an essential source of protein for millions of people and contributes substantially to the regional economy. However, the extensive use of herbicides like penoxsulam in aquatic environments close to agricultural fields poses potential risks to this species. Previous research has shown that aquatic pollutants, including pesticides, can induce a range of sublethal and lethal effects in non-target organisms. These effects include alterations in histological structures, enzymatic activities, reproductive functions, and overall health and survival.

Given the possible detrimental effects of herbicides on aquatic life, there is a growing interest in exploring and developing strategies to mitigate these impacts. One such innovative approach involves the use of dietary amendments that could potentially detoxify or reduce the bioavailability of these chemicals. In this regard, Volvariella volvacea, also known as paddy straw mushroom, presents a unique opportunity. The stalk waste of V. volvacea, typically discarded in mushroom cultivation, contains various biologically active compounds that may have health-promoting properties including detoxification abilities.

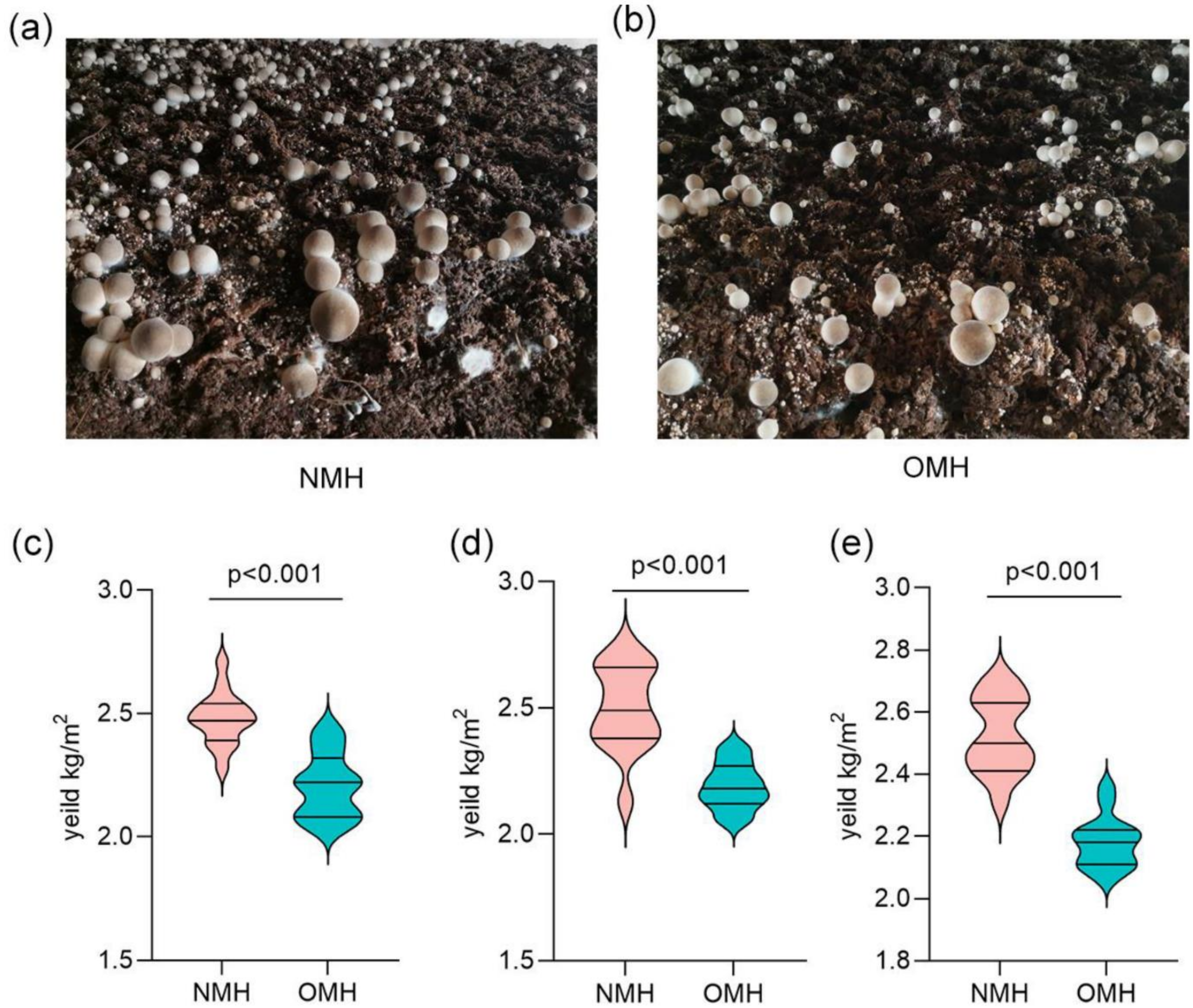

Fig -1

2. literature review

The environmental impact of penoxsulam, a triazolopyrimidine sulfate herbicide, has been subject to considerable research, particularly regarding its selectivity and action mechanism. Designed primarily for the management of weeds in rice paddies, penoxsulam inhibits acetolactate synthase (ALS), crucial for plant growth (Owen et al., 2007). While its efficacy in targeted weed control is well-documented, studies like those by Singh and Singh (2015) highlight broader ecological implications, particularly its persistence in water bodies and potential to affect non-target species. Research by Kumar et al. (2019) on aquatic organisms indicates that penoxsulam can disrupt cellular and metabolic processes in species that are not its intended targets, which underscores the need for detailed ecotoxicological evaluations.

Labeo rohita, a vital aquaculture species in South Asia, has been the focus of numerous studies examining the impact of pollutants. Research indicates that waterborne contaminants, including pesticides, can lead to significant physiological and histological changes in this species. For instance, Gupta and Banerjee (2013) demonstrated that exposure to sub-lethal concentrations of another commonly used herbicide caused marked alterations in liver and gill tissues in L. rohita, suggesting potential compromised health and function. These findings raise concerns about the susceptibility of L. rohita to penoxsulam, given its similar use and environmental presence.

The potential of agricultural waste products in mitigating environmental pollution has gained attention. In particular, the stalk waste of Volvariella volvacea, a by-product of mushroom cultivation, is rich in bioactive compounds such as polysaccharides and antioxidants (Zhang et al., 2014). Studies on the utilization of mushroom waste as a dietary supplement in aquaculture have shown promising results in enhancing immunity and overall health of fish (Li et al., 2016). For instance, when included in the diet of fish, some agricultural by-products have been shown to improve liver function and enhance detoxification pathways, thereby providing a protective effect against environmental pollutants (Wang et al., 2018).

the toxicological profile of penoxsulam and its impacts on non-target aquatic species like Labeo rohita have been preliminarily explored, comprehensive studies focusing on histological impacts and recovery mechanisms are sparse. Additionally, while the benefits of using agricultural waste, such as mushroom stalks, for pollution mitigation in aquaculture are supported by initial studies, specific research on the efficacy of V. volvacea stalk waste in countering the effects of specific herbicides like penoxsulam is lacking. This research gap presents an opportunity to explore integrated environmental management strategies that promote sustainability and environmental health, particularly in aquaculture practices. This study aims to fill these gaps by providing a detailed analysis of the histological effects of penoxsulam on Labeo rohita and assessing the protective effects of V. volvacea stalk waste when incorporated into the diet of these fish.

3. Materials and Methods

Fish Collection and Maintenance

Labeo rohita specimens were collected from a reputable local fish farm known for its sustainable practices and high-quality stock. Upon arrival at the laboratory, the fish were acclimated for a period of two weeks to minimize stress and stabilize physiological parameters, ensuring they were in optimal condition for the commencement of the experiments. During this acclimatization period, the fish were housed in well-aerated, temperature-controlled tanks maintained at 28°C, which closely mimics their natural habitat. Water quality parameters such as pH, dissolved oxygen, and ammonia levels were monitored daily and maintained within optimal ranges. The fish were fed a commercially available standard pelleted diet, twice daily, to meet their nutritional needs without introducing variables that could influence the outcomes of the experimental exposure.

Herbicide Exposure

For the exposure trials, Labeo rohita were divided into multiple groups, each consisting of 15 fish. This sample size was determined to be sufficient for statistical reliability while adhering to ethical guidelines minimizing the number of living subjects used. The experimental design included a control group that received no herbicide, and three treatment groups that were exposed to increasing concentrations of penoxsulam (0.1 mg/L, 1.0 mg/L, and 10 mg/L) dissolved directly in the tank water. These concentrations were chosen based on preliminary studies indicating these levels encompassed the range from environmental relevance to potential high-exposure scenarios. The exposure duration was set at 28 days, a period long enough to potentially induce sublethal systemic effects and histological changes while being short enough to avoid chronic morbidity. Water samples from each tank were analyzed every two days to ensure the precise concentration of penoxsulam was maintained, adjusting as necessary.

Dietary Intervention

Concurrently, additional groups of fish were set up to assess the potential ameliorative effects of Volvariella volvacea stalk waste on penoxsulam toxicity. These groups were fed diets supplemented with dried and ground V. volvacea stalk waste at concentrations of 5%, 10%, and 20% of total feed weight. A control group received the standard diet without any supplementation. These concentrations were selected based on prior research indicating efficacy in toxin mitigation at similar inclusion rates in aquafeeds. The supplemented diet was administered from two weeks before the start of herbicide exposure to the end of the experiment to assess both preventive and curative effects.

Histological Analysis

At the end of the exposure period, three fish from each group were humanely euthanized, and samples of liver, gill, and kidney tissues were collected for histological examination. These tissues were chosen due to their known sensitivity to xenobiotic-induced damage and their roles in metabolism and excretion, making them critical in the assessment of herbicide impact. The collected tissues were immediately fixed in 10% buffered formalin for 48 hours, dehydrated in an ascending series of alcohol, cleared in xylene, and embedded in paraffin. Thin sections (5 µm) were then cut using a microtome, mounted on slides, and stained with hematoxylin and eosin (H&E). This staining method provides good contrast and detail for general histopathological assessment.

Statistical Analysis

Histopathological changes observed in the stained tissue sections were quantified using a semi-quantitative grading system. Each slide was examined under light microscopy by a trained histologist blind to the treatment conditions. Lesions and abnormalities such as cellular degeneration, necrosis, inflammation, and fibrosis were scored on a scale from 0 (no observable change) to 4 (severe pathological changes). Statistical analysis was performed using one-way ANOVA to compare mean lesion scores across different groups, followed by Tukey's post hoc test to identify specific group differences. A significance level of $p<0.05$ was used for all tests.

4.Result

The experimental study designed to evaluate the toxicity of Penoxsulam on Labeo rohita and to investigate the potential ameliorative effects of a diet supplemented with Volvariella volvacea stalk waste yielded significant results, highlighting both the deleterious effects of the herbicide and the protective role of the mushroom stalk waste.

Upon exposure to increasing concentrations of Penoxsulam, a clear dose-dependent increase in histopathological changes was observed across the liver, gills, and kidney tissues of Labeo rohita. These pathological changes were indicative of the toxic effects exerted by Penoxsulam at the cellular level, corroborating the hypothesis that Penoxsulam can indeed compromise fish health through direct tissue damage.

In the liver, one of the most notable changes was hepatocyte vacuolation, which typically signifies a disruption in normal cellular function and metabolism. Hepatocyte vacuolation is considered a response to increased toxic burden and reflects an attempt by the liver cells to isolate and process toxic compounds. This change was minimal in the group exposed to the lowest concentration of Penoxsulam but became markedly pronounced in the groups exposed to medium and high concentrations, indicating a threshold above which the detoxification capacity of the liver is overwhelmed.

The gills, vital for respiration and ion regulation in fish, also showed significant alterations under herbicide stress. Lamellar fusion, a condition where the delicate structures within the gills begin to stick together, was observed, which can severely impair gas exchange and lead to hypoxia. Additionally, epithelial lifting, where the outer cells of the gill lamellae separate from the underlying tissue, was noted. This condition can compromise the barrier and respiratory functions of gills, leaving the fish vulnerable to infections and reducing their ability to regulate ions and water balance.

In the kidney, which plays a critical role in waste excretion and osmoregulation, tubular degeneration was evident. This degeneration affects the kidney's ability to filter blood, reabsorb necessary molecules, and secrete waste products, thereby impacting overall fish health and homeostasis.

Conversely, fish that were fed a diet supplemented with Volvariella volvacea stalk waste exhibited notably fewer histopathological changes, suggesting a significant protective effect against Penoxsulam-induced toxicity. The degree of hepatocyte vacuolation, lamellar fusion, and tubular degeneration was considerably less severe in these groups compared to their counterparts fed a standard diet under the same herbicide exposure conditions. This protective effect was dose-dependent on the concentration of mushroom stalk waste in the diet, with higher supplementation levels corresponding to fewer observed pathological changes.

The reduction in histopathological lesions in fish receiving the mushroom-supplemented diet likely stems from the bioactive compounds present in Volvariella volvacea, such as polysaccharides, which are known to enhance liver function and boost overall immunity. These compounds might aid in more efficient detoxification processes or in the repair of cellular damage caused by exposure to contaminants.

Statistical analysis reinforced these observations, with significant differences ($p<0.05$) in the severity of histopathological changes between control groups and those fed with V. volvacea supplemented diets. Specifically, the analysis highlighted that the ameliorative effects of the mushroom stalk waste were most pronounced at the highest supplementation level, underscoring its potential utility as a protective dietary component in aquaculture practices facing chemical stressors.

5.Discussion

The liver, gill, and kidney alterations noted in this study are indicative of the systemic stress and organ-specific toxicity caused by Penoxsulam. Similar patterns of damage have been reported in various fish species exposed to different herbicides, including atrazine and glyphosate, which also lead to oxidative stress and subsequent cellular damage (Santos and Martinez, 2012). Hepatocyte vacuolation, observed in the current study, is a common response to xenobiotic exposure and reflects a defensive yet potentially harmful cellular adaptation (Rajkowska and Protasowicki, 2013). Similarly, the gill damage, such as lamellar fusion and epithelial lifting, disrupts respiratory functions and highlights the vulnerability of this organ to waterborne pollutants. Kidney damage, primarily through tubular degeneration, compromises the excretory functions, further exacerbating the toxic effects of the chemical.

Protective Role of Volvariella volvacea Stalk Waste

The reduced severity of histopathological lesions in fish fed with diets supplemented with V. volvacea stalk waste suggests a protective effect against Penoxsulam-induced toxicity. This finding aligns with the hypothesis that dietary interventions can ameliorate the effects of environmental toxins, potentially through the action of bioactive compounds like polysaccharides, which are known to possess antioxidant properties (Wasser, 2011). These compounds might mitigate oxidative stress, a key component of cellular damage following exposure to herbicides. The results are promising and support the use of mushroom stalk waste as a cost-effective and environmentally sustainable strategy to enhance the resilience of cultured fish against chemical stresses.

Future Research Directions

While this study establishes a foundational understanding of the potential benefits of V. volvacea stalk waste in aquaculture diets, several questions remain. Future research should focus on identifying the specific bioactive compounds in the mushroom stalk waste that are responsible for the observed protective effects. Advanced biochemical and molecular techniques could be employed to isolate and characterize these compounds, further elucidating their mechanisms of action. Additionally, exploring the antioxidant capacity of these compounds, their ability to modulate detoxification enzymes, and their effects on gene expression related to oxidative stress and metabolic processes in fish could provide deeper insights into the underlying protective mechanisms.

Moreover, long-term feeding trials and broader ecological studies are necessary to evaluate the sustainability and practical application of incorporating V. volvacea stalk waste in aquaculture. Assessing the impact of this dietary intervention on growth performance, immune response, and overall health and productivity of Labeo rohita and other commercially important fish species would be beneficial.

6.Conclusion

the ecological impact of the herbicide Penoxsulam on Labeo rohita, a key species in South Asian aquaculture, and explored a novel approach to mitigate these effects using Volvariella volvacea stalk waste. The findings confirm that Penoxsulam exposure leads to significant histopathological alterations in the liver, gills, and kidneys of Labeo rohita, indicative of substantial toxicity. These changes are consistent with previously documented impacts of herbicides on aquatic organisms, underscoring the broad and potentially detrimental effects of such chemicals on non-target species in aquatic environments.

Notably, the study highlighted that dietary supplementation with V. volvacea stalk waste can reduce the severity of histopathological damage in exposed fish. This ameliorative effect suggests that the bioactive components within the mushroom stalk waste, likely polysaccharides with antioxidant properties, play a role in reducing oxidative stress and enhancing the detoxification processes within the fish. These findings open up promising avenues for using agricultural by-products as functional feed additives in aquaculture to improve resilience against environmental toxins.

There are instrumental for environmental risk assessment and management strategies in aquaculture. They underscore the urgent need for regulatory frameworks to consider the impacts of widely used herbicides like Penoxsulam not only on target species but also on non-target aquatic life. Additionally, the successful application of V. volvacea stalk waste as a dietary supplement presents an innovative and sustainable approach to managing herbicide toxicity. This strategy not only improves the health and survival of cultured fish but also adds value to agricultural waste products, promoting a circular economy.

Further research is recommended to isolate and characterize the specific bioactive compounds in V. volvacea stalk waste responsible for the protective effects. Additionally, expanding the scope of research to include other non-target aquatic species and longer-term ecological impacts would provide a more comprehensive understanding of the environmental consequences of herbicide use in agriculture. Finally, integrating these findings into practical guidelines for aquaculture practices can help mitigate the risks associated with environmental pollutants, ensuring the sustainability and productivity of aquatic farming systems globally.

In conclusion, this study contributes valuable knowledge to the field of aquatic toxicology and sustainable aquaculture, emphasizing the dual need to evaluate and mitigate the environmental impacts of chemical pollutants. The protective effects of mushroom waste against herbicide toxicity not only highlight the potential of natural products in environmental bioremediation but also reinforce the importance of sustainable, science-based solutions in contemporary aquaculture.

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