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ORIGINAL ARTICLE

Genotoxic Effects of Atrazine and Pyrethrum: Insights from DNA Fragmentation Studies in Rohu, *Labeo rohita* (Ham.)

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ABSTRACT

This study investigates the genotoxic effects of Atrazine, a commonly used herbicide, and Pyrethrum, a natural insecticide on DNA integrity in Rohu fish (*Labeo rohita* Ham.*)*. Using agarose gel electrophoresis, we detected significant DNA fragmentation in samples exposed to both chemicals. Pyrethrum caused DNA damage across various tissues—liver, muscle, gills, and kidney—with the most severe effects observed after 28 days of exposure. Atrazine exposure produced similar DNA fragmentation patterns across all tested tissues. Control samples, which were not exposed to either chemical, exhibited intact DNA, suggesting that the DNA damage was due to chemical exposure.

KEY WORDS: Atrazine, Pyrethrum, DNA Fragmentation, Genotoxicity, *Labeo rohita*

INTRODUCTION

Globally, pesticide and herbicide poisoning afflict approximately 3 million people annually, resulting 220,000 deaths. Nearly 99%of these casesstem from third-world nations due to inadequate safeguards against exposure, limited awareness of health hazards, and unrestricted availability of harmful chemicals (Ahmad *et al.* 2009).

Herbicides function through diverse mechanisms, such as disrupting vital biological processes like photosynthesis, mitosis, cell division, enzyme activity, root growth, or leaf development. They also interfere with pigment, protein, or DNA synthesis, damage cell membranes, or induce uncontrolled plant growth (William *et al.,* 1995). Atrazine (ATZ), belonging to the chloro-s-triazine herbicide family, is commonly used on crops like maize, cotton, and wheat before harvesting to combat weeds (Worthing & Walker, 1983). Beyond affecting domestic and wild mammals, birds inhabiting ecosystems adjacent to ATZ-treated fields are also exposed to this chemical while foraging.

Numerous studies have focused on the effects of atrazine exposure on non-target aquatic organisms (Giddings, 2005; Kraak *et al.,* 2014; Albuquerque *et al.,* 2020). Due to its widespread presence in aquatic environments, atrazine can induce sublethal effects on non-target species and disrupt the behaviour, morphology, and physiology of various organisms at environmentally relevant concentrations. Atrazine has been demonstrated to impact reproductive physiology and behaviour in several species. For instance, acute exposure to atrazine has resulted in demasculinization and feminization of male frogs (*Xenopus laevis*), feminization of mussels (*Elliptiocomplanata*), altered sperm quality in zebrafish (*Danio rerio*), and impaired mate odour detection in crayfish (*Faxoniusrusticus*) (Flynn *et al.,* 2013; Belanger *et al.,* 2016).

Atrazine enters the bodies of most aquatic organisms primarily through the gills and can accumulate in various organs and tissues. Accumulation of atrazine has been observed in the liver/hepatopancreas, gallbladder, and ovaries of many fishes- *Danio rerio*, *Tilapia sparrmanii*, *Coregonus fera* and *Cyprinus carpio* (Bautista *et al.,* 2018). Additionally, atrazine has been found to induce DNA damage in cells of tissues where it accumulates. Exposure to atrazine has been associated with biochemical alterations and DNA damage in the cells of the

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hepatopancreas of *D. rerio* and the liver of *Prochilodus lineatus* (Zhu *et al.,* 2011; Santos & Martinez, 2012). DNA damage has also been detected in peripherally located antennule cells of crayfish (*Faxoniusvirilis*) following acute exposures to atrazine (Abdulelah *et al.,* 2020). Morphological and cellular alterations in the hepatopancreas of crayfish have also been observed following exposure to atrazine and its metabolites (Stara *et al.,* 2018; Awali *et al.,* 2019). Pesticide usage is widespread globally, raising significant environmental worries about pesticide residue pollution. Aquatic ecosystems often play crucial roles in agricultural regions by supplying water and drainage services. Unfortunately, contemporary pesticide application methods for crop safeguarding unavoidably result in insecticide fractions infiltrating aquatic environments. Investigations into pesticide presence in aquatic settings have consistently detected traces of these harmful substances in different water bodies. This underscores the potential risk to non-target species inhabiting water catchments in agricultural zones, especially if they possess receptors similar to those of target organisms (Wijngaarden *et al.,* 2005). Pesticides critically damage vital organs and severely alter biochemical parameters in unintentionally exposed aquatic organisms (Das *et al.,* 2013; Pandey *et al.,* 2020).

Studies have highlighted the significant toxicity of pyrethroids to fish (Pandey *et al.,* 2009; Sepici-Dinçel *et al.,* 2009). However, data regarding the genotoxic impacts of synthetic pyrethroids remain contentious, with varying results reported across different experiments depending on the test system or organism utilized (Çavaº & Ergene 2003). Limited information exists on the genotoxic effects of pyrethroid insecticides specifically on fish species (Saillenfait *et al.,* 2015). Nevertheless, some research indicates that synthetic pyrethroids can induce genotoxic effects in fish (Campana *et al.,* 1999; Simoniello *et al.,* 2009).

MATERIAL AND METHODS

Sample Collection

Fishes, *Labeo rohita* Hamilton 1822 (Rohu) were collected from the local fisheries farm of Sultanpur, UP, India. Fishes were sanitized first in 0.05% KMnO4 solutions for 2-4 minutes to avoid any cutaneous infection. The sanitized fishes were kept in a 300 litre plastic tank for 14 to 15 days for acclimatization in *in-vitro* condition. Fishes were fed with wheat flour and mustard cake in a ratio of 3:1 on alternate days. Water with food waste was replaced with freshwater in every 24 hours. Water tanks were covered with transparent nylon net. When the toxicity test was performed the feeding was halted before 24 hours.

Toxicity tests were performed of Atrazine and Pyrethrum at sub lethal concentration for genotoxic analysis. Fishes were exposed with atrazine and pyrethrum for 7, 14, 21 and 28 days at 30% of 96 h LC_{50} (6.42 µg/L of pyrethrum and 3.85 µg/L of atrazine). This experiment was performed with normal control setup.

DNA Extraction

The tissue was grinded using mortar pistil, and then lysis buffers were used for the digestion of the tissue at 56ºC for 1-h and 90ºC for 1-h, subsequently. DNA extraction was performed using Qiagen Kit (Germany). Washing was done in buffer AW1 and AW2 at 1000 RPM centrifugation after all this process elution was performed using elution buffer. The DNA samples were stored at -20ºC for long time storage. For further processing, the DNA was used for agarose gel electrophoresis, the agarose concentration was 1.5% for DNA run and TBE buffer was used for running the DNA in electrophoresis buffer tank at 72 V electric supplies.

Effect of Pyrethrum-

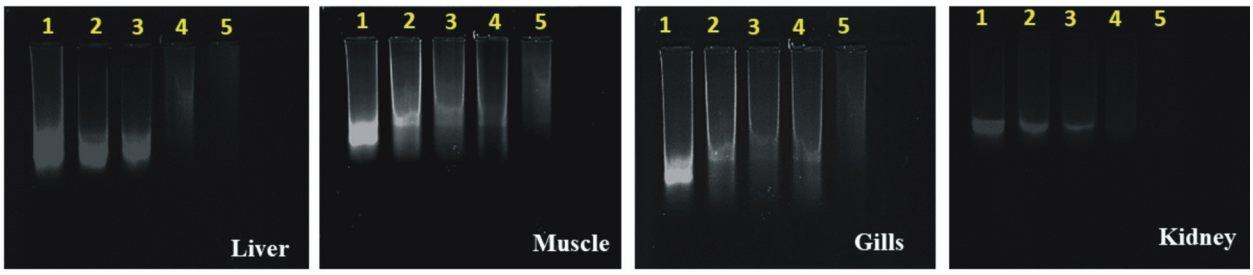


Fig. 1. The first band of each agarose gel image is showing normal control tissue DNA band and from 2nd to 5th band of each gel image is showing after treatment (7th, 14th, 21st and 28th days respectively).

RESULTS

The present study investigates the effects of Pyrethrum and Atrazine on DNA fragmentation by employing agarose gel electrophoresis. Pyrethrum, a natural insecticide, and Atrazine, a commonly used herbicide, were selected for this research due to their widespread use and potential genotoxic effects. Agarose gel electrophoresis, a technique that separates DNA fragments based on size, was utilized to visualize and analyse any DNA damage caused by these substances. By comparing the patterns of DNA fragmentation in samples treated with Pyrethrum and Atrazine to control samples, the study aims to elucidate the extent to which these chemicals may induce DNA damage, which could provide insights into their potential health risks and underlying mechanisms of toxicity.

Genotoxic Effects

Effect of Pyrethrum

In this study, the analysis of DNA fragmentation using agarose gel electrophoresis revealed a significant impact of Pyrethrum on DNA integrity. The control samples (Lane-1), which were not exposed to Pyrethrum, exhibited more intact DNA compared to the treated samples (Lanes 2-5). This suggests that Pyrethrum induces substantial DNA fragmentation, which is evidenced by the increased presence of smaller DNA fragments in the treated lanes compared to the control. This observation underscores the potential genotoxicity of Pyrethrum, as the fragmentation patterns indicate that Pyrethrum disrupts DNA structure, potentially leading to cellular damage.

Further examination showed that the effect of Pyrethrum was most pronounced in the samples treated for 28 days, with notable DNA damage observed across all tissue types analyzed, including the liver, muscle, gills, and kidney. The increased DNA fragmentation in these tissues after prolonged exposure to Pyrethrum suggests a cumulative genotoxic effect, highlighting the chemical's potential for causing cellular damage over extended periods.

Effect of Atrazine

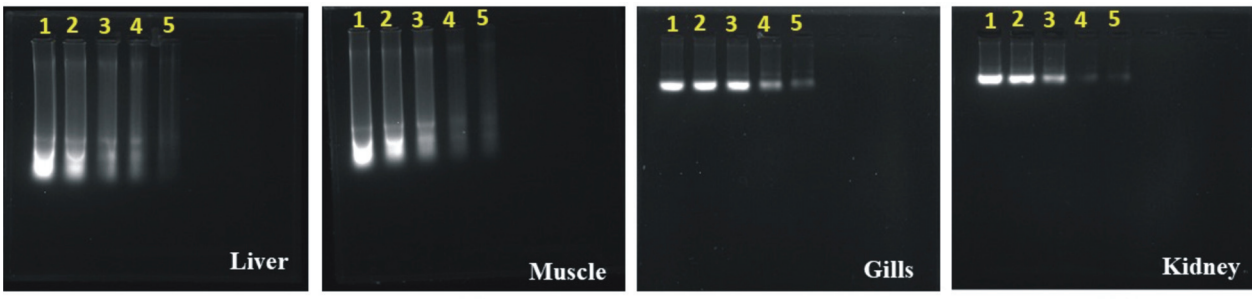
The study found that the impact of Atrazine on DNA damage mirrored the effects observed with Pyrethrum. In control samples, not exposed to Atrazine, DNA remained largely intact across all tissue types—liver, muscle, gills, and kidney—indicating no significant genotoxicity. This baseline observation confirms that the absence of Atrazine results in normal DNA integrity, serving as a reference point for assessing the chemical's impact.

In contrast, the treated samples exposed to Atrazine exhibited notable DNA damage across all analysed tissues. This damage was characterized by increased fragmentation visible in the agarose gel electrophoresis results, reflecting Atrazine's potential to induce significant genotoxic effects similar to those caused by Pyrethrum. The consistent DNA damage across various tissues suggests that Atrazine has a broad and systemic impact, leading to widespread genetic disruption. This pattern of damage underscores the need for further research to understand the mechanisms of Atrazine's genotoxicity and its potential health implications.

DISCUSSION

This study provides crucial insights into the genotoxic effects of Pyrethrum and Atrazine, as demonstrated by the analysis of DNA fragmentation using agarose gel electrophoresis. Our results indicate a significant impact of Pyrethrum on DNA integrity, as evidenced by the substantial DNA fragmentation observed in treated samples compared to control samples. The control samples (Lane-1), which were not exposed to Pyrethrum, exhibited intact DNA, while the treated samples (Lanes 2-5) displayed

Effect of Atrazine-



[A] [B] [C] [D]

Fig. 2. The first band of each agarose gel image is showing normal control tissue DNA band and from 2nd to 5th band of each gel image is showing after treatment (7th, 14th, 21st and 28th days respectively).

an increased presence of smaller DNA fragments. This pattern suggests that Pyrethrum induces considerable DNA damage, consistent with its potential genotoxicity. These findings align with previous research done by Soderlund *et al.* (2002); Pandey *et al.* (2009) demonstrating that pyrethroids, including Pyrethrum, can cause DNA damage and cellular toxicity.

The pronounced effect of Pyrethrum on DNA fragmentation, particularly after 28 d of exposure, indicates a cumulative genotoxic effect. The observed damage across multiple tissues–liver, muscle, gills, and kidney–highlight Pyrethrum's potential for widespread cellular disruption. This finding supports earlier studies (Sheikh *et al.*, 2015) that have reported similar genotoxic effects of pyrethroids on various organ systems (Ullah *et al.,* 2019; Kumar *et al.,* 2015). The prolonged exposure leads to increased DNA fragmentation, emphasizing the need to consider the duration of exposure when evaluating the risks associated with Pyrethrum.

Similarly, the study revealed that Atrazine exerts a genotoxic effect comparable to that of Pyrethrum. Control samples exposed to Atrazine displayed intact DNA, confirming that DNA damage is specifically attributable to chemical exposure. The treated samples exhibited significant DNA fragmentation across all examined tissues, reflecting Atrazine's potential for systemic genotoxicity. This finding is consistent with existing literature, which has reported that Atrazine can cause DNA damage and disrupt cellular function (Solomon *et al.,* 2008; Huang *et al,*. 2015). The consistent pattern of DNA damage across tissues underscores the need for further research to elucidate the mechanisms underlying Atrazine's genotoxic effects and assess its potential health risks.

These results highlight the broad and systemic impact of both Pyrethrum and Atrazine on DNA integrity. The observed DNA damage in various tissues suggests a need for ongoing investigation into the long-term implications of exposure to these chemicals. Future studies should focus on elucidating the molecular mechanisms through which these substances induce DNA damage and their potential effects on human health and environmental ecosystems.

CONCLUSION

This study demonstrates that both Pyrethrum and Atrazine induce significant DNA fragmentation, indicating their genotoxic potential. The agarose gel electrophoresis results revealed substantial DNA damage in treated samples compared to controls, with Pyrethrum causing pronounced effects after 28 days of exposure across multiple tissues. Atrazine, showing a similar pattern of DNA damage, reinforces the concern about its broad and systemic genotoxicity. These findings highlight the need for further investigation into the mechanisms of DNA damage induced by these chemicals and emphasize the importance of assessing their long-term health and environmental impacts.

CONFLICT OF INTEREST

There is no conflict of interest.

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