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Research article

Genotoxicity induced by imidacloprid in aquatic ecosystems: a case study on *Oreochromis niloticus*

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Abstract: Imidacloprid is a widely used insecticide belonging to the neonicotinoid class, which acts on the central nervous system of insects. This study aimed to investigate the genotoxic effects of imidacloprid on *Oreochromis niloticus* using the micronucleus (MN) assay. For this purpose, fish were exposed to 50 and 100 mg/L concentrations of imidacloprid for 24 and 96 hours. Sublethal doses were selected as 1/6 and 1/3 of the 96-hour LC50 value (280 mg/L). The frequencies of micronuclei and nuclear abnormalities (including notched, budding, lobed, and binucleated nuclei) were found to increase significantly in a dose- and time-dependent manner compared to the control groups ($p<0.05$). The highest MN frequency was observed in the 100 mg/L group at 96 hours, reaching a value of 10.8‰. Notched nuclei (11.5‰) and budding nuclei (9.5‰) also exhibited a significant increase. These findings indicate that imidacloprid can cause genotoxic effects even at low concentrations and may pose a substantial risk o aquatic ecosystem. The results emphasize the need for more rigorous biomonitoring of pesticide pollution and underscore the importance of implementing regulatory measures should be taken to protect aquatic environments.

Keywords: *Oreochromis niloticus*, imidacloprid, micronucleus test, genotoxicity, aquatic ecosystem, pesticide pollution, nuclear abnormalities

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Introduction

The use of pesticides in agricultural production is an indispensable method for controlling pests. However, the entry of these chemicals into aquatic ecosystems leads to serious environmental problems (Yıldırım, 2008). Neonicotinoids are particularly significant due to their systemic effects and environmental persistence; among them, imidacloprid is highly effective as it targets the nervous system of insects. Although it is primarily used to manage agricultural pests, it can also negatively impact non-target species (Tomizawa & Casida, 2005). The environmental half-life of imidacloprid in soil and water ranges between 40 and 997 days, and when it reaches aquatic systems, it poses a considerable threat to fish and other aquatic organisms (Bonmatin et al., 2015). While Petković Didović et al. (2022) reported that imidacloprid tends to accumulate more in soil than leach into water, the risk of aquatic contamination remains a pressing environmental concern.

Fish are exposed to pesticides through their gills, cutaneous absorption, and the food chain. This exposure can lead to a range of adverse effects, from genetic damage to physiological disorders (Atamanalp & Yanık, 2001; Helfrich et al., 2009). *Oreochromis niloticus* is widely used not only as a cultured fish species but also as a bioindicator organism due to its sensitivity to environmental pollutants (Dikel, 2009). Especially in agriculturally intensive regions such as Çukurova in Türkiye, this species is considered a suitable model for evaluating the site-specific impacts of pesticide pollution (Figueiredo-Fernandes et al., 2006).

The micronucleus (MN) assay is widely recognized as a practical and reliable method for assessing genotoxicity (Fenech, 2000). Ansoar-Rodríguez et al. (2015) reported that imidacloprid can induce DNA damage, while Guo et al. (2020) demonstrated its genotoxic effects even in human cell lines. Nugnes et al. (2023) emphasized genotoxicity in aquatic organisms related to the production of reactive oxygen species (ROS), highlighting a significant knowledge gap in this research area.

This study aims to contribute novel insights into the effects of pesticide pollution in aquatic ecosystems by investigating the genotoxic effects of imidacloprid on *O. niloticus* through assessment of micronuclei and nuclear abnormalities.

Materials and Methods

Fish Material and Acclimatization

Oreochromis niloticus specimens were obtained from the Freshwater Fish Application and Research Center of Çukurova University, Faculty of Fisheries. The fish measured 10–12 cm in length and weighed 20–25 g. Prior to the experiment, individuals were acclimated for 15 days in 50 L aquaria. During the acclimatization, water temperature was maintained at 25 ± 1°C, pH at 7.2–7.5, and dissolved oxygen levels at 6–7 mg/L. Fifty percent of the water volume was renewed every 48 hours. Fish were fed twice daily with a commercial trout diet containing 35% crude protein. Feeding was suspended 48 prior to the start of the experiment.

Experimental Design and Imidacloprid Exposure

Lighting conditions were adjusted to a natural photoperiod of 15 hours light and 9 hours dark. Dechlorinated tap water was used in the aquaria, and water temperature was maintained at a constant 22.8 °C throughout the experiment. Eight aquaria were used, each containing 12 fish, including two control groups (a negative control and a positive control treated with 5% EMS). Sublethal concentrations of 50 mg/L and 100 mg/L imidacloprid were selected, corresponding to 1/6 and 1/3 of the 96-hour LC50 value (280 mg/L), respectively. Each treatment was conducted in triplicate. Physicochemical parameters of the water were monitored daily. To prevent fish from escaping, the tanks were covered with stretch film (Figure 1).

Micronucleus Assay

Blood samples were collected from the cardiac region of the fish. Peripheral blood smears were prepared on three separate slides per individual, air-dried, and fixed in 95% ethanol for 20 minutes. After fixation, the slides were air-dried again and stained for 20 minutes with 5% Giemsa solution prepared in buffered water. The slides were examined under a Leica DM 500 jşgth microscope at 100× magnification (Maier & Schmid, 1976). A total of 1,000 erythrocytes per fish were scored per fish for the presence of micronuclei and nuclear abnormalities (including notched, budding, lobed, and binucleated nuclei).

Statistical comparisons between groups were performed using Student's t-test with SPSS version 21.

Results

Throughout the experiment, no mortality was recorded in any of the aquaria, including both the control and treatment groups exposed to 50 mg/L and 100 mg/L imidacloprid (each tested in triplicate). The frequencies of micronuclei (MN) observed in erythrocytes of the exposed fish are summarized in Table 1.

Figure 1. Experimental setup

Table 1. Micronucleus frequencies (‰) in *Oreochromis niloticus* following imidacloprid exposure

Exposure Time	Positive Control (EMS)	Negative Control	50 mg/L	100 mg/L
24 hours	7.03	2.38	7.07	9.15
96 hours	6.96	2.96	9.34	10.84

Both 50 mg/L and 100 mg/L concentrations of imidacloprid resulted in statistically significant increases in MN frequencies compared to the negative control group at both 24 and 96-hour exposure durations ($p<0.05$). The highest MN frequency was recorded in the 100 mg/L group at 96 hours, reaching 10.84‰.

The positive control group exposed to EMS also showed significantly elevated MN frequencies compared to the negative control at both exposure durations ($p<0.05$).

Moreover, MN frequencies increased in a concentration- and time-dependent manner, with significantly higher values observed at higher concentration and longer exposure periods ($p<0.05$). Morphological nuclear abnormality analysis also revealed statistically significant increases in all treatment groups compared to the negative control ($p<0.05$). Nuclear abnormalities were observed in some individuals (as illustrated in Figure 2), and their frequencies are summarized in Table 2.

Frequencies of budding nuclei were recorded as follows: 9.16‰ at 50 mg/L for 24 h; 9.38‰ at 96 h; 9.08‰ at 100 mg/L for 24 h; and 9.53‰ at 96 h. Notched nuclei reached the highest frequency at 100 mg/L and 24 h (11.46‰), with other notable frequencies observed at 50 mg/L and 24 h (8.48‰), and at 50 mg/L and 96 h (9.25‰). Lobed nuclei were

absent in the negative control group but were present in all treatment groups: 0.24‰ at 50 mg/L and 24 h; 0.14‰ at 96 h; 0.38‰ at 100 mg/L and 24 h; and 0.30‰ at 96 h. Binucleated cells were detected only in the 50 mg/L treatment groups, with a frequency of 0.05‰.

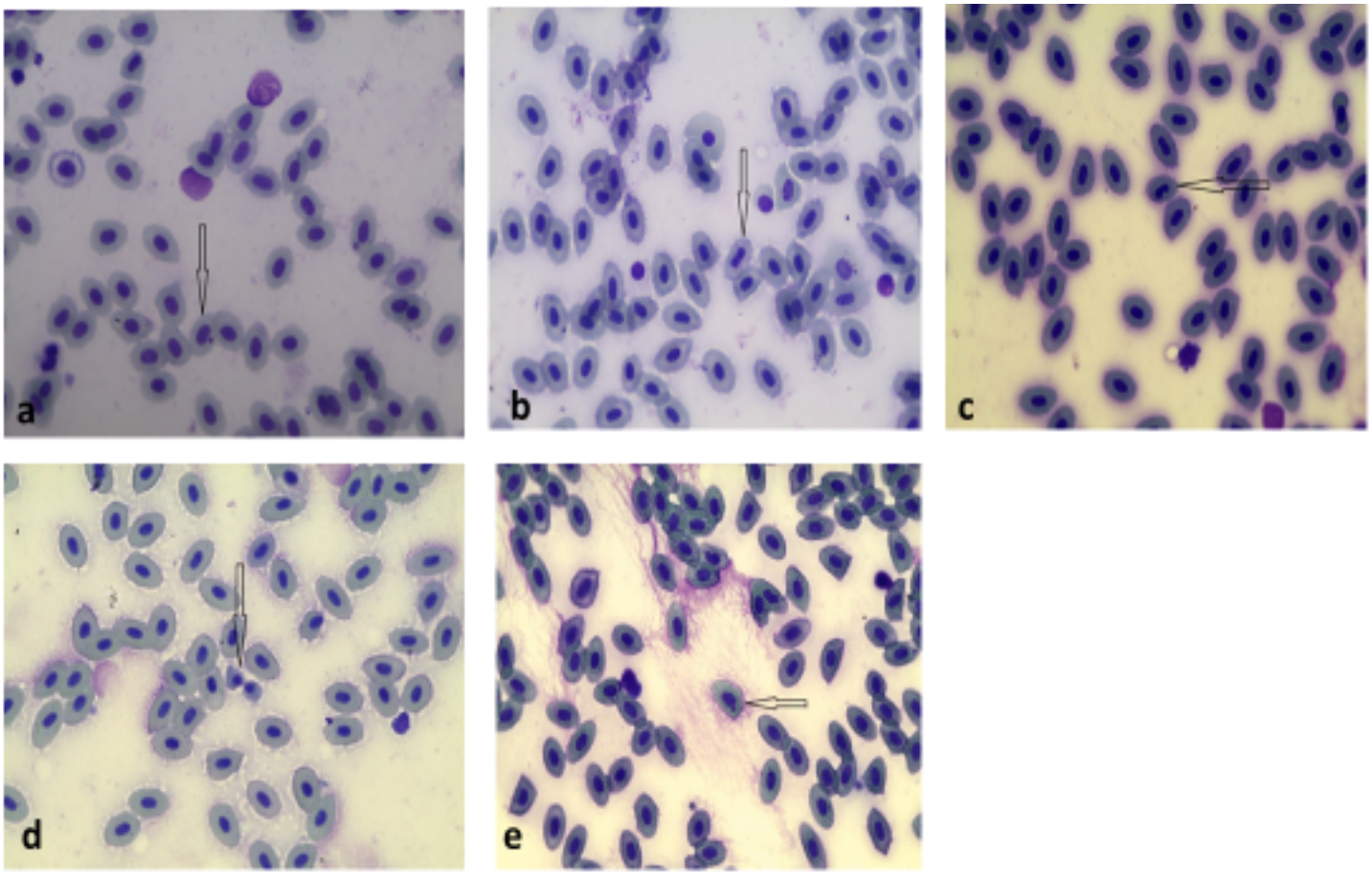

Figure 2. Representative morphological abnormalities: a) Micronucleus, b) Notched nucleus, c) Budding nucleus, d) Lobed nucleus, e) Binucleated nucleus

Table 2. Frequencies of nuclear abnormalities (‰) in *Oreochromis niloticus* following imidacloprid exposure

Anomaly Type	Exposure Time	Negative Control	Positive Control (EMS)	50 mg/L	100 mg/L
Budding Nucleus	24 h	0.00	5.58	9.16	9.08
	96 h	0.00	8.76	9.38	9.53
Notched Nucleus	24 h	0.00	2.08	8.48	11.46
	96 h	0.00	2.96	9.25	9.69
Lobed Nucleus	24 h	0.00	0.88	0.24	0.38
	96 h	0.00	1.08	0.14	0.30
Binucleated Cell	24 h	0.00	0.00	0.05	0.00
	96 h	0.00	0.00	0.05	0.00

Discussion

The findings of this study clearly demonstrate that imidacloprid induces genotoxic effects in *O. niloticus*, as evidenced by the statistically significant increases in micronucleus (MN) frequencies ($p<0.05$). The maximum MN frequency observed in this study (10.84‰) is consistent with previous research findings. For instance, Ansoar-Rodríguez et al. (2015) reported significant increases in MN frequencies after exposing *O. niloticus* to 62.5, 125, and 250 µg/L concentrations of imidacloprid for 96 hours. Similarly, Çavaş and Könen (2008) found that

the herbicides. Trifluralin and Treflan significantly increased MN formation in *O. niloticus* after exposures of 3-, 6-, and 9-day at 1.5 and 10 µg/L. In the present study, imidacloprid was found to induce even higher MN frequencies than EMS, the positive control. Guo et al. (2020) also reported similar genotoxic effects of imidacloprid in human cell lines, further suggesting its broad genotoxic potential.

The observed nuclear abnormalities provide additional insights into the genotoxic effects of imidacloprid. The frequency of notched nuclei reached 11.46‰ in fish exposed to 50 mg/L of imidacloprid for 24 hours, suggesting nuclear envelope instability. The slight reduction to 9.69‰ at 96 hours may indicate a cellular adaptation response over time. The persistent occurrence of budding nuclei points to a potential suppression of DNA repair mechanisms (Shimizu et al., 2000). Although lobed and binucleated nuclei were less frequentlt observed, their occurrence supports the presence of genotoxic stress.

The genotoxic effect of imidacloprid may be attributed to the increased production of reactive oxygen species (ROS) through oxidative stress mechanisms (Nugnes et al., 2023). Mondal et al. (2024) reported that pesticide exposure in fish suppresses antioxidant enzyme activities, which could contribute to underlie the elevated frequencies of MN and nuclear abnormality observed in the present study. The detection of these effects at sublethal concentrations indicates that even low levels of imidacloprid are capable of inducing cellular damage.

From an ecological perspective, the accumulation of imidacloprid in aquatic environments raises significant concern. Goulson (2013) and Morrissey et al. (2015) highlighted the potential indirect effects of neonicotinoids on fish populations, while Yamamuro et al. (2019) reported disruptions in aquatic food webs, suggesting broader ecological consequences. Suzuki et al. (2024) demonstrated imidacloprid-induced toxicity in zooplankton, indicating trophic-level risks that may propagate through the ecosystem. Merga and Van den Brink (2021) further emphasized the heightened vulnerability of tropical aquatic ecosystems to pesticide exposure. In addition, several studies have noted that imidacloprid-induced genetic damage in *O. niloticus* may impair reproduction capacity and compromise overall population health (Depledge, 1998; De Figueirêdo et al., 2024).

In Türkiye, intensive and widespread agricultural activity have resulted in the extensive use of various pesticide, thereby increasing the risk of aquatic ecosystem contamination. This highlights the importance of biological monitoring of pesticide pollution (Tiryaki, 2016). Sánchez-Bayo and Tennekes (2020) emphasized the time-cumulative toxicity of imidacloprid, which aligns with the 96-hour exposure findings observed in the present study. Chará-Serna et al. (2021) also demonstrated that environmental factors, such as sediment composition, can influence the toxicity and persistence of neonicotinoids. Furthermore, the impact of imidacloprid has been documented in non-aquatic organisms, including bees (Karahan, 2015) and amphibians (Akbaş, 2014), indicating its broad ecological toxicity. Its greater genotoxicity potential compared to organophosphates underscores the need for further investigation into its mechanisms of action long-term ecological consequences (Bolognesi, 2003).

Conclusion and Recommendations

This study demonstrated that exposure to imidacloprid significantly increases the frequency of micronuclei (MN) and nuclear abnormalities in the erythrocytes of *O. niloticus*, in a concentration- and time-dependent manner. These findings provide clear evidence of the genotoxic potential of this pesticide in fish.

It is recommended that future studies build upon these findings by incorporating histopathological evaluations and biochemical stress markers to provide a more comprehensive understanding of imidacloprid's toxicological effects. Additionally, further research should examine the impacts of commonly used pesticides on a broader range fish species and other aquatic organisms of ecological and nutritional importance.

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Ethical Approval

Permission was received from Erciyes University Animal Experiments Ethics Committee for this study.

Conflict of interest

The authors have no conflict of interest.

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