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The Impacts of Genotoxıc Damage on Daphnia Magna at Rısk to Penconazole

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ABSTRACT

Penconazole, a fungicide often used in agriculture, is harmful to aquatic species. Although its toxicity to fish is not yet well recognized. Among the triazole fungicides, penconazole(PEN) is among the most widely utilized in a number of nations. In this work, we employed the comet test to investigate the impacts of different Penconazole doses on the fungicide's genetic impact in *Daphnia magna*. During a 10-day period, daphnid were exposed to a controls and four separate dosages of Penconazole (1.25, 1.5, 2.0, and 2.5 mg/ L) according to initially observed values in aquatic habitats. At the end of the experiment, the Comet assay was employed to determine the damage frequency (%), Arbitrary unit (%), and Genetic damage index (%) of tissues. The 2.5 mg L-1 group showed notably greater injury frequencies (50.00±0.10) on *Daphnia magna* ($p<0.001$). Our insights indicated an important rise in DNA strand breakage for Daphnia magna after exposure to penconazole, indicating that the herbicide is genotoxic to daphnids.

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INTRODUCTION

Today's broad industrialization may create a variety of pollutants that have direct and indirect effects on aquatic habitats. Researchers throughout the globe are becoming more worried about the high amounts and diverse kinds of contaminants in today's aquatic ecosystems. Pesticides and other contaminants have led to a wide range of fish health issues. Pesticides are the most toxic to water. Several studies have revealed that pesticides have severe impacts on aquatic environments. Pesticides' adverse effects on aquatic life vary greatly based on the exact chemical, pest, type, species, and so on. Ingestion of pesticides has an instantaneous effect on species, resulting in enormous mortality or destruction of their accessible food organisms (Rohani, 2023).

Other surroundings and pesticides may cause oxidative damage, which results from a cellular equilibrium of oxidative and anti-oxidative stress mechanisms. Antioxidant enzymes and non-enzymatic antioxidants defend tissues against the harmful consequences of oxidative stress (Ergenler & Turan, 2022; Turan & Ergenler, 2023). Accelerated inflammation and altered cellular sensitivity to pathogenic pathogens and toxic chemicals are additional detrimental consequences of oxidative stress, as well. Because of their genotoxic and mutagenic qualities, pesticides applied without protection may induce genomic anomalies and maybe tumor growth

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(Gundogan et al., 2018).

Penconazole, 1-(2-(2,4-dichlorophenyl) pentyl)-1H-1,2,4-triazole, is a systemic fungicide from the triazole family. It is a pesticide containing an irregular Carbon atom that generates two stereoisomers. Penconazole is frequently used to treat illnesses caused by phytopathogenic fungi such as powdery fungal infections in flour, black sigatoka in bananas, mold in fruit such as citrus, and scabs, alternaria leaf spot, and ring rot in apples. Penconazole is a weak base (pH = 5.65), and the acid dissociation of its protonated form is 1.15 pKa, suggesting that neutral penconazole may be the dominant form in normal deposits or soil conditions (Li et al., 2022). As a result, it may have a harmful effect on non-target species, putting environmental safety at risk. Because of the very high concentration of penconazole utilized and the fairly stable structure, non-target species are frequently exposed in aquatic environments (Jia et al., 2020).

In the last few years, penconazole's risk has gained significant attention. Penconazole has been shown to cause cytotoxicity, harm to development, and oxidative damage to the liver. It is shown that initial penconazole treatment causes considerable behavioral damage in zebrafish embryos (Icoglu Aksakal & Ciltas 2018). Goldfish demonstrate severe oxidative damage after 21 days of exposure to penconazole, including SAT, SOD, and GSH (Mu et al., 2016). At the identical time, penconazole exposure caused substantial alterations in the expression of CAT, GPX, and other genes that encode antioxidant enzymes. Penconazole has also been linked to considerable cytotoxicity and an unusual immune reaction in rats (Chaabane et al., 2017). These findings all point to the necessity for a more in-depth investigation of the toxicity consequences of penconazole (Mercadante et al., 2019).

Daphnia has been identified as a keystone species in the food webs of many mainland waters, and it serves as a crucial framework for sustainable, progressive and ecotoxicological studies. Daphnids are very popular for studies due to the requirements indicated above for an appropriate model, as well as its cyclical parthenogenetic life cycle, including diapausing phases. Easy culture of identical cells enables investigations to separate the implications of external and genomic variables. *Daphnia magna* is often used as a model organism to assess contaminants in aquatic ecosystems because it is tolerant of a wide range of chemicals (Felten et al., 2020).The purpose of this research was to assess if the fungicide penconazole generated genotoxicity in the model organism Daphnia magna using the comet test.

MATERIAL AND METHODS

Experimental Design

The Genotoxicology Laboratory at the Faculty of Marine Science and Technology, University of Iskenderun Technical, Iskenderun, Hatay, Turkey, housed and cared for Daphnia magna. The Daphnids were placed in 3-L glass beakers with 2 L of tap water that was aerated and had a 16:8 (light:dark) photoperiod. The results of four different groups (including an observation group

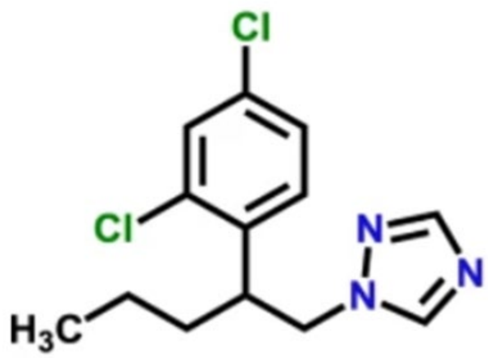

Fig. 1. The chemical structure of penconazole (Takala et al., 2019)

and a pair of test groups) were subjected to a toxicity test. Each concentration of penconazole was tested using three duplicate samples, and twenty daphnias were used per focus, following the Organisation for Economic Co-operation and Development (OECD) Test Guideline 202. The specimens were maintained at a temperature of 20 ± 1 °C, with a dissolved oxygen content of 6.4 ± 0.5 mg/L. The pH value was controlled within the range of 8.2 ± 0.2. The levels for treatment were produced at 0 (control), 1.25, 1.5, 2, and 2.5 mg/L. Overall, a group of twenty newborns, all under one day when were put inside glassware containers with a volume of 100 mL apiece. The pencanozole compounds were prepared as described and underwent continuous stirring throughout the transfer to the test containers. The choice of control and three different pesticide dosages (1.25, 1.5, 2, and 2.5 mg/L) was determined by existing levels found in aquatic settings.

These amounts were used to carry out a 10-day experiment. The comet test was conducted using an enhanced approach based on Cavalcante et al.'s (2008) protocol. The test used a suspension of gill cells, retained cell pellets, and single-cell gel electrophoresis to accomplish the experiment. The slides were analyzed using a fluorescence microscope (Image2M Zeiss) at a magnification of X40. Prior to examination, the slides were stained with ethidium bromide and then neutral with a 0.4 M Tris solution that had been cooled on ice. A total of 100 cells from every specimen were examined directly to assess the nuclei, which were categorized into five types. The damage percentage (% DF), arbitrary values (AU), and DNA damage rating (GDI) were computed for evaluation.

After performing any statistics computations, the evidence underwent an assessment to see whether it met the criteria of regular and homogeneous. Subsequently, a unidirectional analysis of variance was conducted to determine how there were any notable disparities across both treatment groups. The data was evaluated using a one-way ANOVA, and any observed changes were deemed statistically noteworthy at a significance level of P<0.05. (Norusis, 1993).

RESULTS AND DİSCUSSİON

The results of 1.25; 1.5; 2.0; 2.5 mg/L of Pencanozole applied groups as compared to the untreated control fish were presented in Table 1. The table describes the mean as well as the standard deviations of the DNA damage frequency (%), arbitrary units values (AU), and genetic damage index (%) observed in Daphnia magna.

The lowest damage frequencies (%) as 30.00±0.10 was obtained in the control (0 mg/L) group and there was significant change observed in the DNA damage parameters during the experiment (p <0.001). The highest damage frequencies (%) as 50,000±0,10 was obtained 2.5 mg/L groups. In comparison to the control and the other pencanozole groups, the frequency of DNA damage and other damage metrics (AU and GDI) were greater in the 2.5 mg/L

Table 1. The averages and standard deviations for DNA damage in control and different concentrations of penaconazole on the Daphnia magna

Groups (mg L^{-1})	Damage Frequency (%)	Arbitrary Unit AU	Genetic Damage Index (%)
Control	23.285±1,732^{a}	45,333±3,511^{a}	0,453±0,351^{a}
1.25	30.000±0,100^{b}	90,666±3,055^{b}	2,906±0,305^{b}
1.5	32,666±4,163^{bc}	99,666±6,655^{c}	0,996±0,655^{c}
2.0	34,333±1,154^{c}	110,333±1,527^{d}	1,103±0,152^{d}
2.5	50,000±0,100^{d}	150,666±3,511^{e}	1,506±0,100^{e}
P	***	***	***

The data are shown as arithmetic mean ± standard deviation. *Values with different letters in each column indicate significant differences. Indicate significance level between DNA damage Daphnia magna obtained from control and four different concentrations of Pencanozole.

concentration group. However, it was observed that the relatively high damage level of 2.5 mg/L pencanozole was more than fifty percent more than the damage level of the control group.

The past few years have seen the use of a number of effective fungicides; nevertheless, these chemicals have the potential to cause damage to ecosystems and creatures that are not their intended targets.In spite of the fact that penconazole is a chiral triazole fungicide that is frequently used, the differential in toxicity that exists between its various forms has not been investigated. As a result, the growth-related toxicity of penconazole exposure to Daphnia magna was determined in this research. The varied concentrations of penconazole were also considered. The findings demonstrated that Daphnia magna was harmed by penconazole after being exposed to the compound. The developmental toxicity of penconazole was explored by Aksakal & Citaş (2018) in their research. They exposed zebrafish embryos to various concentrations of penconazole (0.8, 1.6, and 2.4 mg/L) four hours after fertilization. The results of their studies were presented in the form of a paper. Several genes were found to be expressed, as well as developing, surviving, and respiration rates, as well as body length, deformity, and other characteristics. The investigation revealed that exposure to penconazole resulted in embryonic toxicity, which manifested itself as a delay in hatching, decreased surviving, and a slower heart rate. In addition, penconazole exposure has been linked to the development of abnormalities such as pericardial edema, edema of the yolk sac, axial deformity, tail malformation, and spinal curvature. After being exposed to penconazole, the findings of the RT-PCR analysis revealed that the levels of some antioxidant genes' messenger RNA were shown to be decreased. In a nutshell, they made the observation that the levels of interleukin 1 beta interferon in zebrafish embryos induced embryonic development toxicity when the embryos were exposed to penconazole.

Farrag et al., (2021)They studied that both male and female red-bellied tilapia of the species Coptodon zillii were subjected to fungicides consisting of 0.8 and 1.6 ug/L penconazole and 7.5 and 15 mg/L copper nanoparticles for a period of three months in the research that was conducted. The examination of the reproductive response was looked down upon. In the groups that were exposed to penconazole and copper nanoparticles, they found that the levels of the hormones estrogen and progesterone had significantly increased. When they examined the testis homogenate, they discovered that there was a notable reduction in the amount of superoxide radicals in the penconazole (I) and penconazole (II) groups, as well as in the amount of catalase (CAT) in the penconazole (I) and copper nanoparticle (I) groups. Nitric oxide (NO) levels were found to have significantly risen, according to their findings. After doing a histological investigation, the researchers discovered that the gonads of fish that had been given fungicides other than the copper nanoparticle (II) group had shown significant degradation. Following the use of fungicides, which were explored by altering gonadal sex hormones and producing redox imbalance and cytopathological abnormalities, these results suggested that C. zillii had a load on its reproductive system.

Chaabane et al., (2016) found that selenium (Se) has a protective effect against penconazole in male Wistar rats. The rats were divided into four groups, with the first group (PEN) not receiving any therapy. After six days, the rats were given 67 mg/kg intraperitoneally from day 7 to day 15. The rats' body weight was measured every two days. In the third group (Se plus PEN), selenium was given daily via meals for 15 days, followed by PEN at a dose of 67 mg/kg every two days.The fourth group (Se) was given Se on a daily basis via food (0.5 mg/kg diet) for a period of 30 days. According to the findings of their research, they demonstrated that Se dropped. Following treatment with PEN, there was a considerable decrease in the amounts of malondialdehyde and protein carbonyl that were present in the heart. This fungicide is responsible for the fragmentation of DNA. Modifications in antioxidant levels were also altered by selenium, including enzymatic antioxidants (superoxide dismutase, glutathione peroxidase, and catalase) as well as non-enzymatic antioxidants (glutathione and vitamin C). Hearts of rats that were treated with Pens. The impact of protection Upon histological examination, they discovered that selenium had become more apparent. The cardiac organ undergoes changes as a result of PEN. After considering all of the evidence, they came to the conclusion that Se could be effective in protecting rats from the oxidative damage caused by PEN. Li et al., (2022) Based on the standard of symptoms of poisons including Daphnia magna, the researchers determined that S-(-)-penconazole exhibited high-grade toxicity after 48 hours (with an EC50 value of 0.1 to 1.0 mg/L). Both raceme and R-(+)-penconazole were evaluated as having a moderate level of toxicity, with an EC50 value of 1.0 to 10 mg/L. A further finding was that R-(+)-penconazole was identified in zebrafish via the examination of their automated engines, respiration, other deformities. In the study conducted by Jia et al. in 2020, it was found that S-(-)-penconazole exhibited significantly greater levels of toxicity. Completeness of all pertinent deficits pertaining to this topic needs to be achieved. The results of our study are consistent with the one of earlier research.

Following examining the susceptibility of this agriculture toxicity toward prevalent pollutants, we might suggest using Daphnia magna as a model organism for ecotoxicological research. This recommendation is based on our findings. Because of this, there is a need for more study on species that utilizes a variety of agricultural pesticides in order to determine whether or not these pesticides are able to get contaminants notice, that is necessary for the development of technologies to track aquatic settings.

CONCLUSIONS

In conclusion, the use of pesticides for diverse objectives may provide significant hazards to water quality and all aquatic organisms. This review research demonstrates that penconazole impacts species in aquatic ecosystems. Bioaccumulation is a process that enables pesticides to accumulate in aquatic organisms, especially in aquatic species. The extensive use of pesticides has resulted in the proliferation of several illnesses in aquatic creatures. Consequently, the frequency of such research should be increased, and biomonitoring should be implemented. Furthermore, it is advisable to reduce pesticide use to preserve a healthy ecology.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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