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Evaluation of the Effects of Imazalil on Genotoxicity and Behavioral Toxicity in *Drosophila melanogaster*

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İmazalil'in Drosophila melanogaster'de Genotoksisite ve Davranışsal Toksisite Üzerine Etkilerinin Değerlendirilmesi

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Abstract

Imazalil (IMZ) is an imidazole and triazole derivative fungicide that is widely used to prevent many diseases in vegetable and fruit fields and to prevent post-harvest spoilage. In this study, the genotoxic potential of IMZ at different concentrations (0.25, 1, 4.5 mM) on *Drosophila melanogaster* was investigated using Somatic Mutation and Recombination (SMART) and Single Cell Gel Electrophoresis (Comet) Assays. The effect of the same IMZ concentrations on behavioral toxicity in *D. melanogaster* was investigated. Larval weight, crawling, and pupa formation success were performed to determine behavioral toxicity. As a result of the study, it was determined that IMZ generally caused a negative effect on *D. melanogaster*. In the SMART test, it was found that the differences between the wing preparations of the individuals obtained as a result of all IMZ concentration applications were not statistically significant compared to the negative control. The damage caused to DNA by IMZ was determined by the Comet test, and a statistically significant increase in DNA damage scores was observed at doses of 1 and 4.5 mM. In the crawling experiment of IMZ on D. melanogaster, a decrease in locomotion occurred due to the increase in dose compared to the control group, and these changes were found to be statistically significant at all application doses. Changes in larval weight were not found to be statistically significant. In the pupa formation success experiment, the decrease at 1 and 4.5 mM doses was found to be statistically significant.

Keywords: Imazalil; *Drosophila melanogaster*; Comet test; Behavioral toxicity; SMART test.

Öz

İmazalil (IMZ), sebze ve meyve tarlalarında birçok hastalığın önlenmesi ve hasat sonrası bozulmanın engellenmesi amacıyla yaygın olarak kullanılan bir imidazol ve triazol türevi fungisittir. Bu çalışmada, farklı konsantrasyonlardaki (0.25, 1, 4.5 mM) IMZ'nin *Drosophila melanogaster* üzerindeki genotoksik potansiyeli Somatik Mutasyon ve Rekombinasyon (SMART) ve Tek Hücreli Jel Elektroforez (Comet) Testleri kullanılarak incelenmiştir. Aynı IMZ konsantrasyonlarının *D. melanogaster'de* davranışsal toksisiteye etkisi de araştırılmıştır. Larva ağırlığı, sürünme ve pupa oluşum başarısı davranışsal toksisiteyi belirlemek için gerçekleştirilmiştir. Çalışma sonucunda, IMZ'nin genel olarak *D. melanogaster* üzerinde olumsuz etkilere neden olduğu belirlenmiştir. SMART testinde, tüm IMZ konsantrasyonu uygulamalarının elde edilen bireylerin kanat preparasyonları arasındaki farkların negatif kontrolle istatistiksel olarak anlamlı olmadığı bulunmuştur. IMZ tarafından DNA'ya verilen hasar, Comet testi ile belirlenmiş, 1 ve 4.5 mM'lık dozlarda istatistiksel olarak DNA hasar skorlarında anlamlı bir artış gözlenmiştir. IMZ'nin *D. melanogaster* üzerinde yapılan sürünme deneyinde kontrol grubuna kıyasla doz artışına bağlı olarak hareket kabiliyetinde azalma meydana gelmiş ve bu değişiklikler tüm uygulama dozlarında istatistiksel olarak anlamlı bulunmuştur. Larva ağırlığındaki değişiklikler ise istatistiksel olarak anlamlı bulunmamıştır. Pupa oluşum başarısı deneyinde ise 1 ve 4.5 mM'lık dozlardaki azalış istatistiksel olarak anlamlı bulunmuştur.

Anahtar Kelimeler: İmazalil; *Drosophila melanogaster*; Comet testi; Davranışsal toksisite; SMART testi.

1. Introduction

Pesticides are chemicals or mixtures of substances commonly used in agriculture to control or prevent fungi, insects, weeds, various plant diseases, microorganisms, and other pests (Kamel and Hoppin 2004, Çali and Kesercioğlu 2010, Akdoğan *et al*. 2012). Unconscious use of pesticides not only affects plants but also pollutes soil and water. This also negatively affects beneficial microorganisms, birds, fishes, human health and non-target organisms (Isin and Yıldırım 2007, Kamal *et al*. 2020). Pollutants such as pesticides, veterinary medicines, antibiotics, and parabens have recently been indicated in many studies to cause contamination in processed foods and agricultural/crop production (Pérez-Ortega *et al*. 2017). Due to advancement in technology, the amount and variety of pesticides has also been increased significantly to increase the yield of agricultural

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products. Therefore, due to their direct and indirect harmful effects on the environment, it is necessary to determine the potential toxic and genotoxic effects of widely used pesticides on non-target organisms before they are released to the market.

Fungicides, an important class of pesticides, are chemical compounds or organisms used to kill fungi or their spores (Haverkate *et al*. 1969). Fungicides have been widely used for more than a century to increase crop yields and maintain food security. Fungal diseases in agriculture cause crop yields to decrease by almost 20% (Lucas *et al*. 2015, Yu *et al*. 2023). According to the data of the National Research Council, it has been reported that a large part of the carcinogenic effects caused by pesticides may be due to fungicides (Schneider *et al*. 1994).

IMZ (1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy) ethyl]-1H-imidazole) is an imidazole and triazole derivative fungicide that has an important place in the prevention and cure of many fungal diseases (Siegel *et al*. 1978, Şişman and Türkez 2010, Çıldır and Liman 2020). IMZ is a slightly yellow to brown solidified oil-colored fungicide with a molecular weight of 297.18 g/mol and is used in agriculture to control fungal pathogens after harvesting, especially fruits such as citrus fruits, pears, and apples (Ghosoph *et al*. 2007, Altieri *et al*. 2013).

More data are needed to determine the negative effects of mutagenic and genotoxic effects of pesticides such as IMZ, which have a widespread field of use, on non-target organisms. In this context, various assays and tests like the Comet test, behavioral tests, and SMART test have been used to determine genotoxicity, behavioral toxicity, and mutagenic effects. The Comet test is a simple, sensitive, and fast technique used to detect DNA damage and repair at the cellular level. The breaks and structural distortions that occur in DNA create comet-like images. DNA damage is determined by the density of the part of the head that makes up the Comet (Dayanıklı 2014). The use of model organisms in conducting toxicity tests is quite common and important. In this context, *D. melanogaster* is a model organism that is widely used in toxicity studies due to its simpler genome structure, life expectancy, and low maintenance cost compared to higher organisms, as well as its similarity in terms of molecular pathways between humans. As a result of genomic studies conducted, it is estimated that about 75% of the genes responsible for the disease in humans are preserved in *Drosophila* (Jeibmann *et al*. 2009, Calap *et al*. 2017, Anushree *et al. 2023*).

There are several reasons why the Comet test is used on *Drosophila* to determine genotoxicity under *in vivo* conditions. *D. melanogaster* hemocytes and lymphocytes in the blood of mammals have the same role. The hemocyte isolation methodology is quite simple. The sensitivity of hemocytes to toxic substances circulating in the hemolymph is quite high and can be directly exposed. So, due to these reasons, it has an important place in genotoxicity studies (Carmona *et al*. 2010, Hersperger *et al*. 2023).

Behavioral tests on different organisms were utilized for the evaluation of the toxic effects of various chemicals and newly synthesized products. The data obtained from these tests are very effective in evaluating the effects of different chemicals in a specific, sensitive, and reliable manner in all fields, especially in basic research. At the same time, behavioral changes are one of the main measurable indicators of chemical exposure in toxicity assessments. For this reason, many different experiments,such as pupa formation and pupa emergence success, pupa position, larval weight, and crawling tests are widely applied to determine behavioral toxicity in *Drosophila melanogaster* (McKellar *et al. 2017,* Bianchini *et al.* 2019, Kurşun *et al.* 2021, Ciğerci et al. 2023).

One of the most common *in vivo* tests used to determine mutation and recombination is the *Drosophila* wing somatic mutation and recombination test, known as SMART (Kurşun *et al*. 2022). The SMART test is an inexpensive and fast test used to detect genetic mutations and variations of somatic recombination. In this test, 3rd instar transheterozygous larvae carrying the recessive *flr* and *multiple wing hair* (*mwh*) genes are used. The genotoxic effects of the agent used cause changes in the imaginal disc cells in larvae. The variations are evaluated according to the mutant trichomes (Kaygısız *et al*. 2017, Nas 2019). For all these reasons, in this study, *D. melanogaster* as a model organism has been used to assess the potential genotoxic effects of IMZ on non-target organisms. Behavioral toxicity has been studied to determine the toxic effects on the organism, SMART has been studied to evaluate mutagenicity and Comet has been studied to evaluate genotoxicity.

2. Materials and Methods

In this experiment, *D. melanogaster* samples obtained from the Akdeniz University Department of Biology Toxicology Laboratory were used. A 12:12 light:dark photoperiod was applied to the colonies and the samples were kept at 25±1°C and 60% humidity.

To determine the doses to be used in the study, doses of 0.01, 0.1, 1, 5, 10 mM were scanned to determine the LD_{50} dose. As a result of the evaluation made with the

probit program the LD_{50} dose was determined as 1 mM. And the lower and upper doses of 0.25 and 4.5 mM, respectively, were determined as the experimental group.

In behavioral experiments and the Comet assay, eggs were obtained by washing with tap water after 8 hours using a fine mesh. Collected *Drosophila* Oregon R+ line eggs were exposed to three concentrations (0.1, 1, 4.5 mM) for IMZ. The exposure was 72±4 h in the 4.5 g of dry *Drosophila* instant medium and 9 mL of IMZ in different concentrations. All the tests were performed in 3 replications and negative control with distilled water (Ciğerci et al. 2023). Imazalil pesticide used in the study was supplied by Sigma Aldrich, Munich, Germany (CAS Number 35554-44-0) and distilled water was used as solvent.

2.1. Comet Assay

The eggs were collected from the 8-hour-old culture medium with *Drosophila* Oregon R+ line. The eggs were put in 4.5 g of instant medium, which applied 9 mL of four different concentrations of IMZ until they reached the third instar larval stage. Approximately 40-60 larvae were taken for all concentrations, and hemocytes were obtained by dissecting the larvae under a microscope in phosphate-buffered saline (PBS) solution containing 0.07% phenylthiourea. The isolated hemocytes were first centrifuged at 300 x g for 10 minutes, 20 µL of cold PBS was added to the resulting pellet, and the hemocytes were mixed in an Eppendorf containing 80 µL low melting point agarose (LMA). Then, this mixture was spread on the slide coated with 1% normal melting point agarose (NMA) the day before and covered with a coverslip. After a while, the coverslips were removed and the preparations were placed in fresh lysis solution (10 Mm Tris, 2.5 M NaCl, 100 mM Na_2EDTA, 1% Trixton X-100, and 1% N-lauryl sarcosinate, pH 10) in the dark for one hour. After the lysis process, the preparations were transferred to cold electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH>13) and waited for 30 minutes. Then electrophoresis was performed at 25 V 300 mA in the dark for 30 minutes. After removing the lysis solution, the slides were embedded into 400 mM Tris (pH 7.5) and neutralized for 5 minutes. This process was applied twice. 70 µL ethidium bromide was used to visualise the cell nucleus. 5 mg of ethidium bromide was dissolved in 25 mL distilled water to prepare the stock solution.

A 10-fold dilution was applied for the experiment. Slides were examined with a fluorescence microscope to visualize fifty comets per slide for scoring by one person to maintain the stability of the results. In the studuy an Olympus Optical Co. Ltd. BX50F-3 fluorescence microscope was used to visualize Comet assay results.The scores of nuclear damages were scored as 0 for no damage to 4 for full damage. All procedures were performed in a dark or red-light environment.

2.2. Behavioral Experiments

2.2.1. Larval Weight

Larvae were weighed before and after exposure. The larvae that were exposed to IMZ were collected with a fine mesh and washed with tap water to prevent any contamination. Then 10 larvae from each replicate were weighed again after 24h of exposure (Kurşun *et al*. 2021). All experiments were performed in triplicate.

2.2.2. Crawling

Solidified agar (2%) was utilized to measure the larval movement. The distance of the larval movement was monitored for one minute and measured (Kurşun et al. 2021). The experiment was carried out with 10 larvae in each repetition and the study was repeated three times. and the average of the data obtained was used for statistical evaluation.

2.2.3. Pupa Formation Success

50 larvae fed on media containing different concentrations of IMZ until the larval stage were recorded, and measurements were calculated using the formula hatching success (number of adults/50) x 100% and the percentage of pupa position according to Fauzi *et al*. (2020) was determined by this method (Fauizi et al. 2020) Measurements were continued untul the end of pupation formation.

2.3. SMART Assay

This assay uses two different strains of *D. melanogaster,* which are *mwh* and *flr*. These two different strains were bred as *flr* females and *mwh* males. Almost 300 *flr* females (to be sure *flr* females are virgin, were collected no more than 6h after hatching) obtained and mated with *mwh* males (Amkiss *et al*. 2021). 125 mL bottles with standard mediums were used. Waited 8h to flies to mate and adults were removed from the mating bottles. After the adults were mated and removed from the bottles, eggs were collected using a fine mesh and placed in test tubes with 9 gr instant medium with 4.5 mL different concentrations of IMZ and then waited for the pupa to hatch. Adults were collected in 4h period to be sure that the individual's wings weren't damaged. Collected adults were stored in 70% alcohol at 4°C until the use. The wings of individuals were placed in a watch glass with Faure's

solutions to collect the wings. 50 ml dH_2O, 30 g gum arabic (Aldrich), 50 g chloral hydrate (Merck) and 20 ml glycerol were used for the preparation of Faure's solution. Stereomicroscopes were used to collect the wings. Wings were collected using lancet and fine tweezers. Wings were placed in pairs into to slides that contained a few µL of Faure's solutions and waited for 24h in a dust-free place. For fixation of the wing preparations, a few drops of Faure's solution were added to the slide and then the wings were carefully added and covered with cover glass. 250 g weight was placed on the preparations and allowed to dry for 24 hours. After 24 hours, the weights were removed and the Faure's solution overflowing to the edge of the preparations was cleaned. (Aşkın and Uysal 2010).

2.4. Statistical Analyzes

Duncan's multiple range test ($p \leq 0.05$) was used to determine significant alterations in the DNA damage data (means ± standard deviations) in the Comet assay. The results obtained from crawling, pupa formation success and larval weight experiments were evaluated with the One-way ANOVA (Kruskal-Wallis) test in SPSS 20 Programme. The results obtained from SMART were evaluated by the MICROSTA program. Statistical analysis of all other results was done by analyzing the variance of the data using SPSS version 15.0 for Windows software. Duncan's multiple range test was used for comparing different concentration groups with the controls.

3. Results

The toxicity and genotoxic potential of IMZ on *Drosophila melanogaster* were investigated using the SMART test and Comet test. Crawling test, larval weight, and pupation success tests were applied to determine the behavioral toxicity of IMZ in *Drosophila melanogaster.*

3.1. Comet Assay

According to the results of the Comet test, it was determined that the amount of DNA damage increased in direct proportion to the increase in IMZ concentration, and this increase was found to be statistically significant when compared to the control group (Figure 1).

3.2. Behavioral Experiments

3.2.1. Larval Weight Assay

In the treatment groups containing IMZ at 0.25 mM and 4.5 mM concentrations, larval weight increased compared to the control, and 1 mM concentration decreased compared to the control. However, these changes were not found to be statistically significant. The results of the larval weight assay in *D. melanogaster* are shown in Figure 2.

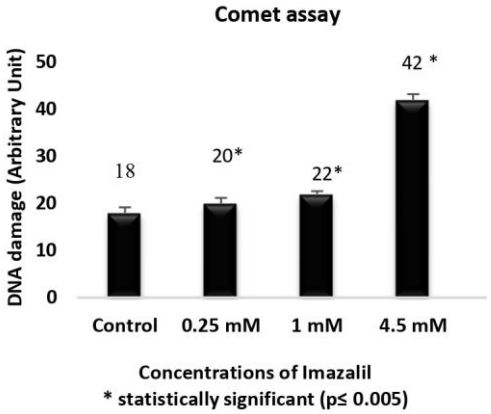

Figure 1. Imazalil-induced DNA damage scores in *D. melanogaster*

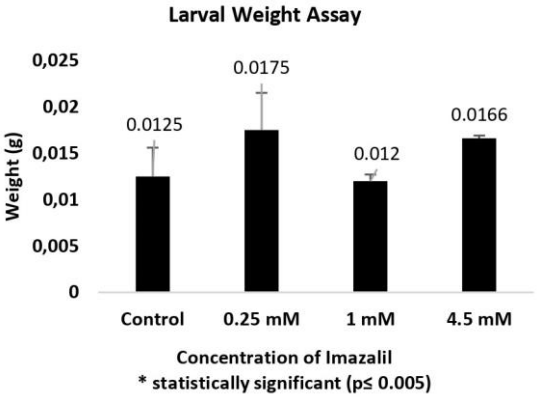

Figure 2. Imazalil-mediated alterations in larval weights of *D. melanogaster*

3.2.2. Larval Crawling Assay

A statistically significant decrease in the movement skills of the larvae was observed in the crawling experiment due to the increase in IMZ concentration (Figure 3).

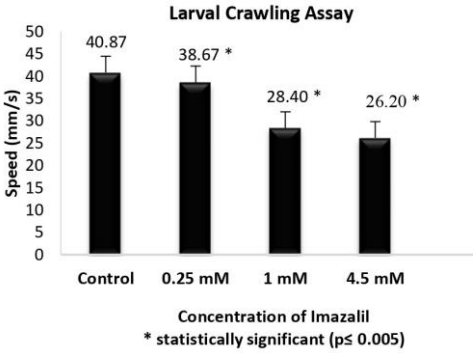

Figure 3. Imazalil-mediated alterations in larval crawling of *D. melanogaster*

3.2.3. Pupa Formation Success

The data of the pupa formation test are given in Figure 4. When the pupa formation success was compared with the control, 0.25 mM application showed a value close to the control group. Depending on the concentration increase, 1 mM and 4.5 mM applications decreased. Except for the 0.25 mM application, these changes were found to be statistically significant.

3.3. SMART Assay

The results obtained from the 0.25, 1, and 4.5 mM applications of IMZ, whose genotoxic properties were evaluated with SMART, are shown in Table 1. In this study, positive results were observed in all clone types when the results of ethyl methane sulfonate (EMS) applications used as a positive control were compared with the results of distilled water application. It was found that the differences between the wing preparations of the individuals obtained as a result of all IMZ concentration applications were not statistically significant compared to the control group. In addition, according to the results of the signed application, there was no increase or decrease in the total clone induction frequency at a certain rate depending on the dose increase.

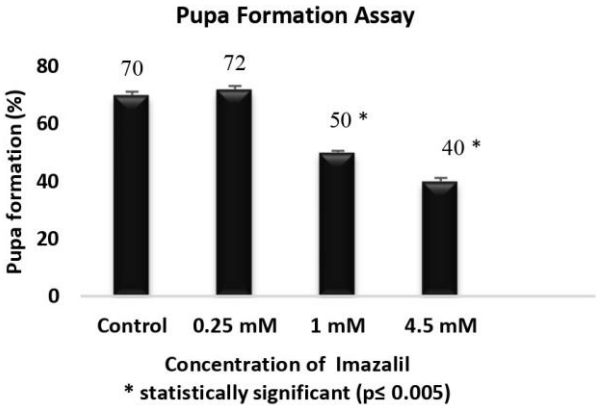

Figure 4. Imazalil-mediated alterations in pupa formation success of *D. melanogaster*

Table 1. Results of SMART, evaluating the genotoxic potential of Imazalil

Experimental groups (mM)	Number of wings (N)	Small single spots (1–2 cells; m =2)			Large single spots (>2 cells; m = 5)			Twin spots (*m*=5)			Total *mwh* spots (*m*=2)			Total spots (*m*=2)		
		No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.
Distilled Water (72h)	40	8	(0.16)		1	(0.02)		0	(0.00)		9	(0.19)		10	(0.19)	
1 mM EMS (72h)	30	60	(2.29)	+	20	(0.87)	+	7	(0.29)	+	80	(3.39)	+	87	(3.48)	+
Imazalil (mM)																
0.25	40	2	(0.05)	-	1	(0.025)	-	0	(0.00)	i	2	(0.05)	-	3	(0.075)	-
1	40	8	(0.2)	i	0	(0.00)	i	0	(0.00)	i	8	(0.2)	i	10	(0.10)	-
4.5	40	8	(0.3)	i	3	(0.075)	i	0	(0.00)	i	9	(0.225)	i	12	(0.3)	i

Fr: frequency; D: statistical results; EMS: ethyl methane sulfonate +: positive; -: not genotoxic; i: non-significant results; m: multiplying factors; confidence level: 0.05.

4. Discussion

The interaction of humans with pesticides and other agricultural drugs is increasing rapidly with the growing technology and pesticide usage in agricultural fields. Pesticides are a crucial part of agriculture, but they contaminate groundwater sources and can be transported with rain or other sources of water to lakes, rivers, and seas. In such cases, pesticides significantly affect soil and aquatic life on non-target organisms. For this reason, it is important to analyze the risks caused by these chemicals in terms of acute or chronic effects.

The fungicide we used in this research has shown the effects on mushroom enzyme cytochrome p450 to inhibit cell wall synthesis. We can see IMZ's usage in different places, such as in citrus after harvest or in veterinary applications for dermatophytes treatment (Altieri *et al*. 2013). That is one of the reasons why we use *D. melanogaster* to evaluate the potential effects, such as behavioral toxicity, Comet test for DNA damage, and SMART test for mutagenic activity, of IMZ to non-target organisms.

Comet test is a fast and reliable test for determining any DNA damage or repair (Gajski *et al.* 2021*)*. The findings of the Comet test showed a significant increase in DNA damage with an increase in the dosage of IMZ compared to the control group. Although there were changes compared to the control group, DNA damage was not found to be statistically significant in any application group. Schwarzbacherova *et al*. (2015) performed the Comet test on human lymphocytes with prosaro (tebuconazole/prothioconazole), a triazole fungicide, in their study and found that a dose of 30 µg/ml of this fungicide caused statistically significant DNA damage. Çıldır and Liman (2020) showed that 0.5, 1, and 2 µg/mL IMZ concentrations caused DNA damage in *Allium* roots, as demonstrated by the Comet test. Pesticide-induced DNA damage can be explained by the accumulation of various free radicals and reactive oxygen species over time (Ciğerci *et al*. 2023). However, although genotoxic damage was observed in the Comet test in some studies due to varying chemicals, application doses and duration of these chemicals, there was no statistically significant change in this study.

IMZ is a member of the azole group of fungicides, and azoles are the most commonly used antifungal agents in clinical medicine (Şeyhan *et al*. 1999, Álvarez *et al*. 2016). Most of the azoles are certified for being hepatotoxic. Posaconazole and ketoconazole are cytotoxic for HepG2 (Hepacellular carcinoma) cell line. When HepG2 cells were exposed for 24 hours to posaconazole and ketoconazole, both decreased the amount of mitochondrial DNA and induced apoptosis (Haegler *et al*. 2017). There is not enough research on IMZ-mediated oxidative stress and we need more data about IMZ's effects (Pereira *et al*. 2020). IMZ also induced oxidative stress on HepG2 and Caco-2 (Human colon carcinoma) cell lines (Silva *et al*. 2022). Moreover, IMZ has also shown developmental toxicity in *Danio rerio* embryos. So, this shows the potential environmental risks of IMZ to non-target organisms (Huang *et al*. 2022). IMZ was applied to lactating female mice and inhibited the genes that are responsible for sex hormone receptors, cholesterol receptors, and synthesis of T cells. (Jin *et al*. 2019). All these researches show similar results to this research that IMZ can be harmful to non-target organisms. The behavioral test is an important test that shows us the observable effects of drugs and chemical agents (Kurşun *et al*. 2021). Behavioral tests give us advantages for finding out the *in vivo* effects of various chemicals and drugs. These tests can be used for the genetic reasons for behaviors and how these genetic mechanisms affect the behaviors of various organisms (McKellar *et al*. 2017).

Bianchini *et al*. (2019) investigated the neurotoxic effects on *D. melanogaster* by feeding 1-2 days old flies with 25 µM thimerosal-supplemented medium.This study observed that thimerosal caused changes in dopamine levels, dopamine energy system and the activity of the rate-limiting enzyme, tyrosine hydroxylazine, on flies. These phenotypes further showed increasing oxidative stress, decreasing cell viability, and survival rate. The dopamine energetic system and speed limiter enzyme tyrosine and hydroxylazine's dysfunction made behavioral anomalies. So, thimerosal has neurotoxicity due to oxidative stress.

In the present study, significant results in locomotor function due to exposure to IMZ were observed. The movement of pupa was decreased with the increasing concentration of IMZ and this showed that there could be a correlation with DNA damage (Ciğerci *et al*. 2023). We found increasing IMZ concentration also makes larval movements slower than in the control group. In addition, if we compare the control group with all experimental groups decreasing the amount of distance covered in the unit time is statistically significant. Larval movements are controlled by a network of neurons. Larvae make peristaltic movements with short pauses while crawling This mechanism is controlled by motor neurons, sensory feedback, and the central pattern of the neural network (Fauzi *et al*. 2020 In the treatment groups containing IMZ at 0.25 mM and 4.5 mM concentrations, larval weight increased compared to the control, and 1 mM concentration decreased compared to the control. However, these changes were not found to be statistically significant. Previous studies have investigated whether imidazole derivatives can induce specific malformations similar to those observed after triazole derivative exposure. Accordingly, post-implantation rat embryos were exposed to determined concentrations (5, 10, 50, and 100 µM) of imidazole (1000 µM) and imidazole derivatives ketoconazole and enilconazole fungicides *in vitro*. Specific malformations are very similar to those observed after triazole exposure detected in culture after hours of exposure (Menegola *et al*. 2006). Farag and İbrahim (2006) revealed the developmental toxicity in rats due to flusilazol exposure. They found that in the medium and high concentrations, there can be changes in the body weight, maternal toxicity, developmental retardation, and there could be skeletal anomalies.

The SMART test is a common method that lets us see if a chemical has any genotoxicity or not. When studying potential drugs or chemical agents, understanding and calculating mitotic recombination as a result of recombinogenic effects is important. The SMART test is based on observing if trans heterozygous larvae's imaginal disk cells heterozygous feature will disappear (Yalçın 2017). When heterozygous in imaginal disk cells disappears, the recessive adverb gene is expressed because of that, the mutant colonies cells will be formed (Spano *et al*. 2001). Kurşun *et al*. (2022) evaluated four different concentrations (1, 2, 5, and 10 mM) of metiram, kresoxim-methyl, propamocarb, and hymexazole fungicides on *D. melanogaster* with the SMART test and reported that these fungicides did not cause genotoxicity. When the results of the study are compared with this study, it is seen that no genotoxic activity was found in terms of the data obtained. However, it should be considered that genotoxic activity can be induced when the ratios of the concentrations used in the studies are increased. In order to fully evaluate this situation, it is necessary to work with more concentration. In some cases, it may become undetectable due to minor DNA damage and its repair.

In conclusion, in this study, it was determined that IMZ induced DNA damage, as a result of the Comet test, caused changes in various behavioral tests, but did not

create a statistically significant change in SMART. The different results obtained in the Comet and SMART test systems are due to the different operating principles of the two test systems. . While the comet test is an indicator of genotoxic damage causing single or double strand breaks, the SMART test is a method that can measure mitotic recombination and it is possible that the results obtained from the two test systems differ from each other. With all this information, further studies on different organisms are required to determine the cytotoxic and genotoxic effects of IMZ.

Declaration of Ethical Standards
The authors declare that they comply with all ethical standards.

Credit Authorship Contribution Statement
Author 1: Investigation, Methodology, Experimental study, Writing
Author 2: Investigation, Methodology, Experimental study, Writing
Author 3: Investigation, Methodology, Experimental study, Writing
Author 4: Investigation, Methodology, Experimental study, Writing
Author 5: Investigation, Methodology, Statistical analysis, Study design
Author 6: Investigation, Methodology, Statistical analysis, Study design
Author 7: Investigation, Methodology, Statistical analysis, Study design

Declaration of Competing Interest
The authors have no conflicts of interest to declare regarding the content of this article.

Data Availability Statement
All data generated or analyzed during this study are included in this published paper.

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