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Investigation of Genotoxic and Development Effects of Tetramethrin on *Drosophila melanogaster*

Burçin YALÇIN[1], Merve GÜNEŞ[2], Ayşen Yağmur BURGAZLI[3], Ghada TAGORTİ[4], Ezgi GOLAL[5], Bülent KAYA[6]✍
[1, 2, 3, 4, 6] Department of Biology, Faculty of Sciences, Akdeniz University, Antalya, Türkiye, [5]Department of Histology and Embryology, Faculty of Medicine, Akdeniz University, Antalya, Türkiye
[1]https://orcid.org/0000-0002-9694-5839, [2]https://orcid.org/0000-0003-3278-0542, [3]https://orcid.org/0000-0003-1657-6808
[4]https://orcid.org/0000-0003-4597-8320, [5]https://orcid.org/0000-0002-7685-7479, [6]https://orcid.org/0000-0002-0491-9781
✉: bkaya@akdeniz.edu.tr

ABSTRACT
The adverse effects of tetramethrin residues, a synthetic pyrethroid used in many insecticide formulations, on environmental health and living organisms are a matter of concern. The aim of this study was to evaluate the genotoxic and developmental effects of tetramethrin in a non-target organism, *Drosophila melanogaster*. Thus, its effect on DNA damage was evaluated using the Comet assay in hemocytes, and its mutagenic and recombinogenic effects were evaluated using *Drosophila* wing SMART. Also, the effects of tetramethrin on *Drosophila* development were evaluated by measuring larval weight, larval length, and fecundity. Results showed that tetramethrin induced a decrease in the larval weight and length only at a high concentration. Moreover, a decrease in fecundity in a dose-dependent manner was observed. According to the Comet assay results, DNA damage was not induced because there was no significant increase in % DNA. However, tetramethrin caused genotoxicity by inducing mitotic recombination in the SMART assay.

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Tetramethrin'in *Drosophila melanogaster*' de Genotoksik ve Gelişim Üzerine Etkilerinin Araştırılması

ÖZET
Birçok insektisit formülasyonunda kullanılan sentetik bir piretroid olan tetramethrin kalıntılarının çevre sağlığı ve canlı organizmalar üzerindeki olumsuz etkileri endişe konusudur. Bu çalışmanın amacı, tetrametrin'in hedef dışı bir organizma olan *Drosophila melanogaster* üzerindeki genotoksik ve gelişimsel etkilerini değerlendirmektir. Bu nedenle *Drosophila* hemositlerinde komet testi kullanılarak DNA hasarı üzerindeki etkisi, *Drosophila* kanat SMART kullanılarak da mutasjenik ve rekombinojenik etkileri değerlendirilmiştir. Ayrıca tetramethrin'in *Drosophila* gelişimi üzerindeki etkileri larva ağırlığı, larva uzunluğu ve yumurta verimi ölçülerek değerlendirilmiştir. Sonuçlar, tetrametrin'in sadece yüksek konsantrasyonda larva ağırlığında ve uzunluğunda bir azalmaya neden olduğunu göstermiştir. Ayrıca yumurta veriminde konsantrasyona bağlı bir şekilde azalma gözlenmiştir. Komet testi sonuçlarına göre, % DNA'da anlamlı bir artış olmadığı için DNA hasarı indüklenmemiştir. Ancak tetrametrin, SMART testinde mitotik rekombinasyonu indükleyerek genotoksisiteye neden olmuştur.

Genetik

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INTRODUCTION

Pyrethroids are synthetic insecticides that have a structure close to pyrethrin whose extract is derived from *Chrysanthemum cinerariaefolium* (United States Environmental Protection Agency, 2019). Nowadays, pyrethroids are one of the most used insecticides to control pests in agriculture, urban and suburban areas (Horton et al., 2011; Saillenfait et al., 2015), with a global market size that will reach $2258 million in 2026 (QYResearch Group, 2021). Of note, Ukraine,

Pakistan, Turkey, Paraguay, and India are the top five countries in terms of pyrethroid use (Li et al., 2017; Kuzukiran et al., 2021). In toxicity studies with different pyrethroids, it has been reported that pyrethroids cause highly toxic effects on non-target organisms. For example, it is highly toxic to many organisms including aquatic invertebrates (Arias et al., 2020; Hartz et al., 2021; Arslan, 2022), nematodes (Shashikumar & Rajini, 2010; Shen et al., 2017; Yuan et al., 2019), insects (Singh et al., 2011; Yan et al., 2011; Ballesteros et al., 2020), fish (Bej et al., 2021; Greno et al., 2021; Beken et al., 2022), plants (Çavuşoğlu et al., 2012), rat (Zhong et al., 2021; Ileriturk et al., 2022; Ileriturk & Kandemir, 2023; Lesseur et al., 2023) and human cells (Barrios-Arpi et al., 2022; Elser et al., 2022).

Tetramethrin, a synthetic pyrethroid with a broad spectrum of action is used in indoor and outdoor environments (Chedik et al., 2017; Mendis et al., 2018; United States Environmental Protection Agency, 2019; 2020). It is effective against flying and crawling insects as a fast knockdown agent that ensures the partial paralysis of insects within a few minutes (United States Environmental Protection Agency, 2020). Although this insecticide is used for pest control, no specific species are targeted, thereby detrimental effects can be observed on non-target insects (Zaller & Brühl, 2019). In a previous study, the honeybee (*Apis mellifera*) and bumblebee (*Bombus terrestris*), which are important pollinators, tetramethrin exposure has been shown to cause modifications in voltage-gated sodium channels in olfactory receptor neurons (Kadala et al., 2011, 2014, 2019). In another study, tetramethrin (70 ng/bee) at non-lethal doses (non-lethal in 48 hours) caused a deficiency in locomotor ability (cleaning brood cells, feeding larvae, etc.) in honey bees and it has been suggested that this deficiency is due to their mode of action on ion channels (Charreton et al., 2015). Chen et al. (2018) studies showed that the knockout of the *Drosophila* Sodium Channel 1 (DSC1) gene in *Drosophila* adults and larvae, causes *Drosophila* to be more sensitive to pyrethroids and that DSC1 channels have important roles in regulating the action of pyrethroids. However, the number of studies investigating the toxic effects of tetramethrin on non-target insects is limited.

It has been shown in different studies that cypermethrin, one of the pyrethroid varieties, causes a significant increase in DNA damage in the brain ganglia and anterior midgut of *Drosophila*, causes Hsp70 expression and tissue damage, and causes larval death at high doses (Mukhopadhyay et al., 2004; 2006). In addition, studies have shown that cypermethrin can cause reproductive toxicity in *Drosophila* in both males and females (Batiste Alentorn et al., 1986; Karataş & Bahçeci 2009). It has been shown that exposure to permethrin and deltamethrin, which are types of pyrethroids, can cause a neurotoxic effect in *Drosophila* (Yan et al., 2011; Abdulbaki & Al-Deeb 2023). Moreover, Cruces et al. (2023) showed that bifenthrin can cause genotoxic effects in *Drosophila*.

Generally, humans are exposed to insecticides through the ingestion of food contaminated with insecticide residues, inhalation of contaminated house dust, and dermal contact with particles adhered to surfaces after domestic use (Corcellas et al., 2017; Simaremare et al., 2021). Simaremare et al. (2021) determined that tetramethrin was the most common pesticide residue among six pesticides with a rate of 78.7% in indoor dust samples compared to 34% in outdoor dust samples. In addition, tetramethrin residues have been detected in the body fluids of some living organisms such as pig milk, human blood, and urine (Zhang et al., 2016; Nozawa et al., 2021; Göl et al., 2023). Thus, these findings highlight the persistence of tetramethrin in the environment, subsequently, humans are also exposed to this insecticide. Therefore, a crucial need to assess the effects of tetramethrin in non-target organisms is recommended.

Drosophila melanogaster is a promising model organism for detecting the non-lethal effects of pesticides (Singh et al., 2011; Yan et al., 2011; Tasman et al., 2021). It is also used to investigate the effects of pesticides on larval development and reproduction (Kissoum et al., 2020). In addition, *Drosophila* is an ideal model organism used in genotoxicity studies, as it has homology with almost 75% of human disease-causing genes (Reiter et al., 2001; Pandey & Nichols, 2011).

Therefore, the present study aimed to assess the effects of tetramethrin on larval development, reproduction, and its genotoxic effects on *D. melanogaster* as a model organism. The mutagenic and recombinogenic effects of tetramethrin in trans-heterozygous individuals (normal winged) and balancer-heterozygous individuals (serrate winged) were assessed using the *Drosophila* wing somatic mutation and recombination test (SMART). Moreover, the potential of tetramethrin to cause DNA single-strand breaks in *Drosophila* hemocytes was evaluated using the single-cell gel electrophoresis assay (Comet assay). In addition, the effects of tetramethrin on the development of *Drosophila* larvae were assessed by measuring the larval weight, larval length, and fertility.

MATERIALS and METHODS

Chemicals

Tetramethrin (Sigma-Aldrich, 97%, CAS No: 7696-12-0) solutions were prepared by dissolving in 3% acetone (Tekkim, 99,5%, CAS No:67-64-1). The rest of the chemicals were obtained from Sigma-Aldrich. Acetone

solvent (3%) and distilled water were used as the negative control. Five different concentrations (0.1, 0.5, 2, 5, and 10 mM) were selected to determine the highest non-lethal tetramethrin concentration. The concentration of tetramethrin that ensures at least 50% eclosion of eggs was determined as 1.33 mM by Probit analysis (IBM SPSS Statistics 20 package program) (Charpentier et al., 2014; Jameel et al., 2019; Anushree et al., 2023). Three different concentrations of tetramethrin (0.1, 0.5, and 2 mM) were used in the assays. The selected concentrations were used based on a survival rate.

Drosophila Strain and Treatment

Drosophila strains were maintained in a standard *Drosophila* medium containing corn flour, sugar, yeast, agar, propionic acid, and ortho-phosphoric acid at 25 ± 1°C under 60% humidity. In all the assays, eggs were collected during 8 h periods from the *Drosophila* strain suitable for the assay and set up in glass vials (5 cm x 14 cm; diameter x height) containing standard *Drosophila* medium. After, the third instar larvae (72 ± 4 h old) were dispensed into plastic vials (3.5 cm x 8 cm; diameter x height) with approximately 4.5 g of instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC, USA), which was rehydrated with 9 mL of three different concentrations of tetramethrin (0.1, 0.5, and 2 mM).

SMART

Drosophila mwh (multiple wing hair) and *flr*^{3} (flare-3) strains were used in the SMART assay (Lindsley & Zimm, 1992). Crosses between males (*mwh/mwh*) and virgin females (*flr*^{3}*/In (3LR) TM3, Bd*^{S}) individuals were performed to obtain trans-heterozygous individuals (*mwh/flr*^{3}, normal winged) and balancer-heterozygous individuals (*mwh/TM3, Bd*^{S}, serrate winged). SMART is an *in vivo* assay that enables the detection of various mutations (deletion, point mutation, and non-disjunction) and recombination in the phenotype (Würgler & Vogel, 1986). Although wings with the normal phenotype (*mwh/flr*^{3}) contain mutant spots resulting from both mutation and recombination, wings with the non-uniform phenotype known as serrate winged (*mwh/TM3, Bd*^{S}) contain only mutation-induced spots resulting from the suppressed recombination of the balancer chromosome (Zordan et al., 1994; Kaya et al., 1999; 2006). The assay was performed as described by Kaya et al. (2004) and Demir et al. (2008). Trans-heterozygous individuals that were treated with three different concentrations of tetramethrin: 0.1, 0.5, and 2 mM (with three repetitions) were scored. Following this, balancer-heterozygous individuals treated with a 2 mM concentration of tetramethrin (Only this concentration at which genotoxicity was induced in trans-heterozygous individuals) were examined in order to determine whether the genotoxicity that occurred after treatment with these concentrations was caused by a mutation or recombination. 1 mM of ethyl methanesulfonate (EMS, Sigma Aldrich, CAS No: 62-50-0) was used as the positive control (Demir et al., 2008; Karadeniz et al., 2011). Wings from trans-heterozygous and balanced heterozygous individuals were separately mounted on slides with Faure solution (30 g gum arabic, 50 g chloral hydrate, 20 mL glycerol, 50 mL distilled water). The wings were scored for the presence of small single spots, large single spots, twin spots, total *mwh* spots, and total spots (Graf et al., 1984). For each concentration, 80 wings (40 individuals) were examined. SMART results were analyzed using the multiple-decision procedure of Frei and Würgler (1988). The conditional binomial test was used according to Kastenbaum and Bowman's (1970) charts ($p < 0.05$) for statistical calculations. To characterize the mutagenic and recombinogenic effects, the frequency of mutant spots was calculated by dividing the number of spots by the total wing number (80 wings, the number of cells inspected in a wing is 24400) for each counted wing (Szabad et al., 1983).

Comet Assay

Drosophila Oregon R+ strain was used in the Comet assay. The third instar larvae (72 ± 4 h-old) were separately treated with three different concentrations of tetramethrin (0.1, 0.5, and 2 mM) with three repetitions. EMS (4mM) was used as the positive control (Sierra et al., 2014). After 24 h, larvae (96 ± 4 h-old) were removed from the treated medium, sterilized with 5 % sodium hypochlorite, and dried with filter paper. Following this, hemocytes were isolated according to the methods of Irving et al. (2005). The cuticle of each larva was disrupted using two fine forceps in cold PBS (120 µl) containing 0.07% phenylthiourea (PTU) in a cavity in the center of the single concave slide. For every 10 larvae, the hemocytes collected in the pit were collected in a 1.5 ml centrifuge tube. Hemocytes were isolated from 60 larvae for each treatment. The tubes were then centrifuged at 1300 rpm for 10 minutes at +4 °C. Finally, the supernatant was removed and the cell pellet was resuspended in 20 µL of cold PBS containing 0.07% PTU (Sierra et al., 2014). The hemocytes were then mixed with 80 µL of low melting agarose (LMA, 0.75%) and were spread over lamellae already coated with normal melting agarose (NMA, 1%). Later, the slides were kept on a cold plate for 10 min and the coverslips were removed. Again, 80 µL of LMA was added and the slides were kept on a cold plate for 10 min. Lysis was performed for 1 h at 4°C in a dark chamber. Then, the slides were kept in a cold electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH > 13) for 30 min. The cells were run in the

same buffer for the next 30 min (25 V and 300 mA). After electrophoresis, the slides were washed with 400 mM Tris buffer (pH= 7.5). This process was repeated two more times for neutralization. The slides were then stained with 50 µL ethidium bromide (60 µg / mL) for 20 min (Sierra et al., 2014). Following this, the slides were examined at 40X magnification on a 480–550 nm wideband excitation filtered and a 590 nm barrier filtered fluorescence microscope (Nikon Eclipse E200) with a charge-coupled device (CCD) camera attachment using (Comet assay IV version 4.11, Kinetic Imaging, UK). Fifty randomly selected comets were analyzed per treatment (Sierra et al., 2014). DNA damage was evaluated according to the tail length (µm), tail intensity (% DNA), and tail moment (the combination of the first two parameters: tail length × % DNA in the tail). Statistical analysis of Comet assay results was performed using one-way ANOVA (Tukey) in the IBM SPSS Statistics 20 package program (SPSS, NY, US) ($p < 0.05$).

Larval Weight, Length, and Fertility Measurement

Drosophila Oregon R+ strain was used to measure larval weight, larval length, and fertility. Third instar larvae (72 ± 4 h-old) were separately treated with three different concentrations of tetramethrin (0.1, 0.5, and 2 mM) and the negative control groups (acetone 3% and distilled water). After 24 h, larvae (96 ± 4 h-old) of each treatment group were separately collected under tap water with a sieve. The weight of larvae was calculated by weighing 30 larvae on a precision scale; the average weight was considered (three repetitions). Lengths of larvae were measured with a cold steel ruler placed on an ice plate to enable a more accurate measurement by restricting larval movement (Parimi et al., 2019). Therefore, 30 larvae from each treatment group were measured one by one with the ruler under a stereomicroscope; the average length was considered (three repetitions). Statistical analysis of larval weight and length measurements was performed using one-way ANOVA (Tukey) in the IBM SPSS Statistics 20 package program (SPSS, NY, US) ($p < 0.05$). To measure fertility, individuals exposed to tetramethrin at the larval stage (72 ± 4 h-old) were allowed to reach the adult stage (approximately 7 days after exposure). After waiting 24 hours for the mating of adult male and female flies, 2 female individuals were selected from each exposed concentration (10 female individuals in total, five repetitions) and transferred to an instant *Drosophila* medium without tetramethrin. Females in the instant *Drosophila* medium without tetramethrin were removed at the end of 48 hours and the eggs' eclosion success was recorded (the average of five repetitions was accepted). Statistical analysis of fecundity was performed with the Mann-Whitney U test after testing its significance with Kruskal-Wallis ($p < 0.05$).

RESULTS and DISCUSSION

SMART results showed that compared to the negative control group (acetone 3%), trans-heterozygous wings (*mwh/flr*^{3}) that were exposed to different concentrations of tetramethrin (0.1, 0.5, and 2 mM) showed a concentration-dependent increase in the small single spot, total *mwh* spot, and total spot frequencies. However, inconclusive results were obtained for all parameters at 0.1 and 0.5 mM concentrations. On the other hand, positive results were reported for the total mwh spot and the total spot at 2 mM concentration (Table 1). The increase obtained for a small single spot at 2 mM indicated that tetramethrin reached its cellular targets (wing imaginary discs) mostly at late developmental stages. Later, balancer-heterozygous individuals treated with a 2 mM concentration of tetramethrin were examined in order to determine whether the positive result observed after treatment with these concentrations in trans-heterozygous wings was due to a mutation or recombination. The results obtained in balancer-heterozygous wings were inconclusive for all categories at 2 mM concentration (Table 1). While single *mwh* spots are formed by a point mutation, nondisjunction, or deletion, twin spots are only formed by recombination. The difference between the total spot frequencies in the trans-heterozygous wings and balancer-heterozygous wings indicates the recombination rate (Frei et al., 1992). The recombination percentages were 15.79% for distilled water, 36.84% for acetone 3%, 44.44% for EMS, and 57.14% for 2 mM tetramethrin (Data not shown in the table). This suggests that tetramethrin mainly induces mitotic recombination at high concentrations.

The Comet assay results showed that compared to the negative control group (acetone 3%), tetramethrin significantly increased the tail length at 0.5 and 2 mM concentrations (Table 2). Moreover, compared to the acetone 3%, tetramethrin increased the tail intensity and tail moment at 2 mM concentration. Of note, only the increase in the tail moment was statistically significant. However, tail intensity (% DNA) is accepted as a better parameter than tail length and moment to evaluate genotoxicity using the Comet assay (Dhawan et al., 2009; Sanchez-Alarcon et al., 2016). Since some xenobiotics cause small breaks, the tail length of the comet may be excessive. Accordingly, since the tail moment is "tail length × % DNA in the tail", the tail moment may appear high due to the high tail length. However, the percentage of DNA in the damaged part of the comet is not high (Kumaravel & Jha, 2006; Sanchez-Alarcon et al., 2016). The results of the Comet assay revealed that the concentrations of tetramethrin used in this study did not cause significant DNA strand breaks in *Drosophila* hemocytes. Genotoxicity was induced at a high concentration in the SMART assay, but not in the

Table 1. SMART results of 72±4 h *Drosophila* larvae after exposure to tetramethrin
Çizelge 1. Tetrametrin maruziyetinden sonra 72 ± 4 saatlik Drosophila larvalarının SMART sonuçları

	Number of wings (N)	Small single spot (1-2 cells; m=2)			Large single spot (> 2 cells; m=5)			Twin spot (m=5)			Total *mwh* spot (m=2)			Total spot (m=2)			Frequency of clone formation (10^5 cells)
		No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.	
Normal Wing (Trans-heterozygous wings – *mwh/flr³*)																	
Distilled water	80	13	(0.16)		2	(0.02)		0	(0.00)		15	(0.19)		15	(0.19)		0.77
Acetone 3%	80	13	(0.16)	i	1	(0.01)	i	1	(0.01)	i	15	(0.19)	-	15	(0.19)	-	0.77
EMS (1 mM)	80	67	(0.84)	+	24	(0.30)	+	9	(0.11)	+	86	(1.08)	+	100	(1.25)	+	4.40
0.1 mM	80	14	(0.18)	i	2	(0.02)	i	1	(0.01)	i	17	(0.21)	i	17	(0.21)	i	0.87
0.5 mM	80	15	(0.19)	i	6	(0.08)	i	0	(0.00)	i	21	(0.26)	i	21	(0.26)	i	1.08
2 mM	80	23	(0.29)	i	3	(0.04)	i	2	(0.02)	i	28	(0.35)	+	28	(0.35)	+	1.43
Serrate Wing (Balancer heterozygous wings – *mwh/TM3*)																	
Distilled water	80	12	(0.15)		1	(0.01)					13	(0.16)		13	(0.16)		0.67
Acetone 3%	76	8	(0.10)	-	1	(0.01)	i				9	(0.12)	-	9	(0.12)	-	0.48
EMS (1mM)	44	24	(0.54)	+	2	(0.04)	i				26	(0.60)	+	26	(0.60)	+	2.42
2 mM	80	11	(0.14)	i	1	(0.01)	i				12	(0.15)	i	12	(0.15)	i	0.62

No: number, Fr: frequency, D: showing the results of the Statistics, +: positive (genotoxic), -: negative (not genotoxic), i: inconclusive, m: multiplication factor, probability level = 0.05, Acetone 3% and distilled water are negative control, EMS (Ethyl methanesulfonate, 1mM) is a positive control.

Table 2. The genotoxic effects of tetramethrin in *Drosophila* hemocytes using the Comet assay
Çizelge 2. Comet testi kullanılarak tetrametrinin Drosophila hemositlerindeki genotoksik etkileri

	Tail intensity (%) [a]	Tail Moment [a]	Tail length (µm) [a]
Distilled water	24.83 ± 2,34	6.43 ± 0.75	66.24 ± 3.43
EMS 4 mM	44.22 ± 2.73***	14.87 ± 1.08***	95.92 ± 2.83***
Acetone 3%	27.41 ± 3.80	3.04 ± 0.44	58.60 ± 3.60
Tetramethrin			
0.1 mM	15.56 ± 2.36	2.63 ± 0,47	59.74± 2.18
0.5 mM	20.57 ± 3.28	3,46 ± 0,68	85.06 ± 4.50***
2 mM	29.20 ± 3.15	8.08 ± 1.31**	102.18 ± 4.28***

[a]Mean ± standard error; 50 comets were counted for each experiment. Acetone 3% and distilled water are negative control, EMS (Ethyl methanesulfonate, 4mM) is a positive control.
* $0.01 < p < 0.05$, ** $0.001 < p < 0.01$, *** $p < 0.001$

Comet assay because these test systems evaluate different genotoxic mechanisms. This shows the importance of investigating the genotoxicity of tetramethrin with different test systems.

Morphological differences in development are important parameters to evaluate the effect of pesticide exposure at levels below lethal concentrations on the organism. Weight and length are the most used parameters to study *Drosophila* larval development (Ormerod et al., 2017). The larval weights after exposure to 0.1, 0.5, and 2 mM concentrations of tetramethrin are provided in Figure 1A. When the average weights of the treatment groups were compared, the weights after exposure to 2 mM concentration (0.0280 g) were found to be significantly lower than those in the control groups (acetone 3%, 0.0351 g, and distilled water, 0.0390 g). The larval lengths are provided in Figure 1B. The larval length was 3.117 mm and 3.367 mm in the control groups (acetone 3% and distilled water, respectively); it was not statistically significant although it decreased to 3.058 mm after treatment with 2 mM tetramethrin. In addition, the increase in larval length and weight at a low concentration (0.5 mM) suggests that it may be an example of hormesis (adaptive response). However, hormetic responses are also generally characterized by increases in reproductive performance (Berry-III & Lopez-Martinez, 2020). On the other hand, herein, no hormesis pattern has been reported in the fertility results. Comparing the fertility results of the treatment groups, it was observed that fertility was decreased in a dose-dependent manner at 0.1, 0.5, and 2 mM concentrations (n=54, n=19, and n=15, respectively, number of individuals eclosion from the egg) compared to the negative control groups (acetone 3% n=80, distilled water n=85) (Figure 1C).

There are limited studies on the genotoxic effects of tetramethrin on non-target organisms (Kim et al., 2005; United States Environmental Protection Agency, 2010; Klopic et al., 2015). Most studies on tetramethrin toxicity have been conducted with aquatic animals (United States Environmental Protection Agency, 2016; Greno et al., 2021). For example, *Lepomis macrochirus* (LC_{50}, 96h 16 µg/L), *Oncorhynchus mykiss* (LC_{50}, 96h 3.7 µg/L) and *Danio rerio* (LC_{50}, 96h 33 µg/L) (Greno et al., 2021). Previous studies on the toxicity of tetramethrin have reported different findings depending on the model organisms and assays used. Dikmen et al. (2018) found that lactate dehydrogenase leakage, which is an indicator of cell damage during apoptosis and necrosis, increased after exposure to 10 and 20 ng / mL of tetramethrin in the RTG2 rainbow trout cell line. Yavuz et al. (2010) investigated the dermal toxicity of different combinations of some pyrethroids (cypermethrin, alphacypermethrin, deltamethrin) and piperonyl butoxide (PBO) and tetramethrin in Wistar rats. The insecticide combinations caused low acute dermal toxicity by causing decreased body weight and feed consumption, increased organ weights, hematological, biochemical and histopathological changes. In another study conducted with the same treatment groups, biochemical changes related to liver, kidney functions and protein metabolism occurred in male Wistar rats (Yavuz et al., 2013). Kim et al. (2005) stated that tetramethrin has an endocrine-disrupting effect on Sprague–Dawley rats. The estrogenic and androgenic activities of tetramethrin revealed that subcutaneous exposure to tetramethrin (5–800 mg / kg/day) resulted in a statistically significant decrease in the absolute and relative uterine wet weights of Sprague–Dawley rats. However, they found that tetramethrin did not exhibit androgenic and antiandrogenic activities. Hence, they suggested that tetramethrin exerts endocrine-disrupting effects on female rats through antiestrogenic activity. Similarly, Klopic et al. (2015) observed that tetramethrin has an endocrine-disrupting effect on human mammalian cells (MDA-kb2) and causes cytotoxicity. In addition, tetramethrin has been classified as a category 2 carcinogen by the Publication Office of the European

Commission (Official Journal of the European Union, 2018). Chedik et al. (2017) found that tetramethrin inhibited various ABC and SLC drug transporters,

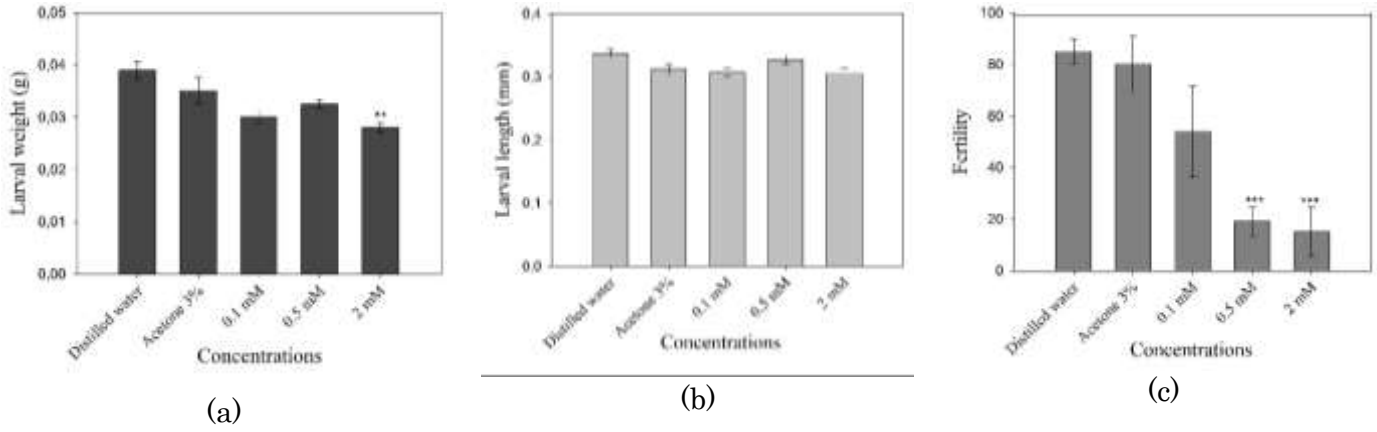

Figure 1. Larval development of *Drosophila* larvae after exposure to tetramethrin (a) Larval weights (g), (b) Larval lengths (mm), and (c) Fertility.

Şekil 1. Tetrametrin maruziyetinden sonra Drosophila larvalarının larva gelişimi (a) Larval ağırlık (g), (b) Larval uzunluk (mm) ve (c) Fertilite.

including multidrug resistance-associated protein (MRP) 2, breast cancer resistance protein (BCRP), organic anion transporter polypeptide (OATP) 1B1, organic anion transporter (OAT) 3, multidrug and toxin extrusion transporter (MATE) 1, organic cation transporter (OCT) 1, and OCT2, at very high concentrations. Mitotic recombination leads to increased loss of heterozygosity (LOH), resulting in genetic instability. Spontaneous mitotic recombination in the *Drosophila* adult midgut has been shown to accumulate in senescent adult intestinal stem cells and frequently gives rise to LOH (Siudeja & Bardin, 2017). LOH is one of the mechanisms that cause the complete loss of function of tumor suppressor genes (Luo et al., 2000). Mitotic recombination is involved in carcinogenesis by causing the upregulation of protooncogenes as well as the loss of tumor suppressor genes (Wang et al., 2007). It has been widely used to test the genotoxicity of different compounds in the SMART developed to measure recombination events that occur during wing formation in *Drosophila* (Siudeja & Bardin, 2017). In this context, considering that mitotic recombination is a mechanism involved in carcinogenesis (Abrahams et al., 2003; Orsolin et al., 2012), our SMART result suggests that tetramethrin may cause carcinogenic effects by inducing recombination at its highest concentration. Apart from tetramethrin, there are also studies investigating the effects of other pyrethroids on non-target Diptera members and *Drosophila*. Abeyasuriya et al. (2017) investigated the effect of the insecticide Pesguard (d-tetramethrin + cyphenothrin), which is commonly used for adult *Aedes mosquito* control, on non-target insects for controlling the incidence of dengue fever in Sri Lanka. That study revealed that the most affected populations were Diptera (36%), Collembola (30%), and Thysanoptera (17%), in the given order. In addition, measurements of the knockdown activity against the mosquito *Aedes albopictus* (n = 417) and the bee *Trigona iridipennis* (n = 122) revealed 83.5% mosquito and 93.5% bee deaths. Thus, Pesguard showed a significant effect on non-target insects. Moreover, Mendis et al. (2018) recorded the coagulation of zebrafish embryos and the lack of both somite formation and heartbeat after exposure to Pesguard. Bifenthrin exposure to *Chironomus tepperi* (Diptera) larvae used for biomarker responses significantly affected the activity of glutathione peroxidase, an oxidative stress enzyme (Ballesteros et al., 2020). Mukhopadhyay et al. (2004) evaluated the *in vivo* genotoxicity of cypermethrin (0.0004, 0.0008, 0.002, 0.2 and 0.5 ppm) in the brain ganglia and anterior midgut of *Drosophila* by Comet method and showed a significant dose-dependent increase in DNA damage. In another study, cypermethrin was found to have adverse effects on reproduction in *Drosophila*, evidenced by Hsp70 expression and tissue damage and in addition,larval death was observed in high dose groups (0.2, 0.5 and 50.0 ppm). They also observed that the negative effects were relatively more pronounced in male flies than in females (Mukhopadhyay et al., 2006). Karataş & Bahçeci (2009) found that cypermethrin did not cause a significant difference in oviposition rates of adult females in *Drosophila*, but the decrease in egg development rate, eggs and early embryonic stages were sensitive to toxic effects. In another study, cypermethrin was tested for the induction of genetic damage to male germ cells in *Drosophila*. No significant increase in the frequency of sex chromosome loss or non-separation was observed

after exposure of male flies to cypermethrin at concentrations up to 20 ppm (Batiste-Alentorn et al., 1986). Yan et al. (2011) found that in *Drosophila* brain, exposure to permethrin at a concentration of 2.5 uM significantly reduced calcium current and cholinergic mini-synaptic current.

CONCLUSION

To our knowledge, this is the first study to assess the genotoxicity of tetramethrin using SMART and the Comet assay and to evaluate the effects of tetramethrin on larval development in *D. melanogaster*. Our results showed that tetramethrin, which is widely used in pesticide formulations, can cause genotoxicity in *Drosophila* (a non-target organism) at high concentrations. Concentrations detected in humans after exposure to environmental tetramethrin are in the nM range (Nozawa et al., 2021). Therefore, the studied tetramethrin concentrations are relatively higher than the amount of tetramethrin that humans will be exposed to from the environment. However, considering the excessive use during spraying that may lead to high exposure, careful preparation of tetramethrin concentrations used for applications is required. Moreover, it should be kept in mind that exposure is not solely related to one pyrethroid or xenobiotic since a mixture of compounds is found in the environment. Consequently, different results can be observed due to tetramethrin interaction with other xenobiotics. In addition, genotoxicity may vary depending on the organism and the exposure route. Therefore, further studies are recommended to understand the genotoxicity of tetramethrin.

Author Contribution Statement

The authors declare the contribution of the authors is equal.

Conflicts of Interest

The authors declare no conflict of interest.

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