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Investigation of methoxyfenozide insecticide genotoxicity with Allium and SMART methods

Fahrettin A. Sırlıbaş[1*] and Zübeyde Kumbıçak[2]

[1]*Nevsehir Haci Bektas Veli University, Institute of Science, Department of Biology, 2000 Evler Mah. Zübeyde Hanım Cad. 50300 Merkez Nevşehir Türkiye.*
[2]*Nevsehir Haci Bektas Veli University, Faculty of Art and Science, Department of Molecular Biology and Genetics, 2000 Evler Mah. Zübeyde Hanım Cad. 50300 Merkez Nevşehir Türkiye.*

***Correspondence** should be addressed to Fahrettin A. Sırlıbaş: f.anilsirlibas@gmail.com

Abstract

Aim of study: To assess the genotoxicity of methoxyfenozide by the Allium test and Somatic Mutation and Recombination Test (SMART).

Area of study: The Institute of Science Laboratories at Nevsehir Haci Bektas Veli University.

Material and Methods: In the Allium test, onion root tips were exposed to methoxyfenozide concentrations of 0.005 mL/L, 0.01 mL/L, and 0.02 mL/L for 24 hours. Post exposure, root tips preparations were analyzed for Mitotic Index (MI) and Chromosome Aberrations (CA). In the SMART, 72 ± 4-hour old transheterozygous larvae, derived from the standard cross of multiple wing hairs (*mwh*) males and flare (*flr*^{3}) virgin females, were exposed to methoxyfenozide at concentrations of 0.1 mL/L, 0.2 mL/L, and 0.4 mL/L in an instant medium. Preparations were made from the wings of emerging adult *Drosophila* to evaluate clone induction frequencies and wing spots.

Main results: The Allium test indicated a concentration-dependent decrease in MI values 5.88 ± 0.40, 4.71 ± 0.44, and 3.97 ± 0.47 at 0.005 mL/L, 0.01 mL/L, and 0.02 mL/L, respectively, compared to the negative control (7.28 ± 0.32). Methoxyfenozide induced common abnormalities such as irregularity, bridges, vagrant, stickiness, and disorientation. The SMART method showed higher clone induction frequencies in both *mwh/flr*^{3} and *mwh/TM3* flies compared to the negative control. Single and twin clones were observed on the wings of transheterozygous flies.

Research highlights: The findings indicate that methoxyfenozide may be genotoxic to both plant and animal systems. However, further research is needed to fully understand the environmental impacts and potential risks to living organisms linked with the agricultural use of methoxyfenozide.

Keywords: allium test; Drosophila; insecticide; root tips; SMART.

Investigación de la genotoxicidad del insecticida metoxifenocida con los métodos Allium y SMART

Resumen

Objetivo del estudio: Evaluar la genotoxicidad del insecticida metoxifenocida mediante el ensayo Allium y la Prueba de Mutación y Recombinación Somática (SMART).

Área de estudio: Laboratorios del Instituto de Ciencias de la Universidad Nevsehir Haci Bektas Veli.

Material y métodos: En el ensayo Allium, las puntas de raíces de cebolla fueron expuestas a concentraciones de metoxifenocida de 0.005 mL/L, 0.01 mL/L y 0.02 mL/L durante 24 horas. Tras la exposición, las preparaciones de raíces se analizaron para determinar el Índice Mitótico (IM) y las Aberraciones Cromosómicas (AC). En la prueba SMART, larvas transheterocigotas de 72 ± 4 horas, obtenidas del cruce estándar entre machos *multiple wing hairs* (*mwh*) y hembras vírgenes *flare* (fl^{r3}), fueron expuestas a metoxifenocida en concentraciones de 0.1 mL/L, 0.2 mL/L y 0.4 mL/L en un medio instantáneo. Se realizaron preparaciones de las alas de *Drosophila* adultas emergentes para evaluar la frecuencia de inducción de clones y la aparición de manchas en las alas.

Principales resultados: El ensayo Allium indicó una disminución dependiente de la concentración en los valores de IM: 5.88 ± 0.40, 4.71 ± 0.44 y 3.97 ± 0.47 para 0.005 mL/L, 0.01 mL/L y 0.02 mL/L, respectivamente, en comparación con el control negativo (7.28 ± 0.32). El insecticida metoxifenocida indujo anomalías comunes como irregularidades, puentes cromosómicos, “cromosomas errantes”,

adherencias y desorientación. La prueba SMART mostró mayores frecuencias de inducción de clones en moscas *mwh/flr*^{3} y *mwh/TM3* en comparación con el control negativo. Se observaron clones simples y gemelos en las alas de las moscas transheterocigotas.

Aspectos destacados de la Investigación: Los hallazgos sugieren que el insecticida metoxyienocida puede ser genotóxico para sistemas vegetales y animales. No obstante, se necesita más investigación para comprender completamente los impactos ambientales y los posibles riesgos asociados con el uso agrícola del insecticida metoxifenocida.

Palabras clave: ensayo allium; Drosophila; insecticida; puntas de las raíces; SMART

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Introduction

Pesticides are chemicals used in agriculture to control pests that reduce crop productivity and quality. On a global scale, between 26% and 40% of potential crop yield is lost annually due to weeds, pests, and diseases. Without crop protection measures, these losses could easily double (FAO, 2017). Approximately one-third of agricultural products are produced through pesticide application. In the absence of pesticides, 78% of fruit production, 54% of vegetable production, and 32% of cereal production would be lost (Tudi et al., 2021). Although the use of pesticides has positive results, they can also have negative impacts on both ecosystems and humans. It is widely recognized that the components within these chemical agents may have direct or indirect adverse effects on DNA and chromosomes (Garcia et al., 2012). The findings of recent *in vitro* studies utilizing blood and urine samples from rice and garlic farmers (Varona-Uribe et al., 2016; Sapbamrer et al., 2019), human peripheral blood cells (Kwiatkowska et al., 2017), and human lymphocytes (Togay et al., 2022) have confirmed that pesticides induce DNA damage. Therefore, it is essential to assess the genotoxic potential of pesticides.

The Allium test is a widely used method for the evaluation of genotoxicity in chemical, physical, and biological agents (Bonciu et al., 2018), and preferred due to its rapid response to genotoxic substances in its highly sensitive roots and clear observation of chromosomal aberrations (Firbas & Amon, 2014). This test evaluates the genotoxicity of chemicals on chromosomes and examines genomic mutation (Bonciu et al., 2018). Furthermore, there is a strong correlation between macroscopic and microscopic effects in the Allium test, indicating high compatibility with mammalian assay systems (Grant & Salamone, 1994; Akyil & Özkara, 2015). The somatic mutation and recombination test (SMART), also known as the wing spot test, evaluates genotoxicity rapidly and economically using *Drosophila melanogaster* somatic cells. This test is based on the loss of heterozygosity (LOH) resulting from genetic damage in the imaginal disc cells responsible for wing formation during the larval period, which is clearly seen as mutant wing spots on adult wings (Graf et al., 1984). The rapid proliferation of imaginal disc cells increases probability of genotoxins interacting with the genome, making SMART a sensitive genotoxicity test (Pitchakarn et al., 2021). This method can identify various type of DNA damage including point mutations, DNA breaks, and mitotic recombination, and it can also detect the activity of promutagens by utilizing strains with a high capacity for converting specific carcinogenic substances into their active metabolites (Graf & van Schaik, 1992; Marcos & Carmona, 2019).

Methoxyfenozide (N-tert-butyl-N'-(3-methoxy-o-toluoyl)-3,5-xylohydrazide) belongs to a new class of insecticides known as bisacylhydrazines and functions as a stomach poison with contact toxicity and ovicidal properties, effectively controlling a range of pests, including thrips, striped armyworms, cotton bollworms, cabbage worms, beet armyworms, and leaf roller moths (Liu et al., 2024). In terms of toxicity, methoxyfenozide exhibits low acute toxicity when administered orally, dermally, or by inhalation to rats. It does not cause skin or eye irritation, nor does it induce skin sensitization (EFSA, 2017). Nevertheless, research has indicated that this insecticide may prove toxic to bees and aquatic organisms, thereby potentially endangering public health (Fisher et al., 2018; Chen et al., 2021). Reverse mutation tests in several strains of *Salmonella typhimurium* gave negative results, and no evidence of chromosomal damage or gene alteration at the *hprt* locus was found in Chinese hamster ovary cells. Negative results were obtained from *in vivo* micronucleus tests performed on mouse bone marrow, consistent with the findings obtained from *in vitro* testing (APVMA, 2002). Given these limitations, it is difficult to draw definitive conclusions about the genotoxicity of methoxyfenozide. Further research utilizing a variety of test systems and organisms is necessary to fully understand the potential genotoxic effects of this insecticide and to determine its safety. Therefore, we examined the genotoxicity potential of methoxyfenozide in both plant and animal systems using the Allium and SMART methods.

Material and methods

Chemicals

Methoxyfenozide (CAS No. 161050-58-4) was used for the assessment of genotoxicity. The chemical structure of methoxyfenozide is illustrated Figure 1. istilled water was used as the negative control, while methyl methanesulfonate (MMS) (CAS No. 66-27-3) was selected as the positive control.

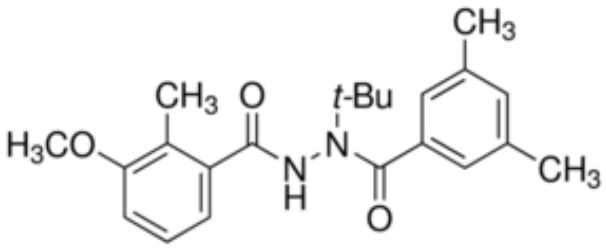

Figure 1. Chemical structure of methoxyfenozide.

A preliminary series of experiments was conducted to determine the concentrations of insecticides to be tested for genotoxicity. These experiments examined the effects of various concentrations of methoxyfenozide on *Allium cepa* root growth and *Drosophila melanogaster* larvae. The *A. cepa* root growth experiments provided insights into specific exposure levels based on plant root growth rates, while *D. melanogaster* larvae were used to assess insect development and survival rates. From these experiments, the appropriate concentration ranges that best represent environmental exposure were identified. All experiments were conducted at room temperature (22±2°C) with 65% relative humidity and three replicates.

Strains

For genetic crossover experiments, two *Drosophila* strains were used: males with multiple wing hairs *(mwh/mwh)* and flare virgin females *(flr³/TM3, BdS*). The *mwh* strain arises from a recessive viable mutation on the left arm of the third chromosome (3-0.3) and is characterized by the production of multiple trichomes per cell. The *flr³* strain is located near the centromere on the third chromosome (3-38.8) and is a recessive mutation that causes flare-shaped wing hairs. All three mutant alleles of flare are recessive zygotic lethal; thus, the *flr³* allele is maintained on a *TM3* balancer chromosome, which carries multiple inversions and a dominant marker (*BdS*) that is lethal in the homozygous state, alters wing shape (Lindsley & Zimm, 1992).

Allium test

Onions were placed in tubes filled with distilled water for 48 hours to promote germination. Then, onions with uniformly germinated roots were put into tubes treated with insecticide concentrations (0.005 mL/L, 0.01 mL/L, and 0.02 mL/L) for 24 hours. The experiments were performed in three replicates. Distilled water was used as a negative control and MMS (10 ppm) as a positive control. The onion roots of both the treatment and control groups were cut down to a length of 1-2 cm. Afterwards, they were kept in a 3:1 solution of ethanol and glycerol and preserved at 4 °C overnight (Fiskesjö, 1985). The root tips were subjected to a Feulgen staining procedure, a fundamental technique for DNA staining. Initially, the root tips were macerated with 1N HCl for 12 minutes to soften the tissues and facilitate the staining process. Subsequently, the root tips were washed with distilled water and stained with Schiff's Reagent for 30 minutes (Feulgen & Rossenbeck, 1924). Four to five stained samples were then placed on a microscope slide and squashed with 45% acetic acid (Rank & Nielsen, 1993). In order to determine the mitotic index (MI), mitotic phase, and chromosomal aberrations (CA), a total of 5000 cells were analyzed, with 1000 cells counted from each of the 5 slides. The MI was calculated as: . Chromosomal abnormalities were assessed in 500 cells selected from these 5000 analyzed cells, with 100 cells evaluated from each slide. The data were analyzed statistically using SPSS version 26.0, and the Tukey test was performed to evaluate the differences between each treatment group and the negative control.

Somatic Mutation and Recombination Test

We carried out the wing spot test following the principle by Graf et al. (1984). Transheterozygous larvae were collected every 8 hours after hatching and transferred to plastic vials containing 1.5 g of Drosophila Instant Medium, which was treated with 5 mL of insecticide at concentrations of 0.1 mL/L, 0.2 mL/L, and 0.4 mL/L. All experiments were performed in three replicates. To prepare the negative control, 5 mL of distilled water were used to dissolve Drosophila Instant Medium. For the positive control, Drosophila Instant Medium was treated with MMS. The flies were collected and preserved in a solution of 70% ethanol. The wings were then detached and mounted on a microscope slide using Faure solution (gumarabic 50 mg, chloral hydrate 30 g, glycerol 30 mL, water 50 mL). This solution is commonly used for the preparation and preservation of microscopic specimens,

providing a clear medium that enhances the visibility and stability of the samples. The wings were examined at 400x magnification to identify any cell clones with abnormally formed wing hairs. The amount and type of spots were recorded. Mutant clones were classified into three categories: (1) small single spots consisting of 1-2 *mwh* cells; (2) large single spots comprising three or more cells; and (3) twin spots containing adjacent *mwh* and *flr*^{3} cells (Graf, 1995).

The statistical significance of the results obtained from the wing mutation test has been evaluated using the multiple decision procedure developed by Frei & Würgler (1988). The procedure is based on two hypotheses: (1) the frequency of mutations in the treated group is no greater than that in the suitable group, and (2) the frequency of induced mutations in the treated group is at least m times higher than the spontaneous mutation frequency observed in the control. Statistical calculations were performed using Kastenbaum & Bowman (1970) conditional binomial test at a 5% significance level.

Results

The study evaluated the genotoxic and cytotoxic effects of methoxyfenozide on *A. cepa* meristematic root cells by monitoring the variables of mitotic index (MI) and chromosomal aberration (CA) rate after 24-hour exposure to different concentrations. The results are presented in Table 1 and Table 2.

Table 1. The effect of a 24-hour exposure to methoxyfenozide on the Mitotic Index in *Allium cepa* root cells.

Concentration (mL/L)	Mitotic Index (%) ±SD	Prophase (%) ±SD	Metaphase (%) ±SD	Anaphase (%) ±SD	Telophase (%) ±SD
N.C	7.28[a]±*0.32*	7.20±2.35	40.51±2.71	32.81±3.71	19.47±4.16
P.C	3.61[a,b]±*0.38*	1.52±0.96	45.92±2.43	29.30±2.37	23.27±0.90
0.005 mL/L	5.88 [a,b,c] ±0.40	8.75±6.50	43.71±0.21	29.86±7.76	17.67±1.45
0.01 mL/L	4.71 [a,b,c]±*0.44*	6.85±5.62	43.64±1.17	32.56±8.49	17.19±4.05
0.02 mL/L	3.97 [a,c] ±0.47	5.20±2.05	46.35±3.11	31.25±4.44	18,91±5.24

SD: Standard Deviation; N.C: Negative Control; P.C: Positive Control; a: indicates groups in which the difference between the negative control group and the groups is statistically significant; b: indicates the positive control group and the groups in which the difference between the groups is statistically significant; c: indicates groups in which the difference between groups is statistically significant according to the 0.005 mL/L concentration ($p \leq 0.05$).

Table 2. Genotoxic effects of methoxyfenozide on *Allium cepa* root cells

Chromosomal Aberrations (±SD)	N.C	P.C	0.005 mL/L	0.01 mL/L	0.02 mL/L
Micronucleus	0.13 ± 0.12	0.87 ± 0.31	0.4 ± 0.2	0.6 ± 0.35	0.67 ± 0.12
Irregular Metaphase	3.13±0.31	6.47±0.50	4.53±0.31	5.07±0.31	5.40±0.53
Sticky Metaphase	2.40±0.40	2.67±0.31	3.53±0.64	3.93±0.50	4.00±0.35
Irregular Anaphase	0.20±0.20	0.33±0.12	0.40±0.53	1.47±0.61	1.40±0.53
Sticky Anaphase	0.40±0.20	0.80±0.40	1.13±0.31	1.20±0.53	1.40±0.35
Fragment	0.13±0.23	0.40±0.20	0.07±0.12	0.73±0.81	1.53±0.31
Vagrant	0.20±0.20	0.33±0.12	0.07±0.12	0.20±0.20	0.27±0.12
Laggard	3.53±0.61	4.20±0.20	5.47±1.42	3.73±0.42	4.67±0.70
Disoriented Anaphase	3.27±0.50	4.47±0.42	3.80±0.53	4.33±0.61	5.13±0.31
Bridge	4.27±0.70	7.47±0.50	4.80±0.53	4.60±0.80	5.20±0.53
c-Mitosis	0.07±0.12	0.20±0.20	0.60±0.20	0.20±0.20	0.27±0.31
Multipolarity	0.33±0,23	0.27±0.12	0.67±0.42	0.93±0.92	1.20±0.20
Total Aberrations (%)	18.07[a]±1.33	28.47[a,b]±0.81	25.47[a,c]±1.17	27.00[a,d]±2.00	31.13[a,b,c,d]±0.95

SD: Standard Deviation; N.C: Negative Control; P.C: Positive Control; a: indicates groups in which the difference between the negative control group and the groups is statistically significant; b:indicates the positive control group and the groups in which the difference between the groups is statistically significant; c: indicates groups in which the difference between groups is statistically significant according to the 0.005 mL/L concentration; d: indicates groups in which the difference between groups is statistically significant according to the 0.01 mL/L concentration ($p \leq 0.05$).

Methoxyfenozide inhibited the MI by affecting the rate of onion root cell division. Mitotic index was determined as 7.28 ± 0.32 in the negative control. At the highest concentration of 0.02 mL/L, MI was 3.97 ± 0.47, indicating that cell division decreased 45% compared to the control. This decrease of 35% was observed at a concentration of 0.01 mL/L, and 19% was observed at a concentration of 0.005 mL/L. Compared to the negative control group, the decrease in MI (%) observed with increasing dose at all concentrations was statistically significant ($p \leq 0.05$). Furthermore, when concentrations were compared with each other, the differences in MI (%) between the 0.01 mL/L and 0.02 mL/L concentrations were not statistically significant ($p \geq 0.05$). However, a significant decrease in MI (%) was observed at both 0.01 mL/L and 0.02 mL/L concentrations compared to the 0.005 mL/L concentration ($p \leq 0.05$) (Table 1).

The study examined the genotoxic effects of methoxyfenozide on onion root cells following a 24-hour exposure. The total CA frequency was 18.07 ± 1.33 in the negative control. In the experimental group, the aberration frequency increased with increasing concentrations, reaching 25.47 ± 1.17, 27.00 ± 2.00, and 31.13 ± 0.95 at 0.005 mL/L, 0.01 mL/L, and 0.02 mL/L, respectively (Table 2). The statistical analysis showed a significant difference ($p \leq 0.05$) between the experimental groups and the negative control group (Table 2). Additionally, when the concentrations were compared, the increase in total aberration (%) between the 0.005 mL/L and 0.01 mL/L concentrations was not statistically significant, whereas the increase in total aberration (%) at the 0.02 mL/L concentration was found to be significant compared to both the negative control and experimental groups ($p \leq 0.05$) (Table 2). Methoxyfenozide mainly induces irregularities during the metaphase phase. Furthermore, methoxyfenozide exposure promotes stickiness and fragmentation. We frequently observed aberrations, such as bridge, vagrant, laggard, stickiness, and disorientation, throughout the anaphase-telophase stage (Figure 2).

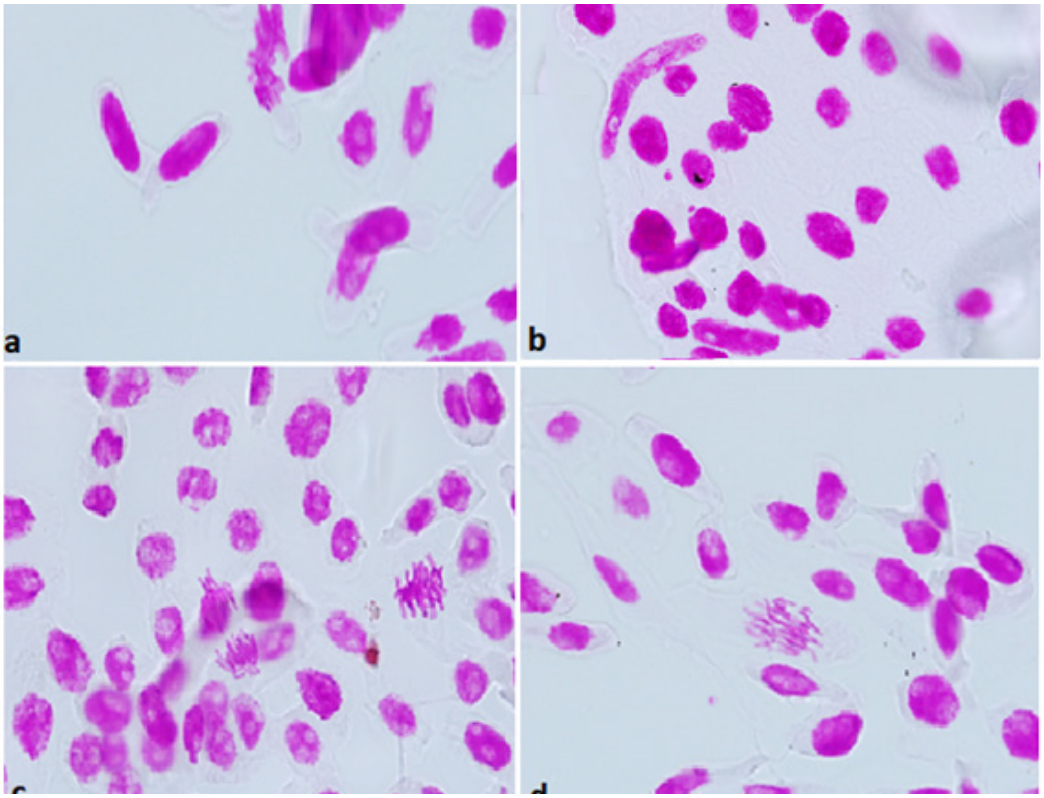

Figure 2. Examples of aberrations induced by methoxyfenozide in *Allium cepa* cells. a. Sticky Metaphase b. Micronucleus c. Vagrant, d. Anaphase bridge.

The results obtained from marker transheterozygous *(mwh/flr³)* and balancer transheterozygous (*mwh/TM3, BdS*) in the SMART experiment are summarized in Table 3. For each concentration and type of wing, 40 pairs were tested for genotoxicity. In the marker heterozygous flies, both small and large spots increased with concentration compared to the negative control. Three twin clones were observed at a concentration of 0.1 mL/L, and one twin clone was observed at 0.4 mL/L. The total number of clones was recorded as 10 in the negative control group and ranged from 15 to 19 in the concentration groups. In the case of balancer heterozygous flies, the total number of clones was 8 in the negative control group and ranged from 7 to 13 in the concentration groups. No twin clones were observed due to the balancer chromosome. The findings suggest that the increase in the total number of clones is statistically insignificant. The frequency of clone induction was higher in normal transheterozygous flies exposed to treatment concentrations compared to the negative control, and it increased with higher treatment concentrations.

Table 3. Frequency and types of wing spots observed in *Drosophila melanogaster* individuals with mwh/flr³ and mwh/TM3 genotypes following methoxyfenozide treatment.

	N	Small single spots (1-2 cells) (m=2)			Large single spots (>2 cells) (m=5)			Twin spots (m=5)			Total mwh (m=2)			Total spots (m=2)			f
Marker Heterozygous		No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D	
Distiled Water	80	8	(0.1)		2	(0.03)		0	(0.00)		10	(0.13)		10	(0.13)		0.51
MMS	80	25	(0.31)	+	5	(0.63)	i	0	(0.00)	i	30	(0.38)	+	30	(0.38)	+	1.54
0.1 mL/L	80	10	(0.13)	i	2	(0.3)	i	3	(0.38)	i	12	(0.15)	i	15	(0.19)	i	0.61
0.2 mL/L	80	12	(0.15)	i	2	(0.3)	i	0	(0.00)	i	14	(0.18)	i	14	(0.18)	i	0.72
0.4 mL/L	80	14	(0.18)	i	4	(0.05)	i	1	(0.01)	i	18	(0.23)	i	19	(0.24)	i	0.92
Balanced heterozygous		No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D	
Distilled Water	80	6	(0.08)		2	(0.03)					8	(0.1)		8	(0.1)		0.41
MMS	80	26	(0.33)	+	4	(0.05)	i				30	(0.38)	+	30	(0.38)	+	1.54
0.1 mL/L	80	7	(0.09)	i	0	(0.00)	-				7	(0.09)	i	7	(0.09)	i	0.36
0.2 mL/L	80	9	(0.11)	i	1	(0.01)	i				10	(0.12)	i	10	(0.12)	i	0.51
0.4 mL/L	80	12	(0.15)	i	1	(0.01)	i				13	(0.16)	i	13	(0.16)	i	0.67

MMS: methyl methanesulfonate (CAS No. 66-27-3); f: Frequency of clone formation per 105 cells, No: number of spots; Fr.: frequency of spots per fly; D: statistical diagnosis according to Frei and Würgler (1988); +: positive; −: negative; i: inconclusive; m: multiplication factor; probability levels, $\alpha=\beta = 0.05$.

Discussion

The potential genotoxic effects of the bisacylhydrazine insecticide methoxyfenozide were investigated employing Allium and SMART methods. Insecticides in this group are effective by disrupting the growth and development processes by affecting the normal functioning of the ecdysone hormone responsible for processes such as cell division, transformation and maturation in the larval period in insects. Methoxyfenozide provides pest control by showing ecdysone effects especially in Lepidoptera larvae, and studies supporting this finding have shown it inhibits the development of *Spodoptera frugiperda* (Smith, 1797) and Asian gypsy moth larvae (Linnaeus, 1758) (APVMA, 2002; Zarate et al., 2011; Wu et al., 2015). Furthermore, research has demonstrated the ovicidal and larvicidal effects of methoxyfenozide against a range of other organisms, including H*armonia axyridis* (Pallas, 1773) (Col., Coccinellidae*), Culiseta morsitans* (Theobald, 1901*)* (Culicidae: Diptera), and *Culex pipiens* L. (Linnaeus, 1758) (Culicidae: Diptera) (Carton et al., 2003; Haouari-Abderrahim & Rehimi, 2014; Hamaidia et al., 2016).

The genotoxic and cytotoxic effects of the insecticide were assessed through the Allium test, employing the mitotic index (MI) and chromosome aberration (CA) variables. The findings revealed a reduction in MI within *A. cepa* meristematic cells after a 24-hour exposure to methoxyfenozide, as compared to the negative control. This decrease in MI could be explained by multiple mechanisms, such as the inhibition of essential processes like microtubule formation, nucleoprotein synthesis, and ATP production during the interphase stage, as well as the suppression of DNA synthesis (Hidalgo et al., 1989). Currently, there is no research demonstrating the effects of methoxyfenozide on MI. However, many researchers have reported that insecticides decrease MI by affecting the rate of cell division (Mesi & Kopliku, 2013; Mesi & Kopliku, 2015; Bıçakçı et al., 2017; Kalefetoğlu Macar, 2021).

The primary effect of methoxyfenozide in onion root tip cells was noted during the metaphase and anaphase-telophase stages. The most frequently recorded abnormalities during the metaphase stage were irregularities and stickiness. Barakat et al. (2010) reported that this spindle abnormality could be attributed to the impact of pesticides on cyclin-dependent kinase activity. Previous research demonstrated these effects of insecticides on metaphase cells. Chauhan et al. (1999a) have reported that deltamethrin exhibits an effect of increasing the number of abnormalities by disrupting the parallel arrangement of microtubules in onion root metaphase cells. Similar effects on the mitotic spindle were observed when the synthetic pyrethroid insecticides cypermethrin and fenvalerate were applied to onion stem cells for 6 and 24 hours (Chauhan et al., 1999b). Cypermethrin also reported to disrupt the structure of spindle fibers and induce irregular metaphase in the root tip cells of *Helianthus annuus* L. (sunflower) (Inceer et al., 2009). Methoxyfenozide also induced bridges, stickiness, laggard chromosomes, vagrant chromosomes, disoriented anaphase, and multipolarity. Chromosomal bridges are a significant marker of genotoxicity. Bridges arise from chromosome breaks, adhesion, and unequal chromatid exchange, leading to the formation of laggards (El-Ghamery et al., 2000). The cause of the stickiness was found to be the subchromatid connections that are formed as a result of misfolded

chromatin fibres between the chromosomes (McGill et al., 1974). Vagrant chromosome an irregularity in the spindle that leads to the production of daughter cells with an uneven number of chromosomes in their nuclei, resulting in an uneven size or irregular shape during interphase. Previous studies have demonstrated the ability of pesticides to induce vagrant formation in onion meristem cells (Yıldız & Arikan, 2008; Sharma & Vig, 2012). The results indicate that methoxyfenozide induces CA formation in onion root tip cells and are consistent with other Allium tests for insecticides.

In the SMART experiment, we observed an increase in clone induction frequencies with an increasing in methoxyfenozide concentrations. Furthermore, we found that the increase in Methoxyfenozide concentration promoted the formation of single spots. Graf (1995) stated that single clones result from point mutations, deletions, translocations, and monosomy. Twin clones were reported to occur solely as a result of mitotic recombination.

Using the wing spot test, researchers examined the mutagenic effects of allethrin, dieldrin, endrin, dimethoate, malathion insecticides, and the ethylenedioxyphenolic compound piperonyl butoxide. Upon analyzing standard and high-bioactivation crosses, it was observed that all of these substances gave negative results (Osaba et al., 1999). Researchers studied the effects of cypermethrin, cyphenothrin, deltamethrin, and permethrin insecticides, along with mixtures of piperonyl butoxide (PBO), on *Drosophila* wing cells. They mixed different ratios of PBO (1:0.25, 1:0.5, 1:0.75, 1:1, and 1:2) with lethal doses of the insecticides and found that they did not cause any harmful genetic effects (Demir et al., 2014).

De Morais et al. (2016) reported mutagenic effects in somatic wing cells exposed to the insecticide fipronil, except at a single concentration. Furthermore, the tumor detection test provided evidence of the mutagenic, recombinogenic, and carcinogenic effects of these insecticides in *Drosophila* somatic cells. A similar investigation was carried out with thiamethoxam (TMX) and actara. While TMX exhibited mutagenicity in the high-bioactivation cross, it was stated that these insecticides were not carcinogenic, unlike the previous study (De Morais et al., 2017). Genotoxicity of deltamethrin and permethrin in *Drosophila* was examined with SMART. The study revealed that deltamethrin exhibited greater toxicity than permethrin during both larval and adult stages. However, the occurrence of twin spots was not observed in the study. The most prevalent mutation was found to be associated with deltamethrin at the highest dose, resulting small single spot (Budak, 2005).

The limited number of studies on the genotoxicity of methoxyfenozide constrains the interpretation of our findings in comparison to previous research. In particular, the absence of studies employing Allium and SMART methods to assess the genotoxicity of methoxyfenozide complicates the comparison of our results. Nevertheless, a study has been conducted to examine the influence of methoxyfenozide on cell proliferation in *Drosophila melanogaster*. As reported in this study, the presence of methoxyfenozide was found to impair the functionality of the ecdysteroid receptor (EcR/USP) (Mosallanejad et al., 2010). Additionally, another study conducted on *Spodoptera frugiperda* Sf9 cells determined that methoxyfenozide causes cell cycle arrest in the G1 phase (Giraudo et al., 2011). Although these findings suggest that methoxyfenozide may disrupt cell proliferation, drawing definitive conclusions regarding its genotoxicity remains challenging.

The genotoxicity of methoxyfenozide has been investigated using the Allium and SMART methods for the first time. Previous studies have indicated that methoxyfenozide was non- genotoxic. However, current results have shown that it may have genotoxic effects in plant and animal systems. Therefore, further research is necessary to better understand the potential environmental effects and health risks associated with the use of methoxyfenozide in farming practices.

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