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Evaluation of the genotoxicity of some standart and eco-friendly detergents with *Vicia faba*

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Abstract. The increasing use of detergents as a result of rapid increase of population and irregular industrialization has a big role in the environmental pollution. Determination of genotoxic effects of some standart and eco-friendly detergents on *Vicia faba* was aimed in this study. Faba beans were treated with detergents for 24, 48 and 72h by using EC50 and 2xEC50 values and analysed by mitotic index (MI) and comet assay. According to the results of root inhibition test, EC50 value for the detergent A1 was 20ml/L, A2 was 50ml/L, B1 was 40ml/L, B2 was 60ml/L respectively. The least decrease in the mitotic index was found in detergent B2 (eco-friendly laundry) and the most decrease was found in detergent A1 (standart dishwasher). Various abnormalities were determined in different phases of mitosis. After comet assay the cells were classified as type 0, type 1, type 2, type 3 and type 4 according to the nuclei damage rates. Type 3 was the most identified cell damage degree in eco-friendly detergents whereas type 4 was the mostly seen in standard detergents. Parameters such as olive tail moment, tail DNA percentage and arbitrary units were also identified by comet assay. All the test results showed that standard detergents cause more genotoxic effects than eco-friendly ones.

Keywords: Comet assay, detergents, DNA damage, chromosome aberations, mitotic index, *Vicia faba*.

INTRODUCTION

Hundreds of chemicals continue to be produced to make our lives easier. Although they are useful in raising our standard of living, we cannot understand the fate of these chemicals without investigating their effects on the living things. Plant biotests have an important role in determining the cytotoxic and genotoxic effects of environmental pollutants, chromosome damage and gene mutations. These studies can contribute to the creation of a database about environmental conditions in various regions of the world. The simple and clear results of the biotests can also be used in public awareness training on the genotoxic effects of environmental pollution.

Recently, pollution from domestic and industrial wastes is increasing and detergents are replaced by a large percentage of effective pollutants (Minareci

ve diğ., 2008). Detergents are mixtures of powder, granule, soft consistency or liquid form, which are preferred for cleaning purposes and which contain anionic surfactants and other cleaning agents as primary cleansers (Egemen, 2000). Laundary detergents are produced in large quantities and used in daily life and industrial activities (Wang et al., 2019). Various physical and chemical analyzes are carried out to determine environmental pollution of the detergents, however, these analyzes alone are not sufficient. Genotoxicity studies are essential to determine the effects of detergents on organisms.

Many plants have been used for cytotoxicity testing, recent reports being of *Allium cepa* L. (Bonciu et al., 2018), *Vicia faba* L., *Zea mays* (Bonea and Bonciu, 2017), and *Drimia indica* (Roxb.) Jessop. (Daphedar and Taranath, 2018). *V.faba* root tips, can be easily obtained in a whole year, its development and storage is easy and inexpensive, the root meristem comprises a high proportion of cells undergoing mitosis and the number of chromosomes is low (2n = 12) and appropriate for accurate and complete counting. The *V.faba* root meristem chromosomal mutation assay is also approved by the International Programme on Chemical Safety (IPCS, WHO) and the United Nations Environment Programme (UNEP) as an effective and standard test for the chemical screening (Youssef and Elamawi, 2020).

The most important point in the root growth inhibition test is the determination of the effective concentration (EC50) which reduces the root length by 50% compared to the control group (Fiskesjö, 1985). In order to determine the toxic and genotoxic effects of environmental pollutants on chromosomes and cell division EC50 values were calculated in many studies (Rank and Nielsen, 1998; Chauhan et al., 1999, Ateeq et al., 2002; Saxena et al., 2005; Arıkan, 2006).

Mitotic activity tests are frequently used in chromosome counting and toxicological research. By the application of this test, information can be provided about the effect of chemical treatment on cell division (Akı and Karabay, 2004).

Cytogenetic methods are widely used in the biological monitoring of populations exposed to mutagenic and carcinogenic compounds. To date, many methods have been used to detect DNA damage. One of them is comet analysis that is widely used because it is a sensitive, fast and reliable method for the detection of various types of DNA damages.

In addition to visual assessment of DNA damage by comet technique, parameters related to tail length, tail moment, and percentage of DNA in the tail can be determined using comet image analysis programs (Collins, 2002). High toxicity may be the cause of the increase in DNA damage. In a study on the genotoxic effects of pollutants in some plants, a relationship between concentration and DNA damage has been demonstrated (Gichner et al., 2006).

There are a few studies that have evaluated the toxicity and genotoxicity of commercial eco-friendly detergents in plant bioassays, their toxic effects assessed generally in *Daphnia magna* (Pettersson et al., 2000), some microalgae (Aizdaicher and Markina, 2006; Azizullah et al., 2011), in *Salmonella* test and in human leukocytes (Pedrazzani et al., 2012).

It was aimed to determine the genotoxic effects of four different dishwasher and laundry detergents, two of which were eco-friendly, on *V. faba* using the root-tip chromosome aberration test of accepted plant biotests. Mitotic index and mitotic abnormalities were investigated with cytotoxicity test and DNA damage was investigated with the single cell gel electrophoresis (SCGE) method which is also called as comet assay. Thus, it will provide new sights to the environmental pollution and indirectly find out the damage caused to the other organisms via the food chain.

MATERIALS AND METHODS

V. faba (2n = 12) seeds were used as research material obtained from local market. The commercial dishwasher (A1 and A2) and laundry (B1 and B2) detergents, two of which were standart named as A1 and B1 and two of which were eco-friendly named as A2 and B2, were used. Chemical content information about detergents was shown in Table 1.

Germination of V. faba seeds and determination of EC50 concentrations

Surface sterilization was performed by selecting dimensionally and morphologically uniform *V. faba* seeds. The seeds were shaken in 70% alcohol for 1 minute, after 15 minutes in a 3% sodium hypochlorite containing 2 drops of Tween-20 for 200 mL, and then washed 4 times with sterile distilled water. Sterilized seeds were kept in sterile distilled water for 12 hours to swell before they were germinated (Hamdy and Hattori, 2006).

Petri dishes used to germinate the seeds were first sterilized in autoclave and then exposed to UV light in a sterile cabinet for 15 minutes. Sterile Whatman papers treated with detergent concentrations were placed into sterile petri dishes placed at regular intervals. 20 pieces of seed were placed in each petri and covered with

Table 1. The chemical contents of the four detergents A1, A2, B1 and B2.

Detergents	Chemical content
A1	15-30% Anionic active substance, Polycarboxylate, Phosphonate, Enzyme (Amylase, Protease), Perfume, Paint, Preservative.
A2	<5% Soap, <5-15 Anionic Substances, <5% Nonionic Substances, Perfumes, Methylisothiazolinone, Water, Raw Materials from Coconut, Palm, Wheat and Potato.
B1	% 5-15 Anionic active substance, Nonionic active ingredient, <5% Phosphonate, Soap, Enzyme, Perfume (Alpha Ionone, Amyl Cinnamal, Benzyl Salicylate, Butylphenyl Methylpropional, Hexyl Cinnamal, Limonene, Linalool), Preservative.
B2	<5% Soap, <5-15 Anionic, <5% Nonionic Substances, Perfume, Methylchloroisothiazolinone, Water, Raw Materials from Coconut, Palm, Wheat and Potato.

A1 and B1 are standard detergents. A2 and B2 are classified as eco-friendly detergents.

Whatman papers treated with detergent concentrations and then put into 24 °C incubator.

The detergent concentrations used in determination of EC50 values for each detergent was listed in Table 2. The root length of the seeds, which were germinated in the incubator for 7 days, were measured for root inhibition test. At the end of one week, the averages of the root lengths were calculated and the decrease in the root lengths were compared with the control group. The test set-ups for calculating the EC50 value were performed in 3 replicates. After the EC50 and 2xEC50 values were calculated, all experiments were carried out on these values.

Table 2. The detergent concentrations used in determination of EC50 values.

Detergent	Concentration (ml/L)	Detergent	Concentration (ml/L)
A1	1	B1	1
	10		10
	20		20
	30		30
	40		40
	50		50
	100		100
	200		200
	400		400
A2	1	B2	1
	10		10
	50		50
	100		60
	200		70
	400		80
			90
			100
			200
			400

Chromosomal abberation test

At the end of 24, 48 and 72 hours, the roots that were approximately 1-2 cm long were cut with the help of sterile scalpel and transferred to Carnoy (3 parts 70 % alcohol, 1 part 45 % glacial acetic acid) fixation fluid. After 24 hours, the root tips were put in 70 % ethyl alcohol and then hydrolised in 1 mL 1 N HCl with the help of the burner flame. The roots that were hydrolyzed in the watch glass were stained with 1-2 drops of 2 % aceto orsein (Merck, Germany). The cover glass was covered on the stained root tip and the crush-smear preparation technique was applied. Photographs of the cells were taken with 40x and 100x lenses to determine chromosome abnormalities with Olympus BX51 photomicroscope. In the calculation of the percentage of mitotic index, 500 cells were counted in each of the 10 slides were prepared for each concentration and 5000 cells were counted for each concentration (Yüksel and Aksoy, 2017). The percentage of mitotic indices was then determined by dividing the number of cells divided by the total cell number and multiplying by 100 for each application concentration (EC50 and 2xEC50), duration (24, 48, 72 h) and their control groups (Souza et al., 2013). The ratios of chromosomal damage such as chromosome bridge, chromosome adhesion, pole shift and laggard chromosome formation in dividing cells were calculated.

SCGE (Single Cell Gel Electrophoresis) Analysis

400 µL cold tris buffer solution and 5-6 root tips (1-2 cm) of *V.faba* were put in petri dishes placed on ice cassettes. The roots were cut gently with the aid of a scalpel until the color of the buffer solution was blurred without taking over the ice cassettes. The resulting suspension was transferred to the ependorf tube and left on ice for 15 minutes to allow the nuclei of the meristematic cells to settle down. The slides were coated with 1% NMP (normal melting point) agarose (ROTH Germany 2267, Sigma, A9539). 100 µL of the nuclei solution was taken close to the bottom of the ependorf tube, and 100 µL

of LMP (low melting point) agarose (ROTH Germany, 6351, Sigma, A9414) was mixed by pipetting in ependorf tube, allowing the nuclei to adhere to the LMP agarose. Nuclei + LMP agarose mixture was poured onto NMP coated slides and closed with lamella immediately. The slides were kept on ice for 10 minutes at +4°C in the refrigerator to stabilize the agarose. Cover glass on the slides were gently removed after taken from the refrigerator. The slides were placed in the same direction and adjacent to each other in the horizontal dark electrophoresis tank, which had been pre-cooled to +4°C with the cold water bath.

A cold SCGE buffer was poured slowly from the edges of the tank and the cover of the tank was closed. The slides were stored in the tank for 20 minutes in order to dissolve the DNA chain. Electrophoresis (CSL-COM 20, 1000 mL, and Cleaver CS-300V power supply) was performed at 27V, 300mA for 25 minutes in order to observe the comets that would occur as a result of different molecular weight DNA fragments. At the end of the electrophoresis, the slides were removed from the tank and placed in the trays and kept in cold tris-HCl 3 times each for 5 minutes for neutralization.

After the neutralization process, the slides were arranged upright on the drying paper and the excess buffer was allowed to move over the slides. The slides were stained with 100 µL of Red Safe dye before drying and left for 5 minutes. They were closed immediately with cover glass. Slides for comet assay were examined by fluorescence microscope Olympus BX51. For each concentration and time, 25 randomly chosen nuclei were analyzed and three slides were evaluated per treatment and the median values of comet parameters for each slide were calculated (Türkoğlu, 2012). The averaged median tail length (µm), percentage of tail DNA (% of DNA in comet tail), olive tail moment (OTM in arbitrary unit) values were calculated using the Kameram Software (Argenit, Turkey) comet assay analysis program for each treatment group.

RESULTS

As a result of the calculations made respectively; EC50 for A1 was determined as 20 ml/L (Fig 1a) and 2xEC50 was 40 ml/L; EC50 for A2 was determined as 50 ml/L (Fig 1b) and 2xEC50 was 100 ml/L; EC50 for B1 was determined as 40 ml/L (Fig 1c) and 2xEC50 was 80 ml/L; EC50 for B2 was determined as 60 ml/L (Fig 1d) and 2xEC50 was 120 ml/L.

It was observed that the percentage of root lengths decreased gradually compared to control in increasing detergent concentrations. It was also observed that the EC50 values of the eco-friendly detergents were higher than the standard detergents (Fig. 1).

The effects of the detergents A1, A2, B1, B2 on mitotic frequency and chromosomal abnormality of V.faba root tip cells

The mitotic index values were decreased in the treatment groups due to increasing time and concentration at the end of 72 hours. Mitotic index values of eco-friendly detergents were higher than the standard detergents (Table 3).

At the end of the seven days, the mitotic index value for the control group was found to be 14.60%, it was 8,90% at 20ml/L and 7,80% at 40ml/L A1 detergent concentration; the mitotic index value for A2 detergent, decreased to 12.80% in 50 ml/L and to 11.50% at 100ml/L concentration; the mitotic index value for B1 detergent, decreased to 8.30% in 40 ml/L concentration and to 8.10% at 80 ml/L concentration; the mitotic index value for B2 detergent, decreased to 11.90% in 50 ml/L concentration and to 10.60% at 100ml/L concentration.

The observed chromosomal abnormalities for all of the detergent treatments of *V.faba* root tip cells were; laggard chromosome, chromosome adhesion, irregular anaphase, chromosomal bridge, chromosome swelling, micronucleus formation, pole shift, C-mitosis, irregular prophase, irregular metaphase and chromosome stickness (Fig 2).

The detection of DNA damage caused by detergents with SCGE

The degree of DNA damage increased in proportion to the concentration and duration, and the degree of DNA damage in the eco-friendly detergents was lower than the standard detergents. The classification of nuclei according to the degree of damage with SCGE yield five types of nucleus; “type 0” means no visible tail, “type 1” means tiny tail , “type 2” means dim tail, “type 3” means a clear tail that is longer than the head diameter and “type 4” means tail is approxiametly three times longer than the head (Fig 3).

In the control groups, a few DNA damaged nuclei, called type 0 and type 1, were observed.

Type 1 and type 2 grade DNA damages were observed at 20ml/L A1 concentration after 24 and 48 hours treatment, and type 3 grade DNA damage was observed to occur more than type 2 after 72 hours. Type 2 grade DNA damages were seen mostly in 24 hours at

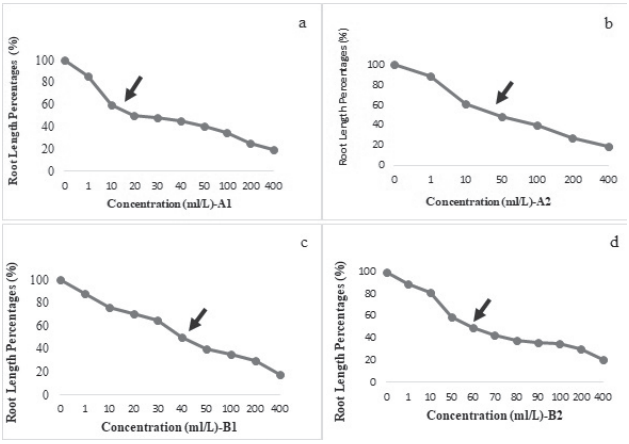

Figure 1. EC50 values determined for the four different detergents, a. detergent A1, b. detergent A2, c. detergent B1, d. detergent B2. Arrows show the EC50 values.

Table 3. Mitotic index percentages calculated for the detergents A1, A2, B1 and B2 after 72 h. treatment.

Detergent	Concentration(ml/L)	MI (%) ± SD*
A1	0	14.60±0.07
	20	8.90±0.08
	40	7.80±0.09
A2	50	12.80±0.11
	100	11.50±0.07
B1	40	8.30±0.09
	80	8.10±0.05
B2	60	11.90±0.04
	120	10.60±0.05

* MI ± SD: average mitotic index percentage±standart deviation.

a concentration of 40 ml/L A1, while type 2 and type 3 grade damages were observed at 48 hours. Type 3 and type 4 grade damages were observed in majority after 72 hours.

Type 0 and type 1 grade DNA damages were detected at 50 ml/L A2 concentration treated for 24 hours. Type 1 and type 2 grade DNA damages were found at the end of 48 hours, and type 3 grade DNA damage was observed when 72 hours were completed. Type 0 and type 1 grade DNA damages were detected at a concentration of 100 ml/L for 24 hours treatment. Type 2 grade DNA damages were observed frequently during 48 hours, and nuclei with DNA damages in type 2 and type 3 were observed at the end of 72 hours.

Type 1 and type 2 grade DNA damages were observed at 40 ml/L B1concentration for 24 hours, while nuclei with type 2 grade DNA damage were observed after 48 hours treatment. Type 3 grade DNA damage was seen after 72 hours. Type 2 grade DNA damage was found in 80 ml/L concentration after 24 hours and type 2 and type 3 grade damage was found after 48 hours treatment. At the end of 72 hours, nuclei with type 4 grade DNA damage were observed. Type 0 and type 1 grade DNA damages were determined at 60ml/L B2 concentration treated for 24 hours. Type 2 grade DNA damage was observed after 48 hours and DNA damage level changed to type 3 after 72 hours. Type 1 and type 2 grade DNA damages were observed at 120 ml/L concentration for 24 hours and type 2 grade DNA damage was observed after 48 hours. Type 3 grade DNA damages were determined after 72 hours.

The effects of different concentrations of detergents on DNA percentage (tail DNA%) were also examined. Tail DNA percentages for the control group of A1 detergent was 2.25% after 24 hours, 3.6% after 48 hours and 4.2% after 72 hours. At a concentration of 20 ml/L A1 detergent, the value increased from 22.4% after 24 hours to 43.6% in 48 hours, reaching 51.8% at the end of 72 hours. Tail DNA damage, which was 40.3% at 40 ml/L concentration in 24 hours, reached 52.3% in 48 hours and 66.7% in 72 hours (Table 5).

The tail DNA percentages was 0.9% after 24 hours in the control group of A2 detergent, 0.7% at 48 hours and 1.5% at 72 hours. At a concentration of 50 ml/L, the value of 17.2% in 24 hours increased to 22.9% in 48 hours. At a concentration of 100 ml/L, it was observed that the value of 29.4% in 24 hours, 38.1% in 48 hours and 49.2% in 72 hours (Table 5).

The tail DNA percentages was 1.8% in the control group of B1 detergent after 24 hours which was, reached 2.3% in 48 hours and 2.6% in 72 hours. At a 40 ml/L B1 detergent concentration, the value of tail DNA was 26.4% at 24 hours, 40.7% at 48 hours, and 50.5% at 72 hours, At a concentration of 80 ml/L, it was observed that the value was 42.6% in 24 hours reached 62.8% in 48 hours and 66.1% in 72 hours (Table 5).

The tail DNA percentages was 0.65% for B2 detergent in the control group of B1 detergent after 24 hours which was reached 0.8 % in 48 hours and 1.27% in 72 hours. At a concentration of 60 ml/L, the value of 18.5% at 24 hours reached 25.9% at 48 hours, and at 72 hours the value of tail DNA was 42.4%. At a concentration of 120 ml/L, it was observed that the value was 32.6% in 24 hours, 46.4% in 48 hours and 50.8% in 72 hours (Table 5).

For all detergent treatments on *V. faba*, the values of the tail were calculated as well as the determination of the percentage of tail DNA that increased in direct proportion to the concentration and duration.

The effect of all detergents on the OTM value at varying concentrations is shown in Table 6.

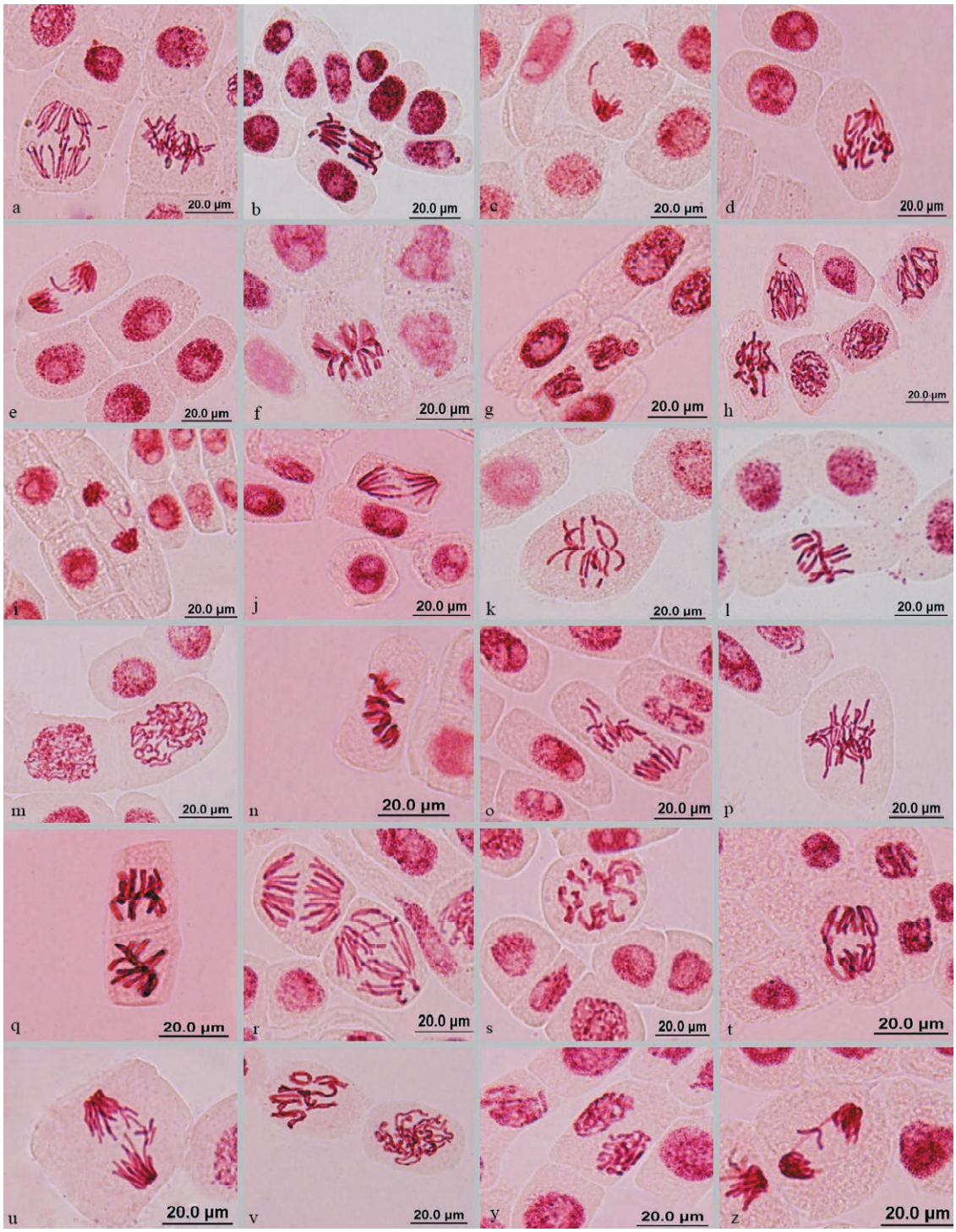

Figure 2. The chromosomal abnormalities caused by detergents in V. faba meristematic cells a. Laggard chromosome in anaphase and chromosome adhesion in metaphase, b. Laggard chromosome in anaphase, c. Laggard chromosome in telophase, d. Irregular anaphase, e. Chromosomal bridge and laggard chromosome in anaphase, f. Chromosome swelling in metaphase, g. Micronucleus formation in cytokinesis, h. Laggard chromosome and chromosome adhesion in anaphase, i. Chromosomal bridge and laggard chromosome in telophase, j. Chromosomal bridge and pole shift in anaphase, k. C-mitosis, l. Laggard chromosome in metaphase, m. Irregular prophase, n. Pole shift and chromosome swelling in metaphase, o. Laggard chromosome in anaphase, p. Irregular metaphase, q. Pole shift in metaphase, r. Irregular anaphase, s. C-mitosis and chromosome stickness, t. Chromosomal bridge in anaphase, u. Laggard chromosome in anaphase, v. C-mitosis and irregular prophase, y. Pole shift in telophase, z. Chromosomal bridge and laggard chromosome in telophase.

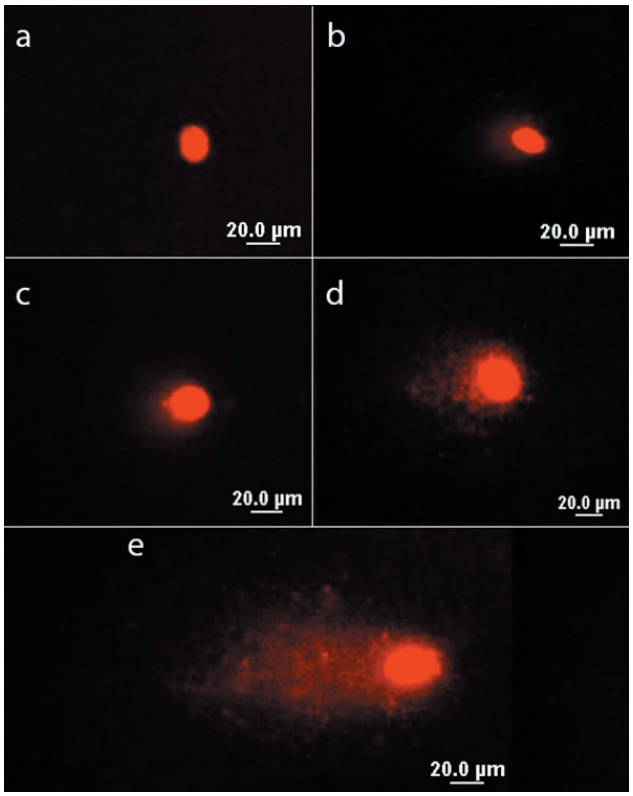

Figure 3. Classification of nuclei according to the degree of damage in *V.faba* with Comet test a) Type 0: undamaged, b) Type 1: tail <0,5 c) Type 2: tail>0,5 or equal to the head, d) Type 3: tail is longer than the head diameter, e) Type 4: tail is approxiametly three times longer than the head.

OTM values at all detergents showed a significant increase due to time and concentrations.

Table 4. The effect of the detergents on chromosomal abnormality percentages in *V.faba*

Detergent	Concentration (mg/L)	Duration time(h)	Chromosomal abnormality(%)±SD*
A1	0	24	0.25±0.27
		48	1.70±0.48
		72	2.70±0.66
	20	24	8.0±0.05
		48	11.0±0.07
		72	14.0±0.07
	40	24	23.2±0.06
		48	26.2±0.07
		72	30.4±0.07
A2	50	24	7.7±0.07
		48	9.2±0.05
		72	8.9±0.54
	100	24	11.8±0.06
		48	13.7±0.49
		72	14.1±0.07
B1	40	24	9.6±0.07
		48	12.2±0.54
		72	15.6±0.06
	80	24	21.1±0.07
		48	32,7±0.05
		72	28.3±0.06
B2	60	24	6.6±0.06
		48	8.3±0.05
		72	9.9±0.06
	120	24	10.8±0.05
		48	12.7±0.07
		72	16.5±0.07

DISCUSSION

V. faba seeds treated with detergents were allowed to grow and the root inhibition test and the values of the root length halving (EC50) by the end of seven days compared to the control group were determined for each detergent separately. It was found that the standard detergents could reduce the root length in lower concentrations than the eco-friendly detergents. Studies on the plant assays revealed that any genotoxic effects manifested in a test sample are likely to result in inhibition of root growth (Yekeen et al. 2017).

Ezemonye et al. investigated the genotoxicity of high anionic surfactant-containing detergents on sweet and salt water shrimps (*Desmoscaris trispinosa* and *Palaemonetes africanus*), it has been shown that increasing detergent concentrations increase the mortality rate compared to the control group (Ezemonye et al., 2009). Wing et al. (2019) demonstrated that both laundry detergents and detergent residue after rinsing showed high cytotoxicity on human bronchial epithelial cell culture.

Pettersson et al. found out the toxic effects of 26 laundry detergents and 5 softeners on *Daphnia magna*, it was reported that the concentration of detergents and the genotoxic effects were correlated with each other (Pettersson et al., 2000). The cytotoxic/genotoxic effects (by mitotic index and micronuclei frequency) of Boron used as detergent additive were reported in root meristems of *V. faba* (Barbefieri, 2016).

The lowest EC50 value was determined in A1 standart detergent that its effective concentration was found as 20 ml/L. According to this finding, A1 standart detergent is the most toxic from all of the other studied detergents and gave damage to the root mitotic cells in

Table 5. The effect of the detergents on Tail DNA percentages in *V. faba*

Detergent	Concentration (ml/L)	Duration Time/ DNA% in tail		
		24 h	48 h	72 h
A1	0	2.25	3.60	4.20
	20	22.40	43.60	51.80
	40	40.30	52.30	66.70
A2	0	0.90	0.70	1.50
	50	17.20	22.90	37.80
	100	29.40	38.10	49.80
B1	0	1.80	2.30	2.60
	40	26.40	40.70	50.50
	80	42.60	62.80	66.10
B2	0	0.65	0.80	1.27
	60	18.50	25.90	42.40
	120	32.60	46.40	50.80

Table 6. The effect of the detergents on OTM values in *V. faba.*

Detergent	Concentration (ml/L)	Duration Time/ OTM value		
		24h	48h	72h
A1	0	0.75	1.2	1.4
	20	7.5	16	17.2
	40	14.7	17.5	22.2
A2	0	0.2	0.3	0.57
	50	6	8.6	12.6
	100	10.8	12.7	16.6
B1	0	0.63	0.8	0.9
	40	8.8	14.3	17
	80	15.2	20.9	22.3
B2	0	0.21	0.26	0.6
	60	7.17	8.7	14.1
	120	11.3	15.4	17

V. faba. The highest EC50 value was determined in B2 eco-friendly detergent that its effective concentration was found as 60 ml/L. Therefore, the lowest amount of damage to root mitotic cells in *V. faba* was caused by B2 detergent. As a result, the chemicals in standard detergents have negative effects on root growth in *V. faba* compared to the chemicals in eco-friendly detergents.

Mitotic index test results showed that there was a reduction in mitoic index percentage with increasing time and concentration in all detergents. The reduction in mitotic index can be explained by the mitodepressive effects of chemicals in detergents, causing errors in normal cell cycle and limiting cell division (Özkara et al., 2015).

A1 detergent was the detergent that decreased the mitotic index percentage by 10.9% compared to the control, while B2 detergent was the detergent that decreased the mitotic index by 2.4% compared to the control. The mean percentage value of mitotic index was higher in the eco-friendly detergents than the standart detergents. It was concluded that the standard detergents influence cell division negatively when the chromosomal abnormalities in the root tip meristematic cells of *V.faba* were determined. Chromosomal abnormalities occur as a result of inability to repair the fractures in DNA double chain (Maluszynska and Juchimiuk, 2005).

In our study, it was determined that chromosomal aberration rate increased significantly in all detergents due to increasing concentration and duration. The most common abnormalies were polar shift, irregular prophase, irregular metaphase, sticky chromosome, c-mitosis and bridge formation. Induction of chromosomal aberrations pointed to potential for genotoxicity (Yekeen et al., 2017). Wang et al. (2019) reported that due to the ability of the detergents that break the lipid-lipid and protein-lipid interactions, membrane proteins and lipids can become soluble in human bronchial epithelial monolayer cell cultures. However, in our study, such a change was not observed visually due to the cell wall in plants but may be observed with cell ultrastructure studies.

The highest chromosomal abnormality was observed in A1 detergent at a rate of 30.4% after a period of 72 hours while the lowest chromosomal abnormality was observed in A2 detergent with a value of 14.1%. The standard detergents were found to have more chromosomal abnormalities than eco-friendly detergents. The chromosomal aberrations observed in this assay suggest that all the detergents exert a mutagenic/cytotoxic effect.

A wide variety of DNA repair mechanisms are available to prevent such damage in the cell nucleus. Replication, transcription and protein synthesis inhibition may occur when these repair mechanisms are ineffective or when very severe DNA damage occurs. However, chromosomal abnormalities and mutations can be seen in the long term treatments (Aksoy, 2017).

The damage caused by the genotoxic effects of detergents was also investigated by comet assay test. The damages were classified in five groups as type 0, type 1, type 2, type 3 and type 4 according to their degree of damage. Type 3 DNA damage was mostly seen in standard detergents at the highest concentrations and durations.

In A1 and B1 detergents, DNA damage from the head of the comet tail reached almost threefold, while eco-friendly A2 and B2 detergents showed DNA damage

of up to twice the size of the tail. As a result, the chemicals in the standard detergents caused much more damage on the DNA of *V.faba* root tip cells than eco-friendly detergents. A1 is the standart detergent that reaches the most intense tail DNA percentage while the A2 eco-friendly detergent has the lowest concentration when treated with the highest concentration and time period. Studies have also shown DNA fragmentation in *V. faba* root apical meristem cells and seedlings exposed to toxic compounds with the highest concentrations and different time periods (Arya et al., 2013; Liu et al., 2015; Ghosh et al., 2016, Iqbal, 2016; Hu et al., 2017; Cortés-Eslava et al., 2018; Youssef and Elamawi 2020).

As a result, it was determined that the percentage of tail DNA in increasing concentrations and durations was higher in standard detergents compared to eco-friendly detergents. The standard detergents caused more genetic damage because they produced more dense DNA-containing tails. As in the tail DNA percentage, olive tail moment (OTM) values showed similar results in terms of increasing concentration and duration. DNA tail percentage was studied with OTM parameters in a another study on the determination of genetic damage on onion root tips treated with 5-100% concentrations of coal ash (Chakraborty et al., 2009). OTM and the percentage of tail DNA correlate well with the concentrations of chemical substances with genotoxic effect and are parameters that give confidence in comet assay evaluations (Kumaravel et al., 2009). In our study, B1 standart detergent had the highest OTM value while the A2 eco-friendly detergent was the lowest. As a result OTM value of eco-friendly detergents remained lower than standard detergents.

Sobrino-Figueroa stated that since detergents are complex mixtures of different substances, in which additive and/or synergistic effects may occur, the deleterious effect caused by the dishwasher detergent was probably due to the combined effects of the ingredients of the detergent. (Sobrino-Figueroa, 2013).

When all results obtained in the study were evaluated in general, it was observed that eco-friendly detergents produced significantly less genotoxic effects on *V.faba* root tip cells than standard detergents. This is due to the fact that in the production of eco-friendly detergents that we have chosen to use in the study, a lower proportion of anionic material and nonionic material is used than standard detergents. In addition, the use of raw materials from coconut, palm, wheat and potatoes in production to reduce the proportion of chemicals in its content has also been a supportive factor in creating less genotoxic damage on living beings.

The results obtained in this study indicate that detergent wastes that reach foodstuffs through food chain cause serious damage to DNA. In light of all these findings, it is obvious that there are serious measures to be taken at the stage of detergent production and use.

Detergents should be treated more carefully in their production due to the negative effects on the life, development and genetics of living things. Detergents that cannot be sufficiently rinsed due to the use of more detergents than necessary are the main reasons that cause residual residues in washing dishes and laundry to cause direct health problems. As it provides only pleasant odor or softness, it should be more sensitive to the consumption of detergents used in addition to cleaning. The amount of waste detergent to be left to the environment should not be increased for such reasons, which is not necessary. Being conscious of consumption and having the consciousness of protecting the environment is extremely important for us to leave a cleaner environment for future generations.

In addition to the measures that consumers can take, there are some points that manufacturers should take into consideration. The products should be subjected to the necessary tests at every stage of production before they are put on sale. The studies on the determination of the harmful effects of detergents on the environment and the environment and the studies to reduce these damages should be made on the basis of the production of raw materials. Particularly in the case of detergents, the amount of phosphate used must be limited and the amounts involved in the water must be removed in phosphate treatment plants. The capacities of the treatment facilities should be increased in order to be able to make a healthier treatment. Any industrialization without taking measures to protect nature should be prevented. Drinking and operating water must be checked periodically. Water quality parameters should be checked regularly by authorized institutions.

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