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Cytotoxic and Genotoxic Effects of Oxyfluorfen on The Somatic Cells of *Allium cepa*

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ABSTRACT

In this work, the cytotoxic and genotoxic influences of oxyfluorfen herbicide were surveyed by examining of mitotic index, mitotic stages, chromosomal anomalies, micronucleus percentage, and comet assay parameters on the somatic cells of *Allium cepa*. The roots were treated with 0.2, 0.4, 0.8 and 1.6 ppm herbicide concentrations with 12, 24 and 36 h. application periods. Mitotic index was noticeably diminished by oxyfluorfen in each application group when matched with their control, except for 0.2 ppm. The percentages of mitotic stages were altered. Oxyfluorfen enhanced drastically the aberrant cell ratio at all application groups and application periods in contrast to their control, excluding 0.2 ppm. Mitotic anomalies were noted as disturbed prophase, stickiness, C-mitosis, chromatid bridges and laggards. The micronucleus was detected at interphase and its percentage was determined in the applied concentrations. Also, the comet assay was employed to examine the single strand breakages. Almost all of the used concentrations of oxyfluorfen increased DNA losses. A positive relationship was discovered between micronucleus occurrence and DNA loss.

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Allium cepa'nın Somatik Hücreleri Üzerine Oxyfluorfen'in Sitotoksik ve Genotoksik Etkileri

ÖZET

Bu çalışmada, *Allium cepa*'nın somatik hücrelerindeki mitotik indeks, mitotik aşamalar, kromozomal anormallikler, mikronükleus yüzdesi ve komet test parametreleri incelenerek oxyfluorfen herbisitinin sitotoksik ve genotoksik etkileri araştırılmıştır. Kökler 0.2, 0.4, 0.8 ve 1.6 ppm herbisit konsantrasyonları ile 12, 24 ve 36 saat uygulama süreleriyle muamele edilmiştir. Mitotik indeks, 0.2 ppm haricinde, kontrol ile eşleştirildiğinde her uygulama grubunda oxyfluorfen tarafından gözle görülür şekilde azalmıştır. Mitotik aşamaların yüzdeleri değişmiştir. Oxyfluorfen, 0.2 ppm hariç olmak üzere, kontrollerinin aksine tüm uygulama gruplarında ve uygulama sürelerinde anormal hücre oranını önemli ölçüde artırmıştır. Mitotik anormallikler, düzgün dağılmayan profaz, yapışkanlık, C-mitoz, kromatid köprüleri ve kalgın kromozomlar olarak kaydedilmiştir. Mikronükleus, interfazda tespit edilmiş ve uygulanan konsantrasyonlarda yüzdesi belirlenmiştir. Ayrıca, comet analizi tek zincir kırılmalarını incelemek için kullanılmıştır. Oxyfluorfen'in kullanılan hemen hemen tüm konsantrasyonları DNA kayıplarını artırmıştır. Mikronükleus oluşumu ile DNA kaybı arasında pozitif bir ilişki keşfedilmiştir.

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INTRODUCTION

Herbicides are commonly used in agricultural areas for controlling weeds (Karaismailoğlu, 2015; Vieira dos Santos et al., 2017). Oxyfluorfen is widely applied as a herbicide utilized pre- and post-emergence to manage annual grass weeds and broad-leaved in areas of the vegetables (EPA, 2002; El-Rahman et al., 2019). It is a diphenyl ether herbicide, and inhibits photosynthesis by impeding chlorophyll creation (Camadro et al., 1991). Also, the major effects obtained from oxyfluorfen analyzes are inhibition of

protoporphyrinogen oxidase's ensuing reticence of heme biosynthesis in living organisms (Stagg et al., 2012). Oxyfluorfen might not processed by the plants and bacteria absorb it at extremely slow percentages (Calderón et al., 2015; El-Rahman et al., 2019).

Plants are useful for monitoring of the toxic effects on living organisms of the substances by analyzing chromosomal abnormalities and micronucleus analysis (Singh et al., 2008; Karaismailoğlu, 2015 and 2017). One of the most popular test plants is *Allium cepa* L. (Karaismailoğlu, 2015; Bonciu et al., 2018), which is one of the most often used plants in genotoxicity assessments because of sensitive and reliable (Grant, 1994).

The comet assay provides data of the thread breaks in genomic DNA, caused by toxic substances. This assay is of the great sensitivity and easiness, and it is frequently used to explain harmfulness. Reports about DNA loss in plants are remarkably few, though the plants are the major receivers of toxic materials including pesticides (Turkoğlu, 2012; Karaismailoğlu, 2015).

The onion is an important plant in cultivation. Oxyfluorfen is used to control weeds such as *Amaranthus retroflexus* L., *Anagallis arvensis* L., *Atriplex patula* L., *Bifora radianis* Bieb., *Bromus sterilis* L., *Chenopodium album* L., *Chrozophora tinctoria* (L.) A.Juss., *Convolvulus arvensis* L., *Digitaria Sanguinalis* (L.) Scop., *Echinochloa colonum* (L.) Link, *Echinochloa crus-galli* (L.) P. Beauv., *Matricaria chamomilla* L., *Sinapis arvensis* L., *Solanum nigrum* L., *Sonchus arvensis* L., *Veronica hederifolia* L. and *Xanthium strumarium* L. in onion agricultural areas at a concentration of roughly 0.4 parts per million (ppm) [Ministry of Agricultural and Rural Affairs of Republic of Turkey (MARA) (2009)]. Though there are researches on the effects of oxyfluorfen on various organisms (Stagg et al., 2012; Dragoeva et al., 2012; Calderón et al., 2015; Vieira dos Santos et al., 2017; El-Rahman et al., 2019), there is currently limited information available on the cytotoxic and genotoxic effects of oxyfluorfen on *A. cepa* (Dragoeva et al., 2012). This article has revealed the effects of oxyfluorfen on the mitotic cell division, somatic chromosomes and DNA material of *A. cepa* with different methods.

MATERIAL and METHODS

Allium cepa (2n = 16), 30–45 mm diameter, were get from a commercial center.

Oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene; $C_{15}H_{11}ClF_3NO_4$; molecular weight: 361.7 g mol^{-1}; CAS No: 42874-03-3] (NCBI, 2021) was obtained from a point of sale and used as stock for analyses. At First, the outer shells of the onions were removed without damaging the primordial roots, and were allowed to grow in tap water at 25°C in the darkness to avoid fluctuations in cell divisions. Once root lengths of onions extended 1–3 cm length, they were exposed to 0.2, 0.4, 0.8 and 1.6 ppm oxyfluorfen concentrations for 12, 24 and 36 h. The applied doses were chosen according to the used concentration in farming areas of oxyfluorfen, which is used with a concentration of roughly 0.4 parts per million (ppm), and its multiples (MARA, 2009). Tap water was used as a control group.

Cellular activities in root tips of onions were stopped with ethanol-glacial acetic acid fixative (3-1), and stored at 4°C overnight. Afterwards, tips were cleaned from fixative with distilled water, and they were hydrolyzed in 1 N HCl at 60°C for 12 minutes and dyed with Schiff's reagent for 2 h. 5 slides were chosen randomly from each treatment group for the mitotic index (MI= number of dividing cells x 100 / total number of cells), micronucleus (MN) frequency, and chromosome aberrations (Kara et al., 1994; Yildiz and Arikan, 2008; Karaismailoğlu et al., 2013; Karaismailoğlu, 2014a, 2014b and 2015).

The comet assay (single cell gel electrophoresis) was performed according to Gichner et al. (2004) and Karaismailoğlu (2015); however, minor changes were made. *A. cepa* root tips subjected to oxyfluorfen (0.2, 0.4, 0.8 and 1.6 ppm) were received on a glass, and were chopped with a blade to get the nuclei in a tampon (Tris). The slides were treated with 50 ml of 0.35% agarose gel in a saline phosphate tampon (pt) spread and dehydrated. Nuclei (20 ml) were attached 200 ml of agarose in pt, and when were spread over the slides. They were stayed for 1 h and slides were rinsed in TAE tampon (40 mM tris-acetate buffer, 1 mM EDTA) to detached the salt. Slides were horizontally accommodated into a gel electrophoresis tank containing electrophoresis tampon and dyed with 70 ml ethidium bromide (20 mg ml^{-1}) for 8 minutes. After, 50 nuclei for each slide were studied. 4 slides were scored for each group. Each picture was classified as for the fluorescence intensity of the comet tail and evaluated with one of the distinct genetic harm index classifications [tip 0 (0), 1 (1–100 arbitrary units = AU), 2 (101–200 AU), 3 (201–300 AU), or 4 (301–400 AU)] (Kocyiğit et al., 2005; Cavas, 2011; Turkoğlu, 2012; Karaismailoğlu, 2015).

The results were evaluated by variance analysis (ANOVA) in SPSS (2008) computer package and grade of importance was determined as $p < 0.05$. The Dunnett multiple-range test was used for finding of statistical importance among the detected variances. The outcomes of statistical examines were given in Tables 1-3.

RESULTS

The effects of different concentrations of oxyfluorfen on mitotic index and phase frequency are given in Table

1. Mostly, the different concentrations of oxyfluorfen substantially decrease the mitotic index of *A. cepa* somatic chromosomes when compared to the control ($p < 0.05$). As an exception, mitotic index does not indicate significant change in 0.2 ppm concentration of oxyfluorfen at all treatment periods in comparison with the control groups (Table 2). As a result of the 1.6 ppm oxyfluorfen application, mitotic index reduce significantly in the treatment times (Table 1). While the mitotic phase percentages are related with the control group in application periods, there are statistically noteworthy results ($p < 0.05$) (Table 1). As the ratio of prophase increase, the rates of metaphase and anaphase-telophase decrease in most of the examined cells. Also, a noteworthy increase in the percentages of anomaly in mitotic phases with increasing oxyfluorfen concentrations is realized (Table 1).

Table 1. The consequences of applications of oxyfluorfen and control on *A. cepa* cell division.
Çizelge 1. *A. cepa hücre bölünmesi üzerine oxyfluorfen ve kontrol uygulamalarının sonuçları.*

Period (h) *(Periyot)*	Concentrations *(Konsantrasyonlar)*	Number of analyzed cells *(Analiz edilen hücre sayısı)*	MI±SD	Cell division stages *(Hücre bölünmesi aşamaları)*					
				Prophase (%) *(Profaz)*		Metaphase (%) *(Metafaz)*		Ana-telophase (%) *(Ana-telofaz)*	
				Total *Toplam*	Aberrant *Anormal*	Total *Toplam*	Aberrant *Anormal*	Total *Toplam*	Aberrant *Anormal*
12	Control	6000	26.19±0.14	27.48	-	41.55	-	30.97	-
	0.2 ppm	6000	26.03±0.17	27.45	-	35.62*	-	26.93*	-
	0.4 ppm	6000	25.44±0.11*	27.49	2.05*	33.45*	2.31*	39.06*	2.88*
	0.8 ppm	6000	21.78±0.23*	30.84*	3.21*	32.79*	3.17*	36.37*	4.26*
	1.6 ppm	6000	16.89±0.32*	32.31*	4.96*	30.65*	3.99*	37.04*	7.35*
24	Control	6000	25.51±0.38	31.88	-	29.13	-	38.99	-
	0.2 ppm	6000	25.43±0.21	33.42*	-	29.15	-	37.43*	-
	0.4 ppm	6000	24.63±0.24*	34.71*	3.02*	32.18*	2.99*	33.11*	4.79*
	0.8 ppm	6000	22.74±0.15*	32.95*	3.94*	29.46*	3.77*	37.59*	5.93*
	1.6 ppm	6000	16.59±0.09*	35.62*	5.87*	33.69*	6.01*	30.69*	8.48*
36	Control	6000	26.10±0.12	30.48	-	32.65	-	36.87	-
	0.2 ppm	6000	25.99±0.22	30.45	-	33.28	-	36.27	-
	0.4 ppm	6000	24.73±0.18*	32.57*	4.71*	29.15*	5.11*	38.28	5.23*
	0.8 ppm	6000	21.94±0.33*	34.51*	6.59*	34.91*	6.78*	30.58*	7.81*
	1.6 ppm	6000	14.05±0.15*	36.68*	10.22*	35.77*	9.05*	27.55*	10.02*

*Dissimilar from control group P<0.05; MI: Mitotic Index; SD: Standart Deviation
* *Kontrol grubundan farklı P <0.05; MI: Mitotik İndeks; SD: Standard Sapma*

Chromosome anomalies have been assessed in dividing cells. The kinds and frequencies of chromosome anomalies caused by herbicide treatments are disclosed in Figure 1 and Table 2. Applications have induced 5 types of common anomalies: disturbed prophase, stickiness, C-mitosis, chromatid bridges and laggards.

The results of the micronucleus test in *A. cepa* applied to control sets and concentrations of oxyfluorfen are given in Figure 1 and Table 3. The frequency of micronucleus has been found to trigger of the application dose and noticed to be markedly different in all treatments when compared to the control ($p < 0.05$), apart from 0.2 ppm. It is noticeably greater at 1.6 ppm than the other doses of oxyfluorfen in all the treatments (Table 3). However, 12, 24 and 36 h treatments of 0.4 and 0.8 ppm concentrations of oxyfluorfen importantly increased the frequency of micronucleus.

The findings of the comet assay are given in Figure 2 and Table 3. DNA damage is noticeably higher in almost all the used concentrations when compared to control group ($p < 0.05$), except 0.2 ppm. 1.6 ppm concentrations have caused the most great genotoxic aberration in comparison of other treatments (Table 3). There is no observed difference between control and 0.2 ppm in the application times.

DISCUSSION

Random or unrestrained utilization of the chemical substances such pesticides has commonly caused to ecological pollution with following in damaging effects on live organisms. Hence, evaluation of the processing paths of oxyfluorfen and its impact on MI, somatic chromosomes and DNA single chain, offer valuable information about the influences of these commonly used genotoxic materials (Karaismailoğlu, 2015). Mitotic index might use as a biological signal in the cell increment, which gauges the ratio of cells in various mitotic phases (Ping et al., 2012; Karaismailoğlu, 2015). The influences of different concentrations of oxyfluorfen on MI in *A. cepa* somatic chromosomes are

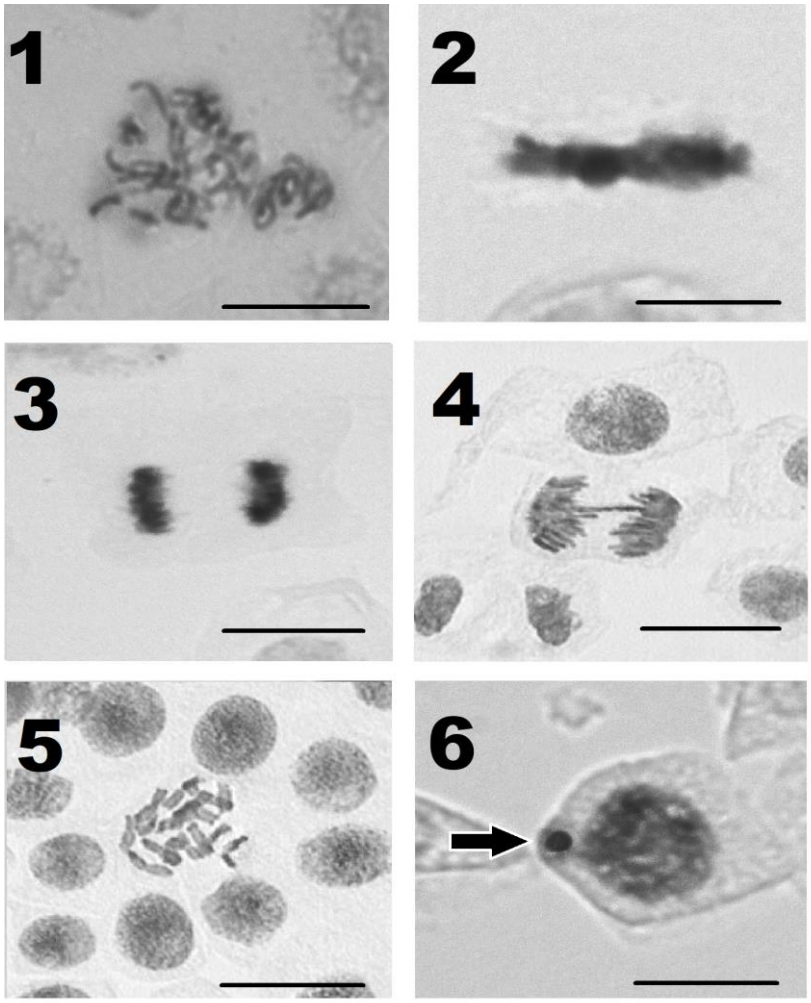

Figure 1. Mitotic deformities produced by oxyfluorfen in somatic cells of *A. cepa*; 1: disturbed prophase, 2-3: stickiness, 4: chromatid bridge, 5: C-mitosis, 6: micronucleus (Scale bars=10 µm).

Şekil 1. A. cepa'nın somatik hücrelerinde oxyfluorfen'in sebep olduğu mitotik şekil bozuklukları; 1: düzgün dağılmayan profaz, 2-3: yapışıklık, 4: kromatid köprüsü, 5: C-mitoz, 6: mikronükleus (Ölçekler=10 µm).

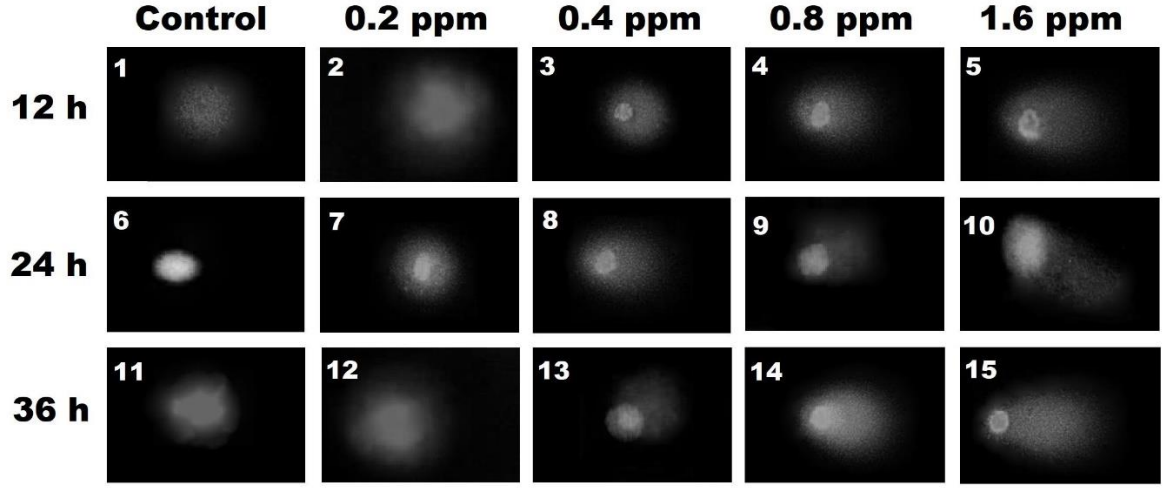

Figure 2. The fluorescence pictures of single cell gel electrophoresis, displaying different concentrations of oxyfluorfen effects on DNA

Şekil 2. DNA üzerinde oxyfluorfen'in farklı konsantrasyonlarının etkilerini gösteren tek hücreli jel elektroforezinin floresans resimleri

Table 2. Type and frequency of abnormalities in chromosomes of *A. cepa* treated with oxyfluorfen and control groups.
Çizelge 2. *Oxyfluorfen ve kontrol grupları ile muamele edilen A. cepa'nın kromozomlarındaki anormalliklerin tipi ve sıklığı.*

Period(h) *(Periot)*	Concentrations *(Konsantrasyonlar)*	Percent of anomalies±SD *(Anormalliklerin yüzdesi±SD)*					Percent of total anomality *(Toplam anormalliğin yüzdesi)*
		Disturbed prophase *(Düzgün dağılmayan profaz)*	Chromatid bridge *(Kromatid köprüsü)*	Stickiness *(Yapışıklık)*	C-mitosis *(C-mitoz)*	Laggards *(Kalgın kromozom)*	
12	Control	-	-	-	-	-	-
	0.2 ppm	-	-	-	-	-	-
	0.4 ppm	1.96±0.19*	1.56±0.12*	0.88±0.11*	1.49±0.12*	0.24±0.04*	6.13*
	0.8 ppm	2.78±0.12*	2.97±0.41*	1.94±0.13*	2.33±0.15*	0.89±0.06*	10.91*
	1.6 ppm	3.44±0.15*	5.13±0.22*	4.55±0.27*	2.96±0.18*	1.73±0.11*	17.81*
24	Control	-	-	-	-	-	-
	0.2 ppm	-	-	-	-	-	-
	0.4 ppm	2.81±0.09*	1.09±0.09*	4.15±0.22*	1.29±0.18*	1.02±0.06*	10.36*
	0.8 ppm	3.15±0.18*	3.63±0.21*	4.26±0.19*	3.12±0.09*	1.85±0.12*	16.01*
	1.6 ppm	3.62±0.26*	5.39±0.27*	5.74±0.12*	4.17±0.31*	2.24±0.15*	21.16*
36	Control	-	-	-	-	-	-
	0.2 ppm	-	-	-	-	-	-
	0.4 ppm	5.12±0.24*	1.33±0.15*	4.61±0.17*	4.01±0.15*	1.56±0.09*	16.63*
	0.8 ppm	6.78±0.18*	3.27±0.12*	6.13±0.15*	4.54±0.18*	2.88±0.27*	23.60*
	1.6 ppm	6.91±0.09*	6.04±0.24*	8.79±0.36*	5.03±0.09*	3.97±0.15*	30.74*

*Dissimilar from the control P<0.05
** Kontrol grubundan farklı P <0.05*

Table 3. The influences of oxyfluorfen on the MN and DNA damage tests.
Çizelge 3. *MN ve DNA hasar testleri üzerinde oxyfluorfen'in etkileri.*

Period (h) (Periot)	Concentrations *(Konsantrasyonlar)*	MN (%)	DNA damage (AU±SD) *(DNA hasarı)*
	Control	-	9.77±1.49
	0.2 ppm	-	9.81±0.75
12	0.4 ppm	0.13*	13.64±1.31*
	0.8 ppm	0.15*	15.15±0.62*
	1.6 ppm	0.16*	19.56±2.88*
	Control	-	10.03±0.15
	0.2 ppm	-	10.21±0.39
24	0.4 ppm	0.16*	20.17±1.65*
	0.8 ppm	0.19*	26.28±2.51*
	1.6 ppm	0.23*	33.47±2.83*
	Control	-	10.45±0.35
	0.2 ppm	-	10.63±0.99
36	0.4 ppm	0.26*	35.76±3.51*
	0.8 ppm	0.30*	39.83±4.15*
	1.6 ppm	0.41*	58.02±3.66*

*Dissimilar from control group P<0.05; MN: Micronucleus; AU: Arbitrary units.
** Kontrol grubundan farklı P <0.05; MN: Mikronükleus; AU: Prosedür tanımlı birim.*

offered in Table 1. Mitotic effect is significantly decreased with increasing oxyfluorfen concentrations at each application period in comparison with the control group (Table 1). Also, MI is not significantly different at application of 0.2 ppm oxyfluorfen (Table 1). Besides, 1.6 ppm of oxyfluorfen is the greatest genotoxic concentration, and has more mitodepressive effect than 0.4, 0.8 and 1.6 ppm concentrations at application periods. If the mitotic index decrease lower 22% in comparison with the control, this causes poisonous impacts on living systems (Karaismailoğlu, 2015). Also, the decreases lower 50% are of the sublethal impacts and they are accepted as toxicity limit (Yildiz and Arikan, 2008). In this work, sublethal impact has found in 1.6 ppm concentration when compared to the control in 12, 24 and 36 h treatments, and sublethal impact valuation are defined as 35.50%, 34.96% and 46.16%, respectively. The obtained outcomes are suitable with the results of the earlier works (Liman et al., 2011; Dragoeva et al., 2012; Karaismailoglu, 2015; Liman and Özkan, 2019).

The effects of oxyfluorfen on cycle phases in the somatic cells of *A. cepa* are showed in Table 1. As the phase rates are matched with the control in all application periods, there are statistically significant outcomes ($p < 0.05$). As the frequency of prophase increase, the frequency of metaphase and ana-telophase decrease in examined cells. The influence on mitotic stages of oxyfluorfen can be ascribed to the inhibition of prophase or hold in the mitotic stages in answer to mitotic pressure (Scolnic and Halazonetis, 2000; Karaismailoğlu, 2015). Furthermore, the applied doses of oxyfluorfen have generally caused a significant increase in the percentages of aberration in mitotic stages in *A. cepa* somatic cells. Similar results have been also reported in previous surveys (Yildiz and Arikan, 2008; Dragoeva et al., 2012; Bonciu et al., 2018; Rosculete et al. 2019).

Chromosome aberrations provide useful information for monitoring the negative effects of environmental contaminants on hereditary material (Carita and Marin-Morales, 2008). The effects of the used oxyfluorfen doses and control applications on mitotic aberrations and percentages of a total anomaly in the *A. cepa* somatic cells are given in Table 2. The detected chromosomal aberrations such as disturbed prophase, stickiness, chromatid bridge, C-mitosis and laggards are displayed in Figure 1. The most frequent types of chromosomal aberration are recorded as stickiness and chromatid bridges (Table 2 and Figure 1), which are chromatid tip anomaly (Badr, 1983; Karaismailoğlu, 2015). Stickiness can occur from deterioration of formation such as depolymerization in genetic material (Mercykutty and Stephen, 1980); however, chromatid bridge comprises disintegration and fusion of chromatid (Shehab and Adam, 1983). Likewise, a high frequency of the disturbed prophase is defined. This might arise from chromatid loss. In C-mitosis, which is the other common type, oxyfluorfen inhibits spindle formation with a colchicine-like effect (Badr, 1983). Enhanced oxyfluorfen concentrations have triggered an increment of the ratio of total anomalies. Genotoxicity of the used herbicide is higher than normal with use of the highest concentration (1.6 ppm) for each application period. These outcomes agree with former studies (Ping et al., 2012; Karaismailoğlu et al., 2013; Karaismailoğlu, 2014b, 2015; Bonciu et al., 2018; Liman and Özkan, 2019; Rosculete et al.,2019; Aydın and Liman, 2020).

Micronucleus analyses are of the crucial role in the review of the genotoxicity influences of the substances (Gebel et al., 1997; Karaismailoğlu, 2014a, 2014b and

2015). The formation and frequency of micronucleus are offered in Table 3 and Figure 1. Mostly, micronucleus frequency has noticeably increased with enhanced oxyfluorfen concentration in comparison with the control, excluding 0.2 ppm at all treatment periods ($p < 0.05$). The micronucleus frequency is significantly greater at the greatest oxyfluorfen concentration (1.6 ppm) than others. Micronucleus happens from microtubules ruptures and corruptions at the polyploidy because of affecting pieces in chromosomes (Konuk et al., 2007; Karaismailoğlu, 2015).

The comet assay which is a fast and vulnerable analysis to evaluate the genotoxic risk of substances is used for finding the genotoxic effect of oxyfluorfen in *A. cepa* somatic cells (Figure 2 and Table 3) (Ribas et al., 1995; Karaismailoğlu, 2015; Srivastava and Singh, 2020). This test lets the definition of DNA strand pieces in single cells. The comet assay has revealed that the used concentrations of oxyfluorfen induce DNA loss in *A. cepa* somatic cells in comparison with the controls. DNA harm happening with oxyfluorfen might be because of growing action of free radicals and triggering DNA strand fractures (Liman et al., 2011). The current survey discovers that there are considerable increment in the percentages of both micronucleus and DNA loss after oxyfluorfen treatments on *A. cepa* somatic cells (Figure 2 and Table 3).

CONCLUSION

As a result, application to oxyfluorfen might present a genotoxylogical risk to genetic material in *A. cepa*. If oxyfluorfen is used in doses below 0.4 ppm, the damage effects on *A. cepa* would be reduced.

Statement of Conflict of Interest

Author has declared no conflict of interest.

REFERENCES

Aydın G, Liman R 2020. Cyto-genotoxic effects of Pinoxaden on *Allium cepa* L. roots. Journal of Applied Genetics, 61: 349-357.

Badr A 1983. Mitodepressive and chromotoxic activities of two herbicides in *A. cepa*. Cytologia, 48(2): 451-457.

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu DH, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV, Schiff S, Slusarczyk J, de Souza CP, Srivastava A, Sutan A, Papini A 2018. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. Caryologia, 71(3): 191-209.

Calderón MJ, Real M, Cabrera A, Koskinen WC, Cornejo J, Hermosín MC 2015. Influence of olive oil mill waste amendment on fate of oxyfluorfen in southern Spain soils. CLEAN-Soil, Air, Water, 43: 1107-1113.

Camadro JM, Matringe M, Scalla R, Labbe P 1991. Kinetic studies on protoporphyrinogen oxidase inhibition by diphenyl ether herbicides. Biochemical Journal, 277: 17-21.

Carita R, Marin-Morales MA 2008. Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. Chemosphere, 72(5): 722-725.

Cavas T 2011. In vivo genotoxicity evaluation of atrazine and atrazine–based herbicide on fish *Carassius auratususing* the micronucleus test and the comet assay. Food and Chemical Toxicology, 49(6): 1431-1435.

Dragoeva A, Koleva V, Hasanova N, Slanev N 2012. Cytotoxic and Genotoxic Effects of Diphenyl-ether Herbicide GOAL (Oxyfluorfen) using the *Allium cepa* test. Research Journal of Mutagenesis, 2(1): 1-9.

El-Rahman GIA, Ahmed SAA, Khalilb AA, Abd-Elhakim YM 2019. Assessment of hematological, hepato-renal, antioxidant, and hormonal responses of *Clarias gariepinus* exposed to sub-lethal concentrations of oxyfluorfen. Aquatic Toxicology, 217: 1-12.

EPA 2002. Oxyfluorfen: Human Health Risk Assessment Health Effects Division (HED) Chapter for Registration Eligibility Decision (RED) Document. Registration Case No. 2490. Registration Case No. 2490. Chemical No. 111601.

Gebel T, Kevekordes S, Pav K, Edenharder R, Dunkelberg H 1997. In vivo genotoxicity of selected herbicides in the mouse bone-marrow micronucleus test. Archives of Toxicology, 71(3): 193-197.

Gichner T, Patková Z, Sza'ková J, Demnerová K 2004. Cadmium induces DNA damage in tobacco roots, but no DNA damage, somatic mutations or homologous recombinations in tobacco leaves. Mutation Research, 559(1–2): 49-57.

Grant WF 1994. The present status of higher plant bioassays for the detection of environmental mutagens. Mutation Research, 310(2): 175-185.

Kara M, Şanda MA, Ateş A 1994. Cytogenetic Effects of the Insecticide Cypermethrin on the Root Meristems of *Allium cepa* L. Turkish Journal of Biology,18: 323-331.

Karaismailoğlu MC, Inceer H, Hayırlıoğlu-Ayaz S 2013. Effects of quizalofop-P-ethyl herbicide on the somatic chromosomes of Helianthus annuus (Sunflower). Ekoloji, 22(89): 49-56.

Karaismailoğlu MC 2014a. Investigation of the cytotoxic and genotoxic effects of *Artemisia annua* methanol extract with the *Allium* test. Ekoloji, 23(91): 64-74.

Karaismailoğlu MC 2014b. Evaluation of potential genotoxic effect of trifluralin in *Helianthus annuus*

L. (Sunflower). Caryologia, 67(3): 216-221.

Karaismailoğlu MC 2015. Investigation of the potential toxic effects of prometryne herbicide on *Allium cepa* root tip cells with mitotic activity, chromosome aberration, micronucleus frequency, nuclear DNA amount and comet assay, Caryologia, 68(4): 323-329.

Karaismailoğlu MC 2017. Assessments on the potential genotoxic effects of fipronil insecticide on *Allium cepa* somatic cells. Caryologia, 70(4): 378-384.

Kocyiğit A, Keles H, Selek S, Guzel S, Celik H, Erel O 2005. Increased DNA damage and oxidative stress in patients with cutaneous leishmaniasis. Mutation Research, 585(1–2): 71-78.

Konuk M, Liman R, Cigerci IH 2007. Determination of genotoxic effect of Boron on *Allium cepa* root meristematic cells. Pakistan Journal of Botany, 39(1): 73-79.

Liman R, Cigerci IH, Akyıl D, Eren Y, Konuk M 2011. Determination of genotoxicity of fenaminosulf by *Allium* and comet tests. Pesticide Biochemistry and Physiology, 99(1): 61-64.

Liman R, Özkan S 2019. Cytotoxicity and Genotoxicity in *Allium cepa* L. Root Meristem Cells Exposed to the Herbicide Penoxsulam. Celal Bayar Üniversitesi Fen Bilimleri Dergisi,15: 221-226.

[MARA] Ministry of Agricultural and Rural Affairs (Turkey) 2009. General directorate of protection and control; plant protection products. Ankara: Ministry of Agricultural and Rural Affairs of Republic of Turkey.

Mercykutty VC, Stephen J 1980. Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test. Cytologia, 45(4): 769-777.

[NCBI] National Center for Biotechnology Information 2021. PubChem Compound Summary for CID 39327, Oxyfluorfen. Retrieved April 4, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Oxyfluorfen.

Ping KY, Darah I, Yusuf UK, Yeng C, Sasidharan S 2012. Genotoxicity of Euphorbia hirta: an *Allium cepa* assay. Molecules, 17(7): 7782–7791.

Rosculete CA, Bonciu E, Rosculete E, Olaru LA 2019. Determination of the Environmental Pollution Potential of Some Herbicides by the Assessment of Cytotoxic and Genotoxic Effects on *Allium cepa*. Int J Environ Res Public Health, 16(1):75-83.

Ribas G, Frenzilli G, Barale R, Marcos R 1995. Herbicide induced DNA damage in human lymphocytes evaluated by the single-cell gel electrophoresis (SCGE) assay. Mutation Research, 344(1–2): 41-54.

Scolnic D, Halazonetis T 2000. Chfr defines a mitotic stress checkpoint that delays entry into metaphase. Nature, 406: 430-435.

Shehab AS, Adam ZM 1983. Cytological effects of medicinal plants in Qatar III. Mitotic effect of water extract of *Anastatica hierochuntico* L. on *Allium cepa*. Cytologia, 48: 343-348.

Singh P, Srivastava AK, Singh AK 2008. Cell cycle stage specific application of cypermethrin and carbendazim to assess the genotoxicity in somatic cells of *Hordeum vulgare* L. Bulletin of Environmental Contamination and Toxicology, 81(3): 258-261.

Srivastava AK, Singh D 2020. Assessment of malathion toxicity on cytophysiological activity, DNA damage and antioxidant enzymes in root of *Allium cepa* model. Scientific Reports, 10: 886-896.

SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.

Stagg NJ, LeBaron MJ, Eisenbrandt DL, Gollapudi BB, Klaunig JE 2012. Assessment of possible carcinogenicity of oxyfluorfen to humans using mode of action analysis of rodent liver effects. Toxicological Sciences, 128: 334-345.

Turkoğlu S 2012. Determination of genotoxic effects of chlorfenvinphos and fenbuconazole in *Allium cepa* root cells by mitotic activity, chromosome aberration, DNA content, and comet assay. Pesticide Biochemistry and Physiology, 103(3): 224-230.

Vieira dos Santos E, Sáez C, Cañizares P, Martínez-Huitle CA, Rodrigo MA 2017. Reversible electrokinetic adsorption barriers for the removal of atrazine and oxyfluorfen from spiked soils. Journal of Hazardous Materials, 322: 413-420.

Yildiz M, Arikan ES 2008. Genotoxicity of quizalofop-P-ethyl herbicide using the *Allium cepa* anaphase-telophase chromosome aberration assay. Caryologia, 61(1): 45-52.