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Investigation of Toxic Effects of the Glyphosate on *Allium cepa*

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ARTICLE INFO

Research Article — Crop Production
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Received: 10 August 2010, Received in revised form: 26 July 2011, Accepted: 19 August 2011

ABSTRACT

In the present study, toxic effects of glyphosate on *Allium cepa* L. (Amaryllidaceae) cells were investigated. For this aim, we used the germination percentage, root length, seedling weight, malondialdehyde (MDA) level, frequency of micronucleus (MN), chromosomal aberrations (CAs) and mitotic index (MI) as indicators of toxicity. In addition to the analyses mentioned above, we also examined changes in the root anatomy of *A. cepa* seeds treated with glyphosate. Glyphosate was applied with three different doses (100, 250 and 500 mg l^{-1}). The results showed significant alterations in the germination percentage, root length, seedling weight, MDA level, MN, CAs and MI frequency depending on treatment doses in the glyphosate treated groups. Glyphosate-exposure significantly reduced the germination percentage, root length and seedling weight in all the treatment groups (P<0.05). But, an increase in the MN and CAs formation (P<0.05) was observed. It was also found that glyphosate has a mitodepressive action on mitosis, and the MI was decreased depending on the dose of applied-glyphosate (P<0.05). Besides, 100, 250 and 500 mg l^{-1} doses of glyphosate significantly enhanced the lipid peroxidation and caused an increase in malondialdehyde (MDA) levels at each dose treatment (P<0.05). Moreover, light micrographs showed anatomical damages such as unclear vascular tissue, unclear epidermis layer, cell deformation, unusual form of cell nucleus (usually flat) and binuclear cells. Each dose of glyphosate caused severe toxic effects on *A. cepa* cells and the strongest toxic effect was observed at the dose level of 500 mg l^{-1}.

Keywords: Germination; Chromosomal aberration; Lipid peroxidation; Micronucleus; Root anatomy

Glifosatın *Allium cepa* Üzerine Toksik Etkilerinin Araştırılması

ESER BİLGİSİ

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Geliş tarihi: 10 Ağustos 2010, Düzeltmelerin gelişi: 26 Temmuz 2011, Kabul: 19 Ağustos 2011

ÖZET

Bu çalışmada, Glifosatın *Allium cepa* L. (Amaryllidaceae) üzerine toksik etkileri araştırılmıştır. Bu amaçla çimlenme yüzdesi, kök uzunluğu, ağırlık kazancı, malondialdehit (MDA) düzeyi, mikronukleus sıklığı (MN),

kromozomal anormallikler (CAs) ve mitotik indeks (MI) parametreleri toksisite indikatörü olarak kullanılmıştır. Bu parametrelere ilave olarak glifosat uygulanmış *A. cepa*'da kök anatomisindeki değişimler de araştırılmıştır. Glifosat uygulamasında 100, 250 ve 500 mg l^{-1} olmak üzere üç farklı doz kullanılmıştır. Sonuç olarak glifosat uygulanan gruplarda çimlenme yüzdesi, kök uzunluğu, ağırlık kazancı, malondialdehit düzeyi, mikronuklues sıklığı, kromozomal anormallikler ve mitotik indeks parametrelerinde doza bağlı olarak önemli değişimler saptanmıştır. Tüm uygulama gruplarında glifosat uygulaması ile çimlenme yüzdesinin, kök uzunluğunun ve ağırlık kazancının azaldığı belirlenmiştir (P<0.05). MN ve CAs oluşumunda ise artış kaydedilmiştir (P<0.05). Ayrıca glifosatın mitozu baskılayıcı etkiye sahip olduğu ve glifosat uygulaması ile mitotik indeksinde doza bağlı olarak azaldığı görülmüştür (P<0.05). Bununla birlikte 100, 250 ve 500 mg l^{-1} glifosat uygulamalarının lipit peroksidasyonunu önemli derecede hızlandırdığı ve tüm doz uygulamalarında MDA seviyelerinde artışa neden olduğu belirlenmiştir (P<0.05). Bununla birlikte ışık mikrograflarından belirsiz vaskuler doku ve epidermis tabakası, hücre deformasyonu, anormal nucleus (genellikle düz), ve binükleer hücre gibi anatomik hasarların meydana geldiği gözlenmiştir. Bu sonuçlar ile her bir glifosat doz uygulamasının *A. cepa* hücreleri üzerine farklı toksik etkiler gösterdiği ve en güçlü toksik etkinin 500 mg l^{-1} doz uygulamasında oluştuğu gözlenmiştir.

Anahtar sözcükler: Çimlenme; Kromozomal anormallikler; Lipit peroksidasyonu; Mikronukleus; Kök anatomisi

1. Introduction

Crop diseases and pests have increased with the development of technology in agricultural production. Thus, the losses in agricultural production due to the diseases and pests have been worked in order to reduce crop losses. A pest is defined as a plant or animal that is harmful to man or the environment (Metin et al 2003).

Pesticides are substances or a mixture of substances used by human society to mitigate the pests and other organisms that affect food production or human health (Ecobichon 2010). They usually act by disrupting some component of the pest's life processes to kill or inactivate it. Pesticides also include substances such as insect attractants, herbicides, plant defoliants, desiccants and plant growth regulators (Draggan 2010).

Despite the tremendous advantages of pesticide use, there is a need for scientific evaluation and control of these products. Pesticides are found as common contaminants in soil, air and water, and on non-target vegetation in urban landscapes. They can be harmful to plants and animals ranging from beneficial soil microorganisms and insects, non-target plants, fish, birds, and other wildlife (Sanders 2010). Besides, human beings are particularly sensitive targets for pesticides. Chronic poisoning in human beings and mammals due to pesticides results from pesticide decay derivatives that are taken with food chain (Metin et al 2003).

Herbicides are a substance used by farmers to kill unwanted plants and can be used to clear roadside weeds, trees and bush (Simmons 2010). They can also kill invasive weeds in parks and wilderness areas which may cause environmental damage. Herbicides are commonly applied in ponds and lakes to control algae and plants such as water grasses (Hamel 2010).

Roundup is the brand name of a systemic, broad-spectrum herbicide produced by the U.S. and contains the active ingredient glyphosate [N-(phosphonomethyl)glycine]. Glyphosate is the most used herbicide in the USA, and Roundup is the number one selling herbicide worldwide since at least 1980 (Ho & Cummins 2010). Glyphosate is effective in killing a wide variety of plants, including grasses, broadleaf, and woody plants. Glyphosate kills the target organisms by inhibiting the enzyme, 5-enolpyruvoyl-shikimate-3-phosphate synthetase (EPSPS), essential for the formation of aromatic amino acids such as phenylalanine, tyrosine and tryptophan (Piesova 2005).

Roundup shows adverse effects in all standard categories of toxicological testing, including medium-term toxicity, long-term toxicity, genetic damage, effects on reproduction, and carcinogenicity. Scientific studies have shown that Roundup formulations and metabolic products cause the death of human embryonic, placental, and umbilical cells *in vitro* at low

concentrations (Richard et al 2005; Benachour & Seralini 2009). Renal and hepatic impairment are also frequent and usually reflect reduced organ perfusion. Respiratory distress, impaired consciousness, pulmonary edema, infiltration on chest x-ray, shock, arrhythmias, renal failure requiring haemodialysis, metabolic acidosis and hyperkalaemia may supervene in severe cases. Bradycardia and ventricular arrhythmias are often present pre-terminally (Bradberry et al 2004).

Besides, the researches demonstrate that glyphosate reduces the growth of beneficial soil-dwelling mycorrhizal fungi. Yousef et al (1996) reported a reduction in sperm production by 50 percent in rabbits exposed to glyphosate. In another study, it was shown that glyphosate causes liver damage in rats, by the leakage of intracellular liver enzymes (Benedetti et al 2006). In many studies, an association between glyphosate use and the risk of non-Hodgkin lymphoma was suggested (McDuffie et al 2001; Hardell et al 2002; Roos et al 2003). Besides, there is direct evidence that glyphosate inhibits RNA transcription in animals at low concentration levels (Marc et al 2005).

The *Allium cepa* assay is an efficient test for genotoxicity and cytotoxicity of environmental contaminants such as pesticides. So in this study we used *Allium cepa* assay for determine the toxic effects of glyphosate. Although there are few published clinical studies on glyphosate in the literature, unfortunately, there are no published a comprehensive data on glyphosate toxicity in plants. The aim of the present study was to evaluate toxicity induced by glyphosate in *A. cepa* cells.

2. Materials and Methods

2.1. Chemicals

Roundup UltraMax (450 g l^{-1} glyphosate) was obtained from Bayer, Istanbul, TURKEY.

2.2. Preparation of root tips

In this study, healthy and proximate equal-sized *A. cepa* L. seeds were selected. The seeds were washed in ultra-distilled water. The seeds in the control and treatment groups were placed into the clean glass beakers. The seeds were divided into four groups (n=25):

Group I (control) was treated with only tap water, for 72 consecutive hours.

Group II was treated with 100 mg l^{-1} dose of glyphosate, for 72 consecutive hours.

Group III was treated with 250 mg l^{-1} dose of glyphosate, for 72 consecutive hours.

Group IV was treated with 500 mg l^{-1} dose of glyphosate, for 72 consecutive hours.

Oral LD50 values for glyphosate are greater than 10,000 mg kg^{-1} in mice, rabbits, and goats (Monsanto Company 1985). The reported 4-hour rat inhalation LC50 values for glyphosate were 5 to 12 mg l^{-1} (Weed Science Society of America 1994). The glyphosate doses used in this study was used according to Rank et al (1993).

2.3. Analysis for germination percentage, root length and seedling weight

For the cytogenetic analysis, when the roots attained a length of approximately 1–2 cm, they were treated with distilled water, and temporary squash preparations were made as described below. The root lengths were determined by radicula formation bases. At the end of 72 h, the root lengths of the germinated seeds were measured with a millimetric ruler. The seedling weight was determined by measuring the differences between the seedling weight before and after glyphosate treatment with a sensitive balance. The germination percentage of the seeds was calculated using the following equation:

$$G\ (\%) = \frac{GS}{TS} \times 100 \quad (1)$$

where G is the germination (%); GS is the germinated seeds; and TS is the total seeds.

2.4. Quantification of lipid peroxidation

The level of lipid peroxidation was determined by measuring the amount of MDA according to Nair & Turner (1984). About 0.5 g of root tissues from the control and treated groups were cut into small pieces and homogenized by the addition of 5 ml of 5% trichloroacetic acid (TCA) solution. The homogenates were then transferred into fresh tubes and centrifuged at 12000 rpm for 15 min at

room temperature. Equal volumes of supernatant and 0.5% thiobarbituric acid (TBA) in 20% TCA solution were added into a new tube and boiled at 96°C for 25 min. The tubes were transferred to an ice-bath and then centrifuged at 10000 rpm for 5 min. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm, 0.5% TBA in 20% TCA solution was used as the blank. MDA contents were calculated using the extinction coefficient of 155 m^{-1} cm^{-1}. Values of MDA contents were taken from measurements of three independent samples, and SD of the means were calculated.

2.5. Micronucleus (MN) assay

The root tips were fixed for 6 h in a Clarke's fixator (3: glacial acetic acid / 1: distilled water), washed for 15 min in ethanol (96%) and stored in ethanol (70%) in the fridge at +4°C until making the microscope slides. The root tips were hydrolyzed in 1N HCl at 60°C for 20 min, treated with 45% CH3COOH solution for 30 min and stained for 24 h in Acetocarmine. After staining, the root meristems were separated and squashed in 45% CH3COOH solution (Staykova et al 2005). For MN analysis, 1000 cells were scored for each slide. Micronucleated cells were examined with a binocular light microscope (Japan, Olympus BX51) at X500 magnification. For the scoring of MN the following criteria were adopted from Fenech et al. (2003). These: (i) the diameter of MN should be tenth of the main nucleus, (ii) MN should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary, (iii) MN should have similar staining as the main nucleus.

2.6. Chromosome obtain and mitotic index

The excised root tips were fixed in Clarke's fixator for 24 h, and kept in 70% alcohol in the fridge at 4°C. These samples were sectioned routinely and stained with Feulgen. For each group 10 root tip squashes were prepared and 500 mitotic cells were counted from random fields on each slide (Inceer et al 2003; Wei 2004). Chromosomal analyses were made in anaphase cells in order to identify chromosome alterations such as chromosome bridges, loops and fragments as well as alterations in the centromere and mitotic spindle disturbances, through the appearance of multipolar anaphases.

The microscopic preparations were analyzed to determine the cell division intensity calculating the MI (%). The latter was determined as a percentage between the number of dividing cells (N') and the total number of cells analyzed (N) in equation:

$$MI\ (\%) = \frac{N'}{N} \times 100 \qquad (2)$$

2.7. Anatomical investigation

To analyze anatomical changes, the root tips were cultivated with different doses (100, 250 and 500 mg l^{-1}) of glyphosate. The root tips were separated and then washed by distilled water 2 times. The cross-section samples of the root tips were taken manually for anatomical studies. These sections were stained by Methylene Blue and cover-slipped with Entellan (Makbul et al 2008). All the photographs were taken with under a binocular light microscope (Japan, Olympus BX51).

2.8. Statistical analysis

The statistical analysis of data was carried out using SPSS for Windows version 10.0 statistical software (SPSS Inc, Chicago, USA). Statistically significant differences between the groups were compared using one-way analysis of variance (ANOVA) and Duncan's test. The data are displayed as means ± standard deviation (SD), and *P* values less than 0.05 are considered "statistically significant".

3. Results and Discussion

3.1. Germination percentage

The results from Table 1 clearly demonstrate that glyphosate has a detrimental effect on the germination of seed. A negative correlation was observed between glyphosate doses and the germination percentage. The germination percentage of the seeds treated with glyphosate was rather different from the control group. The

Table 1-Effect of various doses of glyphosate on the germination percentage of *A. cepa*
Çizelge 1-A. cepa'da çimlenme yüzdesi üzerine farklı dozlardaki glifosatın etkisi

Treatment time, hour	*Groups*[1]	*Number of germinated seeds*	*Number of not germinated seeds*	*Germination percentage, %*
72	Group I	24	1	96a
72	Group II	19	6	76b
72	Group III	15	10	60c
72	Group IV	10	15	40d
P values				<0.001

[1]Each group contains 25 seeds. Group I (control group) seeds were treated with tap water, Group II seeds were treated with 100 mg l^{-1} glyphosate, Group III seeds were treated with 250 mg l^{-1} glyphosate, Group IV seeds were treated with 500 mg l^{-1} glyphosate.
[a-d]: Statistical significance between means was analyzed using one-way ANOVA followed by Duncan's test as a post-ANOVA test (P<0.05). Means with the same letter (vertically) are not significantly different at the P<0.05 level.

highest germination percentage was observed in the seeds of the control group (in proportion as 96%). The lowest germination rate was observed at 500 mg l^{-1} dose of glyphosate. Glyphosate treatment caused a significant decrease in the germination percentage at all the doses. 100, 250 and 500 mg l^{-1} doses of glyphosate caused 24%, 40% and 60%, decrease of seed germination, respectively. These results showed that the effects of glyphosate on the germination percentage depend on its dose, and the germination percentage can be considered as a sensitive indicator for glyphosate toxicity. This information is parallel with the other toxicity datas. Yenish & Young (2000) investigated the effects of glyphosate on seed germination and seedling quality in *Triticum aestivum*. The results showed that glyphosate application fairly reduced percent germination, compared to untreated seeds. In a similar study, Mclaren & Don (2004) investigated the effect of glyphosate in barley crops. Experimental treatment of barley crops with glyphosate resulted a decrease in the levels of germination. In another study, Klingman & Murray (1976) reported a decreased germination percentage of turf grass seeds in the presence of glyphosate and paraquat herbicides.

3.2. Root length and seedling weight

The results related with the root length and seedling weight were given in Tables 2 and 3. Glyphosate treatment significantly decreased the root length and seedling weight of the seeds. The highest root length and seedling weight were determined in the control group at the end of 72 h. The least root length and seedling weight were observed in the seeds treated with 500 mg l^{-1} dose of glyphosate. In control group, final seedling weight of all the seeds increased by about 4.82 g according to initial weight. In the treatment groups, final weights of the seeds increased by about 3.84, 2.48 and 1.47 g according to initial weight at 100, 250 and 500 mg l^{-1} doses of glyphosate, respectively. The differences between treatment groups were statistically significant (P<0.05). Besides, there were significant differences among the control and treatment groups, and differences were statistically significant (P<0.05).

The inhibitory effect of glyphosate on root growth was reported by a few bio-monitoring studies. Many studies were reported that high concentrations of glyphosate may lead to inhibition of root growth in different plant species. Pline et al. (2002) investigated the physiological and morphological effects of glyphosate on cotton seedlings. As a result, they showed that glyphosate inhibited the development of lateral roots at concentrations of 0.01 or 0.1 μM in glyphosate-resistant and non- resistant cotton seedlings. Carlson & Donald (2006) investigated the effect of glyphosate on root development of *Cirsium arvense*. Increasing the rate of glyphosate progressively reduced the total number of root buds more than root biomass.

In previous studies related to the seedling weight, the effect of glyphosate on seed weight

Table 2-Effect of various doses of glyphosate on the root length of *A. cepa*
Çizelge 2-A. cepa 'da kök uzunluğu üzerine farklı dozlardaki glifosatın etkisi

Treatment time, hour	*Groups*[1]	*Min.*	*Max.*	*Average±SD*
72	Group I	4.40	5.90	5.14±0.42a
72	Group II	3.00	4.90	3.93±0.45b
72	Group III	2.70	3.70	3.24±0.25c
72	Group IV	1.50	2.60	2.23±0.23d
P values				<0.001

[1]Each group contains 25 seeds. Group I (control group) seeds were treated with tap water, Group II seeds were treated with 100 mg l^{-1} glyphosate, Group III seeds were treated with 250 mg l^{-1} glyphosate, Group IV seeds were treated with 500 mg l^{-1} glyphosate. All values the mean ± SD.
[a-d]: Statistical significance between means was analyzed using one-way ANOVA followed by Duncan's test as a post-ANOVA test (P<0.05). Means with the same letter (vertically) are not significantly different at the P<0.05 level.

Table 3-Effect of various doses of glyphosate on seedling weight of *A. cepa*
Çizelge 3-A. cepa'da ağırlık kazanımı üzerine farklı dozlardaki glifosatın etkisi

Treatment time, hour	*Groups*[1]	*Seedling Weight*		
		Initial	*Final*	*Weight change*
72	Group I	4.24±0.39e	9.06±0.45a	+4.82
72	Group II	4.22±0.44e	8.06±0.43b	+3.84
72	Group III	4.26±0.46e	6.74±0.51c	+2.48
72	Group IV	4.30±0.40e	5.57±0.35d	+1.47
P values		0.897	<0.001	<0.001

[1]Each group contains 25 seeds. Group I (control group) seeds were treated with tap water, Group II seeds were treated with 100 mg l^{-1} glyphosate, Group III seeds were treated with 250 mg l^{-1} glyphosate, Group IV seeds were treated with 500 mg l^{-1} glyphosate. All values the mean ± SD.
[a-d]: Statistical significance between means was analyzed using one-way ANOVA followed by Duncan's test as a post-ANOVA test (P<0.05). Means with the same letter (vertically) are not significantly different at the P<0.05 level.

was reported. Baig et al. (2003) investigated effect of glyphosate applications on seedling growth of *Pisum sativum*. They showed that glyphosate reduced seedling shoot fresh weight. In a similar study, Jeffery et al. (1981) investigated the effects of glyphosate applied at various stages of maturity to *Zea mays*, *Glycine max*, and *Sorghum halepense* plants. The results showed that glyphosate application decreased seed weight. In another study, Thomas et al. (2005) investigated the effect of glyphosate on reproductive development in *Senna obtusifolia*. As a result, the number of pods, seeds, and total seed weight were reduced by 79, 80, and 81%, respectively. Although the ultimate mechanism of glyphosate toxicity on the seedling weight is completely unknown, it seems plausible that glyphosate act as a blocking agent by interaction with the cell components. This condition may cause significant alterations in nutrient status and nutrient contents of tissues, and may reduce seed weight. Besides, it is known that glyphosate is absorbed by the soil matrix, and it blocks the shikimate pathway, reducing the biosynthesis of aromatic amino acids, followed by the arrest interruption of protein production and a general metabolic disruption of the phenylpropanoid pathway (Marchiosi et al 2009).

3.3. Lipid peroxidation (MDA content)

It is known that chemical stress causes molecular damage to plant cells either directly or indirectly through the formation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl and superoxide radicals (Blokhina et al 2001). Harmful ROS can damage biological molecules such as lipids which are altered by peroxidation. Measurement of MDA levels is routinely used as an indicator of lipid peroxidation under stress conditions (Meng et al 2007). MDA levels in root tips of *A. cepa* was given in Table 4. The results

showed that there was a significantly increase in the MDA levels of the roots exposed to glyphosate. The levels of MDA was increased by 84%, 212% and 263% in 100, 250 and 500 mg l^{-1} glyphosate treated groups, respectively. There was a statistically significant difference between the control and treatment groups (P<0.05) for MDA content. These observations are also in agreement with results reported by Sergiev et al. (2006) and Miteve et al. (2010) on the generation of lipid peroxidation products under glyphosate stress. These researchers reported a significantly increase in the MDA content in the root and leaves treated with different doses of glyphosate. Besides, it is known that glyphosate has a direct effect on the lipid peroxidation and this effect is not reversible (McLaren et al 2004).

3.4. Micronucleus (MN) frequency

In our study, the frequency of MN was also recorded. Only several the MN formation was observed in the control group, but a significant increase in MN formation was observed in all the seeds exposed to glyphosate (Figure 1a). The frequency of MN increased with increasing the glyphosate doses (Table 5). The highest frequency of MN was observed at 500 mg l^{-1} dose of glyphosate. There were statistically significant differences between the MN frequencies of the control and treatment groups (P<0.05). These findings suggest that glyphosate has toxic activity which induced MN formation in the root tip cells of *A. cepa*. No study so far has been undertaken to examine the MN induction role of glyphosate in plant tissues, but there was several studies on animals. Our observations are also in agreement with animal toxicity data reported by other authors. The results of animal studies indicated that herbicides induce MN formation generated by chromosomal, spindle and mitotic apparatus damages. Piesova (2005) investigated the frequency of MN in bovine peripheral lymphocytes after exposure to glyphosate i*n vitro* and reported that glyphosate induced MN formation. In another study, Manas et al. (2009) investigated the genotoxicity induced by glyphosate in Hep-2 cells of mice and reported an increase in the frequency of MN at applied three doses. In our opinion about this matter, glyphosate may enter the cell nucleus and bind to purine and pyrimidine bases or spindle proteins. These interactions may denature spindles and cause a delay in the formation of chromosome-spindle complex, and these conditions may cause MN formation.

3.5. Chromosomal aberrations (CAs) frequency and mitotic index (MI)

The effects of glyphosate on the CAs frequency and MI were given in Tables 6&7. In meristematic root tip cells of the control group seeds, only several CAs were found. But, glyphosate treatment caused an increase in the frequency of CAs in the root tip cells. In this study, CAs was induced by all the doses of glyphosate. Disturbed chromosome was the most common CAs type observed in all the treatments. The CAs types such as fragment (Figure 1b), sticky chromosome (Figure 1c), chromatin bridge (Figure 1d) and unequal distribution of chromatin (Figure 1e) was observed. Besides, glyphosate caused a decrease in MI at all the concentrations. As shown in Table 7, the MI of the treatment groups was very significantly different from the control group (P<0.05). These results revealed that the effect of glyphosate on the MI of *A. cepa* root tip cells depend on its doses. This knowledge is also in the agreement with the results of other authors. Rank et al (1993) investigated the potential genotoxic effect of glyphosate isopropylamine salt in *A. cepa*. The anaphase-telophase *Allium* test showed that glyphosate isopropylamine salt at concentrations of 1.44 and 2.88 mg l^{-1} caused a significant increase in CAs. In a study realized by Dimitrov et al. (2006), the genotoxic effect of glyphosate was compared in plant (*Crepis capillaris*) and mouse bone marrow test systems using CAs and MN. As a result, glyphosate did not induce CAs or MN in either test system. In another study, a laboratory study of human lymphocytes showed an increase in the frequency of sister chromatid exchanges following exposure to high doses of glyphosate (Vigfusson &Vyse 1980).

Table 4-Effect of various doses of glyphosate on malondialdehyde [(MDA) (µmol g^{-1} FW)] content
Çizelge 4-Malondialdehit (µmol g^{-1}) içeriği üzerine farklı dozlardaki glifosatın etkisi

Treatment time, hour	*Groups*[1]	*Min. MDA*	*Max. MDA*	*Average±SD*
72	Group I	8	15	11.36±2.06d
72	Group II	17	25	20.96±2.65c
72	Group III	30	40	35.44±3.29b
72	Group IV	35	47	41.24±3.74a
P values				<0.001

[1]Each group contains 25 seeds. Group I (control group) seeds were treated with tap water, Group II seeds were treated with 100 mg l^{-1} glyphosate, Group III seeds were treated with 250 mg l^{-1} glyphosate, Group IV seeds were treated with 500 mg l^{-1} glyphosate. All values the mean ± SD.
[a-d]: Statistical significance between means was analyzed using one-way ANOVA followed by Duncan's test as a post-ANOVA test (P<0.05). Means with the same letter (vertically) are not significantly different at the P<0.05 level.

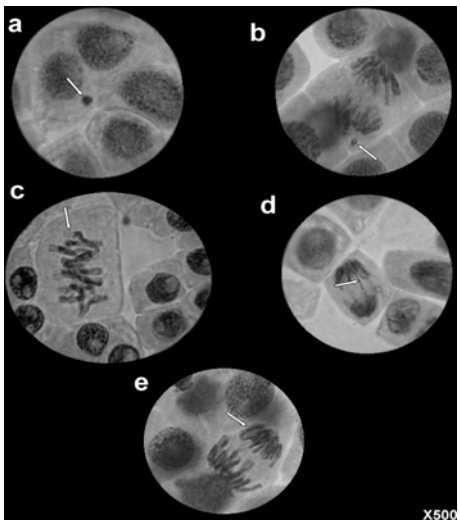

Figure 1-The effects of glyphosate on mitosis of *Allium cepa* root tip cells (a: micronucleus, b: fragment, c: sticky chromosome, d: chromatine bridge, e: unequal distribution of chromatin
Şekil 1-A. cepa kök ucu hücrelerindeki mitoz üzerine glifosatın etkisi (a: mikronukleus, b: fragment, c: yapışkan kromozom, d: kromatin köprüsü, e: kromatinin eşit olmayan dağılımı

Table 5-Effect of various doses of glyphosate on micronucleus (MN) frequency
Çizelge 5-Mikronukleus frekansı üzerine farklı dozlardaki glifosatın etkisi

Treatment time, hour	*Groups*[1]	*Number of scored cell*	*Min.*	*Max.*	*Average±SD*
72	Group I	1000	0	2	00.20±0.50d
72	Group II	1000	10	23	16.52±3.38c
72	Group III	1000	36	55	46.08±4.91b
72	Group IV	1000	48	65	57.24±4.78a
P values					<0.001

[1]Each group contains 25 seeds. Group I (control group) seeds were treated with tap water, Group II seeds were treated with 100 mg l^{-1} glyphosate, Group III seeds were treated with 250 mg l^{-1} glyphosate, Group IV seeds were treated with 500 mg l^{-1} glyphosate. 1000 cells were counted for each group for MN frequency. All values the mean ± SD.
[a-d]: Statistical significance between means was analyzed using one-way ANOVA followed by Duncan's test as a post-ANOVA test (P<0.05). Means with the same letter (vertically) are not significantly different at the P<0.05 level.

Table 6-The frequency of chromosomal aberrations (CAs) induced by various doses of glyphosate, in *A. cepa* root-tip cells

Çizelge 6-A. cepa kök ucu hücrelerinde glifosatın farklı dozları tarafından teşvik edilen kromozom anormalliklerinin frekansı

Groups[1]	*Number of root tips, n*	*Number of scored mitotic cell*	*Fragment*	*Sticky chromosome*	*Chromatin bridge*	*Unequal distribution of chromatin*
Group I	10	500	3.60±2.90d	5.88±1.79d	2.32±1.63d	0.24±0.44d
Group II	10	500	64.64±5.42c	47.80±5.24c	30.44±4.59c	10.00±2.93c
Group III	10	500	81.16±4.01b	60.80±5.20b	41.40±4.34b	20.32±3.41b
Group IV	10	500	113.00±19.31a	72.32±5.91a	50.04±4.93a	27.40±4.86a
P values			<0.001	<0.001	<0.001	<0.001

[1]Group I (control group) seeds were treated with tap water, Group II seeds were treated with 100 mg l^{-1} glyphosate, Group III seeds were treated with 250 mg l^{-1} glyphosate, Group IV seeds were treated with 500 mg l^{-1} glyphosate. Five-hundred cells were analyzed for per root tip (10 root tips/group, for a total of 5000 cells/treatment) for CAs. All values the mean ± SD.

[a-d]: Statistical significance between means was analyzed using one-way ANOVA followed by Duncan's test as a post-ANOVA test (P<0.05). Means with the same letter (vertically) are not significantly different at the P<0.05 level.

Table 7-Mitotic index (MI) of *A. cepa* root tip cells

Çizelge 7-A. cepa kök ucu hücrelerinin mitotic indeksi

Groups[1]	*Number of root tips, n*	*MI*	*%*
Group I	10	776.80±13.99a	7.76
Group II	10	697.32±21.95b	6.97
Group III	10	625.84±16.25c	6.25
Group IV	10	544.40±30.80d	5.44
P values		<0.001	

[1]Group I (control group) seeds were treated with tap water, Group II seeds were treated with 100 mg l^{-1} glyphosate, Group III seeds were treated with 250 mg l^{-1} glyphosate, Group IV seeds were treated with 500 mg l^{-1} glyphosate. The MI was calculated by analyzing 1000 cells/root tip (for a total of 10,000 cells/treatment) and percentage of the MI calculated for each treatment group. All values the mean ± SD.

[a-d]: Statistical significance between means was performed using one-way analysis of variance (ANOVA) followed by Duncan as a post-ANOVA test (P< 0.05). Means with the same letter (vertically) are not significantly different at the P<0.05 level.

3.6. Anatomical observations

The root tissues were microscopically examined to identify anatomical changes caused by glyphosate on *A. cepa* the root tips. When seeds were exposed to glyphosate for 72 h, root cells survived but the root tissues were seriously affected under the presence of glyphosate. The anatomical damages such as unclear vascular tissue (Figure 2a), unclear epidermis layer (Figure 2b), cell deformation (Figure 2b), unusual form of cell nucleus (usually flat) (Figure 2c) and binuclear cells (Figure 2d) were observed when compared to the controls (Figure 3). There were a few studies available on the anatomical structure of plant root tip cells of glyphosate and different chemical agents. For example, Pline et al. (2002) investigated the effects of glyphosate on root morphological of cotton seedlings. As a result, lateral roots of cotton seedlings inhibited by glyphosate appeared shorter and were surrounded by a thick layer of necrotic cells. In a study realized by Carlson & Donald (2006), glyphosate was sprayed at different doses to *Cirsium arvense*. As a result, darkness and softening of cortical tissue in the root and decomposition in some regions were observed. In a similar study, Turkmen et al. (2009) investigated the anatomical alterations induced by petroleum wastewater in *A. cepa* root-tip cells. As a result, the anatomical damages such as an accumulation of chemical compounds in cortex parenchyma, cell death, an unusual form of cell nucleus and unclear vascular tissue was observed.

4. Conclusions

The results of the present study indicated that glyphosate caused to significant toxic effects in

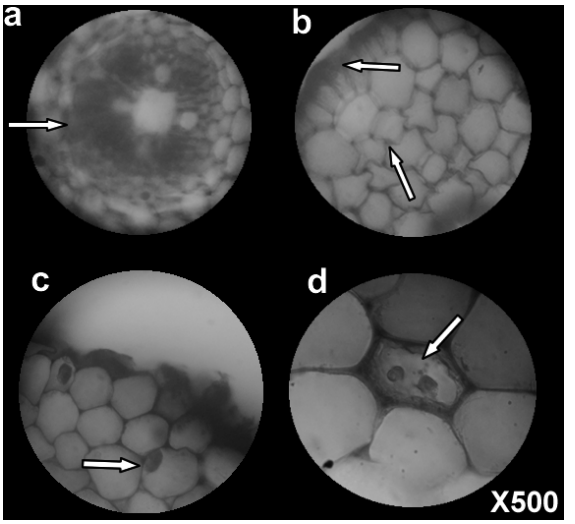

Figure 2-The anatomical alterations induced by glyphosate in *Allium cepa* root tips (a: unclear vascular tissue, b: unclear epidermis layer and cell deformation, c: unusual form (flat) of cell nucleus, d: binuclear cells)
Şekil 2-A. cepa kök ucu hücrelerinde glifosat tarafından teşvik edilen anatomik değişimler (a: belirgin olmayan vasküler doku, b: belirgin olmayan epidermis tabakası ve hücre deformasyonu, c: anormal hücre nukleusu, d: binukleer hücre)

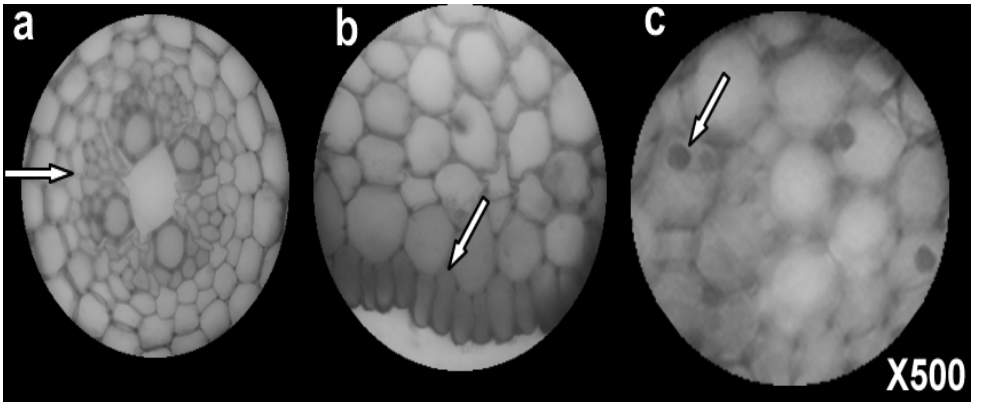

Figure 3-The anatomical appearance of root tips of control group seeds (a: clear vascular tissue, b: clear epidermis layer, c: usual form of cell nucleus (annular)
Şekil 3-Kontrol grubu tohumlarına ait kök ucunda anatomic görünüş (a: belirgin vasküler doku, b: belirgin epidermis tabakası, c: normal hücre nukleusu)

the root cells of *A. cepa*, and this toxic effect induced physiological, anatomical, biochemical, cytological and genetic alterations in *A. cepa*. In conclusion, the analysis of metabolic alterations on plant systems constitutes a simple and reliable technique to detect the toxicity of pesticides. So *Allium* test presented here is useful for rapid screening of herbicides that may be hazardous to organisms. In summary, it can be concluded that less toxic and naturel substances such as acetic

acid, fatty acids, and essential oils should be used in weed management for a safe habitat.

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