

Araştırma Makalesi/Research Article (Original Paper)

Effect of Glyphosate on Some Protective Systems in *Zea mays* L.

Gulcin BEKER AKBULUT^{*1}, Emel YİĞİT², Dilek BAYRAM²

¹Munzur University, Tunceli Vocational School, Department of Organic Agriculture, Tunceli, Türkiye

²Inonu University, Science and Art Faculty, Department of Biology, Malatya, Türkiye

*e-posta: gulcinakbulut@munzur.edu.tr, Tel: 05445440094

Abstract: Glyphosate is an herbicide that is applied after non-selective germination and affects plant growth. In this study, glyphosate was applied to *Zea mays* L. after germination, at a concentration range from 0.017 to 0.145 M in a growth chamber. The effects of this herbicide on some antioxidant enzymes, lipid peroxidation, total chlorophyll and total carbohydrate content were investigated on days 1, 5 and 10 following the application. Results showed that peroxidase (POD), ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) were increased in the groups treated with glyphosate on days 5 and 10, but the activities of reduced glutathione (GSH) and glutathione reductase (GR) were decreased on day 10. Malondialdehyde (MDA) content indicating lipid peroxidation was increased on days 1, 5 and 10. Changes in total chlorophyll and total carbohydrate contents were found to be time-dependent. These increases and decreases in the antioxidant activities, MDA content, total chlorophyll and total carbohydrate content in *Zea mays* L. were determined as a symptom of cytotoxicity caused due to glyphosate.

Keywords: Antioxidant, Glyphosate, Lipid peroxidation, Total chlorophyll, Total carbohydrate

Glifosatın Mısır (*Zea mays* L.)’da Bazı Koruyucu Sistemlere Etkisi

Özet: Glifosat bitki gelişimine etki eden seçici olmayan çimlenme sonrası uygulanan bir herbisittir. Bu çalışmada, glifosat *Zea mays* L.’a çimlenme sonrası 0.017-0.145 M konsantrasyon aralığında iklim odasında uygulanmıştır. Bu herbisitinin bazı antioksidan enzimler, lipid peroksidasyonu, total klorofil ve toplam karbohidrat içeriği üzerindeki etkileri uygulamayı takiben 1., 5. ve 10. günlerde araştırılmıştır. Peroksidaz (POD), askorbat peroksidaz (APX), süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon-S-transferaz aktiviteleri glifosat uygulanan gruplarda 1. 5. ve 10. günlerde artış gösterirken, redükte glutatyon (GSH) ve glutatyon redüktaz (GR) aktivitesi 10. günde azalış göstermiştir. Lipid peroksidasyonunu gösteren malondialdehid (MDA) içeriği 1., 5. ve 10. günlerde artış göstermiştir. Total klorofil içeriği ve total karbohidrat içeriği zamana bağlı olarak değişim göstermiştir. Mısır’da antioksidan aktivite, MDA, toplam klorofil ve karbohidrat içeriğinde artış ve azalışın olması glifosattan dolayı bir sitotoksikite semptomu olarak saptanmıştır.

Anahtar kelimeler: Antioksidan, Glifosat, Lipid peroksidasyonu, Toplam klorofil, Toplam karbohidrat

Introduction

Pesticides are an integral component of modern agriculture and production processes in all agro ecosystems around the world necessitate the application of one or more pesticides (Eash and Bushway 2000; Sikkema et al. 2008). Herbicides, which constitute an important class of pesticides, are used to control weeds in agricultural areas. Although proper use of herbicides provides economic benefits for plant production, herbicide application on a continuous basis creates numerous environmental problems by causing adverse effects on crop growth (Cao et al. 2008; Seiber and Kleinschmidt 2011; Chen et al. 2015). Glyphosate is a post-emergent, systemic, non-selective, broad-spectrum herbicide that has been used to control the growth of annual and perennial weeds and volunteer crops in a wide range of situations (Perez Jones and Mallory Smith 2010). The herbicidal effects of glyphosate are caused due to the inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme from the shikimate pathway (Siehl 1997). Thus, it weakens general metabolic processes such as protein synthesis (Geiger et al. 1986; Maria et al. 2005) and photosynthesis (Bott et al. 2008).

Reactive oxygen species (ROS) are formed when plants are exposed to abiotic stress (Wu and Tiedemann 2002). The POD electron donor is an enzyme used to cleanse H₂O₂ (De Gara 2004). When APX moves with SOD, it plays an important role in cleansing processes (Wang et al. 2004). SOD belongs to a class of metalloproteins that

catalyse the dismutation of superoxide (O_2^-) into molecular oxygen (O_2) and H_2O_2 . GSH is a tripeptide derived from the amino acids glutamic acid, cysteine and glycine (Ogawa 2005). It is a multifunctional intracellular antioxidant and has been demonstrated to be the major thiol-disulphide redox buffer of the cell (Kurutas 2016). GR is an enzyme whose prosthetic group is flavin adenine dinucleotide, and it is found in the cytosol and mitochondria in a dimeric structure. It catalyzes the reduction reaction of oxidized glutathione in the presence of NADPH (Halliwell 1994). In plants, GST genes are promoted by several growth regulators, heavy metals, chlorocarbons and oxidative stress factors. Detoxification of lipid hydroperoxides and fungal toxins, increased tolerance to drought and some mechanisms of antioxidative defense against pathogens are among the natural functions of GST in plants (Schröder 2001).

Oxidative stress induced by various herbicides causes lipid peroxidation by distorting membrane integrity (Lambert and Bondy 1989; Nordquist et al. 1994). Inhibition created in the photosynthetic electron transport causes pigment content and decrease in plant growth (Huang et al. 2006; Huang and Xiong 2009). Carbohydrate content is also affected by this situation (Saladin et al. 2003; Magne et al. 2006).

This study was conducted to investigate the effects of herbicide glyphosate, which is used for controlling the growth of weeds, on some biochemical parameters in *Zea mays* (corn) “Martha F1” culture form. The effects of glyphosate on culture plants were determined by evaluating total antioxidant activities, total chlorophyll and total carbohydrate content, which are known to be important in plant development.

Materials and Methods

Plant materials and treatment conditions

In this study, *Zea mays* cv. “Martha F1” seeds were obtained from May Company. Glyphosate was provided from Safa Company. The samples were grown in perlite, and Hoagland culture solution (Hoagland and Arnon 1938) was used for irrigation. These procedures were carried out in a climate chamber. The temperature of the chamber was set at $23^\circ\text{C} \pm 2^\circ\text{C}$, with ambient air humidity of approximately 60%. The samples were analysed in triplicate. *Zea mays* (21 days old) seedlings of appropriate size were treated with glyphosate after germination (postemergence) at doses 0.017, 0.023, 0.030, 0.039, 0.051, 0.066, 0.085, 0.111 and 0.145 M by spraying. Samples were collected on days 1, 5 and 10 from the treatment groups and analysed.

POD Analysis

POD analysis was described by Peters et al. (1988) and Mac Adam et al. (1992). Enzyme activity was measured at 436 nm for 1 minute by spectrophotometer (Shimadzu UV-1201V).

APX Analysis

APX analysis was assayed according to method of Nakano and Asada (1981) and Çakmak (1994). The oxidation of ascorbate was determined by the change in absorbance at 290 nm ($\epsilon = 2,8 \text{ mM}^{-1} \text{ cm}^{-1}$).

SOD Analysis

The SOD test was carried out according to McCord and Fridovich (1969). Enzyme activity was determined as absorbance change at 550 nm wavelength in 1 minute.

CAT Analysis

The CAT activity was assayed according to Luck (1963). CAT activity was determined by monitoring the decrease in the absorbance at 240 nm as a consequence of H_2O_2 disappearance.

GSH Analysis

GSH content was determined according to Akerboom and Sies (1981). The absorbance change for 1 minute at a wavelength of 420 nm was calculated for the total GSH content.

GR Analysis

GR activity was performed according to the method of Cribb et al. (1989). Enzyme activity was determined as absorbance change obtained in 3 minutes at 405 nm.

Glutathione S-Transferase Analysis

The GST was determined as to Habig et al. (1974). The enzyme activity was assayed as absorbance change obtained in 1 minute at 344 nm.

MDA Analysis

MDA was calculated according to Heath and Packer (1968). The absorbance of the supernatant was measured at 532 nm and 600 nm. The extinction coefficient for MDA is $155 \text{ mM}^{-1} \text{ cm}^{-1}$. The results were expressed as $\mu\text{mol MDA g}^{-1} \text{ FW}$.

Total Chlorophyll Analysis

Extraction and purification of total chlorophyll were made according to De Kok and Graham (1980). The absorbance values were read at 662, 645, 470 nm according to Lichtenthaler and Welburn (1983).

Total Sugar Analysis

The total amount of sugar was determined according to Rosenberg (1980). Glucose values were calculated corresponding to the standard values entered in the Slide program on the computer.

Total Soluble Protein Assay

Soluble protein concentration was measured using bovine serum albumin as standard at 595 nm according to the method of Bradford (1976).

Statistical Analysis

Statistical analysis was made using the SPSS 15.0 software. Duncan's (1955) and *t* tests were used to determine the differences between averages. In the analyses, $p < 0.05$ was considered statistically significant.

Results and Discussion

Changes in Antioxidant Enzyme Activity

Plants exposed to stress may overcome the oxidative stress by the activation of some or all the antioxidant defence systems. POD constitutes one such group of enzymes that scavenges ROS besides having other defensive roles (War et al. 2012). Changes occurring in the amount of POD depending on the days when glyphosate was applied to *Zea mays* leaves after germination were examined, which showed the lowest POD activity in the control group on day 1. POD activity was thereafter increased on days 5 and 10. The highest POD activities of 6.79 and $10.41 \text{ U mg}^{-1} \text{ protein}$ were found respectively in the groups that received 0.051 and 0.066 M glyphosate on day 10 (Table 1). Basantani et al. (2011) also reported similar findings in their study, where an increase in POD activity was observed after glyphosate administration in the two variants of *Vigna radiata* L. Ascorbate is one of the most important antioxidants and reacts directly with hydroxyl radicals, superoxide and singlet oxygen. In addition to its significant role in the regulation of photosynthesis and in light protection, it also plays an important role in the preservation of the activities of enzymes containing metal ions as a prosthetic group against stress (He et al. 2011; Srivastava et al. 2011). Regarding the APX activity after the application of glyphosate to *Zea mays* leaves depending on the application days, the highest APX activity was observed in the group that received 0.039 M glyphosate on day 1, in the group that received 0.085 M glyphosate on day 5 and in the group that received 0.111 M glyphosate on day 10 (Table 1). Similar results were reported by Jiang and Yang (2009), who observed that low concentrations of the herbicide prometryne applied to wheat plants increased the APX activity and it reduced it at low concentrations ($p < 0.05$).

Table 1. Alteration in POD and APX activity in *Zea mays* exposed to glyphosate

Glyphosate (M)	POD ($\text{U mg}^{-1} \text{ protein}$)			APX ($\text{U mg}^{-1} \text{ protein}$)		
	1 st day	5 th day	10 th day	1 st day	5 th day	10 th day
Control	A 3.89 e	A 3.92 e	A 3.94 g	A 0.57 ef	A 0.58 g	A 0.54 h
0.017	C 4.11 cde	B 4.49 d	A 5.08 f	B 0.66 cd	B 0.62 fg	A 1.05 g
0.023	C 3.97 de	B 5.10 c	A 6.41 e	B 0.71 bc	B 0.67 f	A 1.07 g
0.030	C 4.19 cde	B 4.72 d	A 6.56 e	C 0.77 ab	B 0.84 cd	A 1.14 fg
0.039	C 4.33 bc	B 5.19 c	A 6.64 e	B 0.83 a	C 0.79 de	A 1.25 ef
0.051	C 4.95 a	B 6.79 a	A 8.73 d	C 0.62 de	B 0.77 e	A 1.49 d
0.066	C 4.58 b	B 6.68 a	A 10.41 a	C 0.67 cd	B 0.87 c	A 1.28 e
0.085	C 3.96 de	B 5.94 b	A 9.36 c	C 0.52 f	B 0.98 a	A 1.66 c
0.111	C 4.22 c	B 6.29 b	A 9.32 c	C 0.56 ef	B 0.95 ab	A 2.38 a
0.145	C 4.18 cde	B 6.01 b	A 9.94 b	C 0.67 cd	B 0.90 bc	A 1.98 b

The different lower case letters indicate significant differences ($p < 0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of glyphosate according to independent samples *t* tests.

The superoxide radicals formed in plant cells due to stress are transformed to H_2O_2 by the reaction of the SOD enzyme (Dixit et al. 2001; Mittiova et al. 2002). The highest SOD activity of 4.11 U mg^{-1} protein was found in the group that received 0.145 M glyphosate on day 1. SOD activity was increased on days 5 and 10 (Table 2). The lowest CAT activity was detected in the control groups. CAT activity was also increased in parallel with the increase in the number of days. The highest CAT activity of 6.84 U mg^{-1} protein was observed in the group treated with 0.145 M glyphosate on day 10 (Table 2).

Table 2. Alteration in SOD and CAT activity in *Zea mays* exposed to glyphosate

Glyphosate (M)	SOD (U mg^{-1} protein)			CAT (U mg^{-1} protein)		
	1 st day	5 th day	10 th day	1 st day	5 th day	10 th day
Control	A 3.16 f	A 3.15 i	A 3.16 j	A 3.09e	A 3.04 h	A 3.07 i
0.017	C 3.42 de	B 3.70 h	A 4.10 i	C 3.22 d	B 3.32 g	A 3.42 h
0.023	C 3.36 e	B 3.68 h	A 4.36 h	C 3.26 d	B 3.48 f	A 3.82 g
0.030	C 3.45 d	B 3.91 g	A 4.55 g	C 3.26 d	B 3.48 f	A 4.30 f
0.039	C 3.68 c	B 4.20 f	A 4.83 f	C 3.44 b	B 3.90 c	A 4.54 e
0.051	C 3.67 c	B 4.28 e	A 5.16 e	C 3.48 b	B 3.64 e	A 4.87 d
0.066	C 3.75 b	B 4.52 d	A 5.80 d	C 3.37 c	B 3.77 d	A 4.90 d
0.085	C 3.70 bc	B 4.79 c	A 5.94 c	C 3.48 b	B 3.86 c	A 5.40 c
0.111	C 3.68 c	B 4.93 b	A 6.22 b	C 3.54 a	B 4.07 b	A 5.94 b
0.145	C 4.11 a	B 5.24 a	A 6.84 a	C 3.57 a	B 4.70 a	A 6.85 a

The different lower case letters indicate significant differences ($p < 0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of glyphosate according to independent samples *t* tests.

The lowest GSH activity was detected in control groups on days 1, 5 and 10. GSH activity was increased in the groups that received 0.017 – 0.051 M glyphosate on days 5 and 10. However, the GSH activity was decreased in the groups treated with 0.066 – 0.145 M glyphosate on day 10 when compared to that day 5. The highest GR activity of 0.125 U mg^{-1} protein was observed in the group that received 0.085 M glyphosate on day 1. The GR activity was increased on day 5, but it decreased on day 10. The lowest GR activity (0.080 – 0.082 U mg^{-1} protein) was detected in the control groups on days 1, 5 and 10. GST activity was also found to be the lowest in the control groups (Table 3).

Jiang and Yang (2009) investigated the oxidative stress induced by prometryne in wheat and found that it caused significant changes in the activity of antioxidant enzymes including SOD, POD, CAT, APX and GST. Peixoto et al. (2008) examined the effect of three different herbicides, 2,4-D, paraquat and dicamba, on the antioxidant system in potato tubers. They showed that paraquat induced CAT activity at low concentrations but inhibited the same at high concentrations. Paraquat also stimulated SOD activity whereas 2,4-D and dicamba induced the same at high concentrations. Furthermore, GST activity was poorly inhibited by paraquat. These results are in agreement of with our findings.

Changes in MDA

In some situations, the reduction in oxidative stress protection causes changes in lipid peroxidation and free radical formation. MDA is oxidised product of membrane lipids and accumulates when plants are exposed to oxidative stress. Regarding the changes in MDA levels in this study the highest level of MDA on day 1 was found in the group that received 0.066 M glyphosate and the lowest MDA level was detected in the group that received 0.017 M glyphosate. The level of MDA was found to be increased on days 5 and 10. The highest level of MDA of $14.00 \mu\text{mol MDA g}^{-1}$ wet weight was detected in the group treated with 0.145 M glyphosate on day 10 (Table 4). Liu et al. (2009) determined the levels of MDA, O_2^- and H_2O_2 content and antioxidant enzyme activities in cucumber cultivar on which they applied drought stress and paraquat. They detected a change in the levels of MDA, O_2^- and H_2O_2 due to effect of drought. However, they reported that the plants that received paraquat pre-treatment showed lower levels of MDA, O_2^- and H_2O_2 than those of plants that were not pretreated.

Table 3. Alteration in GSH, GR and GST activity in *Zea mays* exposed to glyphosate

Glyphosate (M)	GSH (U mg ⁻¹ protein)		
	1 st day	5 th day	10 th day
Control	A 1.81 e	A 1.84 i	A 1.83 f
0.017	C 1.92 d	B 2.16 h	A 2.89 e
0.023	C 2.14 c	B 2.92 g	A 3.27 d
0.030	C 2.30 b	B 3.10 g	A 3.82 c
0.039	C 2.34 ab	B 3.37 f	A 4.27 b
0.051	C 2.16 c	B 3.61 e	A 4.19 b
0.066	C 2.36 ab	A 5.01 d	B 4.17 b
0.085	C 2.34 ab	A 5.81 c	B 4.67 a
0.111	C 2.40 a	A 6.30 b	B 4.12 b
0.145	C 2.42 a	A 6.71 a	B 4.28 b
	GR (U mg ⁻¹ protein)		
	1 st day	5 th day	10 th day
Control	A 0.082 g	A 0.080 f	A 0.080 g
0.017	C 0.112 de	A 0.334 c	B 0.277 a
0.023	C 0.117 bcd	A 0.376 b	B 0.258 b
0.030	C 0.119 bc	A 0.372 b	B 0.237 c
0.039	C 0.110 e	A 0.398 a	B 0.205 d
0.051	C 0.110 e	A 0.377 b	B 0.206 d
0.066	C 0.121 ab	A 0.405 a	B 0.270 ab
0.085	C 0.125 a	A 0.355 b	B 0.203 d
0.111	C 0.115 cd	A 0.289 d	B 0.183 e
0.145	C 0.104 f	A 0.267 e	B 0.133 f
	GST (U mg ⁻¹ protein)		
	1 st day	5 th day	10 th day
Control	A 0.079 f	A 0.078 g	A 0.78 h
0.017	C 0.091 abcd	B 0.101 f	A 0.134 g
0.023	C 0.092 abc	B 0.136 de	A 0.194 f
0.030	C 0.094 a	B 0.133 e	A 0.199 f
0.039	C 0.090 bcd	B 0.142 c	A 0.285 e
0.051	C 0.093 ab	B 0.154 a	A 0.328 c
0.066	C 0.088 de	B 0.148 b	A 0.358 b
0.085	C 0.090 cd	B 0.136 d	A 0.376 a
0.111	C 0.090 cd	B 0.135 de	A 0.300 d
0.145	C 0.086 e	B 0.135 de	A 0.373 a

The different lower case letters indicate significant differences ($p < 0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of glyphosate according to independent samples *t* tests

Table 4. Alteration in MDA content in *Zea mays* exposed to glyphosate

Glyphosate (M)	MDA ($\mu\text{mol MDA g}^{-1}$ fresh weight)		
	1 st day	5 th day	10 th day
Control	A 5.87 e	A 5.89 h	A 5.77 f
0.017	C 5.78 e	A 5.95 h	B 5.83 f
0.023	C 6.17 d	B 6.56 g	A 7.27 e
0.030	C 6.16 d	B 7.73 d	A 10.58 d
0.039	C 6.71 bc	B 7.14 f	A 11.43 c
0.051	C 6.91 ab	B 8.85 b	A 12.64 b
0.066	C 6.92 a	B 9.20 a	A 12.11 b
0.085	C 6.57 c	B 8.56 c	A 12.31 b
0.111	C 6.52 c	B 7.48 de	A 12.18 b
0.145	C 6.26 d	B 7.29 ef	A 14.00 a

The different lower case letters indicate significant differences ($p < 0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p < 0.05$) for each concentration of glyphosate according to independent samples *t* tests.

Changes in total chlorophyll

In the present study the highest total chlorophyll amount was found in the control group on days 1, 5 and 10. The total chlorophyll levels were as lower as 12.36 $\mu\text{g g}^{-1}$ in the group that received 0.085 M glyphosate on day 1, 9.63 $\mu\text{g g}^{-1}$ in the group that received 0.145 M glyphosate on day 5 and 9.53 $\mu\text{g g}^{-1}$ in the group that received 0.10 M glyphosate on day 10. The decrease in total chlorophyll amount was determined according to the increase in the number of days (Table 5). Ekmekçi and Terzioğlu (2005) investigated the effects of paraquat on oxidative stress in wild and cultivated wheat. They reported that paraquat caused a decrease in chlorophyll (a+b) and carotenoid content at high concentrations. Kana et al. (2004) analysed the effects of the herbicide clomazone on photosynthesis, which was applied before germination to barley (*Hordeum vulgare* L.) leaf. They observed that increased concentrations of clomazone caused a decrease in chlorophyll (a+b) and carotenoid levels. When compared to control, in a study conducted on *Zea mays* plant to which the herbicide mesotrione was applied, it was observed that while KI a, KI b and total chlorophyll levels were decreased on days 5, 10 and 15, the carotenoid levels were increased (Giray Kurt 2007). Similar findings were observed in our study examining the relationship between photosynthetic pigment and antioxidant system wherein a lipid peroxidation resulted in an increase in POD and APX activities from antioxidant enzymes that protect the membrane from peroxidation.

Table 5. Alteration in total chlorophyll in *Zea mays* leaves exposed to glyphosate

Glyphosate (M)	Total chlorophyll ($\mu\text{g g}^{-1}$)		
	1 st day	5 th day	10 th day
Control	A 13.10 a	A 13.10 a	A 13.10 a
0.017	A 13.03 a	B 11.76 b	B 11.79 b
0.023	A 13.06 a	B 11.76 b	B 11.78 b
0.030	A 12.91 ab	B 11.62 b	B 11.63 c
0.039	A 12.78 bc	B 11.33 c	B 11.34 d
0.051	A 12.88 ab	B 11.36 c	B 11.33 d
0.066	A 12.59 c	C 9.51 d	B 9.89 e
0.085	A 12.36 d	B 9.72 d	C 9.57 f
0.111	A 12.60 c	B 9.70 d	C 9.53 f
0.145	A 12.79 bc	B 9.63 d	B 9.57 f

The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

Changes in total carbohydrate

In the present study, total carbohydrate content was decreased on days 5 and 10 (Table 6). Different findings have been observed regarding the changes in carbohydrate content in plants under different stress conditions. For example, it has been reported that the fructose content in the vine plant, which was treated with the herbicide flumioxazin, decreased depending on the applications days whereas the starch content showed a decrease and an increase (Saladin et al. 2003). Magne et al. (2006) reported that administration of the herbicide flazasulphuron to *Vitis vinifera* L. decreased both the starch content (74%) and soluble carbohydrate levels (90%).

Table 6. Alteration in total carbohydrate in *Zea mays* leaves exposed to glyphosate

Glyphosate (M)	Total carbohydrate ($\mu\text{g g}^{-1}$)		
	1 st day	5 th day	10 th day
Control	A 0.57 a	A 0.56 a	A 0.55 a
0.017	A 0.52 a	A 0.50 a	B 0.46 b
0.023	A 0.49 b	A 0.45 b	B 0.37 c
0.030	A 0.47 b	A 0.40 b	B 0.33 c
0.039	A 0.45 b	B 0.37 c	C 0.30 c
0.051	A 0.46 b	B 0.38 c	C 0.28 d
0.066	A 0.50 a	B 0.35 c	C 0.24 d
0.085	A 0.49 b	B 0.37 c	C 0.28 d
0.111	A 0.48 b	B 0.26 d	C 0.16 e
0.145	A 0.45 b	B 0.28 d	C 0.22 b

The different lower case letters indicate significant differences ($p<0.05$) among the different concentrations of glyphosate according to Duncan's tests. The different upper case letters indicate significant differences ($p<0.05$) for each concentration of glyphosate according to independent samples *t* tests

Despite the fact that the plant *Zea mays* has a high economic value and is widely cultivated in our country, it cannot meet the domestic demands due to which production deficit is covered by imports. In this study, conducted in the viewpoint of the type benefits or harms exerted by the herbicides that are commonly used in the biological struggle in our country as in other countries, in fields where culture plant cultivation is common, it was observed that glyphosate causes significant phytotoxicity in *Zea mays*, and has negative effects on the antioxidant system, the pigment system and the total carbohydrate content. The obtained data suggest that improper use of herbicides to control the growth of weeds in plant cultivation lead to serious economic losses and significant adverse effects on the plant's biochemical structure.

Acknowledgements

The study was supported by Inonu University Scientific Research Foundation (24/2010).

References

- Akerboom TPM, Sies H (1981). Assay of glutathione, glutathione disulfide and glutathione mixed disulfide in biological samples, in W.B. Jakoby (Ed.), *Methods in Enzymology*, Academic Press, New York., 77: 373–382.
- Basantani M, Srivastava A, Sen S (2011). Elevated antioxidant response and induction of tau-class glutathione S-transferase after glyphosate treatment in *Vigna radiata* (L.) Wilczek, *Pesticide Biochemistry and Physiology*, 99: 111–117.
- Bott S, Tesfamariam T, Candan H, Cakmak I (2008). Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (*Glycine max* L.) Volker Römheld & Günter Neumann *Plant Soil*, 312: 185–194.
- Bradford MM (1976). A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254.
- Cao J, Guo H, Zhu HM, Jiang L, Yang H (2008). Effects of SOM, surfactant and pH on the sorption–desorption and mobility of prometryne in soils. *Chemosphere*, 70: 2127–2134.
- Chen YL, Zhang S, Yang H (2015). Acceleration of the herbicide isoproturon degradation in wheat by glycosyltransferases and salicylic acid *Journal of Hazardous Materials* 283: 806–814.
- Cribb AE and et al. *Anal. Biochem* (1989) 183: 195–196.
- Çakmak I (1994). Activity of ascorbate-dependent H₂O₂-scavenging enzymes and leaf chlorosis are enhanced in magnesium-deficient and potassium deficient leaves, but not in phosphorus-deficient leaves. *Journal of Experimental Botany* 45: 1259.
- De Gara L (2004). Class III peroxidases and ascorbate metabolism in plants. *Phytochem. Rev.* 3: 195–205.
- De-Kok L, Graham M (1980). Levels of pigments, soluble proteins, amino acids and sulfhydryl compounds in foliar tissue of *Arabidopsis thaliana* during dark induced and natural senescence. *Plant Physiol Bioch.*, 27: 133–142.
- Dixit V, Pandey V, Shyam R (2001). Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). *Journal of Experimental Botany*, 52 (358): 1101–1109.
- Duncan DB (1955). Multiple range and multiple F tests biometrics, 11: 1-42.
- Eash DT, Bushway RJ (2000). Herbicide and plant growth regulator analysis by capillary electrophoresis, *Journal of Chromatography A*, 880: 281–294.
- Ekmekçi Y, Terzioğlu S (2005). Effects of oxidative stress induced by paraquat on wild and cultivated wheats, *Pesticide Biochemistry and Physiology*, 83: 69–81.
- Geiger DR, Kapitan SW, Tucci MA (1986). Glyphosate inhibits photosynthesis and allocation of carbon to starch in sugar beet leaves. *Plant Physiol.*, 82: 468–472.
- Giray Kurt A. (2007). Callisto herbisitinin Mısır (*Zea mays* L.)'ın Martha F1 kültür formunda total glutatyon, glutatyon redüktaz, glutatyon-s-transferaz ve pigment içeriği üzerine etkileri”, Yüksek Lisans Tezi, İnönü Üniversitesi, Fen Bil. Enstitüsü, Malatya,
- Habig WH, Pabst MJ, Jakoby WB (1974). The first enzymatic step in mercapturic acid formation Glutathion S-Transferases. *J. Biol. Chem.*, 249: 7130–7139.
- Halliwell B (1994). Free radicals and antioxidants: A personal view. *Nutrition Reviews*, 52: (8) 253–265.
- He J, Chen F, Chen S, Lu G, Deng Y, Fang W, Liu Z, Guan Z, He C (2011). Chrysanthemum leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation, *Journal of Plant Physiology*, 168: 687–693.
- Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplast, I. kinetics stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125: 180.

- Hoagland DR, Arnon DI (1938). The water culture method for growing plants without soil, Circ. Calif. Agr. Exp. Sta., 347: 461.
- Huang H, Xiong ZT, Li MJ, Li SL, Hunag Y, Gao JQ, Qiu HJ, Mba FQ (2006). Effects of cadmium and herbicides on chlorophyll and soluble sugar content in rice seedlings, Wunhan University. J. Nat. Sci., 3: 742–748.
- Huang H, Xiong ZT (2009). Effects of cadmium, acetochlor and bensulfuron-methyl on nitrogen metabolism and plant growth in rice seedlings. Pest. Biochem. Physiol., 94: 64–67.
- Jiang L, Yang H (2009). Prometryne-induced oxidative stress and impact on antioxidant enzymes in wheat. Ecotox. and Environ Safety 72: 1687–1693.
- Kana R, Spundova M, Ilk P, Lazar D, Klem K, Tomek P, Naus J, Prasil O (2004). Effect of herbicide clomazone on photosynthetic processes in primary barley (*Hordeum vulgare* L.) leaves, Pesticide Biochemistry and Physiology, 78: 161–170.
- Kurutas EB (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state Nutr J. 15: 71.
- Lambert CE, Bondy SC (1989). Effects of MPTP, MPP+ and paraquat on mitochondrial potential and oxidative stress. Life Sci., 44: 1277–84.
- Lichtenthaler K, Welburn AR (1983). Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc, T, 11: 591–592.
- Liu ZJ, Zhang XL, Bai JG, Suo BX, Xu PL, Wang L (2009). Exogenous paraquat changes antioxidant enzyme activities and lipid peroxidation in drought-stressed cucumber leaves. Scientia Horticult. 121:138–143.
- Luck H (1963). Catalase. Methods of Enzymatic Analysis. 885–888.
- Mac Adam JW, Nelson CJ, Sharp RE (1992). Peroxidase activity in the leaf elongation zone of tall fescue. Plant Physiol., 99: 872–878.
- Magne C, Gaëlle S, Clement C (2006). Transient effect of the herbicide flazasulfuron on carbohydrate physiology in *Vitis vinifera* L, Chemosphere, 62: 650–657.
- María N de, de Felipe MR, Fernández-Pascual M (2005). Alterations induced by glyphosate on lupin photosynthetic apparatus and nodule ultrastructure and some oxygen diffusion related proteins. Plant Physiol. Biochem., 43: 985–996.
- McCord JM, Fridovich I (1969). Superoxide dismutase: an enzymic function for erythrocyte (Hemoerythrocyte). J. Biol. Chem., 244: (22) 6049–6055.
- Mittiova V, Tal M, Volokita M (2002). Salt stress induces up-regulation of N efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. Physiologia Plantarum, 115: 393–400.
- Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiology, 22: 867.
- Nordquist RE, Nguyen H, Poyer JL, Carubelli R (1994). The role of free radicals in paraquat-induced corneal lesions. Free Radical Res, 23: 61–71.
- Ogawa K (2005). Glutathione-associated regulation of plant growth and stress responses, Antioxidants & Redox Signaling, 7(7-8): 973–981.
- Peixoto FP, Laranjo JG, Vicente JA, Madeira VMC (2008). Comparative effects of the herbicides dicamba, 2,4-D and paraquat on non-green potato tuber cali. Journal of Plant Physiology 165:1125–1133.
- Perez Jones A. and Mallory Smith C (2010). Biochemical mechanisms and molecular basis of evolved glyphosate resistance in weed species. Glyphosate resistance in Crops and Weeds: History, Development and Management Edited by Vijay K. Nandula Copyright John Wiley & Sons, Inc. 119.
- Peters JL, Castillo FJ, Heath RL (1988). Alteration of extracellular enzymes in Pinto bean leaves upon exposure to air pollutants, ozone and sulfur dioxide, Plant Physiol. 89, 159–164.
- Rosenberg S (1980). Physiological studies of lignocellulose degradation by thermotolerant mold *Chrysosporium prunosum*, Symposium on the biological transformation of lignocellulose, 12: 133–142.
- Saladin G, Magnea C., Cleament C (2003). Effects of flumioxazin herbicide on carbon nutrition of *Vitis vinifera* L., J. Agric. Food Chem., 51: 4017–4022.
- Schröder P (2001). The role of glutathione and glutathione s-transferase in plant reaction and adaptation to xenobiotics. In: Grill D, Tausz M, DeKok LJ (eds), Significance of glutathione in plant adaptation to the environment. Kluwer Academic Publ, Dordrecht, 155–18.
- Seiber JN, Kleinschmidt LA (2011). Contributions of pesticide residue chemistry to improving food and environmental safety: past and present accomplishments and future challenges, J. Agric. Food Chem. 59: 7536–7543.
- Siehl D (1997) Inhibitors of EPSPS synthase, glutamine synthetase and histidine synthesis. In: Roe R, Burton J, Kuhr R, eds. Herbicide activity: toxicology, biochemistry and molecular biology. Amsterdam: IOS Press, 37–67.

- Sikkema PH, Shropshire C, Soltani N (2008) Tolerance of spring barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and wheat (*Triticum aestivum* L.) to saflufenacil, Crop Protection. 1–3.
- Srivastava AK, Srivastava S, Souza SFD (2011). Thiourea orchestrates regulation of redox state and antioxidant responses to reduce the NaCl-induced oxidative damage in Indian mustard (*Brassica juncea* (L.) Czern.), Plant Physiology and Biochemistry, 49: 676–686.
- Wang SH, Yang ZM, Lu B, Li SQ, Lu YP (2004) Copper induced stress and antioxidative responses in roots of *Brassica juncea* L. Bot. Bull. Acad. Sin. 45: 203–212.
- War AR, Paulraj MG, Ahmad T, Abdul Buhroo A, Hussain B, Ignacimuthu S, Sharma HC (2012) Mechanisms of Plant Defense Against Insect Herbivores Plant Signaling & Behavior 7:10, 1306–1320.
- Wu Y, Tiedemann AV (2002). Impact of fungicides on active oxygen species and antioxidant enzymes in spring barely (*Hordeum vulgare* L.) exposed to ozone. Environ. Pollut., 116: 37–47.