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Effects of Glyphosate Herbicide on Photosynthetic Pigments and Antioxidant Enzyme Activities in Corn (*Zea mays L.*) and Wheat (*Triticum aestivum L.*) Varieties

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ABSTRACT

In this study, it was determined that phytotoxic levels of glyphosate herbicide that will be banned in Europe may be at on wheat and corn plants. Biochemical responses due to the toxic effect of glyphosate at different concentrations were determined in corn (*Zea mays* L. cv. Ada 523) and wheat (*Tritucum aestivum* L. cv. Halis) varieties. For wheat and corn varieties, 4 different doses (0, 100, 500 and 1000 µM) of the herbicide were applied to 1-week-old and 15-day-old plants. Hydroponic medium was used for all applications on plants. According to the results obtained; the toxic effect created by glyphosate increased the destruction of pigment in the leaves and significant decreases were detected. While GST, SOD and CAT activities increased in all concentrations of 1-week-old plants treated with glyphosate, only GST activity decreased at 100 µM concentration of wheat leaf and corn root. SOD and CAT activities were increased in 15-day-old wheat and corn plants treated with glyphosate. Only SOD activity decreased in the root part of the maize plant. GST activity was increased in the roots and leaves of the maize plant, while it decreased in wheat leaves and roots at a concentration of 100 µM. As a result, glyphosate was found to be effective at very low concentrations in wheat and corn regardless of age. It was also revealed that 1-week-old corn and wheat crops inhibit more phytotoxic effects than these 15-day-old plants. In other words, young plants were found to be more resistant.

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1. INTRODUCTION

In agricultural terms, herbicides also cause toxicity in cultivated plants [1]. Herbicide toxicity can occur in the leaves, stems, flowers and fruits of plants and may cause symptoms such as intravenous chlorosis, mottled chlorosis, yellow spotting, bruising of the leaves, necrosis and stem death. Due to the reasons arising from herbicide toxicity, the plant remains weak, thus the crop plant remains vulnerable to disease factors, pests and adverse environmental conditions, increasing yield losses [2]. Glyphosate is included as a broad spectrum systemic herbicide used worldwide [3]. Glyphosate; It is a systemic herbicide, broad-spectrum, non-selective with N-phosphonomethyl glycine structure [4]. Glyphosate is take places to acts on the shikimic acid pathway that inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), thus inhibiting the synthesis of other secondary products (phenylalanine, tyrosine, tryptophan) [5]. Generally, it is known that glyphosate is the first active substance used in agriculture [6]. Corn is a highly competitive plant. However, in the early stages of its development, its competitive power with weeds is weak [7]. In this period, combating weeds is very important in terms of high efficiency. It is stated that the yield loss in corn, which is exposed to weed competition during the season, is approximately 42% [8]. Carotenoids are yellow-orange pigments found together with chlorophylls and protect chlorophyll by damping the oxidative energy of singlet oxygen. Herbicides that act as antioxidants and inhibit pigment synthesis cause loss of carotenoids, fragmentation of chlorophyll in light and whitening of plant tissues. Plant height can be shortened by observing necroses in sensitive plants. The properties of pigment synthesis inhibitors can be degraded by microorganisms in the soil [9].

In studies about glyphosate; tomato (*Solanum lycopersicum* L.) were grown for 28 days in soil formulation of a commercial glyphosate (0, 10, 20 and 30 mg/kg $^{-1}$). CAT activity and proline amount increased in shoots and roots, while SOD activity decreased in roots and increased in shoots [10]. Poinsettia (*Euphorbia heterophylla* L.) and soybean plants are plants that have the potential to reduce yield due to herbicide resistance and are difficult to control. Chlorophyll,

carotenoid, lipid peroxidation levels, SOD activity and CAT activity parameters were investigated. Chlorophyll and carotenoid losses were observed [11]. *Salvinia natans* (floating fern) has been shown to be able to activate including increased changes in SOD activity and CAT activity to overcome oxidative stress of 7 days of administered glyphosate (0, 1, 5, 25, 50 and 75 mg/l $^{-1}$) [23]. Glyphosate (0, 10, 20 and 40 µM) was applied to a 14-day-old *A. thaliana* plant. Glyphosate caused a decrease in SOD activity and a general increase in CAT activity [3]. In a study where 0.25 mM concentration glyphosate was applied to pea (*Pisum sativum* L.) seedlings; reduction in chlorophyll (a+b), carotenoids; An increase in SOD and CAT activities was detected [18].

In this study, the effects of glyphosate on wheat and corn varieties of different ages were investigated. Homogeneously grown 1-week-old plants and 15-days-old seedlings; different concentrations of glyphosate (0, 100, 500 and 1000 µM) different concentrations were applied separately for 2 days using the hydroponic method. Pigment analysis was analyzed in leaves of plants treated with glyphosate herbicides. Then, SOD activity, CAT activity and GST activity were analyzed in roots and leaves of plants.

2. MATERIALS AND METHODS

2.1. Application to Plant Material

The seeds were provided by the Sakarya Corn Research Institute in Turkey. Applications to corn (*Zea mays* L. cv. Ada 523) and wheat (*Tritucum aestivum* L. cv. Halis) varieties, which are our experimental material, were carried out in two different periods. The applications were made in 2 different periods; a) It is in the form of herbicide application on 7-day-old germinated seeds, and the targeted parameters for seedlings of certain age growing from them were determined and analyzed. b) In the seedling stage applications; In order to grow corn and wheat seedlings, seeds that are completely homogeneous (uniform) were selected, soaked in tap water and kept in the dark at 23-25 ^{o}C for 6 hours. At the end of this period, the seeds were placed in covered germination boxes where they could breathe and left to germinate in the dark at 23-25 ^{o}C for 3 days. Then the germinated seeds of equal lengths were selected and placed in the channels of the sponge lids at the mouth of the jars filled with previously prepared aqueous nutrient solutions. Here, the seedlings were grown in normal daylight in the long day period (16/8) until they were 15-days-old, and the herbicides were applied using the hydroponic method. Then, the targeted parameters were determined and analyzed for seedlings of certain ages where different herbicides were applied.

2.2. Determination of Chlorophyll (a + b) and Carotenoid Amount

0.5 g of fresh leaf tissue was used separately from all the applied groups. The leaf tissue was crushed with 50 ml of 80% acetone for 3-4 minutes, and a green extract was obtained. This process was continued until the color of the residue became completely colorless, and then it was centrifuged at 9000 rpm for 20 min. Then, in the spectrophotometer, the absorbances of the extracts at 440, 645, 652 and 663 nm wavelengths were read against the blank [12].

2.3. Determination of Superoxide dismutase (SOD; EC 1.15.1.1) Activity

2.6 ml of sod buffer, 100 µl from supernatant of sample, 250 µl epinephrine, 50 µl xanthinoxidase were added to the upper phase glass tubes, respectively. This mixture is vortexed for 30 min. kept in the dark environment. Then, in the spectrophotometer, the absorbance of the extracts at 485 nm wavelengths was read against the blank [13].

2.4. Determination of Catalase (CAT; EC 1.11.3.6) Activity

The upper phase has been taken into epondorphs. 1.9 ml phosphate buffer and 100 µl sample were taken into glass tubes and the absorbance was measured at 240 nm. 1 ml H_2O_2 was added on it and 15 sec. a measurement was made up to 120 seconds [14].

2.5. Determination of Glutathione-S-transferase (GST; EC 2.5.1.18) Activity

GST activity was measured in 100 mM potassium phosphate buffer (pH 6.5), 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 1 mM GSH at 340 nm. Absorbance measurement was started by adding GSH and CDNB to quartz test tubes containing 100 mM potassium phosphate buffer (pH 6.5) and adding reaction tissue samples [15]. Specific activities were determined using an extinction coefficient of 9.6 mM $^{-1}$ cm $^{-1}$.

2.6. Statistical Analysis

The results of the experiment were evaluated statistically with SPSS 15.00 package program. ANOVA (one-way analysis of variance; one-way ANOVA) test was used in the comparison between the control group and experimental groups. Results are given as mean ± standard error. P value for statistical significance level was accepted as $p<0.05$-$p<0.001$.

3. RESULTS AND DISCUSSION

3.1. Changes in Pigment Amount

When the effects of seedling treatments on the amount of chlorophyll (a + b) in the leaves were examined, significant differences were found in all groups where glyphosate herbicide was applied compared to the control group seedlings.

Compared to the control in terms of the amount of photosynthetic pigment in the leaves of the seedlings treated with glyphosate herbicide; A decrease of 7.96%, 17.77% and 40.26% was detected in 100, 500 and 1000 µM concentrations in 1-week-old wheat plant, respectively. A decrease of 5.87%, 10.39% and 16.97% was detected in the concentrations of 100, 500 and 1000 µM, respectively, in 15-day-old wheat seedlings. A decrease of 31.71% and 43.56% was determined at 500 µM and 1000 µM concentrations, respectively, in 1-week-old corn plant. A decrease of 18.01%, 29.79% and 53.31% was detected in the concentrations of 100, 500 and 1000 µM, respectively, in 15-day-old maize seedlings ($p<0.05$-$p<0.001$) (Table 1.).

For carotenoid amounts, in the leaves of the seedlings treated with glyphosate herbicide compared to the control; A decrease of 14.29%, 33.34% and 42.86% was detected in a 1-week-old wheat plant at 100 µM, 500 µM and 1000 µM concentrations, respectively. A decrease of 16.67%, 25% and 33.33% was observed in the 15-day-old wheat seedlings at 100, 500 and 1000 µM concentrations, respectively. A decrease of 11.12%, 27.78% and 44.45% was detected in the 1-week-old corn plant at 100, 500 and 1000 µM concentrations, respectively. A decrease of 8.70%, 17.39% and 34.78% was

detected in the concentrations of 100, 500 and 1000 µM, respectively, in 15-day-old maize seedlings ($p<0.05$-$p<0.001$) (Table 1.).

With the increase in glyphosate ratio, the accumulation of macroelements and microelements in plant tissues decreased, and photosynthetic parameters decreased [16, 17, 18, 11, 19]. Oxidative stress is change to electron transport between PSII and PSI and the NPQ (non-photochemical quenching mechanism) of the affected photosynthetic organisms is rapidly increasing to protect against photoinhibition. Glyphosate, the reduced maximum PSII quantum yield and relative electron transport would contribute to reductions in NPQ and lead to less protection for PSII, which further increased the adverse effects on the PSII reaction centers of the treated plants. When NPQ processes are showing a decrease, plants are seen lower carotenoid contents. Since carotenoids are known to quench ROS to protect against oxidative damage, a decrease in carotenoid content has been observed. For this, they use carotenoids, photosynthesis or chloroplast enzyme reactions [16].

Table 1. Toxic effect of glyphosate herbicide applied to 1-week-old and 15-day-old wheat and corn plants on pigment content

Groups	Chlorophyll (a +b) ($mg.g^{-1}Fw$)	Carotenoid ($mg.g^{-1}Fw$)	Chlorophyll (a + b) ($mg.g^{-1}Fw$)	Carotenoid ($mg.g^{-1}Fw$)
	1-week-old	1-week-old	15-day-old	15-day-old
B-Y-Control	2.29±0.05	0.21±0.01	2.47±0.01	0.24±0.01
B-Y-100 µM	2.11±0.01*	0.18±0.01**	2.32±0.01***	0.20±0.01***
B-Y-500 µM	1.89±0.05***	0.14±0.01***	2.21±0.02***	0.18±0.00***
B-Y-1000 µM	1.37±0.09***	0.12±0.01***	2.05±0.02***	0.16±0.00***
M-Y-Control	2.05±0.08	0.18±0.01	2.70±0.05	0.23±0.01
M-Y-100 µM	1.91±0.01	0.16±0.01*	2.21±0.01***	0.21±0.00***
M-Y-500 µM	1.40±0.05***	0.13±0.01***	1.90±0.01***	0.18±0.00***
M-Y-1000 µM	1.15±0.04***	0.10±0.00***	1.26±0.01***	0.15±0.00***

***: Compared to control; p≤0.05 is important at probability levels (*<0.05; **<0.01; ***<0.001). Average of the data ± SE (n: 10) Wheat: B, Corn: M, Leaf: Y**

3.2. Changes in Superoxide dismutase (SOD; EC 1.15.1.1) Activity

SOD activities of the roots of the seedlings treated with glyphosate herbicide compared to the control; An increase of 21.43%, 26.12% and 29.80% was detected at 100, 500 and 1000 µM concentrations in 1-week-old wheat plant, respectively. An increase of 5.45%, 11.91% and 18.20% was detected in the concentrations of 100, 500 and 1000 µM, respectively, in 15-day-old wheat seedlings. In 1-week-old corn, an increase of 19.02% and 25.14% was detected at 500 µM and 1000 µM concentrations, respectively. A decrease of 5.90%, 8.49% and 12.97% was determined at 100, 500 and 1000 µM concentrations, respectively, in 15-day-old maize ($p<0.05$-$p<0.001$) (Table 2).

SOD activities of the leaves of the seedlings treated with glyphosate herbicide compared to the control; An increase of 11.85%, 16.14% and 20.80% was detected at 100, 500 and 1000 µM concentrations, respectively, in a 1-week-old wheat plant. An increase of 4.86%, 16.46% and 22.77% was detected at 100, 500 and 1000 µM concentrations for 15-day-old wheat seedlings, respectively. An increase of 25.20%, 49.99% and 60.44% was detected at 100, 500 and 1000 µM concentrations in 1-week-old corn, respectively. An increase of 5.75%, 26.54% and 47.11% was detected at 100, 500 and 1000 µM concentrations, respectively, in 15-day-old maize ($p<0.05$-$p<0.001$) (Table 2). While all concentrations of SOD activities were increased in the leaves of the seedlings treated with glyphosate herbicide, the maximum 1-week-old corn plant showed an oxidative effect.

For plants, cell protection defense systems, which are enzymes in SOD activity, are important [20, 21]. SOD enzyme converts O_2^- into H_2O_2; the CAT enzyme decomposes H_2O_2 into H_2O and O_2, whereas APX converts H_2O_2 into H_2O [22].

3.3. Changes in Catalase (CAT; EC 1.11.3.6) Activity

CAT activities in the roots of the seedlings treated with glyphosate herbicide compared to the control; An increase of 4.75%, 14.44% and 34.65% was detected at 100, 500 and 1000 µM concentrations, respectively, in a 1-week-old wheat plant. An increase of 61.43%, 91.04% and 100% was detected in the concentrations of 100, 500 and 1000 µM, respectively, in 15-day-old wheat seedlings. An increase of 7.31%, 10.39% and 15.89% was detected at 100, 500 and 1000 µM concentrations in 1-week-old corn plant, respectively. An increase of 24.74%, 31.28% and 70.63% was found in the 15-day-old maize seedlings at concentrations of 100, 500 and 1000 µM, respectively ($p<0.05$-$p<0.001$) (Table 2).

CAT activities in the leaves of the seedlings treated with glyphosate herbicide compared to the control; An increase of 8.16%, 13.90% and 41.06% was detected at 100, 500 and 1000 µM concentrations in 1-week-old wheat plant, respectively. An increase of 57.20%, 148.14% and 305.18% was detected in the concentrations of 100, 500 and 1000 µM, respectively, in 15-day-old wheat seedlings. An increase of 16.24%, 19.86% and 22.02% was detected in 1-week-old corn plant at 100, 500 and 1000 µM concentrations, respectively. An increase of 7.10%, 11.25% and 15.70% was detected in the concentrations of 100, 500 and 1000 µM, respectively, in 15-day-old maize seedlings ($p<0.05$-$p<0.001$) (Table 2).

When exposed to the herbicide, plants will produce numerous ROS in the cell. ROS can peroxidize membrane lipids by directly or indirectly oxidizing macromolecules may lead to oxidative damage [30]. Plants produce antioxidant

defense systems, including enzymatic and non-enzymatic methods, as a result of ROS accumulation. Antioxidant enzymes play an important role in identifying ROS and oxidant-inducing agents. As the amount of ROS increases, the amount of MDA begins to accumulate. ROS-purifying enzyme activity and MDA content are widely used as markers of plant oxidative stress [23]. SOD is the first defense against ROS and then removed by CAT and peroxidases. Significant reduction in CAT and APX activities are detected in treated plants. It is reduced in plants treated with glyphosate and AMPA. H_2O_2, which increases with SOD activity, is not cleared by the H_2O_2-sakavengen enzyme system, which justifies the H_2O_2 accumulation observed in plants [16]. It has been observed that biochemical analyzes allow the determination of an increased ROS metabolism characterized by oxidative damage (protein oxidation) and an H_2O_2 metabolism with an increase in activity of CAT (peroxisomal enzyme) [3, 24, 25, 26, 27, 19].

3.4. Changes in Glutathione-s-transferase (GST; EC 2.5.1.18) Activity

GST activities of the roots of the seedlings treated with glyphosate herbicide compared to the control; An increase of 30.68% and 51.18% was detected at 100 µM and 1000 µM concentrations, respectively, in a 1-week-old wheat plant. An increase of 49.26% was found in the 15-day-old wheat seedlings at 1000 µM concentrations, respectively. In 1-week-old corn plant, 16.68% decrease, 4.08% and 8.62% increase were detected at 100, 500 and 1000 µM concentrations, respectively. An increase of 78.04% and 94.92% was detected in the 15-day-old corn seedlings at 500 µM and 1000 µM concentrations, respectively ($p<0.05$-$p<0.001$) (Table 2).

GST activities of the leaves of the seedlings treated with glyphosate herbicide compared to the control; A decrease of 18.95%, an increase of 41.82% and 53.71% were detected in 100 µM, 500 µM and 1000 µM concentrations, respectively, in a 1-week-old wheat plant. A decrease of 10.30%, 21.32% and 34.94% was detected in the concentrations of 100 µM, 500 µM and 1000 µM, respectively, in 15-day-old wheat seedlings. An increase of 23%, 29.22% and 35.35% was detected at 100 µM, 500 µM and 1000 µM concentrations in 1-week-old corn plant, respectively. An increase of 35.52%, 68.40% and 96.92% was detected in the 15-day-old corn seedlings at 100, 500 and 1000 µM concentrations, respectively ($p<0.05$-$p<0.001$) (Table 2).

15-day-old corn seedlings had the greatest effect on oxidative stress GST activity plays an important role in detoxification of H_2O_2 and lipid peroxides [28, 5, 29].

Table 2. Toxic effect of glyphosate herbicide applied to 1-week-old and 15-day-old wheat and corn plants on SOD, CAT and GST activities.

Groups	SOD (Unite.g^{-1})	CAT (µg.g^{-1})	GST (µg.g^{-1})	SOD (Unite.g^{-1})	CAT (µg.g^{-1})	GST (µg.g^{-1})
	1-week-old	**1-week-old**	**1-week-old**	**15-day-old**	**15-day-old**	**15-day-old**
B-Y-Control	5.83±0.08	343.33±5.57	2.88±0.05	8.25±0.11	112.06±0.08	9.82±0.11
B-Y-100 µM	6.52±0.03 ***	371.36±4.61 ***	2.34±0.04 ***	8.65±0.08 **	176.16±0.13 ***	8.81±0.01 ***
B-Y-500 µM	6.77±0.17 ***	391.05±3.58 ***	4.09±0,09 ***	9.61±0.04 ***	278.07±0.16 ***	7.73±0.02 ***
B-Y-1000 µM	7.04±0.05 ***	484.30±2.64 ***	4.43±0.02 ***	10.13±0.03 ***	454.06±0.19 ***	6.39±0.03 ***
M-Y-Control	3.66±0.05	324.57±5.78	2.20±0.02	5.21±0.059	256.64±1.61	2.51±0.04
M-Y-100 µM	4.58±0.06 ***	377.28±2.67 ***	2.71±0.01 ***	5.51±0.02 *	274.86±0.60 ***	3.40±0.04 ***
M-Y-500 µM	5.49±0.16 ***	389.01±1.92 ***	2.85±0.03 ***	6.59±0.20 ***	285.52±0.21 ***	4.23±0.02 ***
M-Y-1000 µM	5.87±0.09 ***	396.05±2.29 ***	2.98±0.01 ***	7.66±0.21 ***	296.95±0.33 ***	4.94±0.02 ***
B-K-Control	6.08±0.07	237.97±0.25	1.27±0.01	8.29±0.13	120.62±0.18	2.63±0.10
B-K-100 µM	7.39±0.09 ***	249.28±0.57 **	1.66±0.02 ***	8.74±0.03 **	194.73±0.16 ***	2.41±0.10
B-K-500 µM	7.67±0.03 ***	272.33±0.34 ***	1.79±0.01 ***	9.28±0.11 ***	230.43±0.23 ***	3.47±0.17 ***
B-K-1000 µM	7.89±0.05 ***	320.43±0.52 ***	1.92±0.01 ***	9.80±0.11 ***	319.10±0.12 ***	3.93±0.05 ***
M-K-Control	4.78±0.10	176.13±0.12	1.77±0.02	9.99±0.06	314.90±0.05	2.73±0.20
M-K-100 µM	4.88±0.09	189.00±0.06 **	1.48±0.01 ***	8.46±0.05 **	392.81±0.11 ***	4.13±0.07 ***
M-K-500 µM	5.69±0.10 ***	194.44±0.38 ***	1.85±0.02	8.23±0.07 ***	413.41±0.23 ***	4.86±0.03 ***
M-K-1000 µM	5.98±0.01 ***	204.12±0.25 ***	1.93±0.02 **	7.82±0.02 ***	537.32±0.18 ***	5.32±0.09 ***

***: Compared to control; p≤0.05 is important at probability levels (*<0.05; **<0.01; ***<0.001). Average of data ± SE (n: 3) Wheat: B, Corn: M, Leaf: Y, K: Root**

4. CONCLUSION

As a result, it has been found that glyphosate herbicide, which is not naturally synthesized in plants, is toxic to wheat and corn plants even at very low concentrations. Likewise, it should not be forgotten that the residue amounts of this herbicide may be at phytotoxic levels to wheat and corn depending on the moisture level in the soil, evaporation and insolation, organic matter amount and texture. 1-week-old plants were found to be more resistant than these 15-day-old plants.

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