*Pak. J. Bot.*, 51(2): 421-426, 2019. DOI: 10.30848/PJB2019-2(5)

# CHANGES BASED ON OXIDATIVE STRESS IN METOLACHLOR AND ATRAZINE TREATED MAIZE SEEDLINGS

**SONGUL CANAKCI-GULENGUL[1], OGUZ AYHAN KIRECCI[2] AND FADIME KARABULUT[3]**

*[1]Department of Biology, Fırat University, Elazıg, 23169, Turkey*
*[2]Department of Plant and Animal Production, Hizan Vocational School Bitlis Eren University, Bitlis, Turkey*
*[1]Corresponding author's email: scanakci@firat.edu.tr*

**Abstract**

The present study investigated biochemical effects of Atrazine (0, 200µM , 500 µM and 1000 µM) and Metolachlor (0, 100µM , 500 µM and 1000 µM) concentrations applied to 15-day-old seedlings of three maize varieties (*Zea mays* L., cv. Saccharata, cv. Danona, and cv. Advanta 2898) for 48 hr. Hydroponic environment was preferred for all treatments for the seedlings. Compared to their controls, GSH/GSSG ratio was decreased, SOD activity was increased, and CAT activity was also decreased/decreased in root/leaf of Atrazine treated maize seedlings. While % ratios of fatty acids in leaf was increased for palmitic acid and palmitoleic acid in cv. Saccharata and advanta 2898, but was decreased in cv. Danona. While the rate of linoleic acid was increased in leaves in other two ratios but decreased only in cv. Saccharata; linoleic acid was increased only in cv. Saccharata and decreased in others. Compared to their controls, in root/leaf of maize seedlings treated with Metolachlor; GSH/GSSG ratio decreased/increased, SOD activity decreased/increased, and CAT activity decreased/decreased. While % ratios of fatty acids in leaf showed an exceptional increase and increase for linolenic acid, palmitic acid, and linoleic acid; palmitoleic acid and stearic acid decreased and palmitic acid decreased only in cv. Advanta 2898. Generally, the inhibitive effect of the herbicides Atrazine and Metolachlor elevated in parallel to increased dose (1000 µM).However, Atrazine displayed a more oxidative damagesthan Metolachlor on three maize varieties.

**Key words**: Maize (*Zea mays* L.), Oxidative stress, Atrazine, Metolachlor, Herbicides.

## Introduction

Atrazine is a herbicide from triazine class (Jiang *et al.,* 2016), which is used before plantation of plants to inhibit the development of broad leafed herbaceous weeds. Atrazine, Linuron, Atrazine+Linuron and Atrazine+Metolachlor were applied in corn control weeds before germination. Atrazine becomes intense in root area which is about 125 cm deep. Because half-life of Atrazine is longer compared to metolachlor and Linuron, a great majority of it remains in the environment (Pudelko *et al.,* 1993). Besides, controlling weeds, Atrazine has ecotoxic effects on non-target organisms (crop plants, soil microorganism, and other animals). Thus, this herbicide triggers the formation of reactive oxygen species leading to oxidative stress (Jiang *et al.,* 2016). Even among varieties of the same species may differs in terms of herbicide sensitivity. (Khan *et al.,* 2011).

GSH content was increased as a result of the effect of the herbicides Atrazine and Metolachlor applied to leaves of 30 day-old maize plant (*Zea mays* L. var. Artus) (Hatton *et al.,* 1996). GSH content was also increased by the effect of Metolachlor applied to leaves of 6 and 8 day-old maize plants (Alla & Hassan, 1998). It was reported that when maize plant was treated with 10 µM of metolachlor in a ratio of 18:1 and 18:2 fatty acid contents were elevated and in a ratio of 18:3 fatty acid content was declined in shoots. When 5 µM of metolachlor was applied, 16:0 and 18:1 fatty acid content decreased and 18:3 fatty acid content increased (Wu *et al.,* 2000). Changes were observed in SOD, POD, and CAT enzyme activities in a study where roots and leaves of maize were treated with racemic-metolachlor and s-metolachlor. There was an increase in the treatment of racemic-metolachlor but a decrease in the treatment of s-metolachlor (Xie *et al.,* 2014). Seeds and leaves of *Lactuca sativa* L. cv. Vuka, *Phaseolus vulgaris* L. cv. Zlatko and *Pisum sativum* L. cv. Dunav were treated with 0.2-200 µM concentrations of Metolachlor for 48 hr and SOD and CAT activities decreased (Stajner *et al.,* 2003). In a study, young and mature leaves of pea were sprayed with 23 µM dose of 2,4-D at certain intervals and oxidative damage was identified in proteins and membrane lipids after 72 hr. There was an increase for glutathione content. GSH/GSSG ratio was higher in leaves of mature pea plant. SOD enzyme activity, on the other hand, increased in leaves of young plant. CAT enzyme activity decreased (Pazmiño *et al.,* 2011). SOD, CAT, POD, and antioxidant enzyme activities were determined to increase in roots and leaves of rice plant treated with various doses of Atrazine for 4 days (Zhang *et al.,* 2014). Different doses of Atrazine were applied to 2 varieties of 10-day old maize (*Zea mays* L. Hybrid 351 and Giza 2), GSH content increased in Hybrid 351 at the end of 20$^{th}$ day, on the other hand, it decreased in hybrid Giza 2 maize. SOD and CAT enzymes decreased (Alla & Hassan, 2006). The 21-day-old *Palaemonetes argentinus* plant was kept in hydroponic environment for 1 day after applying 0.4 mg/L$^{-1}$ dose of Atrazine. SOD content was proven to elevate (Griboff *et al.,* 2014). After atrazine was applied at 0-10 mg/L concentrations to leaves of the 2-week-old maize for 3 days, SOD and POD enzymes increased in roots and CAT enzyme increased in leaves (Li *et al.,* 2012). Different concentrations of 2,4-D were applied to maize seeds and GSH/GSSG ratio decreased (Dragicevic *et al.,* 2013). Paraquat was reported to cause increase of SOD, CAT, and ascorbate peroxidase enzymes in *Saccharum* spp. (RB92579, SP83-2847, SP81-3250 and IAC91-5155) (hybrid sugar canes) seedlings (Santos & Silva, 2015). In the study investigating the toxic effect of on leaves of *Triticum aestivum* L., cv. *Mironovskaya* 808 (wheat), *Secale cereale* L. cv. *Estafeta tatarstana* (rye) and *Zea mays* L. cv. *Kollektivnyi* 172MV (maize) seedlings, it was

reported that lipid peroxidation, superoxide anion, total antioxidant activity, catalase and ascorbate peroxidase activities increased (Lukatkin *et al.,* 2013). Kraus *et al.,* (1995) indicated that herbicides paclobutrazol and paraquat led to increase SOD and CAT activity in leaves of *Triticum aestivum* L. cv. Frederick and Glenlea (hybrid wheat) (Kraus *et al.,* 1995). Herbicide chlorimuron-ethyl was reported to lead to decrease SOD content in leaves and roots of *Triticum aestivum* (wheat) seedlings (Wang & Zhou, 2006). Ferns (Azolla spp. viz., Azolla microphylla and Azolla pinnata) were treated with herbicide Pretilachlor (0, 5, 10 and 20 µg $ml^{-1}$), GSH content and GSH/GSSG ratio decreased with increasing dose (Prasad *et al.,* 2016). After herbicide Chrial was applied to leaves of *Arabidopsis thaliana*, GSH content, SOD activity, and CAT activity increased; on the other hand, all declined in the variety Rac DCPP (Chen *et al.,* 2016). SOD and CAT activities increased until 3.2 $mg.kg^{-1}$ of concentration and decreased at 4.8 and higher concentrations in roots and leaves of wheat varieties by applying Simetryne (s-triazine herbicide) (0, 0.8,1.6, 3.2, 4.8, 6.4 and 8.0) (Jiang *et al.,* 2016). After applying Atrazine to roots and leaves of *Pennisetum americanum* seedlings for 68 days, SOD and CAT activity increased and a decline was determined at higher concentrations (Jiang *et al.,* 2016). In the present study, biochemical responses based on toxic effect by different concentrations of synthetic herbicides Atrazine and Metolachlor in three maize varieties were investigated.

## Material and Methods

**Growth conditions of seedlings and experimental design:** Maize (*Zea mays* L., cv. Saccharata, cv. Danona and cv. Advanta 2898) seeds were soaked in tap water for 6 hr, surface was sterilized with 0.5% (m/v) sodium hypochlorite for 30 min, rinsed several times with distilled water and then germinated on moist cotton placed in sterilized dishes. Seeds germinated for three days were sown in plastic pots filled with sand and topsoil. They were grown by being soaked in tap water 3 times for a week. The plants were grown in the controlled growth chambers (having a photoperiod of 16 hr daylight /8 hr night, a temperature of 27±2°C and arelative humidity of 60-70 %), respectively. Seedlings that showed abnormal growth were eliminated. Before the treatment, roots of the seedlings were washed with deionized water and placed between the canals of the sponge lids of jars that contained basic Hoagland nutrient solution (30%). Fifteen-day-old seedlings were used for experimental purpose. When preparing Atrazine stock solutions, it was firstly dissolved in 50 ml of ethyl alcohol and then the final volume was completed to 1 L with distilled water. The nutrient solution was aerated twice a day. Atrazine (0, 200 µM, 500µM and 1000 µM) and Metolachlor (0, 100µM, 500µM and 1000 µM) concentrations prepared in deionized water were used as test solutions. The seedlings were divided into four groups, including 10 seedlings in each one. The test solutions were applied to the roots of seedlings by using Hydroponicenvironment. Four sets of seedlings were placed in jars containing (0, 200µM, 500µM and 1000 µM Atrazine; 0, 100µM, 500µM and 1000µM Metolachlor) solutions and these seedlings were treated with test solutions for 48 hr. After the treatment with Atrazine and Metolachlor, the seedlings were harvested to investigate GSH/GSSG rations, fatty acids, SOD and CAT activityof biochemical parameters.

**Biochemical analysis of seedlings:**GSH/GSSG ratios in plant extracts were determined according to the method of Yilmaz *et al.,* (2009) SOD activity (Mourente, 1999) and CAT activity (Aebi, 1984) were analyzed in liquid portions of plant extracts. Fatty acids were analyzed in solid parts of plant extracts. For the analysis of fatty acids, leaf and root tissues were used and analyzed in gas chromatography (Christie, 1990; Hara & Radin, 1978). Results were determined in terms of weight % of total. Three replicates were maintained for each treatment. All physiological analyses were replicated three times for each treatment. In each analysis, 3 g of leaf tissue and 1.5 g of root tissue were used.

**Statistical analyses:** Results were analyzed using one-way ANOVA (SPSS 15.0 Evaluation Version Production Mode Facility). The difference between the treatments was accepted as significant at the levels of $p<0.01$-0.05. Duncan test (Duncan, 1955)was performed to compare the means. The data which were not statistically significant in all parameters in the present study were not emphasized ($p>0.05$) (Table 1-A and B).

## Results

**The effect of atrazine and metolachlor on GSH/GSSG ratio:** Compared to their controls, there was a considerable decrease in GSH/GSSG ratio in Advanta 2898 (22.57, 28.48, and 35.77%) among the roots of seedlings treated with Atrazine. On the other hand, in leaves, the maximum decrease (18.14, 20.33, and 26.65%) was also determined in Advanta 2898 ($p\leq0.05$) (Table 1-A). The most efficient decrease for Metolachlor treatment (35.48, 52.68, and 69.89%) was determined in Advanta 2898. The most effective increase (10.41, 33.43 and 46.05%) was also observed in leaves of Advanta 2898 ($p\leq0.05$) (Table 1-B).

**The effect of atrazine and metolachlor on SOD and CAT activity:** Compared to their controls, Atrazine treatment led to more effective SOD activity (20.74, 34.29 and 42.87%) increase in roots of Danona seedlings. On the other hand, in leaves, it led to an effective increase (14.28, 52.66 and 72.72%) in Danona ($p\leq0.05$) (Table 1-A). There was a decreased SOD activity in Danona hybrid maize (19.19, 30.46 and 28.86%) in roots of seedlings for Metolachlor treatment. In leaves, a decrease was determined for Saccharata (5.87, 33.50 and 42.38%) ($p\leq0.05$) (Table 1-B). Atrazine treatment not only decreased CAT activity in roots and leaves of all maize seedlings compared to their controls but also led to a more distinct decrease in roots of Saccharata (16.56, 27.13 and 36.34%) and leaves of Danona (28.91, 37.53 and 41.43%). The most effective increase was determined in roots of Danona (35.99, 41.57, and 54.58%) and leaves of Saccharata (6.02, 31.59 and 50.82%) for Metolachlor treatment ($p\leq0.05$) (Table 1-B).

**Table 1. GSH/GSSG ratio, SOD, CAT activity and Fatty acids content changes in the *Zea mays* L. seedlings applied to Atrazine (A) and Metolachlor (B).**

| (A) Atrazine | GSH/GSSG | | SOD (unite/g) | | CAT (µg/g) | | Fatty acid changes ( % of WT ) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 16:00 | 16:01 | 18:00 | 18:02 |
| Groups | Root | Shoot | Root | Shoot | Root | Shoot | Shoot | Shoot | Shoot | Shoot |
| A-Control | 1,51 ± 0,11 | 3,64 ± 0,10 | 19,60 ± 0,47 | 39,79 ± 0,82 | 84,08 ± 1,95 | 44,23 ± 1,36 | 14,12 ± 0,97 | 15,93 ± 0,97 | - | 11,52 ± 1,07 |
| A-200 µM | 1,17 ± 0,31* | 2,98 ± 0,05* | 23,49 ± 0,23* | 42,62 ± 0,49* | 75,94 ± 1,23* | 39,35 ± 1,67* | 16,29 ± 0,98 | 17,74 ± 1,02 | - | 14,07 ± 1,21 |
| A-500 µM | 1,08 ± 0,02* | 2,90 ± 0,06* | 25,01 ± 0,51* | 50,29 ± 0,07* | 64,72 ± 2,16* | 32,61 ± 0,82* | 17,52 ± 0,96 | 18,86 ± 1,31* | - | 14,60 ± 1,27 |
| A-1000 µM | 0,97 ± 0,01* | 2,67 ± 0,01* | 27,09 ± 0,59* | 54,95 ± 0,64* | 57,00 ± 2,31* | 28,09 ± 1,76* | 19,00 ± 1,10* | 20,23 ± 1,44* | - | 15,70 ± 1,63* |
| D-Control | 1,12 ± 0,03 | 3,22 ± 0,03 | 25,90 ± 1,07 | 37,18 ± 1,32 | 201,72 ± 0,18 | 36,66 ± 0,85 | 17,66 ± 2,11 | 15,34 ± 0,59 | - | 12,51 ± 1,23 |
| D-200 µM | 1,04 ± 0,03 | 2,76 ± 0,06* | 31,26 ± 1,11* | 42,49 ± 0,63* | 200,08 ± 0,32 | 26,06 ± 0,79* | 16,85 ± 1,59 | 14,78 ± 0,57 | - | 14,79 ± 1,75 |
| D-500 µM | 0,90 ± 0,04* | 2,67 ± 0,05* | 34,77 ± 0,53* | 56,76 ± 0,99* | 197,09 ± 0,54* | 22,90 ± 0,34* | 16,00 ± 1,53 | 13,42 ± 0,75 | - | 15,57 ± 1,06 |
| D-1000 µM | 0,77 ± 0,01* | 2,06 ± 0,01* | 36,99 ± 0,68* | 64,23 ± 0,76* | 145,39 ± 1,50* | 21,47 ± 0,24* | 15,11 ± 1,36* | 12,46 ± 0,65* | - | 18,02 ± 1,96* |
| S-Control | 1,00 ± 0,09 | 2,47 ± 0,03 | 21,34 ± 1,76 | 34,23 ± 0,69 | 91,24 ± 0,60 | 69,11 ± 0,44 | 11,70 ± 0,93 | 14,61 ± 1,04 | - | 12,22 ± 0,83 |
| S-200 µM | 0,90 ± 0,04 | 2,33 ± 0,01* | 26,02 ± 0,41* | 35,08 ± 0,82 | 76,13 ± 1,09* | 58,14 ± 1,02* | 12,18 ± 1,59 | 15,44 ± 0,96 | - | 11,69 ± 1,25 |
| S-500 µM | 0,75 ± 0,03* | 2,01 ± 0,01* | 27,12 ± 0,36* | 37,13 ± 0,54* | 66,48 ± 0,81* | 54,24 ± 0,66* | 12,61 ± 0,93 | 16,25 ± 1,16 | - | 10,90 ± 1,37 |
| S-1000 µM | 0,66 ± 0,02* | 1,91 ± 0,01* | 28,45 ± 0,67* | 38,89 ± 0,26* | 58,09 ± 0,71* | 41,96 ± 0,41* | 13,90 ± 1,06 | 17,87 ± 0,85* | - | 9,87 ± 1,43 |

*: Compared to the control group at p≤0.05 probability levels. Values are mean of three replicates ± SE (n: 3) Advanta 2898: A, Danona: D, Saccharata: S

| (B) Metolachlor | GSH/GSSG | | SOD (unite/g) | | CAT (µg/g) | | Fatty Acid Changes ( % of WT ) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 16:00 | 16:01 | 18:00 | 18:02 | 18:03 |
| Groups | Root | Shoot | Root | Shoot | Root | Shoot | Shoot | Shoot | Shoot | Shoot | Shoot |
| A-Control | 0,93 ± 0,22 | 3,17 ± 0,38 | 29,12 ± 0,32 | 14,49 ± 0,15 | 62,98 ± 0,31 | 75,72 ± 0,35 | 42,85 ± 1,30 | 2,06 ± 0,29 | 3,06 ± 0,32 | 11,46 ± 0,25 | 47,31 ± 0,40 |
| A-100 µM | 0,60 ± 0,11 | 3,50 ± 0,51 | 26,19 ± 0,60 | 13,80 ± 0,09* | 77,09 ± 0,37* | 82,88 ± 0,29* | 39,16 ± 0,60 | 1,46 ± 0,26* | 2,76 ± 0,32 | 12,32 ± 0,47 | 48,78 ± 0,46 |
| A-500 µM | 0,44 ± 0,06* | 4,23 ± 0,74 | 23,49 ± 1,10* | 12,56 ± 0,22* | 81,13 ± 0,57* | 83,54 ± 0,11* | 36,15 ± 1,72 | 1,26 ± 0,04* | 2,57 ± 0,24 | 12,95 ± 0,13* | 49,53 ± 0,39 |
| A-1000 µM | 0,28 ± 0,05* | 4,63 ± 0,90 | 20,97 ± 1,80* | 12,21 ± 0,02* | 83,55 ± 0,40* | 90,29 ± 0,18* | 34,10 ± 2,41 | 1,27 ± 0,20* | 2,43 ± 0,33 | 13,02 ± 0,36* | 52,09 ± 1,35* |
| D-Control | 0,86 ± 0,18 | 2,34 ± 0,29 | 27,51 ± 1,07 | 15,69 ± 0,06 | 115,61 ± 3,87 | 61,77 ± 0,40 | 37,75 ± 1,64 | 2,52 ± 0,23 | 2,81 ± 0,40 | 11,60 ± 0,55 | 46,22 ± 2,00 |
| D-100 µM | 0,70 ± 0,12 | 3,05 ± 0,31 | 22,23 ± 0,46* | 15,28 ± 0,16* | 157,22 ± 1,15* | 63,63 ± 0,59* | 42,71 ± 1,58 | 2,35 ± 0,13 | 2,52 ± 0,32 | 11,90 ± 0,47 | 49,42 ± 2,00 |
| D-500 µM | 0,54 ± 0,12 | 3,62 ± 0,33* | 19,13 ± 0,51* | 13,71 ± 0,07* | 163,68 ± 3,54* | 65,70 ± 0,63* | 43,87 ± 1,52 | 2,04 ± 0,06 | 2,26 ± 0,26 | 12,20 ± 0,37 | 52,43 ± 1,48* |
| D-1000 µM | 0,40 ± 0,10* | 3,92 ± 0,36* | 19,57 ± 0,20* | 12,35 ± 0,16* | 178,72 ± 0,64* | 69,74 ± 0,16* | 47,62 ± 2,77 | 1,88 ± 0,04* | 2,20 ± 0,37 | 12,68 ± 0,29* | 52,78 ± 1,95* |
| S-Control | 0,88 ± 0,12 | 3,84 ± 0,60 | 28,01 ± 0,34 | 21,61 ± 0,26 | 159,57 ± 0,14 | 68,83 ± 0,33 | 36,18 ± 1,37 | 1,82 ± 0,07 | 2,00 ± 0,31 | 9,15 ± 0,08 | 55,32 ± 2,26 |
| S-100 µM | 0,75 ± 0,13 | 4,37 ± 0,67 | 27,32 ± 0,51 | 20,34 ± 0,17* | 167,87 ± 1,05* | 72,98 ± 0,71* | 40,18 ± 0,71 | 1,60 ± 0,06 | 1,81 ± 0,34 | 9,40 ± 0,06 | 56,53 ± 1,81 |
| S-500 µM | 0,62 ± 0,08 | 4,53 ± 0,69 | 26,71 ± 0,46 | 14,37 ± 0,46* | 175,19 ± 0,33* | 90,58 ± 0,18* | 41,85 ± 1,11 | 1,35 ± 0,16 | 1,56 ± 0,39 | 9,58 ± 0,04 | 57,76 ± 1,54 |
| S-1000 µM | 0,52 ± 0,07* | 4,68 ± 0,67 | 25,68 ± 0,36* | 12,45 ± 0,07* | 180,68 ± 0,62* | 103,81 ± 0,68* | 44,25 ± 0,43 | 1,24 ± 0,12* | 1,54 ± 0,40 | 9,18 ± 0,04* | 61,52 ± 2,51* |

*: Compared to the control group at p≤0.05 probability levels. Values are mean of three replicates ± SE (n: 3) Advanta 2898: A, Danona: D, Saccharata: S

**The effect of atrazine and metolachlor on fatty acid content:** In leaves of seedlings treated with Atrazine; linolenic acid rate exceptionally decreased in Advanta and Danona (10.51, 12.72 and 14.70 % for Advanta ; 15.83 and 21.72% for Danona) but increased in Saccharata (7.55%); on the other hand, linoleic acid rate increased exceptionally (36.28% for Advanta 2898; 44.04% for Danona), there were decreases only in Saccharata. It was determined that palmitic acid rate increased mostly (34.56%) for Advanta 2898 and decreased (14.44 %) for Danona. In roots of maize seedlings treated with Atrazine, palmitic acid increased by 28.71 and 36.33 % for Advanta 2898; decreased by 15.68% for Danona, and increased by 21.56 and 26.56% for Saccharata and linoleic acid increased by 18.61% for Advanta 2898, decreased by 17.07% for Danona, and increased by 22.39% for Saccharata. Palmitoleic acid, stearic acid, and linolenic acid were not identified in roots of maize seedlings. Therefore, data of roots could not be presented since all of the fatty acids were not identified. While there were insignificant decreases for palmitic acid and stearic acid in leaves of seedlings treated with Metolachlor, palmitic acid increased in Danona and Saccharata. In leaves of seedlings treated with Metolachlor, palmitoleic acid decreased (29.12, 38.83 and 38.34% for Advanta 2898; 25.39% for Danona; 31.87% for Saccharata), linoleic acid increased (13 and 13.61% for Advanta 2898; 9.31% for Danona; 7.21% for Saccharata), and linolenic acid increased (10.10% for Advanta 2898; 13.44 and 14.19% for Danona; 11.21% for Saccharata). In roots of seedlings treated with Metolachlor, palmitic acid (8.59 and 16.27% for Advanta 2898; 8.53, 16.34 and 22.82% for Danona; 15.10 and 19.32% for Saccharata) and palmitoleic acid (24.70 and 49.26% for Advanta 2898; 18.89% for Danona; 14.52, 27.19, and 36.18% for Saccharata) decreased, stearic acid (28.72 and 42.45% for Advanta 2898; 32.36% for Danona) and 18:2 (10.48% for Danona; 19.85% for Saccharata) increases were determined ($p \leq 0.05$) (Table 1-B). Generally, declines of response were determined for fatty acids and it was considered that these declines were associated with decreased oxidative stress resistance of seedlings treated with Atrazine. Compared to other two maize varieties, related responses of Advanta 2898 seedlings were more distinctive.

## Discussion

The maximum decrease was observed in Advanta 2898 for Atrazine (Alla & Hassan, 2006) and Metolachlor in terms of GSH/GSSG contents in roots of maize seedlings. In terms of GSH/GSSG contents in leaves of maize seedlings; the maximum decrease for Atrazine was observed in Danona (Alla & Hassan, 2006); whereas, the maximum increase forherbicide Metolachlor was seen in Danona (Hatton *et al.,* 1996; Alla & Hassan 1998; Kaya & Doğanlar, 2016; Pazmiño *et al.,* 2011; Prasad *et al.,* 2016). Glutathione reductase enzyme (GR) converts oxidized glutathione (GSSG) into reduced glutathione (GSH) via a reaction based on NADPH. GSH and GR constitute the compounds of the ascorbate-glutathione metabolism playing a role in plant response to stress (Kaya & Doğanlar, 2016).

In terms of SOD enzyme activity in roots and leaves of maize seedlings; there was the maximum increase in Danona for Atrazine (Zhang *et al.,* 2014; Griboff *et al.,* 2014; Li *et al.,* 2012; Santos & Silva, 2015; Kraus *et al.,* 1995; Chen *et al.,* 2016; Jiang *et al.,* 2016) and the maximum decrease in roots of Danona and in leaves of Saccharata for metolachlor (Xie *et al.,* 2014; Stajner *et al.,* 2003; Wang & Zhou, 2006). Antioxidant system plays a crucial role for protecting cellular compounds from damages of reactive oxygen species produced under stress. Under optimal growth conditions, the production of reactive oxygen species in plant cells is low. However, increased production and accumulation of reactive oxygen species under most of environmental stresses brings along deterioration of cellular hemostasis (Wang *et al.,* 2015). SOD activity increasing under stress conditions indicates that particularly superoxide radical reactive oxygen species are produced excessively. This is because SOD plays a role in eradication of superoxide radical from chloroplasts and superoxide radical is converted into $H_2O_2$. Herbicide toxicity occurs as a result of SOD activation substantially increasing in antioxidant system (Santos & Silva, 2015).

In terms of CAT enzyme activity in roots of maize seedlings; the maximum decrease was observed in Saccharata for Atrazine (Alla & Hassan, 2006). In terms of CAT enzyme activity in leaves of maize seedlings; there was the maximum decrease in Danona for Atrazine. In terms of CAT enzyme activity of maize seedlings; the maximum increase was observed in roots of Danona and in leaves of Saccharata for Metolachlor (Xie *et al.,* 2014; Santos & Silva, 2015; Kraus *et al.,* 1995; Lukatkin *et al.,* 2013; Chen *et al.,* 2016; Jiang *et al.,* 2016). Catalase is found in organelles, called as peroxidase, in all cells of plants and plays a protective role by maintaining $H_2O_2$ ($H_2O + ½ O_2$) at a certain level for the cell. Herbicide exposure of pea, wheat, and maize leaves has led to an increased catalase activity. High concentration of catalase enzyme ensure the minimal damage from stress for plant by detoxifying $H_2O_2$. Increased activity of antioxidant enzymes such as SOD and CAT is a result of detoxification mechanism that provides the decline of lipid peroxidation. Oxidative damage in leaves of seedlings may be considered to cause imbalanced enzymatic activity while increasing SOD and decreasing other enzymes, and elevated $H_2O_2$ in chloroplasts is likely to be associated with this (Santos & Silvas, 2015).

Ivanova *et al.,* (2008) stated that oxidative stress caused decreases in FAME amounts in maize leaves. While palmitic acid (16:0) and palmitoleic acid (16:1)

exceptionally increased in leaves of maize seedlings treated with Atrazine, there was a decrease in Danona. While linoleic acid (18:2) increased in leaves, a decrease was observed for Saccharata. Even though linolenic acid (18:3) decreased in leaves treated with Atrazine, there was an increase for Saccharata. Linolenic acid (Wu *et al.,* 2000) increased, palmitoleic acid decreased, and stearic acid (18:0) content increased exceptionally in leaves treated with Metolachlor; whereas, linoleic acid and palmitic acid increased. Palmitic acid was observed to decrease in Advanta in seedlings treated with Metolachlor. The action mechanism of the herbicide Chloroacetanilid was suggested to occur by inhibiting the synthesis of long-chain fatty acids in plant or inhibiting involvement of unsaturated fatty acids into non-fat structures (Wu *et al.,* 2000). Biochemical and physiological processes were observed to be inhibited by the herbicide Chloroacetanilid in higher plants including cellular growth, mineral intake, cellular division, synthesis of gibberellic acid, lipid, and protein (Wu *et al.,* 2000). While palmitic acid and linoleic acid increased in roots of seedlings treated with Atrazine, there was a decline for Danona. While linoleic acid (Wu *et al.,* 2000) increased, palmitic acid and palmitoleic acid decreased, and stearic acid increased exceptionally in roots of maize seedlings treated with Metolachlor, a decrease was observed for Saccharata. However, data of roots were not presented since all fatty acids were not identified.

Consequently, the synthetic herbicides Atrazine and Metolachlor were determined to be toxic for maize plant even at very low concentrations. Because the related studies are very limited and insufficient, we could not discuss the results of the present study in a wide platform and had to make reference to indirect studies occasionally. We think that further studies would contribute a complete understanding of the issue. In conclusion, high doses of Atrazine according to metolachlor triggered toxic effect, therefore antioxidant responses.

**References**


Aebi, H. 1984. Catalase in vitro. *Method Enzym*., 105: 121-126.

Alla, M.M.N. and N.M. Hassan. 1998. Efficacy of exogenous GA3 and herbicide safeners inprotection of *Zea mays* from metolachlor toxicity. *Plant Physiol. & Biochem*., 36 (11): 809-815.

Alla, M.M.N. and N.M. Hassan. 2006. Changes of Antioxidants levels in two maize lines following atrazine treatments. *Plant Physiol. & Biochem.*, 44: 202-210.

Chen, Z., H. Chen, Y. Zou and Y. Wen. 2016. Stomatal behaviorsre flectenantiose lective phytotoxicity of chiral herbicide dichlorprop in *Arabidopsis thaliana. Sci. the Total Environ.*, 562: 73-80.

Christie, W.W. 1990. Gas Chromato graphyand Lipids. *The Oily Pres: Glasgow*, 302.

Dragicevic, V., M. Spasic, M. Simic, D. Zoran and B. Nikolic. 2013. Stimulative in fluence of germination and growth of maize seedling soriginating fromaged seeds by 2,4-D potencies. *Homeopathy*, 102: 179-186.

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics*, 11: 1-42.

Griboff, J., D. Morales, L. Bertrand, R.I. Bonansea, M.V. Monferran, R. Asis, D.A. Wunderlin and M.V. Ame. 2014. Oxidative stresses ponse induced by atrazine in Palaemonetes argentinus: The protective effect of vitamin E. *Ecotoxicol. & Environ. Safety*, 108: 1-8.

Hara, A. and N.S. Radin. 1978. Lipid extraction of tissues with low-toxicity soluent. *Anal. Biochem*., 90(1): 420-426.

Hatton, P.J., D.J. Cole and R. Edwards. 1996. Influence of plant age on glutathione transferases involved in herbicide detoxification in corn (*Zea mays* L.) and giant foxtail (*Setaria faberi* Herrm). *Pest. Biochem. & Physiol.*, 54: 199-209.

Ivanova, A., A. Krantev, Z.H. Stoynovaand and L. Popova. 2008. Cadmium-Induced changes in maize leaves and the protective role of salicylicacid. *Gen. Appl. Plant Physiol.*, *Special Issue*, 34: 149-158.

Jiang Z., M. Bingbing, K.O. Erinle, B. Cao, X. Liu, S. Ye and Y. Zhang. 2016. Enzymatic antioxidant defense in resistant plant: *Pennisetum americanum* (L.) K. Schum during long-term atrazine exposure. *Pest. Biochem. & Physiol.*, 133: 59-66.

Kaya, A. and Z.B. Doğanlar. 2016. Exogenous jasmonic acid induces stres tolerance in tobacco (*Nicotiana tabacum*) exposed to imazapic. *Ecotoxicol. & Environ. Safety*, 124: 470-479.

Khan, M.I., G. Hassan, I. Khan, K.B. Marwat, N.U. Khan and R. Gul. 2011. Tolerance of chickpea (*Cicer arietinum* L.) cultivars to the major chickpea herbicides. *Pak. J. Bot*., 43(5): 2497-2501.

Kraus, T.E., B.D. Mckersie and R.A. Fletcher. 1995. Paclobutrazol-induced tolerance of wheat leavesto paraquat may involve increased antioxidant enzyme activity. *J. Plant Physiol*., 145: 570-576.

Li, X., T. Wu, H. Huang and S. Zhang. 2012. Atrazine accumulation and toxic responses in maize *Zea mays*. *J. Environ. Sci.*, 24(2): 203-208.

Lukatkin, A.S., A.N. Gar'kova, A.S. Bochkarjova, O.V. Nushtaeva and J.A.T. Silva. 2013. Treatment with the herbicide TOPIK induces oxidative stress in cereal leaves. *Pest. Biochem. & Physiol.*, 105: 44-49.

Mourente, G., D.R. Tocher, E. Diaz, A. Grau and E. Pastor. 1999. Relation ships between antioxidants, antioxidant enzyme activities and lipid peroxidation products during early development in *Dentex dentex* eggs and larvae. *Aquaculture*, 179: 309-324.

Pazmiño, D.M., M. Rodriguez-Serrano, M.C. Romero-Puertas, A. Archılla-Ruız, L.A. Del Rio and L. M. Sandalio. 2011. Differential response of young and adult leaves to herbicide 2,4-dichlorophenoxyacetic acid in peaplants: Role of reactive oxygen species, *Plant Cell & Environ.*, 34: 1874-1889.

Prasad, S.M., S. Kumar, P. Parihar and R. Singh. 2016. Interactive effects of herbicide and enhanced UV-B on growth, oxidative damage and the ascorbate-glutathione cycle in two *Azolla* species. *Ecotoxicol. & Environ. Safety*, 133: 341-349.

Pudelko, J.A., D.L. Wright and I. D. Teare. 1993. A method for salvaging bird damaged pearl millet research. *Fla. Agric. Exp. Stn. Res. Rep*. No: NF 93-12:1-11.

Santos, C.M. and M.A. Silva. 2015. Physiological and biochemical responses of sugar cane to oxidative stres induced by water deficit and paraquat. *Acta Physiol. Plant*, 37: 172.

Stajner, D., M. Popovıc and M. Stajner. 2003. Herbicide Induced oxidative stress in lettuce, beans, pea seeds and leaves. *Biologia Plantarum*, 47(4): 575-579.

Wang, M. and Q. Zhou. 2006. Effects of herbicide chlorimuron-ethyl on physiological mechanisms in wheat (*Triticum aestivum*), *Ecotoxicol. & Environ. Safety*, 64: 190-197.

Wang, Q., X. Que, R. Zheng, Z. Pang, C. Li and B. Xiao. 2015. Phytotoxicity assessment of atrazine on growth and physiology of three emergent plants. *Environ. Sci. Pollut. Res*., 22: 9646-9657.

Wu, J., I.T. Hwang and K.K. Hatzios. 2000. Effects of chloroacetanilide herbicides on membrane fatty acid desaturation and lipid composition in rice, maize and sorghum. *Pest. Biochem. & Physiol.,* 66: 161-169.

Xie, F., H.J. Liuand and W.D. Cai. 2014. Enantio selectivity of racemic metolachlor and S-metolachlor in maize seedlings. *J. Environ. Sci. & Healt Part B: Pest. Food Contaminants, & Agri. Wastes*, 45: 774-782.

Yilmaz, O., S. Keser, M. Tuzcu, M. Guvenc, B. Cetintas, S. Irtegun, H. Tastan and K. Sahin. 2009. A practical HPLC method to measure reduced (GSH) and oxidized (GSSG) glutathione concentrations in animal tissues. *J. Anim. & Veter. Adv.*, 8(2): 343-347.

Zhang, J.J., Y.C. Lu, J.J. Zhang, L.R. Tan and H.Yang. 2014. Accumulation and toxicological response of atrazine in rice crops. *Ecotoxicol. & Environ. Safety*, 102: 105-112.

(Received for publication 8 January 2018)