CATRINA (2020), 22(1): 19-27

Effect of 2,4-Dichlorophenoxy Acetic Acid on Antioxidant Systems in a Non-Target Plant (*Zea mays* L.)

Hanan Mahmoud Abou-Zeid[1*], Salwa Ahmed Abdel-Latif[2] and Ghada Saber Mohamed Ismail[1]
[1]Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt.
[2] Biology and Geology Department, Faculty of Education, Alexandria University, Egypt.

ABSTRACT

This work aimed to study the impact of various concentrations (2, 5, 10 mg L^{-1}) of the herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) on fresh and dry biomasses, photosynthetic pigments, quantum yield of PSII (Fv/Fm) and oxidative stress parameters of 28-day old *Zea mays* leaves. Foliar application of a low dose of 2,4-D significantly promoted the growth biomarkers, whereas, the high doses induced severe disturbances and reduced the growth attributes. In addition, over-accumulation of hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA) were recorded and that was accompanied with a significant increase in activity of superoxide dismutase (SOD), catalase (CAT) and glucose-6-phosphate dehydrogenase (G6PDH). Otherwise there was a significant decline in ascorbate peroxidase (APx), guiacol peroxidase (GPX), glutathione peroxidase (GSHPx) and glutathione reductase (GR). Moreover, a significant decline of the reduced glutathione (GSH) and ascorbic acid (AsA) contents, glutathione redox potential (GSH/GSSG), NADPH and NADPH/$NADP^+$ ratios were recorded. These observations might indicate that high doses of 2,4-D caused a menace to non-target plants through the disruption of antioxidant systems.

Keywords: Antioxidant enzymes, glutathione, hydrogen peroxide, maize, photosynthetic pigments.

INTRODUCTION

The 2,4-dichlorophenoxyacetic acid (2,4-D) is a general herbicide used to manage a variety of weeds with broad leaves. All over the world, it is the third most commonly applied herbicide and is still utilized in Egypt. There is a contradicted thought concerning its half-life and its toxicity in the environment (Samir *et al*., 2015). The wide use of herbicides in intensive agricultural practice has conveyed out serious environmental and health effects. They cause abiotic stress on plants, so they cause phytotoxicity, growth decline, and plant metabolism variations by production of reactive oxygen species (ROS) (Cobb and Reade, 2010).

Highly reactive ROS is causing lipid peroxidation, thus damaging the cell membranes, the disturbance of photosynthesis and numerous protection mechanisms (Kruse *et al*., 2006). 2,4-D is an artificial auxin that is similar in its effect to a natural auxin indole acetic acid (IAA), though, it has long duration owing to their high constancy in the plant and its degradation or inactivation is less than natural auxins in the plant. One of its numerous analogues is well-known in initiating the expression of auxin responsive genes particularly those concerned in ethylene and abscisic acid synthesis; both compounds that are well known to induce the production of ROS, which are accountable for mounting the oxidative stress (Pazmiño *et al*., 2012; Goggin *et al*., 2016). Regrettably, 2, 4-D has non-specific weed targets (Zabaloy and Gómez, 2014); it may reduce growth, incite reproductive problems, create changes in appearance and might cause the non-target species death. Islam *et al*., (2017) reported that short-term exposure to high concentrations of 2,4-D could stimulate DNA damages in the *Phaseolus vulgaris* and *Zea mays* seedlings.

The over production of ROS was induced by biotic and abiotic factors (Czarnocka and Karpinski, 2018). The defense system of plant executes scavenge ROS and reinstate cellular homeostasis. To balance the ROS production and detoxification, plants possess antioxidant-defense systems comprises enzymatic and non-enzymatic mechanisms (Skiba and Wolf, 2017). Alterations in enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APx), guaiacol peroxidase (GPx), glutathione peroxidase (GSHPx) and glutathione reductase (GR) (Agostinetto *et al*., 2016; Harre *et al*., 2018). Non-enzymatic antioxidant compounds comprise phenolic compounds, glutathione, ascorbate (AsA), tocopherol, and proline (Gill and Tuteja, 2010). The ascorbate–glutathione (AsA–GSH) cycle has been considered as one of the most important antioxidant pathways (Noctor and Foyer, 1998).

The major pathway of the production of NADPH is the oxidative pentose phosphate pathway (OPPP) which is significant for biosynthesis and redox steadiness in the plant cells. The majority of NADPH in the cytoplasm is produced by G6PDH and 6-phosphogluconate dehydrogenase (Corpas and Barroso, 2018). G6PDH is essential for maintaining GSH and NADPH homeostasis (Wang *et al*., 2019).

Maize (*Zea mays* L.) is considered as one of the most imperative cereal crops in the globe as it's highly used as food and fodder. The yield of maize is greatly affected by weeds in the field. Strategies efforts have been performed to limit the harmful effects of weeds growing with crop plants. The different defence mechanisms of plants against oxidative stress caused by herbicides might be useful in classifying such herbicides into a product with lower or higher ability of oxidative stress in crops (Nohatto *et al*., 2016).This work aimed to assess the effects of application of different 2,4-D concentrations on growth biomarkers, some physiological attributes and the antioxidant defense system of maize plants.

* *Corresponding author e-mail: Hananmahmoud93@yahoo.com*

MATERIALS AND METHODS

Maize (*Zea mays* L. cv. Nevertity) grains were purchased from the National Research Center, El-Dokki, Giza, Egypt. Grains were subjected to surface sterilization with 0.1% sodium hypochlorite solution for 5 min and then washed several times with distilled water. In plastic pots (12 cm in diameter, 17 cm length) containing soil mixture of clay and sand (1:2 W/W), the grains were sown. Two sets of pots (in triplicates) were placed at natural environmental conditions (photoperiod of 16h/8h light/dark, 31/28±2°C day/night temperature; light intensity PPFD, 23 µmol $m^{-2}s^{-1}$) for 14 days, irrigated every two days intervals using strength one tenth of Hoagland´s nutrient solution (Hoagland and Arnon, 1950) along the experimental period to field capacity. At the day 15 from sowing the first set of pots was sprayed with distilled water and considered as a control and the other set was sprayed with 2,4-D solution (2, 5 and 10 mg L^{-1}). The foliar spray was applied two times at the day 15 and the day 22 from sowing. After 28 days, similar plants were harvested, washed thoroughly from adhering soil particles, gently plotted, and the leaves were quickly saved for estimation of the various growth parameters and chemical analyses.

Plant growth parameters

The leaves were cautiously cleaned by dist.H_2O, dried by tissue paper and weighted for fresh weight (FW) estimation, and for dry weight (DW) the leaves were kept in oven for 72h at 70°C.

Photosynthetic pigments content

The photosynthetic pigments were determined according to the method described by Moran and Porath (1980) using N,N-dimethyl formamide (DMF), total carotenoid content was calculated according to Wellburn (1994) in related to leaf weight.

Measurement of the maximum quantum efficiency of PSII (Fv/Fm)

The ratio of the maximum quantum efficiency of PSII (Fv/Fm) was detected following the method described by Branquinho *et al.*, (1997) using OS-30P pulse modulated (Opti-sciences, Hudson, USA) chloro-phyll fluorimeter. Before every measurement leaves were dark-adapted for 30 min with leaf-clips.

Determination of hydrogen peroxide and malondialdehyde contents

Levels of hydrogen peroxide (H_2O_2) were measured following the method of Sergiev *et al*., (1997). Malondialdehyde (MDA), the lipid peroxidation product was estimated according to method used by Buege and Aust (1978).

Extraction and estimation of antioxidant enzymes activities

Fresh leaves (1g) were homogenized in ice cold 50 mM phosphate buffer (pH 7.5) containing 0.5 mM EDTA with prechilled pestle and mortar. The homogenate was centrifuged at 10,000 xg for 10 min at 4°C, then the supernatants were collected and used for determination the different enzyme activities. Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined following the method described by Giannopolitis and Ries (1977) where its ability to inhibit the photo reduction of nitroblue tetrazolium (NBT) was measured. The activity of catalase (CAT, 1.11.1.6) was assayed following the technique of Zhang *et al*., (2005). Ascorbate peroxidase (APX, 1.11.1.11) activity was assayed according to the method of Boominathan and Doran (2002) following the reduction in absorbance at 290 nm. The activity of guaiacol peroxidase (GPx, 1.11.1.7) was estimated following Urbanek *et al.*, (1991) method. Glutathione reductase (GR, 1.6.4.2) activity was detected using the method of Goldberg and Spooner (1983). However, glutathione peroxidase (GSHPx 1.11.1.9) activity was measured as described by Elia *et al.*, (2003)

Extraction and analysis of ascorbate content and glutathione fractions

Extraction of ascorbate (AsA) and glutathione was performed following the method mentioned by Gossett *et al*., (1994), and it was measured according to Zhang and Kirkham (1996). Reduced glutathione (GSH) and oxidized glutathione (GSSG) contents were assayed according to the methods of Griffith (1985).

Extraction and determination of glucose-6-phosphate dehydrogenase activity

G6PDH (EC 1.1.1.49) extracted and assayed the activity spectrophotometrically at 30°C by monitoring the decrease of $NADP^+$ (ε = 6.22 mM^{-1} cm^{-1}) at 340 nm according to Sgherri *et al*., (2002).

Extraction and determination of $NADP^+$ and NADPH

For HPLC analysis using PerkinElmer series 200 LC and UV/VIS detector 200 LC, USA system set with a 5-µm column (Spheri-5 RP-18; 220×4.6 mm; Brownlee), samples (0.3 g) were extracted either by acid (0.6 M perchloric acid) for nicotinamide adenine dinucleotide phosphate ($NADP^+$) measurement or alkaline (0.5 M potassium hydroxide) extraction for measureement of NADPH. Centrifugation was performed for the extract at 10,000 × g at 4°C for 10 min followed by neutralization by either 0.5 M KOH or 1 M KH_2PO_4 and centrifugation again at 10,000xg at 4°C for 10 min. Twenty µl of the supernatants were employed as declared by Caruso *et al*., (2004).

Statistical analysis

Duncan's multiple range tests by means of SPSS-20 were used for the results statistical analyses following Sokal and Rohlf (1995) method. Data were subjected to one-way ANOVA. Differences between treatment means were considered statistically significant at $p \leq 0.05$.

RESULTS

Plant growth parameters

Both fresh and dry biomasses of 28-day old maize leaves were significantly increased at 2 mg L^{-1} 2, 4-D treatment, the percentage of the increase was 25% and 33%, respectively compared to control. On the other hand, mid (5 mg L^{-1}) and high (10 mg L^{-1}) 2,4-D concentrations significantly decreased the biomass. The decrease in FW and DW of 10 mg L^{-1} sprayed leaves was about 63% and 56%, respectively versus to control (Table 1).

Table (1): The effect of different concentrations of 2,4-D on fresh and dry weight (FW and DW), photosynthetic pigments content and quantum yield of PSII (Fv/Fm) in maize leaves.

Treatment (mg L^{-1})	Biomass (mg $plant^{-1}$)		Chlorophyll content (µg g^{-1} FW)		Carot. Content (µg g^{-1} FW)	Total Pigment content	Fv/Fm
	FW	DW	Chl.a	Chl.b			
0	75±0.32^{a}	18±0.02^{a}	20.7±0.04^{a}	10.4±0.02^{a}	5.3±0.03^{a}	36.7±0.31^{a}	0.791±0.02^{a}
2	94±0.51^{b}	24±0.03^{b}	25.6±0.04^{a}	13.8±0.02^{ab}	5.9±0.03^{a}	38.4±0.32^{a}	0.787±0.02^{a}
5	57±0.22^{c}	15±0.02^{c}	13.0±0.06^{b}	7.6±0.05^{b}	3.3±0.01^{b}	23.9±0.22^{b}	0.704±0.03^{bc}
10	28±0.23^{d}	8±0.02^{d}	7.2±0.03^{c}	4.2±0.04^{c}	1.9±0.03^{c}	13.3±0.06^{c}	0.649±0.02^{c}

Values are means ± SD based on triplicate independent determinations. Different letters indicates significant difference as evaluated by Duncan´s multiple range test.

Photosynthetic pigments content

There was a significant decrease in photosynthetic pigments content of high dose of 2,4-D-sprayed leaves in comparison to unsprayed ones (Table 1). In treatment using 10 mg L^{-1} - sprayed leaves, the decline in Chl.a, Chl.b and Carotenoid content was 65%, 60% and 64%, respectively, compared to control. In addition, the decrease of total pigments content was associated with a decline in Fv/Fm values (Table 1).

Hydrogen peroxide and malondialdehyde contents

The oxidative stress was evaluated from the induction of H_2O_2 generation and lipoxygenation of plasma membranes, as indicated by increasing of MDA content (Figure 1). H_2O_2 and MDA contents in maize leaves sprayed with 5 mg L^{-1} or 10 mg L^{-1} 2, 4-D significantly increased, whereas, those of 2 mg L^{-1} - sprayed leaves insignificantly changed compared to control. The increase of the H_2O_2 and MDA accumulation in 10 mg L^{-1}-sprayed leaves was 2.5- and 3.3-fold of the control, respectively.

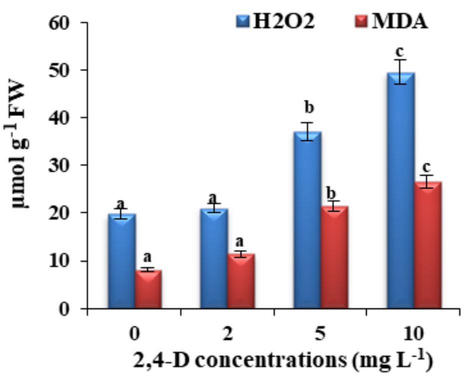

Figure (1): The effect of different concentrations of 2,4-D on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content in maize leaves. Values are means ± SD based on triplicate independent determinations. Different letters means significant difference ($p \leq 0.05$) as evaluated by Duncan´s multiple range test.

Antioxidant enzymes activities

The SOD activity was significantly increased by increasing 2,4-D concentrations. The activity of SOD in 5 mg L^{-1} and 10 mg L^{-1}-sprayed leaves were 4.1- and 6.6-times of controls, respectively, whereas, CAT activity was significantly increased up to 5 mg L^{-1} 2,4-D level, then sharply declined at severe ones. There were significant increases of APx, GSHPx and GR activities of 2 mg L^{-1}-sprayed leaves; which amounted to 40%, 28% and 33%, respectively, with respect to the control plants. Conversely, at mid and sever 2,4-D levels the APx, GSHPx and GR activities in maize leaves were significantly decreased in comparison to non-sprayed plants. The decrease in these enzyme activities reached 45%, 62% and 73% in 10 mg L^{-1}-sprayed leaves, respectively. On the other hand, the GPx activity in 2, 4-D-treated leaves was insignificantly changed (Figure 2).

Ascorbate, glutathione fractions, NADPH and $NADP^+$ contents

Spraying maize leaves with mid and high 2,4-D concentrations significantly declined AsA content compared to control, while the low concentration had almost no effect (Table 2). Total glutathione (TG) and its reduced form (GSH), in the maize leaves, were significantly reduced with increasing 2,4-D concentrations, whereas the oxidized form (GSSG) was signifycantly increased in comparison to untreated leaves. At 10 mg L^{-1} 2,4-D, the decreases of TG and GSH contents were 28% and 56%, respectively, compared to the control. These values were associated with an increase of GSSG content by 62% (Table 3).

In addition, the glutathione redox potential (GSH/GSSG) values in leaves were significantly decreased with increasing the concentrations of 2,4-D (Table 3). At 28 day of the experimental period, the GSH/GSSG in 5 mg L^{-1} and 10 mg L^{-1} 2, 4-D-treated leaves were 1.53 and 0.86, respectively, compared to control leaves (3.17). It is shown that NADPH content and NADPH/$NADP^+$ ratio were significantly declined in 5 mg L^{-1} and 10 mg L^{-1} 2,4-D-treated leaves, while $NADP^+$ content increased compared to control ones. At 10 mg L^{-1} 2,4-D, the decreases of NADPH and NADPH/$NADP^+$ ratio in the leaves were 58% and 73% compared to control, respectively. Simultaneously, an increase in the content of $NADP^+$, reached 51% of the control, was recorded.

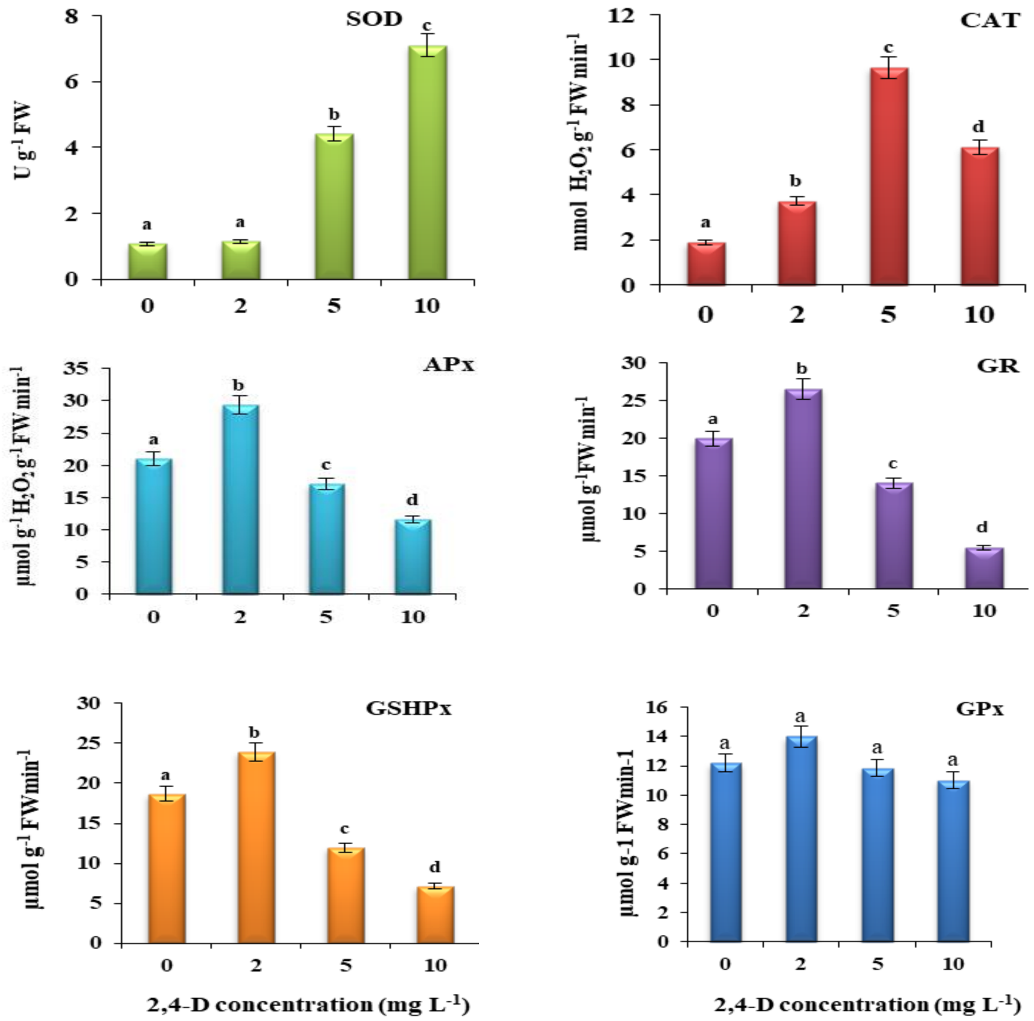

Figure (2): The effect of different concentrations of 2,4-D on activity of antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APx), guaiacol peroxidase (GPx), glutathione peroxidase (GSHPx) and glutathione reductase (GR) in maize leaves. The vertical bars represent standard deviation of the means of three replicates. Different letters indicates significant differences as evaluated by Duncan´s multiple range test.

Table (2): The effect of different concentrations of 2,4-D on NADPH, $NADP^+$, $NADPH/NADP^+$ level and ascorbic acid (AsA) content in maize leaves.

Treatment (mg L^{-1})	Pyridine Nucleotide levels (µg 100 g^{-1} FW)			AsA (µmol g^{-1} FW)
	NADPH	$NADP^+$	$NADPH/NADP^+$	
0	$38.13±0.33^a$	$13.95±0.16^a$	$2.73±0.02^a$	$161±0.34^a$
2	$36.79±0.31^a$	$14.02±0.08^{ab}$	$2.64±0.02^a$	$168±0.33^b$
5	$24.15±0.24^b$	$18.330±.09^{cd}$	$1.32±0.01^b$	$152±0.30^c$
10	$15.99±0.11^c$	$21.07±0.11^d$	$0.76±0.01^c$	$124±0.33^c$

Values are means ± SD based on triplicate independent determinations. Different letters indicates significant difference as evaluated by Duncan´s multiple range test.

Table (3): The effect of different concentrations of 2,4-D on the reduced glutathione (GSH), oxidized (GSSG), total (TG) and the ratio of GSH/GSSG forms in maize leaves.

Treatment (mg L^{-1})	Level of Glutathione (µg g^{-1} FW)			
	GSH	GSSG	TG	GSH/GSSG
0	311±3.2[a]	98±1.2[a]	409±2.8[a]	3.17±0.02[a]
2	296±2.4[a]	102±1.3[a]	398±1.6[a]	2.90±0.07[a]
5	185±1.2[b]	121±1.6[b]	306±1.4[b]	1.53±0.01[b]
10	136±1.4[c]	159±1.4[c]	295±1.8[b]	0.86±0.01[c]

Values are means ± SD based on triplicate independent determinations. Different letters means significant difference as evaluated by Duncan´s multiple range test.

Glucose-6-phosphate dehydrogenase activity

Data in Figure3 revealed that the activity of G6PDH increased significantly upon spraying with different concentrations of 2,4-D herbicide in a dosage contingent manner. The increase of G6PDH activity, in 5 mg L-1 and 10 mg L-1 2,4-D, recorded 2.6-and 4.3-fold of control, respectively.

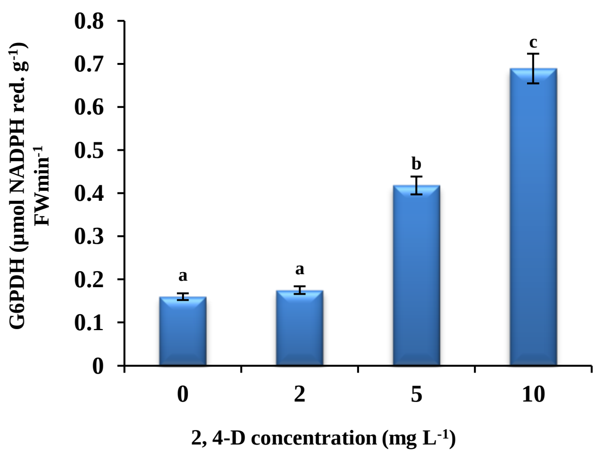

Figure (3): The effect of different concentrations of 2, 4-D on glucose-6-phosphate dehydrogenase (G6PDH) in maize leaves. Values are means ± SD based on triplicate independent determinations. Different letters indicates significant difference as evaluated by Duncan´s multiple range test.

DISCUSSION

Foliar application of low dose (2 mg L^{-1}) of 2,4-D significantly increased the fresh and dry biomasses of 28-day old maize leaves, compared to control, possibly through promoting the growth similar to IAA phytohormone. In accordance with these observations, Xu *et al*., (2013) mentioned that low doses of 2,4-D can act as growth hormone promoting cell division and elongation (as the role of IAA in induction of the growth). Conversely, high 2, 4-D concentrations markedly reduced the growth of maize leaves. These findings might be attributed to generation and accumulation of ROS which are doable for induction of the oxidative damage of the cellular components. The current study clearly demonstrated that there was a significant increase of H_2O_2 and MDA accumulation in highly 2,4-D-sprayed maize leaves, in comparison to control. Cobb and Reade (2010) and Grossmann (2010) reported that some phytotoxicity of herbicides may be related to the generation of ROS and induction of oxidative damage of cellular components as well as accumulation of ABA and ethylene. Thus, the suppers-sion in growth of 2,4-D-treated maize leaves might be related to enhancement of the ABA and ethylene which induce senescence and imbalance of endogenous auxins as well as promoting oxidative damage of plasma membranes and cellular components (Christoffoleti *et al*., 2015).

Although low 2,4-D concentration insignificantly changed the photosynthetic parameters content, while the high concentrations significantly declined the pigments content and quantum yield of PSII (Fv/Fm) values. These observations were accompanied with generation of H_2O_2 and MDA which might reveal the enhancement of oxidative stress of chloroplasts and thylakoid membranes, degradation of pigments and some photosynthetic enzymes and blocking electron transport of PSII. These findings were in accordance with those reported for several plant species such as *Scenedesmus quadricauda* (Wong, 2000), *Oryza sativa* (Wu *et al*., 2010) and wheat (Agostinetto *et al*., 2016). Therefore the reduction of FW and DW in 2, 4-D-treated maize leaves might be attributed also to disorder of the photosynthetic machinery. In accordance with our results, Karuppanapandian *et al*., (2011) and Islam *et al*., (2018) reported that 2, 4-D stimulated oxidative stress symptoms and reduced biomass in mung bean and rice plants owing to the degradation of the synthesis of chlorophyll and DNA.

It has been reported that the activation of ROS-generating oxidases including xanthine oxidase, acyl-CoA oxidase, plasma membranes-associated $NADPH_2$ oxidase and lypoxygenase localized in chloroplasts and peroxisomes (McCarthy-Suarez *et al*., 2011; Siebers *et al*., 2016).Thus, the generation of H_2O_2 and MDA in 2,4-D-sprayed maize leaves, in this study, might be attributed to the enhancement of these cytoplasmic organelles-associated oxidases causing the oxidative stress, and hence led to the chlorosis, senescence and death of maize leaves. It is well known that the generation of ROS required homeostasis equilibrium between the activity of the ROS-generating and the ROS-scavenging enzymatic and non-enzymatic systems (McCarthy-Suarez *et al*., 2011; Nohatto *et al*., 2016).

There was a significant increase of SOD and CAT activities in maize leaves under high 2, 4-D dose, comparing to the control. Similarly, several studies have been recorded the enhancement of SOD and CAT activities in various plant organs under herbicide treatments (Zhang *et al*., 2014; Caverzan *et al*., 2019). Conversely, Laxmi *et al*., (2018) showed a significant decline in field and *in vitro* grown shoots of *Portulaca oleracea* under 2, 4-D treatment. Pazmino *et al*., (2011) stated that, foliar application of 2, 4-D herbicide resulted in a marked upregulation of SOD and CAT activities.

It has been shown that application of phenoxy acetic acid members and other herbicide families markedly inhibited peroxidases activity in a number of plants (Boulahia *et al*., 2016; Harre *et al*., 2018). Compared to the control, APx and GSHPx activities were drastically declined in maize leaves sprayed with 5 and 10 mg L^{-1} 2, 4-D. Moreover, GPx activity was insignificantly changed in the high 2, 4-D dose-applied maize leaves revealing insufficient scavenging of generated H_2O_2. These findings were accompanied by significant increase of H_2O_2 content which indicate the collapses of ROS-scavenging systems, and hence disorder of cellular redox homestasis in maize leaves.

Yang *et al*., (2014) suggested that under stress conditions G6PDH activity is markedly induced for production of NADPH which is involving as a cofactor for several enzymes. Foyer and Noctor (2005) reported that GR-NADPH reduces oxidizing glutathione form to reduced form which introduces directly in the reduction of H_2O_2 in presence of GSHPx and GST enzymes or indirectly *via* AsA-GSH cycle. Although application of 2,4-D resulted in a significant increase G6PDH activity in maize leaves, it significantly declined GR and both GSH and AsA contents. These observations were associated with a significant accumulation of H_2O_2 and might reveal the competition between GR and NADPH-oxidases on the $NADPH_2$ as a reducing agent for GSSG to GSH or $O^{\bullet}$ to H_2O_2. In addition, the decline of GSH content might result in the indirect suppression of AsA-GSH cycle and direct elimination of generated H_2O_2.

It is well known that the ratio of AsA/DHAsA, NAD (P)H/NADP and GSH/GSSG controls the cellular redox homeostasis (Gill *et al*., 2013). In this work, there was a significant decline of GSH and NADPH contents in 2, 4-D- treated maize leaves, and that might be related to decrease of GR activity and increase of plasma membranes-associated NADPH oxidase activity. Moreover, the decrease in both GSH and NADPH was accompanied with a marked decline of GSH/GSSG and $NADPH/NADP^+$ redox state and induction of ROS generation which indicated a disturbance of cellular redox homeostasis and induction of oxidative damage, and hence leaf death.

CONCLUSION

The outcomes of this investigation can suggest that spraying maize leaves with low doses of 2,4-D can act as growth hormone stimulator, whereas high doses alter the cellular homeostasis and elicit the response of the enzymatic antioxidant systems. The disturbance of the antioxidative activities and imbalance of the cellular redox potential leading to enhancement of oxidative damage of cellular components, and hence reduce the growth. ROS overproduction might be considered as the key in the effect of 2, 4-D and may have differential roles in senescence and cell death.

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تأثير 4,2-ثنائى كلوروفينوكسي حمض الخليك على أنظمة مضادات الأكسدة فى نبات غير مستهدف (نبات الذرة)

حنان محمود أبوزيد[1]، سلوى أحمد عبد اللطيف[2]، غاده صابر محمد إسماعيل[1]
[1] قسم النبات والميكروبيولوجي - كلية العلوم - جامعة الإسكندرية - الإسكندرية - مصر
[2] قسم العلوم البيولوجية والجيولوجية - كلية التربية - جامعة الإسكندرية - الإسكندرية – مصر

الملخص العربـــــي

هدف هذا العمل إلى دراسة تأثير تركيزات مختلفة من مبيد الأعشاب 2,4-ثنائى كلورو فينوكسي حمض الخليك (2 ، 5 ، 10 ملى جرام /لتر) على الكتلة الحيوية الغضة والجافة ، محتوى أصباغ البناء الضوئى وكفاءة البناء الضوئى و معايير الإجهاد التأكسدي في أوراق نبات الذرة البالغة من العمر 28 يومًا. أدى رش الورق بجرعة منخفضة من 2،4-D إلى تعزيز العلامات الحيوية للنمو بشكل كبير ، في حين ادي استخدام الجرعات العالية الي انخفاض مؤشرات النمو و حدوث اضطرابات اخري شديدة . أنتجت هذه الحالة تراكمًا مفرطًا لفوق اكسيد الهيدروجين والمالونالدهيد الناتج من أكسدة الدهون والذي كان مصحوبًا بزيادة كبيرة في نشاط أنزيمات السوبرأوكسيد ديسميوتيز ، الكتاليزوكذالك الجلوكوز 6- فوسفات ديهيدروجينيز. كذلك كان هناك انخفاض كبير في إنزيمات أسكوربات البيروكسيديز، الجوياكول بيروكسيديز، الجلوتاثيون بيروكسيديز والجلوتاثيون ريدكتيز. وعلاوة على ذلك فقد تم تسجيل انخفاض كبير في نسبة الجلوتاثيون (المختزل) ،محتوى حمض الأسكوربيك، والقوة التأكسدية لكل من نسبة الجلوتاثيون المختزل إلى المؤكسد ،النيكوتين اميد ثنائى الفوسفات المختزل وكذلك نسبة النيكوتين اميد ثنائى الفوسفات المختزل إلى المؤكسد. خلصت الدراسة إلى أن استخدام الجرعات العالية من 2،4- D ربما تتسبب في تهديد النباتات الغير المستهدفة من خلال اضطراب الأنظمة المضادة للأكسدة.