AcetylCoA carboxylase and antioxidant enzymes activity of sunflower under influence of Super Gallant herbicide by magnetic water

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Abstract

Approaches for reducing the destructive effects of herbicides and increasing water use efficiency have attracted the interest of researchers over the last few decades. This study aims to investigate the protective effects of irrigation with magnetic water (MWT) on acetyl coenzyme A carboxylase (ACCase) and antioxidant enzymes activity of sunflower (*Helianthus annuus* L. cv. Lakomka) against undesirable effects of Super Gallant herbicide. It was conducted in a completely randomized design with four levels of Super Gallant herbicide (0, 25, 50, and 75 ppm) and three replications. Small necrotic spots were observed on some MWT and normal water treated leaves due to the herbicide effect. Although proline content increased in response to herbicide toxicity, MWT plants had significantly higher proline level than corresponding control ones. On the other hand, malondialdehyde content, as a marker of membrane damage, showed dramatic increase due to herbicide treatment. Also, ascorbate peroxidase and catalase activities rose by Super Gallant despite the decline in guaiacol peroxidase activity in herbicide concentration dependent manner. Herbicide reduced activity of ACCase in both roots and shoots significantly, but MWT could ameliorate this effect very well. It seems that MWT may be considered as an appropriate method to cope the negative effects of herbicide super gallant.

Keywords: *Helianthus annuus,* magnetic water, sunflower, Super Gallant, herbicide

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Introduction

Weeds as an agronomic problem are competing with crops for water, nutrients, and space. Using herbicides due to their simple application and low cost is a common method to cope with this problem (Lahmode et al., 2016), but some weeds are herbicide resistant and some herbicides suffer from low efficiency. So, farmers have to raise spraying rates to get enough weed control (Huffman et al., 2016) and this may have undesirable effects on plants as well as environmental pollution. Herbicides as xenobiotic chemicals increase reactive oxygen species (ROS) production through oxidative stress. Their reaction with proteins, nucleic acids, pigments, and lipids leads to membrane lipids damage and inactivation of enzymes which lowers plants' health (Aravind and Prasad, 2005).

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An acetyl coenzyme A carboxylase (ACCase) inhibitor named Fenoxaprop-P-ethyl is a common and effective herbicide for controlling grass weeds (Bi et al., 2016). ACCase (EC 6.4.1.2) carboxylates acetyl coenzyme A using ATP and bicarbonate producingmalonyl coenzyme A (Nikolau et al., 2003). Fatty acid synthesis is inhibited in sensitive grass weeds by ACCase herbicides causing cell injury and plant death (Devine, 2002). They do this by binding to the active site of carboxyl transferase domain of the enzyme. So, the production of phospholipids for new membranes will be inhibited (Kaundun, 2010).

Based on chemical structure, three classes of such herbicides have been described, namely cyclohexanediones (DIMs), phenylpyrazolin (DEN) and oxyphenoxy propionates (FOPs) (Wenger et al., 2012). Super gallant (Haloxyfop-R-methylester) as a selective post-growing and systemic herbicide with emulsion formulation inhibits ACCase activity (Vencill, 2002) and kills sensitive plant tissues after chlorosis and necrosis (Ball et al., 2007).

Magnetic water treatment (MWT) is a widespread useful approach for improving seed germination, seedling vigor and crop yield in different parts of the world. Higher water use efficiency, for example, has been observed for some crops due to MWT effects on some soil and water characteristics (Yadollahpour et al., 2014). Also, significant increase in Ca, K, and Zn content of magnetized water irrigated plants has been reported in comparison with normal water use (ELshokali and Abdelbagi, 2014). Hozayn and Qados (2010) have observed increased level of chlorophyll a, chlorophyll b, carotenoids, total pigments, phenolic compounds, and soluble proteins in chickpea by irrigation with magnetic water. It has been claimed that MWT is a modern technology to save irrigation water and to achieve better yield and quality of canola in reclaimed sandy soils (Hozayn et al., 2016).

Sunflower (*Helianthus annuus* L.) oil has high content of unsaturated fatty acids with no cholesterol, so it has been considered as one of the most important oil crops throughout the world.

Since it seems that MWT can protect the physiological and biochemical characteristics of plants against undesirable effects of Super Gallant herbicide, the present study aimed to investigate the influence of Super Gallant herbicide stress on proline and malondialdehyde (MDA) contents of leaves and roots of sunflower plants irrigated by magnetized water. Moreover, the effects of MWT on the activity of plant enzymes including ascorbate peroxidase (APX), guaiacolperoxidase (GPX), catalase (CAT), and ACCase was also investigated in this study.

Materials and Methods

The experiment was carried out in the growth chamber of the Department of Biology, Urmia University, Urmia, Iran. Sunflower (*Helianthus annuus* L. cv. Lakomka) seeds were sterilized with 10% commercial NaOCl for 5 min and washed thoroughly with distilled water. After germination, the seeds were transferred to plastic pots filled with sterile soil and sand (5:1). Five seeds were planted in each pot. The pots were transferred to growth chamber with day/night temperature of 30 ℃/18 ℃, under 16h photoperiod, and the relative humidity of 70-80%. During the experiment, the pots were irrigated with distilled water (control plants) and magnetized water (treated plants) for the first two weeks and with half strength Hoagland solution from the third week. Distilled water was passed through a magnetic field (170 mT) prepared by ELCLA1 (Italy) to produce magnetized water before it was used to irrigate pots on the same day.

At 4- to 6- leaf stage, different concentrations of Super Gallant herbicide were sprayed on the aerial part of the plants. The experiment ran for 35 day and plants were then harvested for biochemical and physiological analyses. Proline concentrations in leaves and fine roots were determined according to the method introduced by Bates et al. (1973). To determine the content of total protein, the method described by Lowry and colleagues (1951) was used. MDA was measured based on the method introduced by Heath and Packer (1968). Preparation of the extract was performed according to Asada (1992) with a slight modification. ACCase activity was measured using a discontinuous spectrophotometric assay (Willis et al., 2008). The data of the experiment were subjected to analysis of variance (ANOVA) using LSD test at the significance level of 0.05 by SPSS 20.0 software.

Results

The results of this study revealed that proline content increased in all MWT plants (both leaves and roots) in comparison with corresponding control and similar significant increase was observed in the herbicide-treated plants. The lowest (0.076 mg g^{-1}FW) and highest (0.348 mg g^{-1}FW) contents of proline were detected in control roots and combined treatment of MW and 75 ppm Super Gallant, respectively (Fig. I and Table I).

However, in all studied herbicide concentrations, the protein content of the treated plants was higher than that of control plants. It was observed that the protein content of the plants exposed to 75 ppm herbicide concentration was somehow higher than the protein content of control plants (Fig. II and Table I). Furthermore, MWT did not show any significant decline in MDA content of leaves and roots (Fig. III and Table I). MDA content of leaves increased more dramatically (from 0.039 µmol g^{-1}FW in control to 0.086 in 75 ppm Super Gallant) than roots due to the herbicide effect.

The activity of APX and CAT in leaves and roots of the control group was lower than that of MWT-treated plants (Figs. IV and VI and Table II). However, in contrast with roots, the activity of GPX in the leaves of control was higher than the treated plants (Fig. V and Table II). GPX activity in leaves was minimum (5.18 U g^{-1}FW min^{-1}) in MWT-

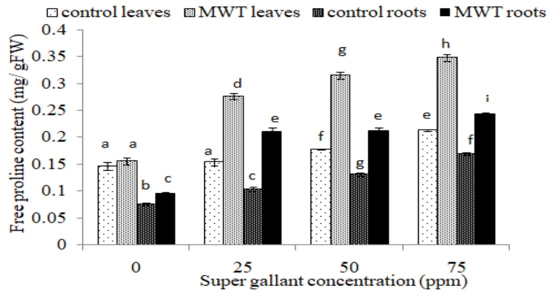

Fig. I. Free proline content in leaves and roots of *Helianthus annuus* under Super Gallant stress; data are shown as means ± SE. Different letters in the indicate significant differences between treatments (P≤ 0.05).

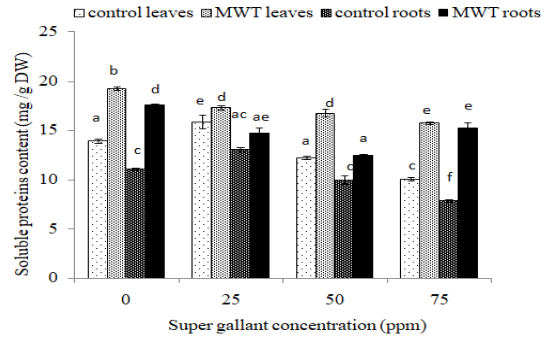

Fig. II. Soluble proteins content in leaves and roots of *Helianthus annuus* under Super Gallant stress; data are shown as means ± SE. Different letters indicate significant differences between treatments (P≤ 0.05).

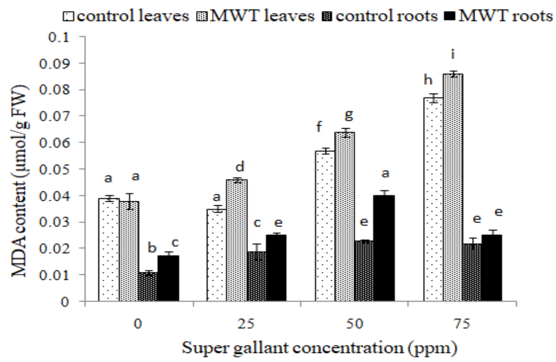

Fig. III. Malondialdehyde content in leaves and roots of *Helianthus annuus* under super gallant stress; data are shown as means ± SE. Different letters in the indicate significant differences between treatments (P≤ 0.05).

Table 1
Analysis of variances of root and leaf proline, soluble proteins and MDA content

Variation source	df	Mean squares					
		root proline	leafproline	root protein	leaf protein	root MDA	leaf MDA
Herbicide (A)	3	0.015**	0.018**	15.397**	19.247**	0.00338**	0.002 **
Magnetic water (B)	1	0.030 **	0.061**	124.305**	108.715**	0.00429**	0.000198**
A * B	3	0.002 **	0.006 **	12.549 **	5.714 **	0.00064**	0.000036 ns
Error	16	0.00025	0.00040	0.372	0.478	0.00011	0.0000186

ns, *, and ** denote no significant difference, significant difference at p≤0.05, and significant difference at p≤ 0.01, respectively.

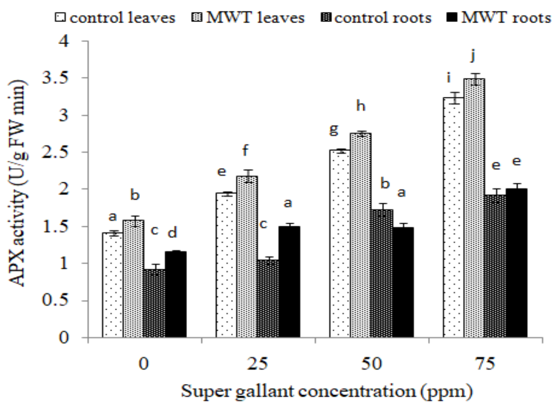

Fig. IV. Ascorbate peroxidase activity in leaves and roots of *Helianthus annuus* under Super Gallant stress; data are shown as means ± SE. Different letters indicate significant differences between treatments (P≤ 0.05).

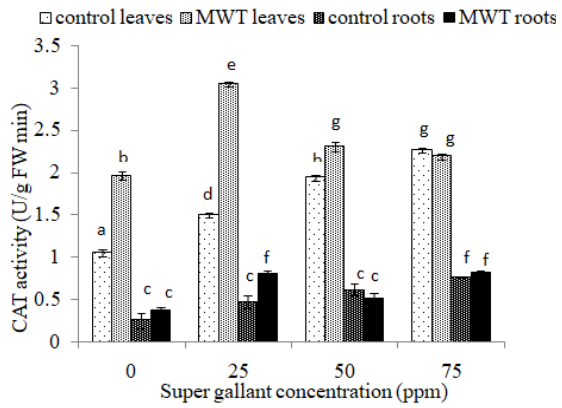

Fig. VI. Catalase activity in leaves and roots of *Helianthus annuus* under Super Gallant stress; data are shown as means ± SE. Different letters indicate significant differences between treatments (P ≤ 0.05).

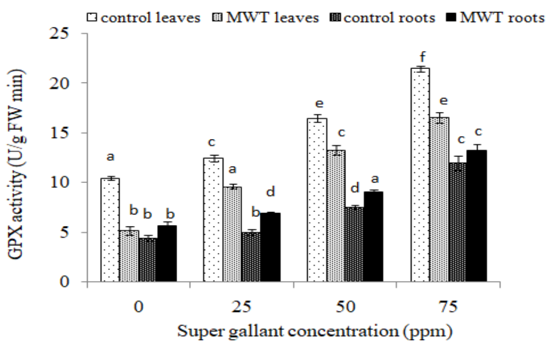

Fig. V. Guaiacol peroxidase activity in leaves and roots of *Helianthus annuus* under Super Gallant stress; data are shown as means ± SE. Different letters indicate significant differences between treatments (P≤ 0.05).

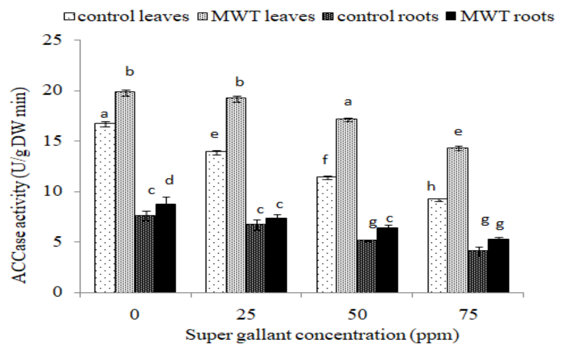

Fig. VII. Acetyl CoA carboxylase activity in leaves and roots of *Helianthus annuus* under super gallant stress. Data are shown as means ± SE. Different letters indicate significant differences between treatments (P ≤ 0.05).

treated plants with no herbicide and maximum (21.5 U g^{-1}FW min^{-1}) in 75 ppm Super Gallant treatment with no MWT. On the other hand, the activity of ACCase in the leaves and roots of the magnetized water treated plants was higher than the control. Shoots showed better response to MWT than roots and maximum activity of the enzyme was observed in MWT plants with no herbicide (18.24 µmol g^{-1} DW). Also, herbicide application caused a significant decline in ACCase activity in concentration-dependent manner (from 16.70 µmol g^{-1} DW for no herbicide control to 9.20 for 75 ppm Super Gallant).

Discussion

Proline as an essential amino acid for metabolism accumulates due to destruction of protein synthesis and reduction of its conversion to proteins (Mittler, 2002). Osmotic balance,

Table 2
Analysis of variances of activity of APX, GPX, CAT and ACCase in roots and leaves

Variation source	df	Mean squares							
		root APX	leafAPX	root GPX	Leaf GPX	root CAT	leaf CAT	root ACCase	leafACCase
Herbicide (A)	3	0.983**	3.830 **	69.024**	141.993**	0.240**	0.772 **	13.563**	47.808**
Magnetic water (B)	1	0.100ns	0.302*	11.985 **	99.064**	0.071**	2.870**	7.010 **	137.09**
A * B	3	0.131*	0.002 ns	0.121 ns	2.273 ns	0.140**	0.736 ns	0.103 ns	1.945 **
Error	16	0.034	0.046	1.334	1.334	0.004	0.019	0.150	1.619

ns, *, and, ** denote no significant difference, significant difference at p≤0.05, and significant difference at p≤ 0.01, respectively.

protection of the protein structure and cell membranes, stabilization of intracellular structure, and removal of free radicals are among proline functions in plant cells (Ain-Lhout et al., 2001). Proline accumulates under stress conditions such as drought, high salinity, high light intensity, UV irradiation, heavy metals (Szabados and Savoure, 2010), and certain herbicides (El-Tayeb and Zaki, 2009; Saladin et al., 2003). It is obvious that the herbicide as a stress factor has raised proline content and MWT can help plants to cope the stress.

Increased synthesis of some enzymes (e.g. antioxidants) and also synthesis of proteins involved in the cell defense system can raise the content of soluble proteins (Choi et al., 2009). On the other hand, it seems that MWT decreases the protein content of the plants to protect sunflower plants against the undesirable effects of Super Gallant herbicide.

Selectivity and permeability of cell membrane declines with stress severity and its health can be detected by the level of MDA as a product of membrane lipids peroxidation and a marker of its damage (Hessini et al., 2009). It seems that herbicide stress can increase the level of MDA due to oxidative effects and membrane injury. MDA content increased in sunflower and tobacco in response to metribuzin and chlorosulphron herbicides, respectively (Kruse et al., 2006; Toteva et al., 2004). However, our findings revealed that MWT cannot protect sunflower plants against the undesirable effects of Super Gallant herbicide on membrane damages.

ROS detoxification during stress conditions can be carried out in plants by the help of antioxidants and other related enzymes. They have important role in preventing oxidative damage (Koffler et al., 2014). So, it appears from our data that MWT treatment increased activity of APX and CAT to cope with the negative effects of the herbicide.

Lipid peroxidation, leakage of electrolytes and small organic molecules, damaging membrane system, and subsequently disorder in various metabolic processes occur if ROS attack membranes due to their inadequate clearance (Semane et al., 2007). Hydrogen peroxide is a toxic compound for cell which must be rapidly converted into H_2O and O_2 by the antioxidative defense system. The induction of CAT activity is necessary for detoxification of hydrogenated peroxide. CAT reduces the production of hydroxyl radicals and protects nucleic acids and lipids. It CAT and peroxidase activities in leaves of wheat were found to increase under influence of herbicides such as isoporturon, phenoxaprop, and sulfuron in a study on the effects of various herbicides on biochemical changes of wheat (Singh et al., 2013). APX can be used as a regulator of intracellular ROS (Kavas et al., 2013) whilst GPX acts in lignification and tannin formation (Choi et al., 2009). Toteva and colleagues (2004) revealed that treatment with chlorsulphron herbicide increases the activity of GPX in sensitive tobacco plants in comparison with the control, which is similar to our findings. It has been reported that activity of antioxidant enzymes SOD and peroxidase increased due to herbicide glyphosate stress (0.01-10 mM) in pea, wheat (Miteva et al., 2005), sunflower (Geetha et al., 2017) and *Vigna radiata* (Basantani et al., 2011). Also, some herbicides can alter phenolic compounds' metabolism. For example, application of butachlor and pendimethalin to wheat seedlings have increased total phenol content and activity of phenylalanine ammonialyase compared with the control (Singh et al., 2017). Higher activity of APX and CAT in leaves and roots of MWT plants in comparison with control group shows positive effects of magnetic water on ROS detoxification. Such studies indicate adaptive responses of plants to oxidative stress induced by herbicides.

Considering higher activity of ACCase in leaves and roots of MWT sunflower plants, it seems that MWT can be an appropriate method to protect sunflower plants against the undesirable effects of Super Gallant herbicide in decreasing the ACCase activity in leaves and roots. Consistent with our findings, Secor and Cseke (1988) have reported a decreased (by about 40%) activity of ACCase in the presence of 1 µM haloxyfop herbicide.

Overall, according to the results of this study, MWT can be a good solution to protect the ACCase activity of plants against Super Gallant herbicide.

Conclusions

Super Gallant is a common herbicide in sunflower production due to tolerance of this crop to selective herbicides. On the other hand, the crop suffers some biochemical and physiological damages induced by Super Gallant, which can reduce the yield and even quality of the crop. Consequences such as membrane damage (as MDA content rise), reduced ACCase activity, increased antioxidant enzymes activity (APX, GPX and CAT), and higher content of proline compared with the control have been observed in the present study. Furthermore, MWT showed some ameliorative effects in our study. The most pronounced result of magnetic water was observed in ACCase activity. For instance, it was raised from 9.20 Ug^{-1} DW min^{-1} to 14.27 and from 4.08 to 5.32 in 75 ppm herbicide-treated leaves and roots due to MWT, respectively. Also, activity of GPX as an antioxidant enzyme in MWT leaves was lower than those irrigated with normal water both under Super Gallant treatment and without it. GPX activity reduced from 21.5 U g^{-1}FW min^{-1} to 16.6 in 75 ppm herbicide-treated leaves due to MWT. It seems that MWT may contribute to the resistance of sunflower to Super Gallant herbicide through its protective effects on ACCase activity and other means. Further investigations are needed to decipher detailed MWT triggered mechanism or pathways to protect seedlings against super gallant.

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