ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 20(1) 2021, 71–80
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2021.1.7
ORIGINAL PAPER
Accepted: 28.01.2020

EFFECTS OF STATIC MAGNETIC FIELD ON GROWTH, SOME BIOCHEMICAL AND ANTIOXIDANT SYSTEM IN LEMON BALM (*Melissa officinalis L.*) SEEDLINGS

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ABSTRACT

The effects of magnetic waves as natural environmental factors on the Earths are not well known on plant growth and development. The present study was carried out to evaluate the effects of static magnetic field (SMF) treatment (4 and 6 mT for 30 and 120 min per day) for 8 days on the biomass production, proline contents and total soluble sugar, phenolic compounds, accumulation of H_2O_2 and MDA along with activity of antioxidant enzymes in lemon balm seedlings. Our results showed that SMF treatments, especially 6 mT and 120 min duration, increased the plant biomass, proline contents, phenolic compounds, H_2O_2 and MDA accumulation, and reduced the contents of total soluble sugars. The SMF application also increased the activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) enzymes compared to untreated seedlings. Our results suggest that SMF treatments induces the antioxidant defense system in the lemon balm seedlings and, by changing the plant metabolism, improves the early vigor of seedlings.

Key words: lemon balm, static magnetic field, antioxidant activity, proline, phenolic compounds

INTRODUCTION

In today's modern life, the effects of magnetic fields (MFs) are growing due to the use of different electrical appliances in the industry, which increases the concern about their effects on various living organisms [Serdyukov and Novitskii 2013]. The mechanism of the effect of MFs on living organisms has not been specifically addressed; however, several reports have shown that various cellular processes can be affected by MFs [Asghar et al. 2016, Sen and Alikamanoglu 2014]. It has been reported that MFs induces oxidative stress in plant cells by disrupting the activity of antioxidant enzymes [Abdollahi et al. 2018].

The antioxidant defense system plays an important role in tolerating adverse environmental conditions. Under stressful conditions, free radicals of RNS (reactive nitrogen species) and ROS (reactive oxygen species), such as NO (nitric oxide) and H_2O_2 (hydrogen peroxide), are produced in plants that alter the activity of antioxidant enzymes involved in the defense system of plants. Reactive free radicals also negatively affect cellular macromolecules such as proteins, nucleic acids and lipids, thereby reducing plant growth [Shao et al. 2008].

In some reports, MFs changes physiological and biochemical traits such as the content of hydrogen per-

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oxide and membrane peroxidation and the activity of antioxidant enzymes in the plant, which suggests that MFs induces oxidative stress in the plants [Haghighat et al. 2014, Abdollahi et al. 2018]. Jouni et al. [2012] showed that static magnetic field treatments in broad bean plants increased the membrane peroxidation and altered the activity of antioxidant enzymes and concluded that increased ROS production damaged DNA. Serdyukov and Novitskii [2013] reported that weak permanent magnetic field increased the malondialdehyde (MDA) contents and changed the activity of antioxidant enzymes in radish seedlings, and indicated that the effect of magnetic field on the antioxidant defense system of the plant depends on the intensity of magnetism field. On the other hand, induction of enzymatic activity during germination by MF has been reported, which can improve post-germination events and early growth of seedling [Iqbal et al. 2016].

Melissa officinalis L. is one of the important medicinal plants of the *Lamiaceae* family, which is native to the western Asia and eastern Mediterranean region and is widely cultivated in Iran [Leung and Foster 1996]. In folk therapy, lemon balm has long been used for properties of herbal aromatic and effects of soothing medicinal [Sari and Ceylan 2002]. Lemon balm is also used in traditional medicine for the treatment of insomnia, influenza, headaches, catarrh and fever. It is suggested that the essential oil of lemon balm has also sedative, antibacterial and spasmolytic properties [Schultze et al. 1992]. Although the effect of MF on improvement of the growth characteristics of different plants was investigated, however, the effect of MF on lemon balm seedlings has not been studied previously.

In the present study, the effects of intensity and duration of MFs exposure on ROS production and activity of antioxidant enzymes, as well as the contents of secondary metabolites of lemon balm seedlings were investigated. The results of this study can show the potential negative effects of FMs on living organisms.

MATERIALS AND METHODS

Plant material. Seeds of lemon balm (*Melissa officinalis* L.) were germinated in petri dishes after surface sterilization. After germination, lemon balm plantlets were treated with magnetic field (MF) 4 and 6 mT for 30 and 120 min per day for 8 days. Seeds of 1 mm root tip were considered as germinated seeds. Control samples were kept in the same conditions but without MF treatments. The experiments were conducted under controlled laboratory conditions at 14/10 h light/dark photoperiod and 24 ±2°C. All samples were irrigated daily with sterile distilled water. At the end of the experiment (8 days), all samples were harvested and stored in –80°C for biochemical measurements.

Magnetic treatment. Helmholtz coils were used to direct the identical magnetic field. To exert the current to the coils, a Variac autotransformer (TSGC2-2 model, the Micro Company, China) was used. SMFs (Static magnetic fields) were produced via base a diode bridge and a capacitor filter among coils and Variac autotransformer to develop direct current. To measure the magnetic field, a Tesla-meter (MG-3002 Model, Taiwan) was applied. The plant samples subjected to magnetic treatments at the center of the coil system every day from 8 am to 11 A.M.

Contents of proline and total soluble sugar. Plant tissue extracted with methanol (95%). After filtration through Whitman No. 1, extract concentrated under reduced pressure at 40°C with a rotary evaporator, then adjusted to 40 cc using methanol. In order to measure proline and total soluble sugar, the methanolic phase of the fresh tissue was used. The protocols of Bates et al. [1973] and Irigoyen et al. [1992] were used to measure contents of proline and total soluble sugar, respectively.

Contents of H_2O_2, lipid peroxidation and DPPH radical scavenging. After centrifugation of the homogenized solution (fresh tissue + trichloroacetic acid) at 12000 rpm for 10 min, the absorbance of the reaction solution (KI (1 M) + potassium phosphate buffer (pH 7.0, 10 mM) + supernatant) was recorded at a wavelength of 390 nm and, according to Velikova et al. [2000], H_2O_2 contents were calculated. Lipid peroxidation was estimated by measuring the malondialdehyde (MDA) contents according to the method of thiobarbituric acid [Heath and Packer 1968]. According to Brand-Williams et al. [1995], the total antioxidant capacity of lemon balm was determined by stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical at 517 nm.

Contents of total phenolic and flavonoids. The total phenolic content was estimated according to the method of Folin-Ciocalteu reagent. The absorbance of the reaction solution (Folin-Ciocalteu reagent, Na_2CO_3 – 7% – and plant methanol extract) was re-

corded at 725 nm after incubation in dark (25°C, 1 h), and calculated as mg gallic acid equivalent/g. The flavonoid contents were measured according to the method of Zhishen et al. [1999] and expressed as milligrams of catechin equivalent per grams.

Activity of antioxidative enzymes. To extract the soluble protein, the fresh tissues were homogenized in phosphate buffer (pH 7.8) with 0.5% of PEG (polyethylene glycol) 4000, 0.1 mM EDTA (ethylenediaminetetraacetic acid), 1% of PVP (polyvinylpyrrolidone) and 2 mM dithiothreitol. Total protein was estimated according to Bradford [1976]. To measure the activity of the SOD enzyme, the absorbance of the reaction mixture (methionine (13 mM), riboflavin (13 μM), nitroblue tetrazolium (63 μM) and protein extract) was read at 560 wavelengths [Giannopolitis and Ries 1977]. By determining the decrease in absorbance of H_2O_2 at 240 nm, the activity of catalase (CAT; EC 1.11.1.6) enzyme was calculated according to Bailly et al. [1996]. Activity of ascorbate peroxidase (APX; EC 1.11.1.11) enzyme was estimated according to Nakano and Asada [1981] by decreasing absorbance at 290 nm during time. Activity of glutathione reductase (GR; EC 1.8.1.7) enzyme was determined according to Carlberg and Mannervik [1985] by decreasing NADPH absorbance at 340 nm.

Statistical analysis. All experiments were repeated three times and each mean was calculated from three independent replicates. Statistical analysis of the results was calculated using SAS 9.1.3 software (SAS Institute, Inc., SAS Campus Drive, Cary, NC, USA) and the mean comparison was performed with a least significant difference (LSD) test (at the 5% level). The correlations among parameters were determined by Pearson coefficients ($p < 0.05$).

RESULTS

Exposure to 4 and 6 mT for 120 min significantly increased total fresh weight of lemon balm seedlings compared to the control plants. However, there was no significant difference in total fresh weight between the control treatment and 4 and 6 mT for 30 min treatments (Fig. 1A). The application of 4 and 6 mT for 120 min increased the total dry weight by 84 and 100%, respectively, compared to control treatment (Fig. 1B). These results showed that 120 min duration was more effective than 30 min duration to improve the growth and biomass production.

The SMF treatments increased proline content compared to non-treated seedlings. The increase in proline content under 6 mT in both 120 and 30 min duration was higher than at 4 mT treatment in same duration (Fig. 2A). There was no significant difference between the control treatment and the seedlings subjected to 4 and 6 mT SMF for 30 min in contents of total soluble sugar. However, the application of 4 mT treatments for 120 min increased the total sugar contents while the 6 mT treatments for 120 min reduced the total soluble sugar contents compared to non-treated plants (Fig. 2B).

Exposure of lemon balm seedlings to different intensities and duration of SMF meaningfully enhanced the contents of total phenolic and flavonoids. The highest total phenolic contents obtained under 4 and 6 mT for 120 min exposure by 25 and 49% increase, respectively than non-treated seedlings (Fig. 3A). The highest increase in content of flavonoids also observed under 4 and 6 mT for 120 min by 61 and 93% respectively, in comparison to non-treated seedlings (Fig. 3B).

The contents of H_2O_2 and lipid peroxidation were measured in lemon balm seedlings under 4 and 6 mT for 30 and 120 min. As shown in Fig. 4, SMF treatments significantly increased the contents of H_2O_2 and MDA compared to untreated plants, and the highest increase recorded in 6 mT and 120 min exposure. Exposure of seedlings to SMF treatments meaningfully increased the DPPH scavenging activity. The highest DPPH scavenging activity observed under 4 and 6 mT for 120 min (Fig. 5).

The application of SMF treatments reduced protein contents compared to untreated treatment. Increasing the intensity and duration of the SMF treatment resulted in a lower protein contents, so that the lowest protein observed under 6 mT and 120 min duration (Fig. 6). Exposure of lemon balm plants to different intensities and duration of SMF significantly increased the activity of SOD and CAT enzymes. The highest SOD activity obtained under 6 mT for 120 min exposure by 74% increase than non-treated plants (Fig. 7A). The highest increase in activity of CAT also estimated under 6 mT for 120 min by 56% in comparison to non-treated seedlings (Fig. 7B). The results of the effects of SMF treatments on the activity of APX enzyme showed

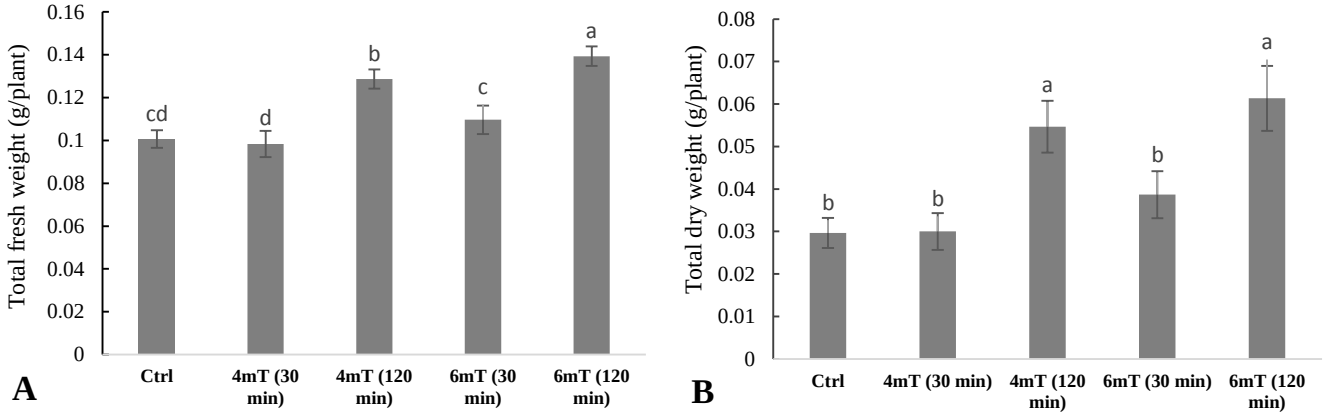

Fig. 1. The effects of SMF treatments on the total fresh weight (A) and total dry weight (B) of lemon balm seedlings. Values (means ±SD, n = 3) followed by the same letter are not significantly different ($p < 0.05$; LSD test)

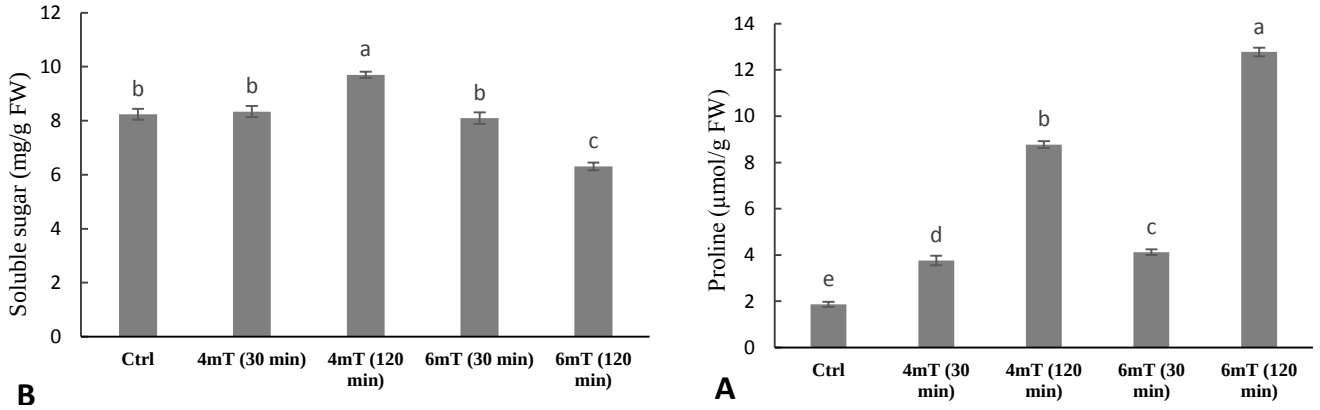

Fig. 2. The effects of SMF treatments on the contents of proline (A) and total soluble sugar (B) of lemon balm seedlings. Values (means ±SD, n = 3) followed by the same letter are not significantly different ($p < 0.05$; LSD test)

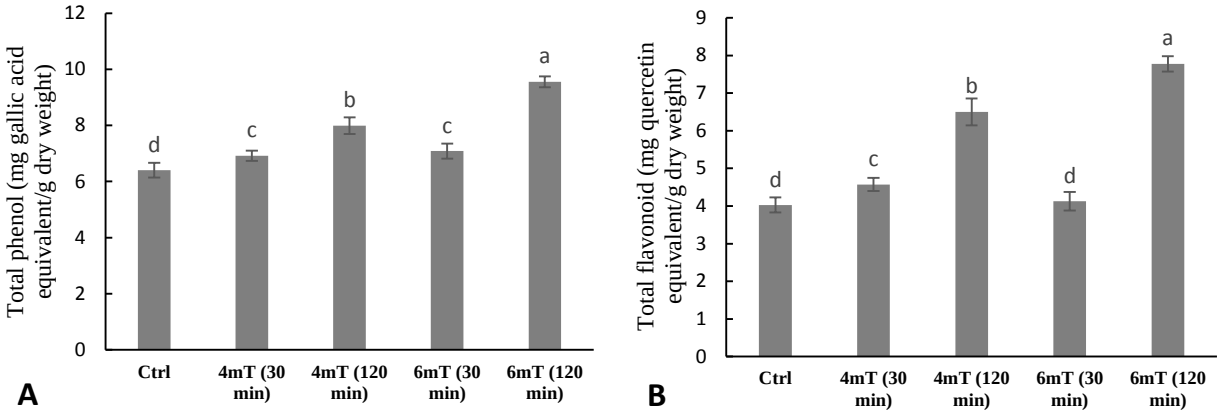

Fig. 3. The effects of SMF treatments on the total phenol (A) and flavonoid (B) contents of lemon balm seedlings. Values (means ±SD, n = 3) followed by the same letter are not significantly different ($p < 0.05$; LSD test)

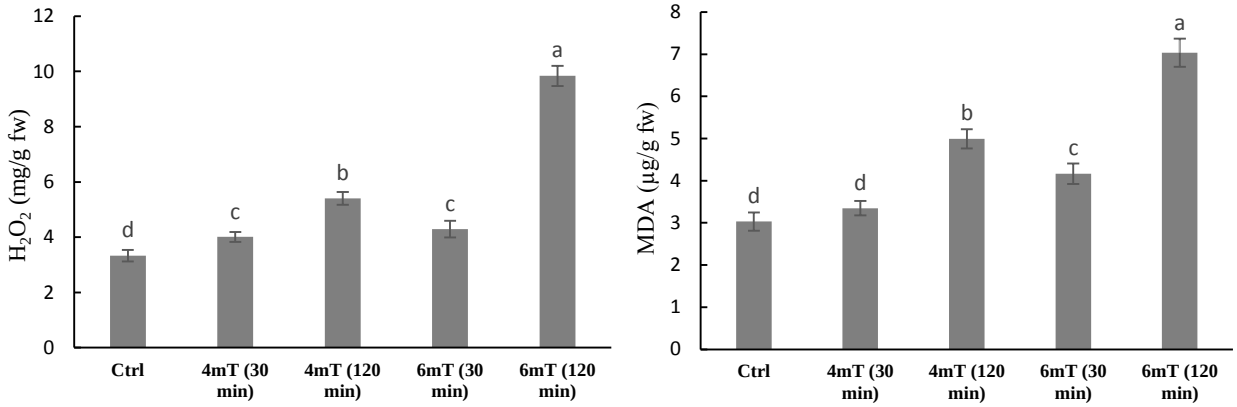

Fig. 4. The effects of SMF treatments on the H_2O_2 (A) and MDA (B) contents of lemon balm seedlings. Values (means ±SD, n = 3) followed by the same letter are not significantly different ($p < 0.05$; LSD test)

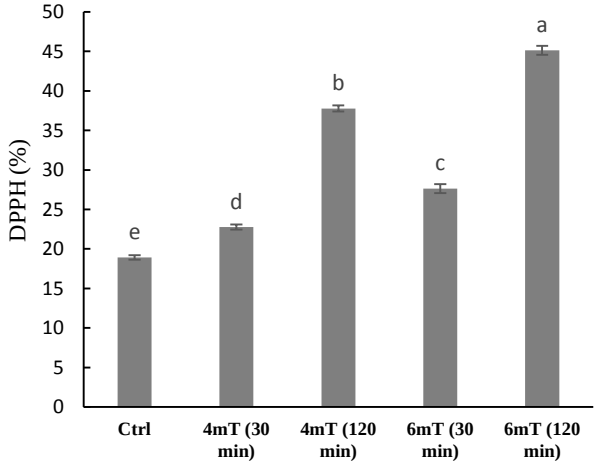

Fig. 5. The effects of SMF treatments on the DPPH scavenging activity of lemon balm seedlings. Values (means ±SD, n = 3) followed by the same letter are not significantly different ($p < 0.05$; LSD test)

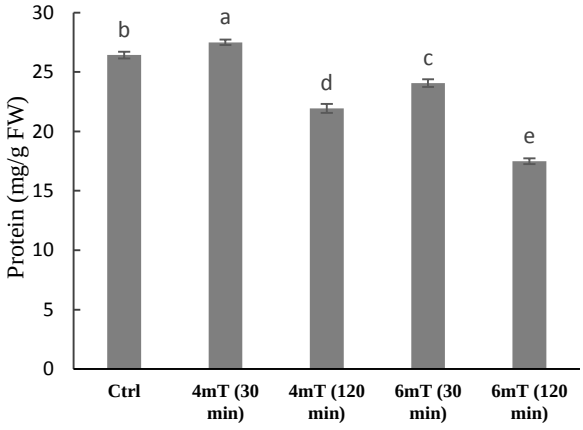

Fig. 6. The effects of SMF treatments on the protein contents of lemon balm seedlings. Values (means ±SD, n = 3) followed by the same letter are not significantly different ($p < 0.05$; LSD test)

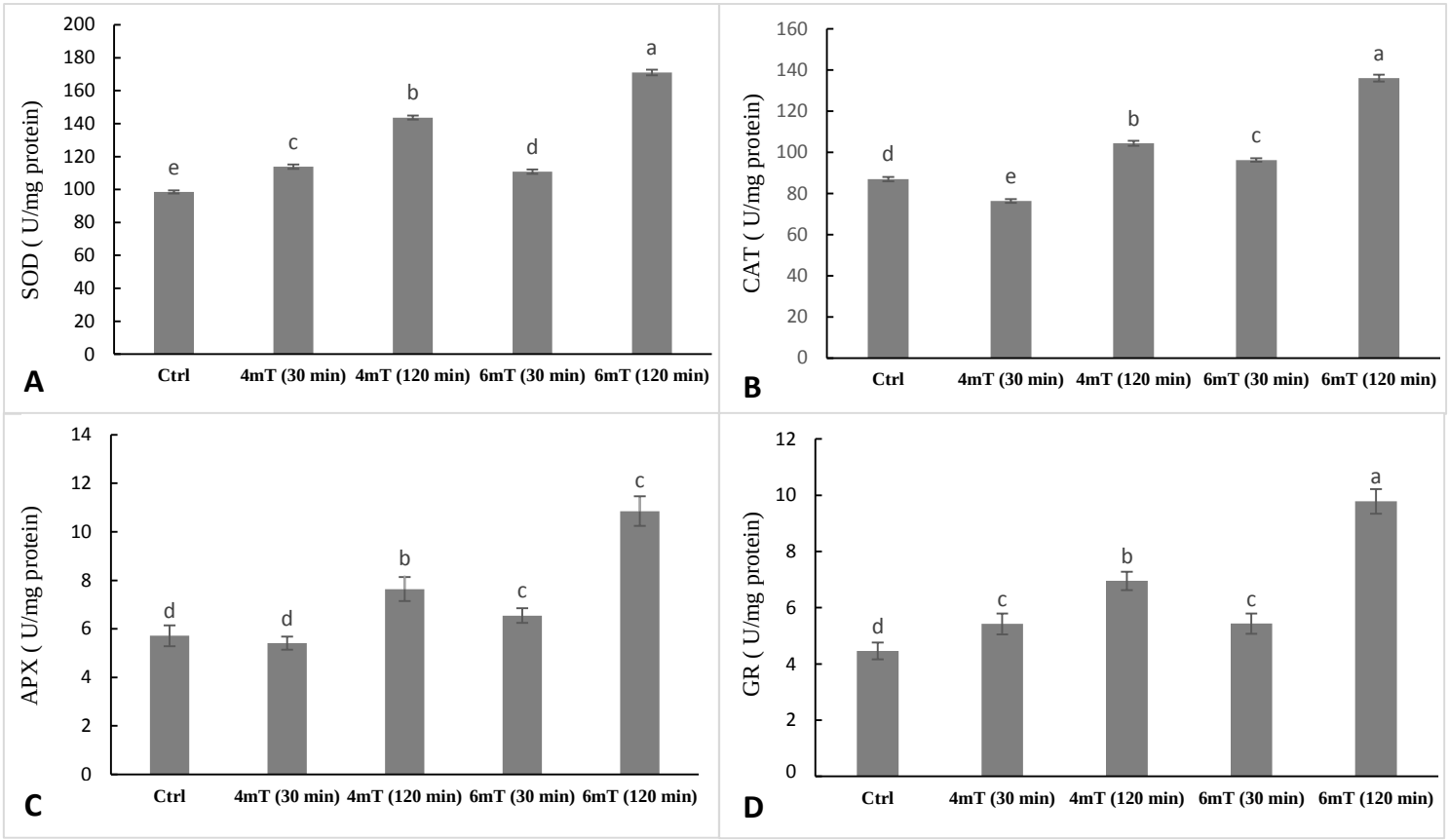

Fig. 7. The effects of SMF treatments on the activity of SOD (A), CAT (B), APX (C) and GR (D) enzymes of lemon balm seedlings. Values (means ±SD, n = 3) followed by the same letter are not significantly different ($p < 0.05$; LSD test)

that the application of 4 mT for 120 min and 6 mT for 30 and 120 min duration increased APX activity by 34, 15 and 90%, respectively, compared to untreated treatment (Fig. 7C). As shown in Fig. 7D, the different intensities and durations of the SMF treatments increased the activity of GR activity compared to untreated conditions, and the highest activity of the GR enzyme observed under 4 and 6 mT for 120 min duration, respectively, by 56 and 119% enhancement than non-treated plants.

Correlation results showed that plant biomass had a significant positive correlation with the activity of antioxidant enzymes, proline content and phenolic compounds and had a negative correlation with protein content. This indicates the effect of secondary metabolites and antioxidant enzymes on the production of purslane biomass under different SMF treatments. There was also a positive correlation between the oxidative stress markers (H_2O_2 and MDA) and the activity of antioxidant enzymes, indicating that increased production of hydrogen peroxide and MDA induced the activity of oxidant enzymes under SMF (Tab. 1).

DISCUSSION

The growth and biomass production of plant are important morphological parameters of the plant, which indicates the establishment of the plants under various environmental conditions. Our results showed that 4 and 6 mT SMF for 120 min increased the growth and biomass production of lemon balm seedlings compared to non-treated plants. The results obtained in this paper are in agreement with the results of Abdollahi et al. [2018] and Verma et al. [2017]. In another report,

Table 1. Correlation between measured traits of lemon balm under different magnetization treatments

	Total fresh weight	Total dry weight	Proline	Soluble sugars	Protein	MDA	H_2O_2	DPPH	Total phenol	Flavonoids	SOD	CAT	APX	GR
Total fresh weight	1.00													
Total dry weight	0.94**	1.00												
Proline	0.94**	0.92**	1.00											
Soluble sugars	–0.30^{ns}	–0.25^{ns}	–0.40^{ns}	1.00										
Protein	–0.94**	–0.91**	–0.95**	0.49^{ns}	1.00									
MDA	0.93**	0.88**	0.97**	–0.54*	–0.98**	1.00								
H_2O_2	0.88**	0.84**	0.95**	–0.67**	–0.93**	0.96**	1.00							
DPPH	0.96**	0.94**	0.98**	–0.34^{ns}	–0.96**	0.97**	0.91**	1.00						
Total phenol	0.90**	0.88**	0.97**	–0.52*	–0.94**	0.96**	0.97**	0.95**	1.00					
Flavonoids	0.92**	0.89**	0.98**	–0.34^{ns}	–0.89**	0.92**	0.91**	0.95**	0.95**	1.00				
SOD	0.92**	0.90**	0.99**	–0.41^{ns}	–0.93**	0.95**	0.95**	0.98**	0.97**	0.98**	1.00			
CAT	0.91**	0.87**	0.92**	–0.58*	–0.99**	0.95**	0.94**	0.92**	0.92**	0.87**	0.90**	1.00		
APX	0.93**	0.89**	0.94**	–0.59*	–0.97**	0.97**	0.97**	0.93**	0.96**	0.92**	0.93**	0.98**	1.00	
GR	0.91**	0.87**	0.97**	–0.55*	–0.92**	0.96**	0.98**	0.95**	0.96**	0.94**	0.97**	0.92**	0.95**	1.00

*significant difference at $p < 0.05$; **significant difference at $p \leq 0.01$, ^{ns} non-significant

De Souza et al. [2006] showed that MFs treatments increased the growth and biomass production of tomato seedlings. It has been suggested that the increase in growth and biomass of seedlings under MFs treatment can be due to the improvement in the efficiency of plant metabolic processes.

Free proline is one of the most important compatible osmolyte in plants, which increases plant tolerance to unfavorable conditions especially oxidative stress [Ghorbani et al. 2018b]. Proline maintains the integrity and permeability of the membrane under stressful conditions, thus maintaining the water content of the cell and improving the tolerance of the plants under stress. It has been shown that proline as a ROS scavenger can also reduce the adverse effects of oxidative stress. Our results showed that SMFs treatments increased the proline contents of lemon balm seedlings, according to Selim and El-Nady [2011] and Abdollahi et al. [2018]. Therefore, proline accumulation under SMFs preserves the osmotic potential of the cells and reduces ROS, and thus improves plant growth. Our results also indicated that the total soluble sugars increased the under 4 mT treatment for 120 min, while increasing the intensity of SMF to 6 mT caused a decrease in contents of total soluble sugar compared to untreated seedlings. Abdollahi et al. [2018] have also reported the decrease in the contents of total soluble sugar under SMF treatment in Almond seedlings. Ashraf et al. [2002] observed that salinity decreased the activity of α-amylase and protease of cotton seed during germination, which decreased the soluble sugar content. Therefore, the decline in the contents of total soluble sugar induced by 6 mT can be due to the decrease of amylase activity under 6 mT SMF.

Phenolic compounds, as the most important secondary metabolites, naturally accumulate in plants, and as non-enzymatic antioxidants, play an important role in plants tolerance to stressful conditions [Ghorbani et al. 2018a]. The induction of secondary metabolite accumulation and alteration in the quality of essential oils of the plant under MFs treatment have been reported [Ghanati et al. 2007, Malinowska et al. 2017]. Our data showed that SMFs treatment increased the contents of total phenol and flavonoid in the lemon balm. Similar results obtained from enhancement of phenolic compounds under MFs treatment by Asghar et al. [2016] and Abdollahi et al. [2018]. In another study, Jouni et al. [2012] showed that SMF treatment reduced the total flavonoids in broad bean plant, indicating that the reduction of the total flavonoids was due to decreased in the activity of polyphenol oxidase. Although secondary metabolites increased in response to MFs treatment as a defense system of plant, it is possible to increase the secondary compounds by using controlled magnetite waves for medical purposes.

Changes in MDA contents resulting from lipid peroxidation as well as DPPH scavenging activity under MFs treatment have been shown in several studies. Our results showed that the SMF treatments increased MDA contents and DPPH scavenging activity, which is consistent with the results of the effect of MF application on wheat [Sen and Alikamanoglu 2014] almond [Abdollahi et al. 2018] and radish [Serdyukov and Novitskii 2013]. Lipid peroxidation as an oxidation stress indicator is stimulated by free radicals, especially hydroxyl radicals. Our results in this study also showed that SMFs treatment changed the activity of antioxidant enzymes, which indicates the role of magnetic field on the induction of plant defense system. The SOD enzyme plays a main role in reducing the toxicity of anion superoxide in the cells, however, by producing hydrogen peroxide, plant cells exposed to toxicity of other free radicals. Increasing activity of SOD enzyme under SMFs treatment is similar to the results reported by Sen and Alikamanoglu [2014] and contrary to the results of Serdyukov and Novitskii [2013] and Abdollahi et al. [2018]. The CAT and APX enzymes, as two important antioxidant enzymes, have an important role in plant adaptation to unfavorable conditions by removing H_2O_2 under stress. Maffei [2014] indicated that MF exposure increased the ROS accumulation and altered the activity of antioxidant enzymes in plants. They also suggested that apoplastic components may act as redox regulators in the process of sensing and signaling magnetic changes. The hydrogen bonding of water molecules may be broken by the polarization of the high-voltage electric field [Chaplin 2005]. It has also been suggested that the electromagnetic field can alter the structure and function of macromolecules by breaking the hydrogen bonding of ultrastructural compounds in plant cells, such as proteins. Depending on the exposure time and the strength of the MF, these structural changes can either denature the protein or alter the activity of the

enzymes [Huang et al. 2006]. Therefore, the greater activity of CAT, APX and GR enzymes in lemon balm plants treated with SMFs is due to the accumulation of H_2O_2 and other free radicals. Similar results have been observed in increasing the activity of antioxidant enzymes in wheat [Sen and Alikamanoglu 2014] and soybean [Asghar et al. 2016] plants under MFs treatment.

CONCLUSION

Various factors such as species, intensity and exposure time of magnetic fields are effective in the plant response to SMFs, however, it is well accepted that magnetic fields induce ROS accumulation in plants. The results of this study showed that treatments of 4 and 6 mT SMF for 30 and 120 min a day (8 days) increased the contents of proline and secondary metabolites and also caused the accumulation of H_2O_2 and MDA and induced antioxidant enzymes activity in lemon balm seedlings; however, the highest increase was observed under 6 mT SMF for 120 min. Therefore, it can be concluded that magnetic fields induce the antioxidant defense system in the Lemon balm plants and, by changing the plant metabolism, increases the tolerance of the plants to oxidative stress.

SOURCE OF FUNDING

The authors acknowledge the financial support of Islamic Azad University, Science and Research Branch, Tehran, Iran.

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