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Alternation of Growth, Phenolic Content, Antioxidant Enzymes and Capacity by Magnetic Field in *Hyssopus officinalis* under Water Deficit

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Abstract
In the present study, the effect of seed priming with magnetic field (MF; 45, 90, 200 and 250 mT for 5, 10, 20 and 30 min) was evaluated in 60-day-old *Hyssopus officinalis* plants grown under 8 days irrigation intervals. The assessments were consisted of biomass, membrane stability, photosynthetic pigments concentrations, polyphenols content, antioxidant enzymes activities and antioxidant capacity. In comparison with the exclusively water-stressed plants, MF-priming significantly altered these parameters, particularly at 200 mT/5 min. At this intensity, the level of biomass, total chlorophyll and polyphenols content increased by 2.2, 2.5 and 7.7 folds, respectively. Furthermore, electrolyte leakage and MDA content decreased by 35 and 33%. Reducing power, DPPH and superoxide anion scavenging activities highly augmented by MF. MF-priming at 200 mT increased catalase (+92%) and ascorbate peroxidase (+2.3 folds) activities. But, the highest activity of guaiacol peroxidase was recorded for MF-primed *H. officinalis* at 90 mT. In conclusion, seed priming with MF increases drought tolerance in *H. officinalis* through protection of cellular membrane integrity, maintenance of photosynthetic pigments content and also alternation of antioxidant enzyme activities. It also improves medicinal properties of the shoots via increasing polyphenols concentration and antioxidant capacity.

Keywords: Drought stress tolerance, hyssop, polyphenols, radical scavenging activity, seed priming.

Abbreviation: Gallic acid equivalents (GAE); Malondialdehyde (MDA); Magnetic field (MF); Millitesla (mT); Reactive oxygen species (ROS); Photosynthetic active radiation (PAR); 1,1-diphenyl-2-picrylhydrozyl (DPPH)

Introduction
Hyssopus officinalis L. or hyssop - belonging to family Lamiaceae - is a medicinal plant that grows in the west of Asia, the districts around the Caspian Sea and Southern Europe. Essential oils extracted from the aerial parts of *H. officinalis* are commonly used in pharmaceutical and/or food industries to produce drugs for diseases of the respiratory system or as a spice to flavor foods (Khazaie et al., 2008). Also, antiviral and antibacterial activities of essential oil

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of this species have been already reported (Reichling et al., 2009).

Under abiotic stresses, such as drought, overproduction of ROS occurs and subsequently leads to oxidative damage. At this case, ROS molecules injure cellular membranes and vital macromolecules, which result in oxidative stress. Nevertheless, plant antioxidants system (including enzymatic and non-enzymatic antioxidants) induce to dampen oxidative stress (Ozgur et al., 2013). This plant defense system controls the detrimental oxidation through deterring the initiation or increment of oxidizing chain reactions (Gill and Tuteja, 2010).

It is well-documented that medicinal plants, as important sources of antioxidants, enable to quench adverse effects of ROS; therefore they seem to be valuable for human health (Krishnaiah et al., 2010). Reports have been already pointed out the correlation of antioxidant properties and polyphenols in several plant species (Ksouri et al., 2007; Gill and Tuteja, 2010). Accordingly, plants with high levels of phenolics or utilization of treatments to augment polyphenols concentration in plants would be useful for medical applications.

Water stress, as one of the major environmental stresses can modify the balance between the generation and scavenging of ROS and negatively affects plant growth and development. Seed priming is known as a competent technique to lessen adverse effects of abiotic stresses. Regarding this point, we investigated the effects of seed priming with different MF intensities and exposure times that might improve drought tolerance in *H. officinalis* and as a result influence antioxidant activity of this medicinal plant.

Yet, several reports indicated the effects of MF in plants. Various researches disclosed the function of MF on improving seed germination traits in crops such as maize, tobacco, cucumber, tomato, pea and lentil or in medicinal plants such as *Salvia officinalis* and *Calendula officinalis* (Aladjadjiyan, 2002; Aladjadjiyan and Ylieva, 2003; Yinan et al., 2005; Flo´rez et al., 2007; Martínez et al., 2009; Flo´rez et al., 2010; Shine et al., 2012; Maffei, 2014). In others, seed priming with electromagnetic has been applied to diminish detrimental effects of abiotic stresses such as drought (Javed et al., 2011; Anand et al., 2012) or salinity (Radhakrishnan et al., 2012; Radhakrishnan and Ranjitha Kumari, 2013; Thomas *et al*. 2013) at seed germination or early growth of seedlings. Moreover, it has been found out that MF-pretreatment of seeds significantly alleviated the deleterious effects of NaCl in *Artemisia sieberi* and *Artemisia aucheri* and also it increased medicinal properties of the mentioned species via increased polyphenols concentration and antioxidant capacity (Azimian and Roshandel, 2015; Roshandel and Azimian,2015).

In the current study, we studied the possible changes in physiological and biochemical characteristics as the consequence of seed priming with MF in *H. officinalis* plants to enhance tolerance and antioxidant capacity under water deficit condition.

Materials and methods

Plant material and preparation

Seeds of *Hyssopus officinalis* were divided in two groups, one group subjected to a MF and the another was untreated. The seeds were moistened under MF exposure. Our preliminary experiments revealed that the interaction effect of MF intensity (45, 90, 200 and 250 mT) and exposure time (5, 10, 20 and 30 min) was not significant. In addition, the first round of experiments showed the irrigation intervals of 8 days reduced the biomass of *H. officinalis* plants to 50%, compared to the other irrigation periods. Thus, the least time of the MF exposure (5 min) and the irrigation intervals of 8 days were used for the main experiments. Primed or unprimed seeds of *H. officinalis* were sown in polystyrene boxes, filled with a potting mixture composed of 50% perlite and 50% fine sand. The plants were irrigated with Hoagland's solution and placed in a greenhouse under controlled conditions (16/8 h light/dark period, 32/25°C temperature, and 1000-1200 µmol m^{-2} s^{-1} photosynthetic photon flux density). For the main experiment treatments included: 1) water deficit treatment (with an irrigation intervals of 8 days), 2) seed priming with 45, 90, 200 and 250 mT at 5 min and then growing the 10-day-old primed seedlings of *H. officinalis* under irrigation intervals of 8 days. Irrigation treatment was lasted for 50 days. At the end of the experiments, the 60-day-old *H. officinalis* plants were sampled to evaluate changes in the dry weight, membrane stability, photosynthetic pigments and total phenolic contents, antioxidant enzymes activities and antioxidant capacity under water deficit condition.

Photosynthetic pigments measurement

The content of total chlorophyll (Chlorophyll a+b) and carotenoids were determined according to the method of Lichtenthaler and Buschmann (2001) with 80% acetone as the solvent.

Chlorophyll a (µg ml^{-1}) $=12.25A_{663} - 2.79A_{646}$; Chlorophyll b (µg ml^{-1}) $=21.5A_{646} - 5.1A_{663}$;

Total chlorophyll (µg ml^{-1}) = Chlorophyll a+b; Carotenoids = [1000A_{470} – (1.82 × Chlorophyll a) – (85.02 × Chlorophyll b)/198; where, A_{663}, A_{645}, and A_{470} represent absorbance values read at 663, 645 and 470 nm wavelengths, respectively.

Evaluation of electrolyte leakage

Leaves were sampled to measure membrane electrolyte leakage according to the method of Campos et al. (2003). Results were expressed as percentage of total conductivity.

Assessment of lipid peroxidation

Lipid peroxidation was evaluated by measuring of MDA concentration in the aerial parts of *H. officinalis* according to the method of Ksouri et al. (2007). MDA concentration was determined using the extinction coefficient 155 mM^{-1} cm^{-1}.

Polyphenol extraction and estimation

Aerial parts of *H. officinalis* plants were shade dried for one week and ground to fine powder. Total phenolic content was estimated using the Folin-Ciocalteu reagent, following Singleton's method with some modifications (Ksouri et al., 2007). Polyphenols concentration of plants (three replicates per treatment) was expressed as mg gallic acid equivalents g^{-1} dry weight through a calibration curve with gallic acid.

Evaluation of DPPH radical-scavenging activity

Antioxidant activity of the extracts was assayed based on the scavenging activity of the stable DPPH free radical (Ksouri et al., 2007). The scavenging activity was stated as IC_{50} (µg g^{-1} dry weight). The DPPH radical scavenging was calculated as:

% Inhibition= $[(A_0-A_1)/A_0]\times100$, where A_0 was the absorbance of the control and A_1 absorbance of extract.

Evaluation of superoxide anion radical scavenging activity

Assay of superoxide anion scavenging activity was based on the method of Kumaran and Joel karunakaran (2006). The antioxidant activity of the extracts was expressed as IC_{50} (µg g^{-1} dry weight). The superoxide radical scavenging activity was calculated using the following formula:

% Inhibition= $[(A_0- A_1)/A_0]\times100$, where A_0 was the absorbance of the control and A_1 was the absorbance of the extract.

Estimation of reducing power

The reducing power of methanolic extracts of shoots of *H. officinalis* was determined according to method of Kumaran and Joel karunakaran (2006). Increase in absorbance of the reaction mixture indicated increased reducing power.

Enzyme extraction and assay

Enzyme extraction procedure was

accomplished according to the method of Chen et al. (2000) with some modification. All of the following operations were performed at 4˚C. The extract was transferred to Eppendorf tubes and kept at -20˚C. Catalase activity was evaluated spectrophotometrically by determining the consumption of H_2O_2 (ε = 39.4 mM^{-1} cm^{-1}) at 240 nm in 50 mM phosphate buffer, pH 7.5 and 200 mM H_2O_2 (Nemat-Ala and Hassan, 2006). Total ascorbate peroxidase activity was evaluated spectrophotometrically according to the method of Kato and Shimizu (1985) at 280 nm in 0.2 mM potassium phosphate buffer, pH 7.5, 15 mM ascorbic acid and 50 mM H_2O_2, as ascorbate (ε = 2.8 mM^{-1} cm^{-1}) was oxidized. Guaiacol peroxidase activity was assayed in 44 mM H_2O_2, and 45 mM guaiacol. The absorption at 470 nm was recorded and the activity was calculated using the extinction coefficient of 26.6 mM^{-1} cm^{-1} (Buchanan and Balm, 2005).

Statistical analysis

The experiments were laid on completely randomized design. The data was analyzed using the software SAS (V. 9.0) and the least significant difference among treatments for each trait was calculated. All measurements were carried out in triplicate. P values less than 0.05 were considered to be statistically significant.

Results

By application of MF, the biomass of plants of *H. officinalis* increased under water deficit (Fig. 1A). At the best case, MF at 200 mT increased total biomass of *H. officinalis* more than 2-fold compared to the exclusively stressed-plants ($p<0.05$) (Table 1). The effect of MF at 90 mT was less than that of at 200 mT, but it increased the value of dry weight by +53% ($p<0.05$). There were no significant differences between the values of primed *H. officinalis* at 45 and 250 mT and unprimed ones.

Data analysis revealed that seed priming with MF (at 200 and 90 mT) significantly ($p<0.05$) increased total chlorophyll content in *H. officinalis* under water deficit condition (Fig. 1B). 200 mT was the most effective intensity that elevated chlorophyll (a+b) concentration by 2.5 folds, whilst MF at 90 mT increased the total chlorophyll content by 67% compared to the exclusively water-stressed plants. Under water deficit condition, applied MF intensities (at 90 and 200 mT) elevated carotenoids content from 16% to 35%, and 200 mT was the best treatment in this regard (Fig. 1C).

Conductivity measurement showed that the electrolyte leakage significantly ($p<0.05$) decreased in the leaves of *H. officinalis* grown from MF-primed seeds (Fig. 2A). The most effective dose of MF was 200 mT followed by 250 mT. MF priming also decreased MDA concentration in the water-stressed plants (Fig. 2B).

Table 1. Analysis of variance for the studied physiological and biochemical parameters in *Hyssopus officinalis* plants grown from primed seeds with different intensity of MF and exposed to water deficit for 50 days

S.O.V	MS df	Dry weight	Total chlorophyll	carotenoids	Electrolyte leakage	MAD content	Total phenolic content	Reducing power
MF intensity	4	0.123**	0.216**	0.00758**	58.77**	0.000297**	0.033**	0.0299**
Error	10	0.054	0.087	0.0053	0.011	0.84	0.92	0.01
CV (%)	-	8.8	4.46	2.65	7.3	9.6	9.9	3.36

**: Significance at 0.01 probability

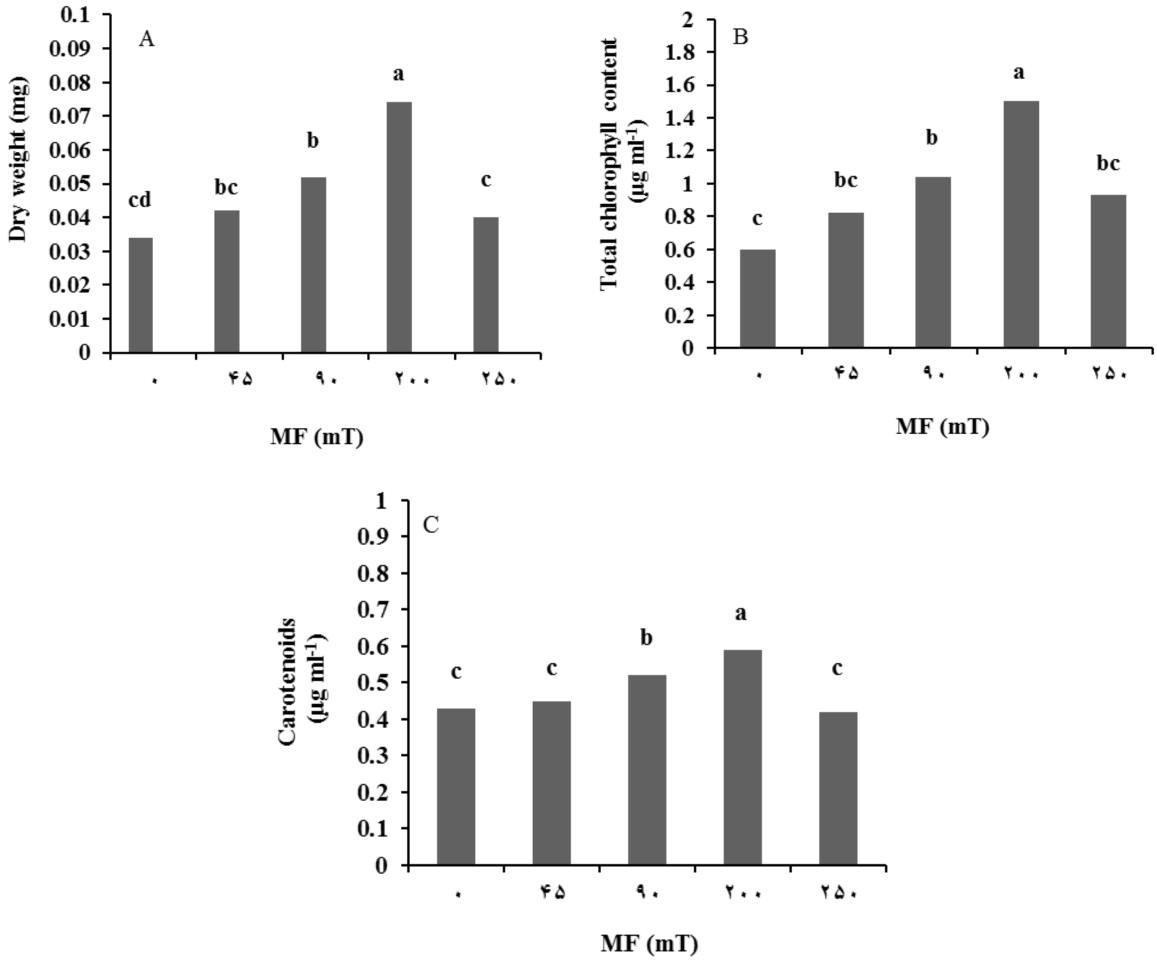

Fig. 1. Effects of seed priming with different intensity of magnetic field (MF) on Dry weight (A); total chlorophyll content (B); carotenoids content (C) of *Hyssopus officinalis* plants grown under water deficit (an irrigation interval of 8 days) for 50 days. Means (three replicates) with the same letter are not significantly different at p<0.05.

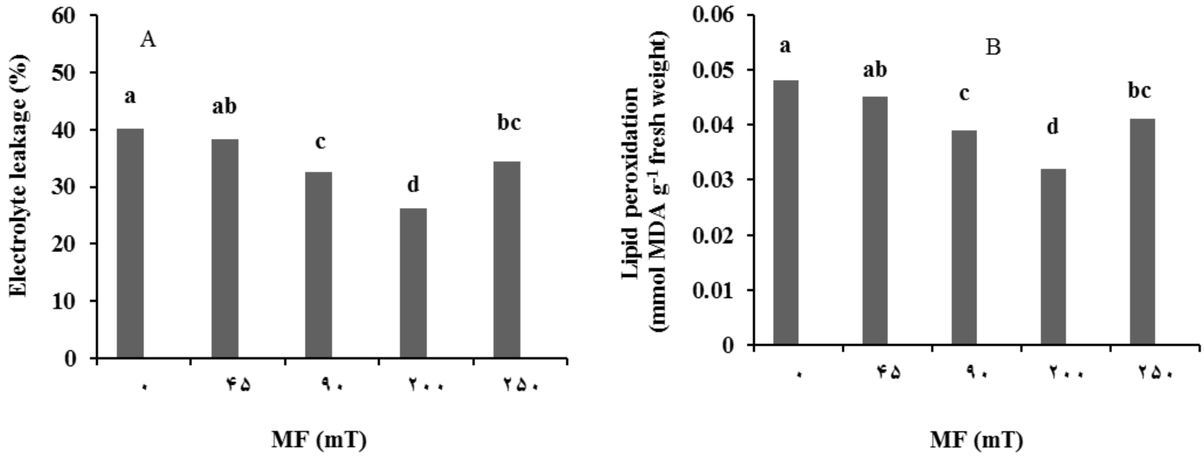

Fig. 2. Effects of seed priming with different intensity of MF on Electrolyte leakage (A) and MDA content (B) of *Hyssopus officinalis* plants grown under water deficit (an irrigation interval of 8 days) for 50 days. Means (three replicates) with the same letter are not significantly different at p<0.05.

The most effective dose of MF was 200 mT that significantly decreased MDA level by 33% ($p<0.05$) followed by 90 mT. Total phenolic content of the shoots was 2.6 mg GAE g^{-1} dry weight under water deficit. Seed priming with MF (at all applied intensities) significantly increased polyphenols concentration in the aerial parts of *H. officinalis* from 3.5 to 20 mg GAE g^{-1} dry weight (Fig. 3A). Under water stress, the highest value of polyphenols (20.3 mg GAE g^{-1} dry weight) was found in the aerial parts of those *H. officinalis* plants that their seeds were already primed with MF at 200 mT (+7.7-fold compared to water stress alone). Polyphenols concentration of the plants grown from primed seeds at 250 mT was at the second level (15.4 mg GAE g^{-1} dry weight), by +5.8-fold compared to exclusively water stress.

DPPH is introduced as a stable free radical. It is routinely utilized to estimate antioxidant activity of plant extracts and expressed as IC_{50} values, the concentration at which radical scavenging activity was 50%. Under water deficit, the IC_{50} value for DPPH-radical scavenging activity of the aerial parts of unprimed *H. officinalis* was 113.5 µg g^{-1} dry weight. However, seed priming with MF improved this trait (Fig 3B). MF, at the most effective intensity (200 mT), significantly ($p<0.05$) increased antioxidant activity of the aerial parts of *H. officinalis* grown from the primed seeds compared to antioxidant activity of plants exposed to water stress alone (Table 2). MF at 200 mT caused a reduction of 45% for IC_{50}.

In unprimed water-stressed *H. officinalis* plants, IC_{50} value for superoxide anion scavenging activity of the aerial parts was 59.6 µg g^{-1} dry weight. However, priming with MF significantly ($p<0.05$) decreased it at 200 and 250 mT (Fig. 3C). At the best case (200 mT), the IC_{50} value reduced to 49.5 µg g^{-1} dry weight.

Data analysis indicated that seed priming with MF (at 200 mT) resulted in elevation of reducing power of the aerial parts of *H. officinalis* ($p<0.05$) (Fig. 3D). The effect of other applied intensities (45, 90 and 250 mT) was not significantly different.

Catalase activity was significantly ($p<0.05$) increased (+92%) in the leaves of *H. officinalis* grown from MF-primed seeds at 200 mT compared to exclusively water stress (Fig. 4A). Other applied intensities (45, 90 and 250 mT) showed no significant effects on catalase activity compared to the control. The activity of ascorbate peroxidase was increased (from 2.1 to 2.3 folds) in the leaves of stressed *H. officinalis* grown from MF-primed seeds at 90 and 200 mT ($p<0.05$) (Fig. 4B). The highest activity of guaiacol peroxidase was recorded in stressed *H. officinalis* plants grown from seeds primed with 90 mT (Fig. 4C). The activity of this enzyme was 2-fold more than that of in exclusively water-stressed *H. officinalis* ($p<0.05$). Guaiacol peroxidase activity in stressed *H. officinalis* plants (primed with 45, 200 and 250 mT at seed germination) were statistically similar to the exclusively water-stressed ones.

Table 2. Analysis of variance for the studied biochemical parameters in *Hyssopus officinalis* plants grown from primed seeds with different intensity of MF and exposed to water deficit for 50 days

S.O.V	MS df	DPPH scavenging	Superoxide radical scavenging	Catalase activity	Ascorbate peroxidase activity	Guaiacol peroxidase activity
MF intensity	4	770.83**	330.83**	0.583**	10.72**	4.89**
Error	10	1.37	12.79	0.075	0.76	0.68
CV (%)	-	9.46	8.95	10.4	10.72	9.55

**:Significance at 0.01 probability

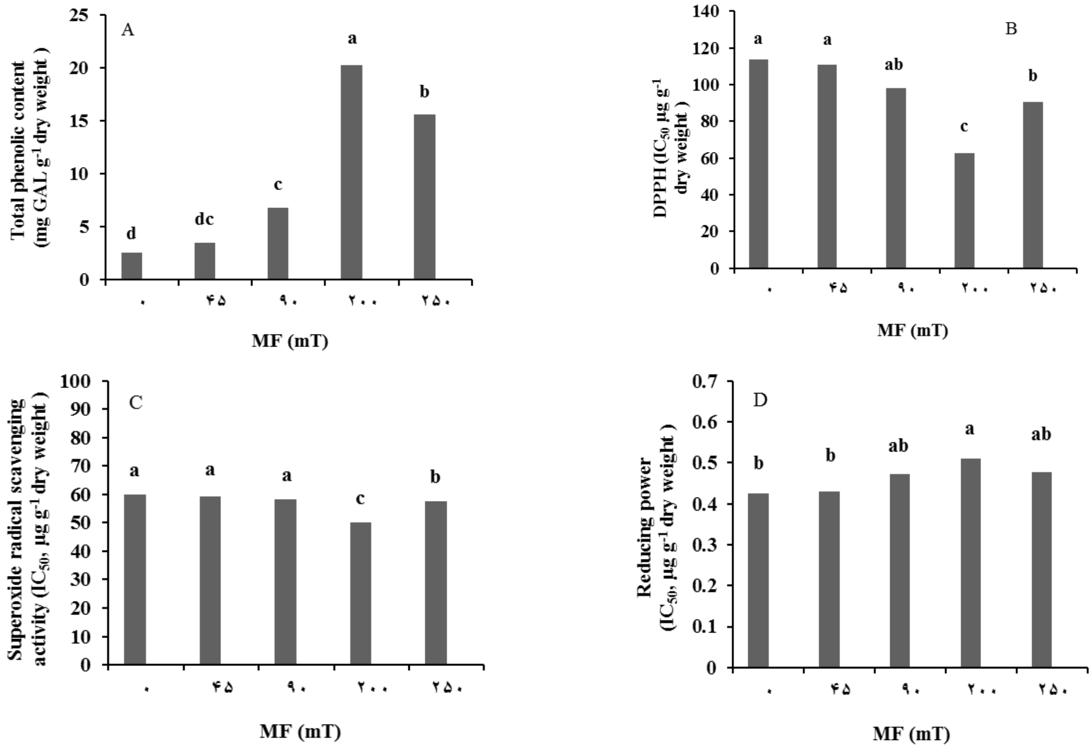

Fig. 3. Effects of seed priming with different intensity of MF on Total phenolic content (A); DPPH radical scavenging activity (B); superoxide radical scavenging activity (C); Reducing power activity (D) of *Hyssopus officinalis* plants grown under water deficit (an irrigation interval of 8 days) for 50 days. Means (three replicates) with the same letter are not significantly different at $p<0.05$.

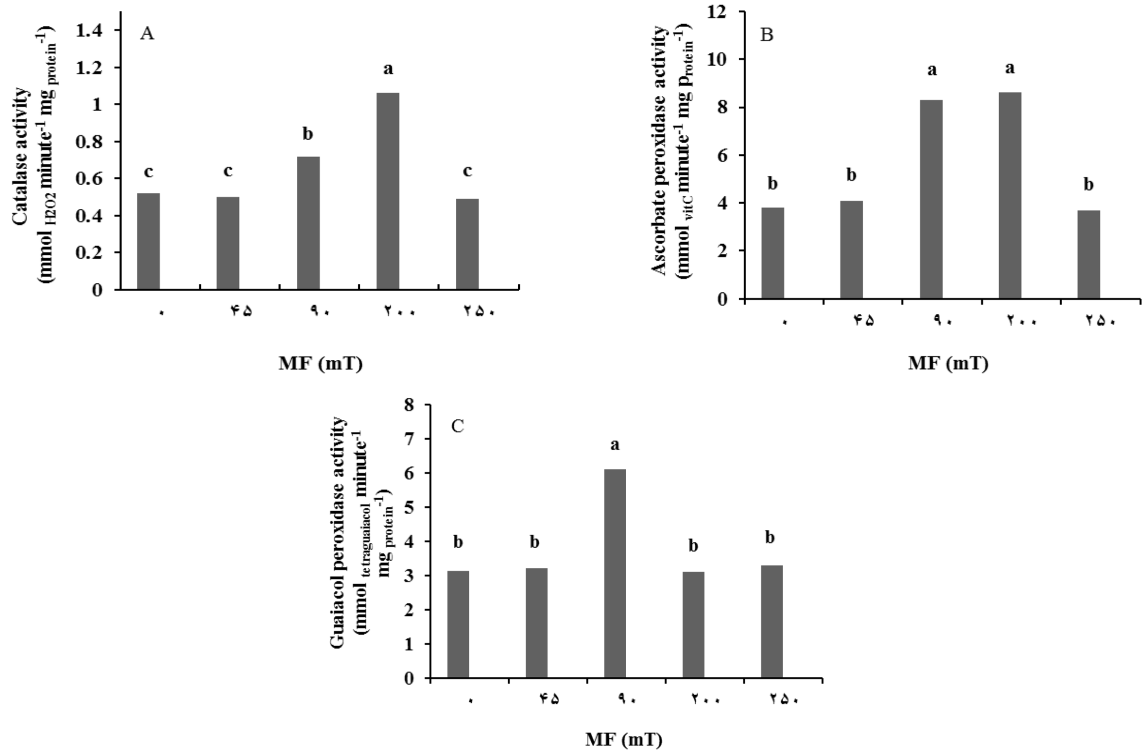

Fig. 4. Effects of seed priming with different intensity of MF on Catalase activity (A); ascorbate peroxidase activity (B); guaiacol peroxidase activity (C) of *Hyssopus officinalis* plants grown under water deficit (an irrigation interval of 8 days) for 50 days. Means (three replicates) with the same letter are not significantly different at $p<0.05$.

Discussion

The theory that seed priming with MF can alter different aspects of plant growth and development has been supported by many previous reports. Previous studies have confirmed the positive effects of MF on germination parameters (Flórez et al., 2010; Shine et al., 2011; Radhakrishnan et al., 2012; Thomas et al., 2013; Radhakrishnan and Ranjitha Kumari, 2013). Moreover, there are some other reports indicating the helpful effects of seed priming with MF on the further stages of plant growth under normal or stressful conditions through improving physiological and phytochemical characteristics (Javed et al., 2011; Azimian and Roshandel, 2015; Radhakrishnan et al., 2012; Roshandel and Azimian, 2015; Thomas et al., 2013). Findings of current study are in line with the findings of previous reports.

Principally, priming is considered as a method to enhance seed physiology and recover seed performance under stressful conditions. Seed priming leads to establish diverse defense mechanisms (such as osmotic adjustment and antioxidant defense system) in seeds to combat environmental stresses. As suggested before, these mechanisms construct a 'priming memory' in seeds that can be employed upon a subsequent salinity stress-exposure and trigger greater stress tolerance in the plants grown-up from primed seeds (Chen and Arora, 2013). Different reports have practically confirmed this finding (Wahid *et al*., 2007; Kumar *et al*., 2010; Hossain *et al*., 2015). However, the molecular details of the associated mechanism(s) are not well understood yet. It is possible that MF indirectly triggers a relevant signal transduction pathway that up-regulate genes involved in different aspects of plant growth (e.g. in synthesizing antioxidants for effective scavenging of ROS produced in cellular metabolism in unstressed or stressed plants).

The current data demonstrated that exposure of the seeds of *H. officinalis* to 200 mT/5 min significantly improved the dry weight and photosynthetic pigments content of the stressed-plants. Likewise in cucumber and soybean seeds, pretreatment with MF at 200 mT improved the germination. But in the former plants the most effective exposure time of MF was 1h (Bhardwaj et al., 2012; Shine et al., 2012). Furthermore, the present research revealed the protective effect of MF on photosynthetic components in *H. officinalis* under water deficit, which is in agreement with the previous reports. It is already reported that seeds of corn cultivars pretreatment with electromagnetic field (100 and 150 mT/10 min) are less influenced by deleterious effects of drought stress via preserving their chlorophyll contents and also quenching harmful photo and non-photochemicals (Javed et al., 2011). However, the efficiency of MF parameters (such as exposure time and intensity) on plant growth and development appear to be species-specific. As suggested, a MF principally affects the growth processes at the cellular and sub-cellular levels; it alters the Ca^{2+} balance, enzyme activities and various metabolic processes (Çelik et al., 2009). Improvement of photosynthetic activities, absorption and assimilation of nutrients has been also reported by Kavi (1977). However, the precise mechanism of the interaction of MF with a living cell remains indistinct.

The present findings showed that MF caused a decrease in lipid peroxidation (MDA content) and membrane electrolyte leakage in *H. officinalis* plants under water deficit condition. The same result has been also reported in mung bean (Chen et al., 2011). Moreover, regarding to the data, MF priming significantly elevated phenolic content and reduced antioxidant activities in the shoots of water-stressed *H. officinalis* plants compared to the exclusively water-stressed ones. There are also some other reports indicating increase in the plant tissue polyphenols under drought conditions (de Abreu and

Mazzafera, 2005; Hura and Grzesiak, 2008). Our results were in agreement with the previous works. Phenolic compounds involve in antioxidant activity due to their aromatic ring containing one or more hydroxyl groups that make them effective hydrogen donors. To estimate antioxidant properties of the shoots of *H. officinalis*, assessment of DPPH, superoxide anion radical scavenging activities and reducing power were assessed. These assessments are commonly used to evaluate antioxidant activity in plants (Kumaran and Joel karunakaran, 2007; Tawaha et al., 2007). In one hand, MF might indirectly triggers the related signal transduction pathways that up-regulate genes associated in producing antioxidants for efficiently scavenging ROS generated in cellular metabolism in unstressed or stressed plants. On the other hand, MF may directly appear as a stress and increase the average radical levels, prolonging their lifetime and raising the risk of radical reactions with cellular components (Çelik et al., 2009). Therefore, the generated radicals due to MF could operate as a signal to induce marked production of antioxidants and increase in radical scavenging activity.

In the MF-primed *H. officinalis* plants, an elevation in radical-scavenging activity occurred in conjunction with alteration in MDA level. MDA is assumed as an appropriate indicator of the oxidative stress resulting from abiotic stresses (Ksouri et al., 2007). The current data suggest that *H. officinalis* plants grown from primed seeds are more tolerant to oxidative damage under water deficit condition. Probably, MF triggers a process to detoxify ROS through an increased radical-scavenging capacity.

Similar to our results, previous reports have demonstrated alternation in the activity of scavenging enzymes (such as catalase, superoxide dismutase, glutathione reductase, glutathione transferase, peroxidase, ascobtate peroxidase, and polyphenol oxidase) by MF (among many: Bhardwaj et al., 2012; Novitskii, 2013; Alema et al., 2014; Haghighat et al., 2014). Hypothetically, change in the activity of antioxidant enzymes is related to buildup of ROS generated by exposure to increased MF.

Conclusion

Findings of present study suggest that seed priming with MF (particularly, at 200 mT) could alleviate the detrimental effects of water deficit in *H. officinalis* plants by employing antioxidant defense system to scavenge ROS and protect cellular membrane stability. Furthermore, increase in the level of photosynthetic pigments leads to stimulate the growth of *H. officinalis* plants under water deficit conditions.

Conflict of interest

The authors declare no conflict of interest for this work.

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