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Effects of Magnetic Field on Growth and Antioxidant Capacity of *Artemisia aucheri* in Normal or Saline Conditions

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ABSTRACT: In the present study, changes of total phenolic concentration and radical-scavenging activity were evaluated in the shoots of *Artemisia aucheri* -a medicinal plant- that had been exposed as seeds to magnetic fields (90 or 200 mT for 5 and 20 min) and that were irrigated for one month with NaCl (150 mM) under glasshouse conditions. Total phenolic content and radical-scavenging activity decreased in *A. aucheri* under salt stress. Magnetic fields (particularly at 200mT for 20min) significantly increased these parameters compared to control, regardless of the presence or absence of NaCl. While salt stress induced MDA accumulation and biomass diminution in *A. aucheri,* magnetic-pretreatment of seeds significantly alleviated the injurious effects of NaCl. Results suggested application of magnetic field would increase salt tolerance in *A. aucheri* through maintenance of cellular membrane integrity. It may also improve medicinal properties of the shoots via increased polyphenols concentration and antioxidant capacity.

Key words: *Artemisia aucheri*, Antioxidant activity, Magnetic field, Salt stress, Seed pretreatment

INTRODUCTION

During Oxidation reactions highly reactive molecules such as reactive oxygen species (ROS) are produced in plant cells leading to start chain reactions which may result in significant damage to cell structures. Although, ROS are generated as a natural byproduct through normal metabolism of plants, however, their concentrations augment greatly as a secondary component of other stresses such as salinity which is known as oxidative stress (Ozgur *et al*., 2013). Currently, the role of antioxidants in reducing damage caused by ROS is well-established (Gill and Tuteja, 2010). Antioxidants can inhibit the initiation or proliferation of oxidizing chain reactions and as a consequence, delay or inhibit the oxidation of lipids, proteins and other vital molecules in cells (Zhengand and Wang, 2001). Free radicals create serious risks for human health due to attenuation of the immune system, alteration in gene expression and induction of abnormal proteins. Consequently, the potential health risks and toxicity of synthetic antioxidants has persuaded researches to introduce novel botanical resources with appropriate antioxidant properties (Ksouri *et al*., 2007). Thus, there is considerable interest in evaluating the capacity of medicinal plants in reducing such free radical-induced tissue injury (Krishnaiah *et al*., 2010).

A number of reports have pointed out the correlation of antioxidant properties and polyphenols in a given plant species (Ksouri *et al*., 2007). Polyphenolic compounds are commonly found in a wide range of both edible and inedible plants, and they have been reported to contain numerous physiological properties, including antioxidant activity (Kähkönen *et al*., 1999). In this regard, finding plant materials rich in phenolics or exerting treatments to enhance polyphenols level in plants could be of considerable interest to the food industry because such plants are good candidates to retard oxidative degradation of lipids and proteins, leading to better preservation of the quality and nutritional value of food. With this respect, in the present research, Artemisia aucheri was selected for study. Artemisia aucheri Boiss., locally named 'Dermaneh-Koohi', is an aromatic plant indigenous to the rangelands of Iran. In traditional medicine, *A. aucheri* is used as an astringent, disinfectant, antiseptic, antiparasitic, and antipoisoning agent (Azadbakht *et al*., 2003).

Salinity, one of the major abiotic stresses around the world, can change the balance between the production and scavenging of ROS in plant tissues through effects on stomatal opening and net gas exchange. With this view, we studied the interactions between the antioxidant capacity and impacts of NaCl in *A. aucheri*. We also investigated pretreatments (i.e. different magnetic field intensities and exposure times) that might improve salt tolerance and so might affect antioxidant capacity during cultivation of medicinal plants.

Positive effects of magnetic field on plant growth have been recognized for a while. For instance, the effects of magnetic field in increasing seed germination, fresh weight and shoot length of maize, tobacco, cucumber and tomato, pea, lentil, medicinal plants like Salvia officinalis and Calendula officinalis plants have also been reported by various workers (Aladjadjiyan, 2002; Aladjadjiyan and Ylieva, 2003; Yinan *et al*., 2005; Flórez *et al*., 2007; Martínez *et al*., 2009a, 2009b; Flórez *et al*., 2010; Shine *et al*., 2012).

Moreover, Radhakrishnan *et al*, (2012) reported the effects of pulsed magnetic field treatment of soybean seeds on calli growth, cell damage, and biochemical changes under salt stress. In another research, Radhakrishnan and Ranjitha Kumari (2013) investigated the influence of pulsed magnetic field on soybean seed germination, seedling growth and soil microbial population. Thomas *et al*. (2013) showed that magnetopriming of dry seeds of chickpea can be effectively used as a pre-sowing treatment for mitigating adverse effects of salinity at seed germination and early seedling growth.

In the present study we investigated: total phenolic concentration and antioxidant capacity in *A. aucheri* plants under: 1) normal condition (with no MF or NaCl treatment), 2) pretreatment by MF, 3) saline condition, and 4) a combination of MF- pretreatment and irrigation with saline solution. Here, we hypothesize that seed pretreatment with a magnetic field can alter phytochemical properties such as antioxidant capacity, which might enhance salt tolerance in plants.

MATERIAL AND METHODS

A. Plant material and preparation

Seeds of *Artemisia aucheri* Boiss. were purchased from Pakan-Bazr (Esfahan, Iran). Seeds were divided in two groups, those to be subjected to a magnetic field and untreated. The seeds were moisture under magnetic field exposure. Our preliminary experiments showed that among the magnetic field treatments of 45, 90, 200 and 300 mT and exposure time of 5, 10, 20 and 30 min, the most effective magnetic field intensity and exposure times were 90 and 200 mT for 5 or 20 min in enhancing the germination of *A. aucheri*. In addition, among the 50, 100, 150, 200 and 250 mM NaCl treatments, application of 150 mM NaCl reduced germination percentage by 50%. Accordingly, for the main experiment treatments included, 1: control (with no MF or NaCl treatment), 2: seed pretreatment with 90 and/or 200 mT at 5 and/or 20 min, 3: irrigation of 14-day-old seedlings with Hoagland solution (pH 6.8) containing 150 mM NaCl (no pretreatment with a magnetic field) and, 4: seed pretreatment with a magnetic field (as in 2) along with irrigation of 14-day-old seedlings with 150 mM NaCl. All treatments with MF were accomplished by a magnetic field generator in the physics lab of Faculty of Sciences at Shahrekord University. Treated or untreated seeds were sown in polystyrene boxes, filled with a potting mixture composed of 50% perlite and 50% fine sand. The plants were raised in a green house under controlled conditions (16/8 h light/dark period, 32/25°C temperature, 60-70% RH and 1000-1200 µmol m^{-2} s-1PAR).The experiments lasted for one month, and at the end of the experiments, 44 days old plants were sampled to determine dry weight production.

B. Estimation of membrane lipid peroxidation

Lipid peroxidation was evaluated by estimating malonyldialdehyde (MDA) accumulation in the aerial parts (Ksouri *et al*., 2007). Fresh samples of shoots (which were completely identical to those samples prepared in 2.1) of *A. aucheri* (250 mg fresh weight) were individually homogenized in 5 ml of 0.1% trichlroacetic acid (TCA). The homogenate was centrifuged at 10000 ×g for 10 min at 4 °C and the supernatant (1 ml) mixed with 5 ml of 0.5% thiobarbituric acid (TBA) prepared in TCA 20%, and incubated at 95°C for 30 min. After stopping the reaction in an ice bath, samples were centrifuged at 10000 ×g for 5 min. The supernatant absorbance was measured at 532 nm and after subtracting the non-specific absorbance at 600 nm, MDA concentration was determined applying the extinction coefficient 155 mM^{-1} cm^{-1}.

C. Polyphenol extraction and estimation

Fresh shoots of *A. aucheri* plants were shade dried for one week and ground to fine powder. A sample (1 g) of this dry powder was extracted with 80% methanol with stirring for 30 min. The extracts were then kept for 24h at 4°C, filtered through a Whatman No. 4 filter paper, and evaporated under vacuum. Phenolic compound were assayed using the Folin-Ciocalteu reagent, following Singleton's method with some modification (Ksouri *et al*., 2007). A sample of extract (0.125 ml, diluted 10-fold) was added to 0.5 ml of deionized water and 0.125 ml of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 ml of 7% Na_2CO_3 solution. The solution was then diluted with deionized water to a final volume of 3 ml and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance of sample reaction was read at 760 nm. Total phenolic concentration of plants (three replicates per treatment) was expressed as mg gallic acid equivalents (GAE) g^{-1} dry weight through a calibration curve with gallic acid.

D. Assay of DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical-scavenging activity

The antioxidant activity of extracts was assayed based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrozyl (DPPH) free radical (Ksouri *et al*., 2007). Methanolic extracts of shoots (2 ml) were mixed with 0.5 ml of 0.2 mM methanolic DPPH, the mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of resulting solution was measured at 517 nm. The scavenging activity was expressed as IC50 (µg g^{-1}dry wt.). The ability to scavenge the DPPH radical was calculated as:

% Inhibition= [(A0-A1)/A0]×100, where A0 was the absorbance of the control and A1 absorbance of extract or standard.

E. Assay of superoxide anion radical scavenging activity

Measurement of superoxide anion scavenging activity was based on the method of Kumaran and Joel karunakaran (2006). The reaction mixture consisted of 50 mM phosphate buffer, pH 7.6, 20 µg riboflavin, 12 mM EDTA and NBT 0.1 mg $3ml^{-1}$, added in that sequence. Reaction was started by illuminating (fluorescent lamp) the reaction mixture with different concentrations of the extract for 90 seconds. Immediately after illumination, the absorbance was read at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes, with reaction mixture were kept in the dark and served as blanks. The antioxidant activity of the extracts was based on IC50 (µg g^{-1}dry wt.). The superoxide radical scavenging activity was calculated using the following formula:

% Inhibition= [(A0- A1) /A0]×100, where A0 was the absorbance of the control and A1 was the absorbance of the extract/standard.

F. Assay of hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of methanolic extracts was measured according to the method of Yuan *et al*. (2005). The reaction mixture (1 ml) consisted of 500 µl aliquots of methanolic extracts of shoots, 1mM $FeCl_3$, 1mM Na_2EDTA, 10 mM H_2O_2, 1 mM L-ascorbic acid, 36 mM 2-deoxy-D-ribose in 25 mM phosphate buffer (pH 7.4). The reaction mixture was incubated for 1h at 37°C, and incubated in boiling water bath for 15 min after addition of 1 ml of 2.8% TCA and 1 ml of 1% TBA. The absorbance was read at 523 nm. The antioxidant activity of the extracts was expressed as IC50 (µg g^{-1}dry wt.). The ability to scavenge hydroxyl radical was calculated using the following formula:

% Inhibition= [(A0-A1) /A0]×100, where A0 was the absorbance of the control and A1 was the absorbance of extract/standard.

G. Reducing power determination

The reducing power of methanolic extracts of shoots of A. sieberi was determined according to method of Kumaran and Joel karunakaran (2006). Different amounts of the extract (50- 1500 µg g^{-1} dry wt.) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide $[K_3Fe(CN)_6]$. Mixture was incubated at 50°C for 20 min, followed by addition of 2.5 ml of 10%TCA, and then centrifuged for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $FeCl_3$, and the absorbance measured at 700 nm. Increase in absorbance of the reaction mixture indicated increased reducing power.

H. Statistical analysis

The experiments were laid in factorial completely randomized design. The data was analyzed using the software SAS (V. 9.0) and the least significant difference (LSD) among treatments for each trait was calculated. All the measurements were carried out in triplicate and were expressed as means of three analysis ±standard error. P values less than 0.05 were considered to be statistically significant. Correlation analyses were carried out using the polynomial regression function in the Excel program 2007.

RESULTS

A. NaCl and MF effects on biomass and lipid peroxidation

By application of MF, biomass of non-stressed plants of *A. aucheri* was significantly stimulated, nearly 2.6-fold at 200mT/20 min (Fig. 1) ($p<0.05$). Under saline condition, dry weight of *A. aucheri* decreased by 48%. However, MF increased dry weight of salinized *A. aucheri* by up to 60% (at 200mT/20 min) and therefore ameliorated the negative impacts of NaCl. Salt stress increased MDA concentration in the aerial parts of *A. aucheri* nearly 1.2-fold compared to control (Fig. 2). MF-pretreatment significantly decreased MDA concentration by 26-36% in salinized plants ($p<0.05$). There was no significant difference between the different MF intensities and exposure times (viz. 90 or 200 mT for 5 or 20 min).

B. Salinity, MF and Antioxidant capacity assay

MF-pretreatment (at all applied intensities and exposure times) significantly increased polyphenol concentrations in the aerial parts of *A. aucheri* in the range of 30.8-49.2 $mgGAEg^{-1}$dry wt., regardless of presence or absence of NaCl ($p<0.05$) (Fig. 3). In the absence of NaCl, the highest value for polyphenols (49.2 $mgGAEg^{-1}$ dry wt.) was obtained by applying of MF at 200 mT/20 min (+59% compared to control). Salt stressed plants of *A. aucheri* (saline control) exhibited a decreased (32%) concentration of polyphenols compared to control, a difference which was also significant ($p<0.05$). Under saline condition, the highest value of polyphenols (47.1 $mgGAEg^{-1}$ dry wt.) was found in the aerial parts of those *A. aucheri* plants that were already pretreated by MF at 200mT/20 min (+52% compared to control). The effect of MF on polyphenols was quite dose dependent as the R2 values obtained were 0.817 for *A. aucheri* plants grown in normal condition and 0.702 for the salinized plants. The stable free radical DPPH is commonly used to evaluate the free radical scavenging ability of plant extracts and antioxidant activity was evaluated as IC50 values, the concentration at which radical scavenging activity was 50%.

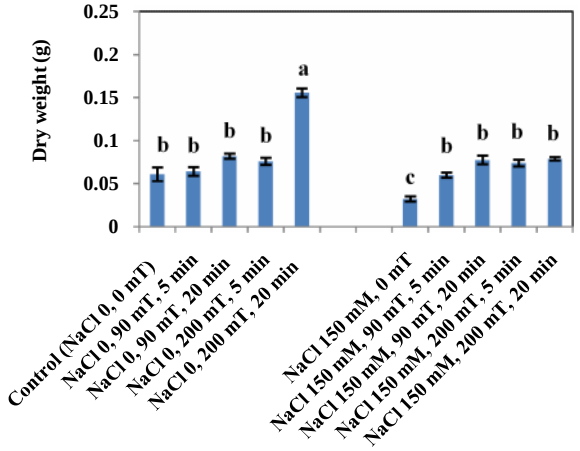

Fig. 1. Dry weight of shoots of 44-day-old *Artemisia aucheri* plants pretreated with a magnetic field and/or irrigated for 30 days with NaCl (150 mM). Means (three replicates) with the same letter are not significantly different at $p<0.05$. Bars represent standard errors.

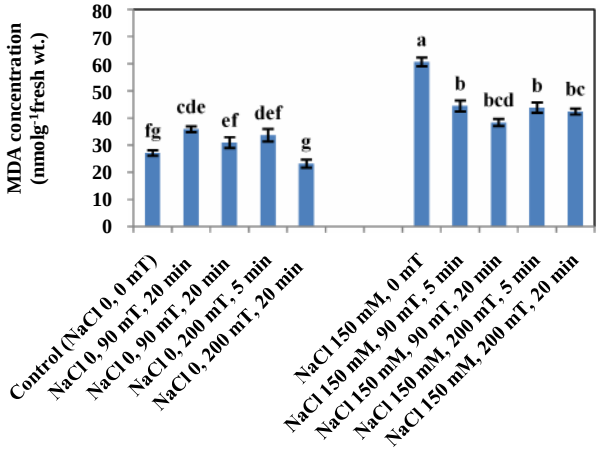

Fig.2. MDA concentration in the fresh tissues of shoots of 44-day-old *Artemisia aucheri* pretreated with a magnetic field and/or irrigated for 30 days with NaCl. Means (three replicates) containing the same letter are not significantly different at $p<0.05$. Bars represent standard errors.

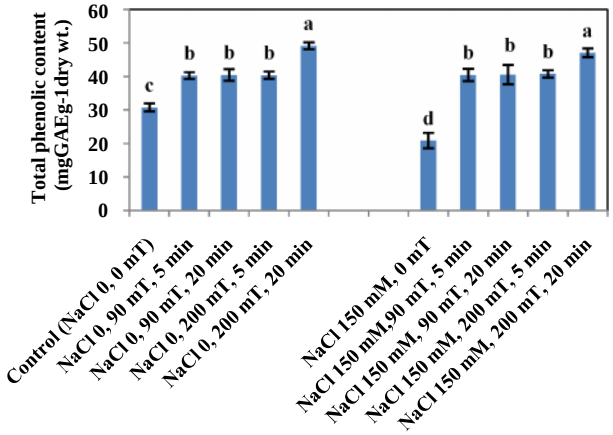

Fig. 3. Total phenolic content in the shoots of 44-day-old *Artemisia aucheri* pretreated with a magnetic field and/or irrigated for 30 days with NaCl (150 mM). Means (three replicates) containing the same letter are not significantly different at $p<0.05$. Bars represent standard errors.

In the absence of NaCl, MF-pretreated plants showed different DPPH-radical scavenging activity at different MF intensities and times, with values (IC50 values ranged from 64.3 to139 µg g^{-1}dry wt.) that were much lower than those of the control (IC50 = 120.4 µg g^{-1}dry wt.) (Fig. 4). Apart from MF intensity, the length of exposure also reduced the IC50 values (a reduction of 47% for IC50 at 200 mT). Although, salt stress, alone caused a reduction of 1.2-fold of antioxidant activity of the aerial parts of *A. aucheri* compared to control, MF-pretreatment improved this parameter particularly at 200mT/20 min in salinized shoots of *A. aucheri* (+41% compared to control). Polynomial regression analysis revealed a relatively good correlation between DPPH radical-scavenging activity (of the aerial parts of *A. aucheri*) and magnetic dose in both normal and saline condition (R2 values were greater than 0.75).

In control plants, IC50 value for superoxide anion scavenging activity of the aerial parts of *A. aucheri* was 203 µg g^{-1}dry wt., but pretreatment with MF significantly decreased the value ($p<0.05$) in a dose-dependent manner (Fig. 5): at the highest dose (i.e, 200 mT/20 min), the IC50 value reduced to76.2 µg g^{-1}dry wt., or 2.7 fold less than control. In the salt-treated plants, superoxide anion scavenging activity of the shoots of *A. aucheri* significantly decreased ($p<0.05$) but pretreatment with MF (particularly at 200 mT/20 min) remarkably augmented superoxide anion scavenging activity of salinized shoots of *A. aucheri* (the IC50 value at 200 mT/20 min was 2.3 fold less than control). Polynomial regression analysis showed a clear correlation between superoxide anion scavenging activity (of shoots of *A. aucheri*) and magnetic dose in both normal and saline condition (R2 values were greater than 0.84).

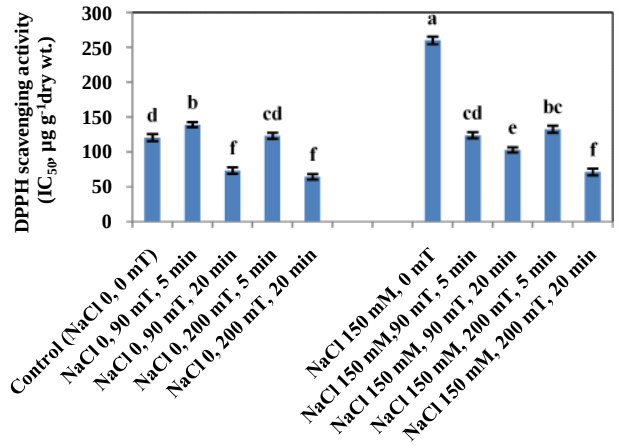

Fig.4. DPPH scavenging activity of the shoots of 44-day-old *Artemisia aucheri* pretreated with a magnetic field and/or irrigated for 30 days with NaCl (150 mM). Means (three replicates) containing the same letter are not significantly different at $p<0.05$. Bars represent standard errors.

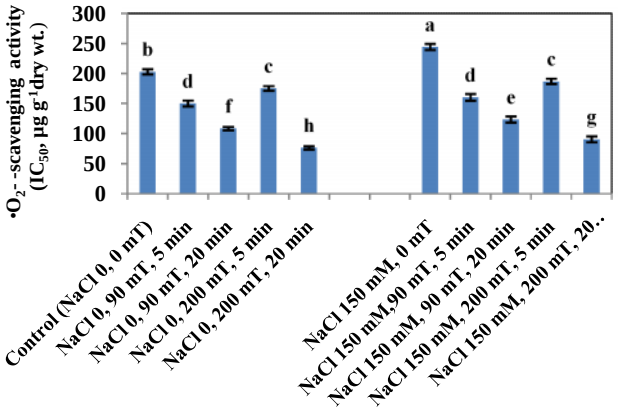

Fig. 5. Superoxide anion radical ($\bullet O_2$)-scavenging activity of the shoots of 44-day-old *Artemisia aucheri* pre-treated with a magnetic field and/or irrigated for 30 days with NaCl (150 mM). Means (three replicates) containing the same letter are not significantly different at $p<0.05$. Bars represent standard errors.

Results showed IC50 for hydroxyl scavenging activity in *A. aucheri* was 189.3 µg g^{-1}dry wt. in non-saline conditions, while it increased to 239.6 µg g^{-1}dry wt. under salt stress (+26.6%), which was statistically significant ($p<0.05$) (Fig. 6). MF significantly increased hydroxyl scavenging activity ($p<0.05$) in pretreated plants. In this case, the IC50 values had a range from 85.7 to 151.2 µg g^{-1}dry wt.- at different intensity or time periods in normal or saline conditions. The most effective MF-pretreatment to increase hydroxyl scavenging activity was 200mT/20min in both non-saline and saline conditions. The correlation between hydroxyl scavenging activity and magnetic dose was greater (R2=0.947) in non-stressed *A. aucheri* than that in salinized plants (R2=0.880).

Salt stress, alone, caused a significant ($p<0.05$) increment of 21% in the IC50 value for reducing power of the methanolic extract of *A. aucheri* compared to control (Fig. 7). Pretreatment with MF significantly decreased the IC50 values in both saline and normal conditions (i.e. increasing in reducing ability). The most effective MF- pretreatment (200 mT/20 min) increased the reducing power by 74% and 64% - in normal and saline conditions, respectively. Also, a considerable correlation was observed for reducing power (of the aerial parts of *A. aucheri*) and magnetic dose in both normal and salinized plants (R2 values were greater than 0.83).

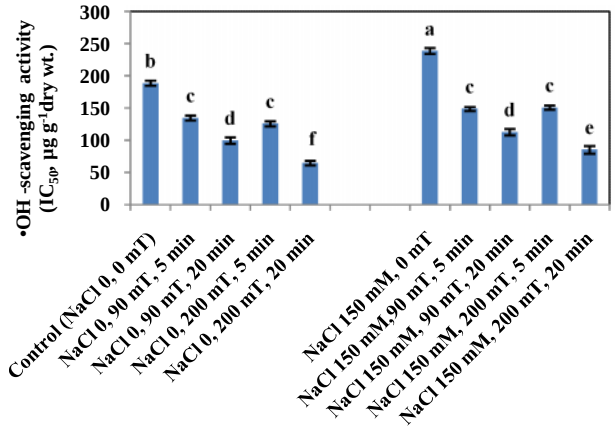

Fig. 6. Hydroxyl radical (•OH)- scavenging activity of the shoots of 44-day-old *Artemisia aucheri* pretreated with a magnetic field and/or irrigated for 30 days with NaCl (150 mM). Means (three replicates) containing the same letter are not significantly different at $p<0.05$. Bars represent standard errors.

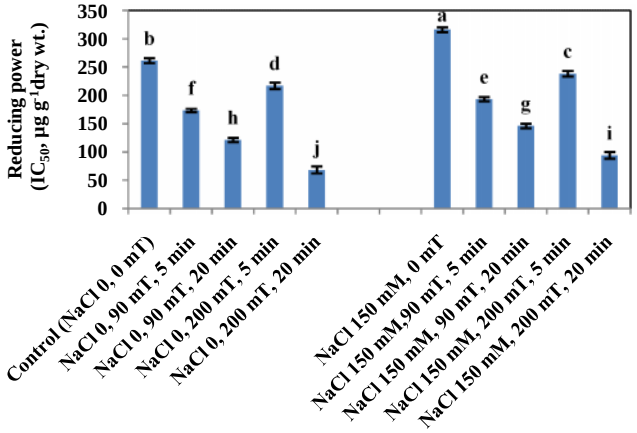

Fig. 7. Reducing power of the shoots of 44-day-old *Artemisia aucheri* pretreated with a magnetic field and/or irrigated for 30 days with NaCl (150 mM). Means (three replicates) containing the same letter are not significantly different at $p<0.05$. Bars represent standard errors.

DISCUSSION

In the current research, changes of antioxidant activity of the shoots of *A. aucheri* were studied under salinity and magnetic treatments. To assay antioxidant properties of *A. aucheri*, estimation of reducing power, DPPH-radical, superoxide anion and hydroxyl radical scavenging activities were utilized. These methods are among the most efficient and commonly used techniques for appraising antioxidant activity in plants (Kumaran and Joel karunakaran, 2007; Tawaha *et al.*, 2007). Our data illustrated, there was a significant difference between the levels of polyphenols in the MF-treated plants compared to control and salt-stressed plants. While salinity decreased significantly total phenolic content in the shoots of *A. aucheri*, MF treatment, principally at 200mT for 20min, ameliorated considerably the negative impacts of NaCl on the levels of polyphenols. Phenolic compounds contain an aromatic ring as part of the molecular structure, with one or more hydroxyl groups. Because of the scavenging ability of their hydroxyl groups which make them effective hydrogen donors, a highly positive relationship between total phenols and antioxidant activity has been reported in many plant species (Vinson *et al*., 1998). Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. Phenolic acids are a major class of phenolic compounds, widely occurring in the plant kingdom especially in fruits and vegetables. The consumption of antioxidant compounds or foods with high levels of these compounds is associated in prevention and reduction of the risk of diseases associated to free radical reactions (Katalinic *et al*., 2010). Nevertheless, polyphenols are not the only constituent involved in antioxidant systems. It has been found that natural antioxidants can be nitrogen-containing compounds (alkaloids, chlorophyll derivatives, amino acids, peptides, and amines), carotenoids, tocopherols or ascorbic acid and its derivatives (Velioglu *et al*., 1998). In the current work, the concentration of carotenoids mirrored variations in the antioxidant capacity with magnetic and/or salt treated Artemisia plants (Data are not shown).

Increase in polyphenol concentration under abiotic stresses, such as salinity, has been mentioned for a number of plants (Agasian *et al*., 2000; Muthukumarasamy *et al*., 2000; Ksouri *et al*., 2007). Nevertheless, in this case, our results were not consistent with the previous reports. As, under salt stress (150 mM NaCl), total phenolic concentration surprisingly reduced (by 32%) in *A. aucheri*. Plants have diverse responses to salt stress. In the case of polyphenols, different responses of plants to salt stress have previously been reported (Ksouri *et al*., 2007). Apparently, the difference in the capacity to accumulate polyphenols is associated to the difference of salt tolerance in plants (Ksouri *et al*., 2007).

Exposure of seeds to a magnetic field, principally at 200mT for 20min, significantly improved the dry weight, MDA concentration, polyphenol concentration and radical-scavenging activity. The advantageous effect of MF was observed in both saline and normal conditions, producing plants with larger vigor or more salt tolerance. Previously, our results revealed that MF significantly increased vigor index in *A. aucheri* seedlings (by 43% compared to control) through increasing the percent of germination and seedling length (data are not shown). Accordingly, it seems exposure of seeds to magnetic fields improves seedling growth parameters toward producing plants with better physiological and phytochemical characters.

Data obtained for the dry weight suggest that increasing in the MF intensity and exposure times would probably augment the values for this parameter. Normally, plants are continually exposed to the Earth's magnetic field of, 50 μT but fields of much greater magnitude are required to influence plant behavior (Adair 1999). However, literature review shows various effects of experimental magnetic field on plants, particularly at the stage of seed germination (amongst: Flórez *et al*., 2010; Radhakrishnan *et al*., 2012; Shine *et al*., 2012; Radhakrishnan and Ranjitha Kumari, 2013; Thomas *et al*. (2013). Kavi (1977) indicated that suitable magnetic treatment increased the absorption and assimilation of nutrients, and ameliorated photosynthetic activities. In most cases, a magnetic field can affect the growth processes at the cellular and subcellular level; alter the Ca^{2+} balance, enzyme activities and various metabolic processes (Çelik *et al*., 2009). Despite these observations, the precise mechanism of the interaction of MF with a living cell remains unclear.

Data analysis reported here revealed a positive correlation between antioxidant properties of the aerial parts in *A. aucheri* and magnetic dose (mT min; polynomials with R2 values greater than 0.75) regardless of salt stress. To our knowledge, the current work would be one of the first studies on the effects of MF on total phenolic concentration and radical-scavenging activity in plants. There are a few reports on the antioxidant enzymes, such as superoxide dismutase and catalase, which indicate the function of these enzymes increases in seedlings following seed-pretreatment by MF (Çelik *et al*., 2009). It is also found that increment of superoxide dismutase activity in MF-treated plants is affected by extension of time exposure (Çelik *et al*., 2009). This finding is consistent with our results.

As a suggestion, it is possible that MF indirectly triggers a relevant signal transduction pathways that up-regulate genes involved in synthesizing antioxidants for effectively scavenging ROS produced in cellular metabolism in unstressed or stressed plants.

MF, by itself may act as a stress and increase the average radical concentration, prolonging their lifetime and augmenting the possibility of radical reactions with cellular components (Çelik *et al*., 2009). In this case, with regard to our results, the produced radicals due to MF could act as a signal to enhance considerably production of antioxidants and cause a dramatic increase in radical-scavenging activity. In the present study, in MF-pretreated plants, an increase in radical-scavenging activities occurred in conjunction with changes in MDA. MDA is presumed as a reliable indicator of the oxidative stress resulting from abiotic stresses, such as salinity (Ksouri *et al*., 2007). The significant increase in MDA concentration in salt-stressed plants compared to the significantly lower MDA concentration in the same plants but already treated by MF suggest that the plants grown from pre-treated seeds are better protected against oxidative damage under salt stress. Probably, MF activates a procedure to detoxify reactive oxygen species by an increased radical-scavenging capacity.

Overall, it could be concluded that seed pretreatment by MF (particularly, at 200mT/20min) contributed to augmenting extractable, active antioxidants from the aerial parts of *A. aucheri* which are useful for food and medicinal application. Also, this pre-treatment seems to be a reliable technique to increase salt tolerance in this species.

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