

Study of some physiological characteristics of two almond species (*Amygdalus scoparia* and *Amygdalus. eburnea*) in response to static magnetic field

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Abstract

Despite the numerous studies on the effect of the magnetic field on plants, the exact mechanism of these effects has not been determined yet. The aim of this study was a comparison between two almond species (*Amygdalus scoparia* and *A. eburnea*) with respect to their responses to the effect of magnetic field on growth and photosynthetic characteristics. For this purpose, 25-day-old almond plants were affected by 10 mT static magnetic field for 4 days and 5 hours for each day. The fresh weight, dry weight, water content and length of ground and aerial parts were measured. Also, the number of leaves, leaf area, percentage of leaf open stomatas, photosynthetic pigments content and finally total phenol content were investigated. The results showed that the fresh weight, water content, percentage of open stomata and shoot length increased compared to the control samples, but the number of leaves and the size of the leaf area did not change. In addition, the content of chlorophyll and carotenoid decreased, but the amount of phenolic compounds increased in *A. eburnea*. It seems that static magnetic fields have receptors in the almond plant that act like phytochromes and induce relevant responses.

Keywords: *Amygdalus*, Carotenoid, Chlorophyll, Phenolic compounds, Static magnetic fields

Introduction

Magnetic fields are present in the daily life both naturally and as a result of human technological activities. Several researches have shown that living organisms show a great deal of sensitivities to magnetic fields, but their effects on biological systems, and in particular the mechanism of these effects, have not yet been well-established (Sen and Alikamanoglu, 2014). It has been, shown in numerous studies that magnetic fields change the content of photosynthetic pigments (Najafi *et al.*, 2014; Sen and Alikamanoglu, 2014; Asghar *et al.*, 2016) and antioxidant compounds (Ghanati *et al.*, 2007, Balakhnina *et al.*, 2015) and cause changes in plant growth rates (Bilalis *et al.*, 2013; Kataria *et al.*, 2017). Three mechanisms have been proposed for the effects of magnetic fields on living organisms: the mechanism of ferromagnetic particles orientation, the mechanism of ion cyclotron and the radicals-pair mechanism (Sen and Alikamanoglu, 2014).

Almond (family Rosaceae) is one of the most popular nuts on a worldwide basis and rank number one in tree nut production. The fruit is an edible kernel which is a commercial product. Moreover, almond has

also been reported to have medicinal values such as anticoagulant activity and promotion of blood circulation (Peng *et al.*, 2015). *Amygdalus scoparia* and *A. eburnea* are two important wild fruit trees in middle Asia. They are grown under variable environmental conditions and possess the tolerance of environmental stresses including drought, low temperatures, harsh winds and ultraviolet radiation (Vafadar *et al.*, 2010). In today's modern world, other stresses such as magnetic and electromagnetic stress have been added to these issues. One of the new problems we face is the installation of high voltage power cables in different roads and mountain areas. The waves emitted from these lines have unknown effects on living organisms (Ghanati *et al.*, 2007).

Nowadays, researches on various species of wild almonds mainly focus on the structure of population and ecology, but the effect of different stresses on this genus is less studied. In relation to the two species, there were no researches in this regard. The purpose of this study was to investigate the effect of static magnetic field on growth and photosynthesis characteristics and also to compare the responses of two almond species.

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Materials and methods

Plant material and magnetic field treatment:

Synchronized almond seeds (two species of *Amygdalus scoparia* and *A. eburnea*) were selected and then about 15 seeds were planted in each pot. Three pots for each treatment groups and three pots for control plants were determined. Seeds of almonds were cultivated in a mixture soil containing sand-clay and humus (2:1). Before planting, the soil was tested for its chemical features in terms of pH, electrical conductivity, and available micro and micronutrients as previously reported (Ghanati *et al.*, 2006). The plants were grown in greenhouse conditions for about 25 days and were irrigated daily with regular water. The greenhouse temperature was $24 \pm 2^\circ\text{C}$ and photoperiodic time was 11 hrs. The photosynthetic photon flux was $58 \mu\text{mol m}^{-2} \text{s}^{-1}$. The plants reached on the 25 st day and then were exposed to magnetic fields for 4 days and each day for 5 hours. The control plants were in the same conditions and far enough away from the magnetic field generator, and only under the influence of the natural magnetic field of the earth. The intensity of the field and the duration of the irradiation period were selected based on the results of previous studies and available resources. At the end of the planting period, the plants were harvested. Then, physiological and biochemical parameters were analyzed.

Magnetic Treatment: The static magnetic field is a kind of magnetic field that is produced by a magnetic field producer. The electrical power was the AC type and was supplied by utilizing a 220 V power source provided with the use of a dynamic transformer. The extreme power was 1 KW. The passing flow was 50 A DC. The arms of the iron core were connected to four circular iron parts covered with a layer of Nickel. To strengthen the system, an electronic board was used, so a uniform field was always accessible. It had two supercoils with 3,000 turns of 3 mm copper wire, which were equipped with several thin layers of iron that were put in the core. To avoid heating, a water flow apparatus around the coils was used. The control group were put in similar moisture, light and temperature conditions, and sufficiently far away from magnetic equipment. The selected magnetic power was 10 mT. The strength of applied SMF was selected because levels of magnetic field near a power transmission line were between 10 mT and 30 mT (Ghanati *et al.*, 2007).

Growth yield: To measure the length of the organs, the plants were randomly selected from each replication and the shoot length and root length were measured by the measuring tape. To measure the leaf area, leaf area meter was used.

For fresh weight, shoot and root of the plant were weighed after harvest. To measure dry weight, shoot and root were dried separately at 60°C for 48 hours and their weight was fixed. Leaf apertures were observed by optical microscopy and randomly taken from several leaf portions to measure the number of stomata, and ultimately the number of open stomata was expressed as

percentages.

The water content of the organs was calculated according to the following equation (Aghaleh *et al.*, 2011):

$$\text{WC [\%]} = [(\text{fresh weight} - \text{dry weight}) / \text{fresh weight}] \times 100.$$

Pigments content: For determination of chlorophyll (Chl) and carotenoid (Car) content, 0.5 g of fresh weight was dissolved in 10 ml of acetone 80% and the solution was filtered with Whatman filter paper. The final volume reached 15 ml and the absorbance of the solution was measured at 470, 646.8 and 663 nm using a spectrophotometer (Shimadzu UV-160, Tokyo, Japan). Finally, the Chl a, Chl b, total chlorophyll content (T Chl) and carotenoids were calculated using the formulas below and was reported in mg/g of fresh weight (Lichtenthaler and Wellburn, 1983):

$$\text{Chl a} = 12.25 A_{663,2} - 2.79 A_{646,8}$$

$$\text{Chl b} = 21.50 A_{646,8} - 5.10 A_{663,2}$$

$$\text{T Chl} = \text{Chl a} + \text{Chl b}$$

$$\text{Car} = (1000 A_{470} - 1.8 \text{ Chl a} - 85.02 \text{ Chl b}) / 198$$

Phenolic compounds content: For determination of phenolic content, dry plant materials (0.1 g) were homogenized in 20 ml of acidic methanol and centrifuged at 3000 g for 15 mins. 4 ml deionized H₂O, 0.5 ml sodium bicarbonate and 0.25 ml foline were added to 0.25 ml extract material. The folin reagent mixture was a mixture of phosphomolybdate and phosphotungstate used for the colorimetric *in vitro* assay of phenolic antioxidants. The absorbance was recorded at 760 nm. Finally, phenolic compounds content was expressed as mg per g of dry weight (Fadda *et al.*, 2014).

Data Analysis: All analyses were performed based on a completely randomized design. The data determined in the triplicate were analyzed by an analysis of variance (ANOVA) using SPSS (version 19). Each data point was the mean of three independent replicates (n=3). The significance of differences was determined according to Duncan's multiple range test (DMRT). P values < 0.05 were considered to be significant.

Results

Investigations on 25-day-old plant showed that the fresh weight of the shoot in both *A. scoparia* and *A. eburnea* species increased significantly under the influence of static magnetic field. Also, the dry weight of the shoot was measured in both *A. scoparia* and *A. eburnea*. The results showed that dry weight of shoot in both species did not change significantly. Under the influence of the SMF, the water content of the aerial parts increased in both *A. scoparia* and *A. eburnea* species significantly. The length of the shoot in the two species *A. scoparia* and *A. eburnea* increased under the influence of SMF and this increase was significant in both species (Table 1).

The effect of SMF on the underground organs of *A. scoparia* and *A. eburnea* species was also investigated. These studies showed that the fresh weight of the root in *A. scoparia* did not change. In *A. eburnea*, a slight

Table 1. Comparison of shoot length, shoot fresh weight, shoot dry weight and shoot water content in control group and SMF treated almonds (two species of *A. scoparia* and *A. eburnea*)

Treatments	Shoot length (cm)	Shoot fresh weight (g plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Shoot water content (%)
<i>A. scoparia</i> (control)	6.33±1.31 ^b	0.723±0.04 ^b	0.166±0.02 ^a	76.2%±2.1 ^c
<i>A. scoparia</i> (10 mT treated)	9.63±1.14 ^a	0.863±0.07 ^a	0.167±0.02 ^a	79.4%±1.4 ^b
<i>A. eburnea</i> (control)	9.25±0.65 ^b	0.368±0.03 ^d	0.059±0.02 ^b	83.1%±0.9 ^b
<i>A. eburnea</i> (10 mT treated)	11.37±0.68 ^a	0.490±0.05 ^c	0.061±0.01 ^b	85.2%±1.5 ^a

Table 2. Comparison of root length, root fresh weight, root dry weight and root water content in control group and SMF treated almonds (two species of *A. scoparia* and *A. eburnea*)

Treatments	Root length (cm)	Root fresh weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Root water content (%)
<i>A. scoparia</i> (control)	13.8±3.24 ^a	0.211±0.08 ^a	0.025±0.007 ^a	88.8%±2.11 ^b
<i>A. scoparia</i> (10 mT treated)	13.1±4.85 ^a	0.212±0.04 ^a	0.025±0.007 ^a	88.1%±3.48 ^b
<i>A. eburnea</i> (control)	12.23±2.72 ^a	0.196±0.06 ^a	0.025±0.007 ^a	87.1±0.62 ^b
<i>A. eburnea</i> (10 mT treated)	15.17±4.28 ^a	0.210±0.06 ^a	0.022±0.008 ^b	89.9%±1.1 ^a

Table 3. Comparison of leaves number, leaf area, open stomata, shoot length/root length ratio and shoot/root fresh weight in control group and SMF treated almonds (two species of *A. scoparia* and *A. eburnea*)

Treatments	Number of leaves (-)	Leaf area (mm ²)	open stomata (%)	Shoot length/Root length ratio (-)	Shoot/root fresh weight (-)
<i>A. scoparia</i> (control)	6±0 ^b	15.1±3.59 ^a	62.5±2 ^a	0.46±0.03 ^b	3.7±0.15 ^b
<i>A. scoparia</i> (10 mT treated)	6±0 ^b	17.1±6.43 ^a	44.4±1.5 ^c	0.74±0.04 ^a	4.3±0.12 ^a
<i>A. eburnea</i> (control)	8±2 ^a	10.2±4.97 ^a	60±1 ^a	0.76±0.03 ^a	1.8±0.15 ^d
<i>A. eburnea</i> (10 mT treated)	9±1 ^a	15.2±6.31 ^a	54.5±1.5 ^b	0.75±0.04 ^a	2.2±0.21 ^c

Table 4. Comparison of chlorophyll a, chlorophyll b, total chlorophyll, carotenoid and phenolic compounds content in control group and SMF treated almonds (two species of *A. scoparia* and *A. eburnea*)

Treatments	chlorophyll a	chlorophyll b	Total chlorophyll	Carotenoids	Total phenolic content
	(mg g ⁻¹ FW)				(mg g ⁻¹ DW)
<i>A. scoparia</i> (control)	7.75±0.14 ^a	7.75±0.99 ^a	15.50±1.24 ^a	2.19±0.66 ^a	304.26±35.25 ^a
<i>A. scoparia</i> (10 mT treated)	6.71±0.32 ^b	6.12±0.92 ^b	12.77±1.4 ^b	2.05±0.38 ^{ab}	290.78±14.33 ^a
<i>A. eburnea</i> (control)	7.54±0.09 ^a	8.15±0.52 ^{ab}	15.69±1.46 ^a	2.19±0.17 ^a	230.26±11.53 ^b
<i>A. eburnea</i> (10 mT treated)	6.66±0.16 ^b	8.71±1.79 ^a	15.22±3.49 ^a	1.93±0.18 ^b	252±10 ^a

increase was observed in the dry weight of the root, but this increase was not significant. The results of the study of the effect of SMF on the dry weight of the root showed that there was no change in *A. scoparia*, but in the species *A. eburnea*, the dry weight of the root decreased significantly. The water content of the root of the two species *A. scoparia* and *A. eburnea* increased under the influence of the static magnetic field, but this increase was not significant in *A. scoparia*. In addition, no significant change was observed in the root length of both species (Table 2).

As shown in Table 3, the number of leaves and leaf area size in both species were not significantly affected by the magnetic field. Although the leaf area size did not change significantly, but the percentage of leaf open stomatas in both species significantly decreased. Finally, the ratio of shoot to root weight in both species

increased compared to the control plants and this increase was significant. The ratio of stem length to root length increased significantly in the *A. scoparia*, but in *A. eburnea* did not change significantly.

The results of photosynthetic pigments measurement showed that in *A. scoparia*, Chl a, Chl b and T Chl content decreased significantly but there was no significant change in carotenoid content. The content of chl a and carotenoid was significantly decreased in *A. eburnea*, but Chl b and T Chl content did not change significantly. Also, the content of phenolic compounds in *A. scoparia* species did not change significantly, but increased in the *A. eburnea* (Table 4).

Discussion

Magnetic fields are one of the physical factors that can affect the function of many cells; however, their exact

mechanism is not yet known. The inhibitory or stimulatory effects of magnetic fields on the growth of plant tissues depend on several factors such as plant species, intensity and type of field, and the duration of the treatment (Maffei, 2014).

Increasing fresh weight and water content and no change in dry weight in two species of almonds indicates that SMF increased water absorption but did not change the plant dry biomass. Magnetic fields appear to affect water absorption by affecting the aquaporins. The studies are in agreement with previous results on *Cuminum cyminum* (Samani *et al.*, 2013) and *Cicer arietinum* (Mridha *et al.*, 2016). Increase in shoot length without any significant change in root length caused an increase in shoot length to root length ratio. The increase in plant height under the influence of the magnetic field has also been observed in *Impatiens balsamina*, *Brassica rapa* and *Lepidium sativum* (Kim *et al.*, 2016). The lack of change in the leaf area size along with the increase in the length of the shoot indicates that no change in plant growth has taken place, and only the height of the 25st-day plant has risen. This can be due to the effect of the field on specific receptors in the plant. Increasing the ratio of shoot to root weight can be due to the decrease of root growth relative to the shoot under the influence of the magnetic field.

Chlorophyll is a very important biomolecule that plays a critical role in photosynthesis. Due to chlorophylls, plants absorb solar energy. Chlorophyll plays an important role in growth and plant adaptation to different environmental conditions (Gossauer and Engel, 1996). Changes in the content of chlorophyll affect the photosynthesis efficiency in the leaves. The effect of magnetic fields in other plants has also been studied. These studies show that chlorophyll is very sensitive to SMFs. Although magnetic fields were conducive to the changes in the amount of photosynthetic pigments in soybean (Asgar *et al.*, 2016), beans (Najafi *et al.*, 2014) and lupin (Zdyrska *et al.*, 2016), the results of Racuciu *et al.* (2008) revealed that the intensity of magnetic fields was effective on the content of photosynthetic pigments. During low intensities, SMFs increase the content of pigments, but at high intensities it decreases (Racuciu *et al.*, 2008). These variations vary according to field strength, type of pigment and plant type (Abdollahi *et al.*, 2012). However, Bilalis *et al.* (2013) has reported that SMFs do not change stomatal conductance. In this study, it was observed that SMF decreased the amount of photosynthetic pigments. Reducing the plant pigments content can be due to reduced pigment production. Some researches have shown that under the influence of SMFs, the amount of some elements such as iron and zinc in the plant is reduced (Hajnorouzi *et al.*, 2011) and this can reduce the pigment biosynthesis. The

decomposition of pigments is another possible reason for reducing them, because the SMFs produce reactive oxygen species and can oxidize the pigments and disable them (Maffei, 2014, Kataria *et al.*, 2017). Magnetic waves appear to affect the content and arrangement of iron element in the cells and thus can affect the cyt b6/f complex in the photosystem II (Nickelsen and Rengstl, 2013), and thus affect photosynthesis. Reducing the number of open stomata in both species can be due to the reduction in the number of photosynthetic pigments and thus reduce the need for carbon dioxide of photosynthesis. Phenolic compounds play an important role in antioxidant activity in cells. These compounds reduce the amount of free radicals in cells by having OH groups (Sies, 2014). Increasing the content of phenolic compounds in *A. eburnea* is associated with no changes in chlorophyll content, but in *A. scoparia*, which does not significantly change the content of phenolic compounds, the content of photosynthetic pigments has decreased. Increasing phenolic compounds seems to increase the scavenging of free radicals in cells (Molassiotis *et al.*, 2006), thus reducing their destructive effects on photosynthetic pigments and other intracellular compounds in *A. eburnea*. Due to the reduction of photosynthetic pigments, it is likely that by increasing the duration of irradiation to more than four days, the amount of plant material also decreases. Reducing the amount of chlorophyll with increasing shoot length and increasing the ratio of shoot to root length indicates that the magnetic field in plants has a specific receptor that is similar to phytochromes because phytochrome is responsible for understanding the shadows in plants, and shade avoidance responses in plants are exactly the same (Rockwell *et al.*, 2006).

Conclusion

In our study it has been found that 10 mT magnetic fields for 4 days, 5 hours each day, increased the water content of the plant, but did not change the dry biomass of the plant. Magnetic fields increased shoot length, but did not change the leaf area. In addition, reducing the amount of photosynthetic pigments along with other data showed that SMFs did not increase the growth in almonds. In general, magnetic fields appear to stimulate receptors that act like a phytochromes. Of course, to achieve this result, numerous researches on different plants and in different stages of growth are necessary.

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