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Influence of Static Magnetic Field Seed Treatments on the Morphological and the Biochemical Changes in Lentil Seedlings (*Lens Culinaris* Medik.)

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Abstract

The static magnetic field has been shown to affect the growth and the biochemical composition of different plant species such as rice and wheat. In this study, lentil seeds were exposed to static magnetic field in a systematic way from low intensity to high intensity to test its effect on lentil growth. After that, the effective magnetic treatments that stimulated or inhibited the growth of lentil seedlings were tested for biochemical changes in lentil seedlings. Lentil seeds were first exposed to a static magnetic field of 1,10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mT for 5, 10, 15, 20, 25, and 30 min. Then, the morphological changes in lentil seedlings were assessed. Some of the effective magnetic treatments were analyzed for the changes in lipid peroxidation and the activity of some antioxidant enzymes in lentil seedlings. The results showed that the effect of magnetic treatment on seedling growth is divided into three groups: growth improvement, growth inhibition and normal growth. Moreover, the magnetic treatment (50 mT for 30 min) that inhibited seedling growth showed a high level of oxidative stress in terms of lipid peroxidation (42.6 µmole malondialdhyde (MDA)/ g fresh mass compared to 20.20 µmole/ g fresh mass of the control). Whereas, the magnetic treatment (20 mT for 20 min) that improved the seedling growth showed the lowest level of lipid peroxidation (5.29 µmole malondialdhyde (MDA)/ g fresh mass compared to 20.20 µmole/ g fresh mass of the control). The magnetic treatment 20 mT for 25 min enhanced lentil growth without a significant lipid peroxidation but showed a significant increase in the activity of catalase and superoxide dismutase 0.57 and 1.09 unit/ mg protein compared to 0.15 an 0.40 unit/ mg protein of the control, respectively. Hence, the priming of lentil seeds with the right magnetic treatment could be an efficient, affordable, and eco-friendly approach for the enhancement of lentil growth.

Keywords: seedling growth; oxidative stress; lipid peroxidation; antioxidant enzymes.

1. Introduction

All living systems, humans, plants, animals, and microorganisms live within the geomagnetic field (GMF). The GMF is considered as a low magnetic field that ranges from 35 to 70 µT (microTesla) (Occhipinti et al. 2014). The magnetic field is a physical factor that can be used to enhance seed germination and plant growth without harming the environment (Pietruszewski and Martínez 2015; Rifna et al. 2019). Recently, many researchers are interested in the study of the effect of the magnetic field on plants' growth and productivity (Maffei et al. 2014). Seed germination was improved after the magnetic treatment of maize (*Zea mays* L.) grains with 125 and 250 milli Tesla (mT) for 1, 10, 20 min and 1 h (Flórez et al. 2007). The pretreatment of okra (*Abelmoschus esculentus*) seeds with a magnetic field of 99 mT for 3 and 11 min also enhanced seed germination (Naz et al. 2012). The magnetic treatment of wheat (*Triticum aestivum* L.) grains and bean (*Phaseolus vulgaris* L.) seeds with 4 and 7 mT for 7 days resulted in the enhancement of seedling growth (Cakmak et al. 2010). Seed germination and seedling growth were significantly improved after seed exposure of melon (*Cucumis melo* L.) to a magnetic field of 100 and 200 mT for 5-20 min (Iqbal et al., 2016a). The same effect was shown after seed exposure of bitter gourd (*Momordica charantia*) to a magnetic field of 25, 50, and 75 mT for 15, 30 and 45 min (Iqbal et al. 2016b). The exposure of soybean (*Glycine max*) seeds to a magnetic field of 50, 75 and 100 mT for 3 and 5 min also resulted in a significant improvement of seed germination and seedling growth (Asghar et al. 2017). The treatment of maize grains by a magnetic field of 100 and 200 mT for 1 and 2 h showed improved chlorophyll content, leaf area, yield, and fresh and dry mass (Anand et al. 2012). Seedling growth of soybean was also enhanced after seed exposure to a magnetic field of 200 mT for 1 h (Baghel et al. 2018). Seed germination and plant growth of Faba Bean (*Vicia faba*) were significantly increased after the magnetic pretreatment of the seeds with a magnetic field of 30 and 85 mT for 15 s (Podleśna et al. 2019). The pretreatment of wheat seeds with an electromagnetic field of 10 and 15 mT for 10 and 15 min resulted in enhanced seed germination, plant growth and productivity (Hussain et al. 2020).

The biochemical responses of plants to the magnetic field were studied in different plant species. A reduction in oxygen radicals was shown in the magnetically treated soybean seeds with 100 and 200 mT for 1 h (Baby et al. 2011). The magnetically treated cucumber seedlings with

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200 mT magnetic field for 1 h showed an increase in superoxide radical and hydrogen peroxide (H_2O_2) (Bhardwaj et al. 2012). Lipid peroxidation and H_2O_2 was increased in shallot seedlings after their exposure to 7 mT magnetic field (Cakmak et al. 2012). A significant reduction in the activity of superoxide dismutase (SOD) in soybean seedlings after seed exposure to a magnetic field of 150 and 200 mT for 1 h was shown (Shine et al. 2012). The activity of the antioxidant enzymes: SOD, catalase (CAT), and guaiacol peroxidase was analyzed in 5-day-old radish seedlings exposed to a magnetic field of 185-650 μT (Serdyukov and Novitskii 2013). The activity of these enzymes was dependent on the intensity of the magnetic field; high intensity resulted in the activation of SOD and CAT. The growing of *Phaseolus vulgaris* L. seeds in a magnetic field of 130 mT for 14 days resulted in a significant increase in guaiacol peroxidase activity (GPOX) in the leaves (Mroczek-Zdyrska et al. 2016). The exposure of rice (*Oryza sativa*) grains to a magnetic field of 25 mT for 60 min resulted in an increased activity of CAT and peroxidase enzymes (Yadav et al. 2018).

Lentil (*Lens Culinaris* Medik.) is a member of family Fabaceae whose seeds are rich in protein. Indeed, lentil is an excellent protein source in poor and developing countries. The highest production of lentil worldwide is in Canada, and then comes India, Turkey, China, Nepal, and Syria (Andrews and McKenzie 2007). Lentil production is challenged by the changing environment and the consequent climate change. Therefore, it is of high importance to find ways to increase the production of this nutritious crop to meet the needs of the growing population, especially in the poor countries.

A few studies tested the effect of the magnetic field on lentil plants (Penuelas et al. 2004; Aladjadjiyan 2010). These studies showed enhancement of seedling growth after the magnetic treatment of lentil seeds. In these studies, only one or two intensities of the magnetic field (150 and 250 mT) were tested for a few selected exposure times (Penuelas et al. 2004; Aladjadjiyan 2010). Therefore, in this study the effect of different intensities of static magnetic field (1, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mT) for different exposure times (5, 10, 15, 20, 25, and 30 min) on the morphological changes in lentil seedlings will be tested. After that, the effective magnetic treatments that caused reduction or increase in lentil growth will be chosen to study the effect of magnetic treatment on some biochemical changes in lentil seedlings.

2. Materials and methods

The experiments of magnetic treatment were done in a biology research lab at Yarmouk University under controlled conditions of temperature, humidity, and light.

3. Plant material

Lentil seeds of genotype international legume lentil 10823 (ILL10823). Lentil seeds of this genotype were purchased from a local farmer of Irbid in the north of Jordan. The genotype of lentil seeds was determined by SSR analysis in the laboratory of Dr. Ayed Alabadallat, Department of Horticulture, Faculty Agriculture, Jordan University.

4. The magnet setup

The static magnetic field (H) was generated by an electromagnet that consists of identical Helmholtz coils (Fig. 1). The current was supplied to the coils by a power supply (HICKOK model number 5055, Hickok Electrical Instrument, Cleveland, OH). The intensity of the magnetic field was measured at several vertical and horizontal positions between the cabs, to find the place of a uniform magnetic field. The magnetic field was changed in two ways. The first way was by varying the current; at a fixed distance between the cabs. The second way was by adjusting the distance between the cabs in a symmetric way. The intensity of the magnetic field was measured in milli Tesla (mT) using magnetic meter model MG-3002 (Wenzhou, ZJ, China). The magnet setup and the magnetic treatments were under controlled laboratory conditions of humidity, temperature and with no interfering sources of radiations.

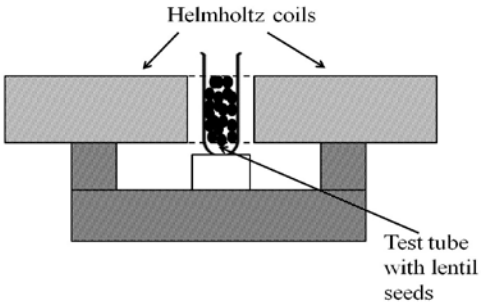

Figure 1. A schematic illustration of the electromagnet setup and the magnetization of lentil seeds.

5. Magnetic treatment of lentil seeds

For the magnetic treatment of lentil seeds, the magnetic field intensity of 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mT was tested. Each magnetic intensity was tested for an exposure time of 5, 10, 15, 20, and 30 min. For each magnetic treatment, three biological replications were treated at the same time. Each replication has 50 seeds in a glass tube. Magnetic treatment was done under controlled laboratory conditions at a temperature (22 ± 2ºC) and relative humidity of 60%.

6. Assessment of seedling growth after magnetic treatment

After magnetic treatment, lentil seeds were sown in a peatmoss: perlite mix (1:1) at 24°C, 70% humidity, 585 μmol m^{-2} s^{-1} light intensity and 16h light/16 h dark in a growth chamber. They pots were completely randomized in the growth chamber. After 7 days of growth in, 30 control lentil seedlings and 30 seedlings of magnetically treated seeds were sampled. Then, shoot length, root length, and seedling fresh mass were measured. After that, lentil plants were dried in the oven at 80 ºC for 1 day and then their dry mass was determined.

7. Analysis of biochemical changes in lentil seedlings under effective magnetic treatments

For the biochemical analyses, the magnetic treatments that resulted in an increase or decrease in the growth of

lentil seedlings (effective magnetic treatments) were selected (Table 1). The biochemical analyses in this study are the accumulation of malondialdhyde (MDA) and enzyme assay of some antioxidant enzymes: CAT (CAT 1.11.1.6), APX (APX 1.11.1.1), and SOD (SOD 1.15.1.1). For lipid peroxidation and antioxidant enzymes analyses, the shoots, and roots of 15 lentil seedlings were sampled separately. They were pooled into 3 biological replicates. All Plant samples were immediately frozen in liquid nitrogen.

Table. 1. Selected magnetic treatments for the biochemical analyses.

Magnetic treatment	Intensity of magnetic	Duration of
D1	20	20
D2	20	25
D3	40	30
D4	50	30
D5	70	5
D6	70	10
D7	70	25
D8	100	10
D9	100	25

8. Assessment of lipid peroxidation

Lipid peroxidation was determined by the quantification of MDA according to Heath and Packer (1968). Briefly, homogenization of samples in trichloroacetic acid (TCA) of 2.5 ml of 20% (w/v) was followed by centrifugation. The supernatant was mixed with thiobarbituric acid in TCA and boiled at 95ºC for 30 min. The reaction was stopped by cooling and centrifuged. Finally, the absorbance of the mixture was read at 532 nm and 600 nm. After subtracting the non-specific absorbance at 600 nm, the MDA concentration was determined by its extinction coefficient of 155 mM-1 cm-1.

9. Enzyme assay of CAT

The activity of CAT enzyme was determined according to Aebi (1984). In brief, the samples were homogenized in 1 ml extraction buffer Aebi 1984, and centrifuged. A volume of 20 µL of the shoot extract or 60 µL of the root extract was mixed with 50 mM potassium phosphate buffer (pH 7.0) and 15 mM H_2O_2. The reduction of H_2O_2 was then measured spectrophotometically at 240 nm for 2 min (UV-Visible Spectrophotometer-M51, Bell engineering S.R.L, Italy). The extinction coefficient of CAT is 43.6 $mM^{-1}cm^{-1}$.

10. Enzyme assay of APX

The activity of APX enzyme was determined according to Nakano and Asada (1981). The samples were homogenized in 1 ml extraction buffer (Nakano and Asada 1981) and then centrifuged. After that, the activity of APX was assayed by mixing 25 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate and 20 µL of the shoot extract or 60 µL of the root extract. The reaction was started by adding 1 mM H_2O_2 and APX activity was measured at 290 nm. The extinction coefficient of APX is 2.8 $mM^{-1}cm^{-1}$.

11. Enzyme assay of SOD

The activity of SOD enzyme was determined according to Beauchamp and Fridovich (1971). The samples were homogenized in 1 ml extraction buffer (Beauchamp and Fridovich 1971). The activity of SOD in the extract was assayed by mixing 50 mM potassium phosphate buffer (pH 7.8), 19.79 mM L-methonine, 0.025 % Triton X-100, 0.114 mM p- nitroblue tetrazolium (NBT) and 50 µL of the plant extract to a final volume of 1 ml. The reaction was started by adding 20 µL riboflavin solution (0.044 mg/ml). One unit of SOD activity is defined as the amount of enzyme that inhibits NBT reduction by half in comparison with the negative control (tubes lacking the enzyme).

12. Statistical analysis

The results of the morphological and biochemical changes in the control and the magnetically treated groups were statistically analyzed by One-way ANOVA and means were compared by Tukey's test at $p < 0.05$ using Minitab 17 (Minitab, LLC, USA). The results were expressed as the mean value ± standard deviation.

13. Results

The effect of the different magnetic treatments on the shoot and root length of lentil seedlings can be classified into 6 categories: a significant increase in both the shoot and root length, an increase in the shoot length without affecting the root length, no effect on the shoot length with an increase in the root length, a significant decrease in the shoot length with an increase in the root length, a significant increase in the shoot length with a decrease in the root length and a significant decrease in both the shoot and root length (Table 2). The highest increase in the shoot length was shown at 40 mT for 5 min (5.07 cm compared to 3.37 cm of the control). The highest root length was at 90 mT for 5 min (6.55 cm compared to 4.92 cm of the control).

Most of the magnetic treatments (1, 10, 20, 30, 40, 70, 90, and 100 mT) resulted in a significant decrease in the fresh mass of lentil seedlings for all or some of the exposure durations (Table 2). The magnetic field of 50 mT resulted in a highly significant increase in the fresh mass of lentil seedlings at an exposure time of 5, 15, 20, and 25 min (0.32, 0.39, 0.41, and 0.37 g compared to 0.20 g of the control, respectively). The magnetic field of 60 mT also resulted in a highly significant increase in the fresh mass of lentil seedlings at an exposure time of 5, 15, 20 and 30 min (0.30, 0.32, 0.33, and 0.27 g compared to 0.20 g of the control, respectively).

In general, the biomass (dry mass) of lentil seedlings was not significantly changed in response to most magnetic treatments (Table 2). The exposure of lentil seeds to 10 mT for 10, and 30 min showed a significant decrease in the biomass of lentil seedlings compared to the control seedlings (0.028 g compared to 0.034 g of the control). A significant decrease in the biomass was also shown in response to 50 mT for exposure time of 5, 20, 25, and 30 min (0.029, 0.028, 0.029, and 0.026 g compared to 0.034 g of the control, respectively).

Table 2. Morphological changes in lentil seedlings after seed exposure to different magnetic treatments.

Magnetic treatment					
Magnetic field intensity (mT)	Time of exposure (min)	Shoot length (cm)	Root length (cm)	Fresh mass (g)	Dry mass (g)
1	0	3.37 ± 1.11^{BC}	4.92 ± 2.10^{A}	0.20 ± 0.09^{A}	0.034 ± 0.010^{B}
	5	2.67 ± 0.79^{D}	2.60 ± 1.12^{C}	0.15 ± 0.03^{B}	0.034 ± 0.009^{AB}
	10	3.60 ± 1.40^{AB}	3.45 ± 1.76^{BC}	0.16 ± 0.03^{B}	0.032 ± 0.007^{B}
	15	2.75 ± 0.75^{CD}	2.73 ± 1.07^{BC}	0.15 ± 0.03^{B}	0.034 ± 0.008^{AB}
	20	3.33 ± 0.91^{ABCD}	3.83 ± 2.09^{BC}	0.17 ± 0.04^{AB}	0.035 ± 0.009^{AB}
	25	3.13± 1.29^{BCD}	3.05 ± 1.57^{BC}	0.16 ± 0.03^{B}	0.040 ± 0.012^{A}
	30	4.02 ± 1.32^{A}	4.13 ± 1.38^{AB}	0.16 ± 0.03^{B}	0.034 ± 0.010^{B}
10	0	3.37 ± 1.11^{A}	4.92± 2.10^{ABC}	0.20 ± 0.09^{A}	0.034 ± 0.009^{AB}
	5	3.20 ± 1.18^{A}	3.83 ± 1.48^{C}	0.16 ± 0.03^{B}	0.029 ± 0.01^{BC}
	10	3.40 ± 1.42^{A}	5.98 ± 2.13^{A}	0.17 ± 0.03^{AB}	0.028 ± 0.01^{C}
	15	3.63 ± 1.11^{A}	4.32 ± 1.47^{BC}	0.16 ± 0.04^{B}	0.031 ± 0.008^{ABC}
	20	3.55 ± 1.05^{A}	5.03 ± 1.74^{ABC}	0.17± 0.04^{AB}	0.038 ± 0.021^{A}
	25	3.84 ± 1.30^{A}	5.02 ± 2.04^{ABC}	0.18 ± 0.03^{AB}	0.031± 0.007^{ABC}
	30	3.92 ± 1.02^{A}	5.42 ± 2.05^{AB}	0.18 ± 0.05^{AB}	0.028 ± 0.007^{C}
20	0	3.37 ± 1.11^{C}	4.92 ± 2.10^{B}	0.20 ± 0.09^{A}	0.034 ± 0.009^{A}
	5	3.77 ± 1.15^{BC}	4.78 ± 1.36^{AB}	0.15 ± 0.03^{B}	0.035 ± 0.009^{A}
	10	4.18 ± 1.12^{B}	4.75 ± 1.65^{AB}	0.17 ± 0.04^{AB}	0.034 ± 0.006^{A}
	15	3.88 ± 0.10^{BC}	5.23 ± 1.69^{AB}	0.18 ± 0.04^{AB}	0.035 ± 0.009^{A}
	20	3.66 ± 1.23^{BC}	6.21 ± 1.64^{A}	0.17 ± 0.03	0.033 ± 0.008^{A}
	25	5.17 ± 1.24^{A}	5.09 ± 1.40^{AB}	0.16 ± 0.03^{B}	0.031 ± 0.010^{A}
	30	3.47 ± 0.94^{BC}	4.93 ± 1.71^{AB}	0.17 ± 0.03^{AB}	0.034 ± 0.009^{A}
30	0	3.37 ± 1.11^{D}	4.92 ± 2.10^{A}	0.20 ± 0.09^{A}	0.034 ± 0.009^{A}
	5	4.80 ± 1.42^{A}	3.22 ± 1.58^{BC}	0.16 ± 0.03^{B}	0.032 ± 0.010^{A}
	10	4.03 ± 0.93^{ABC}	3.05 ± 1.04^{BC}	0.15 ± 0.03^{B}	0.032 ± 0.006^{A}
	15	4.47 ± 1.49^{AB}	4.00 ± 1.49^{AB}	0.16 ± 0.03^{B}	0.032 ± 0.005^{A}
	25	3.30 ± 0.85^{CD}	2.58 ± 1.16^{C}	0.14 ± 0.03^{B}	0.030 ± 0.006^{A}
	30	4.38 ± 0.93^{AB}	2.32 ± 0.68^{C}	0.17 ± 0.03^{AB}	0.036 ± 0.007^{A}
40	0	3.37 ± 1.11^{C}	4.92 ± 2.10^{A}	0.20 ± 0.09^{A}	0.034 ± 0.009^{A}
	5	5.07 ± 1.30^{A}	3.87 ± 1.62^{ABC}	0.16 ± 0.03^{B}	0.032 ± 0.010^{A}
	10	3.63 ± 1.03^{BC}	4.87 ± 1.77^{AB}	0.15 ± 0.03^{B}	0.032 ± 0.006^{A}
	15	4.08 ± 1.57^{B}	2.57 ± 1.36^{CD}	0.15 ± 0.03^{B}	0.034 ± 0.009^{A}
	20	3.27 ± 1.28^{BC}	3.55 ± 1.51^{BCD}	0.16 ± 0.03^{B}	0.031 ± 0.007^{A}
	25	3.42 ± 1.01^{BC}	4.06 ± 1.45^{AB}	0.15 ± 0.03^{B}	0.031 ± 0.005^{A}
	30	2.20 ± 0.54^{D}	2.23 ± 0.72^{D}	0.12 ± 0.02^{B}	0.031 ± 0.007^{A}
50	0	3.37 ± 1.11^{A}	4.92 ± 2.10^{A}	0.20± 0.09^{C}	0.034 ± 0.009^{A}
	5	3.33 ± 0.93^{AB}	4.98 ± 1.88^{A}	0.32 ± 0.11^{B}	0.029 ± 0.007^{BC}
	10	2.97 ± 1.27^{AB}	6.15 ± 5.78^{A}	0.15 ± 0.03^{D}	0.033 ± 0.006^{AB}
	15	2.67 ± 0.86^{B}	4.28 ± 1.99^{A}	0.39 ± 0.07^{A}	0.032 ± 0.008^{ABC}
	20	3.15 ± 0.89^{AB}	5.95 ± 1.81^{A}	0.41 ± 0.07^{A}	0.028 ± 0.007^{BC}
	25	3.40 ± 0.82^{AB}	5.17 ± 2.00^{A}	0.37 ± 0.02^{AB}	0.029 ± 0.006^{BC}
	30	3.40 ± 1.73^{AB}	5.80 ± 1.60^{A}	0.18 ± 0.03^{CD}	0.026 ± 0.006^{C}
60	0	3.37 ± 1.11^{B}	4.92 ± 2.10^{A}	0.20 ± 0.09^{B}	0.034 ± 0.009^{A}
	5	4.27 ± 1.70^{A}	4.68 ± 1.82^{A}	0.30 ± 0.11^{A}	0.030 ± 0.006^{AB}
	10	3.38 ± 1.12^{B}	5.30 ± 1.73^{A}	0.16 ± 0.03^{B}	0.032 ± 0.006^{AB}
	15	3.40 ± 0.91^{AB}	4.63 ± 2.44^{A}	0.32 ± 0.11^{A}	0.031 ± 0.007^{AB}
	20	2.82 ± 1.19^{B}	4.10 ± 1.47^{A}	0.33 ± 0.12^{A}	0.029 ± 0.006^{B}
	25	3.58 ± 1.18^{AB}	5.25 ± 2.00^{A}	0.17 ± 0.07^{B}	0.032 ± 0.007^{AB}
	30	3.65 ± 1.08^{AB}	5.17 ± 1.76^{A}	0.27 ± 0.09^{A}	0.031 ± 0.007^{AB}
70	0	3.37 ± 1.11^{A}	4.92 ± 2.10^{A}	0.20 ± 0.09^{A}	0.034 ± 0.009^{A}
	5	2.50 ± 0.92^{B}	3.30 ± 1.95^{A}	0.14 ± 0.03^{B}	0.034 ± 0.010^{A}
	10	2.58 ± 1.10^{B}	4.68 ± 1.78^{A}	0.15 ± 0.04^{B}	0.037 ± 0.012^{A}
	15	2.60 ± 1.24^{B}	3.60 ± 1.68^{A}	0.15 ± 0.04^{B}	0.033 ± 0.010^{A}
	20	2.18 ± 1.05^{B}	5.25 ± 1.95^{A}	0.13 ± 0.03^{B}	0.030 ± 0.006^{A}
	25	2.17 ± 0.78^{B}	4.90 ± 1.95^{A}	0.14 ± 0.03^{B}	0.032 ± 0.006^{A}
	30	2.28 ± 0.63^{B}	5.08 ± 2.06^{A}	0.14 ± 0.03^{B}	0.034 ± 0.008^{A}
90	0	3.37 ± 1.11^{A}	4.92 ± 2.10^{B}	0.20 ± 0.09^{A}	0.034 ± 0.009^{A}
	5	2.58 ± 0.86^{B}	6.55 ± 2.15^{A}	0.17 ± 0.04^{AB}	0.033 ± 0.007^{A}
	10	2.62 ± 0.87^{B}	5.38 ± 2.25^{AB}	0.16 ± 0.03^{B}	0.032 ± 0.006^{A}
	15	2.10 ± 0.96^{B}	4.40 ± 2.18^{B}	0.16 ± 0.04^{B}	0.032 ± 0.007^{A}
	20	2.65 ± 1.52^{B}	6.07 ± 2.56^{AB}	0.17 ± 0.04^{AB}	0.035 ± 0.008^{A}
	25	2.17 ± 0.76^{B}	4.55 ± 2.52^{B}	0.16 ± 0.04^{B}	0.032 ± 0.007^{A}

	30	2.92 ± 1.49^{AB}	5.67 ± 2.86^{AB}	0.17 ± 0.04^{AB}	0.030 ± 0.005^{A}
100	0	3.37 ± 1.11^{A}	4.92 ± 2.10^{A}	0.20 ± 0.09^{A}	0.034 ± 0.009^{AB}
	5	2.63 ± 1.19^{B}	4.63 ± 1.65^{A}	0.16 ± 0.04^{AB}	0.037 ± 0.007^{AB}
	10	2.40 ± 1.21^{B}	4.03 ± 2.14^{A}	0.15 ± 0.04^{B}	0.031 ± 0.006^{BC}
	15	2.18 ± 0.73^{B}	4.40 ± 1.73^{A}	0.16 ± 0.04^{AB}	0.038 ± 0.011^{A}
	20	2.22 ± 0.78^{B}	4.43 ± 2.15^{A}	0.17 ± 0.03^{AB}	0.036 ± 0.006^{AB}
	25	1.85 ± 0.98^{B}	4.63 ± 2.03^{A}	0.16 ± 0.04^{AB}	0.029 ± 0.004^{C}
	30	2.27 ± 0.77^{B}	5.45 ± 2.20^{A}	0.16 ± 0.04^{AB}	0.032 ± 0.006^{ABC}

Different letters indicate statistically significant values following Tukey's test at $p < 0.05$. Each value represents the mean of thirty (n = 30) replicates ± standard deviation.

MDA in the shoot of lentil seedlings of the magnetically treated seeds was not significantly changed in most of the magnetic treatments (Table 3). MDA was significantly decreased in the root of lentil seedlings of the magnetically treated seeds with 20 mT for 20 min (D1) (5.29 µmole/ g fresh mass) compared to 23.93 µmole/ g fresh mass of the control. The root of lentil seedlings of the magnetically treated seeds with 50 mT for 30 min (D4) also showed a significant decrease in MDA concentration (10.44 µmole/ g fresh mass) compared to 23.93 µmole/ g fresh mass of the control. The magnetic treatment 50 mT for 30 min (D4) also resulted in a significant increase in MDA in the shoot of lentil seedlings (42.6 µmole/ g fresh mass) compared to 20.20 µmole/ g fresh mass of the control (Table 3).

The activity of CAT did not change significantly in the shoot of lentil seedlings of the magnetically treated seeds compared to the control seedlings (Table 3). The root of lentil seedlings of the magnetically treated seeds with 20 mT for 25 min (D2) showed significantly high CAT activity (0.57 unit/ mg protein) compared to 0.15 unit/ mg protein of the control (Table 3). The magnetic treatment 100 mT for 25 min (D9) also resulted in a significant increase in CAT activity in the root of lentil seedlings (0.30 unit/ mg protein) compared to 0.15 unit/ mg protein of the control.

The activity of APX activity was not significantly changed in the shoot of lentil seedlings of the magnetically treated seeds (Table 3). The magnetic treatment of lentil seeds with 70 mT for 5, 10, and 15 min (D5, D6, and D7) significantly increased APX activity in the root (9.18, 8.77, and 6.69 unit/ mg protein) compared to 2.46 unit/ mg protein of the control.

The activity of SOD was not significantly changed in the shoot of lentil seedlings from magnetically treated seeds (Table 3). The activity of SOD was significantly high in the root of lentil seedlings of the magnetic treatments D2 (20 mT for 25 min) and D3 (40 mT for 30 min) 1.09 and 1.00 unit/ mg protein compared to 0.40 unit/ mg protein of the control.

Table 3. Biochemical changes in lentil seedlings after seed exposure to effective magnetic treatments.

Magnetic treatment	**MDA (µmole/ g fresh mass)**		**CAT (unit/ mg protein)**		**APX (unit/ mg protein)**		**SOD (unit/ mg protein)**	
(Magnetic field (mT), time (min))	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
Control	20.20 ± 4.35^{B}	23.93 ± 5.10^{ABC}	0.16 ± 0.05^{AB}	0.15 ± 0.009^{C}	3.20 ± 0.43^{AB}	2.46 ± 1.13^{D}	0.32 ± 0.10^{ABC}	0.40 ± 0.17^{C}
D1 (20, 20)	20.70 ± 5.54^{B}	5.29 ± 0.27^{E}	0.20 ± 0.04^{AB}	0.08 ± 0.010^{C}	6.13 ± 0.79^{AB}	3.25 ± 1.39^{CD}	0.48 ± 0.17^{A}	0.27 ± 0.10^{C}
D2 (20, 25)	19.80 ± 2.95^{B}	23.50 ± 3.44^{ABC}	0.16 ± 0.01^{AB}	0.57 ± 0.050^{A}	3.56 ± 1.54^{AB}	4.05 ± 2.62^{CD}	0.46 ± 0.12^{A}	1.09 ± 0.33^{A}
D3 (40, 30)	23.37 ± 4.55^{AB}	31.57 ± 1.58^{A}	0.11 ± 0.04^{B}	0.16 ± 0.050^{C}	2.05 ± 1.46^{B}	5.47 ± 1.57^{ABCD}	0.39± 0.13^{ABC}	1.00 ± 0.37^{AB}
D4 (50, 30)	42.6 ± 20.8^{A}	10.44 ± 0.32^{DE}	0.33 ± 0.15^{A}	0.13 ± 0.05^{C}	2.13 ± 0.11^{A}	1.85^{D} ± 0.95	0.12 ± 0.09^{C}	0.21 ± 0.13^{C}
D5 (70, 5)	19.52 ± 1.68^{B}	17.00± 4.00^{CD}	0.08 ± 0.01^{B}	0.14 ± 0.006^{C}	5.39 ± 3.08^{AB}	9.18 ± 0.93^{A}	0.16 ± 0.04^{BC}	0.21 ± 0.02^{C}
D6 (70, 10)	20.20 ± 4.35^{B}	23.93 ± 5.10^{ABC}	0.11 ± 0.01^{B}	0.14 ± 0.03^{C}	8.11 ± 2.81^{A}	8.77 ± 2.54^{AB}	0.20± 0.04^{ABC}	0.25 ± 0.09^{C}
D7 (70, 25)	17.05 ± 3.52^{B}	19.56 ± 5.18^{BCD}	0.17 ± 0.02^{AB}	0.15 ± 0.010^{C}	7.24 ± 2.79^{AB}	6.69± 1.26^{ABC}	0.26± 0.02^{ABC}	0.47 ± 0.17^{BC}
D8 (100, 10)	10.57 ± 2.62^{B}	13.62 ± 4.79^{CDE}	0.18 ± .03^{AB}	0.09 ± 0.015^{C}	6.69 ± 3.82^{AB}	4.40 ± 0.37^{CD}	0.35± 0.05^{ABC}	0.48 ± 0.27^{BC}
D9 (100, 25)	21.83 ± 4.60^{AB}	30.11 ± 2.47^{AB}	0.28 ± 0.13^{AB}	0.30 ± 0.06^{B}	4.37 ± 2.92^{AB}	5.47 ± 1.77^{BCD}	0.41 ± 0.12^{AB}	0.39 ± 0.09^{C}

Different letters indicate statistically significant values following Tukey's test at $p < 0.05$. Each value represents the mean of three (n = 3) biological replicates ± standard deviation.

14. Discussion

In the present study, lentil seeds were exposed to different magnetic treatments and the seedling growth was assessed in terms of shoot and root length, seedling fresh and dry mass. The results revealed growth stimulatory, inhibitory, and neutral magnetic treatments. This is consistent with the results of the previous studies, which showed the enhancement and the inhibition of the growth of different plant species such as wheat, barley, maize, chickpea, sunflower, and lentil after seed exposure to a static magnetic field (Pittman 1977; Martinez et al. 2000 ; Flórez et al.,2004; Flórez et al. 2007; Vashisth and Nagarajan 2008; Martínez et al. 2009; Aladjadjiyan 2010; Vashisth and Nagarajan 2010; Asgharipour and Omrani 2011; Carbonell et al. 2011; Harb and Abu Aljarayesh 2013). Overall, there is an effective magnetic treatment in terms of the magnetic field intensity and the duration of exposure that results in the maximum enhancement of plant growth. Many factors determine this effective magnetic treatment. Plant species and its genotype, seed quality and its physical and physiological characters are major factors. In addition, the environmental conditions at

the time of magnetic treatment and even the time of the year are very crucial determinants of the magnetic effect (García Reina et al. 2001). The exact mechanism of growth changes in response to static magnetic field is not clear, but some biochemical changes such as the ROS and their scavengers might play an important role in plants' responses to magnetic treatment.

In general, most of the magnetic treatments in this study did not result in lipid peroxidation and the accumulation of MDA. But, one growth stimulatory magnetic treatment showed the lowest concentration of MDA in the root of lentil seedlings, and one inhibitory magnetic treatment has the highest concentration of MDA in the shoot of lentil seedlings. The effect of the static magnetic field on the accumulation of reactive oxygen species (ROS) and the oxidative stress were studied in a few plant species such as radish, soybean, and cucumber. The accumulation of ROS results in lipid peroxidation. Lipid peroxidation is mainly caused by Hydroxyl radical (OH•), which is produced from H_2O_2 by Fenton reaction (Gill and Tuteja 2010). Lipid peroxidation and the accumulation of MDA are indicative of oxidative stress, which at high concentrations has harmful effects on plant growth. Indeed, lipid peroxidation and the accumulation of MDA had negative impact on plants' resistance to different stresses such as drought and heat, due to the reduced ability of scavenging the accumulated ROS under stress (Liu and Huang 2000; Liang et al. 2003; Song et al. 2016). Therefore, the improvement of the seedling growth of lentil shown in this study for some magnetic treatments could be explained by the alleviation of the accumulation of ROS and the consequent oxidative stress. Indeed, reduced lipid peroxidation in the magnetically treated radish seedlings was a positive factor that enhanced the seedlings' growth (Novitskii et al. 2015). Moreover, the significant decrease in superoxide radical in soybean plants after seed exposure to 100 and 200 mT magnetic field for 1 h was suggested as an explanation for the enhanced plant growth (Baby et al. 2011). In contrast to this, the treatment of cucumber seedlings with 200 mT magnetic field for 1 h showed a significant increase in superoxide radical and H_2O_2, which was suggested as the cause of growth stimulation of cucumber seedlings (Bhardwaj et al. 2012). The discrepancy of the results could be explained by the different plant species that were tested, the method and the conditions of application of the magnetic field, the time of the day and the time of the year during which the magnetic treatment was applied. In addition, many other internal and external factors could play a role in the plant's response to magnetic treatment.

The activity of the antioxidant enzymes: APX, CAT and SOD was not changed in the shoot of lentil seedlings in response to magnetic treatments. But significant differences in the activity of these enzymes were shown in the root of lentil seedlings under many magnetic treatments. The activity of CAT was significantly increased in the root of lentil seedlings under one stimulatory and one inhibitory growth magnetic treatments. The activity of APX was significantly increased in the root in lentil seedlings under some growth inhibitory magnetic treatments. Yet, other stimulatory and inhibitory magnetic treatments showed no change in APX activity. APX is required for the fine scavenging of H_2O_2 under conditions of oxidative stress, whereas CAT is required for the bulk scavenging of H_2O_2 under the same conditions (Gill and Tuteja 2010). The changes in the activity of antioxidant enzymes under stress conditions are controversial (Abogadallah 2010). The high activity of antioxidant enzymes in the stress tolerant genotypes is a positive indicator of stress tolerance, whereas their high activity in the stress sensitive genotypes is an indicator of a significant oxidative stress. Therefore, in the present study, the high activity of CAT under the inhibitory magnetic treatments could be an indicator of elevated oxidative stress, whereas its high activity under the stimulatory magnetic treatments could be an indicator of alleviated oxidative stress. Our results showed high activity of SOD in the root of lentil seedlings under two magnetic treatments. The previous studies showed discrepancy in the effect of magnetic treatment on the activity of SOD. The activity of SOD was increased in the magnetically treated suspension-cultured tobacco cells (Sahebjamei et al. 2007), whereas it was reduced SOD in the magnetically treated maize and soybean plants (Shine and Guruprasad 2012; Asghar et al. 2017). Hence, changes in the activity of SOD after seed exposure to a magnetic field are dependent on the plant species and the intensity and the duration of magnetic treatment.

15. Conclusion

The results of this study showed three effects of the magnetic treatments on the growth of lentil seedlings: stimulatory, inhibitory, and neutral. In general, most of the effective magnetic treatments did not cause significant changes in the accumulation of MDA and the activity of antioxidant enzymes in the shoot of lentil seedlings. Most of the biochemical changes were shown in the root of lentil seedlings under magnetic treatments. One growth stimulatory magnetic treatment (20 mT for 20 min (D1)) significantly decreased the concentration of MDA and showed normal activity of antioxidant enzymes in the root of lentil seedlings. The pre-sowing treatment of lentil seeds with the effective magnetic treatment in terms of the magnetic field intensity and the duration of exposure could be an affordable and eco-friendly method for the improvement of lentil growth. Further detailed dissection of the interaction between the magnetic treatment and the growth of lentil seedlings at the molecular, biochemical, and physiological level is recommended.

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