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The effects of the magnetic field on germination and seedling growth of chickpea (*Cicer arietinum* L.) and sunflower (*Helianthus annuus* L.)

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Manyetik alanın nohut (*Cicer arietinum* L.) ve ayçiçeği (*Helianthus annuus* L.) çimlenmesi ve fide büyümesine etkileri

Abstract: Organisms interact with their environment and effects of environmental factors vary depending on ecology and tolerance levels. However magnetic field is an inevitable factor for all organisms. The aim of the study was to investigate the effects of different magnetic field (MF) applications on germination percentage, pigment content and antioxidant capacity of two important agricultural plant (Sunflower and Chickpea) species. Initially, seeds were exposed to 5 mT, 10 mT and 20 mT magnetic field generated by Helmholtz coil for detection of germination effects. Then seedling test was survived at the same conditions. MF was applied 20 minutes for every day at the same time period. According to germination results, MF application to sunflower and chickpea seeds was resulted with increase in germination percentage compared to control. 20 mT application caused decrease in shoot length of sunflower seedlings. On the contrary, 20 mT MF application resulted with increase in shoot length of chickpea seedlings. All magnetic field strengths increased carotenoid levels in chickpea seedlings. Also, MF application affected the phenolic and flavonoid contents of sunflower and chickpea seedlings. Depending on the increase in secondary metabolites, DPPH and FRAP activities varied. As a conclusion, MF application contributed to effect on plant metabolism and it has the potential to be used in agricultural applications.

Key words: Magnetic field, Helmholtz coil, germination, magnetic field response

Özet: Organizmalar çevreleriyle etkileşim halindedir ve çevresel faktörlerin etkileri ekoloji ve tolerans düzeylerine bağlı olarak değişmektedir. Ancak manyetik alan tüm organizmalar için kaçınılmaz bir faktördür. Çalışmanın amacı, farklı manyetik alan (MF) uygulamalarının iki önemli tarım bitkisi (Ayçiçeği ve Nohut) türünün çimlenme yüzdesi, pigment içeriği ve antioksidan kapasitesi üzerine etkilerinin araştırılmasıdır. Çimlenme etkilerinin tespiti için ilk olarak tohumlar Helmholtz bobini tarafından oluşturulan 5 mT, 10 mT ve 20 mT manyetik alana maruz bırakılmıştır. Daha sonra fide testi için de aynı şartlarda hazırlanmıştır. MF her gün aynı saat diliminde 20 dakika uygulanmıştır. Çimlenme sonuçlarına göre ayçiçeği ve nohut tohumlarına MF uygulamasının kontrole göre çimlenme yüzdesinde artışa neden olduğu görülmüştür. 20 mT uygulaması ayçiçeği fidelerinde sürgün boyunda azalmaya neden olmuştur. Buna karşılık 20 mT MF uygulaması nohut fidelerinde sürgün uzunluğunun artmasına neden olmuştur. Tüm manyetik alan kuvvetleri nohut fidelerinde karotenoid düzeylerini arttırmıştır. Ayrıca uygulama ayçiçeği ve nohut fidelerinin fenolik ve flavonoid içeriklerini de etkilemiştir. Sekonder metabolitlerdeki artışa bağlı olarak DPPH ve FRAP aktiviteleri farklılık göstermiştir. Sonuç olarak MF uygulamasının bitki metabolizması üzerine etkisi olduğu ve tarımsal uygulamalarda kullanılma potansiyeline sahip olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Manyetik alan, Helmholtz bobini, çimlenme, manyetik alan tepkisi

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1. Introduction

The nutritional needs in the World are increasing due to the continuous increase in the population. For this reason, natural and alternative methods or applications are being researched to increase food quality and yield. Static magnetic field, one of the methods used in recent years, is used to improve plant development (Pentoś et al., 2022).

Paleogeographic records revelated that the geomagnetic field has been effective on Earth for at least 4.2 billion years (Tarduno et al., 2015). Plant genome, exposure time, energy level and target distance to energy source affect the biological effects of MF (Baghel et al., 2018). Plants are not completely resistant to all kinds of environmental changes due to their physiological, molecular and genetic structures, and they must modify depending on their genetic potential under changing conditions. Recently, some researchers have focused on the use of MF to improve agronomical characters (Shabrangy et al., 2021), and also MF affects phytochemical content of plants (Nasiri et al., 2022). However, our knowledge is limited about how MF effect on metabolism.

An important question is the MF has any distinctive effects on biological systems. In literature studies, it has been stated that MF affects various plant functions, such as growth, development, protein biosynthesis and enzyme activity. In addition, MF has positive effects on plant characteristics; such as shoot development, seed germination, fresh weight and plant height, fruit yield per plant and average fruit weight, increasing photosynthetic pigment content; and intensifying cell division, as well as water and nutrient uptake (De Souza et al., 2006; Sarraf et

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al., 2020; Tirono et al., 2021). On the contrary, in some cases, application of magnetic field caused a decrease in germination parameters. It has been found that weak electromagnetic fields suppress the growth of plants and reduce cell division (Belyavskaya, 2004; Kornarzyński et al., 2020). MF can change the antioxidant enzyme activities (Sahebjamei et al., 2007; Alikamanoğlu and Sen, 2011). Exposure of seeds with MF caused the increase in amylolytic enzymes activity in seeds and seedlings of pea (Podleśny et al., 2021).

MF has been shown to significantly improve plant development in various species, including sunflower (*Helianthus annuus* L.) (Vashisth and Nagarajan, 2010), rice (*Oryza sativa* L.) (Fl'orez et al., 2007), and chickpea (*Cicer arietinum* L.) (Vashisth and Nagarajan, 2008). Improving germination and growth rates is crucial for sustainable horticulture, particularly in the Mediterranean region.

The aim of this study was to investigate the how different static magnetic intensities (0 mT, 5 mT, 10 mT and 20 mT) affect to germination rate, shoot and root length, pigment content and antioxidant capacity of two important commercial plants chickpea (*Cicer arietinum*) and sunflower (*Helianthus annuus*). Also i) to create a constant and controllable magnetic effect, unlike the variable intensity in magnetic field studies with different magnets, ii) to reveal the state of the magnetic field in different vegetables and to determine their comparative responses. iii) to determine their effects on germination and development physiology and to determine their agricultural application potential.

2. Materials and Method

2.1. Plant materials and growth conditions

Helianthus annuus L. (TD0005 AA) and *Cicer arietinum* L. (3660 OC) seeds were used for MF applications. Uniform and undamaged seeds were selected and sterilized with 3 % sodium hypochlorite for 10 min. Seeds were sowed in sterile glass jars (8 x 5 x 5.5 cm) with bi-layered sterile filter paper on the bottom of each sterile glass jar. Filter papers were watered with 5 mL of Hoagland's solution. 10 seeds were placed into each jar with five replicate. Jars were incubated in controlled plant growth room (25±1 °C, 16/8 photoperiod, 2500 lux light intensity and 70% humidity).

2.2. Magnetic field treatment for germination and seedling growth

Seeds were exposed to different MF intensities (0 mT, 5 mT, 10 mT and 20 mT) for 20 minutes after planting for every day. Power supply (DC: direct current) and Helmholtz Coil were used for generation of magnetic field of 5 mT (22.4 V), 10 mT (34 V) and 20 mT (41.8 V). Generated MF was measured regularly by a Teslameter during the exposure period (Fig. 1). At the end of the 5th day of application, germination percentage was calculated for each group.

After five days, seeds were considered as germinated and calculations (germination percentage) were made, for seedling applications seeds were exposed to same strength and duration magnetic field for ten days. At the end of the period, samples were harvested by liquid nitrogen and stored in ultra-freezer (-80 °C) for further analysis.

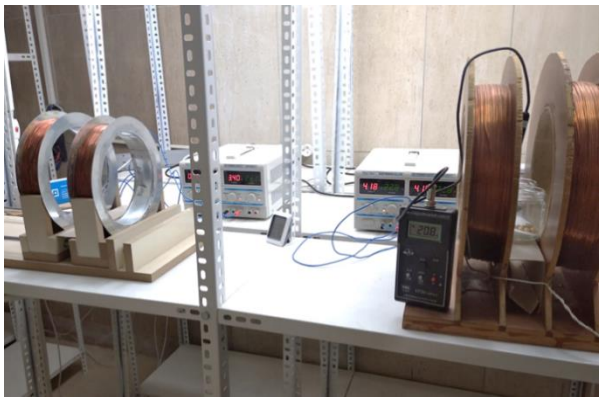

Figure1. Magnetic field apparatus and application methodology

2.3. Physiological parameters

Fresh weight, dry weight, turgid weight, length of soot and root tissues were measured at the end of the 10th day. The relative water content of the seedlings was calculated according to equation based on the three measured values (dry, fresh and turgor weight) (Hu et al., 2010).

Relative Water Content (%) = [(Fresh Weight-Dry Weight)/ (Turgid Weight-Dry Weight)] x 100

2.4. Pigment content

Chlorophyll a, b and carotenoids were determined by using spectrophotometer. For chlorophyll content, leaf sample (0.1 g) was homogenized by using 10 ml (80 %) acetone. Chlorophyll and carotenoid contents were assessed by determining absorbance at 480, 645 and 663 nm (Arnon, 1949). The amounts of photosynthetic pigments were calculated according to the equations below and expressed in mg/g.

Chlorophyll-a = [ΔA663 x 12.70 - Δ A645 x 2.69] [(V/1000)*W]

Chlorophyll-b = [ΔA645 x 22.90 - Δ A663 x 4.68] [(V/1000)*W]

Total chlorophyll = [ΔA645 x 20.2 + Δ A663 x 8.02] [V/1000)*W]

Carotenoids = [ΔA480 + Δ A663 x 0.114 - Δ A645 x 0.638/112.50] [(V/1000)*W]

{ΔA: The absorbance value at the specified wavelengths, V: the final extraction volume in ml, W: the amount of plant taken for analysis in g}.

2.5. Total Phenolic and Total Flavonoid Content Analysis

Folin-Ciocalteu assay was performed to determine the total phenolic content (TPC) at 600 nm. TPC of samples was expressed as mg of gallic acid equivalent per gram dry weight (mg GAE/g DW) (Dalar and Konczak, 2013).

The total flavonoid content (TFC) assay was performed at 510 nm. Results of TFC were presented as mg of rutin equivalent per gram of dry weight (mg RE/g DW) (Dalar and Konczak, 2013).

2.6. Antioxidant activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) activity was performed according to Konczak-Islam et al. (2003). 75 µL of the sample solution used for extraction and 75 µL of 0.4

mM DPPH solution were mixed and left at room temperature for 2 min. The decrease in absorbance of the prepared mixture at 517 nm was measured using a microplate reader. Measurements were made three times. DPPH inhibitions were calculated using the following equation (Moraes-De-Souza et al., 2008).

DPPH activity (%) = [(absorbance control − absorbance of samples) / absorbance of control] × 100

Fluorescence recovery after photobleaching (FRAP) activity was performed according to Sudha et al. (2012). 10 µL of the prepared plant extracts were placed in a 96-well microplate and 3 mL of FRAP reagent (300 mM acetate buffer, 20 mM $FeCl_3$ and 10 mM 2,4,6-tri-s-triazine (TPTZ) solution) was added. After 4 min of incubation, absorbance was measured at 593 nm using a microplate reader. The total reducing capacities of the extracts were expressed as µ mol of iron (Fe^{2+}) per gram of dry weight (µmol Fe^{2+}/g DW) based on an iron sulphate standard (Fe_2SO_4) curve against a blank control.

2.7. Statistical analysis

Samples were presented as mean value and standard error. Samples were compared using one-way analysis of variance (GraphPad Prism 8.0 One Way Anova). Statistical difference was accepted as $p \leq 0.05$.

3. Results

The seeds began to germinate five days after starting the application. The number of germinated seeds were monitored daily for five days, and changes were observed. At the end of the fifth day, germination rates of sunflower and chickpea seeds were evaluated compared to the control group. Figure 2a demonstrates the germination percentage of sunflower seeds either control or magnetic field exposed conditions. Germination of the control seeds was 68%, which increased to 97, 98 and 98 % by applications of 5/10/20 mT magnetic field, respectively. However, germination rate of chickpea for were calculated as 54 % for control and 85, 80, and 89% for 5/10/20 mT magnetic field treatments, respectively (Fig. 2b).

The longest shoot length was measured in 5 mT treated sunflower seedlings (7.64±0.40). On the other hand, the shortest and significant shoot length was measured in 20 mT treated sunflower seedlings (5.66±0.16). The length of shoot of the sunflower seedlings in 5 and 10 mT treatments were measured as 7.64±0.40 and 7.21±0.28, respectively. The root length was measured as 10.21±0.93, 10.11±1.44, 7.15±0.68 and 9.30±0.53 in control and different MF applications 5, 10 and 20 mT, respectively (Fig. 3a).

According to Figure 3b, the longest shoot length of chickpea seedlings was found in 20 mT application as 2.00±0.04. Control seedlings had the shortest shoot length (1.35±0.24). Shoot length of other MF applications, 5 and 10 mT, was found as 1.70±0.04 and 1.80±0.17, respectively. Chickpea seedlings had different root lengths. While the longest root legth (2.75±0.67) was in 20 mT application, the shortest root length (1.88±0.22) was measured in control seedlings (Fig. 3b).

Pigment concentrations of samples was determined as µg/g (Table 1). According to findings, the highest pigment content of sunflower plant was found in 20 mT MF application. On the other hand, the lowest pigment content was determined in 10 mT MF application.

According to chickpea pigment content results, the highest pigment content except carotenoids was found in 20 mT MF application. The lowest pigment content was determined in control chickpea samples. (Table 1) Data about total phenolic, flavonoid and antioxidant activities were presented in Table 2. According to results, 20 mT MF applied sunflower plant had the highest total phenolic content and FRAP activity. The highest total phenolic content was determined in 10 mT MF application. The highest DPPH activity was measured in control sunflower seedlings.

According to findings, 20 mT MF applied chickpea plant had the highest total phenolic content and FRAP activity. The highest total phenolic content and DPPH activity were determined in 10 mT MF application (Table 2).

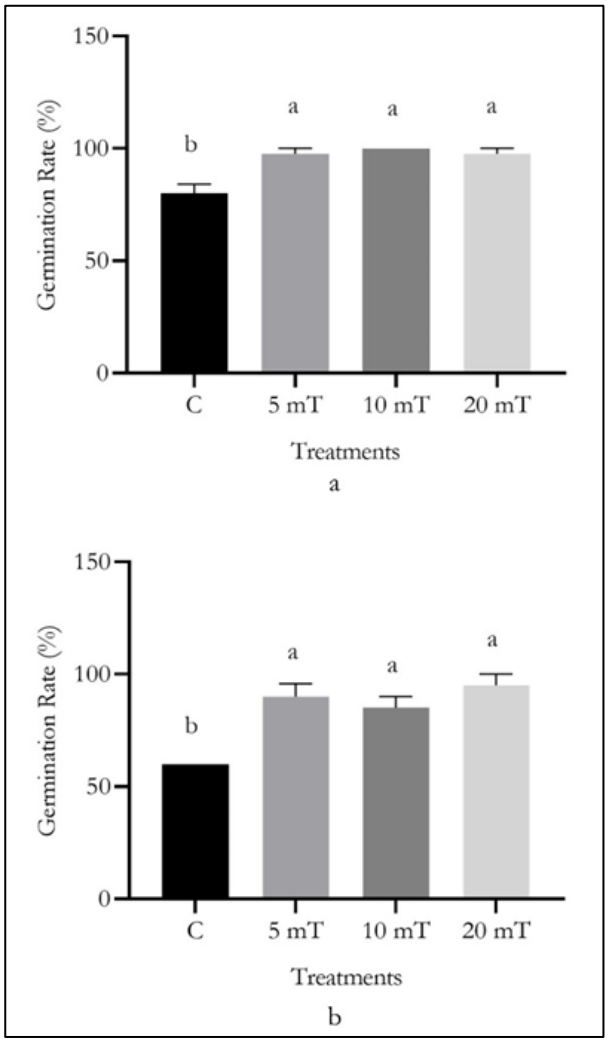

Figure 2. Effects of magnetic fields on germination rate (%). a: *H. annuus*, b: *C. arietinum*. Different letters on the same column exhibited statistically significant different between groups (P=0.0007).

4. Discussions

MF has been demonstrated to increase a range of plant physiological responses, including germination, blooming time, photosynthesis, biomass, cryptochrome activation, and shoot growth (Maffei, 2014). Magnetic field caused

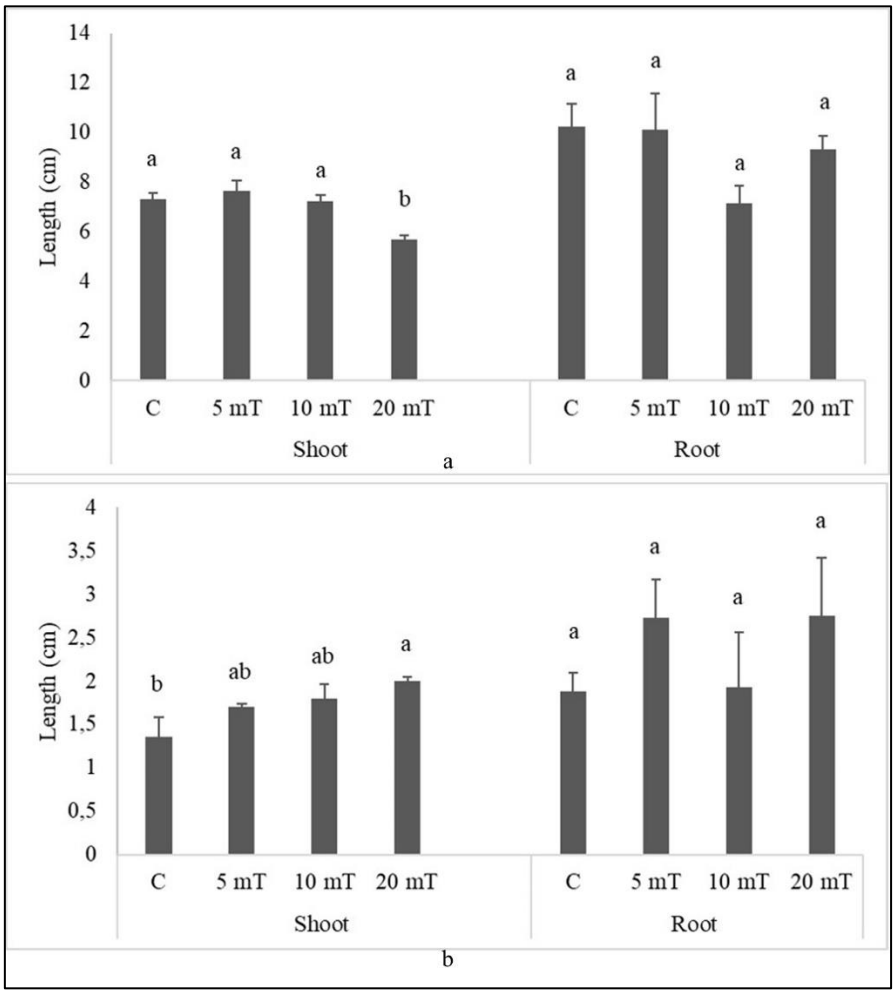

Figure 3. Effects of magnetic fields on shoot and root length (cm). a: *H. annuus*, P=0.0001 for shoot, b: *C. arietinum*, C vs. 20mT for shoot: P=0.0393 Different letters on the same column exhibited statistically significant difference between groups.

significantly increase in the germination rate of sunflower and chickpea seedlings in different rates. This revealed that MF strength is a critical factor affecting the seed germination especially in chickpea. Furthermore, exogenous MF can impact plant transcriptome, proteome, and metabolome profiles based on its strength and frequency (Herranz et al., 2013; Jin et al., 2019; Islam et al., 2020). The effective MF strength that stimulates growth and germination varies depending on the plant type employed, as well as the MF strength, frequency, and exposure period (Ercan et al., 2022). Among the evaluated MFs, this study discovered that 20 mT is the most effective, increasing germination by 33%. External MF stimulates the maximum activity of proteolytic enzymes, including α-amylase (Ramakrishna and Rao 2005). It is possible that MF treatment may improve germination and speed of germination by increasing the activity of proteolytic enzymes (Vashisth and Nagarajan 2010). The study indicated that the magnetic field had a statistically significant influence on germination in chickpea seeds rather than sunflower seeds.

Table 1. Effects of magnetic fields on pigments concentrations (µg/g).

		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoid
	Control	222.33 ± 13.85^{a}	236.16 ± 51.39^{a}	458.34 ± 65.16^{a}	6.34 ± 0.12^{a}
	5 mT	241.78 ± 26.54^{a}	236.50 ± 44.73^{a}	478.13 ± 70.84^{a}	6.55 ± 0.38^{a}
H. annuus	**10 mT**	217.70 ± 12.56^{a}	206.69 ± 15.39^{a}	424.27 ± 27.47^{a}	5.34 ± 0.35^{a}
	20 mT	242.07 ± 13.15^{a}	236.80 ± 12.85^{a}	478.72 ± 25.96^{a}	7.00 ± 0.34^{a}
	P value	0.672	0.910	0.863	0.108
	Control	84.90 ± 1.09^{a}	108.78 ± 2.10^{a}	193.62 ± 2.97^{a}	2.48 ± 0.05^{b}
	5 mT	110.35 ± 6.58^{a}	135.51 ±7.42^{a}	245.78 ± 13.95^{a}	3.22 ± 0.19^{a}
C. arietinum	**10 mT**	111.64 ± 12.09^{a}	129.08 ± 13.09^{a}	240.64 ± 25.17^{a}	3.33 ± 0.13^{a}
	20 mT	113.54 ± 4.00^{a}	141.60 ± 4.17^{a}	255.05 ± 8.17^{a}	3.31 ± 0.10^{a}
	P value	0.068	0.080	0.077	0.004

Table 2. Effects of magnetic fields on total phenolic, flavonoid content and antioxidant activities.

		Total Phenolic (mg/g)	Total Flavonoid (mg/g)	DPPH (%)	FRAP (μ mol Fe^{2+}/g)
	Control	3.82 ± 0.09^{b}	3.39 ± 0^{b}	84.57 ± 0.18^{a}	1.89 ± 0.02^{b}
	5 mT	3.92 ± 0.03^{b}	3.35 ± 0.09^{b}	81.56 ± 0.88^{b}	2.21 ± 0.03^{a}
H. annuus	**10 mT**	3.95 ± 0.03^{ab}	4.41 ± 0.27^{a}	83.19 ± 0.50^{ab}	2.27 ± 0.02^{a}
	20 mT	4.24 ± 0.11^{a}	3.45 ± 0.03^{b}	83.38 ± 0.44^{ab}	2.33 ± 0.07^{a}
	P value	0.005	<0.0001	0.010	0.038
	Control	9.01 ± 0.03^{c}	1.79 ± 0.06^{b}	65.29 ± 0.29^{b}	2.77 ± 0.04^{b}
	5 mT	9.24 ± 0.04^{b}	1.40 ± 0.03^{b}	66.07 ± 0.49^{b}	2.85 ± 0.02^{ab}
C. arietinum	**10 mT**	10.01 ± 0.04^{a}	2.41 ± 0.27^{a}	75.65 ± 0.35^{a}	2.97 ± 0.04^{a}
	20 mT	10.02 ± 0.01^{a}	2.02 ± 0.13^{ab}	60.59 ± 0.71^{c}	2.98 ± 0.05^{a}
	P value	<0.0001	0.017	<0.0001	0.004

In agricultural use, it can be used to accelerate germination, speed up growth, and increase yield of chickpea seeds. Different MF intensities and durations increase the germination rate (Suarez et al., 2017; Shabrangy et al., 2021; Sharma et al., 2021). The reason for this is that exposure of seeds to MF improves seed membrane integrity, reduces cellular leakage and electrical conductivity. In germinating seeds, enzyme activities of α-amylase, dehydrogenase and protease were significantly higher in MF treated seeds compared to control group. Higher enzyme activity in MF treated sunflower seeds could trigger rapid germination and early viability of seedlings (Vashisth and Nagarajan, 2010). Florez et al. (2007) stated that there was a significant increase in germination of chickpea seeds exposed to static MF. The researchers connected their findings to amylolytic enzymes (α-β amylase) or hydrolysis or the expression of hormones (indole, gibberellins, or zeatin). Variable magnetic fields can affect variations in hormone concentrations, enzyme activity, ion transport across the cell membrane, DNA synthesis, or transmission (Strasak et al. 2002).

Shoot and root length are important parameters for physiological development under different growth conditions. According to findings obtained from this study, MF application caused significantly decrease in shoot length of *H. annuus* according to control, 5 mT and 10 mT applications (Fig. 3). On the other hand, MF application caused significantly increase in shoot length of *C. arietinum* compared to control group. There was no difference between root lengths of seedlings. It was reported that applied MF intensities increased the growth parameters and plant development in the early stages of growth in the sunflower plant, and this could be due to the changes in the Ca^{+2} level induced by MF (Florez et al., 2007). In another study conducted with *H. annuus* L., the effect of constant MF in systems with and without iron nanoparticles (Fe-NPs) was investigated, and it was reported that Fe-NPs and MF caused a decrease in germination parameters of seeds in most cases (Kornarzyński et al., 2020). In addition, Belyavskaya (2004) determined that weak electromagnetic fields suppressed the growth of plants, reduced cell division, and concentrated the protein.

As in our study, it was emphasized that MF applications increased shoot length in most of the MF studies on chickpea (Bhattacharya and Barman, 2011; Mridha and Nagarajan, 2014; Sharma et al., 2021). In our study, it was determined that the shoot length increased compared to the control, especially in parallel with the increasing magnetic field strength. In a similar study, Vashisth and Nagarajan (2008) reported that total root length doubled in 1-month-old plants grown from chickpea seeds treated with 100 mT for 1 h. In a study, the effects of MF on the growth parameters of *H. annuus* L. were investigated and it was stated that different MF intensities did not have a significant effect on the fresh weight of the leaves (Peyvandi et al., 2013). Similarly, in the study, it was determined that the MF did not cause any change in the root and shoot relative water content of sunflower and chickpea seedlings.

Application of MF with different intensities to sunflower and chickpea seedlings did not cause changes for pigment concentrations in the seedlings except carotenoid content. There was a significant increase in the carotenoid concentration of all MF applied seedlings compared to the control group (Table 1). MF treatment enhances photosystem II (PSII) efficiency, photosynthetic pigments (chlorophyll a and b), and the performance index, as well as leaf gas exchange performance (Yano et al., 2004; Rochalska and Orzeszko-Rywka, 2005; Baghel et al., 2018; Tirono et al., 2021). A similar study showed that magnetized seeds irrigated with magnetized water increased seed performance in terms of total photosynthetic pigments (chlorophyll a, b and carotenoids) (Abdul et al., 2010). In our study, no significant difference was detected. MF caused similar effect on pigment content (carotenoids, chlorophyll a, b, and total pigments), whereas carotenoids and chlorophyll a were more affected than chlorophyll b (Shine et al., 2012). Similarly, a significant increase was observed in carotenoid, especially in chickpeas compared to the control group in our study. The MF intensity, exposure time and plant genome are important parameters for pigment content.

In sunflower and chickpea seedlings, it was determined that the application of MF caused increase in antioxidant capacity. According to Table 2, total phenolic content of 20 mT MF applied *H. annuus* was significantly increased compared to control and 5 mT applied plants. Additionally, 10 mT treated seedlings of sunflower had statistically more total flavonoid content than control, 5 mT and 20 mT applied seedlings. On the other hand, 5 mT application of *H. annuus* caused importantly decrease in DPPH activity according to control. FRAP activity of all MF treated sunflower seedlings was determined significantly higher than control.

Total phenolic content of all MF treated *C. arietinum* seedlings was determined significantly higher than control.

Additionally, 10 mT treated seedlings of chickpea had statistically more total flavonoid content than control and 5 mT applied seedlings. Moreover, 10 mT application of chickpea caused importantly increase in DPPH activity according to other applications. FRAP activity 10 mT and 20 mT treated *C. arietinum* seedlings was determined significantly higher than control (Table 2).

There is a correlation between total phenolics and antioxidant capacity in many plant species (Rainha et al., 2011). Phenolic acids and flavonoids are known as typical phenolics with antioxidant activity. In our study, it was determined that MF application caused increase in antioxidant capacities of sunflower and chickpea seedlings according to control in the light of the results of total phenolic and flavonoid contents and DPPH and FRAP activities.

MF application caused increase in germination rate of *H. annuus* and *C. arietinum*.While MF treatment was resulted with increase in shoot length of chickpea seedlings, MF treatment caused decrease in shoot length of sunflower seedlings. MF did not affect the root length. Chlorophyll a and chlorophyll b contents were not affected from MF, however MF caused increase in carotenoid content. Additionally, MF application caused increase in antioxidant capacity of sunflower and chickpea seedlings. In the light of these findings, MF application could have been used as agricultural purposes.

Because the magnetic field affects diverse channels, its impacts on living things are assessed differently. Some view the magnetic field as an environmental stressor that hinders plant growth, while others see it as a potentially useful tool for enhancing agricultural practices.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

Concept: Ö.B., Design: Ö.B., Data Collection or Processing: S.G., Ö.B., Analysis or Interpretation: Ö.B., S.G., Literature Search: S.G., Writing: Ö.B., S.G.

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