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Impacts of three different magnetic field applications on seed germination and seedling development of *Melissa officinalis* L.

Canan ÜLGEN[1], Arzu UÇAR TÜRKER[*2]
ORCID: 0000-0002-8272-3370; 0000-0001-9617-6673

[1] Şırnak University, Technology and Research Centre, 73000 Şırnak, Türkiye
[2] Bolu Abant Izzet Baysal University, Faculty of Science and Art, Department of Biology, 14030 Bolu, Türkiye

Abstract
The herb *Melissa officinalis* L., most commonly recognized as lemon balm, has been used as a treatment for problems with the brain and central nervous system, headaches, nervousness, digestive disorders, respiratory and circulatory conditions, various types of cancer and rheumatism. This investigation was designated to compare the impacts of 3 discrete magnetic field strengths (50 mT, 100 mT and 150 mT) with various time periods of exposure (5 min, 15 min, 30 min, 1 hr and 3 hr) on *M. officinalis* seed germination (rate and onset) and seedling growth (length of shoot-root, weight of fresh-dry and water content). The seeds, which were surface sterilized in petri plates, were subjected to different applications, including exposure to various magnetic fields and control (without exposure). Germination was assessed by observing the radicular protrusion. The number of germinated seeds was recorded over 20 days. In all 3 magnetic field applications, exposure to magnetic field for 1 hr caused the highest number of seed germination (36%, 52% and 50% for 50 mT, 100 mT and 150 mT, respectively) comparing to control (28%). The onset of seed germination was earlier with magnetic field application (day 7) compared to control (day 11). Growth elements and water contents of the seedling were assessed in order to understand the impact of different magnetic field applications. Implementation of 100 mT and 150 mT magnetic field for 1 hr increased shoot and root length in addition to fresh weight of the seedlings. The maximum dry weight increase was provided by 150 mT-5 min, 15 min and 30 min. The highest water content (%) was observed with exposure to 150 mT (68%) and 100 mT (66%) for 1 hr compared to control (44%). Magnetic field intensity of 100 mT and 150 mT for 1 hr was the most efficient in the seed germination and seedling growth.

Keywords: germination, growth, lemon balm, magnetic field, *Melissa officinalis.*

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Üç farklı manyetik alan uygulamasının *Melissa officinalis* L.' nin tohum çimlenmesi ve fide gelişimi üzerine etkileri

Özet
Oğul otu olarak da bilinen *Melissa officinalis* L. bitkisi, beyin ve merkezi sinir sistemi ile ilgili sorunlar, baş ağrıları, sinirlilik, sindirim bozuklukları, solunum ve dolaşım rahatsızlıkları, çeşitli kanser türleri ve romatizma tedavisinde kullanılmaktadır. Bu araştırma, *M. officinalis* tohum çimlenmesi (hızı ve başlangıcı) ve fide büyümesi (sürgün-kök uzunluğu, yaş-kuru ağırlık ve su içeriği) üzerine çeşitli maruz kalma süreleri (5 dk, 15 dk, 30 dk, 1 s ve 3 s) ile 3 farklı manyetik alan şiddetinin (50 mT, 100 mT ve 150 mT) etkilerinin karşılaştırılmasını amaçlamaktadır. Petri kaplarında yüzey sterilizasyonu yapılan tohumlar, çeşitli manyetik alan maruziyeti ve kontrol (maruz bırakmadan) olmak üzere farklı uygulamalara tabi tutulmuştur. Çimlenme, radiküler çıkıntı gözlemlendiğinde değerlendirilmiştir. Çimlenen tohumların sayısı 20 gün boyunca kaydedilmiştir. Üç manyetik alan uygulamasının hepsinde, 1 saat boyunca manyetik alana maruz kalma, kontrol (%28) ile karşılaştırıldığında en yüksek sayıda tohum çimlenmesine (50 mT, 100

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +903742545138; Fax.: +903742534642; E-mail: turker_a@ibu.edu.tr
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mT ve 150 mT için sırasıyla %36, %52 ve %50) neden olmuştur. Tohum çimlenmesinin başlangıcı, manyetik alan uygulamasıyla (7. gün) kontrole (11. gün) kıyasla daha erken olmuştur. Farklı manyetik alan uygulamalarının etkisini anlamak için fidelerin büyüme elementleri ve su içerikleri karşılaştırılmıştır. 1 saat süreyle 100 mT ve 150 mT manyetik alan uygulaması fidelerin taze ağırlığına ek olarak gövde ve kök uzunluğunu artırmıştır. En fazla kuru ağırlık artışı, 150 mT-5 dk, 15 dk ve 30 dk ile sağlanmıştır. Kontrole (%44) kıyasla en yüksek su içeriği (%) 1 saat süreyle 150 mT (%68) ve 100 mT (%66) maruziyetinde gözlemlenmiştir. 1 saat boyunca 100 mT ve 150 mT manyetik alan yoğunluğu, tohum çimlenmesi ve fide büyümesinde en etkili olmuştur.

Anahtar kelimeler: çimlenme, büyüme, manyetik alan, *Melissa officinalis*, oğul otu.

1. Introduction

Melissa officinalis L., generally recognized as lemon balm, is an aromatic plant indigenous to the Eastern Mediterranean area, Western Asia, Southern Europe, Caucasus, and Northern Iran [1, 2]. It has a wide distribution in the Mediterranean, Marmara and Black Sea regions of Turkey and is locally distributed in Central and Southeastern Anatolia regions [3]. It is widely cultivated in Europe and is extensively researched because of its chemical constituents and various therapeutic properties [1, 2, 4]. *M. officinalis* has a long history of usage in folk therapy due to its calming and herbal aromatic qualities. It is also used to treat fever, colds, headache, toothache and insomnia in folk medicine [4, 5]. It has tannins, phenolic acids, flavonoids, triterpenes, essential oils, and triterpenes [1, 2]. In general, living things are beings affected by Earth's magnetism. Magnetic and electromagnetic treatments are a technique used in agriculture to improve the germination of seeds and increase crop and yield [6]. Particularly, low-level magnetic field is reportedly stated to positively affect plant seed germination, growth, and content [7-18]. The investigations demonstrate the advantageous impacts seen on magnetically treated plants under various circumstances, depending on applications like exposure period and constant or variable magnetic field strength [6]. Applications of magnetic fields in agriculture can be utilized to increase the quality of its products and yield [6, 9]. Our previous study revealed the impact of 2 different magnetic field strengths (50 mT and 100 mT) with various exposure durations (1 h, 3 hr, 6 hr, 12 hr, 24 hr, 48 hr, 72 hr, 144 hr and 240 hr) on *M. officinalis* seed germination [16]. It was found that a magnetic field increased the germinated seed frequency and decreased the time needed for the germination of seeds [16]. Consequently, our goal was to evaluate the efficacy of three different magnetic fields strengths (50 mT, 100 mT and 150 mT) with discrete exposure times (5 min, 15 min, 30 min, 1 hr and 3 hr) on seed germination and seedling growth regarding shoot-root length, fresh-dry weights, and water content.

2. Materials and Methods

2.1. Magnetic Field Settings

Block magnets (neodymium) with dimensions of 100 x 50 x 5 mm were utilized to generate three different magnetic fields: 50 ± 5 mT, 100 ± 5 mT and 150 ± 5 mT. The magnets were immobilized on the aluminum stand side by side to create the magnetic intensity (Figure 1A, B, C and D). Teslameter (Compensation®) was handled to determine the strength of the magnetic field [6].

2.2. Germination

M. officinalis L. subsp. *officinalis* L. seeds were collected at Bolu, Turkey and "Flora of Turkey and the East Aegean Islands" was used to identify the plant [3]. The seeds were cleaned with an antibacterial soap, washed with distilled water, and surface sterilization was performed after 15 minutes of shaking in 0.1% $HgCl_2$ for 15 minutes, cleaned three more times with sterilized water, sterilized in 70% ethanol for 1-2 minutes, and then cleaned three more times with sterile water [16]. Completely sterilized seeds were put into sterile and disposable petri plates with Murashige and Skoog's minimal organics medium [19], which contains 30 g/l sucrose (Caisson®) and 8 g/l agar (BD-Bacto®) (pH 5.7, autoclaved at 121°C and 105 kPa for 20 minutes). Each petri plate contained ten seeds, and ten petri plates (90 mm) were utilized for each treatment. Seeds were properly aligned in petri dishes to be exposed to the most accurate magnetic field strengths (Figure 1E). Surface sterilized seeds in petri plates were placed into 3 different magnetic field strengths (50 mT, 100 mT and 150 mT) exposing to 5 different durations (5 min, 15 min, 30 min, 1 hr and 3 hr). Radicle protrusion was the sign to assess the germination. For a period of 20 days, the number of germinated seeds was recorded. For seedling development, germinated seeds were placed into Magenta containers (GA-7 Vessel, Sigma-Aldrich®) including MSMO medium for a further six weeks to allow for shoot elongation and root formation (Figure 1F). After 6 weeks of culture, shoot and root length, fresh and dry weights and water content were recorded (Figure 1G).

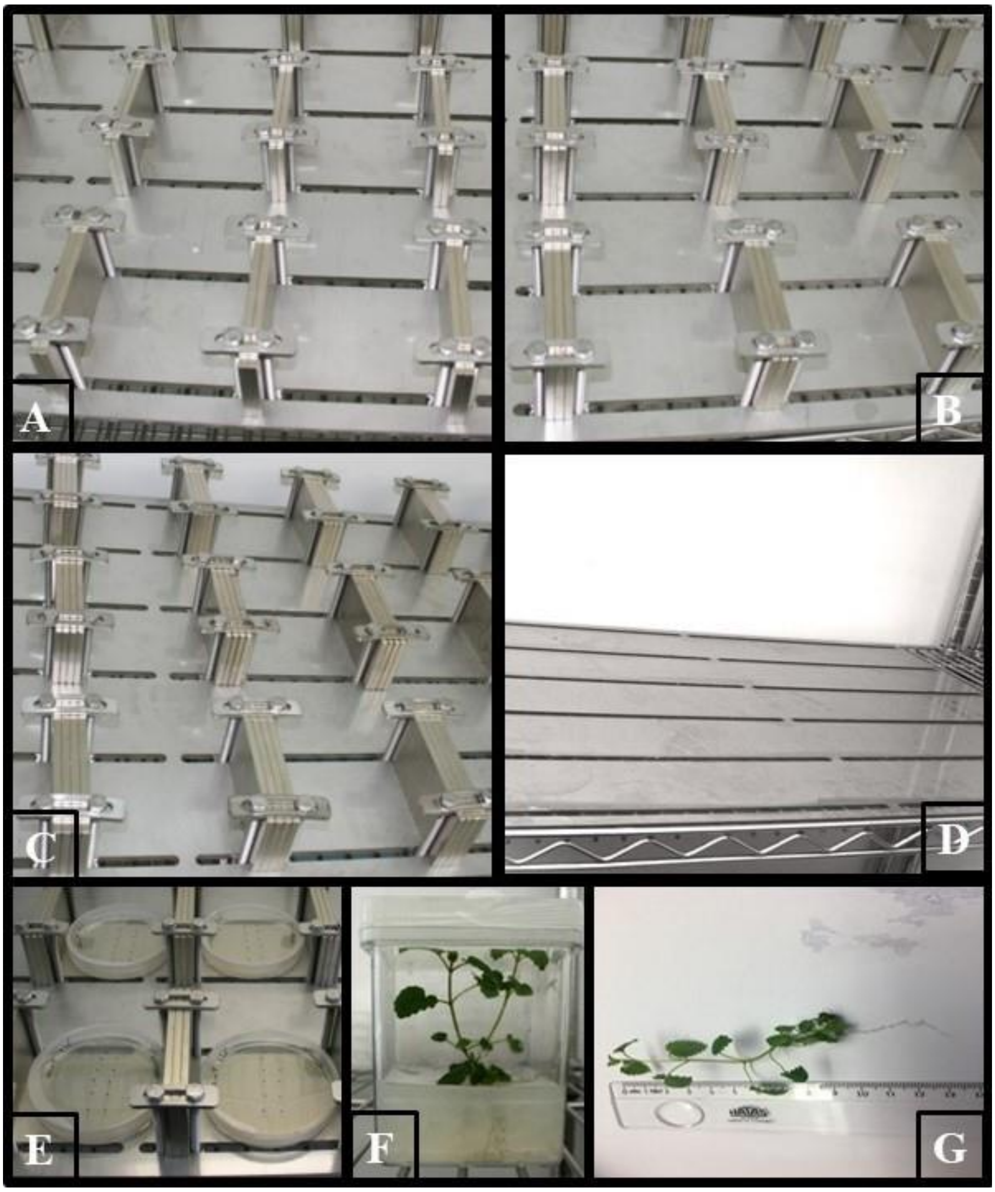

Figure 1. Magnetic field setup-(A) 50 mT, (B) 100 mT and (C) 150 mT, (D) Control; (E) Seed alignment demonstrated in magnetic field applications; (F) Seedling exposing 1hr-100 mT magnetic field after 6 weeks; (G) Shoot-root length measurement of the seedlings after magnetic field exposures

2.3. Statistical analysis

All the data were first tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test) to meet statistical demands [20]. Then, the results were analyzed using ANOVA by a completely randomized design and multiple range parametric tests (Duncan's) in SPSS version 26 (SPSS Inc, Chicago, IL, USA). The table's data was given as a mean number with standard error (SE). Differences between means were considered significant when $P<0.05$.

3. Results

M. officinalis seeds were magnetically treated for varying exposure times (5 min, 15 min, 30 min, 1 hr, and 3 hr) to static magnetic fields with an intensity of 50 mT, 100 mT, and 150 mT. When compared to unexposed seeds (control), it was observed that the strength of the magnetic field and the length of exposure time had a profound impact on lemon balm seed germination. When the radicle started to protrude, seeds were regarded to have germinated. Magnetic field intensity of 100 mT and 150 mT with all exposure times enhanced the germination frequency comparing to control group (non-exposed seeds-28%). Lemon balm seeds required a 1 hr exposure to 50 mT, 100 mT, or 150 mT to germinate, with 36%, 52%, and 50%, respectively, being the best germination rates. With a 100 mT magnetic field applied for one hr, the maximum seed germination was noted (52%) that is 1.9-fold increase regarding control (Figure 2 and 3). It was also noteworthy that magnetic field treatment to the seedlings accelerated germination. The time required for seed germination was reduced with magnetic field utilizations, with seed germination occurring in 7th day with all magnetic field applications (except for 50 mT with 3 hrs-8th day) and 11th day with non-treated seeds (Figure 2 and 3).

In order to understand the effect of 3 different magnetic field strength with various exposure durations, the shoot and root lengths, fresh and dry weights, and water content of the seedlings were compared (Table 1). Application of 100 mT magnetic field for 1 hr enhanced shoot and root lengths. Furthermore, 150 mT treatment for 1 hr was also effective in shoot and root length increments. Fresh weight raised the most with 150 mT and 1 hr application. Second, 100 mT treatment for 1 hr increased fresh weight successfully. The maximum dry weight increase was achieved with the application of 150 mT with 5 min, 15 min and 30 min exposure times. All applied magnetic field intensities with various durations increased water holding capacities comparing to no magnetic field exposure-control (44%). Water content (%) results showed that 1 hr exposure to 150 mT (68%) and 100 mT (66%) increased water holding capacity at most (Table 1).

4. Conclusion and Discussion

Magnetic field intensity and exposure duration to magnetic field were crucial components for lemon balm seed germinations. Lemon balm seeds dealt with magnetic field germinated more quickly. Germination rate was higher for magnetically treated lemon balm seeds. Also, germination time was significantly reduced when seeds were exposed to magnetic fields. Magnetic field intensities of 100 mT and 150 mT for 1 hr were proven to be the most effective in seed germination and seedling growth. All magnetic field applications increased the amount of water in the seedlings. The magnetic field strength and duration, which are the most effective in germination and growth also caused more water retention. Our findings revealed that the fresh weight rose because it retained more water after 1 hr exposure to 100 mT and 150 mT. On the other hand, dry weight increase was the most efficient with 5 min exposure duration of 150 mT magnetic field.

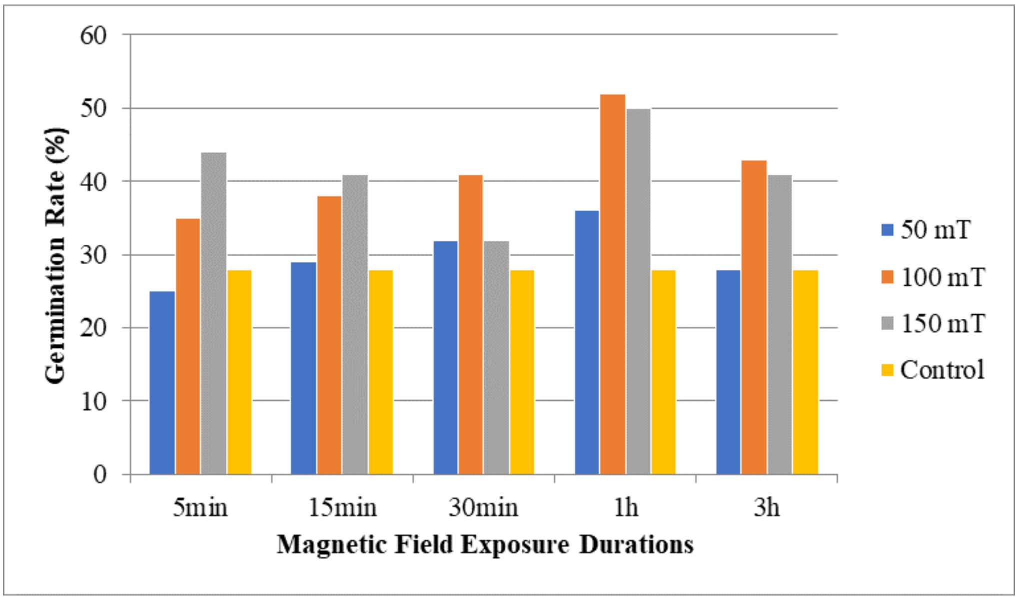

Figure 2. Influences of different applied magnetic fields with different durations on seed germination rate (%) of *M. officinalis*

Similar to present study, comparison of 50 mT and 100 mT magnetic field with discrete exposure durations (1 hr, 3 hr, 6 hr, 12 hr, 24 hr, 48 hr, 72 hr, 144 hr and 240 hr) in *M. officinalis* seed germination in our previous study [16] showed that among the different duration times, 1 hr exposure showed the best germination rate and seed germination, and onset of seed germination occurred on the 7th day with both magnetic field treatments. In line with this result, Reina and Pascual [7] showed that the applied magnetic field increased water uptake rate, which could explain the rise in germination of treated lettuce seedlings. Their explanation was that variations in intracellular Ca^{2+} levels and ionic current density across the cellular membrane due to magnetic field treatments generated changes in osmotic pressure and the capacity of cellular tissues to absorb water. Water intake was reported to increase with magnetic field treatments in multiple investigations [7] which is supposed to promote cell development. In chickpea, magnetic field treatments increased germination of the seeds, germination speed, the length of seedling, and dry weight, according to research by Vashisth and Nagarajan [21]. They came to the conclusion that [21] Ca^{2+} ion separation and subsequent increase of free cell Ca^{2+} level may be caused by the mobility of ions in magnetic field (ion-cyclotron resonance). An early mitotic cell cycle entry signal could be provided by an elevated Ca^{2+} ion concentration. The cell's increased Ca^{2+} ion concentration may be the cause of the increased biomass in the seedlings.

Table 1. Influences of 3 different magnetic fields with different durations on growth parameter and water content in *M officinalis*

Magnetic Field Exposures	Shoot length (mm)	Root length (mm)	Fresh weight (gr)	Dry weight (gr)	Water content (%)
50 mT					
5 min	55±1.8^{ef}	31±3.1^{ef}	1.178±4.5^{f}	0.577±1.2^{c}	51
15 min	45±2.4^{f}	36±2.3^{e}	1.320±1.3^{e}	0.673±2.1^{bc}	49
30 min	49±1.9^{f}	37±4.1^{e}	1.509±3.4^{d}	0.679±0.6^{bc}	55
1 h	70±4.5^{d}	49±0.5^{cd}	1.782±1.9^{bc}	0.695±2.9^{b}	61
3 h	59±2.7^{e}	35±1.6^{e}	1.634±2.1^{cd}	0.752±0.8^{b}	54
100 mT					
5 min	69±1.7^{d}	44±2.5^{d}	1.358±1.8^{e}	0.578±1.5^{c}	57
15 min	80±2.9^{c}	58±3.8^{bc}	1.730±2.1^{c}	0.694±2.3^{bc}	59
30 min	90±3.0^{bc}	50±2.27^{b}	1.846±1.0^{b}	0.673±1.8^{bc}	64
1 h	**108±2.8^{a}**	**73±2.3^{a}**	**2.187±4.3^{ab}**	**0.735±1.4^{b}**	**66**
3 h	97±3.4^{b}	56±4.6^{bc}	1.812±2.7^{b}	0.656±4.2^{bc}	63
150 mT					
5 min	75±1.8^{cd}	47±1.1^{cd}	2.161±1.8^{ab}	0.908±3.4^{a}	58
15 min	74±3.6^{cd}	53±2.7^{c}	1.767±2.5^{bc}	0.848±1.4^{ab}	52
30 min	82±0.7^{c}	62±6.5^{b}	1.929±0.9^{b}	0.890±0.9^{ab}	55
1 h	94±4.2^{b}	72±4.1^{ab}	2.327±1.2^{a}	0.745±2.1^{b}	68
3 h	91±3.6^{bc}	65±0.7^{b}	2.234±1.6^{ab}	0.820±2.1^{ab}	63
Control (no exposure)	39±0.6^{f}	29±1.6^{f}	1.154±3.1^{f}	0.651±0.7^{bc}	44

The impact of magnetic field implementations (80 mT, 100 mT, and 170 mT) for 1 min, 3 min, 5 min, 10 min, 15 min, 20 min and 25 min on tomato seedling germination and growth was examined by Souza Torres et al. [22]. With some magnetic field applications, an enhancement in germination rate and seedling height was recorded, and the stimulus varied according to the magnetic field strength levels and exposure period. Similar to our study, one previous study evaluated that germination rate of *M. officinalis* seeds treated with 3.8-4.8 mT magnetic field was higher comparing to control [12]. However, unlike our findings, the seedlings remained short in length comparing with control in their study [12].

Different magnetic field treatments promoted the germination rate in numerous other plants, similar to our findings: Gholami and Sharafi [23] applied magnetic fields of 125 mT or 250 mT for varying lengths of time on wheat seeds and reported the stimulating effect of different magnetic doses on germination compared to the control. Rice and maize seeds subjected to 125 mT and 250 mT stationary magnetic fields showed an increase in early growth phases and an early sprouting [13, 14]. In another study with wheat, it was determined that root dry and fresh weight increased, and the germination rate raised at the end of magnetic field application [24]. Aladjadjiyan and Ylieva [25] found that magnetic field caused an increase in seed germination percentage in tobacco plant. It was reported that seedlings grown from magnetically treated wheat germ (180 mT) collected more water and grew faster than untreated controls [26]. Kavi [27] determined that the moisture absorption capacity of soybean seeds increased when exposed to a magnetic field of 300 mT [28-30]. In both *Salvia officinalis* and *Calendula officinalis*, the application of a magnetic field increased the percentage of germination in the treated group relative to the untreated group [15].

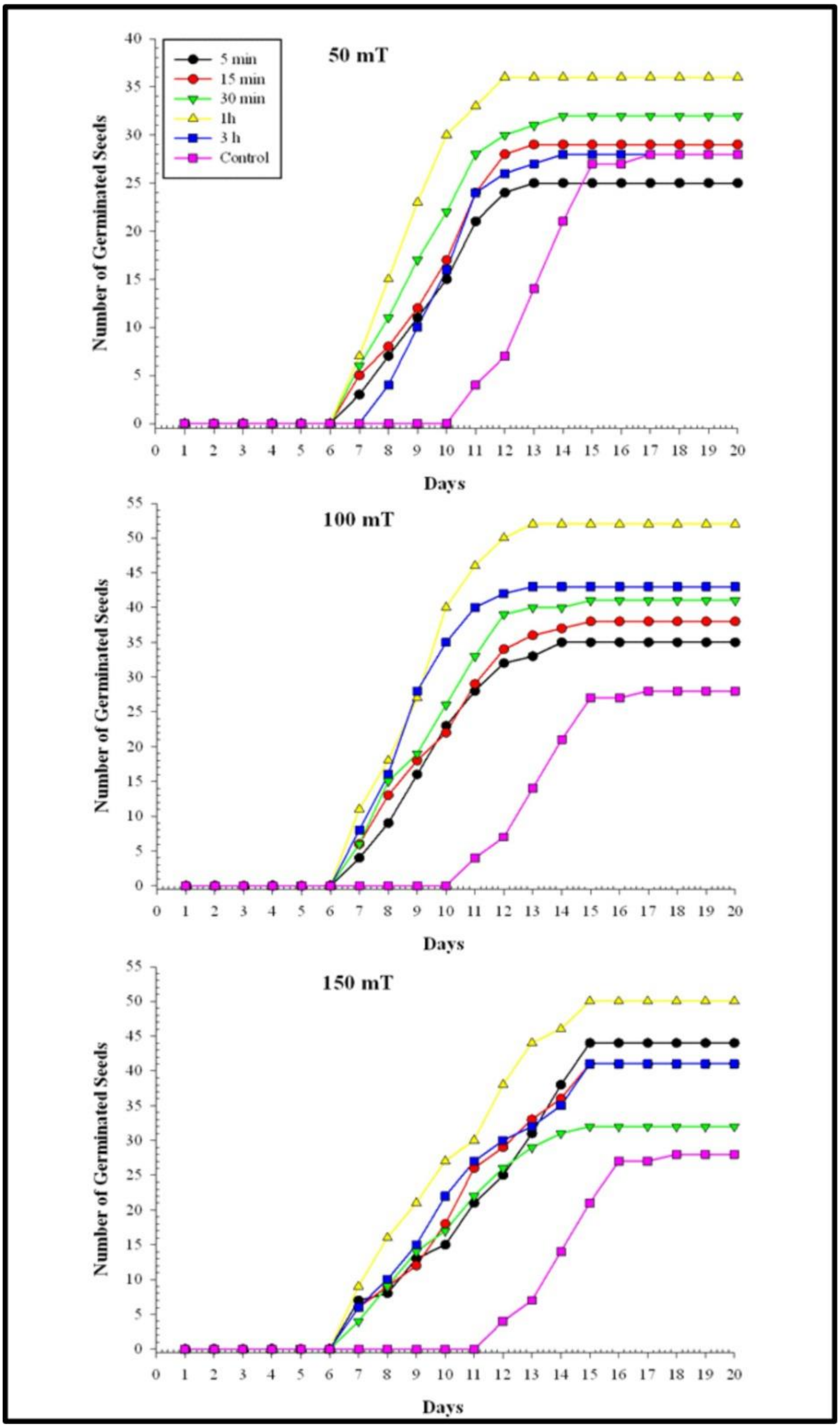

Figure 3. Influences of different applied magnetic fields with different durations on seed germination of *M. officinalis*

In consistent with our findings, Racuciu et al. [11] reported the stimulatory effect of the magnetic fields (50 mT-250 mT) in *Zea mays* seedlings and plant lengths were higher for all exposed samples. Racuciu et al. [10] evaluated the impact of varied magnetic field applications (50-250 mT) on the growth and development of maize seedlings during a 14-day period. They claimed that low magnetic field (50 mT) stimulated growth by increasing fresh tissue mass, length of plant, chlorophyll ratio, assimilatory pigments, and average nucleic acid level. On the other hand, they discovered that high magnetic field utilizations (100 mT-250 mT) inhibited growth. Racuciu [9] showed that the treatment of seedlings with a 10 mT magnetic field for 4 hr had a substantial effect on the length of *Zea mays* plants, and this might be attributable to an improved capacity for water storage following magnetic field exposure. In contrast to our findings, Ghanati et al. [8] reported a decline in the development of *Ocimum basilicum* L. (basil) subjected to 30 mT magnetic field for 6 days and 5 hr each day.

It can be concluded that positive effect of stationary magnetic field can be used in agriculture sector for lemon balm to increase of germinated seeds and their biomass (fresh or dry weight).

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