Original Research

Effect of Magnetic Field Treatment on Germination of Medicinal Plants *Salvia officinalis* L. and *Calendula officinalis* L.

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Abstract

Great development in medicinal, spice, and aromatic plant crops has occurred in Mediterranean countries due to their high added value as a consequence of the reappearance of phitotherapy, among other reasons. The main objective of this study is to determine the effects of magnetic treatment, in addition to the geomagnetic field, on germination of *Salvia officinalis* L. and *Calendula officinalis* L. seeds. This objective has a practical application in agricultural science: to obtain the early growth of both plants. Groups of 100 seeds were exposed to a 125 mT stationary magnetic field generated by magnets at different times, whereas the other group of 100 seeds was subjected to a magnetic pre-treatment, and non-exposed seeds were used as control. Germination tests were performed under laboratory conditions. The selected germination parameters were: time for the first seed to germinate (T_1), time to reach 10-75% germination (T_{10}, T_{25}, T_{50}, and T_{75}), mean germination time (MGT), and number of germinated seeds (G_{max}), all provided by the Seed calculator software package.

The germination parameters recorded for *Salvia officinalis* L. seeds for each treatment and pre-treatment were lower than corresponding control value. Among the various treatments, chronic exposure to 125 mT provided best results; the MGT was significantly reduced compared to control, parameters (T_1-T_{50}) were also significantly reduced for most treatments. Results obtained for *Calendula officinalis* L. seeds showed that germination parameters were reduced, in most cases, for magnetic treatment versus control, and all parameters of germination were reduced for pre-treatment versus control. The best results were obtained from chronic exposure. Results indicated that magnetic field application enhanced germination rate and percentage of germinated seed on the treated group compared to the non-exposed in both cases.

Keywords: *Calendula officinalis* L., germination, magnetic treatment, magnetic field, *Salvia officinalis* L., seedling

Introduction

In recent decades, physical techniques based on the application of magnetic fields are being developed in the agricultural sector. In recent years the number of bioelectromagnetism research reports focusing on the investigation of magneto sensitivity of living organisms has increased. Plants mean an attractive model for the study of biological effects of magnetic fields [1]. Studies on rice and onion showed that magnetic pre-treatment improved the

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germination and seedling vigour of low viable seeds [2]. Magnetic field pre-treatment had also a positive effect on cucumber, such as stimulating seedling growth and development [3]. An increased rate of germination of cereal seeds exposed to magnetic field has been obtained; greater albumin, gluten and starch contents in wheat seeds exposed to magnetic fields were obtained [4, 5]. A possible mechanism associated with magnetism to accelerate tomato ripening was proposed [6]. A great development of crops of medicinal plants, spices and aromatic plants is taking place in Mediterranean countries due to their high added value as a consequence of the reappearance of phytotherapy, among other reasons. Salvia and calendula plants are used as spices, but due to its essential contents they have some medicinal properties. They are cultivated for the food, herbalist, cosmetics, and liqueur industries [7].

In previous studies, we found that 125 mT and 250 mT magnetic treatment produces a biostimulation on the initial growth stages and an increase of the rate of germination of several seeds such as rice, wheat, and barley [8-11]. We studied the effect of germination of maize seeds and concluded that the time required for germination recorded for each magnetic treatment were lower than corresponding control values, thus the rate of germination of treated seeds was higher than the untreated seeds rate. Growth data measured on the 7th and 10th days after seeding allowed them to corroborate the effect observed in germination tests; significant differences between length and weight of maize seedlings subjected to a 125 mT and 250 mT magnetic field at different times versus control were obtained [12]. A positive response in grass seeds has been observed; exposure to magnetic field provided earlier germination, increased the number of germinated seeds, reduced the germination rate, and increased root length for *Festuca arundinacea* Schreb. and *Lolium perenne* L. seeds [13]. Recently, the 125 mT and 250 mT magnetic field exposure to pea and lentil seeds has been studied; the growth parameters (total and stem weight, total and stem length) measured on days 7 and 14 were increased; consequently, seedlings from seeds magnetically treated grew taller and heavier than control ones. Increased root development was also observed [14].

The main objective of this study was to evaluate the effects of magnetic treatment on germination of *Salvia officinalis* L. and *Calendula officinalis* L. seeds, by exposing the seeds to 125 mT magnetic field at different periods of time and pre-sowing exposure. This objective has a practical application in agricultural science: to obtain the early growth of medicinal plants.

Material and Methods

Germination tests were conducted to study the effect of a stationary magnetic field of around 125mT on seed germination. The test was performed in summer under laboratory conditions with natural light and an average temperature of 20±2ºC for *Salvia officinalis* L., and 25±2ºC for *Calendula officinalis* L. Seeds of uniform size and shape without visible defects and malformation were selected.

Five groups of 100 *Salvia officinalis* L. seeds were exposed to magnetic field by varying the exposure time (A1-A5). Another group of 100 seeds was subjected to magnetic pre-treatment (P1) for the 24-hour previous sowing. A static magnetic field was generated by permanent ring magnets with 125 mT strength. Geometrical characteristics of the ring magnet are external diameter 7.5 cm, internal diameter 3 cm, and height 1 cm. Analogous rings to the ring magnets, manufactured with the same material but without magnetic induction, were used as blind (Control). Times of exposure were: 10 min (A1), 20 min (A2), 1 h (A3), 24 h (A4) or chronic exposure (A5). An experimental design using four replicates (n=4), with 25 seeds in each, was carried out. Thus, groups of 100 seeds were subjected to each magnetic treatment, and analogous groups were used as control. Two groups of one hundred *Calendula officinalis* L. seeds each were exposed to 125 mT for 24 h (A4) or chronic exposure (A5) and were compared with control (C1). Another group of 100 seeds was subjected to a magnetic pre-treatment (P1) for the 24-hour previous sowing and were compared with control (C2).

The germination test was performed according to the guidelines issued by the International Seed Testing Association [15] with slight modifications. Seeds were germinated by placing 25 seeds per Petri dish on filter papers soaked with 12 ml of distilled water. The seeds were placed around a circular line; in this way, all the seeds were subjected to the same magnetic field strength when the Petri dish was placed on top of a permanent magnet. To obtain dose A5 Petri dishes were placed on the magnets for all the entire experiment, then the seeds were chronically exposed. To obtain the other doses the Petri dishes were placed on the magnets for the corresponding times of 10 min, 20 min, 1 h, and 24 h (A1-A4) or pre-sowing magnetic treatment (P1). After that, they were placed on a blind-ring without magnetic induction. The control group of Petri dishes was located on blind-rings since the beginning of the experiment; then the seeds were not exposed to a magnetic field.

Experimental groups A1-A5, P1 and control C ran simultaneously for the germination test. For each treatment the number of germinated seeds was registered three times per day for the necessary time to achieve the final maximum percentage of germinated seeds (G_{max}). Seeds were considered as germinated when their radicle was at least 2 mm long. The selected germination parameters were: time for the first seed to germinate (T_1), time to reach 10, 25%, etc. germination (T_{10}, T_{25}, T_{50}, and T_{75}), the number of germinated seeds (G_{max}), correlation coefficient (R^2), and the mean germination time (MGT), all of them were provided by the Seed calculator software package developed for seed germination data analysis by Plant Research International.

Statistical Analysis

Data of germination obtained from the magnetic treatments were compared by the use of the t-student value and the p-values were calculated to test for significant differences between each treatment and the control using the seed calculator software for seed germination data analysis.

Table 1. Germination parameters determined for *Salvia officinalis* L. seeds exposed to 125 mT stationary magnetic field, expressed as mean ±standard error. Exposure times were 10 min (A1), 20 min (A2), 1 hour (A3), 24 h (A4), chronic exposure (A5), pre-treatment (P1), and not exposed seeds (C).

Dose	R^2	G_{max} (%)	Time (hour) $\overline{x}\pm SEM$					
			T_1	T_{10}	T_{25}	T_{50}	T_{75}	MGT
C	0.98	47 ±4.4	72.96 ±0.72	78.96 ±0.72	83.04 ±0.72	88.80 ±0.96	97.44 ±1.92	95.28 ±2.16
A1	0.96	60 ±4.3	34.08 ±9.36**	57.36 ±9.36*	72.96 ±7.20	90.96 ±5.28	109.9 ±5.52*	91.92 ±5.76
A2	0.86	63 ±4.4**	27.36 ±1.44****	48.24 ±2.40****	62.64 ±2.88****	79.68 ±3.36**	97.68 ±4.08	80.64 ±3.36**
A3	0.94	56 ±4.3	25.44 ±7.44**	46.80 ±11.0**	61.68 ±7.92**	78.96 ±4.08*	96.96 ±1.20	79.68 ±3.60**
A4	0.97	69 ±1.9**	23.04 ±2.88****	44.16 ±2.64****	59.76 ±2.16****	79.20 ±2.40**	101.4 ±4.56	81.84 ±2.88***
A5	0.98	58 ±5.0	22.56 ±6.24****	41.28 ±2.88****	54.96 ±2.40****	72.48 ±4.32**	92.64 ±6.48	75.60 ±2.64***
P1	0.94	65 ±5.3*	25.20 ±8.16**	47.04 ±11.3**	62.4 ±8.16**	80.64 ±4.56	99.84 ±2.16	81.60 ±5.76*

G_{max}: number of germinated seeds (%); MGT: Mean germination time; T_1, T_{10}, T_{25}, T_{50}, T_{75}: time required for 1, 10, 25, 50, and 75% of the seeds to germinate expressed in hours. R^2: correlation coefficient. Asterisks indicate differences *versus* control: ****($p<0.001$): very strongly significant; ***($0.001<p<0.01$): strongly significant; **($0.01<p<0.05$): significant; *($0.05<p<0.1$): differences.

Results and Discussion

Results of *Salvia officinalis* L. Seeds

Germination parameters calculated for salvia seeds are recorded in Table 1. Results show that the percentage of germination (G_{max}) was higher for magnetically treated seeds. Parameters T_1 and T_{10} were significantly reduced on all magnetic treatment; then, the onset of the germination occurred earlier. Value of T_1 of seeds not exposed to magnetic field was 72.96 h, while this value was 23.04 h for A4, 22.56 h for A5, and 25.20 h for pre-treatment (P1). Significant reductions were also obtained for T_{25} parameter. The mean germination time (MGT) of salvia seeds was significantly reduced when seeds were exposed to magnetic field; the greatest differences between treated seeds and control were obtained when seeds were treated for 24 h and chronically exposed (81.84 h for A4, 75.60 h for A5 versus 95.28 h for control). In addition, the other parameters evaluated, T_{50} and T_{75}, were also reduced. As a consequence, the percentage and rate of germination of salvia seeds exposed to a 125 mT stationary magnetic field were increased.

In Figs. 1 a, b and c the curves of cumulative germination of *Salvia officinalis* seeds are plotted for both the magnetic treatment applied seeds and control. It shows that in all cases, the germination curve of control is underneath the curves of treated seeds. Then control germination rate is lower than the rate corresponding to all magnetic doses. The percentage of germination of control seeds is always below corresponding to all with magnetic treatments applied.

Results of *Calendula officinalis* L. Seeds

Germination parameters calculated for *Calendula officinalis* L. seeds are recorded in Table 2. Results show that germination parameters were reduced, in most cases, for magnetic treatment (A4, A5) versus control (C1), and all parameters of germination were reduced for pre-treatment (P1) versus control (C2). The best results were obtained for chronic exposure (A5).

Fig. 2a shows the curves of cumulative germination of *Calendula officinalis* L. seeds exposed to 125 mT for 24 h (A4), chronic exposure (A5), and control (C1). Fig. 2b shows the curves of cumulative germination of *Calendula officinalis* L. pre-treated seeds (P1) and control (C2). Germination rate of untreated seeds is lower than the rate of magnetic treatment seeds. Percentage of germination of control seeds is also below that corresponding to magnetic treatments applied.

Results obtained for both medicinal plants match other studies regarding the influence of a stationary magnetic field on seed germination and plant growth, which reveals that magnetic treatment produces an improvement on per-

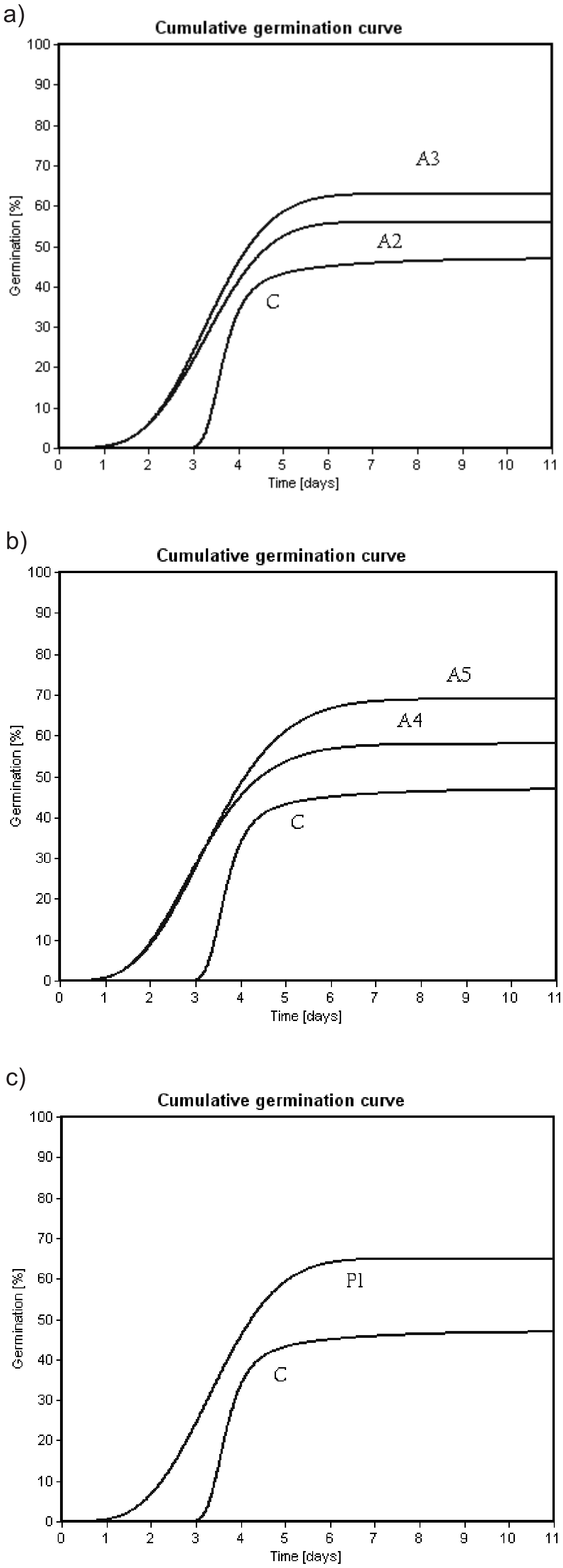

Fig. 1. Cumulative germination curves of *Salvia officinalis* L. seeds subjected to 125 mT stationary magnetic field at different times, a) Doses A2: 20 min, A3: 1 hour, and control, C; b) Doses A4: 24 h, A5: chronic exposures and control C; c) Magnetic pre-treatment (P1) for 24 hour before sowing and control curve C.

centage and rate of germination of exposed seeds. An increase in germination and shoot development when exposed maize seeds to 150 mT magnetic field for 10, 15, 20, and 30 minutes was obtained [16]; similar results with tobacco seeds were obtained [17]. The induction of primary root curvature in radish seedlings in a static magnetic field was observed, and roots responded tropically to the static magnetic field, with the tropism appearing to be negative, these roots responded significantly to the south pole of the magnet [18]. A positive effect of the magnetic treatment on the germination and emergence of bean cultivars was confirmed; plant emergence from magnetized seeds was 2-3 days earlier compared to the control, the yield was increased due to the higher number of pods per plant [19]. Length of young plants of maize exposed to a magnetic field varying from 50 to 250 mT were reported [20] to be higher than control for all exposed samples, linear correlation was found to describe the average length dependence on the magnetic energy, and after 11 days the total fresh substance mass was weighted as well as dry substance mass.

Seeds of maize (*Zea mays* L.), chickpea (*Cicer arietinum* L.) and sunflower (*Helianthus annus* L.) were exposed to different magnetic fields and were sown in a greenhouse. The maximum enhancement in seedling growth and root characteristics was observed under different combinations of magnetic field and duration time of exposure. Among the three species, it was noticed that protein-rich chickpea required less exposure to magnetic energy, followed by starch rich maize seeds and lipid rich sunflower seeds to attain the required enhancement for seedling growth. The root characteristics of the plants showed an increase in root length, root surface, and root volume. In another study, significant increase in germination, seedling vigour, and shoot/root growth of one-month-old plants of chickpea seeds exposed to static magnetic fields was also published. Seeds of chickpea (*Cicer arietinum* L.) were exposed in batches to static magnetic fields of from 0 to 250 mT strength in steps of 50 mT for 1-4 h in steps of 1 h for all fields. Results showed that magnetic field application enhanced seed performance in terms of laboratory germination compared to unexposed control; combinations of field strength and time of exposure: 50 mT for 2 h, 100 mT for 1 h, and 150 mT for 2 h exposures gave best results [21, 22]. On the other hand, results obtained suggest that both magnetic treatment of water and magnetic treatment of seeds have the potential to improve early seedling growth and nutrient contents of pea and chickpea seedlings, [23].

Growth of the germinated seedlings of *Vicia faba* was found to be enhanced by the application of power frequency magnetic fields (100 μT) as evidenced by mitotic index and ^{3}H-thymidine uptake [24]. In Austria and Germany, electric railways are powered by a frequency of 162/3 Hz. Then, sunflower and wheat seedlings were exposed to 162/3 Hz sinusoidal 20 μT (rms) vertical magnetic fields. Results showed that sunflower seedlings exposed to experimental magnetic fields showed small but significant increases in total fresh weights, shoot fresh weights, and root fresh weights. Also, experimentally treated wheat

Table 2. Germination parameters determined for *Calendula officinalis* L. seeds exposed to 125 mT stationary magnetic field, expressed as mean ±standard error. Exposure times were 24 h (A4), chronic exposure (A5), unexposed seeds (C1), pre-treatment (P1) and unexposed seeds (C2).

Dose	R^2	G_{max} (%)	Time (hour)					
			T_1	T_{10}	T_{25}	T_{50}	T_{75}	MGT
C1	0.99	72 ±6.7	25.2 ±2.64	36.48 ±1.68	44.4 ±0.96	54.00 ±0.48	65.28 ±0.96	56.16 ±0.24
A4	0.98	71 ±4.4	27.36 ±2.40	37.44 ±2.88	44.40 ±3.84	53.04 ±3.94	63.36 ±4.56	55.20 ±4.80
A5	0.99	80 ±4.3	28.32 ±2.16	36.48 ±1.68	41.76 ±1.44	48.72 ±1.20****	57.36 ±1.68****	50.88 ±1.68***
C2	0.99	80 ±4.7	14.88 ±2.88	21.60 ±3.12	25.92 ±1.44	30.96 ±0.48	36.24 ±1.68	31.20 ±0.72
P1	0.99	84 ±3.3	10.56 ±3.84	17.28 ±2.16**	21.84 ±0.48**	27.84 ±1.44	34.80 ±3.60	29.04 ±1.68

G_{max}: number of germinated seeds (%); MGT: Mean germination time; T_1, T_{10}, T_{25}, T_{50}, T_{75}: time required for 1, 10, 25, 50, and 75% of the seeds to germinate expressed in hours. R^2: correlation coefficient. Asterisks indicate differences versus control: ****(p<0.001): very strongly significant; ***(0.001<p<0.01): strongly significant; **(0.01<p<0.05): significant; *(0.05<p<0.1): differences.

exhibited marginally, but significantly higher root fresh and dry weights, total fresh weights, and higher germination rates [25]. A study to determine the effects of an electromagnetic field from a high voltage transmission line on the yield of agricultural crops cultivated underneath and near the transmission line was conducted. For 5 years, experiments with winter wheat and corn were carried out near the 380 kV transmission line Dürnrhor (Austria)-Slavetice (Czech Republic). Different field strengths were tested by planting the crops at different distances from the transmission line. The wheat grain yields were 7% higher (averaged for 5 years) in the plots with the lowest field exposure than in the plots nearer to the transmission line. The responses of the plants were more pronounced in years with drought episodes during grain filling than in humid years. No significant yield differences were found for corn yields, [26]. Effects of high-frequency, continuous wave (CW) electromagnetic fields on mung beans (*Vigna radiata* L.) and water convolvuluses (*Ipomoea aquatica* Forssk.) were studied at different growth stages (pre-sown seed and early seedling). The frequency used in the experiments was 425 MHz, the field strengths were 1 mW, 100 mW, and 10 W, and the exposure durations were 1, 2, and 4 h. Results show that germination enhancement is optimum for the mung beans at 100 mW/1 h power-duration level, while for water convolvuluses the optimum germination power-duration

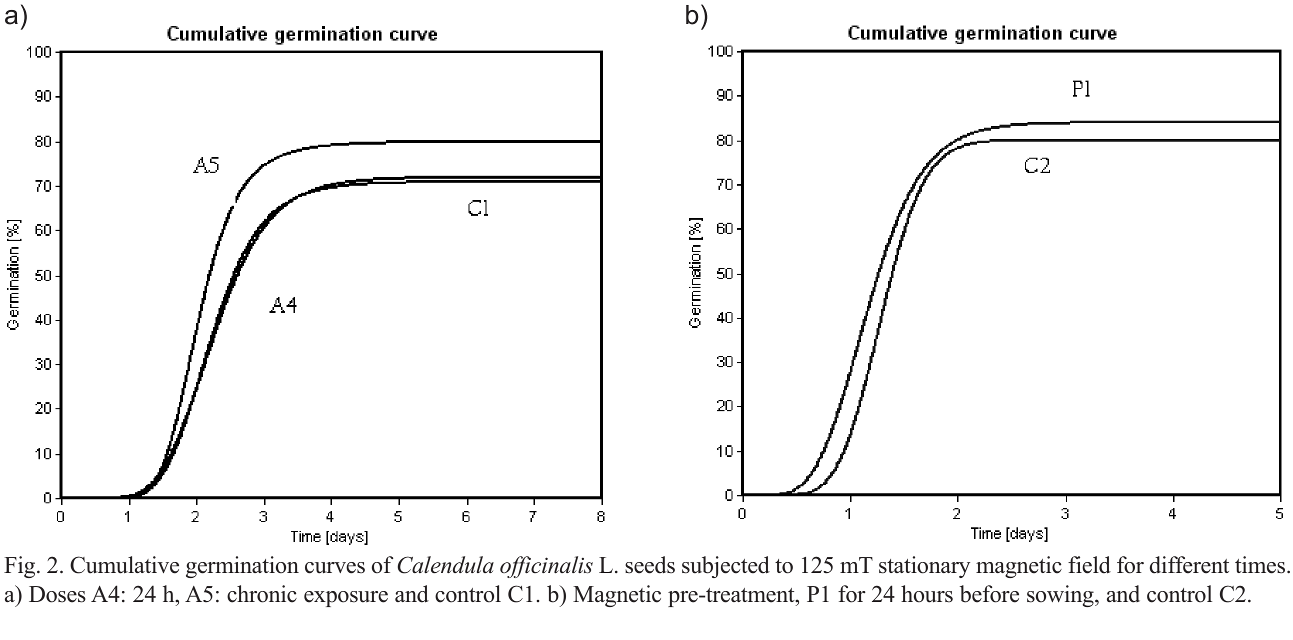

Fig. 2. Cumulative germination curves of *Calendula officinalis* L. seeds subjected to 125 mT stationary magnetic field for different times. a) Doses A4: 24 h, A5: chronic exposure and control C1. b) Magnetic pre-treatment, P1 for 24 hours before sowing, and control C2.

level was 1 mW/2 h. When both seed types were exposed at the early sprouting phase with their respective optimum power-duration levels for optimum seed growth, water convolvuluses showed growth enhancement while mung bean sprouts showed no effects [27]. The effect of magnetic field forces on asparagus seed germination and seedling growth was studied. Results showed that in a magnetic field, asparagus seeds imbibed and germinated more rapidly. Seed germination percentage and epicotyl and hypocotyl lengths were also significantly higher. In addition, the number of germinated *Ocimun basilicum* seeds, and the length of radical and primary stem were significantly higher with a static magnetic field [28, 29].

Plants react in many different ways to geomagnetic fields, either strong continuous fields or alternating magnetic fields, physiological investigations were pursued and any magneto-response was immediately obvious. Two mechanisms for magneto-reception are currently receiving major attention: the "radical-pair mechanism" consisting on the modulation of singlet-triplet inter-conversion rates of a radical pair by weak magnetic fields, and the "ion cyclotron resonance" mechanism [30].

Conclusion

The mean germination time (TMG) and parameters T_1-T_{75} were reduced for all magnetic doses applied, and rate of treated seeds is higher than the control one. Germination rate and percentage of germination of control seeds is also below that corresponding to magnetic treatments applied. In summary, stationary magnetic fields could be used as a physical technique to improve the germination of *Salvia officinalis* L. and *Calendula officinalis* L. seeds.

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