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Enhancing *Aloe Vera* growth and secondary metabolite production using an alternating magnetic field

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Abstract

A magnetic field can be used as a physical elicitor to increase secondary metabolites in medicinal plants. In this research, the effects of alternating magnetic stress on the production of flavonoids and growth parameters of *Aloe vera* were studied. The number of weeks, magnetic flux density, and exposure time are effective parameters that have been changed in this experiment. To apply an alternating magnetic field, a system was designed and built, including a Helm Holtz coil, auto-transformer, multi-meter, and Tesla meter. Control samples were grown without a magnetic field. All samples were kept in a growth chamber at a temperature of 24 ± 2 °C and a 16-hour light, 8-hour dark photoperiod. A factorial experiment based on RCD was used to test magnetic field effects on selected traits. According to the obtained results, the magnetic field was effective in secondary metabolite production. 38.44 mg/g DW flavonoid was produced with a flux density of 1.5 mT and 45 min daily exposure times for two weeks. The maximum number of leaves was observed in 0.5 mT and 135 min per day exposure times. In addition, the tallest plant was produced after three weeks with 0.5 mT. The week, exposure time, and magnetic flux density have a second-order interaction. Depending on various factors, the magnetic field can be harmful or beneficial for Aloe vera. In order to achieve the desired result, the optimum field should be used.

1. Introduction

Magnetic fields promote secondary metabolite production and can be used as a new approach to increase their amount. Magnetic fields also affect plant growth. Biophysics methods can affect the growing process of plants through energy transformation. Biological systems are capable of responding to stress or applied external factors. The plant growth will be affected by monochromatic light, electricity, magnetism, and noise. This technology is called Electro-culture. *Aloe vera* plants, with different metabolites, are one of the most special cases in food and pharmaceutical research. However, there are few examples of using magnetic fields to increase seed germination and plant growth (Baghel *et al.*, 2019; Bezerra *et al.*, 2023).

A magnetic field can influence ion exchange across the cell membrane. When materials with magnetic properties are present within the cell, they can enhance the effectiveness of this magnetic field. Understanding the mechanisms

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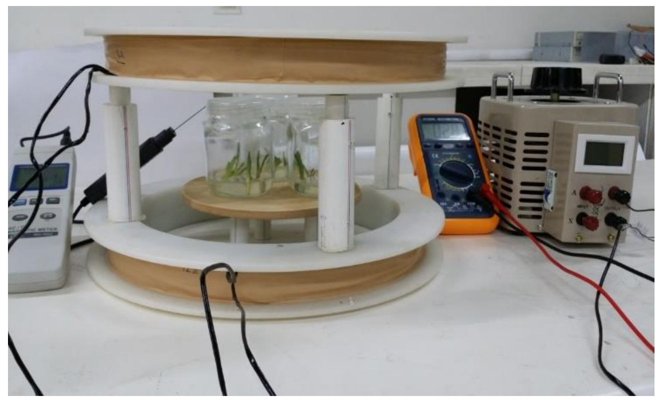
behind this effect on cell behavior in an electric culture demands further research by biologists and specialists in related fields. Most studies examining the impact of magnetic fields on germination utilize magnets or direct currents, as these are generally simpler to generate. However, alternating magnetic fields may produce distinct effects; for example, at a frequency of 50 Hz, the north and south poles switch positions 50 times per second. In this research, the effects of alternating magnetic stress on the production of flavonoids and growth in *Aloe vera* were studied in tissue culture conditions.

2. Materials and methods

Plants were prepared and obtained from the Iranian Research Organization for Science and Technology's greenhouse. The apical meristem of *Aloe vera* was used as an explant. The plants were washed under running water for 30 min, and their surface was disinfected using 70%(v/v) of ethanol for a min, followed by 15% aqueous sodium hypochlorite solution for 20 min, then rinsed three times in sterile distilled water. Plant samples were kept in a growth chamber at a temperature of 24 ± 2 °C and 16 hr light/8 hr dark photoperiod in Murashige and Skoog medium. The different parameters studied included a three-week time period (1, 2, and 3), magnetic flux density (0.5, 1.5, and 4.5 mT), and exposure time (15, 45, and 135 minutes). Control samples were grown without a magnetic field. Finally, growth parameters and secondary metabolic content (flavonoids) were measured as the active ingredient. In order to apply an alternating magnetic field, a system was designed and constructed, including a Helm Holtz coil, auto-transformer, multi meter, Tesla meter, and the position of the samples. The Helm Holtz coil had a radius and average height of 18 cm and consisted of two coils designed to produce a uniform magnetic field. The pulleys, made from polyethylene and shaped with Teflon machining, served as the foundation for each coil. Around each pulley, 500 turns of copper wire, with a 1 mm diameter, were wound. A sinusoidal alternating magnetic field was generated by applying a 50 Hz alternating current to the coils, connected in a series circuit. An electrical transformer (PDGC2-2, Micro Company, China) with a single coil for voltage regulation managed the current flowing through the coils. A multi-meter (VC9805, Gilsun Company, China) was placed in series between the transformer and Helm Holtz coil to monitor current. A Tesla meter (MG-3002, Lutron, Taiwan) was used to measure and detect the magnetic field.

Figure 1: System for Applying an Alternating Magnetic Field to a Plant



2.1. Quantitative analysis of morphological traits

Growth and developmental characteristics were measured, including plant height (cm) and number of leaves.

2.2. Determination of flavonoid content

Total flavonoid was measured using the method in Xu et al. (2005). According to this protocol, we used 80% ethanol to release and extract flavonoids from 0.2 g of finely dried powder with 20 ml of *A. vera* leaf. Quantitative determination of the flavonoids was done using $AlNO_3$ reagent as described by Khan et al. (2018). The O.D.of the samples was measured at 510 nm wavelength by a spectrophotometer. The standard curve was plotted using quercetin. Each sample was assayed three times, and the average value was taken.

2.3. Statistical analysis

A factorial experiment with a completely randomized design was conducted and replicated three times to evaluate growth parameters and flavonoid content. Each experimental unit consisted of three plants. Data analysis was carried out through ANOVA in MiniTab17, and Tukey's test was applied for mean comparisons in all experiments (Radhakrishnan et al., 2012).

3. Results and discussion

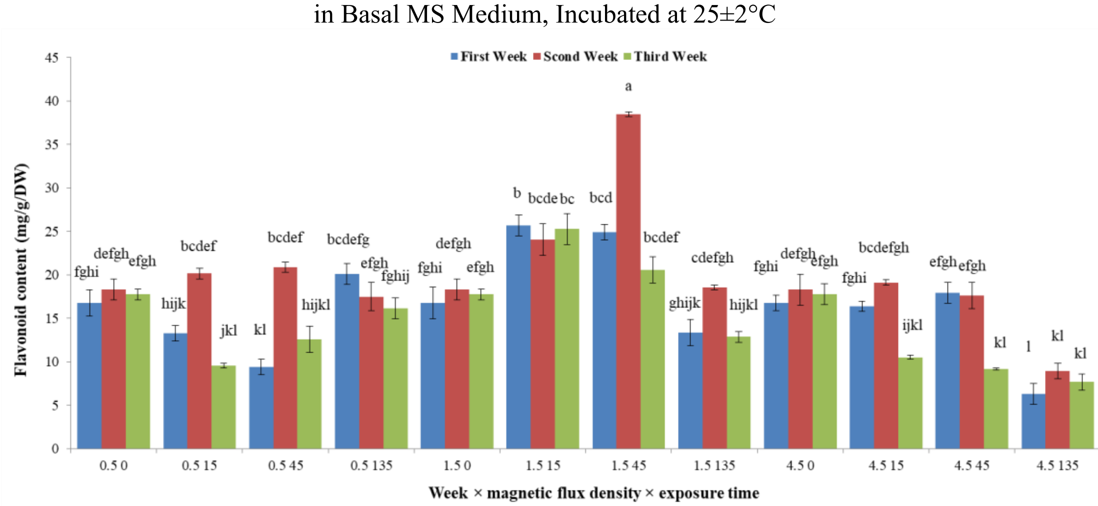
The results show that the magnetic field effectively elicits secondary metabolite production (Table 1). Duration of use, exposure time, and the magnetic flux density have a second-order interaction. The negative and positive bio-stimulations were found in some combinations of time (week) and magnetic flux density, highlighting the importance of finding a combination of parameters to produce favorable bio-stimulation effects in different treatments.

Table 1: *Analysis of Variance for Flavonoid Content, Height, and Leaf Number in an Alternating Magnetic Field*

S.O.V	Df	Ms. of flavonoid (mg/g DW)	Ms. of plant height (cm)	Ms. of leaf number
Time (week)	2	169.94**	2.34**	0.173**
Magnetic flux density (MFD)	2	357.99**	0.63**	0.086**
Exposure time (ET)	3	109.88**	0.0.049**	0.03**
MFD ×Week	4	5.90 n.s	0.039**	0.003n.s
ET ×Week	6	37.11**	0.012 n.s	0.004 n.s
ET × MFD	6	125.58**	0.163**	0.044**
ET × MFD × Week	12	22.13**	0.009 n.s	0.003 n.s
Error	36	2.63	0.006	0.002

Variance analysis to 1% level of significance, for flavonoid production in *A. vera*, showed that treating a plant for two weeks with 45 minutes daily exposure time to the magnetic field at 1.5 mT magnetic intensity produced 38.44mg/g DW flavonoid in *Aloe vera*, which was 56.37 % more than the control (Fig 2). There are negative and positive bio-stimulations depending on the magnetic flux density (MFD). By increasing MFD, the flavonoid production was decreased.

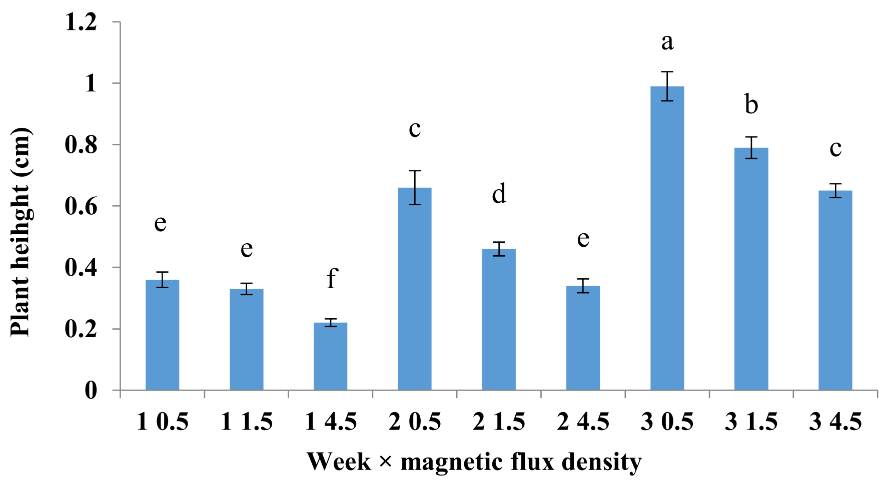
Figure 2: Effects of Week, Magnetic Flux Density, and Exposure Time Combination on the Flavonoid Content of A. Vera in Basal MS Medium, Incubated at 25±2°C



Note. Values are the mean of three experiments. 0.5 0 (0.5 mT, 0 minutes exposure time), 0.5 15 (0.5 mT, 15 minutes exposure time).

Additionally, a positive value for height was observed among the treated plants, with the application of 0.5 mT MFD for three weeks having the best results (Fig 3).

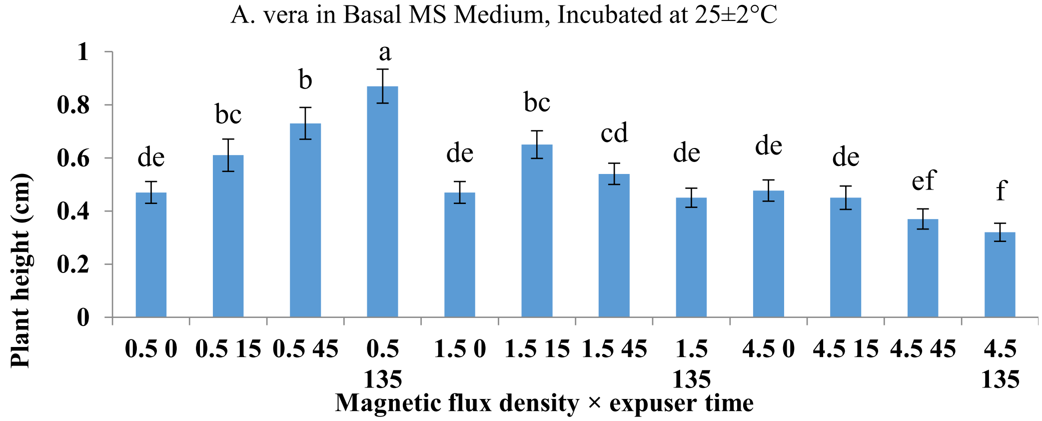
Figure 3: Effects of Magnetic Flux Density and Exposure Time Combination on the Plant Height of A. vera in Basal MS Medium, Incubated at 25±2°C



Note. Values are the mean of three experiments.1 0.5 (I week, 0.5 mT), 1 1.5 (1 week, 1.5 mT)

The results showed that a daily exposure of 135 minutes at 0.5 mT MFD significantly increased plant height compared to the control (Fig 4). Plant height improvement was generally higher than the control across most treatments, except at a magnetic flux density of 4.5 mT. Overall, *in vitro* experiments indicated that explant height varied based on the different combinations of exposure duration and MFD, as well as their interaction.

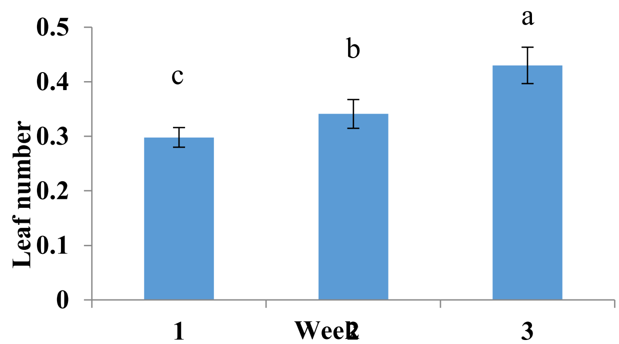
Figure 4: Effects of Magnetic Flux Density and Exposure Time Combination on the Plant Height of A. vera in Basal MS Medium, Incubated at 25±2°C



Note. Values are the mean of three experiments. 0.5 0 (0.5 mT, 0 minutes exposure time), 0.5 15 (0.5 mT, 15 minutes exposure time)

Furthermore, a three-week treatment led to a higher number of leaves than a one-week treatment (Fig 5). Leaf count tended to increase at 135 minutes of daily exposure with a 0.5 mT MFD treatment (Fig 6).

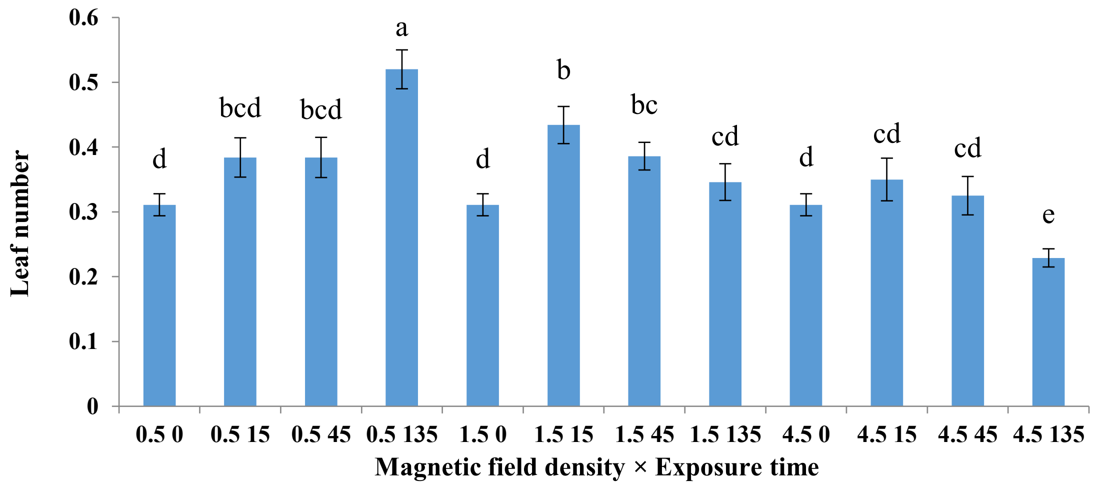
Figure 5: Effects of the Week on the Leaf Number of A. Vera in Basal MS Medium, Incubated at 25±2°C.



Note. Values are the mean of three experiments.

The findings suggest that the effects of the alternating magnetic field on leaf count depend on the interaction between magnetic flux density and exposure time. As both MFD and exposure time increased, the impact of the magnetic field on growth changed accordingly.

Figure 6: Effects of Magnetic Field Density and Exposure Time Combination on the Leaf Number of A. Vera in Basal MS Medium, Incubated at 25±2°C



Note. Values are the mean of three experiments. 0.5 0 (0.5 mT, 0 minutes exposure time), 0.5 15 (0.5 mT, 15 minutes exposure time)

The effect of magnetic field (MF) exposure varies depending on the concentrations of ions like magnesium and sodium. These ions interact with cellular components and resonate at frequencies optimal for DNA and RNA polymerases as well as adenosine triphosphate (ATP) catalyzing enzymes (Mghaiouini et al., 2020). Plants exposed to adverse conditions, including MF, salinity, and drought stress, immediately activate antioxidant defense systems to counter toxic reactive oxygen species (ROS), which leads to an increase in the activity of free radical ions (Vian et al., 2016; Kaur et al., 2021). "Weak (low) magnetic field" (WMF) generally refers to intensities between 100 nT and 0.5 mT (Hajnorouzi et al., 2011; Ercan et al., 2022).

Studies on the effects of MF on *Zea mays* seeds have shown that a low static magnetic field (50 mT) significantly enhances fresh tissue mass, nucleic acid levels, chlorophyll ratios, and levels of assimilatory pigments, while higher magnetic field intensities (100–250 mT) inhibit these parameters (Martinez *et al.*, 2017). In this study, low magnetic flux densities (0.5 and 1.5 mT) promoted flavonoid production and growth. Similarly, in date palm seedlings, a low magnetic field positively affected photosynthetic pigments, whereas high doses reduced chlorophyll a, chlorophyll b, carotenoids, and total pigment levels (Dhawi & Al-Khayri, 2009; Racuciu et al., 2015).

Sunita et al. (2017) observed that magneto-priming with a static magnetic field (200 mT for 1 hour) mitigates salinity's adverse effects on seedling germination and vigor by enhancing water uptake and hydrolytic enzyme activity. Furthermore, an increase in iron content was found in WMF-treated onion plants, while pulsed magnetic field (PMF) treatment triggered adaptive responses in salt-stressed calli, enhancing secondary metabolites such as alkaloids, saponins, and flavonoids. This aligns with our findings, where exposure to 1.5 mT magnetic flux density increased flavonoid production; however, higher MFD levels reduced flavonoid content in *A. vera*.

In this study, the maximum leaf count was observed with a 0.5 mT magnetic flux density and 135 minutes of exposure, consistent with Kataria et al. (2019) who reported that WMF impacts photosynthesis and reduces chlorophyll content in kidney bean leaves. Structured magnetized irrigation systems have been shown to improve yield, chlorophyll content, plant height, and biomass production. Magnetized water can thus be considered a valuable, safe, and cost-effective approach for improving yield while conserving water resources (Omid, 2016; Sarraf et al., 2020).

WMF exposure influences cell cycle duration by delaying the presynthetic (G1) phase while leaving other phase durations unaffected compared to geomagnetic field (GMF) controls (Mohammadi et al., 2018). Osmotic pressure measurements have also shown that cell sap from WMF-treated plants has higher osmotic pressure than GMF controls (Xia et al., 2024). Research has shown that phosphorus levels in citrus leaves increase with magnetic water treatment (Taimourya et al., 2017). Bukhari *et al.* (2021) reported that in *Helianthus annuus*, 50 mT MF treatment for 45 minutes enhanced germination growth and antioxidant activity. Menegatti et al. (2019) observed that a 200 mT magnetic field in passion fruit enhanced germinating energy, fresh weight, and shoot length. These findings agree with our results, where 135 minutes of daily exposure at 0.5 mT MFD stimulated plant growth. In *Oryza sativa*, 125 mT power frequency magnetic fields enhanced growth, supported by increased mitotic index and 3H-thymidine uptake (Florez *et al.*, 2019), a pattern similarly observed in *A. vera* in this research. In *Chlorella fusca*, applying 60 mT MF throughout cultivation increased biomass and carbohydrate content by 20.5% and 24.8%, respectively (Deamici *et al.*, 2016). For large-scale cultivation of *Salvia officinalis* seeds, a 15 mT, 5-minute treatment resulted in longer, heavier radicles (Abdani Nasiri *et al.*, 2018).

In summary, our *in vitro* experiments indicate that both flavonoid content and explant growth respond to different time exposures at MF intensities from 0.5 to 4.5 mT. Growth characteristics under a 0.5 mT magnetic field for 135 minutes were positively affected, while higher intensities (4.5 mT) reduced height, leaf count, and flavonoid content compared to controls. Thus, using magnetic fields as physical elicitors to boost active ingredient production in medicinal plants shows potential for future research.

4. Conclusion

Alternating magnetic field systems may serve as non-chemical, non-invasive, and non-destructive stimulants for valuable medicinal plants in greenhouses, growth chambers, manufacturing plants, and laboratories. The effects of magnetic fields on *Aloe vera* secondary metabolism appear variable, being either beneficial or harmful depending on field strength and exposure conditions.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Open access

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Not Applicable

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

Majid Masoumian: Supervisor, Conceptualization, Formal Analysis, Methodology, Writing – Review & Editing. Mohammad Zangi: Sample Collection, Laboratory Work, Data Collection.Rouzbeh Abbaszadeh: Co-Supervisor, Validation, – review & editing.

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