



Influence of Magnetic Fields on Thymol Biosynthesis in *Thymus vulgaris* L. Callus Tissues

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ABSTRACT

This study demonstrated the positive effect of the magnetic field (MF) on the germination of thyme *Thymus vulgaris* L. seeds, seedlings growth and initiated the callus from its stem parts. The results showed the success of cultured seedling stem pieces on Murashige and Skoog (MS) medium supported by Kinetin (K) and 2,4-Dichlorophenoxy acetic acid (2,4-D) with 0.5 and 1.0 mg/L respectively in high percentage of callus initiation, reaching 80%.

Exposure of thyme seeds to a magnetic field led to a high germination rate and seedlings growth, Also, when exposed its stem pieces to MF for different periods led to an increase in the callus initiation percentage, reaching its highest 95% at a period of 60 minutes. The study also proved the effective role of the MF in stimulating the growth of callus cells to grow reaching to produce the secondary metabolic compounds by increasing the callus cells, oil content, accompanied by an increase in the percentage of thymol (the main component of thyme oil, which has medicinal benefits) recording the highest oil content of 3.49% and thymol content of 42.01% for callus cells exposed to MF at 60 minutes compared to the unexposed callus group (control) with 1.94% callus oil content and 38.98% of thymol content.

Keywords: Magnetic field (MF), thyme (*Thymus vulgaris*), callus, oil content, thymol compound.

INTRODUCTION

Thymus vulgaris L. a member of the genus *Thymus* L. which belongs to the family Lamiaceae, which has around 220 genera and 4000 species, grows well in a dry, temperate, sunny, and hot climate (Vouillamoz and Christ, 2020). Thyme grows as a perennial, wild, and woody flowering shrub, reaching a height between 5 to 30 cm. It is characterized by its small green leaves, which range in length from 2.5 to 5 mm, and its pink or purple flowers that bloom in clusters. It has been widely utilized in traditional medicine for centuries due to its strong antimicrobial, antiviral, and antiseptic effects (Hosseinzadeh *et al.*, 2015; Waheed *et al.*, 2024). According to the studies thyme contains approximately 56.53% monoterpenes, 28.69% monoterpene hydrocarbons, 5.04% sesquiterpene hydrocarbons, and 1.84% oxygenated sesquiterpenes (Almanea *et al.*, 2019) and it is a rich source of flavonoids and phenolic antioxidants, including zeaxanthin, lutein, apigenin, luteolin and thymonin (Dauqan and Abdullah, 2017). The main constituent of thyme essential oil is thymol (2-isopropyl-5-methylphenol) Fig. (1), a primary monoterpene phenolic compound that contribute significantly to thyme's antioxidant properties. It is also known as hydroxy cymene, a white crystalline compound, characterized by its strong aromatic odor (Gholami-Ahangaran *et al.*, 2020).

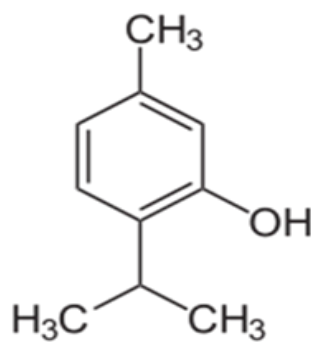


Fig. 1: The chemical structure of thymol compound (Waheed *et al.*, 2024).

Thymol essential oil is fully soluble in alkaline aqueous solutions, organic solvents, and alcohols. However, its solubility in water at neutral pH is relatively low. Additionally, thymol is utilized in medications targeting the respiratory and digestive systems, as well as in wound healing, and fungal and bacterial infections (Alagawany *et al.*, 2021).

The magnetic field is a type of force that forms in the space surrounding a magnetic material or a conductor carrying an electric current (Marycz *et al.*, 2018). Additionally, plants on earth are affected by the earth's natural magnetic field, which typically ranges in strength between 50 and 60 μ T (Minorsky, 2007). Many studies have investigated the effects of magnetic field (MF) exposure on seed germination, with several researchers confirming its beneficial role in promoting germination (Occhipinti *et al.*, 2014 ; Sarraf *et al.*, 2020). Another study's findings that magnetic fields may help reduce oxidative stress in plants by enhancing the activity of antioxidant enzymes such as peroxidase, polyphenol oxidase (PPO), superoxide dismutase (SOD), and catalase (CAT) within plant tissues (Dhawi, 2014 ; Mansure and Yahya, 2024 ; Al-Tai and Mohammed, 2024).

This study aimed to explain the role of magnetic field (MF) on the growth of thyme seedlings cells resulting on callus tissues and demonstrated its effect on their callus cells oil content and the most benefit compounds as thymol compound content.

MATERIALS AND METHODS

Preparation of callus initiation MS (Murashige and Skoog, 1962) medium:

To prepare the MS medium of callus initiation and growth follow the same steps of MS method steps with the addition of the plant growth regulators Kinetin and 2,4-D at concentrations of (0.5 and 1.0) mg/L individually and in combination to stimulate the initiation and growth of thyme callus (El-Banna, 2017).

Production of sterile thyme seedlings:

Thyme plant seeds are surface sterilized through immersed them in a 3% of commercial sodium hypochlorite solution (NaOCl) for 10 minutes with continuous agitation (Al-Nuaimi *et al.*, 2012).

Subsequently, the seeds are rinsed twice with sterile distilled water. The sterilized seeds are cultured on the surface of 30 ml of MS medium, with 5 seeds per bottle and transferred in an incubation room under dark conditions at 24 ± 2 °C. After seed germination, which required 3 days from the time of planting, the seedlings are transferred to photoperiod conditions of 16 hours of light at an intensity of 1500 lux and 8 hours of darkness.

Callus initiation and growth:

Stem explants at about 2.0 cm were taken from two-week-old seedlings and cultured by placed them on the surface of MS solid medium alone (control) and the MS media (supplemented with K and 2,4-D at concentrations of 0.5 and 1.0 mg/L). All samples were incubated in the culture room under controlled conditions of 25 ± 2 °C and a photoperiod of 16 hours per day with a light intensity of 1500 lux.

Exposure of the callus to a magnetic field:

To study the effect of the static magnetic field on the initiation and growth of callus from seedlings stem used ring-shaped magnets (with a magnetic flux density of 1500 Gauss) from Central Scientific Company/ USA and exposed durations of 30, 60 and 90 minutes in the Physics Department Laboratories, College of Science, University of Mosul. These magnets are fixed to the outer surface of the flask at a distance of 1.5 cm from the cultured stem explants Fig.(2). After 15 days of culture, the callus initiation percentage and fresh weight of the callus were measured.



Fig. 2: Thyme initiation callus under the effect of magnetic field.

The effect of the magnetic field on callus initiation rate:

The initiation percentage of the callus growth on standard MS alone (control) which supplemented with the K and 2,4-D at concentrations of 0.5 and 1.0 mg/L of them after 15 days from seedlings stem explants cultured exposure to the magnetic effect.

The effect of the magnetic field on fresh weight of callus:

The fresh weight of the callus developed from thyme seedlings stem explants is determined for all exposure and un exposure samples by calculating the difference between the weight of the culture containers containing only the medium and their weight after 20 days of the callus is initiation.

Determination the oil content of stem callus under the effect of the magnetic field:

The oil content of the thyme callus cultures for all treatment as:

- A. Callus after 20 days of growth on MS medium supplemented with K and 2,4-D at concentrations of 0.5 and 1.0 mg/L (control).

- B. Magnetic callus after 20 days of growth on MS medium supplemented with K and 2,4-D at concentrations of 0.5 and 1.0 mg/L exposed to MF at 30 min.
- C. Magnetic callus after 20 days of growth on MS medium supplemented with K and 2,4-D at concentrations of 0.5 and 1.0 mg/L exposed to MF at 60 min.
- D. Magnetic callus after 20 days of growth on MS medium supplemented with K and 2,4-D at concentrations of 0.5 and 1.0 mg/L exposed to MF at 90 min.

All types of oil above extracted by Soxhlet (VELP SCIENTIFIC, SER 148, Italy) solvent extractor (Palmieri *et al.*, 2020) according to the following steps (Mahdi and Yahya, 2022):

1. The callus dried in a thermal oven (Gallen Kamp oven BS Model, England) at 50°C for 45 minutes.
2. 5g from each dried sample is placed inside the extraction cell tube (Thumble) and in a flask filled with petroleum ether as solvent for 3 h. (Al-Unis, 2019).
3. The extraction device is connected to the solvent to extract oil from the samples using petroleum ether solvent. The extraction cell tube containing the sample is inserted into a flask filled with the solvent, and then the extraction device is disassembled, the petroleum ether is separated from the oil by vacuum distillation; the extraction process is repeated until the required amount is obtained, finally, the extracted oil is calculated using the following equation (Al-Hajjar, 2015).

$$\text{Oil concentration (\%)} = (\text{Weight of extracted oil} / \text{Weight of sample}) \times 100.$$

The effect of magnetic field on the concentration of the thymol compound in the thyme callus using Gas Chromatography (GC) technique:

The Gas Chromatography (GC) technique used for separating thymol compound from all callus samples mention above by using of a gaseous mobile phase, and the sample introduced into the separation column as its gaseous state. The stationary phase represented by a copper column (30 meters in length), and a mobile phase (inert gas such as argon or helium). At the same time, they carry the active compounds present in the sample, with temperatures inside the GC device sometimes reaching up to 200°C. The samples are introduced by direct injection at the top of the separation column using a special rubber septum. The gaseous mobile phase carried the sample through the column, where its components are separated and then detected at the end using the GC device (Shimadzu, Q 2010, Japan). The analysis is conducted under operating conditions using helium as the carrier gas with a purity of 99.999% and a flow rate of 1 mL/min. The temperature program ranged from 50°C to 280°C. The GC analysis is conducted under the following conditions: an Elite-1 column made of fused silica (HP-5MS, 30 mm × 0.25 mm) operating in electron impact mode at 70 eV. An injection volume of 0.5 µL is used, with the injector temperature set at 250°C and the ion source temperature at 280°C. The oven temperature was programmed starting at 60°C (isothermal for 2 minutes), then increased at a rate of 10°C/min to 270°C, followed by a rate of 5°C/min to 290°C, and finally held isothermally for 9 minutes at 310°C.

The compounds are identified by comparing the mass spectra of the unknown component (Thymol) with reference data stored in the National Institute of Standards and Technology (NIST) database to confirm the structure, name, and molecular weight of the tested substances. The measurements are carried out at the University of Technology, Ibn Al-Bitar Center, Baghdad.

RESULTS AND DISCUSSION

Production of sterilized seedlings:

The results of surface sterilization of thyme seeds using 3% commercial NaOCl solution demonstrated its efficiency in producing healthy seedlings with high efficiency and vitality Fig. (3). Sterilization plays essential role in producing healthy plant for successful vegetative propagation (Muresan *et al.*, 2021). Moreover, the findings highlight the importance of sterile solutions, their concentrations, and the sterilization period, in addition to maintaining a balance between plant cell

viability and the effective elimination of contaminants. These two-week-old seedlings are then used as a source for obtaining explants to induce callus initiation.



Fig. 3: Thyme seedlings grown on MS medium after 15 days.

Callus initiation

The results indicated that the use of plant growth regulators K and 2,4-D in this study had a clear effect on the variation in callus initiation and growth from thyme seedling explants cultured on MS solid medium depending on the type and concentrations of growth regulators used (Table1). The addition of either K or 2,4-D alone slightly stimulated (almost failed to induce) callus. The results indicated that the MS solid medium supplemented with 0.5 and 1.0 mg/L of K and 2,4-D respectively was the most effective treatment for inducing and promoting callus initiation from seedlings stem explants, achieving 80% which considered as standard medium for thyme callus initiation (Table1).

So, the initiation of callus from any plant seedlings explants depending on the type and concentration of growth regulators and results obtained clearly indicated that callus formation was affected by the combination between auxin and cytokinin type. A ratio of auxins and cytokinin's greater than one in the nutrient medium was effective for the induction of callus in different plants (Nikam and Savant, 2009).

Table 1: Effect of K and 2,4-D on callus initiation percentage from seedlings stem explants of *Thymus vulgaris* after 15 days of growth.

MS solid medium Supplemented with: (mg/L)	Callus Initiation Percentage (%)		
K	0.0	0.5	1.0
2,4-D			
0.0	50	61	63
0.5	65	70	72
1.0	74	80	76

-Each value represented average of five replicates.

The Effect of magnetic field on callus initiation and growth.

The results showed a clear variance in the effect of magnetic field on the initiation percentage of the thyme callus according to the time of exposure (Table2), the exposure to the MF at 60 minutes gave the highest callus initiation on standard MS solid medium reached to 95 % Fig. (4-C) compared to the initiation percentage recorded for the callus un exposure to the MF (control) at the 80% Fig. (4-A). The results indicate the positive effect of the magnetic field in enhancing callus growth and induction which attributed to its division and differentiation. Studies have shown that the

magnetic field increases mitotic activity in meristematic cells, as documented in common bean plants (Mroczek-Zdyrska *et al.*, 2021). The increase in the exposure time to 90 minutes had a negative effect on callus initiation, reducing it to 55% Fig. (4-D). This indicates that prolonged exposure has a negative effect, which varies depending on the plant species (Sarraf *et al.*, 2020).

Table 2: The effect of magnetic field on the thyme callus initiation after 15 days of growth on standard solid medium.

Time of Magnetic Field Exposure (minutes)	Callus initiation Percentage %
0.0 (control)	80
30 minutes	85
60 minutes	95
90 minutes	55

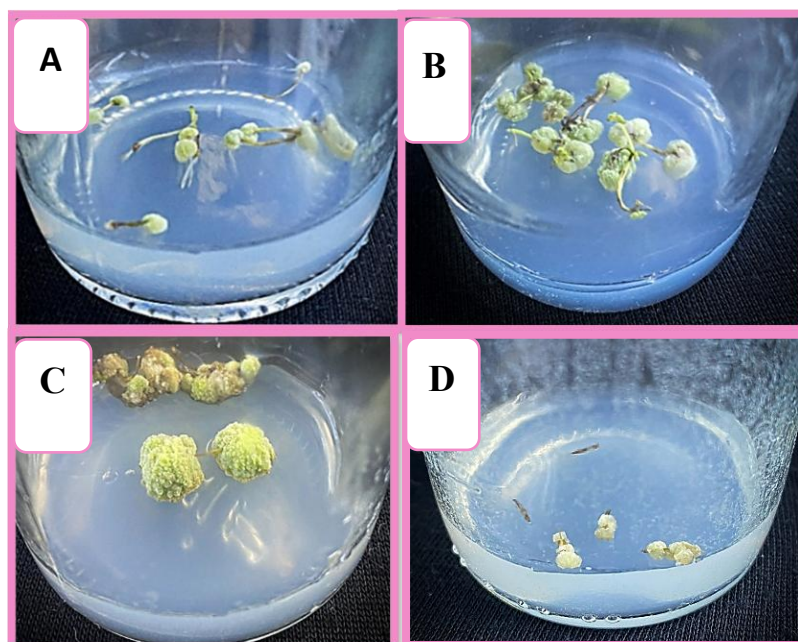


Fig. 4: Thyme seedlings stem callus initiation after 15 days of growth on standard MS medium for:
A. Normal callus (control). B. Magnetic callus at 30 min.
C. Magnetic callus at 60 min. D. Magnetic callus at 90 min

The Effect of magnetic field on the callus fresh weight of thyme seedlings stems.

The results indicate a difference in the average fresh weight of thyme seedlings stems callus in response to different durations of magnetic field exposure. Exposure to the magnetic field for 60 minutes resulted in the highest average fresh weight of 5.56 g after 30 days of culture. This was followed by treatment the with 30 minutes of magnetic field exposure, which yielded an average callus fresh weight of 3.63 g. In contrast, the callus exposure to 90 minutes showed negative effects on the callus fresh weight which reached to 1.12 g compared to the callus cultured on the MS solid medium without magnetic field exposure recorded the lowest fresh weight of 1.26 g. These results are attributed to the positive effect of the magnetic field in increasing the fresh weight of the callus (Hassanpour, 2024). The magnetic field stimulates cell division and affects cell membrane permeability, thereby enhancing the absorption of nutrients and plant hormones from the culture medium, which contributes to accelerating growth and biomass accumulation (Nasiri *et al.*, 2022).

However, prolonged exposure may lead to cellular stress, resulting in a reduction in callus growth (Kahrizi *et al.*, 2013).

The Effect of magnetic field on the thyme callus oil content

Results showed a significant increase in oil content of thyme stem callus exposed to the magnetic field, with the highest oil content of 3.49% recorded in the callus exposed for 60 minutes. Compared to the unexposed callus which showed the lowest oil content, reaching 1.94% (Table 3).

Additionally, an oil content of 2.14 % was recorded in the callus exposed to the magnetic field for 30 minutes. While the negative effect of magnetic field on the oil content of the stem callus observed on callus exposed to 90 minutes recorded the lower content at 1.22 %. These results suggest that exposure to magnetic fields can promote oil production in thyme callus, with the effect varying according to the treatment conditions. Previous studies have indicated that exposing aromatic plants to magnetic fields can activate metabolic pathways involved in the synthesis of secondary compounds, thereby enhancing the production of essential oils (De Souza *et al.*, 2014). Moreover, research has demonstrated that magnetic fields can affect the activity of enzymes and proteins responsible for the biosynthesis of aromatic compounds, which contributes to a higher percentage of essential oils in medicinal plants such as thyme and mint. Furthermore, certain studies have reported that subjecting plants to low-intensity magnetic fields for controlled periods can improve nutrient absorption efficiency, ultimately improve essential oil yield and increase its concentration in plant tissues (Hozayn and Qados, 2010; Yahya, 2022).

Table 3: Effect of magnetic field on the fresh weight of thyme seedlings stem callus

Time of Magnetic Field Exposure (minutes)	Callus Fresh Weight Average (gm)
0.0 (control)	1.26
30	3.63
60	5.56
90	1.12

- Each value represented average of five replicates

Extraction and determination of the thymol compound on the callus oil using Gas Chromatography (GC) technique.

GC analysis of the oil extracted from the thyme seedlings stem callus revealed the presence of thymol as the major compound at different concentrations in all samples significantly the standard thymol measured by GC techniques Fig. (5).

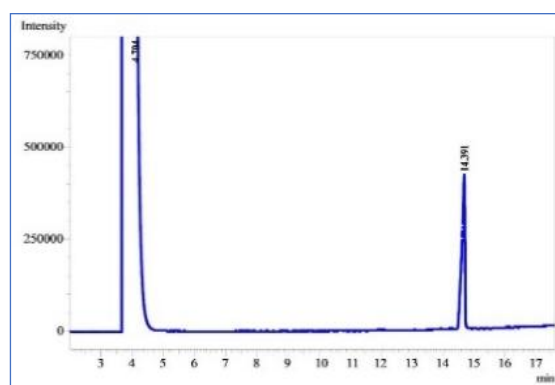


Fig. 5: GC analysis curves of Standard thymol compound.

Also, the results of exposing the stem callus to the magnetic field showed an indistinct variance in thymol concentration among the samples, depending on the duration of exposure, and showed

differences in the peak area of thymol extracted under different magnetic field exposure durations (Table 4).

Table 4: Oil content % of the seedlings stems callus of thyme (*Thymus vulgaris* L.) plant extracted after 20 days of growth on standard MS medium.

Time of Magnetic Field Exposure Minutes	Thyme Seedlings Oil Content %
0.0 (control)	1.94
30	2.14
60	3.49
90	1.22

The results referred that thymol compound appeared at the highest peak area in the callus exposure to magnetic field at 60 minutes reached to 1.9652% with thymol concentration at 42.01% Fig. (6-A), while the callus exposed for 30 minutes recorded a lower peak area of 1.9622% and 39.22% of thymol con. compared to the area of the of the unexposed callus (control) at 0.2388% of 38.98% thymol con. Fig. (6-B). These results indicate that exposing thyme callus to a magnetic field can directly influence thymol production. The magnetic field likely acts as a stimulant for specific metabolic responses in callus cells, serving as a potential catalyst that enhances the biosynthetic pathways responsible for essential oil formation, ultimately increasing the accumulation of active compounds such as thymol (Radhakrishnan, 2019; Saykat *et al.*, 2024).

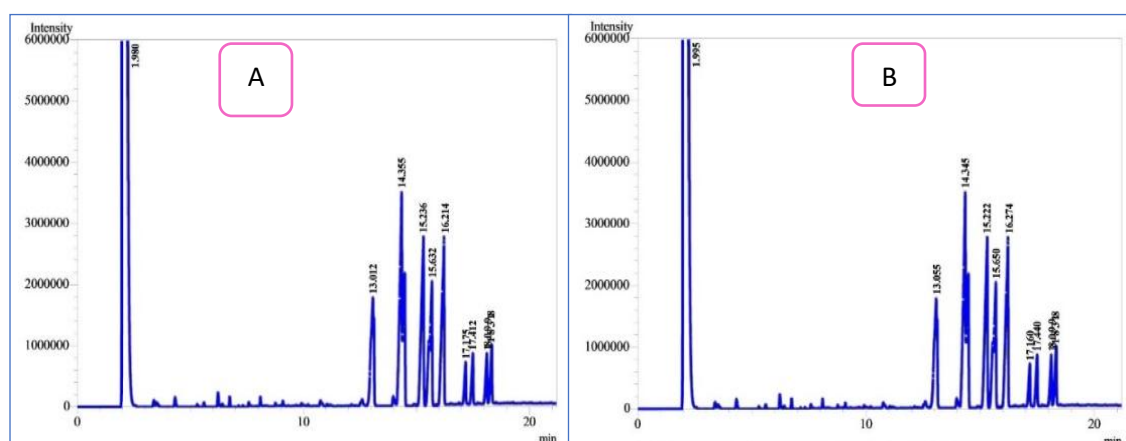


Fig.6: A. Oil extracted from thyme callus grown on standard MS medium (control).

B. Oil extracted from thyme callus grown on standard MS medium exposure to MF at 60 min.

While the decreasing on the thymol compound concentration observed on the thyme callus exposure to MF at 90 minutes recording 30.01% (Table 5). As indicated by some studies, the effective-ness of magnetic field effect on seeds depends also on the species, the cultivar and the time of exposure (Teixeira da Silva and Dobránszki, 2016).

Table 5: GC analysis of thymol compound identified from thyme (*Thymus vulgaris*) callus oil.

Time of magnetically treated thyme callus Min.	R. time(min)	Area (mv.s)	Area %	Callus thymol con. %
0	14.391	2807446	0.2388	38.98
30	4.355	31256025	1.9622	39.22
60	14.345	38520165	1.9652	42.01
90	14.300	31256005	0.1832	30.01

CONCLUSIONS

The current study succeeded in explain the prominent role of one of the types of physical influences present in our environment, represented by exposure to the magnetic field, in increasing the efficiency and medical value of medicinal plants with important pharmaceutical products, including the thymol compound with several medical benefits from the medicinal thyme plant, at the level of callus tissue cells.

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تأثير المجال المغناطيسي على محتوى مركب الثايمول لأنسجة كالس نبات الزعتر *Thymus vulgaris* L.**جمانة عبد الجبار احمد**

قسم علوم الحياة/ كلية العلوم/ جامعة الموصل

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الملخص

أثبتت هذه الدراسة التأثير الإيجابي للمجال المغناطيسي (MF) على إنبات بذور نبات الزعتر *Thymus vulgaris* L. ونمو بادراته وعلى استحداث ونمو الكالس من أجزاء سيقان هذه البادرات. أظهرت النتائج نجاح زراعة قطع سيقان البادرات على وسط Murashige and Skoog مدعماً بمنظمات النمو 2,4-Dichlorophenoxy acetic acid (2,4-D) و Kinetin (K) بتركيز 0.5 و 1.0 ملغم/لتر لكل منهما على التوالي في إعطاء نسبة استحداث عالية للكالس بلغت 80%. ان تعريض بذور نبات الزعتر للمجال المغناطيسي أدى الى إعطاء نسبة انبات ونمو عالية لتكوين بادرات جيدة النمو، وان تعرض قطع سيقانها للمجال المغناطيسي بفترات تعرض مختلفة أدى الى زيادة في نسبة الاستحداث بلغ اعلاها عند التعرض للمجال بفترة بلغت 60 دقيقة بإعطاء نسبة الاستحداث وصلت إلى 95%. كما اثبتت الدراسة الى الدور الفعال للمجال المغناطيسي في تحفيز نمو خلايا الكالس على النمو بها الى انتاج مركبات الايض الثانوي من خلال زيادة محتوى خلايا الكالس من الزيت مترافقا مع زيادة نسبة مركب الثايمول (المكون الأساسي لزيت الزعتر ذو الفوائد الطبية العالية) مسجلاً أعلى محتوى زيتي بنسبة 3.49% ومحتوى ثايمول بلغ 42.01% لخلايا الكالس المعرضة ل MF عند 60 دقيقة مقارنة بمجموعة الكالس غير المعرضة (المقارنة) بنسبة 1.94% من محتوى زيت الكالس و 38.98% من الثايمول.

الكلمات الدالة: المجال المغناطيسي، نبات الزعتر، الكالس، المحتوى الزيتي، مركب الثايمول.