

EFFECT OF MAGNETIC FIELD ON THE GROWTH, MULTIPLICATION AND CONCENTRATION OF THE VOLATILE OIL OF *ROSEMARY OFFICINALIS IN VITRO*Z. M. Abdul Husain
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ABSTRACT

This experiment was conducted at the plant tissue culture laboratory – Dept. of Horti.- Coll. of Agricultural Engineering Sciences – Univ. of Baghdad, in order to study the effect of magnetic field in the propagation and production of volatile oil compounds of rosemary plant *Rosmarinus officinalis*. Factorial experiments within completely randomized design with thirty replications were used. The results indicated an increase in the treatment of exposure to south pole for 30 days with the highest mean of the shoots number, fresh and dry weight and total chlorophyll content (7.5 shoots.plant⁻¹, 6634 mg, 769.5 mg and 160.6 mg.100 g⁻¹) respectively. While the control treatment produced the lowest value of shoot number, fresh and dry weight and total chlorophyll content (5.7 shoots.plant⁻¹, As shoots length, the exposure to south pole for 30 days, gave the shoot length (6 cm), while the exposure treatment to north pole for 20 days gave mean branch length of 2.25 cm. In terms of the effect of magnetic field on the concentration of volatile oil compounds, the results indicate that the exposure to south pole for 30 days significantly higher than the other treatments in the following compounds: linalool, Terpinene, linderol, Limonene cymene, and camphor penene. The 30-day north pole exposure was significantly higher in the two compounds: merycenen and sabinene.

Keywords: micropropagation, rosemary, secondary metabolites, magnetic technique, volatile oil

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تأثير المجال المغناطيسي في نمو وتضاعف وتركيز الزيت الطيار لنبات اكليل الجبل (*Rosmarinus officinalis*) خارج الجسم الحي

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باحثة

قسم البستنة-كلية علوم الهندسة الزراعية-جامعة بغداد

الشركة العامة للصناعات الهيدوليكية-وزارة الصناعة والمعادن

المستخلص

نفذت التجربة في مختبر زراعة الانسجة النباتية - الدراسات العليا- كلية علوم الهندسة الزراعية - جامعة بغداد بهدف دراسة تأثير المجال المغناطيسي على نمو وتضاعف وتركيز مركبات الزيت الطيار لنبات اكليل الجبل نفذت كتجربة عاملية وفق التصميم العشوائي الكامل (CRD) بثلاثين مكرر . اظهرت النتائج تفوق معاملة التعريض للقطب الجنوبي لمدة 30 يوم باعطاء اعلى معدل لعدد الافرع والوزن الطري والجاف ومحتوى الكلوروفيل الكلي حيث بلغ 7.5 فرع.نبات⁻¹ و6634 ملغم و769.5 ملغم و160.6 ملغم.100 غم⁻¹ بالترتيب بينما اعطت معاملة المقارنة(من دون التعريض للمجال المغناطيسي) اقل معدل لعدد الافرع والوزن الطري والجاف ومحتوى الكلوروفيل الكلي بلغ 5.7 فرع.نبات⁻¹ و4064 ملغم و471.4 ملغم و98.70 ملغم.100 غم⁻¹. اما بالنسبة لطول الافرع فقد اعطت معاملة التعرض للقطب الجنوبي المدة 30 يوم اعلى معدل لطول الافرع بلغ 6سم بينما اعطت معاملة التعريض للقطب الشمالي لمدة 20 يوم اقل معدل بلغ 2.25 سم . اما من ناحية تأثير المجال المغناطيسي على تركيز مركبات الزيت الطيار فالنتائج تشير الى ان التعريض للقطب الجنوبي للمدة 30 يوم تفوق معنوياً على باقي المعاملات في زيادة المركبات التالية : - linalool Terpinene و linderol و Limonene cymene و camphor penene في حين ان معاملة التعريض للقطب الشمالي لمدة 30 يوم تفوق معنوياً في زيادة المركبين التاليين merycenen و sabinene.

الكلمات الافتتاحية: الاكثار الدقيق، اكليل الجبل، المركبات الثانوية، التقنية المغناطيسية، الزيوت الطيارة

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INTRODUCTION

Rosmary (*Rosmarinus officinalis* L.) is a medicinal plant that is an perennial herb with fragrant that follows the Lamiaceae family, has dense growth and strong perpendicular spherical branches. It is used in the preservation of foods for its anti-bacterial and anti-oxidant effect, in addition to improving its flavor (16), for the effectiveness of volatile oil, the plant is used in aromatherapy (10). The leaves and apical meristem used in the treatment of nervous weakness, depression, blood circulation, accompanying fainting, improvement of digestion, headache, rheumatism, cold, treatment of arthritis, and treatment of hair loss, rejuvenation and growth by stimulates blood flow in the scalp. Beside, it is used in manufacture of soap and shampoo (13,26). The propagation through tissue culture has been advantages, not limited to a certain planting date, and small space required to produce large number of plants, as well as the control to disease infections. As for the production of secondary compounds in this technique, it leads to an increase in the amount of active substance as well as to give a substance of high purity. The propagation and the increase of active substances may be influenced by several factors, including the physical, such as the magnetic field (30). Magnetism is the most effective property in the plant; the earth is a magnet with a magnetic field of 0.5 gauss (22). All the materials on the ground acquire magnetic materials that vary according to their internal atomic structure. The internal molecules of living organisms follow this magnetic field (18). The growth and development of the plant is influenced by the magnetic field as the plants respond to this field and the importance of the results obtained on the role of the magnetic field in improving the growth of field plants encouraged the study of the effects of magnetic properties in the growth of plant parts in vitro (20), exposed the parts of potatoes grown in vitro to a magnetic field with a magnitude of 6,4,2 militeslas, the intensity of 4 militeslas was the best in improving the growth rate and there is no effect of polarity. While successful of other researchers (7), when they exposed parts of soybeans grown in vitro to the media of

Gamborg to a magnetic field using metal magnets 2.9-4.6 militeslas for three periods 2.2, 6.6 and 19.8 seconds in increasing branches compared to non-magnetic-exposed parts. The percent of branches formations was 86.96% compared to 61.9% for non-magnetic exposure parts, and the duration of 2.2 seconds was the best in increasing fresh weight and concentration of chlorophyll exposed to the magnetic field compared to non- exposed. Lenuta *et al.*(19) grew potato branches invitro and exposed it to a magnetic field using metal magnets for 14 and 28 days days in both cases, which improved vegetative growth and increased pigment of chlorophyll a, b and carotenoids. Al-Bashi (2) studied the effect of the exposure period of the gardenia branches grown on MS medium to the magnetic field of the south or North Pole with intensity of 200 militeslas in the growth and multiplication of branches. During the multiplication stage, the branches were exposed for 0, 10, 20, 30 or 40 days. After 8 weeks of branching, results showed that 20 days of exposure to south increased significantly branches number, fresh weight and branches lengths compared to the control treatment. The treatment of exposure to the North Pole increased significantly the number of branches for 10 days and significantly increased the length of the branches for 20 days exposure compared with the treatment which is did not exposed to the magnetic field. A number of studies were revealed that the magnetic field increased the growth of callus and the production of active compounds invitro The aim of this study is to demonstrate the effect of the magnetic field (northern and southern) poles on the growth, multiplication and increase of active compounds in vitro.

MATERIALS AND METHODS

This study was carried out in the plant tissue culture laboratory - post graduate - College of Agricultural Engineering Sciences - University of Baghdad. The plants were obtained from the Research Unit of Medicinal and Aromatic Plants of the same college. Apical buds were taken from two-year-old plants for the propagation purposes. All tools, such as blades, tweezers and petri dishes, were sterilized using an Autoclave at 121 ° C, 1.04 kg. cm⁻² pressure for 30 minutes, As well as

ethyl alcohol with concentration of 99% was used in the sterilization of the tweezers and blades, Bunsen burner was used after each use. The medium was sterilized by Autoclave vacuum cleaner at 121 ° C and under pressure of 1.04 kg.cm⁻² for 15 minutes. The medium was cooled at room temperature until it was ready for use. Apical buds were washed with running water and a little liquid soap, then washed well and left under tap water for 30 minutes. Explants were sterilized using bleaching agent (Fas - Active ingredient 6%) at a concentration of 0.9% for 15 minutes with continuous stirring and then washed with distilled water for five minutes three times to remove bleaching effect. The Apical buds were then inoculated on MS medium containing 0.5 mg. L⁻¹ of BA with 0.1 mg.L⁻¹ of NAA and then transferred to MS medium containing 0.5 mg. L⁻¹ of BA for the purpose of multiplication. The shoots (1 cm) were exposed to oval metal magnets with an intensity of 2000 gauss per magnet piece for north and south poles for three durations 10, 20, or 30 days. Thirty replicates for each treatment were used. The magnetic pieces were mounted on the outer surface corresponding to the shoots by the transparent adhesive tape and 1 cm away from shoots. After 6 weeks; measurements were taken including the number and length of branches, the fresh and dry weight and the concentration of the main compounds of volatile oil.

Extraction and estimation of volatile oil%

Extraction of the volatile oil was done using a Clevenger device and water distillation. Moist sample was placed in a 1 liter flask connected to the device and aliquot to the sample 250 ml distilled water was added and then distilled for three hours at 100 C° (27). The samples were placed in sealed tubes and placed in a dark box. The samples were then stored in the refrigerator at a temperature of 4 °C in the laboratory for qualitative analyzes (3).

Separation of volatile oil components using gas chromatography

GC was used for the separation of the volatile oil components of the Rosemary plant model Shimadzh Zolo, connected to the Flame ionization detector using helium gas as a carrier gas with a flow velocity of 1 ml.minute⁻¹, and the use of an HP-5MS separation

column of 30 m x 0.25 mm. Fifty mL of oil was taken and diluted with ethyl ether to 0.5 ml and injected with the device. The injector temperature and the detector were 250°C and 320 °C respectively. The column temperature was regulated from 70 °C to 250 °C at rate of a temperature of 10 °C.min⁻¹. All measurements were taken using the internal data processing program. The components were detected by mass spectrometry. The following compounds were found: Camphenen, Limonene, Penene, Camphor, Cymene, Linalool Merycenen, Linderol, Terpinene, and Sabinene.

Statistical analysis

Statistical analysis of the data was performed by using ANOVA we applied factorial within completely randomized design with thirty replications. Least significant differences (LSD) were used to compare between means at 0.05level.

RESULTS AND DISCUSSION

Effect of magnetic field on vegetative growth

Shoots number

The results of statistical analysis reveal that there are no significant differences between magnetic poles on shoots number (Table 1). The south and north pole gave 6.7 and 6.33 shoot.plantlet⁻¹ respectively. The results indicate a significant effect for time of exposure on shoots, the exposure time for 30 days produced the highest shoots number (7.35 shoot.plantlet⁻¹) which significantly exceeded that of 10 and 20 days (6.55 and 5.65 shoot.plantlet⁻¹) respectively. The results of the interaction revealed that all treatments were significantly higher than control treatment. The highest shoot number was at exposure to south pole for 30 days (7.5 shoot.plantlet⁻¹), whereas the control treatment produced the lowest shoot number (5.7 shoot.plantlet⁻¹)

Table 1. Effect of magnetic field on shoots number of Rosemary plants (shoot.plantlet⁻¹)

poles	Exposure Time (day)			Poles mean
	10	20	30	
S	5.90	6.70	7.50	6.70
N	5.40	6.40	7.20	6.33
LSD		N.S		N.S
mean	5.65	6.55	7.35	
LSD		0.633		
Control		5.70		
LSD		0.874		

Shoot length (cm):

The results in table 2 show non significant differences between magnetic poles on shoots length. The south pole exceeded north pole giving 4.92 and 3.23 cm respectively. The results indicates a significant effect of exposure time on shoot length, the exposure time for 30 days produced the highest shoot length of 5.50 cm ,which significantly exceed than 10 and 20 days (2.9 and 3.8 cm) respectively. The interaction between magnetic poles and time of exposure revealed that exposure to south pole for 30 days had a higher shoot length (6 cm) which did not significantly differed from exposure to south pole for 20 days and for north pole for 30 days (5.4 and 5.0 cm) respectively. The lowest shoot length occurred after exposure to north pole for 20 days (2.25 cm).

Table 2. Effect of magnetic field on shoot length (cm) of Rosemary plants

poles	Exposure Time (day)			Poles mean
	10	20	30	
S	3.35	5.40	6.00	4.92
N	2.45	2.25	5.00	3.23
LSD	N.S			1.09
mean	2.90	3.82	5.50	
LSD	1.887			
Control	2.48			
LSD	1.781			

Shoots fresh weight (mg)

The results in Table 3 shows that the effect of exposure to magnetic poles, the south pole gave the highest shoot fresh weight (5591 mg), whereas the north pole had 5209 mg fresh weight. The Table shows that the effect of the time of exposure caused a significant difference between the periods 10, 20 and 30 days. The 30 days exposure significantly exceeded to the 10 and 20 days, which had the highest rate of fresh weight of 6232 mg, while the treatments of 10 or 20 days had 5335 and 4633 mg respectively. The interaction between poles and durations was significant; the exposure treatment for south pole for 30 days higher than other treatments, the highest rate of fresh weight was 6634 mg, while the control treatment showed the lowest average fresh weight of 4064 mg.

Table 3. Effect of magnetic field on shoot fresh weight (mg) of Rosemary plants

poles	Exposure Time (day)			Poles mean
	10	20	30	
S	4920	5220	6634	5591
N	4347	5451	5830	5209
LSD	586.5			338
mean	4633	5335	6232	
LSD	414.7			
Control	4064			
LSD	587.04			

Shoots dry weight (mg): The results in Table 4 indicated that the effect of exposure to magnetic south pole significantly superior to the north pole with a mean dry weight of 649 mg (Table 4). The results indicate a significant effect exposure time on dry weight, the exposure time for 30 days gave the highest dry weight of 723 mg , significantly exceeded that of 10 and 20 days (619 and 537 mg) respectively. The results of the interaction between magnetic poles and time of exposure revealed that the treatment of exposure to South pole for 30 days caused a higher dry weight (769 mg) which is significantly different from other treatments, whereas the control treatment showed the lowest dry weight (471 mg).

Table 4. Effect of magnetic field on shoots dry weight (mg) of Rosemary plant

poles	Exposure Time (day)			Poles mean
	10	20	30	
S	571	605	769	649
N	504	632	676	604
LSD	68.0			39.3
mean	537	619	723	
LSD	48.1			
Control	471			
LSD	68.1			

Chlorophyll a content (mg.100gm⁻¹):

The results of statistical analysis revealed that there is no significant effect between magnetic poles on chlorophyll a content (Table 5), the south and north pole gave 74.59 and 74.89 mg.100g⁻¹ respectively. The results indicates a significant effect for time of exposure on chlorophyll a content , the exposure time for 30 days gave the highest chlorophyll a content

(84.08 mg.100g⁻¹) significantly exceed that of 10 and 20 days (78.15 and 61.93 mg.100g⁻¹) respectively. As for the effect of interaction between poles and durations, the results showed a significant difference between the treatments. The exposure treatment to north pole for 20 days produced the highest content of 85.73 mg.100g⁻¹, which did not differ significantly from the exposure treatment for South pole for 30 days at 85.43 mg.100g⁻¹, while the lowest treatment was the 10-day exposure to North pole of 56.10 mg.100g⁻¹, which was not significantly different from the control treatment, which was 51.17 mg.100g⁻¹.

Table 5. Effect of magnetic field on chlorophyll a content (mg.100gm⁻¹) of Rosemary plant

poles	Exposure Time (day)			Poles mean
	10	20	30	
S	67.77	70.57	85.43	74.59
N	56.10	85.73	82.73	74.86
LSD		6.957		N.S
mean	61.93	78.15	84.08	
LSD		4.919		
Control		51.17		
LSD		6.36		

Chlorophyll b content (mg.100gm⁻¹):

As for the leaves chlorophyll b content, the results showed no significant differences between magnetic poles (Table 6). The results also indicates significant differences between exposure duration to magnetic field, the duration of 30 days exceeded other durations by giving the highest concentration of 73.78 mg. 100g⁻¹ fresh weight. As for as interaction between treatments, the highest treatment was exposure to South pole for 30 days (75.17 mg .100g⁻¹), while control treatment gave a minimum content of 47.53 mg .100g⁻¹

Table 6. Effect of magnetic field on chlorophyll b content (mg.100gm⁻¹) of Rosemary plant

poles	Exposure Time (day)			Poles mean
	10	20	30	
S	54.70	65.07	75.17	64.98
N	53.73	66.47	72.40	64.20
LSD		N.S		N.S
mean	54.22	65.77	73.78	
LSD		3.841		
Control		47.53		
LSD		4.964		

Total chlorophyll content (mg.100gm⁻¹):

The total chlorophyll was not significantly differed between the poles (Table 7). The south pole recorded 139.57 mg. 100 g⁻¹, while the north pole was 139.06 mg. 100 g⁻¹. While, there are significant differences between exposure duration, the exposure for 30 days was significantly higher than 10 and 20 days with total chlorophyll content of 157.87 mg. 100g⁻¹ compared to 143.92 and 116.15 mg.100g⁻¹ for the 20 and 10 days respectively. For the effect of interaction between the electrodes and the duration of exposure, the results indicate that the superiority of the exposure treatment to the south pole for 30 days (160.6 mg.100g⁻¹), whereas the lowest treatment was exposure to north pole for 10 days and control treatment (109.83 and 98.70 mg.100g⁻¹) respectively

Table 7. Effect of magnetic field on total chlorophyll content (mg.100g⁻¹) of Rosemary plant

poles	Exposure Time (day)			Poles mean
	10	20	30	
S	122.47	135.63	160.60	139.57
N	109.83	152.20	155.13	139.06
LSD		4.62		N.S
mean	116.15	143.92	157.87	
LSD		3.27		
Control		98.70		
LSD		4.29		

The results of Tables 1-7 indicates that the positive effect of the magnetic field in the studied vegetative traits could be due to the influence of the magnetic field on some of the plant's biological activates such as facilitating

water movement inside plant cells, that contact the food media by improving the permeability of the cellular membranes as well as increasing the ion exchange through the change of the osmotic potential outside and inside the cell (25,28). Exposure of plants to the magnetic field may cause increased absorption of mineral and its concentration in the leaves (15). As well as the role of the magnetic field in reducing the resistance of cellular walls to elongation of the cells during the growth process and thus facilitate their growth and development (23). Magnetic field plays a role in increasing the amount of RNA and chlorophyll within plant cells (8). Barefoot and Reich (1992) reported that exposure of the callus with the media to the magnetic field exhibit water enough energy to arrange random water ions in a regular form and thus will give him a high capacity to penetrate the walls of cells . This means that the magnetic field affects the bonds angles between the oxygen and hydrogen atoms by the water molecule and the water properties of water groups from 10-12 molecules to 6-7 molecules which leads to arrange water molecules in one direction and thus easy to enter into the cellular membranes and increase growth. The significance of the magnetic field is also the affected of Enzyme activity, protein synthesis, cell efficiency and thus its production (17). Many studies have agreed that exposing the vegetative parts grown invitro to the magnetic field improve vegetative growth as has been reported by other researchers (4,5,12,21,29).However, this study not agrees with (7,11,14).

Volatile oil components:

The results listed in table 8 indicate that exposure to south pole for 30 days significantly exceeds other treatments for the following compounds: linalool Terpinene linderol, Limonene cymene and camphor penene. While treatment of exposure to the North pole for 30 days significantly higher in the increase of the following compounds merycenen and sabinene. The concentration of the compound camphenen increased significantly when exposed to the North pole for 20 days. A reference to Table (8) indicates that the exposure of branches to the magnetic field led to increase the compounds of oils effective compared to non-magnetized and that may be due to the effect of the magnetic field to improve the metabolism of the plant or through its role in increasing the production of growth regulators especially cytokanins and auxins (6, 24),This reflects positively on increased absorption of nutrients and increased cell division, thereby increasing the levels of major compounds of active oils, These results are consistent with the findings of (24) in their ability to increase the amounts of alkaloids extracted outside the living body by exposing the Tangerine callus to the effect of the magnetic field. As well as the study carried out by (6) in its ability to extract additional quantities of active compounds from the callus of *Nigella sativa* plant exposed to magnetic field and cultivated in the media of MS and is equipped with Kin growth regulators and 2.4-D. In addition , it is agree with (1), which showed that exposure of the callus to the magnetic field has led to increased compounds of oils effective compared to the callus not exposed to the magnetic field.

Table 8. Effect of the magnetic field on volatile oil compounds concentration of Rosemary plant

Treatments	Camphene	Limonene	Penene	camphor	Cymene	Linalool	merycenen	linderol	Terpinene	sabinene
10S	1.68	0.50	12.58	8.38	1.12	0.70	1.09	2.56	0.10	7.77
20S	0.97	0.34	10.20	4.42	1.59	0.97	0.96	1.34	0.14	5.40
30S	0.24	0.76	17.02	12.70	3.28	2.10	0.73	3.88	0.37	13.45
10N	0.96	0.40	13.00	9.30	1.11	0.50	0.24	2.31	0.12	4.10
20N	2.40	0.52	10.51	8.24	2.87	1.76	1.48	2.51	0.35	12.95
30N	2.34	0.73	13.74	12.53	2.40	1.48	1.82	3.83	0.35	17.54
control	0.20	0.04	0.43	0.83	0.26	0.17	0.95	0.26	0.03	0.30
LSD	0.07	0.09	0.07	0.07	0.018	0.095	0.018	0.018	0.018	0.09

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