EFFECT OF GROWTH REGULATORS ON ESSENTIAL OILS COMPOSITION OF *Lavandula officinalis* TISSUE CULTURES.

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ABSTRACT

Shoot tips and leaves from *Lavandula officinalis* L. of mature greenhouse-grown plants were inoculated aseptically on callus initiation medium. Callus cultures were subcultured on MS-media supplemented with different growth regulators. The influence of growth regulators (2,4-D, BA, NAA and kinetin) on composition of the volatile compounds has been analyzed individually by gas chromatography (GC) for both types of callus tissues as well as the chemical analysis of *in vitro* produced plant leaves in comparison with the intact mature plants. Results obtained indicated that application of BA enhanced greatly the growth of shoot-tips derived callus which accounted about two times of that shown in MS-free-hormone medium, while application of kinetin is the most suitable regulating substance for enhanced leaves derived callus growth. Results also indicated that no essential oils were synthesized in shoot-tips and leaves derived callus originated from medium free from any growth regulators, while application of all growth regulators investigated enhanced markedly the biosynthesis of essential oils, which varied greatly at different extents. However, comparison of components, content of the oils and chemical composition between *in vitro* produced plants with those of leaves from plants growing in their natural habitat revealed that an increase in essential oil concentration accounted by 17% in *in vitro* plant and there is a profound differences in the composition of essential oils, but no considerable differences was observed in chemical composition.

INTRODUCTION

Lavender *Lavandula officinalis* L. is a small shrub with grey-green leaves and usually colored flowers. It originated around the Mediterranean, the Middle East, and Northern Africa. Yet, there are varieties found also in Canary Islands, India, and the great area of Asia Minor. Lavender is one of the most frequently used essential oils in aromatherapy. It is of great importance due to its contents of essential oils which are widely used in pharmaceutical preparations, perfumery and cosmetics (Font-Quer, 1978).

In recent years, there has been a revived interest in the cultivation of aromatic and medicinal plants. In most cases, however, methods are not easily available to improve these species by traditional breeding methods. The uses of tissue culture techniques may be overcome most of problems associated with the cultivation and production of some aromatic plants (Chaturvedi *et al.*, 1982). A major barrier to establishing tissue culture procedures for the commercial production of secondary metabolites has been the inability of

cultured plant cells to produce significant amounts of these compounds (Whitaker and Hashimoto, 1986). Recently, interest in this area is being stimulated by possible application of techniques such as single cell culture or using specific additives and genetic manipulation to enhance the biosynthesis of these compounds. Cell cultures permit large-scale production of useful substances without field planting (Arrebola *et al*., 1997). However, organized or differentiated cultures often accumulate high levels of secondary metabolites (Mulder-Krieger *et al*., 1988). The yield of many secondary products in plant tissue cultures is influenced by the composition of the nutrient medium particularly the type and concentration of the growth regulators. In this respect, the effect of some hormonal and nutritional factors on monoterpene synthesis in *Rosemarinus officinalis* and *Cnidium officinale* callus cultures were investigated (Tawfik *et al*., 1992, and Shin and Park, 1994). Accumulation of terpenoids by cell suspensions of *Lavandula angustifolia* was studied by Banthorpe *et al*. (1995)

Different authors used plant tissue culture techniques for production of essential oil and to compare between the *in vitro* cultures and the corresponding plants. In this context, production of monoterpene by tissue cultures of several *Mentha* species was reported by Charlwood and Charlwood, (1983). Moreover, Ceniza *et al*. (1992) studied the fatty acid composition of endosperm from coconut fruits as well as endosperm derived callus. Also, the lipids of the biomass of *Ruta graveolens*, grown *in vivo* and *in vitro* was studied (Asilbekova *et al*., 1993). Becker and Blechschmidt (1995) compared the sesquiterpenoid pattern of field collected samples of *Jamesoniella autumnalis* from several habitats with the *in vitro* cultures derived there from. The essential oils of transformed shoot cultures of *Pimpinella anisum* and their corresponding intact plant were compared by Salem and Charlwood, (1995). Recently, Rady and Youssef, (1999) compared the essential oil and fats from *in vitro* cultures and leaves of intact plants of *Laurus nobilis*. Also, Youssef and Rady, (2000) compared the essential oil content and composition of salt-stressed *Rosemarinus. officinalis* callus cultures and intact plant leaves.

This work aims to study the influence of different growth regulators in the growth media on the composition of the volatile compounds for both types of lavender callus tissues. Comparison of components, content of the oils and chemical composition between *in vitro* produced plants with those of leaves from plants growing in their natural habitat was investigated.

MATERIALS AND METHODS

Plant material:

Stem cuttings of *L. officinalis* L. were planted and grown in pots for 4 months in the greenhouse of National Research Centre. Giza, Egypt.

Preparation of explants:

Shoot tips and leaves from *Lavandula officinalis* L. plants var. *delphinensis* were gathered from the greenhouse, cut to about 2 cm in length and washed with tap running water. Under aseptic conditions, explants were immersed in 70 % ethanol for 30 (sec.) and transferred to a solution of 65 % Clorox (containing 5.25 % sodium hypochlorite) for 20 min, then finally washed four times with

distilled sterilized water.

Culture media, culture conditions and initiation of callus cultures:

Segments (1-1.5 cm) were placed onto MS (Murashige and Skoog, 1962) basal medium supplemented with 5.37 μM α-naphthalene acetic acid (NAA) + 4.44 μM 6-benzylaminopurine (BAP) (callus initiation medium). After one month, initiation of callus tissues was observed and cultures were transferred to the same medium for three months to obtain a profuse amount of callus tissues. Shoot tips and leaf derived callus tissues were transferred to MS media supplemented with different types of growth regulators, 2,4-dichlorophenoxyacetic acid (2,4-D), NAA, Kinetin (kin) and BA. The concentration of each hormone in the each medium was 22.620 μM. The media tested were as the follows : MS1 = MS basal MS2 = MS + 22.620 μM 2,4-D; MS3 = MS + 22.620 μM NAA; MS4 = MS + 22.620 μM Kin; MS5 = MS + 22.620 μM BA.

MS medium contained 3 % sucrose, 100 mg / l inositol and gelled with 0.7 % agar (Fluka BioChemica 05040) as the basal medium. The pH of the media was adjusted to 5.8 using 1 N of either NaOH or HCL, then autoclaved at 121°C at a pressure to 1.2 kg cm^{-2} for 20 min. Cultures were incubated in a growth chamber at 25 ± 2°C under a 16-h photoperiod (irradiance of about 40 μmol m^{-2} s^{-1} provided by cool white fluorescent lamps).

Culture growth :

For the determination of callus growth, callus tissues (250 ± 50 mg) derived from the two explants types were grown in the different media and incubated at the same conditions and data of fresh and dry weight were recorded after one month of cultivation. Data for callus growth were subjected to statistical analysis as described by Snedecor and Cochran, (1967). Five replicates were used for each treatment.

Establishment of micropropagated plants:

Shoot-tip explants of mature greenhouse-grown *Lavandula officinalis* L. plants were inoculated aseptically on Murashige and Skoog's (MS) medium supplemented with 4.5 μM BAP for shoot proliferation. After elongation of the shoots (within 30 days) the axillary buds developed and formed new shoots which excised and subcultured on multiplication media (MS + 4.5 μM BAP) for shoot multiplication. For root induction, individual shoots were transplanted onto rooting medium (MS + 9.8 μM of 3-indolebutyric acid (IBA)). The rooted plantlets were acclimatized in a controlled environment growth chamber. Plantlets were successfully transferred to the greenhouse and showed normal morphology when grown to maturity.

This part of study was performed to obtain plants produced from *in vitro* culture. The produced plants were subjected to essential oils analysis and compared with the essential oils of *in vivo* plants.

Extraction of essential oil:

The intact plant and *in vitro* produced plant leaves (500 g) was

separately subjected to hydrodistillation in order to yield the essential oil according to the Egyptian pharmacopoeia (1984). The callus tissue (10 g) was immersed in 100 ml CH_2Cl_2 at 5° C for 24 hr. The extract was concentrated to 20 ml and hydrodistilled at atmospheric pressure for 10 min to yield the essential oil according to Nabeta *et al*. (1983).

Gas chromatography (GC) analysis:

The essential oil components were analyzed by GC. The essential oil of each callus tissue types exposed to different growth media, as well as leaves of the intact plant and *in vitro* produced plants was individually fractionated by GLC with Varian, VISTA Series 6000, and FID detector. Stainless steel 3 % OV-101 (2m 1/8) column was used with temperature program of 80° to 200° at 4° /min. The injector and detector temperature were maintained at 180° and 220°, respectively. Nitrogen was used as a carrier gas at flow rate of 50 ml / min. The relative percent of each compound was determined according to the peak area by Varian 4270 integrator. The identification of the different components in the volatile oil was known by matching their retention times (Rt) with those of the authentic samples under the same conditions.

Chemical analysis:

Total carbohydrate percent and total soluble sugars in the dried material were determined according to Dubois *et al*., (1956). Total nitrogen was determined (on a dry matter basis) using the modified Micro-Kjeldahl method according to A.O.A.C. (1980). Potassium and phosphorous were determined according to the procedure described by Cottenie *et al*. (1982).

RESULTS AND DISCCUSION

Callus cultures growth:

Data in Table (1) show the influence of different growth regulators in the media on growth (fresh and dry weights) of shoot-tips and leaves derived callus tissues. Data clearly indicate that all the growth regulators investigated enhanced the callus growth at different extent and this hold true for both shoot tips and leaves derived callus. It could be observed that shoot-tip highest callusing (2.62 g.) was obtained when grown on MS-medium containing BA, which accounted about two times of that shown in MS-basal medium (control). The presence of 2,4-D or NAA in the medium gave the lowest values of callus growth of shoot-tip derived callus.

On the other hand, leaves derived callus showed the best values (2.65 g.) for callus growth when grown on medium containing kinetin followed by 2,4-D while the presence of BA or NAA in the media gave values lesser than the other treatments. The dry weights of both types of callus tissues closely paralleled the fresh weights responses.

In general, minimum callus growth of both types of callus tissues was observed in MS-free hormone medium, whereas the media containing BA or Kin was the best in stimulating callus growth of shoot-tip and leaves derived callus respectively. From the above results it could be concluded that although the growth regulators added to the media have the same concentration but it

had different effect on callus growth. In this respect Shin and Park (1994) reported that, callus cultures derived from *Cnidium officinale* shoots had a higher callus growth rate when grown in medium containing NAA than 2,4-D

Table (1): Growth of shoot-tips and leaves derived callus cultures of lavender after 4 weeks of cultivation on different growth media.

Growth regulator (concentration μM)	Callus growth (g.)			
	Shoot-tips derived callus		Leaves derived callus	
	* FW	** DW	FW	DW
MS basal	1.23 ± 0.332	0.13 ± 0.117	1.31 ± 0.107	0.14 ± 0.005
2,4-D (22.620)	1.59 ± 0.096	0.16 ± 0.018	2.27 ± 0.130	0.18 ± 0.011
NAA (22.620)	1.81 ± 0.130	0.17 ± 0.006	1.63 ± 0.051	0.14 ± 0.012
Kin (22.620)	1.96 ± 0.240	0.18 ± 0.008	2.65 ± 0.086	0.22 ± 0.005
BA (22.620)	2.62 ± 0.058	0.21 ± 0.021	1.92 ± 0.063	0.17 ± 0.008

Values are the means ± SE *** Fresh weight** **** Dry weight**

Essential oils constituents of shoot-tip callus cultures:

The results of GLC analysis of the essential oils from lavender shoot-tip derived callus cultures grown under different MS media are shown in Table (2). No essential oils were synthesized in shoot-tips derived callus originated from MS1 medium (control) free from any growth regulators. Total hydrocarbon terpenes ranged from 5.6 to 53.5 %, while the oxygenated compounds ranged between 46.5 to 94.4 % for callus tissues grown under different MS media. Accumulation of 1,8-cineol is enhanced in callus grown on medium containing NAA, while it existed in minute proportion ranging between (0.0 - 0.45 %) due to application of other growth regulators. Linalool is the most predominant fraction in essential oils originated from callus grown on medium containing kinetin, while its value reached only 12.54 % with BA. Application of BA activated the biosynthesis of lavandulyl acetate (23.8 %) compared with NAA (10.36 %) and 2,4-D (3.11 %). Moreover, the biosynthesis of α-Caryophyllene is promoted to great extent ranged between 38.38 - 47.83 % due to application of 2,4-D and BA.

Generally, if essential oils having total oxygenated compounds are desired, then application of kin is recommended in case of shoot-tips derived callus tissues as it gave (94.4 %) oxygenated compounds and only 5.6 % total hydrocarbon. Also, the choice of plant organ from which callus is derived is of great importance for production of lavender essential oil having the most desirable constituent such as linalool (88.67 %) which was stimulated within shoot-tips derived callus pretreated with kinetin.

Several authors have studied the effect of different factors on the essential oil constituents in plant tissue culture. Banthorpe *et al*. (1995) established callus lines of *Lavandula angustifolia* and found negligible amount of these terpenoids accumulated in derived cell suspension. They also added that pretreatment of callus tissues by pulse-feeding with mevalonate had little effect on terpenoid accumulation, but the derived cell suspension stored monoterpenoids at concentration *ca*. 103-fold those of controls; further

accumulation were observed following the use of a 2-phase culture medium. Also, Svoboda *et al*. (1995) found that callus of *Tanacetum vulgare* had volatile oil containing 80 - 90 % thujone on MS media supplemented with plant growth regulators (NAA, BA, kinetin, 2,4-D, GA_3 and thidiazuron). Small amounts of volatile oil were detected in callus tissues of both species. No glandular compartments or other compartments were detected in callus cultures. On the other hand, no compounds were detected in callus tissues grown in MS1 (medium without growth regulators). This result is in accordance of our previous study (Rady and Youseef, 1999) on *Laurus nobilis* callus tissues grown in medium free-hormone.

Table (2): Comparison of constituents of essential oils of shoot-tip derived callus cultures grown on MS medium with different growth regulators.

Component	Relative percent				
	MS1	MS2	MS3	MS4	MS5
α-Pinene	---	---	---	---	---
β-Pinene	---	---	---	---	0.38
1,8-Cineol	---	---	18.7	0.45	0.22
α-Terpinene	---	4.69	2.44	5.6	5.29
Linalool	---	40.21	18.12	88.67	12.54
Camphor	---	1.04	1.46	3.59	---
Linalyl acetate	---	1.43	6.86	1.69	3.14
Geraniol	---	2.02	0.93	---	6.8
Lavandulyl acetate	---	3.11	10.36	---	23.8
Geranyl acetate	---	---	0.31	---	---
α-Caryophyllene	---	45.51	38.38	---	47.83
Sesquiterpenes	---	---	---	---	---
β-Bisabolene	---	---	---	---	---
Total identified compounds (%)	---	98.01	97.56	100	100
Total hydrocarbon	---	50.2	40.82	5.6	53.5
Total oxygenated	---	47.81	56.74	94.4	46.5

MS1 = MS free hormone **MS2 = MS + 22.620 μM 2,4-D**
MS3 = MS + 22.620 μM NAA **MS4 = MS + 22.620 μM Kin**
MS5 = MS + 22.620 μM BA

Essential oils constituents of leaves derived callus cultures :

Data presented in Table (3) show that application of all the investigated growth regulators enhanced greatly the biosynthesis of oxygenated compounds which ranged between 60.7 % and 87.85 %, however the existence of oxygenated compounds in relatively high proportion is desired in most essential oils. It could be observed that 1,8-cineol responded positively and showed remarkable accumulation between 41.06, 50.35 and reached 68.42 % due to application of NAA, BA and 2,4-D respectively. Linalyl acetate (36.63 %) and geraniol (15.78 %) are the main components of essential oil of callus grown on medium with kin. In general, lavender essential oil extracted

from leaves derived callus tissues grown in medium containing 2,4-D is characteristic by high level of 1,8-cineol (68.42 %). Also, application of NAA resulted in essential oil having 1,8-cineol (41.06 %) and α-Caryophyllene (23.9 %) as predominant components.

In this respect, different authors have studied the *in vitro* production of essential oils in different plants. Banthorpe *et al*. (1986) studied the ability of plant callus cultures to synthesize and accumulate lower terpenoids and reported that *Pinus radiata* callus cultures accumulated alpha and beta-Pinene at levels similar to those in the parent stem and needles. They also added that all cultures yielded cell free extracts containing prenyltransferase and an isomerizing system with activities from 3-fold to 400-fold greater than those extracted from the parent plants or seedlings and all contained the enzymes necessary for synthesis of the lower terpenoids. The effect of some hormonal and nutritional factors on monoterpene synthesis in *R. officinalis* and *Cnidium officinale* callus cultures were investigated. Essential oil of rosemary has been shown to be affected by composition of nutrient media (Tawfik *et al*., 1992) who found that maximum beta-pinene levels were obtained from *R. officinalis* callus cultures grown on MS medium with 19 gm / l sucrose, while borneol levels decreased at the highest levels of sucrose. Ca^{2+} concentration in the culture media affected the yield of camphore, 1,8-cineole, linalool and bornyl acetate. Shin and Park (1994) found that NAA induced a higher callus growth rate and essential oil production than 2,4-D. They added that the essential oil compositions were influenced by light in the cultivated callus tissues of *Cnidium officinale*. Recently, Rady and Youssef, (1999) found that callus tissues of *Laurus nobilis* were capable of producing 1,8-cineol and α-pinene when grown on MS medium containing 0.01 mg / l NAA and 0.3 mg /l BAP.

Essential oils constituents and contents of *in vivo* and *in vitro* produced plants :

The comparison between the composition of essential oils originated from *in vitro* and *in vivo* lavender plants is taken into consideration in the present study to throw some light on the probable alteration that may be produced due to application of tissue culture techniques in production of medicinal and aromatic plants. The relative percent of the main components of essential oils obtained from *in vitro* produced plants and *in vivo* plants were shown in Table (4). It could be observed that tissue culture techniques stimulate the biosynthesis of oxygenated components (71.62 %) compared with those of *in vivo* plants (57.03 %) and vise versa concerning the total hydrocarbons.

Table (3): Comparison of constituents of essential oils of leaves derived callus cultures grown on MS medium with different growth regulators.

Component	Relative percent				
	MS1	MS2	MS3	MS4	MS5

α-Pinene	---	---	---	---	---
β-Pinene	---	---	---	---	---
1,8-Cineol	---	68.42	41.06	2.77	50.35
α-Terpinene	---	6.4	3.45	8.56	6.15
Linalool	---	2.31	5.64	2.12	3.22
Camphor	---	1.61	2.69	2.98	6.86
Linalyl acetate	---	1.8	1.73	36.63	6.1
Geraniol	---	2.87	0.95	15.78	4.06
Lavandulyl acetate	---	3.29	3.06	11.53	9.14
Geranyl acetate	---	1.19	5.57	3.74	1.26
α-Caryophyllene	---	8.27	23.9	6.07	2.96
Sesquiterpenes	---	---	0.78	0.88	1.34
β-Bisabolene	---	---	1.12	0.42	0.29
Total identified compounds (%)	---	96.16	89.95	91.48	91.73
Total hydrocarbon	---	14.67	29.25	15.93	10.74
Total oxygenated	---	81.49	60.7	75.55	87.85

MS1 = MS free hormone **MS2 = MS + 22.620 μM 2,4-D**
MS3 = MS + 22.620 μM NAA **MS4 = MS + 22.620 μM Kin**
MS5 = MS + 22.620 μM BA

A profound differences in the composition of essential oils of *in vitro* and *in vivo* lavender plants were observed. Data revealed that α and β- pinene, 1,8-cineol, linalool and β-Bisabolene showed variable increases in *in vitro* plants compared with in vivo ones. The most profound change was achieved in case of 1,8-cineol as it increased from 22.62 % to 35.80 % followed by linalool which increased from 16.26 % to 23.6 % in *in vivo* and *in vitro* plants respectively. The increases in the fractionated components occurred at the expense of geraniol which decreased from 6.5 % (*in vivo*) to 0.1 % *in vitro*, and sesquiterpenes which declined from 10.81 % to only 2.1 % and α-Caryophyllene which drastically decreased from 28.4 % to only 4.92 % *in vivo* and *in vitro* plants respectively.

Data in Table (4) also showed the essential oil (%) of lavender intact plants as well as *in vitro* produced plants. It could be observed that tissue culture techniques enhanced the accumulation of essential oil by 17% as the concentration of essential oil increased from 0.24% (in vivo) to 0.28% (in vitro). From the above results, it could be concluded that the preferential between *in vitro* and *in vivo* production of lavender plants is controlled by the desirable components needed to be biosynthesized and accumulated in lavender herb.

In this connection, Katagi *et al*. (1983) studied the essential oil in adventitious shoots from cultured *Lavandula vera* cells. They found that five components (beta-pinene, limonene, 1-8-cineol, camphor and borneol) were identified in the shoots although the amount of 1,8-cineol was less than that reported for intact leaves. Webb *et al*. (1984) reported that shoots regenerated from *Lavandula angustifolia* and *R. officinalis* callus cultures accumulated monoterpenes characteristic of the parent tissue, such compounds could not

be detected in undifferentiated callus maintained under a variety of conditions. It was reported also that *in vitro* cultures of *Jamesoniella autumnalis* had almost the same composition (qualitatively and quantitatively) in sesquiterpenes as the respective material from natural habitats (Becker and Blechschmidt, 1995). The composition of the oil obtained from some micropropagated plants which belong to *Lamiaceae* family and plants in the same phenological stage which were collected in the field was compared (Arrebola *et al*., 1997). They found that oil obtained from micropropagated plants was less complex than that of intact plants. Qualitative differences were only found for the minor constituents. The essential oil yield was always higher in the micropropagated plants.

Table (4): Comparison of constituents and content of essential oils of *in vitro* produced and field grown *L. officinalis* plants.

Component	Relative percent	
	***In vitro* produced *L. officinalis* plants.**	**Field grown *L. officinalis* plants.**
α-Pinene	4.19	---
β-Pinene	3.78	0.83
1,8-Cineol	35.80	22.62
α-Terpinene	0.21	0.8
Linalool	23.6	16.26
Camphor	2.45	2.42
Linalyl acetate	3.36	1.33
Geraniol	0.1	6.5
Lavandulyl acetate	2.73	2.48
Geranyl acetate	4.1	5.42
α-Caryophyllene	4.92	28.4
Sesquiterpenes	2.1	10.81
β-Bisabolene	4.35	0.72
Total identified compounds (%)	91.17	98.59
Total hydrocarbon	19.55	41.56
Total oxygenated	71.62	57.03
Essential oil %	0.28	0.24

Chemical analysis of *in vivo* and *in vitro* produced plants :

Data in Table (5) revealed that in vitro produced lavender plants had the largest contents of carbohydrates (19.2 %), soluble sugars (5.9 %), total protein (13.93 %), whereas it had the lowest values of phosphorus and potassium contents 0.32, 1.96 % respectively than that are found in field grown plants.

Table (5): Comparison of chemical composition of *in vitro* produced and

field grown *L. officinalis* plants.

	***In vitro* produced plants.**	**Field grown plants.**
Total carbohydrates %	19.2	18.7
Total soluble sugars %	5.9	4.67
Total protein %	13.93	12.71
Phosphor %	0.32	0.39
Potassium %	1.96	2.11

In conclusion, from the above results one can concluded that no compounds were detected in callus tissues grown in free-hormone medium so, growth regulators in the growth media had essential effect on the accumulation of essential oil. Also, composition of the essential oils from both types of callus tissues grown onto different media were different. These differences could be due to the type of growth regulators added to the culture medium and may be related to the type of explant used. However, both types of callus tissues grown in medium containing NAA showed oil constituents similar to those of intact plant leaves. In general our findings may draw the attention to marvelous influence of growth regulators in enhancing the biosynthesis and accumulation of lavender essential oils in shoot-tips and leaves derived callus and one can choose the growth regulators suitable for the production of essential oil having the maximal any desired constituents.

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تأثير منظمات النمو على مكونات الزيت الطيار فى مزارع أنسجة نبات اللافندر.

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في هذا البحث تم تأسيس مزارع الأنسجة لنبات اللافندر وذلك بزراعة قطاعات من الأوراق والقمم الخضرية من النبات الحقلي وزرعت علي بيئة استحداث تكوين المزارع الخلوية. ثم نقلت هذه المزارع إلى بيئات تحتوى على هرمونات مختلفة ومنفصلة ذات تركيز متساوى لدراسة تأثير هذه الهرمونات (2,4-D, BA, NAA, kinetin) على مكونات الزيت الطيار باستخدام تكنيك الفصل بكروماتوجرافى الغاز. كذلك تمت مقارنة نباتات اللافندر الناتجة معمليا بنباتات الصوبة من ناحية المحتوى الكيماوى ومحتوى ومكونات الزيت الطيار. أشارت النتائج إلى أن وجود الهرمونات وخاصة بنزيل الأدنين أدى إلى زيادة نمو المزارع الخلوية الناشئة من القمم الخضرية بينما كان لوجود الكينيتين فى البيئة تأثير كبير فى زيادة نمو المزارع الخلوية الناشئة من الأوراق. كما أشارت النتائج إلى أن المزارع الخلوية النامية على بيئة بدون هرمونات لم تعطى نتيجة إيجابية بينما كان لوجود منظمات النمو فى البيئات الأخرى تأثير كبير في تنشيط عملية التمثيل الحيوى للزيت الطيار والتى اختلفت مكوناته بصورة كبيرة فى المزارع الناشئة من القمم الخضرية بالمزارع الناشئة من الأوراق باختلاف منظم النمو المستخدم. كما لوحظ أنة ليس هناك اختلافات كبيرة فى المكونات الكيميائية بين نباتات المعمل ونباتات الصوبة بينما كان هناك اختلافات كبيرة في محتوي الزيت العطرى حيث زاد بنسبة ١٧% في نباتات المعمل وكذلك اختلافات واضحة في مكونات الزيت الطيار بينهما.