SEASONAL CHANGES OF DRUG YIELD AND ESSENTIAL OIL CONTENT IN Salvia officinalis L. PLANTS.

Mazrou, M.M., Eraki, M.A. and Afify, M.M.
Faculty of Agriculture, Minufiya University, Shebin El-Kom.

" التغيرات الموسمية فى كل من محصول العقار ومحتوى الزيت الطيار فى نبات المريمية "

متولى مسعد مزروع ـ محمد عبد الله عراقى ـ محمد موسى عفيفى
كلية الزراعة ـ جامعة المنوفية ـ شبين الكوم

ملخص البحث

أجريت التجربة بمزرعة كلية الزراعة بشبين الكوم فى موسمين متتاليين ١٩٨٦ ، ١٩٨٧ لدراسة تأثير مواعيد الحصاد على كل من محصول العقار ونسبة الزيت الطيار ومكوناته وكذلك محصول الزيت ، وأدت التجربة الى النتائج الآتية :ـ

١ ـ أدى حصاد النباتات فى شهر سبمتبر الى زيادة معنوية فى كل من طول النبات وعدد الأفرع وقد وصل ارتفاع النبات فى هذا الموعد الى ٣ ,٥٩ سم و ٦٧ ,٥٧ سم على التوالى وكان عدد الأفرع ٧ ,٧١ ، ٧٠ فرع فى عامى ١٩٨٦ ، ١٩٨٧ .

٢ ـ ازداد كل من الوزن الطازج وكذلك الوزن الجاف لكل من الأوراق والساق وكذلك محصول العشب لنباتات المريمية بتقدم النباتات فى العمر ووصل الى أقصاه فى ميعاد الحصاد الخامس فى شهر سبتمبر فى موسمى ١٩٨٦ ، ١٩٨٧ وقد وصل الوزن الجاف للأوراق الى ٦ ,١٠٠ ، ٠٩ ,١١٧ جم / نبات فى عامى ١٩٨٦ ، ١٩٨٧ على التوالى ، ووصل وزن الساق الجاف الى ٧٢ ، ٠٣ ,٤٨ جم / للنبات والعشب الجاف الى ٦ ,١٧٢ ، ١٢ ,١٦٥ جم / للنبات فى الموسمين على التوالى .

٣ ـ ارتفع محتوى الزيت الطيار فى أوراق النباتات بتقدم النبات فى العمر ووصل لأعلى نسبة مئوية فى ميعاد الحصاد الثانى فى شهر يونيو فى عام ١٩٨٦ ، شهر يوليو ١٩٨٧ ثم تناقص تدريجيا بتقدم النبات فى العمر .

٤ ـ لوحظ حدوث تغيرات فى مكونات الزيت الطيار خلال موسم النمو حيث كان محتوى الزيت الطيار من كل من (ألفا وبيتا ـ بنين ، السنيول) عاليا فى ميعاد الحصاد الأول وتناقص محتوى الزيت الطيار من تلك المواد بتقدم النبات فى العمر ، حيث وصلت لأدنى مستوى لها فى ميعاد الحصاد الخامس ، بينما سلكت التربينات الكيتونية ـ ثنائية الحلقة (البـاى ـ سيكلك ـ تربين ـ كيتون) سلوكا معاكسا حيث ازداد محتوى الزيت التيار من هذه المركبات حتى وصلت لأقصى قيمة لها وهـى ١,٧٢٪ (ثيوجون وكامفور) فى ميعاد الحصاد الخامس .

٥ ـ ارتفع محصول الزيت الطيار الناتج من كل من الأوراق والعشب خلال أشهر (يونيو ويوليو) فى عامى التجربة وتناقص تدريجيا بتقدم النبات فى العمر ، حيث انخفض بصورة واضحة فى مواعيد الحصاد التالية .

ABSTRACT

Salvia officinalis L. plants were harvested on 30 May, 30 June, 30 July, 30 August and 30 September in the two experimental seasons. The obtained results showed that harvesting Salvia officinalis L. at the end of September resulted in the highest yield parameters such as plant height, number of branches/plant, fresh and dry weights of leaves, stems and total herb. On the other hand the highest oil percentages in the dry herb were 1.96% and 1.11% during the summer months (June and July) in the two seasons, respectively. The highest essential oil yield (7.69 and 6.22 cc./m^2) was obtained during June 1986 and July 1987, respectively. The highest cineole content (9%) was obtained from the first harvest, whereas the bicyclic trepene ketones (α, β-Thujone and Camphor), increased generally with increasing the plant age. The highest content of these ketones (72.1% α, β-Thujone and Comphor) was obtained at the end of September.

INTRODUCTION

Salvia officinalis L. is one of the most important aromatic plants of the Family Labiateae. This importance due to its essential oil content, which have many uses in both pharmaceutical and

food industries. The essential oil of Salvia officinalis L. is usually added to our foods, such as soups, meats and sausage. Hodison and Crisan (1975) reported that sage oil had a fungicidal activity against Embellisia allii and Alternaria tenuis. Cherevalyl et al. (1980) stated that sage oil extract was active against Staphylococci and bacilli bacteria.

The production of the essential oil from sage plants depends not only on the drug yield but also on the essential oil content. Therefore it is very important to determine the optimal harvesting time of sage plant, which gaurantee the highest drug and essential oil yield under our enviromental conditions. In the review of literature there are different opinions about the optimal harvesting time of Salvia officinalis L. plants. Heeger (1956) and Freudenberg and Caesar (1954) reported that sage plants contained the highest essential oil content at the preflowering stage. On the other hand, Strepkov (1938) stated that the highest essential oil content in the leaves of Salvia sclarea L. was found at the end of the flowering stage, then decreased gradually and reached its minimum at the end of the growing season. Palic et al. (1981), mentioned that the essential oil content in Satureja plants decreased continously with the progressive growth of the plants during the growing season. Putievsky and Ravid (1982), reported that the highest essential oil yield of Majorana vulgare L. was obtained during the summer months. Mautner (1981) reported that the chemical structure of the essential oil obtained from sage plants was influenced by both climatic conditions and the developmental stage of the plant.

From the previous mentioned results obtained by the different authors it could be noticed that both essential oil content in sage plants as well as its composition differs according to enviromental conditions and developmental stage of the plant. Moreover, the most of these publications are dealing with the essential oil percentages in sage plants.

Hereat, it was very necessary to make an attempt through this study to determine the optimal growing stage for harvesting Salvia officinalis L. plants, which realize the highest production of the essential oil. In this study the changes in drug yield, essential oil percentages and it composition during the vegetation of sage plant were studied.

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Farm of the Faculty of Agriculture at Shebin El-Kom, during two successive seasons of 1986 and 1987. The purpose of these experiments was to determine the optimal developmental stage for harvesting Salvia officinalis L. plants. The seeds were obtained from the research station of Justus Liebig University, Giessen, Fedral Republic of Germany. The seeds were cultivated in the nursery on 15, October for each seasons. The seedling of 2-3 pairs of leaves were transplanted on 15th February in plots 2x2 m. Each plot contained four rows of 50 cms apart. The planting distance was 40 cms. The plants were fertilized with 100 Kg/feddan urea (46% N), 200 Kg/feddan Calcium superphosphate (15.5%) and 100 Kg/feddan Potassium sulphate (50% K_2O). The amount of N, P and K fertilizers were added as two side dressings. The first addition was after one month from transplanting, the second addition was after one month from the first one.

The plants were harvested at the following dates in the two seasons: 30 May, 30 June, 30 July, 30 August, 30 Sept.

The experiments were planned in a complete randomized block design with three replicates. For each harvesting time the following data were recorded, plant height, number of branches/plant, fresh and dry weights of leaves, stems and total herb. The obtained results from each harvest were statistically analyzed.

Determination of the essential oil percentages:

The essential oil percentages were determined in the air dried samples of leaves, stems and total herb for each harvesting date. The distillation of the essential oil and the determination was that described in the British pharmacopoea (1963). The obtained results were calculated and recorded as volume of oil/certain weight of the air dried sample of the plant according to the following equation:

$$\text{Essential oil percentage} = \frac{\text{observed oil in graduated tube}}{\text{weight of sample (gms)}} \times 100$$

Determination of the essential oil composition:

The essential oils obtained from the leaves of each harvesting time in the second season were analysed by a GCV Pye Unicam gas chromatograph equipped with dual flame ionization detectors. The chromatographic seperation conditions were the same as those of El-Wakeil et al. (1986). Standard chromatograms of authentic compounds were used to characterize the unknown samples by comparing the retention times. The peak areas were measured by triangulation and the percentages of the volatile oil compounds was calculated as the ratio of the partial to the total area.

RESULTS AND DISCUSSION

The obtained data of the two experimental seasons 1986 and 1987 indicated clearly that both drug yield and essential oil content were influenced by harvesting time and consequently with the developmental stage of Salvia officinalis L. plants.

a) Vegetative growth:

The reported data in Table (1) show clearly that both plant height as well as the number of branches per plant increased gradually with increasing the plant age; and reached its maximum

Table 1: The effect of harvesting date on the growth of Salvia officinalis L.

Harvest date	Plant height in cms	No. of branches/ plant	Fresh weight of plant organs in gms/plant			Fresh weight in C./feddan *		
			leaves	stems	herb.	leaves	stems	herb.
			First Season 1986.					
30, May	29.00	30.00	105.20	31.70	136.90	22.10	6.70	28.80
30, June	38.70	48.00	237.97	63.43	301.40	50.00	13.30	63.30
30, July	43.30	61.67	296.93	88.60	385.50	62.40	18.60	81.00
30, Aug.	53.30	70.00	321.60	92.40	414.00	67.50	19.40	87.30
30, Sept.	59.30	71.70	350.90	175.80	526.70	74.00	37.00	111.10
L.S.D. 5%	6.88	14.36	107.30	25.74	128.82	22.53	5.41	27.05
L.S.D. 1%	10.01	20.90	156.04	37.43	187.40	32.77	7.86	39.35
			Second Season 1987.					
30, May	33.00	32.00	115.50	34.89	150.39	24.26	7.33	31.58
30, June	38.67	49.00	261.76	69.78	331.54	54.97	14.65	69.62
30, July	48.33	51.67	333.88	103.39	437.27	70.11	21.71	91.82
30, Aug.	53.33	64.67	386.04	115.18	501.22	81.07	24.19	105.26
30, Sept.	57.67	70.00	418.12	120.12	538.24	87.81	25.22	113.03
L.S.D. 5%	2.85	12.20	137.31	29.16	158.56	28.84	6.12	33.30
L.S.D. 1%	4.13	17.74	199.73	42.40	230.63	41.94	8.90	48.43

* C. = 100 Kg.

at the end of September in the two seasons. The plant height ranged between 29-59.3 cms. and between 33-57.67 cms. in the seasons of 1986 and 1987 respectively, whereas the number of branches per plant rose from 30 to 71.7 in the first season and from 32 to 70 in the second one. These results could be attributed to cell growth and multiplication during the growing season (Semon and Chapman, 1961).

The recorded data in Table (1) and Table (2) indicate that both fresh and dry weights of plant organs as well as the total herb production followed the same trend of plant height and number of branches per plant. Both fresh and dry weights of leaves and stems as well as the total herb production increased significantly, when the plants were harvested at the end of September compared to the first harvest on 30 May in the two seasons. Assuming the control value is equal 100%, the increment in the total drug yield during the growing period could be expressed as follow:

The increment in the total dry herb of Salvia officinalis L.

Plants during the growing season:

Harvesting date	First season 1986	Second season 1987
30, May	100.0%	100.0%
30, June	233.8%	235.3%
30, July	350.7%	362.9%
30, August	383.1%	415.6%
30, September	512.7%	443.5%

From the previous above mentioned results it could be noticed that the growth rythm of Salvia officinalis L. plants followed the curve of growth, which is controlled with genetical factors and enviromental conditions.

Table 2: The effect of harvesting date on the drug yield of Salvia officinalis L.

Harvest date	Drug yield/plant in gms.			Drug yield in C./feddan		
	leaves	stems	herb	leaves	stems	herb
			First Season 1986.			
30, May	26.33	7.43	33.76	5.50	1.60	7.10
30, June	61.10	17.83	78.90	12.80	3.70	16.60
30, July	85.17	33.07	118.24	17.90	7.00	24.90
30, Aug.	93.60	35.80	129.40	19.60	7.50	27.20
30, Sept.	100.60	72.00	172.60	21.10	15.30	36.40
L.S.D. 5%	26.68	8.70	33.96	5.60	1.83	7.13
L.S.D. 1%	38.81	12.67	49.39	8.15	2.66	10.37
			Second Season 1987.			
30, May	28.88	8.88	35.23	6.06	1.76	7.82
30, June	68.07	19.53	87.60	14.30	4.10	18.40
30, July	96.83	38.23	135.07	20.35	8.03	28.38
30, Aug.	110.99	43.77	154.76	23.31	9.19	32.50
30, Sept.	117.09	48.03	165.12	24.59	10.09	34.68
L.S.D. 5%	38.94	8.43	44.09	8.18	1.77	9.26
L.S.D. 1%	56.65	12.26	69.14	11.90	2.57	14.52

From the obtained results it could be also concluded that Salvia officinalis L. plants must harvested at the end of September to realize the highest total drug yield as shown in Table (2).

b) The essential oil content:

The resported data in Table (3) show clearly that the essential oil percentages in the leaves increased from 1.7% in the first harvest on 30, May and reached its maximum 2.4% in the second one, then decreased gradually and reached its minimum value 0.75% in the last harvest at the end of September of the first growing season. The essential oil content in sage leaves followed the same trend of the first season, otherwise the highest essential oil percentage 1.2% was recorded in the third harvest at the end of July as shown in Table (3).

From the above mentioned results it could be noticed that the highest essential oil percentage in the leaves were obtained during the summer months (June and July) in the two growing seasons, then decreased with progressive growth of the plants. These results may be due to the dilution effect of oil concentration as a result of the increment in the drug yield. These results are in agreement with the findings of Palic et al. (1981), who reported that the essential oil percentages in Satureja plants decreased gradually with increasing the plant age.

The reported data in Table (3) show clearly that the essential oil content in the stems of sage plants decreased gradually with increasing the plant age in the two experimental seasons. The highest oil percentages in the stems were recorded in the first harvest, whereas at the end of vegetation in the two seasons the stems contained only traces from the essential oil. These results could be attributed to the increment of wood formation with progressive growth of the plants and concequently a reduction in the

Table 3: The effect of harvesting date on the essential oil percentage in Salvia officinalis L.

Harvest date	First season 1986			Second season 1987		
	leaves	stems	herb	leaves	stems	heherb
30, May	1.70	1.00	1.55	1.00	0.40	0.92
30, June	2.40	0.40	1.96	1.00	0.20	0.83
30, July	1.40	0.20	1.06	1.20	0.20	1.11
30, Aug.	1.20	0.10	0.90	0.80	0.10	0.53
30, Sept.	0.75	Traces	0.43	0.60	Traces	0.42

essential oil content in the stems. These results are in accordance with the findings of Piprek et al. (1981).

It is evident from the data in Table (3) that the leaves of all harvests in the two experimental seasons contained more essential oil than the stems. These results are in agreement with the findings of Piprek et al. (1981) on lanyana plants and Mazrou (1978) on Ocimum basilicum L.

The obtained results indicate that the essential oil percentage in the dry of sage plants followed the same trend of the essential oil content in the leaves as shown in Table (3).

From the above mentioned results it could be noticed that the highest essential oil content in both leaves and total herb of Saliva officinalis L. plants was obtained during Summer months (June and July) in the two growing seasons, and reached its minimum values in Autmn. These results may be due to the high metabolic activities during Summer months, which produce more essential oil as a result of these physiological processes, whereas the reduction in the essential oil content in the advantages stages of growth could be explained through the increment in the woody tissues of the stems. These results are in agreement with the findings of Palic et al. (1981) and Piprek et al. (1981).

c) Essential oil composition:

The results obtained from the investigation of the essential oil in the leaves of sage plants showed that during the different developmental stage of growth occured not only changes in the total oil percentages but also a clearly changes in the essential oil constituents were observed.

The results obtained from GLC study of sage-leaves essential oil at the different stages of growth in the second experimental season are shown in Table (4) and Figs. (1-5). These results showed the presence of 16 peaks in the chromatograms. Only ten peaks were identified, α and β-pinene as terpene hydrocarbons, myrcene as an unsaturated cyclic hydrocarbons, lemonene as a Monocyclic terpene, cineole, Terpenyl-acetate, α and β-Thujone, camphor as Bicyclic terpene ketones and carvone as a monomcylcic terpene ketone. The main compounds which characterised the essential oil of sage plants were α- and β-Thujone and camphor. Brieskorn and Dalferth (1964), Mautner (1981) mentioned that the essential oil of Salvia officinalis plants contains mainly pinene, cineol, α , β-Thujone and camphor at different concentrations.

The reported data in Table (4) and Figs. (1-5) showed that there was a clearly variation in the essential oil composition during the different stages of growth. The highest value of terpene hydrocarbones (20.06%) α , β-pinene was recorded in the first harvest on 30, May then decreased gradually and reached its minimum (6.53%) in the last harvest on 30, September. Both the myrcene and lomonene showed no clear trend during the different stages of growth, whereas the c coneole compound generally decreased from the first harvest at the end of May and reached its minimum at the last harvest. The cineole content varied between 9% and 1.53% in the essential oil of sage plants. The total content of the main oil constituents bicyclic terpene ketones (α , β-Thujone and Camphor) generally increased by increasing the plant age. The lowest value of these compounds (52.86%) was recorded at the begining of the growing period, whereas the highest value (72.1%) of these compounds together was observed at the end of September.

From the previous results it could be noticed that the contents of α, β-pinene as well as cineole in the essential oil of sage plants

Table 4: The effect of harvesting date on the essential oil composition in the leaves of Salvia officinalis L. in the second season 1987.

Harvesting date	30,May	30,June	30,July	30,Aug.	30,Sept.
	%	%	%	%	%
1. Unknown	0.38	0.20	0.49	0.67	0.28
2. ∝ - pinene	5.72	12.15	12.07	10.00	4.86
3. ß - pinene	14.34	7.71	4.93	5.07	1.67
4. Myrcene	3.05	2.56	1.35	1.20	3.19
5. Limonene	1.75	0.49	0.86	2.74	0.83
6. Unknown	4.58	1.48	1.20	3.33	0.69
7. Cineol	9.00	8.70	6.16	8.40	1.53
8. Terpenyl-acetate	0.46	0.10	0.49	1.00	1.53
9. ∝, ß-thugone	27.46	27.17	36.95	26.00	46.67
10. Camphor	25.40	30.90	24.63	31.73	25.43
11. Unknown	0.92	0.99	0.25	0.53	0.69
12. Unknown	1.83	2.47	1.85	5.07	4.17
13. Methyl chavecol	0.61	2.47	2.22	1.45	1.94
14. Carvon	1.22	2.47	1.23	1.45	1.94
15. Unknown	1.37	----	----	-----	----
16. Eugone	1.91	0.10	2.83	2.67	4.58

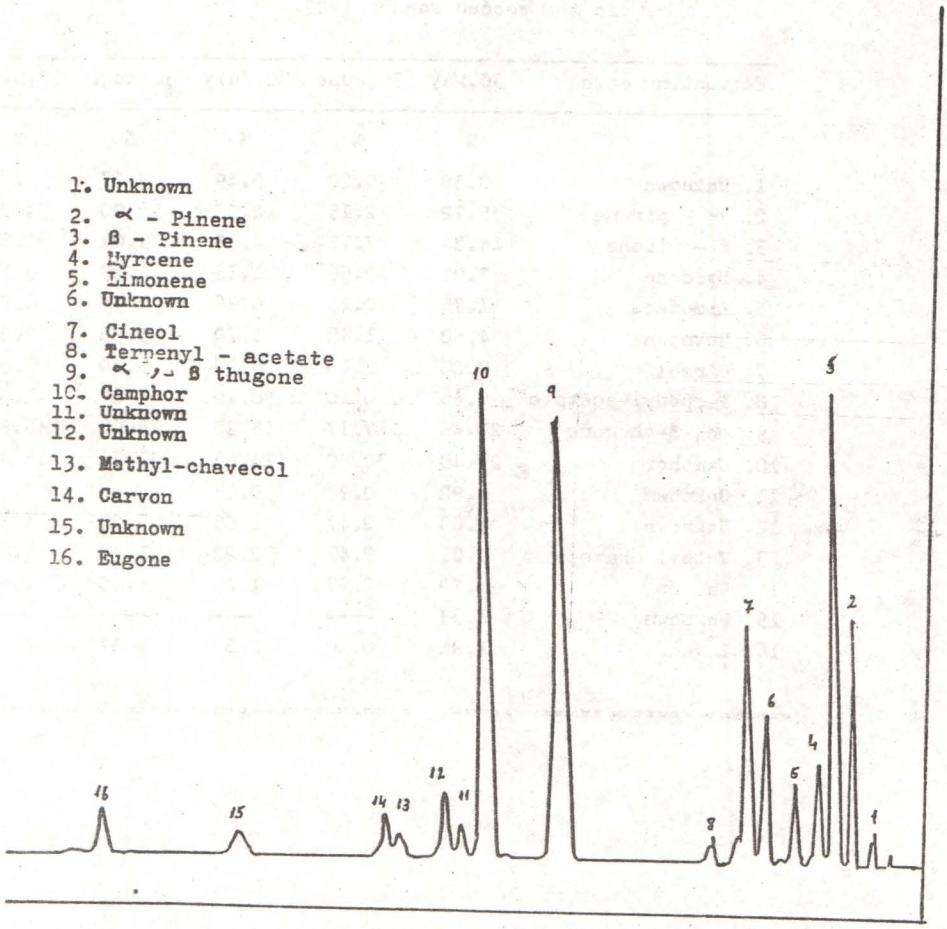

Fig. 1: The essential oil composition of the first harvest on 30, May, 1987.

1. Unknown
2. ∝ - pinene
3. ß - pinene
4. Myrcene
5. Limonene
6. Unknown
7. Cineol
8. Terpenyl-acetate
9. ∝ , ß - thugone
10. Camphor
11. Unknown
12. Unknown
13. Methyl chavecol
14. Carvon
15. Unknown
16. Eugone

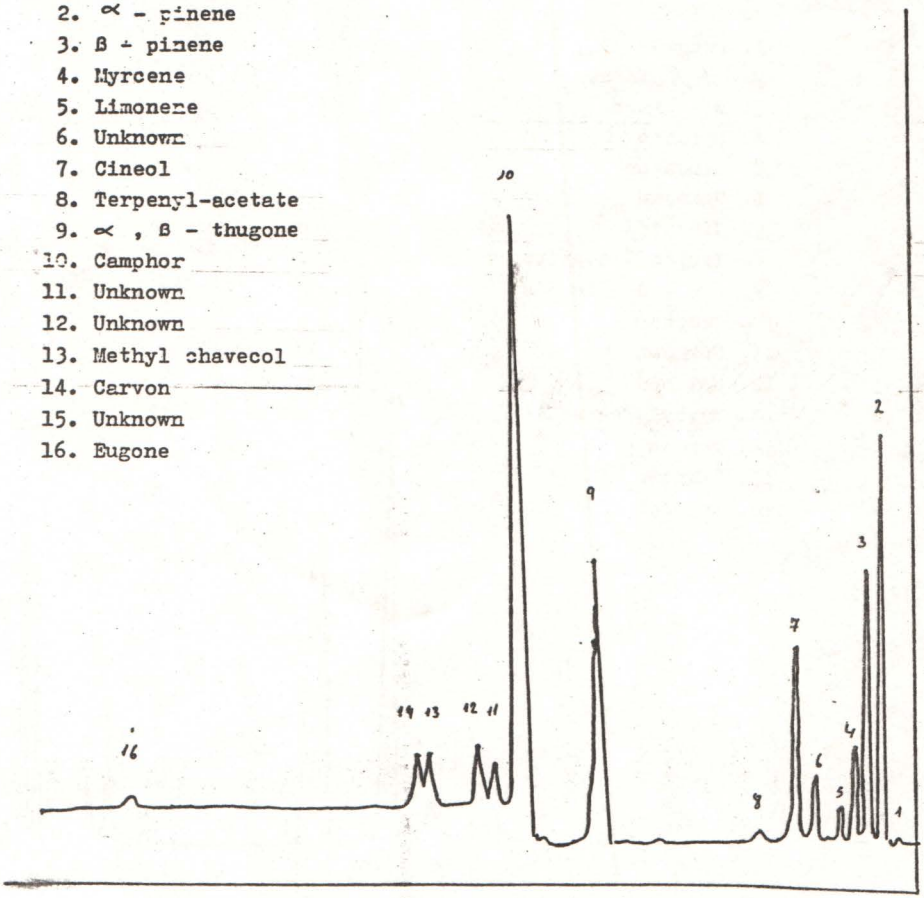

Fig. 2: The essential oil composition of the second harvest on 30, June, 1987.

1. Unknown
2. ∝ - pinene
3. ß - pinene
4. Myrcene
5. Limonene
6. Unknown
7. Cineol
8. Terpenyl-acetate
9. ∝ , ß - thugone
10. Camphor
11. Unknown
12. Unknown
13. Methyl chavecol
14. Carvon
15. Unknown
16. Eugone

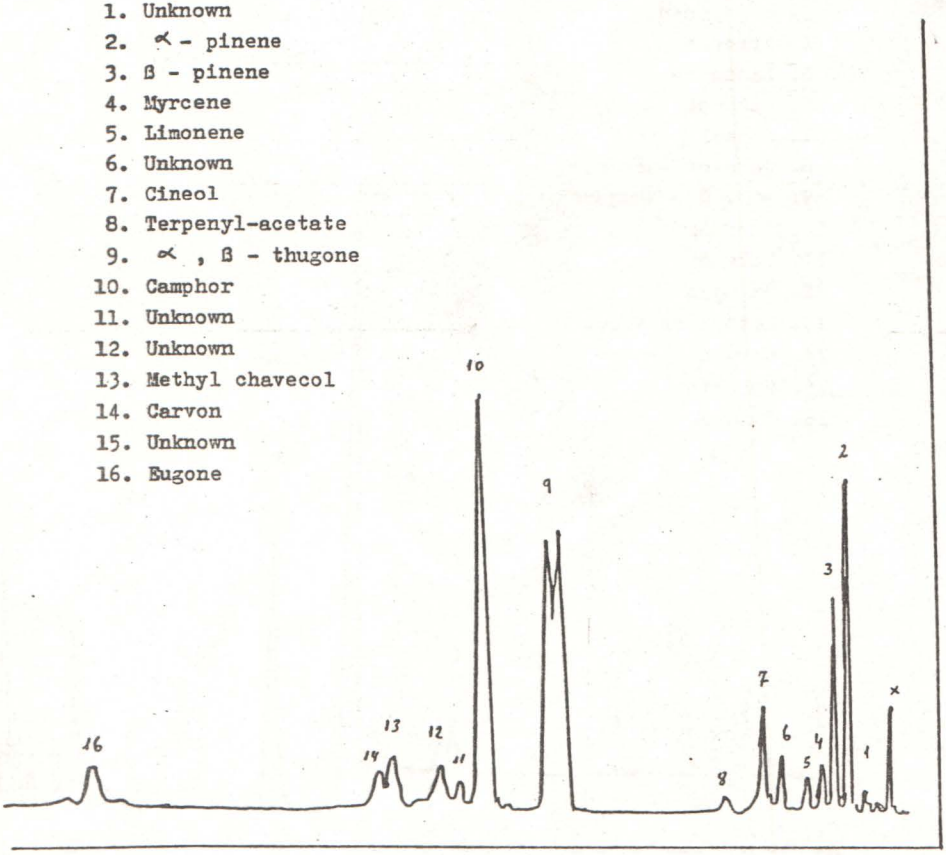

Fig. 3: The essential oil composition of the third harvest on 30, July, 1987.

1. Unknown
2. ∝ - pinene
3. ß - pinene
4. Myrcene
5. Limonene
6. Unknown
7. Cineol
8. Terpenyl-acetate
9. ∝ , ß - thugone
10. Camphor
11. Unknown
12. Unknown
13. Methyl chavecol
14. Carvon
15. Unknown
16. Eugone

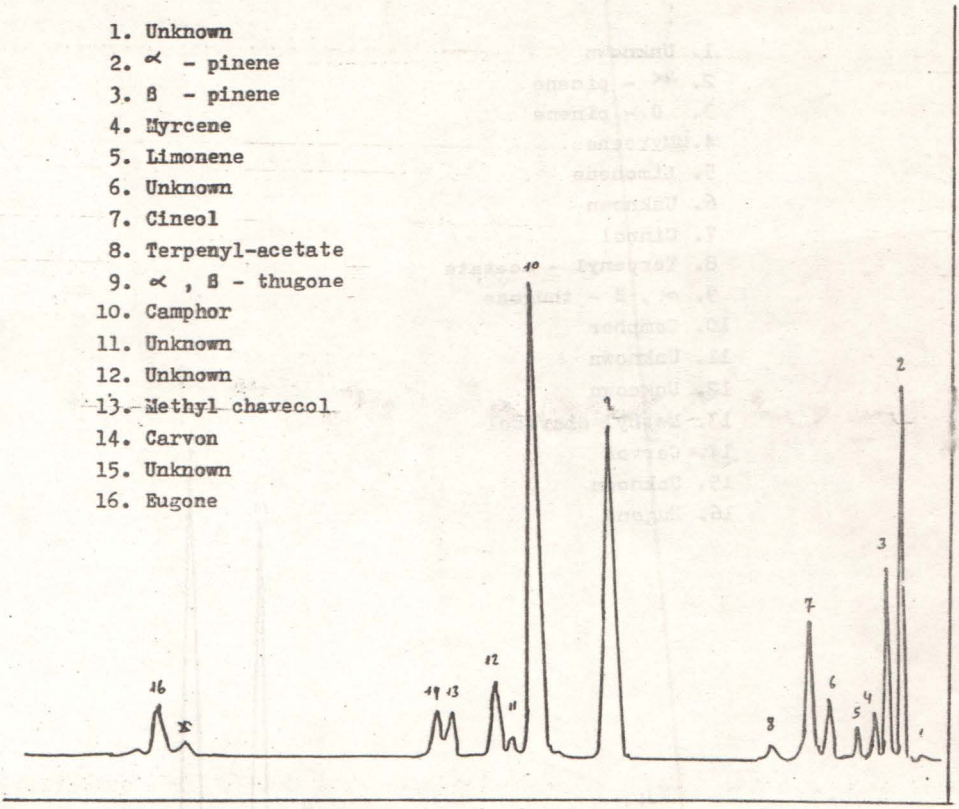

Fig. 4: The essential oil composition of the fourth harvest on 30, August, 1987.

1. Unknown
2. ∝ - pinene
3. ß - pinene
4. Myrcene
5. Limonene
6. Unknown
7. Cineol
8. Terpenyl - acetate
9. ∝, ß - thugone
10. Camphor
11. Unknown
12. Unknown
13. Methyl chavecol
14. Carvon
15. Unknown
16. Eugone

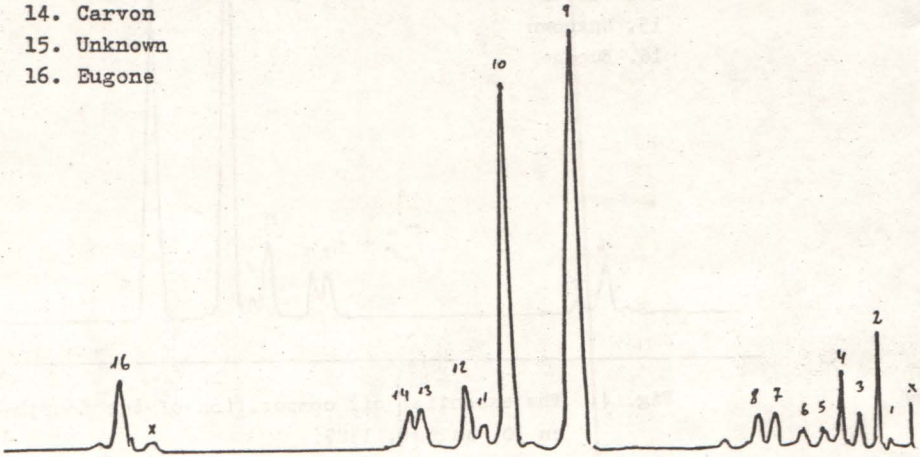

Fig. 5: The essential oil composition of the fifth harvest on 30, September, 1987.

in the earlier stages of growth were higher than those obtained in the advantages stages of growth. On the other hand the bicyclic trepene ketones content increased generally with increasing the plant age. These results may be due to the differences in the physiological activities and climatic conditions during the growing season of the plants. Mautner (1981) reported that the differences in composition of the essential oils obtained from Salvia officinalis plants could be explained through the changes in both climatic conditions and developmental rythm of the plant during the growing seasons.

Generally the bicyclic terpene ketones contents in essential obtained from the leaves of Salvia officinalis L. grown under the Egyptian environmental conditions, were higher than those obtained by Mautner (1981), who found that the content of both α, β-Thujone and camphor together was about 58% in the essential oil, whereas the leaves of sage plants grown in Egypt contained about 72.1% bicyclic-trepene ketones (α, β-Thujone and Camphor). These results could be attributed to differences in the environmental condition. Rovesti (1976) mentioned that the composition of essential oil obtained from Ocimum suave varies with the altitude and humidity of the plant location with increasing the altitude and consequently decreasing humidity the Ketones content in the essential oil increased.

d) The yield of the essential oil:

The reported data in Table (5) indicate clearly that the essential oil obtained from sage leaves increased gradually from the first harvest and reached its maximum in the second and third harvesting dates during the Summer months in the experimental seasons of 1986 and 1987 respectively. In the followed harvests of the two seasons, the obtained essential oil from the leaves was reduced and reached its minimum in the last harvest on 30th September.

Table 5: The effect of harvesting date on the essential oil yield of Salvia officinalis L.

Harvest date	Essential oil yield in cc/m^2 1986			Essential oil yield in cc/m^2 1987		
	leaves	stem	herb	leaves	stem	herb
30, May	2.24	0.38	2.63	1.45	0.17	1.62
30, June	7.33	0.36	7.69	3.42	0.20	3.62
30, July	5.95	0.33	7.28	5.83	0.39	6.22
30, Aug.	5.63	0.18	5.80	4.03	0.22	4.25
30, Sept.	3.80	----	3.80	3.52	----	3.52
L.S.D. 5%	2.30	0.16	2.42	2.11	0.18	2.15
L.S.D. 1%	3.35	0.23	3.53	3.06	0.27	3.12

The essential oil yield obtained from the leaves ranged between 2.21 and 7.33 cc/m² in 1986 and between 1.45 to 5.83 cc/m² in the second one.

From the previous mentioned results it could be noticed that the highest essential oil production, was obtained from sage leaves during the Summer months in the two seasons, whereas the lowest oil yield was obtained at the end of September. These results may be due to the changes in both the dry weights of leaves and their essential oil content during the growing season. These results are in accordance with the findings of Putievsky and Ravid (1982) on Origanum vulgare L. and Mazrou (1978) on Ocimum basilicum, who reported that the highest essential oil yield from these plants was obtained in Summer, whereas the essential oil production was greatly reduced in Autumn months.

It is evident from data in Table (5) that the essential oil production from the stems of sage plants followed the same trend of the essential oil content in the stems in the two seasons. The obtained essential oil from the stems varied betwee 0.0 and 0.38 cc/m² and between 0.0 to 0.39 cc/m² in the seasons of 1986 and 1987 respectively.

The data in Table (5) indicate clearly that the total essential oil production increased with increasing the plant age and reached its maximum (7.69 cc/m² and 6.22 cc/m²) in Summer months (June and July) in the seasons of 1986 and 1987 respectively. These results could be attributed the high essential oil content in the herb at this time. On the other hand the total essential oil production was reduced in the followed harvests in the two seasons and reached its second minimum at the end of September. These results may be due to the decrease of essential oil percentages with increasing the plant age.

From the previous mentioned results it could be concluded that Salvia officinalis L. plants must be harvested during Summer months (June and July) to obtaine the highest essential oil yield.

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