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EFFECT OF HARVEST DATE AND STEAM DISTILLATION TIME ON ESSENTIAL OILS OF THREE *Eucalyptus* species GROWING IN EL-KASSASIN REGION

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ABSTRACT

In Egypt *Eucalyptus* species are presently used as poles, windbreak and firewood but not for oil extraction. Egyptian imports of eucalyptus oils are increasing from year to another. This study was aimed to increase the *Eucalyptus* species value as non- wood products by determining the suitable harvest date and the time of steam distillation on yield and contents of the essential oils of *Eucalyptus* species . This work was carried out in 2001 on 13 yr-old trees irrigated by drip irrigation in soil under reclamation.

The yields of essential oils extracted from dried leaves of *Eucalyptus citriodora*, *E. gomphocephala* and *E. camaldulensis* growing at the Experimental Farm of El-Kassasin, 80km. East of Cairo were studied. The highest oil yields were obtained in summer, followed by spring and winter with the lowest yield in autumn for both *E. citriodora* and *E. gomphocephala*, while, the highest yield of *E. camaldulensis* was obtained in spring followed by autumn and summer, with the lowest yield in winter.

Twenty components were identified by GC/MS. In the three *Eucalyptus* species 90% of the isolated oils were obtained after 90 min. distillation and contained maximal amounts of citronellal 71.3% for *E. citriodora* oil and 52.9% and 31.2% ,1.8 cineole for *E. gomphocephala* and *E. camaldulensis* oils, respectively.

At longer distillation times, further quantities of oils collected became negligible and the proportions of the main constituents fell significantly. The main components were citronellal, isopulegol and citronellol for *E. citriodora*. While, the principal leaf oil components of *E. gomphocephala* were 1.8 cineole, P-cymene, allo – aromadendrene, aromadendrene and B-pinene. The most abundant essential oils of *E. camaldulensis* were 1.8- cineole (eucalyptol), P-cymene, alpha- pinene and beta pinene. The results from this study indicated that it is possible to use *Eucalyptus* species grown in reclaimed land in El-Kassasin region to produce essential oils of high economic value with *E. citriodora* as an interesting source of citronellal. The oil of *E. gomphocephala* is a potential source of 1.8 cineole and P-cymene,while, *E. camaldulensis* is rich in 1.8 cineole and Alpha pinene.

Key words: *camaldulensis, citriodora ,distillation, essential oils, eucalyptus, gomphocephala, harvest date.*

1.INTRODUCTION

The genus *Eucalyptus*, Myrtaceae family comprises more than 600 species. The plant, which is a native to Australia, is widely distributed throughout the world. Different species are used for afforestation, improvement of marshlands, and as ornamental trees in towns and al roadsides. (Chalchat *et al.* 2000).

Many species yield valuable timber, firewood, charcoal and row material for paper and pulp industry and to some extent for essential oil and tanning materials.

The oil may be roughly divided into three classes of commercial importance: (1) the medicinal oils which contain substantial amounts of eucalyptol (also known as cineole) with antibacterial activity against gram-positive and negative microorganisms and antifungal activity against *C. albicans*. (2) the industrial oils, containing terpenes, which are used for flotation purposes in mining operations; (3) the aromatic oils such as *E. citriodora*, which are characterized by their aroma[Grieve, (2000) and Oyedeji *et al.* (1999)].

The production of eucalyptus oils in Egypt is not known and commercial plantations have never been established, especially for oil

production. According to the reports of the Egyptian Exports Development Center, Egyptian imports of eucalyptus oils increased from $ 57.000 in 1965 to $ 405.000 in 2000. This reflects the importance of expanding the growing *Eucalyptus* species of high oil productivity, to reduce oil imports.

Accordingly, research should be carried out concerning Eucalyphus to solve the various related problems which include the cultural requirements of the trees leading to maximum yield of the desired products. We studied the more abundant species in Egypt; *Eucalyptus camaldulensis* Dehnh, *E. citriodora* Hook and *E. gomphocephala* Dhen. These trees are presently used for poles, windbreak and firewood. However it is desirable to find ways of increasing the value of these trees. The study of oil composition may realize this goal.

Adhikari *et al.*,(1992) showed that essential oil and eucalyptol contents in leaves of *E. camaldulensis* growing in Nepal increased during the period from March to September. Fechtal *et al.*, (1995) reported that the highest cineole yield was obtained between 40 to 60 minutes of rectification time for *E. camaldulensis* growing in Morocco.

Bignell *et al.*, (1996) indicated that the principal leaf oil components of *E. gomphocephala* growing in Australia were alpha-pinene (0.2 - 31%), beta pinene (0 -12.5%), 1.8 cineole (0.2 - 46%), P-cymene (0 - 20.8%) and aromadendrene (0 - 12.5%).

Shieh and Shieh (1996) concluded that the highest yields of essential oil in leaves of *E. camaldulensis* growing in Taiwan were obtained in spring, followed by autumn and summer with the lowest yields in winter.

Muchori *et al.*, (1997) indicated that the essential oil of *E. camaldulensis* was rich in 1.8-cineole (eucalyptol) (> 60%). The main component of *E. citriodora* was citronellal (>70%) in Kenya.

Moudachirou *et al.*, (1999) reported that yields of essential oils from dried leaves of *E. camaldulensis* and *E. citriodora* grown in different locations in Benin and collected from February to August were in the range (0.6-1.4%) and (2.3 – 5.9%), respectively. A total of 28 components was indentified by GC/MS. About 90% of the isolated oils were obtained after 1 hour of distillation, and it was concluded that for industrial purposes a distillation time of 45 minutes would be adequate . The main essential oil constituent of *E. citriodora* was

citronellal (55.1 – 89%) and for *E. camaldulensis* was 1.8 cineole (31 – 72.5 %).

Chalchat *et al.*, (2000) reported that the essential oil from *E. camaldulensis* contained 43 constituents of which limonene (4.1 – 10.2 %) 1.8 cineole (42.6 – 64.1 %), gamma terpinene (4.8 – 6.2 %), P-cymene (11.6 – 24.8 %), trans pinocorveol (1.4 – 4.0 %), and alph – terpineol (3.7 – 4.8 %), were the most representative. In *E. citriodora*, of the 18 constituents identified citronellal and isopulegol were the most abundant (approx. 78 and 6 %, respectively) in Mali country.

Dunlop *et al.*, (2000) showed that the principal components of *E.camaldulensis* oil were almost independent of environmental and seasonal conditions. Essential oils contained monoterpenoids and sesquiterpenoids (Australia).

Benayache *et al.*, (2001) indicated that essential oil of *E. camaldulensis* and *E. gomphocephala* growing in Algeria contained alpha – pinene (2.8 – 24 %) beta pinene (0.2 – 3.6 %), limonene (1.5 – 4.5 %), myrcenel (0.1 – 1.1 %), 1.8 – cineole (eucalyptol), (3.5 – 81 %) , allo – aromadendrene (0.1 – 8.8 %) and globulol (0.2 – 5.7 %) as principal leaf oil components.

2. MATERIALS AND METHODS

The oils were isolated at the middle of every month in 2001 during four seasons from the dried leaves of (*E. gomphocephala* Dhen, *E. citriodora* Hook and *E. camaldulensis* Dehnh)trees planted in 1989 from one year old seedlings. The seasons were winter (January, February and March), spring (April, May and June), summer (July, August and September) and autumn (October, November and December) . The trees were growing at the Experimental Farm of El – Kassasin Horticultural Research Station, Horticulture Research Institute, Agriculture Research Center. The soil is sandy loam and

Table (1) Range and means of total height of *E.* species 13 year- old trees.

E. species	Tree height (m.)	
	Range	**Mean**
E. camadulensis	18.0 – 21.8	19.9
E. gomphocephala	14.4 – 21.4	19.4
E. citriodora	11.4 – 14.5	13.0

alkaline (pH 8.1) under reclamation .The distance between trees was 5 x 5 meters . The range and means of total height of the trees are presented in Table (1). The soil of the experimental farm had physical and chemical properties as shown in Table (2). Six samples of leaves were collected at different parts of each sampled tree every (2 - 2.5 m.) depending on the total height of the tree. The leaves were mixed together as one sample. Every sample containing the young and mature leaves. The leaves were dried at room temperature in shade. The leaves (200 – 500 g) were bulked and the samples were homogenized. The oil samples were obtained by steam distillation. The oils were analyzed by gas chromatography on a Delsi 121 instrument

Table (2): The physical and chemical properties of the sample site soil.

I	Mechanical analysis		%	
	Coarse sand		70.75	
	Fine sand		13.40	
	Silt		3.80	
	Clay		7.40	
	$CaCO_3$		4.50	
	Organic matter		0.15	
	Textural Class		Sandly loam	
II	Chemical analysis			
	Level of available nutrients:			
	Nitrogen in ppm.		20	
	Phosphorus in ppm		6	
	Potassium in ppm		67	
	Soil reaction (pH)		8.1	
III	Soluble ions in paste extract (mEq./100gm. soil):			
	Cations		Anions	
	Mg^{++}	0.65	Cl^-	0.93
	Ca^{++}	0.81	Hco_3^-	0.75
	K^+	0.33	Co_3^-	0.11
	Na^+	0.95	So_4^-	0.44

fitted with a CP wax – 52 CB column (25 m x 0.25 mm.) ; film thickness 0.1 µm with a temperature gradient programmed from 50 c° (5 min isothemo) to 220 c° at 2 c° / min. The temperatures of the injector and detector were 240 c° and 255 c°, respectively. The carrier gas was nitrogen. The constituents were identified by co – injection of standard substances and comparison of mass spectra. Our mass spectra data were obtained by GC/MS using a Sigma 300 chromatograph fitted and an HP 5970 300 mass pectrometer. Temperatures were set as above, ASTA (1985).

3.RESULTS AND DISCUSSION

3.1. Influence of harvest date on oil percentage

Table (3) indicates that, the maximum oil percentages of *E. citriodora* and *E. gomphocephala* were recorded in summer samples (5.350 and 3.750 v/w), respectively followed by spring (2.830, 1.627 v/w) and winter (2.360, 1.554 v/w), respectively. The minimal percentages of oil were (1.640 and 1.120 v/w), in autumn respectively. In contrast, the maximum oil percentage was obtained in spring for *E. camaldulensis* (3.300 v / w) followed by autumn, summer and winter (1.866, 1.801 and 1.543 v/w), respectively. These results showed that the oil percentage obtained during the four seasons depended on the harvest time for the three *Eucalyptus species*. These results are in agreement with those obtained by Adhikari *et al.*(1992), Shieh and Shieh (1996), Moudachirou *et al.*, (1999), and Dunlop *et al.*, (2000).

Table (3): Oil percentages (v/w) obtained by steam distillation during the harvest seasons in 2001.

Harvest season	*E. citriodora*	*E. gomphocephala*	*E. camaldulensis*
Winter	2.360	1.554	1.543
Spring	2.830	1.627	3.300
Summer	5.350	3.750	1.801
Autumn	1.640	1.120	1.866

3.2. Oil compound percentages of *Eucalyptus* species

The analytical results are collected in Table(4).In the summer season for *E.citriodora* and *E.gomphocephala* and in the spring season for *E. camaldulensis*, the chemical compositions of the samples of three *Eucalyptus* species showed that the principal compositions of *E. citriodora* were citronellal (71.30%), isopulegol (7.60 % and citronellol (8.60 %. The main constituents of *E. camaldulensis* were 1.8 cineole (52.90 %), alpha – pinene (8.30) %, P – cymene (9.40) % and Beta – pinene (6.30) %. For *E. gomphocephala*, the main constituents were 1.8 cineole, P–cymene, allo– aromadendrene, aromadendrene, globulol, beta–pinene and alpha– phellandrene with percentages of 31.20, 15.60, 8.70, 6.60,5.60,5.40 and 5.10, respectively. These results are in agreement with Bignell *et al.*, (1996), Muchori *et al.*,(1997), Mouduchirou *et al.*, (1999), Chalchat *et al.*, (2000) and Benayache *et al.* (2001).

3.3- Influence of distillation time on extracted oil

The volumes of oils collected at regular time intervals during the extraction of three *Eucalyptus* species are given in Table (5). Their time course is depicted in (Figure 1). Oil fell down markedly after 30 to 90 min; beyond that time, further distillation afforded only a 10 % increase of oil. After 30 and 45 min the isolation rates were about 50 % and 65 %, respectively. Hence, for industrial purposes a distillation time of 45 or 60 min, would seem reasonable or certainly no more than 90 min.

For the oils of the three species, the percentages of characteristic components varied widely between 45 and 90 min distillation time (Figure 1). These results showed that an oil of high quality can be obtained if the extraction time does not exceed 90 min. These results are in harmony with Fechtal *et al.,* (1995), and Moudachirou *et al.,* (1999).

Table (4): Percentage of compounds in oils of three *Eucalyptus* species from El – Kassasin region during 2001.

Compound	*E. citriodora*	*E. gomphocephal*	*E. camaldulensis*
	Summer season		Spring season
Alpha-pinene	0.16	4.20	8.30
Camphene	0.14	2.30	0.30
Beta - pinene	0.50	5.40	0.36
Myrcene	0.20	1.20	0.10
Alpha phellandrene	-	5.10	3.20
Limonene	0.20	4.60	2.20
1.8 cineole	2.50	31.20	52.90
Gamma terpinene	-	0.80	2.10
P – cymene	-	15.60	9.40
Citronellal	71.30	1.30	-
Pulegol	2.60	-	-
Linalool	1.50	0.50	-
Isopulegol	7.60	-	-
B – caryophllene	1.00	-	-
Aromadendrene	-	6.60	3.70
Allo aromadendrene	-	8.70	0.50
Citronelly lacetate	1.00	-	-
Alpha – terpineol	-	2.80	3.80
Citronellol	8.60	-	-
Globulol	-	5.60	4.20
Total percentages	97.30	95.90	97.00
Unidentified Compounds	2.70	4.10	3.00

Table (5): Extracted oil (v/w) and δ – oil (v/w) as affected by time of distillation in 2001.

Time (min)	*E. citriodora*		*E. gomphocephala*		*E. camaldulensis*	
	Oil (ml.)	δ– Oil	Oil (ml.)	δ– Oil	Oil (ml.)	δ– Oil
15	1.0	1.0	0.8	0.8	0.5	0.5
30	2.3	1.3	1.8	1.0	1.5	0.9
45	3.4	1.1	2.5	0.7	2.2	0.7
60	4.3	0.9	3.1	0.6	2.7	0.5
90	4.9	0.6	3.5	0.4	3.0	0.3
120	5.2	0.3	3.7	0.2	3.2	0.2
210	5.4	0.2	3.8	0.1	3.3	0.1
330	5.4	t.	3.8	t.	3.3	t.
Total	-	5.4	-	3.8	-	3.3

t.=trace

3.4. Effect of distillation time on the main components of oils

Tables (6, 7, 8) and Figures (2, 3, 4) indicate the amounts of main components in oil fraction at time intervals during distillation of *E. citriodora* and *E. gomphocephala* in the summer season and during spring for *E. camaldulensis*. In the three *Eucalyptus* species 90 % of the isolated oils were obtained after one-hour distillation. At longer isolation times furthur quantities of oils collected became negligible and the main constituents fell down significantly.

The difference in chemical composition and oil content between *Eucalyptus* species might be due to the physiological changes associated with leaf maturation. The extent of these changes depended on the interaction between genotype and environmental conditions. Although genotypic variation was great, there was detectable variation attributable to site, season and year. There was a relationship between seasonal patterns and climatic factors. Part of the observed variation in oil content with time was attributed to variability in leaf maturity. The relative superiority in oil yielding capacity of certain individual trees was maintained throughout, despite substantial variation from non-genetic sources. This consistency in ranking is of advantage to tree breeders wishing to make selections amongst plantations of differing age, on different sites and at different times of the year. However, even with careful sampling controls, absolute values will vary substantially and progeny testing will be necessary to further assess the potential of the initial selections; Doran and Bell (1994).

Table (6): Main components % obtained at different times during oil distillation of *E. citriodora* (summer season 2001).

Time (min)	Main components %		
	Citronellal	Isopulegol	Citronellol
15	71.0	5.6	1.8
30	77.7	5.3	3.0
45	78.2	4.8	4.5
60	52.4	3.9	2.8
90	13.8	2.1	1.0
120	5.2	1.3	0.6
210	2.3	1.2	0.3
330	1.1	0.6	0.2

Table (7): Main components % obtained at different times during oil distillation of *E. gomphocephala* (summer season 2001).

Time (min)	Main components %		
	1.8 cineole	P- cymene	Allo - aromadendrene
15	41.2	13.2	4.8
30	36.2	18.5	9.7
45	26.4	11.3	15.5
60	17.5	8.6	10.8
90	8.9	4.4	6.1
120	4.7	3.1	4.3
210	3.2	2.0	2.6
330	3.0	0.6	1.8

Table (8): Main components % obtained at different times during oil distillation of *E. camaldulensis* (spring period 2001).

Time (min)	Main components %		
	1.8 cineole	Alpha pinene	P - cymene
15	46.5	4.6	4.2
30	41.2	11.0	9.2
45	30.5	14.3	7.2
60	20.2	19.0	6.0
90	9.0	10.1	4.4
210	3.1	3.5	1.3
330	2.4	2.2	1.0

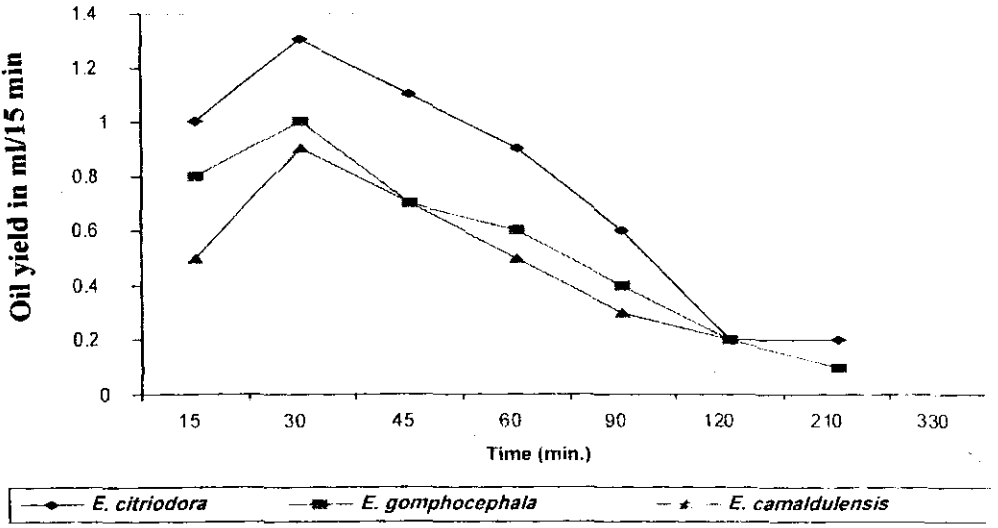

Fig. (1): Extracted oil in ml/ 15 min. during distillation .

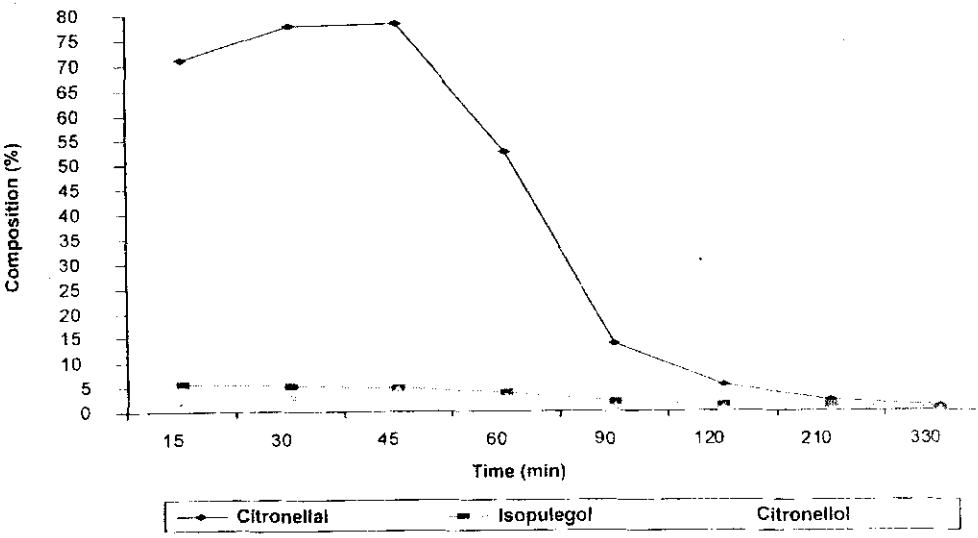

Fig. (2): Main components % obtained at different times during oil distillation of *E. citriodora* (summer season 2001).

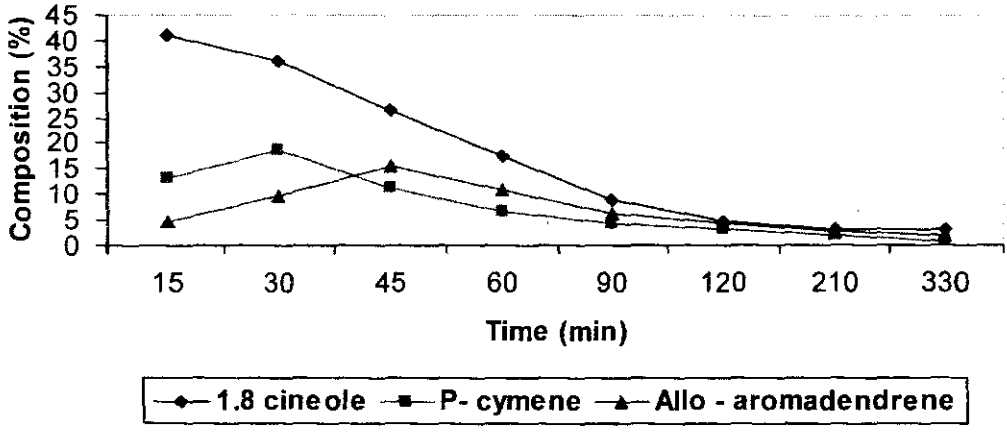

Fig.(3) : Main components % in oil fraction of *E. gomphocephala* obtained at time intervals during steam distillation (summer season2001).

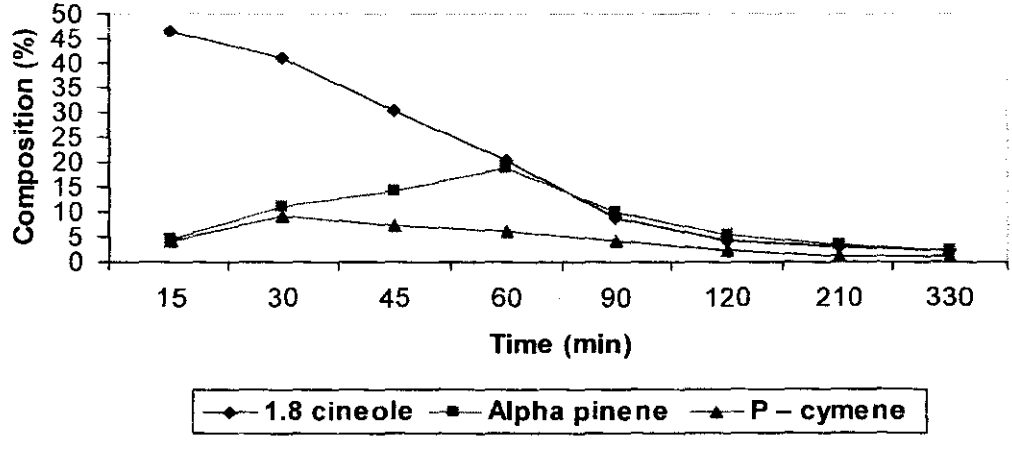

Fig.(4): Main components % in oil fraction of *E. camaldulensis* obtained at time intervals during steam distillation (spring season 2001).

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تأثير وقت الحصاد وزمن الاستخلاص علي الزيوت الطيارة لثلاثة أنواع من الكافور النامية في منطقة القصاصين

صفوت لبيب مكسيموس

قسم الغابات – معهد بحوث البساتين – مركز البحوث الزراعية –جيزة

ملخص

تستخدم انواع الكافور المختلفة النامية في مصر كمصدات للرياح و كدعامات و لإنتاج الفحم. ويمكن زيادة قيمة هذه الاشجار من الناحية الاقتصادية اذ ما اعطيت اهميه لناتج الزيوت التى يمكن استخلاصها من هذه الانواع . علما بأن قيمة واردات مصر سنويا من هذه الزيوت فى زيادة مطرده. لذا تهدف هذه الدراسة لزيادة القيمة الفعلية لاهم انواع الكافور النامية فى مصر و التقليل من قيمة الواردات من زيوت الكافور وذلك بمعرفة كمية الزيت الكلية و أهم ما به من مكونات الخاصة بكل نوع و ايضا معرفة الموسم الذى يعطى اكبر كمية زيت وكذلك الزمن الاقتصادى لعملية الاستخلاص.

أجريت هذه الدراسة خلال عام ٢٠٠١ على ثلاث انواع من الكافور هى الليموني – الجمفوسيفلا – العادى و المنزرعة عام ١٩٨٩ بالمزرعة البحثية لمحطة بحوث البساتين بالقصاصين التى تبعد حوالى ٨٠ كم شرق القاهرة و التابعة لمعهد بحوث البساتين – مركز البحوث الزراعية . تروى هذه المزرعة بالتنقيط وهى تعتبر مناطق تحت الاستصلاح .

أوضحت نتائج الدراسة أن كمية الزيوت الطيارة تتوقف علي موسم الحصاد ، ووجد أن أكبر كمية محصول زيت يمكن الحصول عليها في فصل

الصيف ثم تتدرج فى الانخفاض خلال فصل الربيع ثم الشتاء وكان أقلها إنتاجا فـــي فصل الخريف وذلك بالنسبة لكل من الكافور الليموني والجمفوسيفلا.

أما الكافور العادي فإن أعلى إنتاجاً للزيت الطياركان في فصل الربيع وتقل كميته تدريجيا فى فصلى الخريف و الصيف وكان أقلها إنتاجاً في فصل الشتاء.

و لقد أظهر التحليل الكروماتوجرفي إمكان فصل عشرين مكونـــاً للزيـــت الطيار موضع الدراسة وأن أكثر من ٩٠% من كمية هذه المكونات يمكن فصلـــها خلال الساعة و النصف الأولى من عملية التقطير . ومن الناحيـــة الاقتصاديـــة يكتفي بهذه المدة حيث أن باقي كمية المكونات الناتجة يمكن إهمالها نظراً لضآلتها.

كان زيت الكافور الليموني أكثر إحتواء على الـــcitronellal وذلك بنســبة ٧١,٣% بينما كان 1.8 – cineole بنسبة ٥٢,٩% ، ٣١,٢% فىنوعـــي الكـــافور العادى والجمفوسيفلا ، علي الترتيب.

وكانـــت أكثـــر المكونـــات الموجودة في زيت الكافور الليموني تركيزا هي Citronellal, Isopulegol and Citronellol

أما الكافور الجمفوسيفلا فأن أكثر المكونات تركيزاً في زيته هي 1.8–Cineole,P-Cymene, Allo-Aromadendrene, Aromadendrene, وأيضاً Beta – Pinene

بينما يتميز الكافور العادى باحتواء زيته الطيار علي 1.8- Cineole, Beta Pinene وأيضاً Alpha – Pinene, P- Cymene وذلك بنسب أكبر من باقي المكونات الأخرى.

ويتضح من هذه الدراسة إمكانية نجاح هذه الأنواع الثلاثة في الأراضي تحت الاستصلاح بجمهورية مصر العربية كمصدر للزيوت العطرية عالية الجودة و ذات قيمة اقتصادية كبيرة وكان أكثرها تفوقاً الكافور الليموني ثم الجمفوســـيفلا ويليـــه الكافور العادى .