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Original Article

The Best Harvesting Time of Three *Eucalyptus* Leaves to Obtain More Oil and 1,8-Cineole Contents

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Abstract

The seeds of three *Eucalyptus* species (Myrtaceae) (Origin: Australia), including:, *Eucalyptus loxophleba* Benth., *Eucalyptus microtheca* F.Muell. and *Eucalyptus largiflorens* F.Muell. were cultivated in 1993-1994 at two research stations (Shushtar and Dezful) in Khuzistan province (western south region of Iran) and a research station (Kashan) in Isfahan province (Central region of Iran). These species have good adaptability to climatic condition of this area (dry warm). The leaves of these species were collected in middle of the four seasons (spring, summer, autumn and winter) at two consecutive years, in order to find out the best harvesting time with the highest oil yield and 1,8-cineole percentage. After drying the plant materials in shade, their essential oils were obtained by hydro-distillation. The oils were analyzed by GC and GC/MS devises. The results showed that the harvesting time had significant effects on oil yield and 1,8-cineole content of the *Eucalyptus* species. The main components of the harvested oils consisted of 1,8-cineole and α-pinene. The highest oil yields of *E. loxophleba* Benth. in all localities were obtained from the samples collected in autumn and winter. The highest percentage of 1,8-cineole for Dezful site was extracted from the winter (70.2%) and autumn (63.2%) samples, For Kashan sits, was extracted from the winter (55.5%) and spring (58.1%) samples and for Shushtar site, was extracted from the summer samples (53.6 and 49.6%). The highest oil yield of *E. microtheca* F.Muell. was obtained in spring from Dezful and Shushtar localities and in autumn from Kashan locality. For both Shushtar and Dezful sites, the highest percentage of 1,8-cineole was obtained in winter and summer samples whereas for Kashan site, was obtained in summer samples. The highest oil yield of *E. largiflorens* F.Muell. was obtained in autumn and winter for Dezful and Shushtar samples and summer for Kashan samples. The highest percentage of 1,8-cineole was extracted in summer and winter for Shushtar samples, in spring and winter for Dezful samples and winter for Kashan samples.

Key words: *Eucalyptus loxophleba* Benth., *Eucalyptus microtheca* F.Muell., *Eucalyptus largiflorens* F.Muell., season.

Introduction

There are over 700 different species of *Eucalyptus* in the world, of which at least 500 produce a type of essential oil. The leaves and oils of many *Eucalyptus* species are especially used for respiratory ailments such as bronchitis and croup [1-8], and the dried leaves are smoked like tobacco for asthma in some countries. Some of the *Eucalyptus* species are also used for feverish conditions (malaria, typhoid, cholera, etc.) and skin problems like burns, ulcers and wounds. Aqueous extracts are used for aching joints, bacterial dysentery, ringworms, tuberculosis, etc. and employed for similar reasons in western and eastern medicine. Some of the *Eucalyptus* oils and their main component (1,8-cineole) are largely employed in the preparation of liniments, inhalants, cough syrups, ointments, toothpaste and pharmaceutical flavorings also used in veterinary practice and dentistry. *Eucalyptus* oils are also used as fragrance component in soaps, detergents and toiletries, little used in perfumes. The oils of *Eucalyptus* species have also

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antioxidant properties [7] and anti-inflammatory effects [9].

Literature search showed several examples of variation in the essential oil content and composition of some *Eucalyptus* species at different seasons. Ammon and coworkers reported that there was a general decrease in the percentage of cineole in the oils of *E. polybractea* and *E. viridis* during the winter months [9].

The effect of harvesting period on the oil production was examined on five *Eucalyptus* species (*E. camaldulensis, E. globulus, E. globulus* ssp. *Maidenii, E. melliodora* and *E. bosistoana*) of Moroccan origin. For some species oil yield seemed to depend on the harvesting season. The 1,8-cineole content of the oils also varied during the year [19].

Study of seasonal variation in the composition of essential oil of *E. camaldulensis* in Pakistan showed that the major compound was 1,8-cineole (47.7-52.6%). The oil yield was minimum in Apr. (0.97%) and maximum in Oct. (1.25%) [10].

Seasonal variation in the volatile leaf oils of individual trees of *E. ovata* and *E. camphora* was investigated. The trees sampled included several chemical forms, and individuals varied characteristically and differently over the sampling period. Variation is best explained by a leaf ageing effect rather than a strictly seasonal effect. Individual trees were readily distinguished in spite of the observed variation and with appropriate sampling; chemosystematic studies should not be affected [11].

Study of seasonal variation in the volatile oil and 1,8-cineole content of three *Eucalyptus* species in Iran (*E. porosa, E. leucoxylon and E. camaldulensis*) showed that, for obtaining the 1,8-cineole rich oil from *E. porosa* leaves, the best harvesting time is spring, while for E. *leucoxylon* and *E. camaldulensis* var. *obtusa* autumn and winter are the best times in Shushtar and Dezful, respectively [12].

Here are many other references about changes in the oil content and composition of *Eucalyptus* species in different countries [13-16].

In change, researches carried on by Brooker and coworkers showed that seasonal variation in cineole production of *E. kochii* and *E. plenissima* was only slight, meaning that time of harvesting was not important, although in plantations it might need to be done at the optimum time for regeneration[1]. So for, finding the best harvesting time to obtain more quantity and quality of the oil (more oil yield and higher percentage of cineole), study of these variations is necessary.

In this paper, we will examine the seasonal changes in the volatile oil and cineole content of three cultivated and adapted *Eucalyptus* species: *E. loxophleba* Benth., *E. microtheca* F.Muell and *E. largiflorens* F.Muell from central and western south region of Iran.

Materials and Methods

Plant material

The seeds of some Eucalyptus species (Origin: Australia) were cultivated in the years 1993-1994 in three research stations: Shushtar and Dezful in Khuzistan province (in the west south of Iran) and Kashan in Isfahan province (Center of Iran). These species have good adaptability to the climatic condition of this area (dry warm). Fresh leaves of the three adapted *Eucalyptus* species were collected in middle of spring, summer, autumn and winter, during two consecutive years (2005-2006). The voucher specimens have been deposited in the national herbarium of Iran (TARI).

Oil extraction

Air-dried leaves of all samples were subjected to hydro-distillation for 2.5 h using a Clevenger-type apparatus. The oils separated from water, dried over anhydrous sodium sulfate and stored in sealed vials at low temperature before analysis.

GC and GC/MS analysis

GC analysis was performed, using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 40°C for 5 min and then programmed to 280°C at a rate of 4°C/min. Injector and detector (FID) temperature was 290°C; helium was used as carrier gas with a linear velocity of 32 cm/s. The percentage of the compounds was calculated by the area normalization method, without considering response factors.

GC-MS analyses was made by a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.) and its characteristics were as follows: oven temperature was 40°C to 250°C at a rate of 4°C/min, transfer line temperature 260°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, Ionization energy 70 eV; scan time 1 s and mass range of 40-300 amu. Oil components were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature [17-18]. The retention indices were calculated for all

volatile constituents using a homologous series of n-alkanes [19].

Results and Discussion

The oils of *E. loxophleba* Benth., *E. microtheca* and *E. largiflorens* were found to be pale yellow liquids. The oil yields of each species are shown in Table1 and Figures1-3. The highest oil yields of *E. loxophleba* in all localities were extracted from the autumn and winter samples. The highest oil yields of *E. microtheca* were obtained from the spring samples for Dezful and Shushtar sites and from the autumn samples for Kashan site.

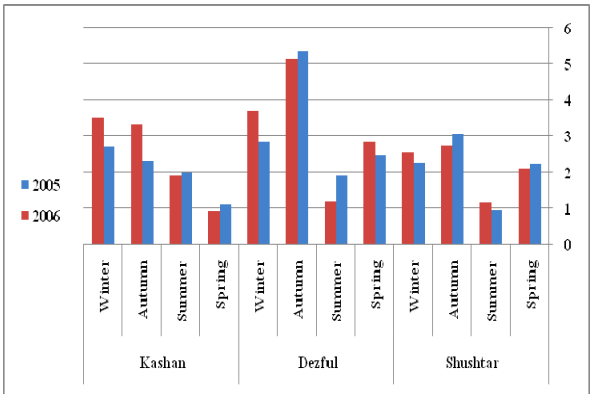

Fig 1 Essential oil yields of *E. loxophleba* Benth. in three locations and at four seasons

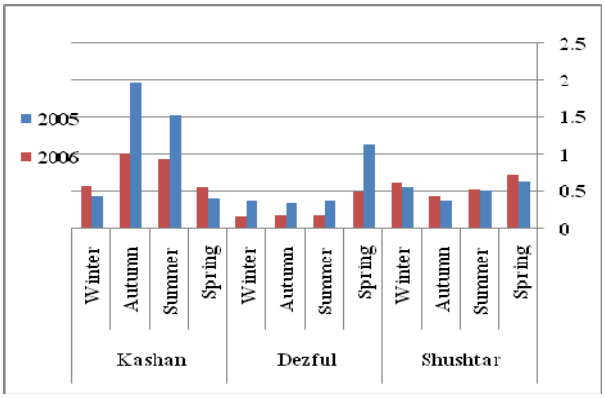

Fig 2 Essential oil yields of *E. microtheca* F.Muell. in three locations and at four seasons

The highest oil yields of *E. largiflorens* were obtained from the autumn and winter samples for Dezful and Shushtar sites and from the summer samples for Kashan site. Thirty-five components were identified in the oils of *E. loxophleba*. The major components were 1,8-cineole and α-pinene during two consecutive years and at all of the harvesting times. The chemical compositions of the oil of *E. loxophleba* at the three studied sits are presented in Table 2. The components are listed in order of their elution on the DB-5 column. As shown in Table 2, the high amount of 1,8-cineole in Shushtar was at summer during two consecutive years. Although, the oil yields were obtained at autumn, but the quality of essential oils was maximum at summer during two consecutive years. The high amount of 1,8-cineole in Dezful was at winter at the first studying year, whereas it was the highest at autumn and winter respectively, at the next year. The high amount of 1,8-cineole in Kashan was at winter summer respectively at the first year, whereas it was the highest at spring in the next year. Our research during the two consecutive years showed that to identify the best harvesting season in order to obtain a highest percentage of essential oil with a highest content of 1,8-cineole from *E. loxophleba* leaves, in Shushtar and Kashan sites, needs enough investigation for certain declare, whereas for Dezful site, the best harvesting time was winter .

Thirty-four compounds were identified in the oil of *E. microtheca* during the two consecutive years. The main components were 1,8-cineole and α-pinene and at the harvesting seasons. The chemical composition of the oil of *E. microtheca* is presented in Table3. The components are listed in order of their elution on the DB-5 column. As shown in Table 3, the highest amount of 1,8-cineole in Shushtar site was measured at winter and autumn seasons respectively at the first year of trial, whereas it was the highest at summer for the next year. The percentage of 1,8-cineole was low in Shushter site. The highest amount of 1,8-cineole in Dezful site was measured at summer for the first year of the trial and it was the highest at winter for the next year. The highest amount of 1,8-cineole in Kashan site was at summer and then winter for the first year of the trial, whereas it was the highest at summer and spring respectively for the next year. Our findings after the two consecutive years showed that to obtain the highest percentage of essential oil with high content of 1,8-cineole from the leaves of *E. microtheca* it couldn't suggest special time certainly for Shushtar site, but for Dezful site it needs enough investigation for certain declare due to high variation in the quality of essential oils during the two consecutive years and for Kashan site the best harvesting season was summer (Fig 2).

Thirty-four components were identified in the oil of *E. largiflorens*. The main components were 1,8-cineole and α-pinene during the two consecutive years and at all of the harvesting seasons. The chemical components of the oil of *E. largiflorens* are presented in Table4. The components are listed in order of their elution on the DB-5 column. As shown in Table 4, the highest amount of 1,8-cineole in Shushtar site was found at summer at the first year of the trial and it was the highest in winter at the next year.

Table 1 Oil yields of three *Eucalyptus* species at different seasons.

Species	Oil Yield (%) Shushtar								Oil Yield (%) Dezful								Oil Yield (%) Kashan							
	2005				2006				2005				2006				2005				2006			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Eucalyptus loxophleba	2.23	0.95	3.08	2.28	2.10	1.16	2.73	2.53	2.46	1.91	5.37	2.86	2.84	1.20	5.15	3.71	1.10	2.00	2.33	2.70	0.9	1.91	3.31	3.50
Eucalyptus microtheca	0.63	0.51	0.37	0.55	0.74	0.53	0.43	0.62	1.14	0.37	0.34	0.37	0.50	0.18	0.18	0.16	0.40	1.52	1.98	0.43	0.55	0.94	1.02	0.57
Eucalyptus largiflorens	1.3	1.6	2.2	2.8	1.4	1.6	1.6	1,8	1.2	0.8	2.1	1.3	1.2	0.8	2.3	1.4	1.22	1.47	1.40	1.14	1.21	2.24	1.28	1.12

Table 2 Variation in the essential oil components of *Eucalyptus loxophleba* in different seasons.

Peak No.	Compound	Shushtar								Dezful								Kashan							
		2005				2006				2005				2006				2005				2006			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
1	Isopentyl acetate	-	-		-	-	-	-	-	0.4	0.3	-	-	-	-	-	-	-	-		-	-	-	-	-
2	no name	20.5	-	20.7	11.8	29.1	-	24.5	18.7	25.7	26.0	31.4	2.3	25.9	25.4	12.4	25.5	-	-		-	-	-	-	-
3	Isopentyl formate	-	-		-	-	-	-	-	-	-		-	-	-	-	-	0.4	0.6	-	-	0.3	0.3	0.9	0.4
4	5-hydroxy pentanal	-	-		-	-	-	-	-	-	-		-	-	-	-	-	28.6	12.3	18.3	8.2	18.0	14.3	25.6	27.2
5	tricyclene	-	-		-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	0.2	-	-	0.5	0.5
6	α-pinene	9.7	5.8	10.6	11.3	19.9	9.8	11.4	3.3	4.1	4.7	6.7	-	3.9	9.6	0.4	1.5	1,8	7.7	5.2	10.4	9.1	6.3	5.3	1.2
7	β-pinene	-	0.1	-	-	0.3	0.1	0.2	-	-	-		-	-	-	-	-	-	-	-	-	1.0	-	-	-
8	myrcene	-	0.2	-	-	-	0.2	-	-	-	-		-	-	-	-	-	-	-	-	-	0.2	-	-	-
9	α-phellandrene	-	2.7	-	-	0.2	2.5	0.2	-	-	-	-	-	-	0.3	0.4	-	-	-	-	-	0.6	-	-	-
10	p-cymene	9.0	6.0	1.3	0.6	t	6.2	0.1	0.6	-	0.4	-	-	-	0.2	2.2	0.4	0.7	2.5	1	3.5	4.7	1.3	0.7	0.8
11	limonene	1.9	1,8	-	1.4	-	1,8	-	-	-	0.4	-	-	-	-	2.4	-	0.3	2.4	-	-	1.0	1.2	0.7	1.1
12	1,8-cineole	39.8	53.6	34.0	49.4	26.2	49.6	35.6	39.9	49.4	44.0	40.2	70.2	48.0	45.1	63.2	61.8	33.5	48.0	39.6	55.5	58.1	28.3	25.4	24.1

Table 2 (Continue)

Peak No.	Compound	Shushtar								Dezful								Kashan							
		2005				2006				2005				2006				2005				2006			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
13	γ-terpinene	-	0.4	-	-	0.1	0.4	0.1	-	-	0.1	0.5	0.1	0.1	0.3	0.3	-	0.2	-	-	-	0.2	0.6	-	-
14	terpinolene	-	-	0.5	0.7	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
15	Isopentyl isovalerate	-	0.3	-	0.4	0.1	0.3	0.2	-	-	-		-	-	-	-	-	-	0.2	-	-	0.2	-	-	-
16	Trans pinocarveol	4.9	3.6	1.6	3.5	2.5	3.4	3.0	4.5	2.0	2.4	0.5	4.4	2.2	0.2	5.9	2.8	3	3.2	3	2.5	1.1	2.2	1.5	2.8
17	pinocarvone	1.1	0.9	-	0.9	0.4	0.8	0.6	1.0	0.5	0.5	-	0.9	0.4	0.5	1.2	0.6	0.8	1.0	0.8	0.9	0.4	2.2	0.5	0.8
18	borneol	-	0.5	-	-	-	0.7	0.2	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
19	Terpinen-4-ol	-	1.3	-	-	0.2	1.4	0.2	-	-	0.1	-	-	0.4	0.5	0.8	-	0.7	0.6	-	-	0.5	-	-	-
20	α-terpineol	-	0.4	-	-	0.1	0.5	0.1	-	-	0.2	-	-	0.1	0.1	0.5	-	-	-	-	-	-	-	-	-
21	α-terpinyl acetate	-	-		-	-	-	-	-	-	-		-	-	-	-	-	-	0.2	-	1.2	-	-	-	-
22	α-gurjunene	-	-		-	-	-	-	-	-	-		-	-	-	-	-	4.9	-	-	-	-	0.8	-	-
23	E-caryophyllene	-	0.2	8.7	-	-	0.2	0.2	0.3	-	-		-	-	-	-	-	-	0.2	-	-		-	0.7	-
24	α-guaiene	5.5	0.6	1.2	4.8	6.7	0.5	5.6	1.1	3.3	4.6	5.9	4.2	3.1	4.9	2.5	1.5	-	4.9	10.5	5.0	1.2	12.8	14.4	6.5
25	Allo-aromadendrene	0.9	0.1	1.3	0.7	0.8	0.1	0.7	0.3	-	-	1.1	0.6	0.4	0.8	0.5	0.1	-	0.9	1.3	0.9	-	2	1.7	1.0
26	viridiflorene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	2.3	-	-
27	β-selinene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9	-	-	-	0.4	-	-

Table 2 (Continue)

Peak No.	Compound	Shushtar								Dezful								Kashan							
		2005				2006				2005				2006				2005				2006			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
28	bicyclogermacrene	-	0.4	1,8	-	-	0.4	-	-	-	-	1.4	0.2	-	1.2	0.5	0.1	-	-	-	-	-	-	-	-
29	spathulenol	1.0	0.6	1.3	1.2	0.4	0.6	0.3	2.2	-	0.2	-	1.2	0.8	0.5	0.4	0.2	2	0.9	-	2.7	-	1.3	-	-
30	globulol	-	4.0	7.1	7.7	4.5	3.8	5.7	0.8	4.7	4.3	3.4	6.5	5.1	2.9	1,8	2.5	0.8	5.1	10.5	6.0	0.6	9.9	11.2	14.5
31	viridiflorol	3.4	1.1	1.9	1.7	1.0	1.3	0.3	-	1.0	0.5	1.1	0.6	1.0	0.8	2.0	0.3	11.5	t	2.2	1.7	t	2.1	3	3.1
32	γ-eudesmol	1.9	0.6	1.0	0.6	0.5	0.7	0.5	0.3	3.6	0.1	0.1	0.5	0.2	0.4	-	0.1	0.9	0.1	0.7	-	-	2.6	1.3	-
33	β-eudesmol	4.4	0.2	1.7	2.2	0.5	0.2	1.2	0.5	1,8	1.9	1.9	0.4	0.4	2.0	1.7	1.6	-	0.5	3.5	-	-	3.8	-	6.9
34	α-eudesmol	2.4	0.2	2.1	1.0	0.8	0.2	3.3	0.5	-	0.1	2.0	3.1	4.8	1.5	-	-	3.2	0.4	-	1.1	-	3.4	1.5	2.3
35	Cyclocolorenone	-	-		-	-	-	-	-	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total		99.7	84.8	99.1	100	94.3	85.7	94.2	74	99.1	90.8	97.3	95.2	97.7	96.6	100	99	93.3	92.9	96.6	99.6	97.1	92.4	94.4	95.2

RI = Retention indices in elution order from DB-5 column
t = trace = less than 0.05%

Table 3 Variation in the essential oil components of *Eucalyptus microtheca* in different seasons.

Peak No.	Compound	Shushtar								Dezful								Kashan							
		2005				2006				2005				2006				2005				2006			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
1	α-thujene	0.4	0.2	-	1..3	0.1	-	0.3	0.3	-	0.1	-	-	-	1.6	-	-	0.4	-	0.1	-	-	-	0.5	-
2	α-pinene	12.5	14.1	6.0	11.4	16.3	18.6	20.1	18.6	12.0	15.5	0.6	11.2	10.2	14.6	0.2	10.6	10.7	7.4	10.3	11.4	19.4	16.15	9.2	10.3
3	camphene	0.2	0.3	-	-	0.1	0.4	0.4	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	β-pinene	6.0	5.2	2.9	2.2	9.3	10.0	9.0	7.3	-	0.8	0.3	0.5	-	0.7	0.2	0.3	10.5	0.2	7.1	2.2	5.6	3.2	16.3	3.2
5	myrcene	0.5	0.4	-	0.3	-	0.5	0.6	0.5	-	0.6	-	-	-	-	-	0.2	0.6	0.2	0.1	-	0.4	0.3	0.7	-
6	α-phellandrene	7.8	2.8	-	0.4	0.4	1.2	3.7	2.9	7.1	-	-	0.5	6.8	0.7	-	0.2	7.3	-	0.5	0.4	0.9	2	3.2	0.6
7	α-terpinene	0.5	0.1	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	p-cymene	14.8	7.9	12.0	9.5	11.4	t	19.8	22.9	20.4	0.4	5.9	20.4	17.4	16.7	7.9	1.1	12.4	2.8	12.6	12.6	3.5	3.8	10.4	10.4
9	limonene	4.6	2.7	1.2	3.9	-	-	-	-	3.2	6.0	0.2	-	3.2	-	0.2	-	4.2	2.8	0.1	2.2	2.2	2.7	3.6	3
10	1,8-cineole	26.7	34.5	48.0	52.1	24.3	42.2	23.5	23.0	16.9	63.4	25.3	21.5	21.8	41.1	19.3	75.4	34.0	57.5	24.4	45.3	53.4	55.5	30.3	47.4
11	Z-β-ocimene	0.3	0.5	-	-	6.5	-	0.1	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	γ-terpinene	2.1	3.7	2.9	1.2	4.0	2.1	6.5	0.4	-	0.3	2.1	0.2	-	1.7	2.1	0.2	1.2	0.4	1.6	1.2	-	0.9	1.6	1.6

Table 3 (Continue)

Peak No.	Compound	Shushtar								Dezful								Kashan							
		2005				2006				2005				2006				2005				2006			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
13	terpinolene	0.7	-	-	0.4	0.6	0.2	0.4	1.3	-	1.0	5.1	0.2	-	0.6	7.1	0.2	-	-	-	-	-	-	-	-
14	Isopentyl isovalerate	0.2	0.6	0.6	0.5	-	1.0	0.8	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Endo-fenchol	0.2	-	1.3	-	1.0	0.4	0.4	5.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Cis-P-menth-2-en-1-ol	0.3	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Trans pinocarveol	1.9	3.8	6.7	1.9	-	2.0	1,8	2.8	1,8	0.9	5.6	8.4	1.9	1.1	6.6	1.5	1.6	3.5	1.6	3.2	1,8	1.5	0.8	3.7
18	pinocarvone	0.7	1.1	3.4	0.8	1.1	0.2	0.7	0.7	-	0.2	0.7	2.9	-	0.3	0.8	0.5	0.7	1.2	1.0	1.5	0.8	0.5	-	1.7
19	Borneol	0.4	0.4	1.5	-	-	0.9	0.5	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	Terpinen-4-ol	3.2	2.0	2.2	1.4	-	0.3	2.3	1.0	2.9	1.4	2.2	0.9	2.7	4.0	2.2	0.7	1,8	0.7	1.5	1.3	0.5	0.9	2.3	1.5
21	cryptone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-	1	0.1	-
22	α-terpineol	1,8	0.3	2.4	1.2	-	3.1	2.1	0.6	-	0.8	0.4	0.5	-	0.7	0.4	0.7	2.1	0.5	2.9	1.4	0.8	-	1,8	1.2
23	myrtenal	0.6	2.1	-	-	3.1	2.5	0.2	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	α-terpinyl acetate	-	-	-	-	-	-	-	-	2.3	-	1.3	0.6	2.4	0.9	1.3	0.1	-	-	-	-	-	-	-	-
25	α-guaiene	1.2	1.7	4.1	2.2	2.3	2.3	1.1	1,8	4.8	0.7	16.5	4.5	3.6	3.5	14.3	0.5	1.5	4.4	0.3	1.1	3.4	-	2.8	1.2

Table 3 (Continue)

Peak No.	Compound	Shushtar								Dezful								Kashan							
		2005				2006				2005				2006				2005				2006			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
26	Allo-aromadendrene	0.5	0.3	-	0.8	1.4	0.4	0.2	0.4	-	0.2	1.6	2.1	-	0.5	1.6	-	-	-	1.5	-	0.6	-	-	-
27	viridiflorene	0.3	0.3	-	0.7	0.8	0.6	0.1	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	bicyclogermacrene	-	-	-	-	-	-	-	-	7.0	0.2	1,8	-	6.8	-	1,8	-	-	-	-	-	1.3	-	-	-
29	spathulenol	0.4	0.2	-	2.4	0.6	0.2	2.7	1.7	7.2	1.4	3.7	7.4	7.8	6.0	2.7	1.3	1.1	5	0.3	1.6	0.5	4.1	3.0	1.4
30	globulol	4.6	3.8	4.2	3.3	-	3.8	0.4	0.2	11.1	0.2	16.5	7.2	10.6	1.1	17.1	1.3	5.2	1.3	7.8	8.1	2.7	1.1	4.5	5.6
31	viridiflorol	0.8	0.5	-	0.6	0.6	0.6	0.2	-	-	-	2.2	0.8	-	0.3	2.1	-	1.0	0.6	0.6	-	-	0.5	1.2	-
32	β-eudesmol	0.8	0.3	-	-	0.9	0.4	0.2	0.3	-	-	0.5	0.5	-	0.3	0.6	-	-	-	-	-	-	-	-	-
33	α-eudesmol	0.9	0.3	-	0.6	2.4	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	α-cadinol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	1.0	-
Total		95.9	90.1	99.4	99.1	87.2	94.4	98.4	97.3	98.1	94.1	92.5	90.3	99.8	96.4	88.5	94.8	96.2	88.5	84.5	93.5	97.8	97.3	93.3	92.8

RI = Retention indices in elution order from DB-5 column

t = trace = less than 0.05%

Table 4 Variation in the essential oil components of *Eucalyptus largiflorens* in different seasons.

Peak No.	Compound	Shushtar								Dezful								Kashan							
		2005				2006				2005				2006				2005				2006			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
1	α - thujene	-	-	1.9	0.7	-	0.5	1.9	1.0	0.4	0.4	1.0	0.6	1.3	1.2	0.5	0.3	0.3	0.3	0.3	-	0.4	0.6	0.8	-
2	α - pinene	4.3	21.0	7.7	7.0	3.7	7.3	6.1	5.0	4.0	6.8	5.3	7.3	5.0	7.9	5.5	4.8	2.4	12.1	12.6	18.4	8.3	10.5	4.6	10.2
3	sabinene	-	-	1.1	-	0.2	-	0.8	0.5	0.4	0.1	0.9	-	-	-	1.0	0.2	0.3	0.7	0.4	-	0.2	0.6	0.7	-
4	β -pinene	-	0.8	5.3	0.7	1.4	2.1	2.2	0.5	4.2	12.6	6.8	13.3	11.5	13.8	10.4	8.6	0.2	0.8	0.5	-	0.9	0.6	0.6	1.2
5	myrcene	-	0.3	1,8	0.7	0.5	0.6	1.9	0.8	0.6	0.4	0.8	0.4	1.1	0.9	-	0.2	0.3	0.9	-	-	0.4	0.6	-	-
6	α–phellandrene	-	-	3.2	1.7	1.2	1.2	5.9	1.7	0.9	0.4	0.6	1,8	1.7	0.6	0.4	-	1.5	1.7	0.7	-	0.9	2	-	-
7	α-terpinene	-	-	-	-	-	-	-	-	0.4	2.0	-	0.1	0.3	-	-	0.2	0.2	0.8	-	-	-	-	-	-
8	p-cymene	4.5	0.6	1.6	5.4	-	0.5	0.9	8.7	5.8	6.9	9.0	1,8	0.4	3.7	12.8	11.1	17.4	9.6	13.1	10.2	11.4	5.8	22.4	8.1
9	limonene	3.3	1,8	1,8	13.1	-	-	-	-	2.1	-	9.2	-	-	-	-	-	6.5	-	2.2	1.7	-	1.6	3.7	-
10	1,8-cineol	45.5	58.4	49.0	53.3	59.5	65.1	55.8	66.2	53.0	27.0	48.2	38.7	53.4	53.5	50.4	59.1	37.5	41.3	46.7	50.9	63.8	64.2	36.0	65.8
11	γ-terpinene	-	0.2	1.5	0.8	0.9	0.3	1.6	0.4	0.8	0.4	-	3.7	0.7	0.3	0.2	0.2	0.4	0.7	0.5	3.5	0.3	0.4	0.4	-
12	terpinolene	-	-	-	-	-	0.3	1.0	0.7	0.8	0.3	-	-	0.3	0.2	0.2	0.1	0.3	0.8	-	-	-	-	-	-
13	β-thujone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-

Table 4 (Continue)

Peak No.	Compound	Shushtar								Dezful								Kashan							
		2005				2006				2005				2006				2005				2006			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
14	Cis-p-Menth-2-en-1-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.2	-	-	-	-	1.1	-
15	α-camphol enal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	1,8	-	5.4
16	Trans-pinocarv eol	1.4	4.8	1.3	1.1	2.9	1.7	0.6	2.3	1.7	4.4	1.3	3.3	1,8	1.7	2.4	2.4	-	-	-	-	1.3	1,8	-	5.4
17	Trans-p-menth-2-en-1-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.1	1.1	2.9	-	-	-	1.3	-
18	pinocarv one	-	1.4	0.3	2.4	-	2.7	3.2	3.3	0.5	1.5	-	1.4	0.5	0.7	0.2	1.1	-	-	0.9	-	0.5	0.5	-	2.4
19	terpinen-4-ol	1.6	-	4.1	-	5.4	3.4	1.9	0.7	3.1	2.7	2.9	3.1	3.0	2.9	0.6	1.3	3.6	1.9	1.9	1.4	2.3	1.4	4.6	1.0
20	cryptone	2.7	0.3	3.8	-	0.4	0.8	0.8	0.6	2.6	8.1	3.1	7.6	4.0	4.8	2.4	3.7	-	-	3.9	2.5	2.4	-	-	-
21	neo isoverbe nol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.1	0.8	-	-	-	-	9	3.7
22	α - terpineol	1.0	0.5	1.3	-	1.2	0.9	0.4	0.4	1,8	1.5	1.0	3.7	1.0	0.7	0.5	0.5	1.5	0.3	0.8	0.6	0.4	0.7	0.8	0.8
23	Myrenol	-	-	-	-	-	-	-	-	1.0	4.8	-	0.4	0.3	1.0	3.7	1.4	-	-	-	-	-	-	-	-
24	cuminald ehyde	0.9	0.1	1.1	-	1.0	0.5	0.4	0.3	0.8	2.4	1.0	2.0	1.1	1.2	1.4	0.8	3.6	1.1	1.6	1.2	0.7	-	3.1	-

Table 4 (Continue)

Peak No.	Compound	Shushtar								Dezful								Kashan							
		2005				2006				2005				2006				2005				2006			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
25	piperitone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-	-	-	-
26	thymol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9	-	-	-	-	-	-	-
27	α - terpinyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.7	0.8	-	-
28	α - guaiene	1.2	0.6	0.5	2.4	0.3	4.3	3.0	1.5	0.6	0.2	-	0.4	0.5	-	1.5	-	-	-	1.2	1.9	0.6	0.7	-	-
29	bicyclogermacrene	1.6	1.6	0.9	1.7	-	1.2	2.9	1.0	1,8	0.1	2.0	-	1.0	-	0.9	-	0.4	0.9	-	-	-	2.9	-	-
30	spathulend	12.8	0.3	2.4	3.5	6.2	0.1	0.3	-	5.6	6.6	3.3	3.8	3.4	1,8	2.8	1.2	6.7	3.4	6.1	2.3	1,8	2.4	8.3	-
31	globulol	7.7	0.2	1.7	4.5	3.3	0.2	0.5	-	3.5	0.9	1.9	1.4	2.2	-	0.3	0.2	2.4	2.7	3.7	2.3	1.6	2.0	1,8	-
32	β-eudesmol	4.8	0.9	0.2	-	-	-	-	-	-	-	1.7	0.5	0.2	-	0.1	-	-	-	-	1,8	-	-	-	1.4
33	α - eudesmol	2.8	-	0.2	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-
34	cyclocolorenone	3.9	-	-	-	1.3	0.4	1.7	0.3	2.9	-	-	-	0.9	-	0.2	-	-	-	-	-	-	-	-	-
Total		100.0	93.8	92.7	99.0	89.4	94.1	93.8	95.9	99.3	90.5	100.0	95.3	95.9	96.9	98.4	97.4	98.1	82.1	100.0	98.7	98.7	100.0	100.0	100.0

RI = Retention indices in elution order from DB-5 column

t = trace = less than 0.05%

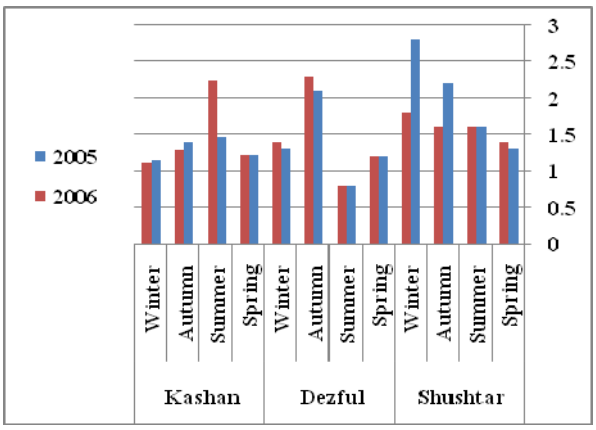

Fig 3 Essential oil yields of *E. largiflorens* F.Muell. in three locations and at four seasons

The highest amount of 1,8-cineole in Dezful site was at spring at the first year of the trial and it was the highest at winter at the next year. The highest amount of 1,8-cineole in Kashan site was at winter at the both years. Our findings showed that to obtain a high percentage of essential oil with high content of 1,8-cineole from *E. largiflorens* leaves, winter was the best for Shushtar site, but for Dezful and Kashan site, it needs longer period research for certain declare due to high variation of essential oils quality after the two consecutive years (Fig 3). Therefore, our research showed that winter is the best harvesting season for *E. largiflorens* in Shushtar, whereas for Dezful and Kashan it needs more study for certain declare. Variation in oil yields of the three *Eucalyptus* species are shown in figures1-3.

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