Essential oil constituents of different organs of fennel (*Foeniculum vulgare* var. *vulgare*).

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ABSTRACT: Essential oil constituents of different organs of fennel (*Foeniculum vulgare* var. *vulgare*). The fennel (*Foeniculum vulgare* Mill.var. *vulgare*) is an annual herbaceous plant, whose seeds are very used in the homemade medicine and pharmaceutical industry. The fennel seeds produce yellow-clear aromatic essential oil, used in the production of several licorous drinks and of perfumery, with action carminative and stimulant. Therefore, another structure of plants (steams, leaves, flowers bud, inflorescences) also production essential oils with commercial interest. An experiment was carried out at farm of the Agronomical Sciences College – UNESP, in Botucatu, Brazil. The statistical design used was completely randomized block with 3 replications and 7 treatments. The treatments were different harvest times. The seedling were sowings in summer of the 2001 (January) and harvested every 14 days, until the complete maturation during one year (total of seven harvests). The results presented the highest value of trans-anethole (78.25%) during the summer in dry seeds, limonene (42.30%) in spring in steams/leaves and fenchone (16.98%) in green seeds in autumn.

Key words: fennel, phenological phases, seasons of year, medicinal plants.

INTRODUCTION

The renewed interest in natural products, rather than synthetic agents, has again focused attention on plants as a source of flavouring compounds (Yaylayan, 1991). Fennel is a plant belonging to the Umbelliferae (Apiaceae) family, a medicinal native plant from Mediterranean used as antispasmodic, appetite stimulant, stomachic, diuretic, anti-inflammatory, anti-diarrheic, against colic and as a lactation promoter. Several components of the essential oil from this plant have important applications, namely, fenchone is used as counterirritant; limonene is used as solvent, resins, wetting and dispersing agent; *trans*-anethole is used for flavoring agent in perfumery, in cosmetics, in soap, pharmaceutical aid (flavor); metyl-chavicol or estragole is used in perfumeries and as flavor in foods and liquors; a-pinene, used in manufacture of camphor, insecticides, solvents, perfume bases (Marotti *et al*., 1993; Piccaglia & Marotti,1993; Cavaleiro *et al.,*1993).

However, the study of a plant as a source of flavoring compounds requires the analysis of not only its seeds but also other parts of the plant. For this reason, some authors have reported comparison between fruit oil and herb oil from sweet fennel (Embong *et al.,*1977); components of oil produced from stems, leaves, flowers and the whole plant (Baldrich *et al.,* 1986); the volatile components of leaves, stems and fruits (Miura *et al*., 1986); and the chemical composition of the essential oils obtained from various parts of the bitter Turkish fennel plant (Akgül & Bayrak, 1988).

The objective of this work was to identify the chemical composition of the essential oil of *Foeniculum vulgare* var. *dulce* in other structure of plants (stems, leaves, flowers bud, inflorescences) with potential commercial interest.

MATERIAL AND METHOD

Samples and extraction

The study was carried out in the Experimental Farm of the Department of Plant Production, Horticulture Section, São Paulo State University, Campus of Botucatu, Brazil In field. The statistical design was in completed randomized blocks with seven treatments and three replications. Stem and leaves, inflorescences in anthesis, flowers buds and green, mature (yellow seeds) and dry seeds phase were collected. The essential oil was isolated by hydrodistillation from fresh in Clevenger apparatus; a total of 100 g of fresh plants parts were used in each extraction.and 50 g of the seeds in dry base (SILVA,1991). The extraction proceeded for three

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hours. The essential oil composition of the extracts was determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). Triplicate analyses were performed.

Soil analysis

Soil samples were taken at a depth of 20 cm from randomly chosen points at each of the four experiment sites. All samples were analyzed in the laboratory of the Departamento de Recursos Naturais - Ciências do Solo, da Faculdade de Ciências Agronômicas - UNESP - Campus de Botucatu (Natural Resources Department – Soil Sciences, Faculty of Agronomic Sciences – UNESP). The results of the chemical analysis are shown in Table 1.

TABLE 1. Results of the chemical analysis of the soil samples, expressed in volume of air-dried fine soil.

Experiment	pH at $CaCl_2$	O.M.	P	$H^+ + Al^{3+}$	K^+	Ca^{2+}	Mg^{2+}	SB	CEC	V
		g/dm^3	mg/dm^3	---------- Miliequivalent 100 cm^{-3} of soil ----------						(%)
1, 2, 3 and 4	4.8	20	16	18	3.2	12	5	20	39	53

Soil preparation, liming and fertilization

Soil was prepared by ploughing and harrowing. Fertilizer and lime application were made according to the results of soil analysis. Five Kg/m^2 of cattle manure and 2,6t/ha of dolomitic lime (PRNT100%) were applied to the soil. Pits of 10 cm in depth received the following nutrients: 2 Kg/m^2 of K as potassium chloride (K_2O), 1 Kg/m^2 of N as urea ($CO(NH_2)_2$) and 15 kg for each plantation (470 m^2) of P as single superphosphate (P_2O_5). As the organic matter was only regular, it was applied 5 kg/m^2 of cattle manure together with the mineral fertilizers. Cover fertilizations were made trough urea application at 30, 60 and 90 days after planting (1°, 2º, 3 º and 4º experiments).

Hydrodistillation Extract

The essential oil yield was determined by hydrodistillation in Clevenger apparatus. For essential oil extraction was used 100 g of plants parts, and 50 g of seeds. The extraction was performed at boiling heat of water over three following hours, and the oil content being recorded.

Gas chromatography-MS identification

The chemical composition analysis of the essential oil was performed in a gas chromatograph connected to a mass spectrometer (CG-EM, Shimadzu, QP-5000), running at 70 eV, equipped with fused silica DB-5 column (30 m x 0.25 mm x 0.25 μm), helium as carrier gas (1.0 mL/min), injector at 240° C, detector at 230° C. The samples were solubilized in ethyl acetate (P.A. Merck, lote K25488323837; 0,03g of oil / 1 ml of solvent and 1 mL solution was injected, split: 1/30 in the temperature program as follows: 50° C (5 min) - 180°C, 5° C/min.; 180º-280º, 10 ºC/ min. The chemical compound identification was based on comparison of their mass spectra with data of CG-EM system (Nist 62.lib) and literature reference (Mclafferty & Stauffer 1989) and retention index of Kovats (Adams, 2001).

The retention index (IR) of the substances were achieved by co-elution with one succession homologate of n-alcano (C9H20 – C2%H52, Sigma – Aldrich, 99%), application equation of Van den Dool e Kratz, 1963.

RESULT AND DISCUSSION

The yield of essential oils was high in green seeds and dry seeds in summer and autumn (table 2 and 3).

The main compounds of essential oils from plant parts were *trans-anethole* in dry seeds in Summer (78.25%); *limonene* in steams/leaves in Spring (42,30%); *Fenchone* in green seeds in Autumn (16,98%) and Summer (15,08 %); *methyl-chavicol* in green seeds in Autumn (3,57%); and to inflorescences in anthesis phase in summer α-*pinene* (2,24 %), γ-*terpinene* (1,14 %) and *myrcene* (1,24%) (tables 6, 7, 8 and 9). In the Summer was found 1.78% β-*ocimene* in leaves and 44,61% of *exo-fenchyl acetate* in leaves and stem.

The high proportion of *trans-anethole* funded in dry seeds and in other plant organs, are not in agreement with those obtained from essential of different parts of Turkish bitter fennel oils that presented high proportion of α-phellandrene (30,3%) in leaves and 16,9 % in stems, the lower concentrations of this compounds in essential oil from seeds (Akgül & Bayrak, 1988).

The proportion of *fenchone* in the volatile fraction of both leaf and stem extracts was of a similar order and smaller than for seed extract in studied of Gillén & Manzanos (1996); whereas was different in

the proportion in this study, where it was obtained higher proportion of fenchone in green seeds than in mature and dry seeds, leaves, stems, flowers buds and inflorescences in anthesis. This was found to be the case for limonene as well. These results are not in agreement with previous reports of essential oils of bitter and sweet fennel of the author foresaid.

Methylchavicol and *exo-fenchyl-acetate* was found in leaves, stems, flowers buds, inflorescences in anthesis and green and mature seeds (Tables 5 and 6).

TABLE 2 - Yield essential oil (%) of 36 plants of fennel (*Foeniculum vulgare* var. *vulgare*) in Summer

Harvest	days after plantation	Leaves and Stems (%)	Flowers buds (%)	Inflorescences in Anthesis (%)	Green Seeds (%)	Mature Seeds (Yellow) (%)	Dry Seeds (%)
1ª	158	0,27	0,83	0,97	-*	-	-
2ª	172	0,22	0,69	0,69	-	-	-
3ª	186	0,21	0,68	0,79	3,77	-	-
4ª	200	0,28	0,37	0,99	3,69	-	-
5ª	214	0,30	1,03	1,37	3,67	1,57	2,80
6ª	228	0,29	2,18	1,36	3,37	1,50	2,23
7ª	242	0,26	0,00	0,68	0,90	0,00	2,28
	Média	**0,26**	**0,96**	**0,98**	**3,08**	**1,54**	**2,44**

(-) traces

TABLE 3 -Yield essential oil (%) of 36 plants of fennel (*Foeniculum vulgare* var. *vulgare*) in Autumn

Harvest	days after plantation	Leaves and Stems (%)	Flowers buds (%)	Inflorescences in Anthesis (%)	Green Seeds (%)	Mature Seeds (Yellow) (%)	Dry Seeds (%)
1ª	158	0,32	0,70	0,76	-	-	-
2ª	172	0,40	0,74	0,93	-	-	-
3ª	186	0,18	-	0,68	3,00	-	-
4ª	200	0,30	-	0,93	3,00	2,20	3,20
5ª	214	0,20	-	0,72	2,60	-	2,00
6ª	228	0,30	-	0,80	-	-	2,20
7ª	242	0,28	0,42	0,90	1,80	-	1,80
	Médias	**0,28**	**0,62**	**0,80**	**2,60**	**2,20**	**2,30**

(-) traces

TABLE 4- Yield essential oil (%) of 36 plants of fennel (*Foeniculum vulgare* var. *vulgare*) in Winter

Harvest	days after plantation	Leaves and Stems (%)	Flowers buds (%)	Inflorescences in Anthesis (%)	Green Seeds (%)	Mature Seeds (Yellow) (%)	Dry Seeds (%)
1ª	158	0,23	0,65	0,78	-	-	-
2ª	172	0,27	-	1,07	-	-	-
3ª	186	0,32	-	1,31	-	-	-
4ª	200	0,35	-	1,10	-	-	-
5ª	214	0,33	1,10	1,43	-	-	-
6ª	228	0,25	-	1,22	-	-	-
7ª	242	0,37	-	1,37	-	-	-
	Média	**0,30**	**0,88**	**1,18**	-	-	-

(-) traces

TABLE 5- Yield essential oil (%) of 36 plants of fennel (*Foeniculum vulgare* var. *vulgare*) in Spring

Harvest	days after plantation	Leaves and Stems (%)	Flowers buds (%)	Inflorescences in Anthesis (%)	Green Seeds (%)	Mature Seeds (Yellow) (%)	Dry Seeds (%)
1ª	158	0,15	0,77	0,82	-	-	-
2ª	172	0,13	0,85	0,80	-	-	-
3ª	186	0,23	0,27	0,78	-	-	-
4ª	200	0,23	0,39	0,48	-	-	-
5ª	214	0,25	0,75	0,82	-	-	-
6ª	228	0,13	0,75	0,72	-	-	-
7ª	242	0,12	0,74	0,73	-	-	-
	Médias	**0,18**	**0,64**	**0,74**	-	-	-

(-) traces

TABLE 6- Essential oil composition of different organs of *Foeniculum vulgare* var. *vulgare*, in Summer of 2001.

Compounds	Leaves and Stems (%)	Flowers buds (%)	Inflorescences in Anthesis (%)	Green Seeds (%)	Mature Seeds (Yellow) (%)	Dry Seeds (%)
1 – α-Pinene	1,95	1,55	2,24	1,27	0,28	1,19
2 – Myrcene	0,97	0,77	1,24	0,93	0,50	0,72
3 – Limonene	34,48	20,78	19,41	5,26	2,74	3,67
4 – Fenchone	2,65	3,07	7,22	15,08	15,14	13,98
5 - Metyl-chavicol	1,74	2,47	2,33	2,56	2,50	2,45
6 - Cys-Anetole	1,34		0,49	-	-	-
7 – Trans-anethole	53,95	66,75	59,77	73,81	77,67	78,25
8 - β-Ocimene	1,18	1,08	0,86	-	-	-
9 - γ- Terpinene	0,69	1,11	1,14	0,57	-	-
10 – Endo Fenchyl -acetate	1,06		0,53	-	-	-
11 – Exo Fenchyl -acetate	3,06	1,25	1,75	-	-	-

(-) mean not sample

TABLE 7- Essential oil composition of different organs of *Foeniculum vulgare* var. *vulgare*, in Autumn of 2001.

Compounds	Leaves and Stems (%)	Flowers buds (%)	Inflorescences in Anthesis (%)	Green Seeds (%)	Mature Seeds (Yellow) (%)	Dry Seeds (%)
1 – α-Pinene	1,43	0,90	1,89	1,16	0,41	0,29
2 – Myrcene	0,97	0,60	1,09	0,95	0,49	0,41
3 – Limonene	34,83	18,95	19,99	6,70	3,32	2,32
4 – Fenchone	1,65	1,35	7,15	16,98	14,9	4,03
5 - Metyl-chavicol	1,66	1,87	2,11	3,57	-	-
6 - Cys-Anetole	-	-	-	-	-	-
7 – Trans-anethole	53,84	71,09	63,65	69,67	76,01	77,26
8 - β-Ocimene	0,66	0,75	0,70	-	-	-
9 - γ- Terpinene	0,98	0,84	0,82	0,56	-	-
10 – Endo Fenchyl -acetate	-	-	-	-	-	-
11 – Exo Fenchyl -acetate	2,91	1,36	1,60	3,35	4,24	-

(-) mean not sample

TABLE 8 - Essential oil composition of different organs of *Foeniculum vulgare* var. *vulgare*, in Winter of 2001.

Compounds	Leaves and Stems (%)	Flowers buds (%)	Inflorescence in Anthesis (%)	Green Seeds (%)	Mature Seeds (Yellow) (%)	Dry Seeds (%)
1 – α-Pinene	1,56	0,95	2,15	-	-	-
2 – Myrcene	1,20	0,63	1,34	-	-	-
3 – Limonene	41,34	17,96	22,22	-	-	-
4 – Fenchone	1,80	1,75	9,47	-	-	-
5 – Metyl-chavicol	1,49	2,09	1,90	-	-	-
6 - Cys-Anetole				-	-	-
7 – Trans-anethole	46,11	74,75	58,56	-	-	-
8 - β-Ocimene	1,49	0,89	0,86	-	-	-
9 - γ- Terpinene	1,46	0,82	1,24	-	-	-
10 – Endo Fenchyl -acetate	1,11			-	-	-
11 – Exo Fenchyl -acetate	4,25	1,7	1,79	-	-	-

(-) mean not sample

TABLE 9 - Essential oil composition of different organs of *Foeniculum vulgare* var. *vulgare*, in Spring of 2001.

Compounds	Leaves and Stems (%)	Flowers buds (%)	Inflorescence in Anthesis (%)	Green Seeds (%)	Mature Seeds (Yellow) (%)	Dry Seeds (%)
1 – α-Pinene	1,56	1,20	2,53	-	-	
2 – Myrcene	1,38	0,77	1,55	-	-	
3 – Limonene	42,30	22,79	23,42	-	-	
4 – Fenchone	3,70	4,80	8,07	-	-	
5 – Metyl-chavicol	1,63	2,73	2,20	-	-	
6 - Cys-Anetole	0,23			-	-	
7 – Trans-anethole	44,61	63,44	57,18	-	-	
8 - β-Ocimene	1,78	1,19	1,55	-	-	
9 - γ- Terpinene	0,29	1,03	1,55	-	-	
10 – Endo Fenchyl -acetate	3,19			-	-	
11 – Exo Fenchyl -acetate	44,61	1,21	2,66	-	-	

(-) mean not sample

CONCLUSION

The best period for growing fennel to obtain the compound *Trans-anethole* in dry seeds was the Summer (78.25 %); for *limonene* in steams/leaves was the Spring (42,30 %), and *Fenchone* in green seeds in Autumn (16,98%) followed of the Summer (15,08%).

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