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Drug Yield and Essential Oil of *Thymus vulgaris* L. as in Influenced by Ecological and Ontogenetical Variation*

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Abstract: Drug yield and volatile oil content and its components of *Thymus vulgaris* were investigated during the different growing periods under both lowland and mountainous conditions. The highest drug yields were obtained from lowland conditions during the post flowering stage. Also, it has been determined that environmental conditions have significantly influenced the harvest dates. The 17 components were identified in the essential oil of Thyme. Essential oil content and its components were significantly affected by both climatic and ecological conditions and various harvest dates.

Ekolojik ve Ontogenetik Varyabilitenin Esas Kekiğin Drog Verimi ve Uçucu Yağ Oranına Etkisi

Özet: Esas kekiğin kuru herba verimleri ile uçucu yağ oranı ve bileşenleri farklı gelişme devrelerine göre ova ve yayla koşullarında incelenmiştir. En yüksek kuru herba verimleri çiçeklenme sonrasında ve ova koşullarında saptanmıştır. Ayrıca çevre koşullarının hasat tarihlerini önemli derecede etkilediği gözlenmiştir. Esas kekiğin uçucu yağında 17 bileşen saptanmıştır. Uçucu yağ ve bileşenleri iklim ve ekolojik koşullar ile farklı hasat zamanlarından etkilenmiştir.

Introduction

Thyme (*Thymus vulgaris* L.) is a perennial *Labiatae* of the Mediterranean region, which has been used for centuries as spice, home remedy, drug, perfume and insectiside. In Medicine, it is used as antispasmolytic, antibacterial, antifungal, secrotolytic, expectorant, antiseptic, anthelmintic and antitusive as reported by other authors (1-4).

More than fifty plants are named and used as "thyme" in Turkey. Most of them belong to the genus *Thymus*, but some of them to other genera of *Lamiaceae* such as *Origanum*, *Majorana*, *Satureja* and *Thymbra* (5).

Thymol and carvacrol constituted the main phenolic compound of Thyme oil. The major nonphenolic compounds were linalool and p-cymene (6). Thyme oil with high thymol content strongly inhibited the bacterial growth. Also, thymol has the higher activity against fungi, followed by carvacrol and geraniol, but linalool, terpineol and thujone exhibited the least effect (3).

The market demand for thyme is rather high, yearly estimates running at about 500 tonnes in USA and 1000 tonnes in Europe. Owing to a general popularity of the use of natural substances instead of synthetic compounds, an increase in that demand is predictable (5).

The yield of plant material, the essential oil content and quantitative composition of *T. vulgaris* can be influenced by harvest time, ecological and climatical conditions (7, 8). It has been reported that a fairly tight correlation exists between the soil type and the chemotypic structure of the thyme population growing on it. Where the soil type varies, distinct differences among chemotypes can be found over a few metres (9).

Since the altitude can also be considered as a major factor influencing the physiological and chemical responses of plants, a correlation is attempted between the altitude where aromatic plants occur and their yield in essential oils. Taking into account their altitude distribution and chorology it is concluded that *Thymus* is a Balkan element. Balkan elements never pass to the oil-rich category. However, relatively high values of yield occur in a wide range of altitudes, from 600 to 1900 m (in Greece). Concerning the other chorological groups, it was found that their range of essential oil yield is fairly

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limited. For Balkan and Eurasiatic elements, in particular, yield does not seem to be related to altitude as in the case of the Mediterranean elements. Knowledge of the factors such as genetic or environmental, that influence the essential oil content is insufficient, and the same holds for the role they exerted and now exert in the ecological complex. Altitude seems to play a role in the case of oil-rich and oil-intermediate aromatic plants, affecting their essential oil content. It does not seem to influence oil poor plants (10).

Drug yield, essential oil content and composition in *Thymus vulgaris* plants showed big variation from years to years because of perennial plants (11).

Objective of this study was to determine the most suitable harvest time and growing conditions for obtaining maximum drug yield, essential oil and essential oil components in Çukurova conditions.

Material and Methods

Thymus vulgaris seeds were transferred from Germany, then they were grown in Medicinal and Aromatic Plants Garden at the Field Crops, Department of Çukurova University, Agriculture Faculty.

Thymus vulgaris L. is a perennial plant. It is a gynodioecious species with both hermaphrodite and female individuals occuring in populations. Reproduction are by seeds and vegetative parts. *Thymus vulgaris* can live about 10 to 15 years. Pollination is mainly by bees and the proportion of self-pollination was assumed to vary from 0 to 80 per cent in natural conditions and to be dependent on both the genotype and the immediate environment. The migration of pollen and seeds occurs over short distances and is thus likely to permit genetic differentation of populations.

In this research, *Thymus vulgaris* plants were reproduced by root cuttings. The cuttings were transplanted into sandbox in greenhouse in December. Then seedlings were transplanted from greenhouse to field in May. They were planted in rows 25 x 50 cm. Field trials were established according to randomized block design in different ecological conditions (Adana: Lowland, 18 m; Pozantı: mountainous, 1200 m. above sea level). Field trials were designed as split block design with four replications. Locations and cutting frequencies were considered as main and subplots, respectively.

Monthly mean temperatures, relative humidity and total rainfalls in Adana and Pozantı ecological conditions during the experiment years are presented in Table 1.

As shown in Table 1, distribution of the mean temperatures of Adana and Pozantı over the experiment period were parallel, however mean temperature of Adana were higher than Pozantı, in generally.

When compared the locations for relative humidity significant differences were found between Adana and Pozantı. The relative humidity in Adana during summer months were higher than Pozantı, while relative humidity during winter months were higher in Pozantı.

In both locations, rainfalls were very low especially in July and August.

The soil of experimental areas in both Adana and Pozantı are classified as sandy loam, but soil of Pozantı was calcerous than Adana.

Three different harvest frequencies were examined during three years in Adana and Pozantı conditions. In order to study ontogenetic variation plants were harvested in pre-flowering time, full-flowering time and post flowering time. The plants only could not be harvested in 1986, because plant heights were very short (12).

5 kg/da nitrogen and phosphorous were applied before planting and after each harvest. Plants were irrigated when necessary. All data were subjected to the analyses of variance according to a split plot design.

The plant material was dried at room temperature. The content of essential oil in the dried leaves of (20 g) from each harvest was determined with a steam distillation apparatus.

Samples of the essential oils were analyezd by GC Carlo Erba 6000. Vega 2 Column: FS-SE-54-DF-025; 50x0.25 mm. Injection temp. 230°C, detector temp. 250°C, column temp. 80-220°C, flow rate 1.5 ml/min.

Result and Discussion

Drug yields in Adana were found to be significantly higher than Pozantı in all harvest times and years (13, 14) (Table 2). Drug yields in Adana were not influenced by years. Drug yields of Pozantı in 1987 and 1989 showed same trend however drug yields in 1988 was lower than other years.

The highest drug yield was observed at the post reproductive time in Adana and in all experiment years. Dry matter has increased from pre-flowering to post-flowering stages, because of available day-length, temperature and sunlight (14, 15, 16).

Table 1. Temperature, relative humidity and rainfall data during four years at Adana and Pozantı ecological conditions.

		ADANA			POZANTI		
Months		Mean temp. (°C)	Rel. Hum. (%)	Rainfall (mm)	Mean temp. (°C)	Rel. Hum. (%)	Rainfall (mm)
January	1986	11.1	66.0	107.2	4.1	73.0	91.7
	1987	11.1	69.0	153.1	4.3	69.0	42.8
	1988	10.5	57.0	46.6	3.6	69.0	18.1
	1989	8.3	50.0	65.8	–0.4	68.0	79.2
February	1986	12.3	73.0	67.8	5.2	75.5	86.3
	1987	12.9	61.0	40.8	6.3	65.0	29.7
	1988	11.3	63.0	130.6	5.1	71.0	93.2
	1989	11.1	47.0	2.1	3.6	58.0	0.0
March	1986	15.2	59.0	4.0	8.6	64.0	8.3
	1987	10.4	63.0	200.0	2.6	70.0	197.3
	1988	12.2	68.0	164.3	6.5	70.0	147.3
	1989	15.0	65.0	57.4	9.8	61.0	43.5
April	1986	19.9	62.0	8.4	14.5	61.0	24.0
	1987	16.1	66.0	15.8	10.4	59.0	45.9
	1988	17.6	64.0	27.1	13.0	62.0	90.6
	1989	21.0	57.0	–	16.4	48.0	0.0
May	1986	20.0	67.0	50.3	14.0	68.0	76.4
	1987	20.5	67.0	38.2	16.0	61.0	34.4
	1988	22.3	66.0	62.9	18.0	53.0	47.4
	1989	23.4	57.0	0.1	18.3	50.0	53.6
June	1986	25.3	66.0	13.8	20.3	62.0	40.9
	1987	25.2	65.0	3.9	21.4	49.0	20.2
	1988	25.2	69.0	60.5	20.8	54.0	32.5
	1989	25.0	68.0	18.2	21.3	52.0	15.3
July	1986	27.9	72.0	–	26.0	54.0	–
	1987	28.2	68.0	55.8	26.0	37.0	5.1
	1988	30.1	57.0	–	27.0	34.0	8.9
	1989	28.3	69.0	–	26.5	39.0	3.2
August	1986	28.8	72.0	–	25.7	64.0	–
	1987	28.4	65.0	48.4	25.3	38.0	4.4
	1988	29.1	64.0	–	25.2	40.0	4.2
	1989	28.2	70.0	–	25.5	48.0	0.0
September	1986	27.0	64.0	3.6	21.2	63.0	24.0
	1987	26.6	59.0	–	21.7	34.0	0.0
	1988	26.2	57.0	0.5	21.2	42.0	20.9
	1989	26.1	59.0	29.1	20.7	50.0	37.4
October	1986	21.9	60.0	60.3	14.0	67.0	25.9
	1987	20.7	54.0	76.5	13.7	49.0	67.9
	1988	20.3	64.0	107.1	13.5	68.0	111.1
	1989	20.3	61.0	106.8	13.8	62.0	78.2
November	1986	13.7	58.0	57.9	6.6	70.0	55.8
	1987	15.0	65.0	70.7	7.4	66.0	60.7
	1988	12.6	61.0	114.8	5.9	72.0	93.4
	1989	–	–	–	8.4	71.0	142.0
December	1986	10.9	61.0	83.7	4.3	65.0	–
	1987	11.7	63.0	201.3	4.8	71.0	231.4
	1988	11.4	71.0	90.9	5.4	74.0	109.6
	1989	11.0	62.6	131.3	4.0	68.0	23.6

Table 2. Drug yields of *Thymus vulgaris* L. depending on the growing sites and development stages (kg/ha).

Harvest Times	1987 Adana	1987 Pozantı	1987 Average	1988 Adana	1988 Pozantı	1988 Average	1989 Adana	1989 Pozantı	1989 Average
Pre-flowering	7230 ab	3140 d	5185 d	7080	2650	4865 b	7090	2880	4985 c
Full-flowering	7000 b	3570 c	5285 b	7300	2730	5015 b	7280	3260	5270 b
Post-flowering	7540 a	3850 c	5695 a	7680	2990	5330 a	7680	3380	5530 a
Average	7257 a	3520 b	5390	7350 a	2790 b	5070	7350 a	3173 b	5262
L.S.D. (%5)*Region	200.75**			1540.30**			920.42**		
L.S.D. (%5)*Harvest	220.05**			190.20**			270.66**		
L.S.D. (%5)*Interaction	320.35**								

Pozantı region is drought and hot windy in summer because of high altitude, 1200 m, the sunshine in Pozantı is so strong that the plants become dwarf. Also their leaves are more smaller, thicker and leathery than those of Adana (14, 17, 18).

As a result *Thymus* plants were adapted to Pozantı conditions in such away that dwarfing and lower yields than those of Adana yields.

In Table 3, it was observed that essential oil (0.32-0.83%) and its component changed by different ecological regions, harvest time and years (6,18, 19).

The great differences in essential oil contents in Adana and Pozantı were found among the harvest even though they were harvested at the same development stage.

The highest oil content occured at the start of flowering in Pozantı and Adana experiments in 1989. Also, the highest oil content occured at full-flowering stage Pozantı in 1987 and Adana in 1988 (13). For this reason, maximum essential oil contents depended not only on flower development, but also temperature, relative humidity and duration of sunshine, air movement and rainfall (19, 20).

In Adana, maximum percentage of *Thymus vulgaris* essential oil (0.83%) was found at post-flowering stage in the first year, but in Pozantı, it was found at full-flowering stage in the first year.

The 17 components were identified in *Thymus vulgaris* essential oil such as α-pinen, β-pinen, sabinen, myrcen, limonen, p-cymol, cineol, γ-terpinen, linalool, terpineol, borneol, linaly-acetat, geraniol, bornyl-acetat, thymol, carvacrol, β-caryophyllen (Table 3). *Thymus vulgaris* essential oil was found to be rich in thymol, carvacrol, linalool and p-cymene (6). Thymol (7.44-16.58%) and carvacrol (2.46-4.55%) constituted the main phenolic compounds of thyme oil. The major compound was p-cymene (24.36-51.26%) (5).

According to a proposed biogenetic pathway that includes these compounds, γ-terpinene is the component involved in the aromatization process which results in the formation of p-cymene, the precursor of possible oxygenated derivatives: thymol, carvacrol. It may be assumed that the sequence in this process is as follows γ-terpinene, p-cymene, thymol or carvacrol.

Comparison of the analytical data of the oils in years shows only slight changes in the qualitative composition but considerable differences in the quantitative data particularly for p-cymene, linalool, carvacrol and thymol (5).

In all experiment years, generally, thymol content was maximum in full-flowering stage then decreased in post-flowering stage in both regions. In generally the maximum carvacrol contents were observed at post-flowering stage.

α and β-pinen, sabinen, myrcen, limonen, γ-terpinen, borneol, linalyl-acetat, geraniol, bornyl-acetat and β-caryophyllen compounds were found only in traces.

Today, linalyl-acetate is well known to rearrange during hydrodistillation forming artefacts mainly represented by linalool and other terpene alcohols, namely α-terpineol, nerol, geraniol and their acetyl esters (21).

Small amounts of terpineol were determined in the 1988-1989 oil. Whereas this compound was not found in the 1987 oil. This can be attributed to the plant age (6). Because in 1987, the plants were only two years old.

In spite of slight ontogenetical variation, essential oil composition is not qualitatively affected by environmental conditions, only, amount of essential oil components were changed, but these changes did not show clear trend.

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Table 3. Average essential oil content (%) and its components (%) in different region and development stages of *Thymus vulgaris* L.

Diff.ecolo. condition	Harvest time		Ess. oil	α-Pinen	β-Pinen	Sabinen	Myrcen	Limonen	P-cymol	Cineol
						1987				
Adana	Pre-flowering	6.4.87	0.32	0.40	0.24	1.11	0.21	0.22	32.40	1.57
Adana	Full-flowering	7.5.87	0.66	0.39	0.43	1.98	0.25	0.27	31.79	1.35
Adana	Post-flowering	30.6.87	0.83	0.35	0.19	1.31	0.33	0.26	35.56	1.63
Pozantı	Pre-flowering	–	–	–	–	–	–	–	–	–
Pozantı	Full-flowering	7.7.87	0.71	0.34	0.49	0.79	0.30	0.23	35.56	1.63
Pozantı	Post-flowering	28.7.87	0.60	0.38	0.18	0.78	0.26	0.24	37.58	1.55
						1988				
Adana	Pre-flowering	5.4.88	0.47	0.26	0.24	1.62	0.03	0.44	34.92	0.86
Adana	Full-flowering	20.5.88	0.53	0.32	0.52	0.48	0.35	0.28	40.45	1.88
Adana	Post-flowering	31.6.88	0.44	–	0.18	0.75	0.23	0.32	25.62	1.54
Pozantı	Pre-flowering	10.6.88	0.56	0.88	0.56	0.52	0.20	0.25	51.26	1.27
Pozantı	Full-flowering	15.7.88	0.34	0.47	0.37	0.64	0.26	0.30	24.86	1.62
Pozantı	Post-flowering	10.8.88	0.70	0.61	0.65	0.88	0.14	0.21	42.31	0.78
						1989				
Adana	Pre-flowering	15.4.89	0.60	0.70	0.32	1.34	0.13	0.23	51.18	1.48
Adana	Full-flowering	10.5.89	0.50	0.55	0.43	0.51	0.06	0.24	46.35	0.75
Adana	Post-flowering	1.7.89	0.50	0.66	0.21	0.87	0.12	0.25	38.30	1.08
Pozantı	Pre-flowering	12.6.89	0.60	0.56	0.46	0.67	0.30	0.22	46.29	1.28
Pozantı	Full-flowering	17.7.89	0.40	0.20	0.06	0.52	0.26	0.22	24.36	1.12
Pozantı	Post-flowering	11.8.89	0.50	0.10	0.11	0.72	0.03	0.17	28.54	1.23

γ-Terpinen	Linalool	Terpineol	Borneol	L.acetat	Geraniol	Bornylacetat	Thymol	Carvacrol	β-Carpophyllen	Σ
					1987					
0.25	1.60	–	0.23	0.00	0.26	0.31	7.44	2.46	0.43	46.80
0.51	1.94	–	0.59	1.12	0.97	0.41	13.45	2.58	0.84	55.72
0.39	2.48	–	0.70	0.37	0.38	0.66	11.24	4.55	0.47	63.24
–	–	–	–	–	–	–	–	–	–	–
0.43	1.95	–	0.80	3.86	1.58	0.64	11.77	3.01	0.91	62.97
0.28	1.85	–	0.49	1.72	1.19	0.67	9.49	2.77	0.38	59.75
					1988					
2.12	2.68	1.99	0.39	0.47	0.45	0.36	16.58	3.02	1.32	67.75
0.31	2.39	1.75	0.47	1.34	1.01	0.26	15.34	3.34	0.88	71.37
0.57	2.19	1.87	1.00	1.08	1.05	0.22	12.46	4.15	0.82	54.41
0.30	1.59	1.45	0.37	0.24	0.66	0.24	8.86	3.54	0.39	72.58
0.44	2.00	1.89	0.42	8.61	2.54	0.17	12.29	2.94	1.48	61.30
0.53	3.20	2.48	0.46	0.22	0.58	0.06	12.19	3.71	0.38	69.39
					1989					
0.24	1.73	1.32	0.41	0.24	0.34	0.42	11.44	3.64	0.56	75.72
0.39	1.77	1.44	0.28	1.11	0.54	0.04	11.41	2.80	0.53	69.20
0.32	2.34	2.32	0.33	1.96	1.43	0.17	10.60	3.50	0.65	65.11
0.31	1.87	1.68	0.36	0.27	0.50	0.16	11.63	3.88	0.68	70.82
0.30	2.20	2.59	0.39	9.19	2.99	0.16	14.32	3.64	1.52	46.04
0.18	2.46	1.84	0.44	1.92	1.57	0.19	12.60	4.05	0.81	56.96

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