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Effects of Different Harvest Stages on Yield and Essential Oil Content of Thyme (*Thymus kotschyanus* Boiss. & Hohen)

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Effects of harvest at the beginning of flowering, full flowering and after full flowering on yield and essential oil of *Thymus kotschyanus* were evaluated under Diyarbakir ecological conditions during 2002–03 and 2003–04 growing seasons. Harvest times had significant effect on plant height, fresh and dry herbage, dry leaf yield, essential oil and yield. Maximum thymol (48.9%) was obtained when the harvesting was done at the beginning of flowering.

Key Words: *Thymus kotschyanus*, Harvest time, Dry leaf yield, Essential oil, Thymol.

INTRODUCTION

Turkey is regarded as an important gene-centre for Lamiaceae. The family is represented in Turkey by 45 genera, 546 species and 731 taxa. The rate of endemism in the family is 44.2%. *Thymus* represented in Turkey[1] consists of 57 taxa and the number of endemic is 20. *Thymus kotschyanus* is a perennial, herbaceous shrub belonging to family *Lamiaceae* and is found abundantly as wild plant in South East Anatolian region. *T. kotschyanus* has three subspecies, var. *glabrescens*, var. *kotschyanus* and var. *eriophorus*[2].

The genus *Thymus* has been credited with a long list of pharmacological properties, such as spasmolytic, antiseptic, antitussive, expectorant, etc.[3] Thyme oils are widely used in dietary supplementation due to their antioxidant capacity[4] and also present high antimicrobial effects[5-7].

Chemical polymorphism with thymol as main component of essential oil is characteristic of many *Thymus* species[8, 9]. Five chemotypes of *T. pulegioides:* fenchone, linalool, citral/geraniol, thymol and carvacrol were described in Slovakia[10]. Tümen *et al.*[11] reported that *T. atticus* and *T. roegneri* species contain 37.15 and 58.23% thymol, 7.76 and 8.59% carvacrol, 5.84 and 2.92% borneol and 9.0 and 12.94% *p*-cymene as major components, respectively. Salgueiro *et al.*[8] reported that *T. zygis* has 22.—38.0% thymol and 15.—35.0% *p*-cymene. However, Başer[1] reported that *T. kotschyanus* var. *glabrescens* contains 65% carvacrol. Similarly, Rasooli and Mirmostafa[3] reported that, *T. pubescens* contains 48.75–64.79% carvacrol, *T. serpyllum* contains γ-terpinene (21.9–22.69%), *p*-cymene (21.12–22.68%) and thymol (18.73–18.68%) as major components. Seasonal variations affect the ratio of these components. Sefidkon *et al.*[12] reported the variations in the composition of essential oil at the beginning of flowering and at full flowering stages of *T. kotschyanus* with range of 0.28–1.80% and

essential oil content includes carvacrol (40.74–61.23%), thymol (7.51–26.92%), γ-terpinene (3.72–8.25%), *p*-cymene (3.28–6.74%) and borneol (1.33–4.52%).

The biosynthesis of secondary metabolite is primarily controlled genetically and is strongly influenced by environmental factors including agricultural practice, which have a critical effect on quantitative and qualitative traits. Spacing and harvesting schedule can be a very effective factor in this area[13]. Another important factor influencing thyme oil production is harvesting time and climate as phenol content at full flowering, which vary from year to year[7, 14].

To our knowledge, an investigation of the agronomic properties of *T. kotschyanus* has not been reported to date. The aim of the present study was to determine the optimal harvest time of wild thyme *T. kotschyanus* to obtain the highest essential oil components.

EXPERIMENTAL

The study was conducted under Diyarbakir ecological conditions (latitude 37°53′ N and longitude 40°16′ E, 680 m above sea level) at the Department of Field Crops, Faculty of Agriculture, Dicle University during 2001–02, 2002–03 and 2003–04 growing seasons. The seeds used in the study were collected from the wilds of South-east Anatolia, Turkey.

T. kotschyanus seeds were grown under high-tunnel in December 2001 to raise a nursery. The seedbed was prepared by using soil, sand and farmyard manure in 1 : 1 : 1 ratio. Field trial was conducted using randomized block design with three replications. When the seedlings attained a height of 10–15 cm, they were transferred to the field in April 2002, such that each plot was arranged as 4 rows with a plant density of 30 × 20 cm. No harvesting was done during 2001–02. Harvesting was done at three different stages of development, *i.e.*, in the pre-flowering, full flowering and after full flowering stages. The plots were weeded and irrigated when needed.

Plant height, fresh and dry herbage yield, dry leaf yield, essential oil content, and essential oil yield were evaluated. The harvested plants were dried under shade at an airy place for one week. Plant height (in centimetres) and essential oil content (in millilitres per 100 g-v/w) were measured for each harvest stage. Total yields were also measured as described below:

Total fresh herbage yield: Crops at each developing stage were harvested and weighed for every plot.

Total dry herbage yield: Fresh herbage samples (1 kg) were taken and dried at 35°C for 48 h to determine dry herbage yield.

Total dry leaves yield (drug leaves) was determined by separating the leaves and stems in the dry herbage samples.

Data obtained were analyzed statistically, using MSTAT-C computer program, and means were separated by using LSD test ($P < 0.05$).

Isolation of the essential oils

Essential oil of each plot of wild thyme (*T. kotschyanus*) was extracted by Clevenger type apparatus for 3 h following British Pharmacopoeia. The essential oils were stored in glass bottles at 4°C until analysis. Voucher specimens of *T. kotschyanus* were stored at the herbarium of medicinal and aromatic plants, located in the Faculty of Agriculture (DUZF 0044), Dicle University.

The GC analysis was performed on a Varian 3400 apparatus equipped with Supelcowax-10 fused silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness). Initial column temperature was kept at 60°C for 5 min and programmed to 200°C at a rate of 3°C min^{-1} and held for 10 min at 200°C. The injector and detector (FID) temperature were 250° and 300°C, respectively. Carrier gas was nitrogen and flow rate was 1 mL min^{-1}. Split ratio was adjusted to 30 mL min^{-1}. The injected volume of sample was 1 μL. Analyses were performed in three replications.

The components were identified by referring to voucher component R_f (retention time) values determined by Telci *et al.*[15]. The identification of components was based on comparison of their relative R_f values with those of authentic standards.

RESULTS AND DISCUSSION

Harvest times affected all investigated parameters ($P < 0.05$) (Tables 1 and 2). *T. kotschyanus* is widely spread in nature. Plant height is unstable. Producers prefer high plants, as low plants affect fresh and dry leaf yield. In this study, the highest plant height was obtained at the pre-flowering stage (15.43 cm).

Harvest times affected ($P < 0.05$) the fresh, dry herbage and dry leaf yield. Average of the two years showed that the highest fresh and dry herbage yield and dry leaf yield was obtained at full flowering stage and the lowest after full flowering stage (Tables 1 and 2). Studies made on *T. vulgaris* emphasized that the dry matter gradually increased from pre-flowering to after full flowering stage[16]. These increases may be exploited with plant leaves in the active vegetative period that contain more water than the other stages.

TABLE-1
EFFECTS OF HARVESTING TIMES ON PLANT HEIGHT, FRESH AND DRY HERBAGE YIELD

Harvest time	Plant height (cm)			Fresh herbage yield (t ha^{-1})			Dry herbage yield (t ha^{-1})		
	2002–03	2003–04	Mean	2002–03	2003–04	Mean	2002–03	2003–04	Mean
Pre-flowering	16.06	14.80	15.43a*	12.0	11.4	11.7ab	3.58	3.34	3.46ab
Full flowering	15.00	14.53	14.77ab	14.0	12.4	13.2a	4.02	3.74	3.88a
After full flowering	13.90	13.93	13.92b	9.5	10.5	10.0b	2.84	2.93	2.89b
Mean	14.90	14.40		11.9	11.4		3.48	3.34	
LSD (0.05)	Harvest times: 0.9310			Harvest times: 2.362			Harvest times: 0.7278		

*Means followed by the same letter are not significantly different ($P < 0.05$).

Essential oil means of harvest times varied between 0.41–0.76%, and the highest oil content was obtained from pre-flowering stage (Table-2). Essential oil contents of the study are compatible with Sefidkon *et al.*[12] (0.28–1.80%) and lower than that of Meriçli[17], who reported 2.3% oil content. The variation between

TABLE-2
EFFECTS OF HARVESTING TIMES ON DRY LEAF YIELD, ESSENTIAL OIL CONTENT AND ESSENTIAL OIL YIELD

Harvest time	Dry leaf yield (t ha^{-1})			Essential oil content (%)			Essential oil yield (L ha^{-1})		
	2002–03	2003–04	Mean	2002–03	2003–04	Mean	2002–03	2003–04	Mean
Pre-flowering	2.49	2.55	2.52ab*	0.80	0.72	0.76a	19.7	18.5	19.1a
Full flowering	3.02	2.74	2.88a	0.48	0.50	0.49b	14.7	13.4	14.1ab
After full flowering	2.03	2.10	2.07b	0.41	0.40	0.41b	8.4	8.4	8.4b
Mean	2.51	2.46		0.57	0.54		14.3	13.4	
LSD (0.05)	Harvest times: 0.6093			Harvest times: 0.2063			Harvest times: 6.013		

*Means followed by the same letter are not significantly different ($P < 0.05$).

harvest times with respect to oil content was found statistically important (Table-2). The highest essential oil content resulted from plants harvested at the beginning of flowering. This could explain that the oil synthesis starts with the active vegetative growth of the plants and decreases with the cessation of growth (Table-2). Özgüven and Tansi[16] reported that *T. vulgaris* essential oil content showed differences according to localities and harvesting times. As they had found high essential oil content at one locality at the beginning of flowering. Contrarily, they found high essential oil content at full flowering stage at the other locality. Mentioned researchers explain that this situation could result from temperature, duration of sunshine, air movement, rainfall and flower development stages.

Like oil content, the highest oil yield was obtained at the beginning of flowering stage. This is due to high yields of fresh and dry biomass and high oil content at this stage, as herb yield and essential oil production are directly connected with biomass production[13].

The results of essential oil components analyses are shown in Table-3. The analyses confirmed the presence of thymol, carvacrol, *p*-cymene, limonene, γ-terpinene, α-pinene and myrcene as main components in *T. kotschyanus* at all harvest times. Low levels of α-pinene, limonene at the beginning of flowering increased towards fruit ripening periods (Table-3). The highest thymol (48.9%) was obtained when the harvesting was done at the beginning of flowering with the lowest concentration obtained when the harvesting was done after full flowering stage. Moreover, the percentage of carvacrol was the highest at the beginning of flowering, which decreased at full flowering and increased again after full flowering stage. The results show that harvest times have significant effect on the percentage of essential oil components. Differences between harvest times show that oil production and oil components are metabolically regulated. Badi *et al.*[13] reported that harvesting time affected thymol and carvacrol content such that the highest yields were found in plants harvested at the beginning of flowering. These results are in agreement with those obtained in this study.

TABLE-3
EFFECTS OF HARVESTING TIMES ON ESSENTIAL OIL COMPONENTS OF *T. KOTSCHYANUS* (average of two years)

Essential oil components	Content (%)					
	Pre-flowering	Min-Max	Full flowering	Min-Max	After full flowering	Min-Max
α-Pinene	5.2 ± 0.576	3.8–6.2	7.3 ± 1.345	5.4–10.2	11.9 ± 1.294	10.0–15.5
Myrcene	3.9 ± 0.437	3.1–4.8	11.4 ± 1.552	8.8–14.2	nd	—
Limonene	3.7 ± 0.459	3.2–4.7	8.7 ± 0.803	7.2–10.4	20.9 ± 1.608	18.7–24.5
γ-Terpinene	4.1 ± 0.376	3.2–4.5	6.3 ± 0.450	5.5–7.2	4.8 ± 0.568	3.2–5.8
Cineole	4.2 ± 0.287	3.2–4.4	nd		nd	—
p-Cymene	7.1 ± 0.499	6.2–8.0	2.9 ± 0.528	1.0–3.6	3.6 ± 0.296	3.1–4.2
γ-Terpineol	nd	—	3.2 ± 0.520	2.7–5.0	nd	—
Linalool	nd	—	0.6 ± 0.051	0.5–0.72	2.6 ± 0.202	2.1–2.9
Caryophyllen	nd	—	0.3 ± 0.038	0.26–0.40	1.7 ± 0.320	1.1–2.3
Camphor	nd	—	0.5 ± 0.032	0.40–0.52	0.6 ± 0.079	0.46–0.84
Borneol	nd	—	0.2 ± 0.025	0.14–0.24	0.6 ± 0.090	0.44–0.84
Geranyl acetate	nd	—	nd	—	0.8 ± 0.096	0.61–1.1
(+)-Carvone	0.5 ± 0.102	0.35–0.76	0.7 ± 0.06	0.66–0.92	1.4 ± 0.080	1.3–1.6
Thymol	49.4 ± 3.088	43.6–54.5	41.5 ± 2.76	32.8–46.1	31.1 ± 2.007	27.1–35.4
Carvacrol	20.9 ± 1.871	18.0–24.3	15.5 ± 2.451	10.82–20.45	18.4 ± 2.387	13.9–22.8

nd: non-determined

Kasumov[18] reported that essential oil of *T. kotschyanus* includes 35.48% thymol, 17.74% *p*-cymol, 11.65% carvacrol, 8.83% α-pinene and 6.5% α-terpineol. Rustaiyan *et al.*[19] determined that *T. kotschyanus*, *T. pubescens* and *T. carmanicus* contain 38.0, 37.9 and 40.8% thymol, respectively.

Sefidkon *et al.*[12] reported that the highest oil yield is obtained at full flowering stage. This confirmed that *Thymus* species could be selected according to major components of oil as a chemotype: thymol or carvacrol type. Plants of *Lamiaceae* family have a wide range of variations according to the compositions of essential oils. These variations may be due to like effects of different locations, growing and environmental conditions or genotype of plants.

Variations in the results could be attributed to the wild nature of seeds, as they were collected from wild. It is believed that evaluation of ontogenetic variation and genotypic variability could be an important technique for selecting plants for the production of economically important secondary metabolites.

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