

Effect of Harvest Time on Yield and Quality of *Thymus vulgaris* L. Essential Oil in Isfahan Province, Iran

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Summary

Thyme (*Thymus vulgaris* L.) is a perennial sub shrub belonging to family *Lamiaceae*. Thyme essential oils have been used for many thousands of years, especially in food preservation, pharmaceuticals, alternative medicine and natural therapies. In order to determine the effect of harvest time on quality and quantity of essential oil in thyme, this experiment was done in a randomized complete block design with three replications at research station of Islamic Azad University, Khorasgan (Isfahan) during 2010-2011. Plants were harvested in five stages: before blooming, beginning of blooming, 50% blooming, full blooming and fruit set. The essential oils obtained from the phenological stages of thyme were analyzed by using GC/MS. The results obtained in our study showed that the phenological stages had very significant effects ($P < 0.01$) on essential oil yield and percentage as well as thymol percentage and yield. The highest essential oil content of thyme (2.42%) was extracted at the beginning of blooming stage. Analysis and identification of components showed that thymol is the main compound in all samples. The highest thymol content of thyme (74.8%) was extracted at the full blooming stage. According to the results of this research, harvesting the thyme at 50% blooming stage have maximum essential oil quality and quantity in Isfahan province.

Key words

Thymus vulgaris, phenological stages, GC-MS, essential oil yield and percent, thymol

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Introduction

Thyme (*Thymus vulgaris* L.) is a perennial sub shrub belonging to family *Lamiaceae*. It is commonly grown wild throughout the Mediterranean region (Spain, France and Italy) (Stahl-Biskup, 2002). Yield and quality of essential oil varies according to the genetic make-up of plant material, crop maturity at harvest, environment and distillation practice. Some 90% of the thyme oil of world trade is produced in Spain (Blanco et al., 1998). Recent studies have showed that thyme have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities (Stahl-Biskup, 1991). Over 60 compounds have been identified in *T. vulgaris*, including thymol [5-methyl-2-isopropylphenol] and carvacrol [5-isopropyl-2-methylphenol] with small quantities of 1,8-cineole, borneol, geraniol, linalool (characteristic of the family *Lamiaceae*), bornyl, linalyl acetate, thymol methyl ether and α -pinene (Masada, 1976). The accumulation of the essential oil takes place in gland hairs, which are distributed on the surface of the epidermis of the aerial parts of the plant. The content of the essential oil can depend on origin, climate, harvest, as well as on drying and storage conditions (McGimpsey et al., 1994; Venskutonis, 1997).

Omidbaigi et al. (2010) reported that the most suitable time for harvesting of lemon thyme (*Thymus × citriodorus* (Pers.) Schreb) to achieve the maximum yield of essential oil production is at fruit set stage. Sefidkon et al. (2009) concluded that the beginning of blooming is the best for obtaining the highest oil content and thymol percentage in thyme. Harvesting time of thyme may differ according to location (Rey, 1991). Aim of the present work was to evaluate the effect of harvest time on yield and quality of *Thymus vulgaris* L. essential oil in Isfahan province.

Materials and methods

This experiment has been conducted in glasshouse and in field at Islamic Azad University, Khorasgan (Isfahan), Iran (32° 38' N and 51° 47' E, 1550 m above sea level) in 2010. The experiment was carried out as factorial experiment using randomized complete block design with three replications. Climate in this province varies from semi-dry to semi-humid. Plants were harvested in five stages: before blooming, beginning of blooming, 50% blooming, full blooming and fruit set. Seeds of thyme were planted on 7 January 2010 in glasshouse at Islamic Azad University, Khorasgan (Isfahan). The soil of the field was clay loam with: pH 7.37, total N 0.75%, total P₂O₅ 35 ppm, total K₂O 452 ppm and EC of 4.69 ds/m.

Rooted cuttings have been transplanted into the open field on 15 March 2010 at Islamic Azad University Khorasgan (Isfahan). They were planted in rows 50 cm apart with inter-row spacing of 20 cm. Data were recorded on some growth characters: plant height (cm), diameter (cm), fresh and dry herb weight (kg/ha), oil percentage, oil yield (kg/ha), thymol percentage and thymol yield (kg/ha).

Plants were cut at a height of 10 cm above soil levels and dried in a shaded area. were extracted by Hydrodistillation of the dried parts of the plants (100 g) was done by using a modified Clevenger-type apparatus for 2 h. The oils were dried over anhydrous sodium sulfate and kept at -4°C until chromatographic analysis. Yield was calculated as volume (mL) of essential oil per 100 g of dry plant matter.

Gas chromatography-mass spectrometry (GC/MS) was carried out by use of Thermoquest 2000 GC coupled with Thermofinnigan Mass system and a DB-1 capillary column (30 m × 0.25 mm; 0.25 μ m film thickness). The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. Mass range was from m/z 35–375 amu.

Analysis of variance of the results was conducted using the MSTAT-C & SPSS program, and means of the results were compared using the Duncan's Multiple Range test at the 5% significance level.

Results and discussion

Analysis of variance for different parameters is shown in Table 1. Generally, the tallest plants (28.66 cm) were obtained at blooming stage and the lowest plants (22 cm) were obtained at before blooming stage (Table 2).

The highest plant diameter (73.66 cm) was achieved in plants harvested at fruit set stage and the lowest plant diameter (19.77cm) was achieved in plants harvested at beginning of blooming stage (Table 2).

The results obtained by statistical analysis showed that the effects of phenological stages were not significant for fresh and dry herbage weight (Table 1).

Results of fresh weight/plant (Table 2) revealed that the highest fresh weight/plant (4964 kg/ha) was obtained at 50% blooming stage and the lowest (2238 kg/ha) was obtained at before blooming stage. The highest dry herbage weight (2564 kg/ha) was obtained at 50% blooming stage and the lowest (1354 kg/ha) was obtained at before blooming stage (Table 2).

The results obtained by statistical analysis showed that phenological stages had very significant effect ($P < 0.01$) on essential oil yield and percentage (Table 1).

The highest oil percentage (2.42%) was achieved in plants harvested at beginning of blooming stage and the lowest oil percentage (1.35%) was obtained at before blooming stage (Table 2).

The highest oil yield (43.4 kg/ha) was achieved in plants harvested at 50% blooming stage and the lowest oil yield (18 kg/ha) was obtained at before blooming stage (Table 2).

The results obtained in Statistic analysis showed that the effects of phenological stages had very significant effect ($P < 0.01$) on thymol percentage and yield (Table 1).

Analysis and identification of components showed that thymol was main compound in all samples. The highest thymol content (74.8%) was achieved in plants harvested at full blooming stage and the lowest thymol content (31%) was obtained at beginning of blooming stage (Table 2). The highest thymol yield (24.24 kg/ha) was achieved in plants harvested at 50% blooming stage and the lowest thymol yield (9.73 kg/ha) was obtained at before blooming stage (Table 2).

Omidbaigi et al. (2010) reported that harvest time had a significant effect on fresh and dry weight of aerial parts of *T × citriodorus* in ($P < 0.01$) probability level. According to their results maximum amount of fresh and dry weight of aerial parts of *T × citriodorus* plant was obtained at full flowering and fruit set stages.

Table 1. Analysis of variance for different parameters

S.V	d.f	Plant height	Diameter	Fresh herbage	Dry herbage	Oil Content	Oil Yield	Thymol Content	Thymol Yield
Block	2	6.49 n.s	16.28 n.s	135.94 n.s	2.54 n.s	0.000372 n.s	0.17 n.s	1.305 n.s	0.326 *
Treatment	4	19.47 **	1900.03 **	346.16 n.s	79.84 n.s	0.493 **	342.2 **	726.542 **	102.26 **
Error	8	8.01	83.63	132.03	61.34	0.000214	0.053	0.36	0.048

n.s., *, **: non-significant, significant at 0.05 and 0.01 probability levels, respectively

Table 2. Effect of harvesting time on fresh and dry herbage yield, content and yield of oil, thymol

Harvesting time	Plant height (cm)	Diameter (cm)	Fresh herbage (kg/ha)	Dry herbage (kg/ha)	Oil Content (%)	Oil yield (kg/ha)	Thymol Content (%)	Thymol yield (kg/ha)
Before blooming	22 b	20 c	2239 b	1354 a	1.35 e	18.00 e	54.1 c	9.73 e
Beginning of blooming	28.66 a	19.77 c	3548 ab	1602 a	2.42 a	38.92 b	31 d	12.06 d
50% blooming	25.33 ab	66.22 a	4964 a	2564 a	1.72 b	43.4 a	55.9 b	24.24 a
Full blooming	23.22 ab	45.22 b	4058 ab	1494 a	1.55 d	23.39 d	74.8 a	17.5 c
Fruit set	24.11 ab	73.66 a	4658 a	2164 a	1.62 c	35.1 c	56.03 b	19.67 b

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

Sefidkon et al. (2009) showed that different stages of plant growth had significant effect on oil yield of *Thymus vulgaris* L. The highest oil yield was obtained at beginning of flowering stage (1.18% w/w).

Naghdi Badi et al. (2004) reported that the maximum yield of dry and fresh herbage, yield and content of oil and thymol yield from *Thymus vulgaris* L. were obtained at beginning of blooming stage. Maximum thymol content was observed at the beginning of blooming.

Omidbaigi et al. (2010) reported that harvest time had no significant effect on the essential oil of *T. citriodorus*. Although the highest essential oil content (2.21%) was extracted at beginning of flowering stage there was no significant difference among different phenological stages.

Sefidkon et al. (2009) showed that the percentage of thymol at the beginning and at full flowering stage was the same, but at the beginning of flowering was lower.

McGimpsey et al. (1994) reported that the highest oil and phenol contents resulted in plants harvested after full blooming stage. This variation may be referred to the seasonal variations that affected chemical constituents, yield and content in thyme.

Naghdi Badi et al. (2004) and Jordan et al. (2006) found that wild-growing thyme in Jordan had higher concentrations of essential oil (5.40%) than it was recorded in Egypt (1.07%), Chile (0.39%), Iran (1.4%) and Belarusia (1.75%).

A report on thyme grown in northern Italy indicated that phenol content at full flowering stage varied from year to year (Piccaglia and Maroti, 1991). McGimpsey et al. (1994) have shown that seasonal variation has a significant effect on the yield and composition of thyme oil. Oil yield and phenol content peaked after flowering had finished. Harvesting time can be suggested according to area and its environmental conditions (Rey, 1991). Environmental differences among sites may cause differences in monoterpene production (Thompson et al., 2003).

Conclusions

According to the results of this project optimum harvest time for *Thymus vulgaris* L. on the basis of quantity/quality of essential oil and thymol yield is at 50% flowering stage. Agricultural factors have a critical effect on quantitative and qualitative characteristics of thyme which finally result in plant growth and yield increment.

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