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Full Length Research Paper

Effects of phenological stages on herbage yield and quality/quantity of oil in garden thyme (*Thymus vulgaris* L.)

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Garden thyme (*thymus vulgaris*) belongs to Lamiaceae family. Thyme is widely used in pharmacy, cosmetics and Food industries of developed countries; it is a strong disinfectant with antioxidant. In order to determine the effects of phenological stages on herbage yield and quality/quantity of oil in Garden thyme, this study was conducted in research farm of Khorasgan (Isfahan) branch of Islamic Azad University in 2009 to 2010 years using a randomized complete blocks design with three replications. Plants were harvested in five phenological stages, that is, in the vegetative stage, beginning of blooming, 50% blooming, full blooming and fruit set stages. Oils were extracted by hydrodistillation for 3 h, of the aerial parts using Clevenger-type apparatus. Thymol percentage was determined by GC/MS. Results showed the significant effects of phenological stages on essence and thymol yield and percentage. Mean comparison showed that beginning of blooming has significantly the highest essence efficiency (2.42%). According to GC/MS analysis, thymol was the main compound of essence in all-phenological stages. On the whole, it is recommended to harvest this plant in 50% blooming to gain the highest essence and thymol yield as well as fresh and dry herbage.

Key words: Garden thyme, phenological stages, fresh and dry herbage, essence and thymol.

INTRODUCTION

Garden thyme (*Thymus vulgaris* L.) is belonged to mint family (Lamiaceae). The origin of this plant is Mediterranean region, North of America, and some parts of Asia (Stahl–Biskup and Saez, 2002). Thyme has a wooden plant, short, perennial and Grey colored with C3 metabolism system that will be 30 to 50 cm tall depending the climate of growth region (Stahl-Biskup, 1991; Ozguven and Tansi, 1998).

At present time, this plant is cultivated in large scale in Iran. Evidently, thyme continues to command an important place in expanding world market. Thyme volatile phenolic oil has been reported to be among the top 10 essential oils (Letchamo and Gosselin, 1996), showing antibacterial, antimycotic, antioxidative, natural food preservative, and mammalian age delaying properties (Letchamo and Gosselin, 1996; Jackson and Hay, 1994). The biosynthesis of secondary metabolites, although controlled genetically, is affected strongly by environmental influences. Agricultural factors have a critical effect on quantitative and qualitative characteristics of thyme, which finally result in plant growth and yield increment. The phenological stages can be very effective factor in this area (Mirahmadi et al., 2010; Omidbaigi et al., 2005; Golparvar et al., 2011).

In Sefidkon et al. (2009) study about effects of harvest stages and various methods of hydrodistillation on essence efficiency of garden thyme, phenological stages had significant effect on essence efficiency. Mean comparison results showed that the highest efficiency belonged to beginning of blooming (1.18%) and vegetative phase had the lowest (0.86 %). In other research, the highest efficiency belonged to full blooming (1.71%) whereas fruit set stage (0.18%) had the lowest (Hornok, 1991). Also, some studies showed that the

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highest herbage yield and essence of garden thyme were obtained in lower elevations and in full blooming stage (Hudaib and Aburjai, 2007; Ozguven and Tansi; 1998).

The effect of seasonal regime on amount and essence components of garden thyme was studied in New Zealand and the highest essence yield was obtained in December (22.8 lit/ha) after blooming. Essence components showed also lots of changes in a 13 months period. The highest level of phenol compounds, that is, thymol and carovacrol was observed in summer after blooming stage (McGimpsey et al., 2006). Jordan et al. (2006) in a study about effects of phenological stages concluded also that in hymalian thyme (*Thymus hyemalis* Lange.) the highest percentage of thymol and carvacrol were obtained in beginning of blooming. Nejad-Ebrahimi et al. (2008) find that the lowest essence percentage and the highest percentage of carvacrol were obtained in vegetative phase.

Omidbaigi et al. (2005) with studying effect of various harvest times on quality and quantity of thyme (*Thymus critriodorus* (pres.) Schreb) showed that the highest essence yield (2.21%) was obtained in beginning of blooming. The most extent compound in essence was geraniul which its lowest (54.21%) and highest (72.48%) amounts in essence were obtained in seed set and vegatative stages, respectively. Khorshidi et al. (2010) Investigated four various stages in two region to assess the effect of climate and phenological stages on essence percentage of Denaian thyme (*Thymus daenensis* Celak.). Results showed higher essence percentage in full blooming stage in both regions (3.4 and 2.93% in Malaayer and Hamedan, respectively). Mirahmadi et al. (2010) reported that the highest mean of essence percentage in *T. daenensis* Celak. was obtained in full blooming stage (3.4%) whereas fruit and seed set stages had the lowest (2.17%). Gharabaghian thyme (*Thymus fedtschenkoi*) showed similar results (full blooming: 2.94%; fruit and seed setting: 0.66%).

Therefore, this study was achieved to assess the effect of phenological stages on herbage yield and quality/quantity of oil in thyme as well as to determine the best harvesting time in garden thyme (*T. vulgaris* L.).

MATERIALS AND METHODS

The study was conducted in research farm of Khorasgan (Isfahan) branch of Islamic Azad University in 2009 to 2010 years using a randomized complete blocks design with three replications. Phenological stages that is, in the vegetative stage, beginning of blooming, 50% blooming, full blooming and fruit set stages were considered as treatments. Seeds of *T. vulgaris* L. were sown in green house at 2010 January. Small seeds were mixed with gravel to facility and uniformity in sowing (one part seeds plus two part gravel). Seeds were sown in 0.5 cm depth. The amount of used seeds was 0.8 to 1 g/m^2. There was no disease during growth in green house. Seedlings transplanting was done at March 2010.

The research farm is located in east of Isfahan (32° 38'N 51°47'E) with 1550 m elevation. Region climate was dry or cold dry according to Demartin and ambergay methods, respectively. Mean annual precipitation is 114.5 mm and mean temperature of region is 15.1°C. Maximum temperature in July was 42.6°C and minimum in January was 17.25°C. Evapotranspiration of this region is 1723.25 mm per year. Soil texture was silty-loam with 1.5% of organic carbon, 0.02% of nitrogen, 20 p.p.m of available phosphorus, 504 p.p.m of available potassium, pH=7.37, acidity of 7.8 and 3.5 mmohs/cm electrical conductivity in 0 to 30 cm depth.

Rooted cuttings had been planted in rows 50 cm apart with inter-row spacings of 20 cm apart. Experimental units consist of four rows of 3 m length. Irrigation was done every 4 to 6 days using furrow method. Weed control was conducted during growing season. Any pest was observed on thyme plants.

Traits that is, fresh and dry herbage (kg/ha), essence percentage (essence weight obtained from 100 gr. dry matter), essence yield (dry herbage × essence percentage (kg/ha)), thymol percentage and thymol yield (thymol percentage × essence yield (kg/ha)) were measured for every experimental unit.

Sampling was done from middle rows of each plot by eliminating border effects. Fresh herbage was recorded as biomass wet weight. These samples were dried in shaded area in room temperature with appropriate ventilation for four days and then measured as dry herbage. Wooden parts were separated and 100 g of dried biomass was prepared to essence hydrodistillation.

Oils were extracted by hydrodistillation for 3 h, of the aerial parts using Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate and kept at –4°C until it was analyzed. Qualitative and quantitative analyses of oils were performed by Shimadzu gas chromatography model 15A, equipped with a FID detector and fused silica capillary column (OV-101, 25 m × 0.2 mm). GC analytical conditions were: injector temperature: 230°C, oven temperature: 175°C (isothermal), and detector temperature: 230°C. The percentage of the thymol was computed from GC (FID) peak areas with using the area normalization method (Shibamoto, 1987).

Analysis of variance of data was done based on randomized complete blocks design model. Then existence of significant difference between means of phenological stages were evaluated by Duncan's new multiple range test (DNMRT) at 5% probability level. $SPSS_{16}$ program was used to statistical analysis.

RESULTS AND DISCUSSION

Analysis of variance (Table 1) showed the significant effect of different phenological stages on essence percentage, essence yield, thymol percentage and thymol yield. Mean comparison of treatments (Figure 1) showed that fresh herbage of vegetative phase had significant difference with the other stages. The highest fresh herbage (4964 kg/ha) resulted in 50% blooming and the lowest (2238 kg/ha) obtained from vegetative phase (Figure 1). Naghdibadi et al. (2004) found that various stages of growth had significant effect on wet weight. Harvest mean comparison results showed the highest wet weight (17.70 ton/ha) was obtained in flowering initiation whereas full flowering had the lowest (15.56 ton/ha).

The highest dry herbage (2564 kg/ha) was related to 50% blooming and vegetative phase showed the lowest (1354 kg/ha). Although, differences among the means of different phenological stages were not significant for dry herbage (Figure 1). According to Naghdibadi et al. (2004) reports, effects of various harvest times were not significant on dry herbage and the highest quantity of dry herbage (5.63 ton/ha) was resulted in seed setting stage

Table 1. Analysis of variance of phenological stages for percentage and yield of essence and thymol in garden thyme (*Thymus Vulgaris* L.).

Source of variance	df	Fresh herbage	Dry herbage	Essence percentage	Essence yield	Thymol percentage	Thymol yield
Block	2	$135.94^{n.s}$	$2.54^{n.s}$	$0.000372^{n.s}$	$0.17^{n.s}$	$1.305^{n.s}$	0.326^{*}
Treatment	4	$346.16^{n.s}$	$79.84^{n.s}$	0.493^{**}	342.20^{**}	726.542^{**}	102.26^{**}
Error	8	132.03	61.34	0.000214	0.053	0.36	0.048

ns ,* and **: non-significant and significant at 5% and1% probability levels, respectively.

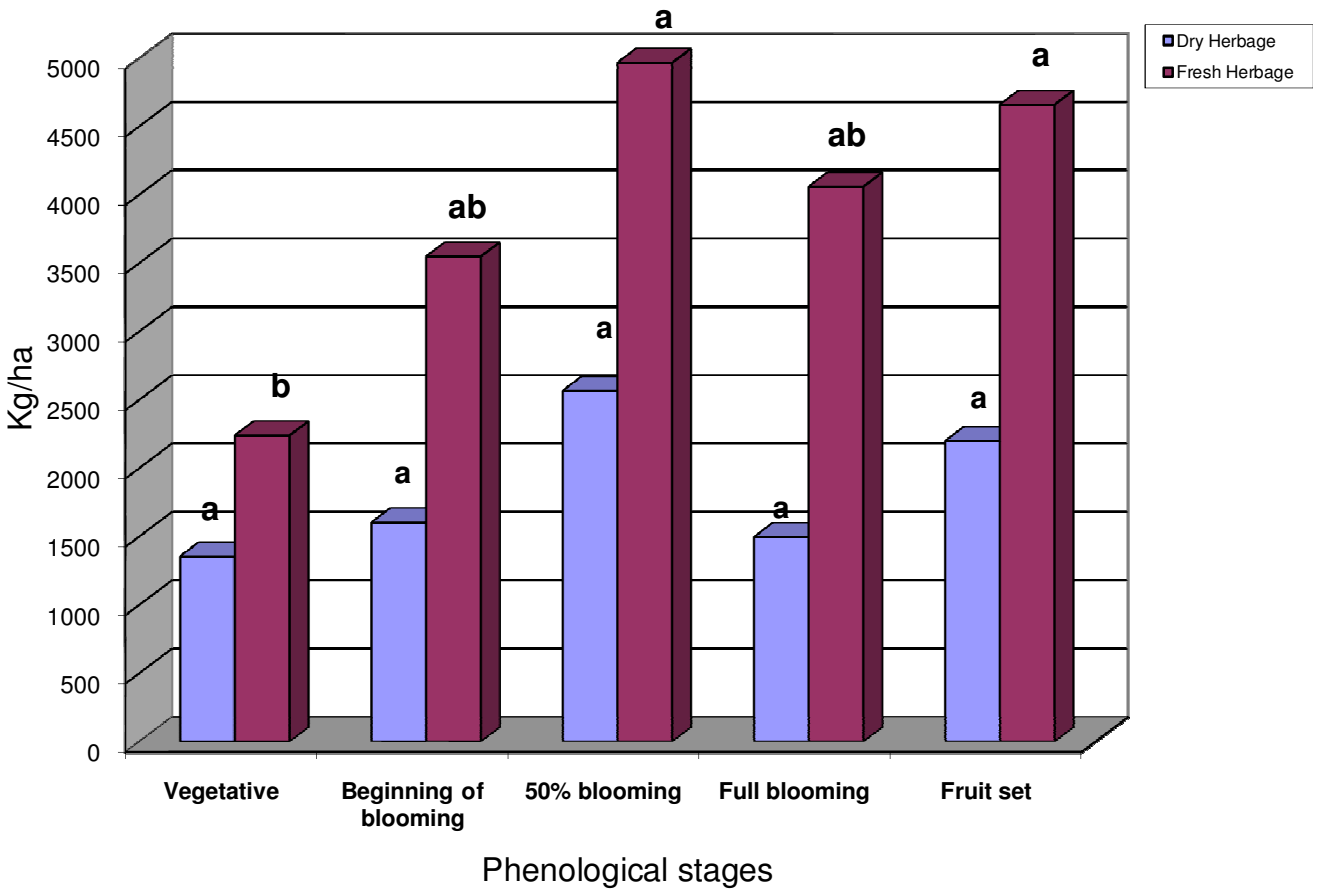

Figure 1. Mean comparison of phenological stages for fresh and dry herbage (Kg/ha) in Garden thyme (In each trait, means with the same letter (s) have no significant difference according to Duncan's new multiple range test).

Table 2. Mean comparison of phenological stages for percentage and yield of essence and thymol in garden thyme (*Thymus vulgaris* L.).

Phenological stage (treatments)	Essence percentage (%)	Essence yield (kg/ha)	Thymol percentage (%)	Thymol yield (kg/ha)
Vegetative	1.35^{e}	18.00^{e}	54.1^{c}	9.73^{e}
Beginning of blooming	2.42^{a}	38.92^{b}	31^{d}	12.06^{d}
50% blooming	1.72^{b}	43.4^{a}	55.9^{b}	24.24^{a}
Full blooming	1.55^{d}	23.39^{d}	74.8^{a}	17.50^{c}
Fruit set	1.62^{c}	35.1^{c}	56.03^{b}	19.67^{b}

In each column, means with the same letter (s) haven't significant difference according to Duncan's new multiple range test.

whereas flowering initiation phase had the lowest (5.54 ton/ha). Ozguven and Tansi (1998) showed that various harvesting times have significant effect on dry biomass weight and the highest dry matter was obtained in seed setting phase.

Studying the ascending trend of fresh and dry herbage changes (Figure1) showed that fresh herbage had an obvious increasing from vegetative phase to 50% blooming that plus the length of plant growth it could be because of shiny days with high temperatures, specifically in July and August because days are longer in this period and then radiation is more. In full blooming stage, weight had a little decrease that was probably because of late summer low temperatures (Letchamo and Gosselin, 1996)

Mean comparison of various phenological stages (Table 2) showed that the most essence percentage (2.42% volume per weight) was related to beginning of blooming and vegetative phase had the least (1.35% volume per weight). Also, the most essence yield (43.2 kg/ha) and the least (18.7 kg/ha) belonged to 50% blooming and vegetative phase, respectively (Table 2).

Naghdibadi et al. (2004) showed that different harvesting times did not affect essence yield but significantly affected essence percentage at 5% probability level. Results of mean comparison expressed that the highest essence yield (115 kg/ha) was resulted in beginning of blooming and fruit set stage had the lowest essence yield (101.8 kg/ha). The highest essence efficiency (20.8%) belonged to beginning of blooming whereas full flowering stage had the lowest (1.8%).

Result of Sefidkon and Rahimibidgoli (2002) about *T. kotschyanus* Boiss showed that full flowering was the best time to gain the highest essence amount. Nejad-Ebrahimi et al. (2008) in their study on *Thymus caramanicus* Jalass observed that the lowest essence was obtained in vegetative phase (before flowering).

Evaluation of the variations happened in essence percentage reveal that this plant has less essence in vegetative phase (Table 2) but after transition to blooming stage will have obvious increase in essence amount, and then with blooming completion, essence amount will reduce obviously that can be because of different external/internal factors. This phenomenon is not only important for essence amount but also is interesting from other aspects like changes in amount of some of its components. Environmental factors like temperature, humidity, light, location, soil etc are important but this is essential to know that clearing up effects of environment will not reduce the role of genetic factors which may be themselves affected by environment. Thompson et al. (2003) mentioned high temperatures as the reason of higher essence production of garden thyme. According to results of Cristina et al. (2008), high temperatures could limit photosynthesis in *T. vulgaris* L. and also with changing absorption of nutrients from soil would sway organic matter production, sugar and amino acids. In this situation, plant feels stress and with reduction in activity of primary metabolites cycles activates secondary metabolites (essence) to resist against stress which it increases essence as result. They also mentioned that low temperature was an effective factor in reduction of essence production in garden thyme.

According to extent reports, garden thyme in Iran has 0.8 to 2.6% essence. The best harvest time is different in various regions. In this study, the most essence percentage (2.42%) was obtained in flowering start that is in agreement with results of Naghdibadi et al. (2004) in Karaj, and also with foundings of Sefidkon et al. (2009) on garden thyme, and Omidbaigi et al. (2005) on *Thymus citriodoruspers* Scherb. The most thymol percentage (74.8%) was obtained in full blooming but the least (31%) belonged to beginning of blooming (Table 2). Also, 50% blooming stage had the most thymol yield (24.15 kg/ha) whereas vegetative phase had the least (10.12 kg/ha) (Table 2). Researches on garden thyme conducted by Naghdibadi et al. (2004) showed

that the highest thymol amount (47.99%) and thymol yield (55.28 kg/ha) were related to flowering initiation. Changes regime of thymol percentage showed that this plant had low thymol in vegetative phase but with passing vegetative phase and entrance to flowering phase, an obvious reduction in thymol amount occurred, then in full flowering, thymol amount increased highly that its reason could be various environmental and genetic factors. Jordan et al. (2006) in research of various harvesting times effects on essence quality and quantity of Himalayan thyme (*T. hyemalis*) observed that the highest density of gamma-terpinene (starter of p-cymene synthesis) was related to full flowering but the highest densities of thymol and carvacrol were obtained from full flowering and start of fruit ripening. The highest densities of alcohols, Ketons and steres were obtained in vegetative phase. Sefidkon et al. (2009) observed that thymol amount was increased gradually in garden thyme from the first of vegetative phase to full flowering.

In conclusion, the results showed that to reach the highest amount of essence in *T. vulgaris* L., it is better not to harvest in vegetative phase because the best time for the highest essence amount is beginning of blooming, but for obtaining the highest essence yield, thymol yield, fresh and dry herbage, harvesting in 50% of flowering is better than other phenological stages. Research about aromatic and medicinal plants which are not native of Iran, especially frequently used plants like garden thyme and evaluating qualitative and quantitative yield of essence and chemical compounds of these species on farm situation is necessary. Planting a medicinal plant will be economical if its secondary metabolites production were in a favorable amount. Considering the daily increasing in thyme use for Pharmacia industries and high request for scientific production, it is recommended to harvest this plant in 50% blooming in Isfahan region (Iran) and similar areas.

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