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In order to evaluate the possibilities of cultivation of Khorasan thyme (*Thymus transcaspicus*), an experiment was conducted in the years 2006 and 2007 under field conditions at the Research Station of the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. Irrigation and organic manure as organic fertilization were employed within a split-plot design with three replications. Irrigation intervals were imposed at three intervals of 2, 3 and 4 weeks, which were allocated to main plots and three levels of organic manure at 10, 20 and 30 t ha^{-1} were allocated to subplots. All other practices were based on organic manner cultivation. Plant biomass and its components as proportion of leaf, stem and flowers and also essential oil percentage and yield were measured at final harvest. Phenological cycles were recorded during the two years of the experiment. Results indicated that increasing application of organic manure beyond 10 t ha^{-1} did not show any significant effect on plant biomass. Increasing irrigation intervals in the second year of trial, significantly ($p < 0.05$) reduced plant biomass. This was also true for stem, leaf and flower content in dry matter. Essential oil content and yield in response to organic manure and irrigation showed no particular trend. Phenological cycles were completed in 192 days equivalent to 3300 degree-days in the first year and 172 days equivalent to 3050 degree-days in the second year. The constituents of essential oil under field conditions were 25 components, which were 43.1% of the total essential content with thymol as the main constituent.

Keywords: domestication; Khorasan thyme; low input cropping system; phytochemical characteristics

Introduction

Over-utilization of medicinal and aromatic plants from natural habitats has damaged plant communities and hence, has caused a serious threat to these valuable plants (Hoareau and Da Silva 1999). It has been estimated that 25% of vascular plants in the world are facing extinction in the future (Kala 2000). Another estimate has indicated that at present 21% of total plant species of the world are endangered

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(Bramwell 2003), which includes 15,000 medicinal and aromatic plant species (Schippmann et al. 2006). Since medicinal and aromatic plants include diverse species with a wide range of life-cycle, morphological features and medical purposes, sustainable utilization of these plants should be based on diverse methods of management according to their natural habitats. It must also consider the social and economic aspects of the human communities surrounding these habitats. In other words, it is very important to consider the needs of stakeholders involved in order to be able to come to a sustainable basis of plant utilization (Siebert 2004).

For cultivation of medicinal and aromatic plants under field conditions, different management practices are required including method of propagation, time of planting, plant density, nutrient and water requirements and competition with weeds. However, among these practices, nutrient and water requirements, which may interact with plant secondary metabolites, should be dealt with cautiously (Najafpoor Navaei et al. 1996). It has been noticed that higher amounts of available nutrients in the form of mineral fertilizer may reduce the content of these metabolites (Schippmann et al. 2006). Therefore to supply the amount of nutrient needed, organic fertilizers may be more appropriate for this type of plant (Najafpoor Navaei et al. 1996). There are reports which show that the application of organic fertilizers to medicinal and aromatic plants including *Thymus vulgaris*, *Mentha piperita, Marrubium vulgar* and *Corianderum sativum* has improved yield and chemical composition (O'Brien and Barker 1996; Herrera et al. 1997; Casimir et al. 2002).

Although water is a limiting factor for plant production (Bannayan et al. 2008), a mild water stress has been shown to increase secondary metabolites in medicinal and aromatic plants (De-Abreu and Mazzafera 2005). In fact, production of these metabolites has been considered as a defense mechanism in response to environmental stress (Gulen and Eris 2004) including herbivory (Tucker and Maciarello 1994).

Khorasan thyme (*Thymus transcaspicus*) belongs to the family of Lamiaceae, containing a wide range of plant species (Mozaffarian 1998). This plant is native to the north-east of Iran and Turkamanistan (Rechinger 1982) and grows in mountains of altitude 1700–2800 m (Rechinger 1982). The essential oils of 162 taxa of the genus *Thymus* have been chemically investigated revealing about 360 different volatile components; among these, the terpenes lead by almost 75% (Stahl-Biskup and Saez 2002). In the genus *Thymus*, their pleasant aroma and flavor as well as their potent pharmacological activities assert them as one of the most popular genus widely used in medicinal and non-medicinal fields including flavoring agents, culinary herbs and herbal medicines. It is also used in perfumes as well as being a commercial source of the monoterpene thymol (Stahl-Biskup and Saez 2002). Our initial ethnobotanical investigation of this species showed that this plant is used widely by the local communities for different purposes. The most common names for this plant in the local area are Rafigh-e-Anokh and Anokhe Karan. Leaves and twigs containing flowers are the main parts used to cure illnesses like cold and cough, as spice and condiment and its aromatic water is used for different purposes separately or mixed with other herbs. Late spring and early summer collection from the wild is a normal practice by local people.

The purpose of the present study was to evaluate the performance of Khorasan thyme under different levels of irrigation and organic manure levels.

Material and methods

Experimental site

This study was conducted in the 2006 and 2007 growing seasons at the experimental station of the College of Agriculture, Ferdowsi University of Mashhad (latitude: 36.15°N, longitude: 59.28°E, 928 m) in the central part of Khorasan province, Iran. During the period of the study, climatic conditions were characterized by an annual average temperature of 16.5°C and 15.5°C and average annual rainfall of 202 and 263 mm in 2006 and 2007, respectively. Monthly maximum and minimum temperatures and precipitation of both years of the experiment are shown in Figure 1. The soil texture in the first 30 cm of the experimental field was silty-loam with pH 7.9 and an EC of 3.2 dS m^{-1}. Field soil contained 0.2% nitrogen, along with 3.8 mg kg^{-1} phosphate and 319 mg kg^{-1} potassium.

Experimental details

Seeds were sown under glasshouse conditions in small pots (10 cm height and 8 cm diameter) in a medium of mixed leaf litter, sand and loamy soil and then the

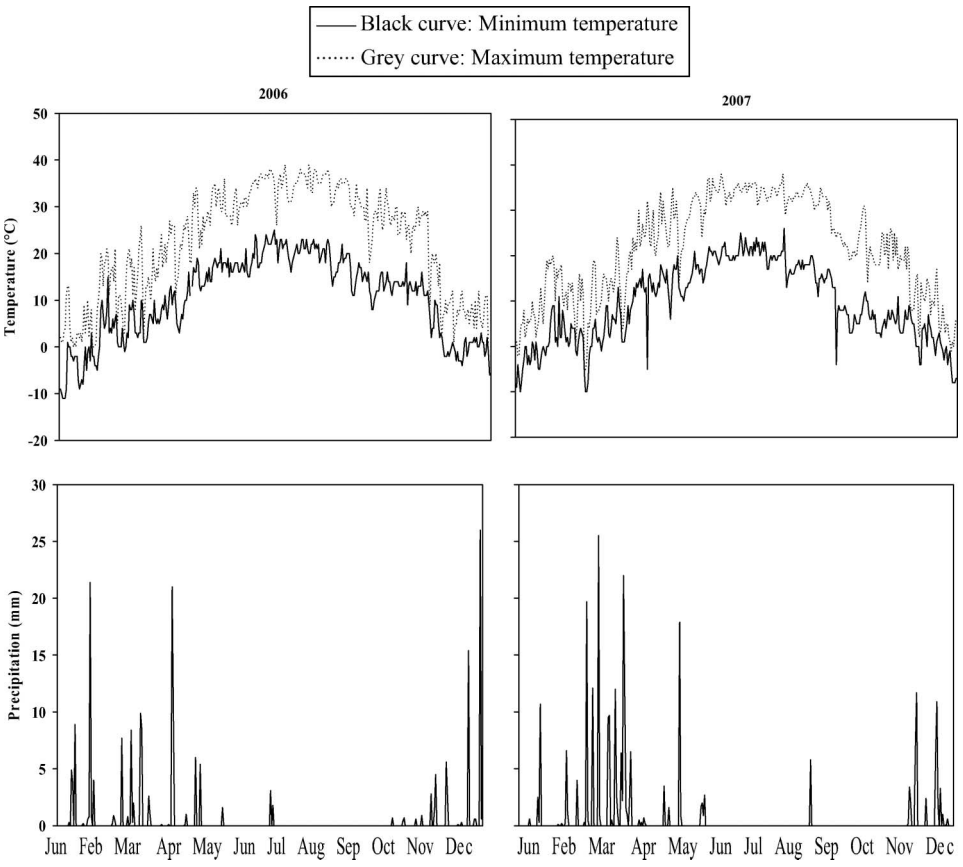

Figure 1. Daily values of maximum and minimum air temperature (°C) and precipitation (mm) in years 2006 and 2007.

66-day-old seedlings were transplanted to the experimental plots. Glasshouse temperature was adjusted at $24 \pm 2°C$ during the day and $15 \pm 2°C$ at night. For uniform application of water, a mist system was used for irrigation of pots. These seedlings were transplanted in the field on 10 May 2006 as spring cultivation with 5 seedlings m^{-2} in plots of 4 m^2.

All these treatments were arranged in split-plot design with three replications in which irrigation intervals were allocated in main plots and organic manure as subplots. Subplots were 2 × 2 m and plants were transplanted in rows 50 cm apart and 40 cm between plants on the rows.

In both years of the experiment, three levels of irrigation with 2, 3 and 4 weeks intervals were applied in the field. However in 2006, the first irrigation was employed immediately after transplanting then two irrigations were also applied with intervals of 3 and 6 days for uniform establishment of seedlings. Experimental plots were irrigated up to field capacity (26.5% θ) with1.5 cm of water (675 l) in each irrigation.

Different quantities of four years composted cattle manure 10, 20 and 30 t ha^{-1} were applied three months prior to transplanting on 5 February 2006. The chemical properties of organic manure were 53% organic matter, 0.43% nitrogen along with 764 mg kg^{-1} phosphate and 3891 mg kg^{-1} potash. EC (dS m^{-1}) and the pH of the manure were 12.7 and 8.4, respectively.

Phenological observations were carried out in each plot and the occurrence of each phenological stage (Table 1) was considered when 50% of the plants in the central rows reached the specified phase. Durations of the intervals between phases were measured in calendar days and in growing degree day (GDD) using the following formula (Mcmaster and Wilhelm 1998):

$$\mathrm{GDD} = \sum_{i}^{n} [(\mathrm{T_{max}} + \mathrm{T_{min}})\ 2] - \mathrm{T_b}$$

where T_b is the base temperature, T_{min} and T_{max} were the minimum and maximum air temperature (°C), respectively.

For the final harvest, one cut on 16 August 2006 and two cuts on 28 April and 14 August 2007 were performed. All harvests were carried out at full blooming stage. At each harvest, from 0.5 m^2 area in each plot, plants were harvested from the soil surface and dried under shaded conditions. Dry matter yield and plant parts (leaf, stem and flower) proportions, except at the second harvest in the second year when the flower number was low and ignored, were determined, and proper chemical analysis were conducted. Simultaneous investigations were carried out on the natural habitants close to the experimental site for the purpose of comparison of some of plant criteria. Samples from the natural habitat were obtained at the same time that plants were harvested from the experimental site as to be consistent for the plant development stage.

Essential oils from the dried aerial parts were isolated by hydrodistillation using a Clevenger-type apparatus for 3 h. The oil was dried with anhydrous sodium sulphate and stored in a sterilized vial at 4°C for further gas chromatography (GC) analysis and gas chromatography-mass spectrometry (GC-MS). GC-FID analysis of thymol content (main oil component) was conducted using a Thermoquest-Finnigan instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at a constant flow

Table 1. Phenological stage of *T. transcaspicus* under field condition.

Growing stage	Seed planting: transplanting	Transplanting: vegetative growth	vegetative growth: early blooming	Blooming: early flowering	Early flowering: full flowering	full flowering: early seed maturity	early seed maturity: maturity	Seed maturity: end of life cycle
Year 2006								
Development stage duration (day)	66	56	17	14	11	18	48	28
GDD*	702.5	1067.5	343.5	291.5	219	334.5	729.5	296.5
Year 2007								
Development stage duration (day)	–	–	27	10	–	25	61	49
GDD	–	–	253.5	125	–	371.5	993.5	1306

*GDD: Growing degree day.

of 1.1 ml min^{-1}. The oven temperature was raised from 60–250°C at a rate of 8°C min^{-1} and held for 20 min. The injector and detector (FID) temperatures were kept at 250°C and 280°C, respectively.

Data analysis was made using SAS statistical software (SAS Institute 2002) and means were compared by Duncan's Multiple Range test at $p < 0.05$.

Results and discussion

Crop development

Our observations showed that in the first year of the experiment (2006), the total duration of plant growth and development from transplanting to maturity was 192 days equivalent to 3300 GDD (Table 1). However, in the second year (2007) up to the second cut when the growth was initiated, from the established plants of first year, it lasted 172 days equivalent to 3050 GDD. Established plants of the second year showed longer reproductive growth duration, from early flowering to the end of plant life-cycle, both in days and GDD measures, compared to the first year plants. A growth analysis sampling during the reproductive stage may provide more information on the exploitation of this plant. As was expected, the employed treatments did not show any effect on development duration.

Plant biomass

Increasing the amount of applied organic manure by more than 10 t ha^{-1} showed a negative effect on biomass production in the first year and similar results obtained in the second year (Table 2). Among various concerns of using fertilizers, including their optimum rate, time and location of application and their interaction with other environment and management factors (Anwar et al. 2007), our field observation

Table 2. Interactive effects of irrigation interval and manure on herbage biomass and oil production of *T. transcaspicus* in two years of experiment (2006–2007).

Irrigation intervals (weeks)	2006			First cut 2007			Second cut 2007		
	Manure supply (t ha^{-1})								
	10	20	30	10	20	30	10	20	30
Dry matter (g m^{-2})									
2	37.25^{ab*}	29.61^{ab}	19.94^{ab}	61.23^{a}	63.93^{a}	57.82^{a}	36.92^{a}	37.66^{a}	38.33^{a}
3	38.95^{ab}	11.82^{b}	19.30^{ab}	51.00^{a}	51.76^{a}	50.39^{a}	27.30^{ab}	19.15^{ab}	34.27^{ab}
4	47.76^{a}	19.59^{ab}	18.51^{ab}	54.88^{a}	46.49^{a}	44.65^{a}	15.12^{ab}	12.40^{ab}	3.55^{b}
Essential oil (%)									
2	2.46^{a}	2.02^{a}	1.82^{a}	0.97^{ab}	1.14^{ab}	1.10^{ab}	0.82^{a}	1.19^{a}	0.96^{a}
3	1.97^{a}	1.99^{a}	2.16^{a}	0.86^{b}	0.94^{ab}	0.90^{ab}	0.69^{ab}	0.72^{ab}	0.71^{ab}
4	2.27^{a}	2.16^{a}	1.96^{a}	1.20^{a}	1.07^{ab}	1.13^{ab}	0.17^{c}	0.30^{bc}	0.12^{c}
Essential oil yield (g m^{-2})									
2	0.91^{ab}	0.63^{ab}	0.37^{ab}	0.55^{a}	0.72^{a}	0.65^{a}	0.81^{a}	0.86^{a}	0.72^{ab}
3	0.76^{ab}	0.25^{b}	0.44^{ab}	0.46^{a}	0.45^{a}	0.48^{a}	0.44^{ab}	0.38^{ab}	0.53^{ab}
4	1.24^{a}	0.43^{ab}	0.36^{ab}	0.73^{a}	0.50^{a}	0.52^{a}	0.31^{ab}	0.22^{ab}	0.05^{b}

Means in each column followed by the same letter are not significantly different ($p < 0.05$), using Duncan's Multiple Range test.

indicated plants loss mostly due to root decay at higher rates of manure application. It may be postulated that the high amount of organic manure in the first year, which is the period of plant establishment with smaller root structure, might have had a negative effect on the plant growth. This has also been reported for other similar plants (Koocheki et al. 2004). In another experiment with *Nepeta binaludensis* there was no difference of plant biomass with the application of 10, 20 and 30 t ha^{-1} organic manures (Najafi 2006). Plant biomass was higher in the second year of the experiment particularly at the first cut (Table 2).

Increasing the irrigation intervals showed no significant ($p > 0.05$) effect on plant biomass in the first year and the first cut of the second year, except the second cut of the year 2007. In this cut, with increasing the irrigation intervals, the plant biomass was reduced (Table 2). In general, higher irrigation intervals resulted in lower plant biomass, although not significant; but the negative impact of longer irrigation intervals was more than the negative impact of higher amounts of manure. Therefore, it appears that this species is not able to tolerate high organic matter in the soil, particularly where irrigation water is limited. Drought stress reduced growth and yield of *Rosmarinus officinalis* and this has been associated with lower photosynthetic rate due to reduced stomatal conductance (Leithy et al. 2006). There are other reports which confirm the negative effect of drought stress on dry biomass production of medicinal and aromatic plants such as *Lippia graveolens* (Dunford and Vazquez 2005), *Mentha peperita* (Charles et al. 1990), *Hypericum brasiliense* (Zobayed et al. 2007), *Rosmarinus officinalis* (Delffine et al. 2005), and *Nepeta binaludensis* (Najafi 2006). As a general rule, it is expected that with increasing plant irrigation intervals, the proportion of stem in total dry matter increases and accordingly the proportion of leaves and flowers decrease. This type of effect has been reported for forage and rangeland crops (Colom and Vazzana 2002); however, we did not realize such an effect on medicinal and aromatic plant like Khorasan thyme (Figure 2). Singh et al. (2002) also found that increasing the ratio of irrigation water to cumulative pan evaporation from 0.8–1 increased plant biomass and also leaf to stem ratio of *Pogostemon cablin*.

Oil production

As shown in Table 2, essential oil yield was not affected by cattle manure nor irrigation intervals. This was somehow in contradiction with some literature (Najafi 2006; Hussein et al 2006; Habibi 2007) in which a positive effect of organic fertilizers on essential oil percentage and yield has been previously reported. Increasing irrigation interval from 2–3 weeks decreased the oil percentage; however, a further increase of irrigation interval from 3–4 weeks increased the oil content. Ram et al. (2006) reported that increasing water availability reduced essential oil percentage of *Mentha arvensis* but total essential oil yield was increased. The latter was reported to be due to the positive effect of available water on plant biomass, i.e. a dilution effect.

Interactive effects of irrigation interval and manure

Table 3 shows the interactive effects of irrigation intervals and organic manure on plant biomass, essential oil percentage and oil yield. Decreasing the irrigation interval from 4 and 3 weeks to 2 weeks showed the highest positive interaction of the two treatments on foliage biomass in both years (Table 2). At the second cut of 2007,

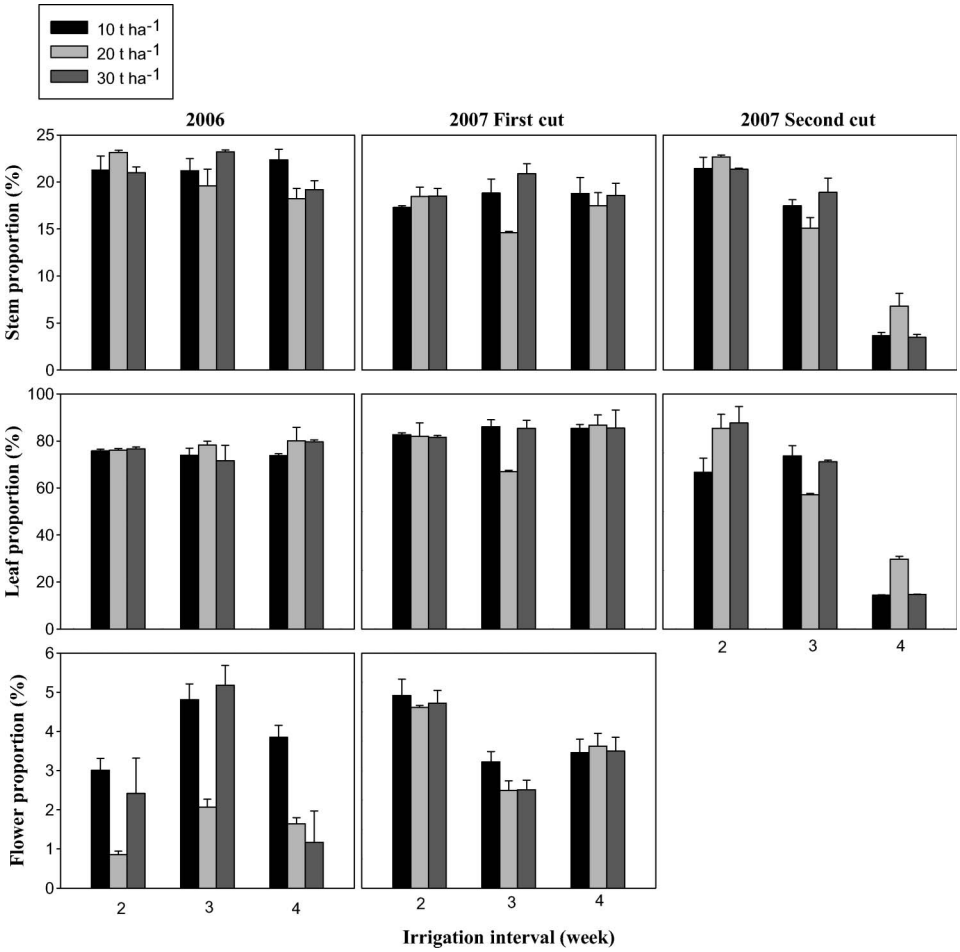

Figure 2. Effects of irrigation intervals and manure on plant part proportion (%) in years 2006 and 2007 (error bars indicate the standard errors).

Table 3. Comparison of agronomic properties and phytochemical characteristics of *T. transcaspicus* growing under field condition and natural habitats using 25 plant samples.

Characteristic	Natural habitat	Cultivated condition
Aerial part dry matter (g m^{-2})	29	35
Essential oil (%)	1.8	1.2
No. of essential oil constituents	51	25
Thymol content (%)	48.2	43.1
Plant life cycle duration (day)	201	182
Plant life cycle duration (GDD)	3331	3175

increasing the applied manure more than 10 t ha^{-1} increased the production of biomass as the irrigation intervals decreased. At the first cut of both years (2006 and 2007), the oil percentage and total oil production increased as the irrigation interval increased from 2–4 weeks across all manure levels. This result was not consistent for

the second cut of year 2007. It seems that after the first cut, the plants are not able to tolerate any water deficit and increasing irrigation interval more than two weeks would result in biomass reductions, oil percentage and total oil production across all manure levels (Table 2).

A comparison of plants under field conditions and natural habitats is shown in Table 3. It seems that the plant biomass per unit area was higher under cultivated conditions, but essential oil percentage and constituents and also thymol content were higher in natural habitats. The constituents of essential oil under field conditions were 25 components, which were 98.2% of the total essential oil content (0.81 g m^{-2}) with thymol as the main constituents. Our recent study on phytochemical characteristics of this species revealed that essential oils of *T. transcaspicus* from different natural habitats varied considerably (1.3–2.3%) possibly due to different environmental conditions and therefore two distinct chemotypes were identified. Thymol and carvacrol were the main constituents and plants from natural habitats contained higher amounts.

Conclusion

In summary, it appears that under optimal conditions of soil water, a lower application (10 t ha^{-1}) of manure would produce high yield. Higher application of manure would result in higher yield when there is less available water (4 weeks irrigation interval vs. 2 weeks). As we could not monitor any specific trend of response to water and manure inputs under field conditions, it seems that in the process of domestication more selection is needed for the plant to be adapted under cultivation practices. In general, it may not be unusual for a medicinal and aromatic plant which has not been grown to respond in such ways to applied inputs under field conditions, and this was the first attempt to experience the process of cultivation of this species. Further investigation is needed in order to fully exploit the potential of this species under field conditions.

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