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ORIGINAL ARTICLE

Effect of Different Ecological Environments on Growth and Active Substances of Garden Thyme

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ABSTRACT

In order to evaluate the effect of cultivation areas on growth and essential oils of garden thyme (*Thymus vulgaris)*, a medicinal plant, an experiment was conducted in a randomized complete block design with three replications. Treatments were included Estahban, Shiraz and greenhouse conditions. Hydrodistillation was used to isolate the essential oils and chemical analyses were performed by GC and GC-MS. Twenty nine components were identified in essential oils of thyme. The major components were thymol, γ-terpinene, *p*-cymene, terpinolene and carvacrol. The results showed significant differences in fresh and dry weights, essential oil yield and essential oil efficiency.The maximum fresh weight, dry weight, essential oil yield and essential oil efficiency were achieved on greenhouse conditions and the minimum amount on Shiraz area.

Key words: *Thymus vulgaris*, greenhouse, medicinal plants, thymol, carvacrol

Introduction

Thyme (*Thymus vulgaris* L.) is a perennial plant belonging to the Lamiaceae family. The green part of thyme plant constitutes the most popular herbal medicine and spice, used in all developing countries. The beneficial effects of thyme are well known from ancient times and consumption of its extract is recommended all over the world [1]. It is used as water extracts for its pharmacological activities and thus, have a very important role in phytotherapy [30]. Recently, thyme has become one of the most important medicinal plants used as a natural additive in poultry and livestock feeding studies [5,13]. Such studies have shown that thyme plant could be considered as an alternative natural growth promoter for poultry instead of antibiotics [20].

Essential oil content of thyme have been reported from 0.32% [25] to 4.9% [7]. Thymol and carvacrol, which are the principal constituents of thyme oil [2,12] have been reported to act as antioxidant [9,17,19], antimicrobial agent [8,28], antifungal agent [18] treatment for respiratory tract diseases [15], wound healing, a stomachic carminative, diuretic, urinary disinfectant and vermifuge [6].

The composition and quantity of essential oil from a particular species of thyme plant could be markedly affected by harvesting season [2], geographical environment and other agronomical factors [16,21].

Researchers have revealed that major volatile constituents obtained from the aerial parts of the plant are geranial, linalool, carvacrol, thymol and trans-thujan-4-ol/terpinen-4-ol [22,23,24,26,27]. In samples of thyme were collected during the flowering

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period in eastern Morocco (Taforalt) in May, essential oil yield was 1.0% and camphor (38.54%), camphene (17.19%), α-pinene (9.35%), 1,8-cineole (5.44%), borneol (4.91%) and β-pinene (3.90%) were the major oil components [14]. However, characteristic compounds of *T.vulgaris* essential oil are thymol (44.4 – 58.1 %), *p*-cymene (9.1-28.5%), γ-terpinene (6.9 – 18.9%) and carvacrol (2.4-4.2%) [3,4,10,11].

This study focuses on influence of three different ecological environments on growth and active substances of thyme.

Materials and Methods

This study was conducted on three different ecological environments: Shiraz, state of Fars, Iran (29°40' N, 52°27' E; 1662 m above sea level), Estahban, state of Fars, Iran (29°632' N, 54°142' E; 1760 m above sea level) and experimental greenhouse of Islamic Azad University, Estahban Branch, Iran (29°632' N, 54°142' E; 1760 m above sea level). Breeded seeds were sown and the plants were transplanted in pots (20×20×20) containing 1/3 soil, 1/3 sand and 1/3 peat (v/v). Experiment was carried out using a randomized complete block design (RCBD) with three replications. Each replicate contained 10 pots. Plants were harvested at prebloom stage from the surface of pots in order to determine shoot fresh weights, and were dried at 30° C for shoot dry weight measurements.

Isolation of essential oils was performed using hydrodistillation of 20 g sample of dried shoots using a Clevenger-type apparatus over 3 hours. The oils were dried over sodium sulphate and the yield of the essential oils (w/w) and oil efficiency were calculated.

Gas Chromatography (GC) analysis was performed on an Agilent technologist model (6890 USA) series II gas chromatograph equipped with flame ionization detector and capillary column HP-5 (30 m ´ 0.25 mm, 0.25 μm film thicknesses). The chromatographic conditions were as follows: The oven temperature increased from 60 to 240ºC at a rate of 3ºC/min. The injector and detector temperatures were 240 and 250ºC, respectively. Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s. The samples were injected using split sampling technique by a ratio of 1:50. Quantitative data was obtained from electronic integration of peck areas without the use of correction factors.

Essential oil was also analysed by Hewlett-Packard GC-MS (model 6890 series II) operating at 70e V ionization energy. Equipped with a HP-5 capillary column (phenyl methyl siloxane (30 m ´ 0.25 mm, 0.25 μm film thickness) with He as the carrier gas and a split ratio of 1:50. The retention indices for all the components were determined according to the Van Den Doll method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI- AP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and mass finder 3 libraries or with the published mass spectra.

Results and Discussion

Different ecological environments resulted in significant differences (Table 1). The maximum shoot fresh weight (28.40 g/plant) was achieved on greenhouse conditions which was significantly different when compared to other treatments. The minimum shoot fresh weight (23.60 g/plant) was observed in Shiraz treatment.

The highest shoot dry weight (7.29 g/plant) was shown in greenhouse conditions which was significantly different when compared to other treatments. The lowest shoot dry weight (5.96 g/plant) was achieved on Shiraz treatment.

The yield of essential oil was maximum (1.37%) in greenhouse conditions which was not significantly different when compared to Estahban conditions. Essential oil efficiency was maximum (96.20 mg/plant) in greenhouse conditions.

Twenty nine components were identified in essential oils of thyme (Table 2) which representing 99.60%, 93.11%, and 97.54% of the oil of Estahban, Shiraz and greenhouse sample respectively. The major constituents of Estahban sample were thymol (58.46%), γ-terpinene (15.06%), *p*-cymene (8.41%), carvacrol (2.07%) and terpinolene (2.05%). The major components of Shiraz sample were thymol (51.76%), *p*-cymene (11.04%), γ-terpinene (7.67%), terpinolene (2.89%) and carvacrol (2.78%). The major components of greenhouse sample were thymol (53.45%), *p*-cymene (12.37%), γ-terpinene (7.88%), terpinolene (3.12%) and carvacrol (2.76%).

Ozguven and Tansi [25] indicated that different ecological conditions and harvesting time affect the yield and components of thyme oil. Yanive and Palevitch [31] showed that environmental conditions influence on qualitative and quantitative characteristics of active substances. Other researchers have revealed that major volatile constituents obtained from the aerial parts of the plant are geranial, linalool, carvacrol, thymol and trans-thujan-4-ol/terpinen-4-ol [22,23,24,26,27].

Table 1: Effect of different ecological environments on shoot fresh and dry weights, oil yield and oil efficiency of thyme.

Treatment	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Oil yield (%)	Oil efficiency (mg/plant)
Estahban	24.78b	6.03b	1.29a	76.40b
Shiraz	23.60b	5.96b	1.16b	66.00b
Greenhouse	28.40a	7.29a	1.37a	96.20a

In each column, means with the same letters are not significantly different at 5% level of Duncan's new multiple range test.

Table 2: Amounts of the chemical components of thyme oil in different ecological environments.

No	Component name	RI	% in Estahban oil sample	% in Shiraz oil sample	% in Greenhouse oil sample
1	α-Thujene	928	0.91 ± 0.15	0.54 ± 0 .01	0.64 ± 0.05
2	α-Pinene	934	0.61 ± 0.14	0.56 ± 0.01	0.76 ± 0.09
3	Camphene	950	0.44 ± 0.09	0.49 ± 0.01	0.48 ± 0.06
4	β-Pinene	974	1.30 ± 0.29	1.44 ± 0.01	1.73 ± 0.15
5	1-Octen-3-ol	976	1.24 ± 0.25	0.94 ± 0.07	0.76 ± 0.18
6	Myrcene	990	t	0.08 ± 0.00	0.07 ± 0.02
7	α-Phellandrene	1002	0.16 ± 0.03	0.11 ± 0.00	0.11 ± 0.03
8	α-Terpinene	1015	1.47 ± 0.27	0.91 ± 0.02	1.02 ± 0.27
9	*p*-Cymene	1024	8.41 ± 0.32	11.04 ± 0.20	12.37 ± 1.55
10	1,8-Cineole	1033	0.73 ± 0.10	1.19 ± 0.02	1.11 ± 0.22
11	γ-Terpinene	1057	15.06 ± 4.15	7.67 ± 0.13	7.88 ± 1.66
12	Cis-sabinene hydrate	1061	0.88 ± 0.18	1.09 ± 0.01	1.24 ± 0.32
13	Terpinolene	1087	2.05 ± 0.35	2.89 ± 0.04	3.12 ± 0.52
14	Linalool	1098	0.08 ± 0.06	0.46 ± 0.26	0.71 ± 0.11
15	Camphore	1143	1.04 ± 0.16	1.83 ± 0.06	2.01 ± 0.16
16	Borneol	1161	0.22 ± 0.10	0.45 ± 0.24	0.57 ± 0.08
17	Thymyl methyl ether	1237	0.43 ± 0.25	0.79 ± 0.01	0.79 ± 0.10
18	Carvacrol methyl ether	1241	0.33 ± 0.13	0.92 ± 0.05	1.11 ± 0.08
19	Thymol	1290	58.46 ± 3.04	51.76 ± 0.69	53.45 ± 3.29
20	Carvacrol	1303	2.07 ± 0.28	2.78 ± 0.04	2.76 ± 0.57
21	β-Bourbonene	1385	t	0.16 ± 0.00	t
22	β-Caryophyllene	1417	1.80 ± 0.07	2.66 ± 0.04	2.75 ± 0.56
23	α-Humulene	1454	0.62 ± 0.73	0.22 ± 0.13	0.19 ± 0.11
24	Germacrene-D	1482	0.23 ± 0.16	0.23 ± 0.01	0.32 ± 0.14
25	Valencene	1493	0.13 ± 0.04	0.26 ± 0.20	0.39 ± 0.13
26	α-Muurolene	1497	0.28 ± 0.11	0.36 ± 0.05	0.78 ± 0.23
27	γ-Cadinene	1510	0.31 ± 0.08	0.86 ± 0.05	0.06 ± 0.01
28	δ-Cadinene	1522	0.21 ± 0.20	0.23 ± 0.01	0.24 ± 0.13
29	Caryophyllen oxide	1581	0.13 ± 0.80	0.18 ± 0.02	0.11 ± 0.09
	Total (%)		99.6	93.11	97.54

RI, retention index
All data are means of three replications ± SD
t, trace (<0.05%)

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