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Full Length Research Paper

Diversity of *Thymus daenensis* Celak in Central and West of Iran

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The plant *Thymus* is one of the largest and the most famous genus of *Lamiacae*. *Thymus* species are commonly used as herbal tea, condiment, spice and medicinal plants. *Thymus daenensis* Celak is an endemic species grown in Iran. The production of secondary metabolites in different ecosystems is affected by genetic and environmental factors. This study was conducted to determine variation of plant properties and effect of ecological factors on plant properties in different populations of *T. daenensis* in different regions of Iran (Isfahan, Chaharmahal and Bakhtiari Provinces) on April to June 2009. The results showed that height plant and date of flowering between populations of *T. daenensis* had significant difference (P ≤ 0.05). Also, our investigation showed that there is a positive and linear relationship between content of thymol as major component with elevation. So we can conclude that the best place for the production of quantity effective materials this plant in order to attain the best results, is the height between 2400 to 2800 m above sea level.

Key words: *Thymus daenensis,* ecological factors, variation, population.

INTRODUCTION

The genus *Thymus* belongs to the family Lamiaceae and includes nearly 215 herbal and small shrub species in the world. The mediterranean area is said to be the origin of this kind of genus (Cronquist, 1988; Heywood, 1993; Morales, 2002). Among 215 species of this genus grown in the world, 14 species are distributed in Iranian flora (Jalas, 1982; Stahl-Biskup and Saez, 2002), which *Thymus carmanicus* Jalas., *Thymus daenensis* subsp. *daenensis* Celak. and *T. daenensis* subsp. *lancifolius*, *Thymus persicus* (Roniger ex Reach. F.) and *Thymus trautvetteri* Klokov and Desj.-Shost. are endemic (Rechinger, 1982). The Persian and local names of *Thymus daenensis* are ''Avishan-e-denaee'' and ''Ooshon-e-kohi'', respectively (Ghasemi Pirbalouti, 2009; Mozaffarian, 2006). *T. daenensis* generally grows in high altitude places in the submontane (Figure 1).

Thymus species are well known as medicinal plants because of their biological and pharmacological properties. In traditional medicine, leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea, ahntiseptic, antitussive and carminative as well as treating colds (Amin, 2005; Ghasemi, 2009; Zargari, 1990). Thymus oils and extracts are widely used in pharmaceutical, cosmetic and perfume industry also for flavoring and preservation of several food products (Bauer et al., 1997).

Pervious works showed that essential oil of *T. daenensis* leave and flower exhibited antimicrobial activities against *Candida albicans* (Ghasemi et al., 2009a), *Listeria monocytogenes* (Ghasemi et al., 2009 b), *Campylobacter jejuni* and *Campylobacter coli* (Ghasemi et al., 2010a) and *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Ghasemi et al., 2010b). Ghasemi et al. (2009)

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Figure 1. Areal plant and flowers of *T. daenensis* subsp. *Daenensis*.

reported that the essential oils of *T. daenensis* (MIC ≥ 50 % = 0.63 µl ml^{-1}) showed higher of inhibition against the *Saprolegnia parasitica* than the other extracts.

The results of studies shown that plants growth in ecosystems is affected by genetic and environmental factors such as species (genetic), climate, edaphic, elevation and topography (Pourohit and Vyas, 2004). Each of these factors can have dominant effect on the quantity and quality of natural products in herbs. Recent studies showed that the two way effect of genetic variation has meaningful effect on the function of the plant and will help in adaptation and natural density. The variety of height plant can be affected by genetic and ecological factors such as precipitation, temperature, plant competition, nitrogen content in soil and etc (Letchamo et al., 1995) For example, results of the study showed that increase elevation from the sea level, variation in soil, and different amounts of K, N and Ca in the soil is among the most important factors in the spread and amount of volatile materials of the *Thymus serpylloides* in Southeastern Spain (Letchamo et al., 1995).

The aim of this research was to study variation of plant properties and effect of ecological factors on plant properties in different populations of *T. daenensis* in different regions in Southwest and Central Iran (Isfahan, Chaharmahal and Bakhtiari Provinces).

MATERIALS AND METHODS

Study site

The study carries out in Isfahan, Chaharmahal and Bakhtiari provinces, Central and Southwest of Iran, which are two provinces of the most important native areas. These are located between latitude 30º 42′ N and 33º 37′ N. The elevations range between 1009 and 3889 m above sea level. The natural vegetation is rangeland and oak forest; most of the areas are used for agriculture.

Meteorological information was obtained from 14 variation weather stations located within the study area and the surrounding zones. The variables taken into account were maximum and minimum daily average temperature, monthly precipitation, monthly relative humidity and number of freezing days. The number of years registered at the weather stations ranged from 15 to 20. Average values for each variable per 10-day calendar period were calculated. This regional thermal gradient was generated by a regression model that took into account the elevation and temperature of weather stations located in center and west of Iran.

Soil physical and chemical such as pH, Ec, texture, OC %, content of N, P, K and etc characteristics were taken from a soil-sampling from 15 regions of Isfahan, Chaharmahal and Bakhtiari provinces.

The slope and elevation information were obtained from the

Table 1. Geographical and environmental conditions.

Row	Region	Altitude (m asl[1])	Latitude	Longitude
1	Yancheshmeh	2450	N 32°37'19.5"	E 50°43'15.0"
2	Sabz-e-koh	2292	N 31°49.0'0.03"	E 50°51'51.3"
3	Larak	2370	N 32°34'52.3"	E 50°39'35.0"
4	Koh-e-sheida	2394	N 32°37'22.2"	E 50°34'46.9"
5	Azadegan	2094	N 32°40'33.6"	E 50°29'06.0"
6	Noorjamaloo	2330	N 32°39'22"	E 50°32'39.3"
7	Shahrekord	2045	N 32°21'06.1"	E 50°53'11.6"
8	Dezak	2298	N 32°06'25.4"	E 51°03'08.9"
9	Farsan	2539	N 32°19'0.1"	E 50°32'11.4"
10	Tomanak	2762	N 32°31'13.4"	E 50°37'23.3"
11	Bardeh	2572	N 32°33'59.5"	E 50°29'32.6"
12	Sheikhshaban	2747	N 32°35'1.2"	E 50°38'19.2"
13	Koohrang	2479	N 32°27'59.6"	E 50°17'18.0"
14	Daran	2303	N 32°56'56.1"	E 50°26'44.4"
15	Semirom	2302	N 31°40'34.9"	E 51°33'13.2"

digital elevation model (DEM) using two well-known GIS software packages ILWIS. This array was geo-referenced using a metric UTM coordinate system and the geometric correction was carried out in the GIS ILWIS.

Plant material

Aerial parts (up to ~ 5 cm, 0.05 to 0.2 kg) of *T. daenensis* subsp. *daenensis* Celak., (four individuals from each population) were collected at the early flowering stage on April to June 2009 from 15 localities in Isfahan, Chaharmahal and Bakhtiari Provinces (Iran) in the following regions Table 1. Each sample was labeled and its location was recorded using a global positioning system (GPS, Garmin) receiver. Samples of the plants were identified by regional floras and authors by floristic and taxonomic references (Ghahreman, 2000; Rechinger, 1982) and deposited at the Herbarium of Researches Centre of Medicinal Plants and Ethno-veterinary, Islamic Azad University, Shahrekord Branch, Iran.

Extract preparation

Approximately 100 mg of ground sample were accurately weighed into a 250 ml tube, and extracted with 30 ml methanol high performance liquid chromatography (HPLC grade) with the aid of sonication for 30 min. The resulting mixture was transferred to a 100 ml volumetric flask. The residual solid was further extracted with 50 ml of the same methanol/water mixture with sonication.

Preparation of standard solution

Stock standard solutions were prepared by accurately weighing 22.3 mg of thymol reference standard into separate 50 ml volumetric flasks and dissolving in acetonitrile/water (50:50, v/v) with the aid of sonication. Working standard solutions, 1 to 5 ml, were prepared by dilution from the stock standard solution.

HPLC analysis

In order to determine the content of thymol, we use the commonest method which is in compliance with HPLC. We pass the above mentioned solution through filter 0.45 µm and inject it into HPLC model Kanauer, Germany. An HP 1000 series liquid chromatograph system comprising vacuum degasser, quaternary pump, autosampler, thermostatted column compartment, and diode array detector was used. Column Machery-NAGEL, Nucleosin-100-5 C_{18}, Loop 20 µl was maintained at 30°C.

Solvents used for separation were HPLC grade water (eluent A) and HPLC grade acetonitrile (eluent B). The gradient used was: 0 to 5 min, linear gradient from 70 to 30% B; 13 min, linear gradient from 42 to 58% B, maintain at 70 to 30% B until 30 min. The flow rate was 1.0 ml min^{-1}. Detection wavelength was 330 nm. The sample injection volume was 20 µl. The chromatographic peaks of thymol was confirmed by comparing their retention times and ultra-violet (UV) spectra with that of their reference standard. Working standard solutions were injected into the HPLC and peak area responses obtained. Standard graphs were prepared by plotting concentration versus area. Quantification was carried out from integrated peak areas of the samples using the corresponding standard graph.

RESULTS

The results revealed clear variation in date of flowering from wild thyme plant grown at different environmental geographical regions of Iran. It completes its vegetative growth during the months of April and May and starts to blossom in May to mid June. Blooming continues until mid July. In July it starts to produce fruits and seeds mature (Table 2).

The results showed variation in the values of height plant from wild thyme plant grown at different geographical regions in Iran. The highest height plant in all investigated samples was recorded in Dezak region (29.3 cm) (Chaharmahal and Bakhtiari province), while the lowest was observed in Farsan Region (10 cm) (Chaharmahal and Bakhtiari province) are shown in Table 2.

Table 2. Plant height, date of flowering and content of thymol collected from *T. daenensis* in geographic different.

Row	Region	Plant height (cm)	Date of flowering	Thymol (mg/g extract)
1	Yancheshmeh	16.3	29 May	20.86
2	Sabz-e-koh	13.5	27 May	13.89
3	Larak	17.8	3 June	25.94
4	Koh-e-sheida	15.6	7 June	21.32
5	Azadegan	14	5 June	18.28
6	Noorjamaloo	12.3	5 June	23.02
7	Shahrekord	19.7	1 June	23.05
8	Dezak	29.3	1 June	19.39
9	Farsan	10	31 May	25.01
10	Tomanak	18	5 June	32.96
11	Bardeh	15.9	6 June	30.37
12	Sheikhshaban	16.5	10 June	35.89
13	Koohrang	15.3	15 June	21.66
14	Daran	12	5 June	25.51
15	Semirom	13.5	4 June	14.47

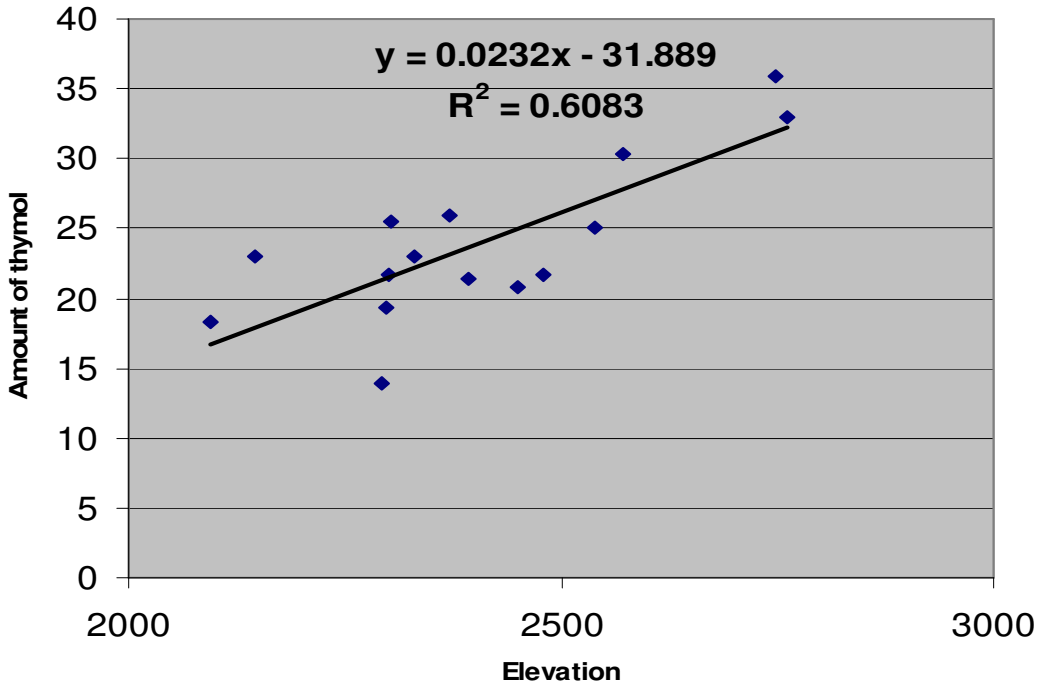

Figure 2. Linear regression between altitude and content of thymol.

Our investigation showed that high variation in content of thymol from *T. daenensis* populations grown at different geographical regions in Isfahan, Chaharmahal and Bakhtiari provinces, Iran. The highest content of thymol in all investigated samples was recorded in Sheikhshaban Region (35.89 mg/g methanol extract) (Chaharmahal and Bakhtiari province), while the lowest was observed in Sabz-e-koh Region (13.89 mg/g methanol extract) (Chaharmahal and Bakhtiari province) (Table 2). Sheikhshaban Region is high altitude in study area (2762 m above sea level).

The results of present study showed that a positive and linear relationship (R^2= 0.61) between content of thymol as major component with elevation (Figure 2). The results of the correlation showed that some of the geographic, climatology and edaphic factors had no significant effects

on content of thymol and carvacrol.

DISCUSSION

Several earlier studies on another Iranian *Thymus* spp. showed that the main components of the oils were carvacrol and thymol. Nickavar et al. (2005) reported that the hydrodistillation of the aerial parts of *T. daenensis* subsp. *daenensis* have twenty six components, which represented about 99.7% of the total detected constituents. The major constituents of the oil were thymol (74.7%), p-cymene (6.5%), b-caryophyllene (3.8%) and methyl carvacrol (3.6%) (Nickavar et al., 2005). *T. daenensis* subsp. *daenensis*, monoterpene phenols were also the most abundant compound group of this oil (72.7%). In this study, comparison of the content of thymol of *T. daenensis* subsp. *daenensis* extract in different geographic regions showed that there are some qualitative and quantitative differences between fifteen regions in Chaharmahal, Bakhtiari and Isfahan provinces, Iran.

These chemical differences can be most probably explained by the variability of the genetic and the existence of different chemotypes. Chemotype *T. daenensis* of Sheikhshaban Region may be a potential thymol-rich source for commercial cultivation. Also, we can conclude that the best place for the production of quantity effective materials this plant in order to attain the best results, is the height between 2400 to 2800 m above sea level.

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