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The Effects of Changes in Altitude on Quantity of Essential Oil, Carvacrol and Thymol of Thymus kotschyanus Boiss in Central Province (Arak)

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Abstract

The purpose of this research was to study the effects of regional altitude on essential oil, Carvacrol and Thymol of *Thymus kotschyanus* Boiss. In order to this goal, aerial parts of this species and top 30 cm soil samples of from four regions in Central Province (Arak) of Iran was collected. Essential oil samples were extracted by hydrodistillation method. Percentage of essential oil with soil pH has a significant positive relation and there is significant negative relation with altitude. Evaluating environmental factors, we found regional altitude and soil pH as the important factors influencing the production of oil. This was conclude that ecological factors are important and should be taken into account for cultivation of *Thymus kotschyanus.* The results showed that the highest and lowest percentage of essential oil were observed of 2150 and 1750 meters altitude respectively.

Keywords: Essential Oil; Thymus *Kotschyanus;* Carvacrol; Thymol

Introduction

Medicinal herbs are said to plants, which in certain organs such as root, stem, leaf and flower contain active ingredients and directly or indirectly have a therapeutic effect [1]. Iran enjoys a history of illustrious history in medicine, geographic and climatic potential aptitudes (11 out of 13 global climates, daily temperature range of 50^{o}C and 300 sunny days per year) and has more than 7500 plant species that about 10 to 15% of them are known as medicinal [2]. Essential oil plants are plants, which depending on ecological and climatic conditions of every region have different richness and diversity. Each essential oil properties changes depending on the species, climate conditions of plant growth location and also sampling time and the time of harvesting the organ containing essential oil [3]. In general, the effective factors on active ingredients of medicinal plants could be separated into four factors; the ecological, genetic, management and stages of plant growth [4]. Quality and quantity of active ingredients of medicinal plants is mainly influenced by genetic factors, but environmental factors of growth location can also have an important effect [5]. Of the most important ecological factors affecting the quantity and quality of active ingredient in medicinal plant, the weather and climatic conditions, edaphic factors and geographical conditions are mentionable [6]. Each of these factors can have a significant influence on the quantity and quality of essential oils and accordingly, the researchers try to present the different methods to produce more active ingredient [7]. Based on conducted research, the environmental factors of growth location of medicinal plants influence on these plants in three aspects; the effect on the total amount of active ingredient, composing elements of the active ingredients and the amount of dry weight yield of plant. Therefore internal and external factors have a significant impact on yield and essential oil compositions [8].

The mint family (Lamiaceae) is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide. This family has an almost cosmopolitan distribution. Many members of the family best know the Lamiaceae for the essential oils common. Many biologically active essential oils have been isolated from various members of this family. These plants are frequently aromatic in all parts and include many widely used culinary herbs, such as thyme. The genus Thymus L. belongs to the Nepetoideae subfamily of Lamiaceae family is a well-known aromatic herb and consists of about 215 species of herbaceous perennials and small shrubs in the world. The Mediterranean region can be described as the center of the genus [9-11]. Thymus, with the common Persian name of "Avishan or Azorbe," [12] is presented in Iranica flora by 14 species, including: T. *daenensis*, T. *carmanicus*, T. *fallax*, T. *persicus*, T. *trautvetteri* ,T. *kotschyanus*, T. *pubesens* ,T. *nummularius*, T. *transcaspicus* T. *eriocalyx*, T. *caucasicus*, T. *migricus*, T. *transcaucasicus* and T. *fedtschenkoi* [13-15]. Thyme is a familiar name and for a group of plants that all of them belong to mint family (Lamiaceae). Among the important genus of Lamiaceae, is the Thymus genus (Thymus), which has about 250 species worldwide and 31 species in Iran. The distribution of this species in the world is in Turkey, Caucasus and Iran countries and in term of ecology in mountain areas it grows from 650 to 3900 meters height [16]. Thyme is native plant of Asia and Europe that includes 400 species worldwide and 31 species in Iran [17]. Thymus there are dozens of species in the world that obviously 14 species of it has been identified in Iran and its Iranian excusive species is *Thymus kotschyanus* Boiss and the most famous species of this genus is common thyme or *Thymus vulgaris*. One of the valuable species of thymus genus that grows mainly in mountainous altitude is a plant with the scientific name *Thymus kotschyanus* Boiss & Hohen and its Persian name is mountainous thyme that the main region of this species is in the Mediterranean regions. T. *kotschyanus* is a perennial plant. It grows up to 20cm of height. On the small wooden branches, dark, green sharp and pointy leaves grow. The aromatic leaves are used as spice and medicine. The white flowers are scented. This species grows in mountainous regions and although is dispersed almost all over the world, but actually accumulates in Mediterranean region. This species has the largest dispersion in Iran [18]. Thyme with the scientific name *Thymus kotschyanus* Boiss & Hohen is the most known and the most important species in terms of quantity and quality of essential oil. This species is the most well-known species of Thymus genus in Iran [16]. It is a bush plant, almost straight, short stature. Stem with many ramifications, hyper--branched, the length of flowering branch 6-12 cm with short fluffs, lying or wide and dense, dispersed or fluff less

with many red sticky secretory glands. Prominent vervures on the underside of leaves, inflorescence often pile, compressed in dense capitol. Peduncle is almost equal to calyx. Flowers pink to purple and sometimes white. The flowering time is from late spring to mid-summer [9]. This plant grows in steeps and rocky areas [19]. The plant reproduction is by seed and cuttings or root division. Its vegetative growth starts in early April and the flowers appear in late June; its seed is formed in early July and after ripening, is scattered around. Region of Thyme is very broad in Iran and often is specific in mountainous. The number of species reported in Iran is 31 that have the greatest distribution in the North and West of the country in Gilan, Mazandaran, Azerbaijan, Hamedan, Kermanshah, Kordestan, Lorestan, Chaharmahal and Bakhtiari, Kerman, Isfahan, Khorasan, Central, Tehran, Kohkilooyeh and Boyer Ahmad, Semnan, Zanjan, Qazvin, Arak and Yazd [20]. Thyme is a plant that naturally grows in semi-arid regions to the warm mild at high temperatures and intense sun radiation and adapts with cold to mild Mediterranean climate and loamy-sandy soil. The amount of water, soil and light regimes significantly change the growth of thyme colonies (dry weight of roots and shoots) and the interaction between light and soil moisture observed [21]. Nowadays, thyme has changed from a traditional herb to a serious drug rational phytotherapy. It is incredible wellspring of iron, calcium, manganese, vitamin K, likewise upgrades blood flow, and pushes an invigorating impact for the entire system. This herb-invigorated activity on anxious framework made it as a cure for physical and mental weakness and additionally for diminishing insomnia. The remedial potential of Thymus is due to the presence of flavonoids, thymol, carvacrol, eugenol, phenols, luteolin and tetramethoxylated. Its controls numerous valuable effects, such as, antispasmodic, antimycotic, mammalian age-delaying properties, bactericides, antiseptics, antioxidants, anthelmintic properties and has late been recommended as substitute as cancer prevention agent [22-24]. Moreover, infusion and decoction of aerial parts of Thymus species are used to produce tonic, carminative, digestive, antispasmodic, anti-inflammatory and expectorant and for the treatment of colds in Iranian traditional medicine [25,26]. Recent studies have shown that Thymus species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities [13,26-28]. On the other hand, Thymus species are commonly used as herbal tea, flavoring agents (condiments and spices) because of their biological and pharmacological properties (Burnett *et al.*, 2005 and Stahl-Biskup).

Materials and Methods

This experiment was carried out in four climatic zones of Arak city in May 2014. Then on each height level plant, collecting in flowering phenological stage was done from three points by random systematic method from the considered plants, so that three samples and total of 12 samples were taken in each region. Sampling time was the middle of July 2014.

Table 1: Specifications of studied regions

Regions	Marzijeran	Hosseinabad	Zamenjan	Gherdoo
Altitude(m)	1750	1850	1950	2150
Latitude	34° 08' 13.6" N	34° 02' 31.5" N	34° 01' 44.7" N	34° 02' 13.7" N
Longtitude	49° 38' 11" E	49° 47' 27.9" E	49° 37' 13.4" E	49° 41' 36.8" E

Soil sampling

In order to determine the physical and chemical properties of soil and its relationship with the quantity and quality of essential oil of studied species, from the regions soil in every region, one sample from around the plant root and in depth of 0-30 cm collected to transport to the laboratory in order to evaluate the physical and chemical parameters.

Determining the soil texture

Determining the soil texture (clay, silt and sand percentage) was performed using a hydrometer. Finally, with the help of soil texture triangle, tissue of all samples in-

dicated and determined that the most texture type of the area is loam type.

Measuring soil measure acidity

For measuring the acidity of soil (pH), first, from all of the soil samples, the saturated mud is prepared. Finally, it was determined that all samples are alkaline.

Measuring the electrical conductivity of the soil

The aim of measuring the electrical conductivity of soil-saturated extract is measuring the dissolved salts in it. Electrical conductivity is directly proportional to the amount of ions in the environment, so by passing an electrical current through an electrolyte or extract, its dissolved minerals can be realized. In order to measure the electrical conductivity of saturated mud samples using Extractor soil, extraction from the soil was conducted for two hours the extracts were collected in a glass container. Then by the electrical conductivity meter, the obtained electrical conductivity of the extracts was measured.

Measuring the soil absorbable phosphorus

Soil phosphorus was measured according to Olsen method. To do this, 2.5 g of each soil sample was weighed and poured into 1000 ml flask. The 0.3 gr activated charcoal plus 42-gr half-normal sodium bicarbonate (with pH 8.5) was added to it and it was put on a shaker to be stirred for 30 minutes. Then solution was passed through an extraction filter. The obtained extracts were poured into flasks of 50 ml and was mixed with the indicator which is a combination of 100 cc distilled water, 25 cc 4 M sulfuric acid, 7.5 cc Ammonium heptamolybdate, cc15 ascorbic acid and 2.5 cc *Antimony potassium tartrate* that produced different intensities of blue color. The darker blue color indicates higher levels of phosphorus. Then, phosphorus was measured by spectrophotometer at a wavelength of 270 nm.

Measuring soil nitrogen

Measuring soil lime

For measuring the lime, the method of Calcimetery was used. Titration operation was conducted as follows: 1 gr of each soil sample with 10 ml of hydrochloric acid were poured into a 250 ml Erlenmeyer flask and the Erlenmeyer was placed on the electric heater until the solution boiled and then removed from the heater until to be cooled. Then with distilled water, the solution volume was increased to 100 ml and then 5 drops of phenolphthalein indicator was added to the solution then the solution was titrated using sodium hydroxide by Burette (special apparatus for titration) until the solution color to be violet. Finally, the obtained number is read using Burette.

Method of extracting essential oils from plant

For extraction of essential oil of *Thymus kotschyanus* is used the water distillation method by Clevenger apparatus for 4 hours.

Method of identifying compounds

Detection of essential oil compounds using (GC/MS) at the Chromatography Laboratory of Tabarestan Institute of Genetics and Biotechnology of University of Agricultural Sciences and Natural Resources of Sari city was done. This device is a combination of two methods that is used for the isolation and analysis of chemical compounds existing in essential oil. In examining the essential oil of considered samples, the gas chromatography model HP - 7890 and mass spectrometer Model 5975 HP – made in Agilent Company as well as the column separation HP-5ms (0.25 mm×0.25 mμ×30m) were used. Temperature of injection valve was 250^{oC} and also planning the column temperature was from 60 to 250^{oC} with the speed of 5^{oC} per minute. Also the required time length for separating each sample was determined 67 minutes.

Statistical analysis

In order to statistical analysis of data obtained from SAS and SPSS software version 16 was used. In order to compare the characteristics of soil and essential oil in all of the four studied regions, the one-way ANOVA was used. To compare the difference of mean of soil data and also the essential oil data, multi-way Duncan test was used.

Results and Discussion

By studying the flowering stage essential oil of Th. *Kotschyanus*, totally 23 combinations in essential oil of this organ of this plant was observed that the compounds Car-

vacrol 65.27% and Thymol 32.01% had the highest percentage respectively (Table 2).

The results of analysis of variance test on data of the percentage of compounds of essential oil of flowering stage of plant showed that the region had significant effect on yield of essential oil, compounds of Thymol, γ-Terpinene, Camphene, α-pinene, Caryophyllene oxide, 4-Terpineol, Linalool, α–Terpinene, Carvacrol, β–Pinene, O–-cymene, Geraniol, α-Terpineol, Eugenol, Trans– Caryophyllene, Camphor, 3–Octanol and α– Thujene. However, did not have significant effect on compounds of essential oil of these plants that were in the four regions (Table 2). Studying the compounds percentage of Thymol in different heights and regions.

Review the table of comparison of mean of percentage of composition Thymol in essential oil of Th. *kotschyanus* in regions and different altitudes showed that between the height of 2150 m (site Jordo) with 1950 m (Zamen jan region), 1850 meters (opposite the University) and 1750 meters (Mrzyjan region); there was significant difference in the percentage of composition of Thymol in the essential oil (Table 2 and Figure 2).

Between the heights of 1950 m (Zamenjan region) with a height of 1850 meters (opposite the University) and 1750 meters (Marzijan region), significant difference was observed in the percentage of composition of Thymol in the essential oil. Also between the height of 1850 meters (opposite the University) and 1750 meters (Mrzijan region) there was no significant difference.

The highest percentage of Thymol of essential oil belonged to the height of 2150 m (Jordo region) with 44.45% and the lowest percent belonged to the height of 1750 (Mrzijan region) with 22.83% percent (Table 2 and Figure 1).

Table2: Analysis of variance of different compounds in the essential oil of *Thymus kotschyanus* in different regions

Coefficient of variation	Experimental error	Effect of region	Replicationeffect	Sources of variations
-	6	3	2	Degree of freedom
5.01	0.0053	0.16328 **	0.121906 **	Essential oil yield
4.26	1.8721	308.941 **	74.8831 **	Thymol
3.29	0.0052	0.85780 **	0.3094 **	Borneol
27.25	0.0819	0.81070 **	0.181 ns	γ -Terpinene
101.2	0.0816	0.11680 *	0.0588 ns	Camphene
58.31	0.0625	0.76410 **	0.0444 ns	α-pinene
65.49	0.1082	0.32130 ns	0.2491 ns	Linalool
57.11	0.0823	0.09190 **	0.0379 ns	α-terpinene
71.32	0.2708	1.14090 *	0.5686 ns	Caryophyllene oxide
5.51	0.0001	0.00178 **	0.00025 **	β -Pinene
3.12	0.0003	0.05158 **	0.02334 **	4-Terpineol
4.78	2.4207	399.480 **	23.914 **	Carvacrol
57.98	0.0786	0.18620 ns	0.6575 *	β -Myrcene
131.82	0.0823	0.11067 ns	0.006616 ns	Carvacryl acetate
211.8	0.0854	0.05875 ns	0.07602 ns	α-Humulene
32.74	0.0890	0.77392 *	0.02026 ns	O-cymene
38.97	0.0905	0.41943 *	0.02298 ns	Geraniol

5.32	0.0036	0.59100 **	0.09256 **	trans-Caryophyllene
67.28	0.1053	0.25618 ns	0.25374 ns	Eucalyptol
3.53	0.0001	0.00066 **	0.00023 **	3-Octanol
2.71	0.0001	0.00059 **	0.00035 **	α-Thujene
6.81	0.0001	0.01134 **	0.00107 **	α-Terpineol
5.75	0.0001	0.00324 **	0.000432 **	Eugenol
3.41	0.0001	0.00087 **	0.000327 **	Camphor

ns: non-significant * significant at 95% ** significant at 99%

Studying the mean of percentage of Thymol compound in the essential oil at altitudes and different regions showed that the percent of this combination has increased from the height of 1750 (Mrzijan region) up to 2150 m (Gherdoo region) (Figure 1).

Studying the table of comparison of mean of percentage of Carvocrol composition in essential oil of Th. kotschyanus in different regions and altitudes showed that between the height of 2150 m (Jordo region) with 1950 m (Zamen jan region), 1850 meters (University opposite) and 1750 meters (Mrzijan region) there was significant difference in the percentage of composition of Carvocrol in the essential oil.

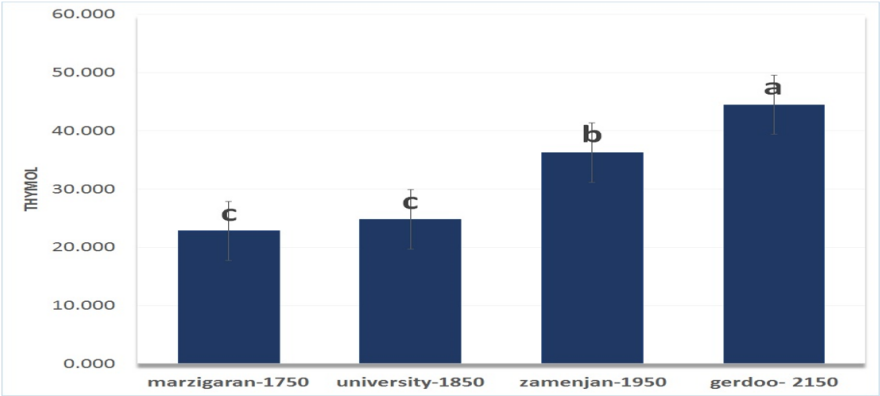

Figure 1: Comparison of average of Thymol composition in *Th. kotschyanus* in different regions

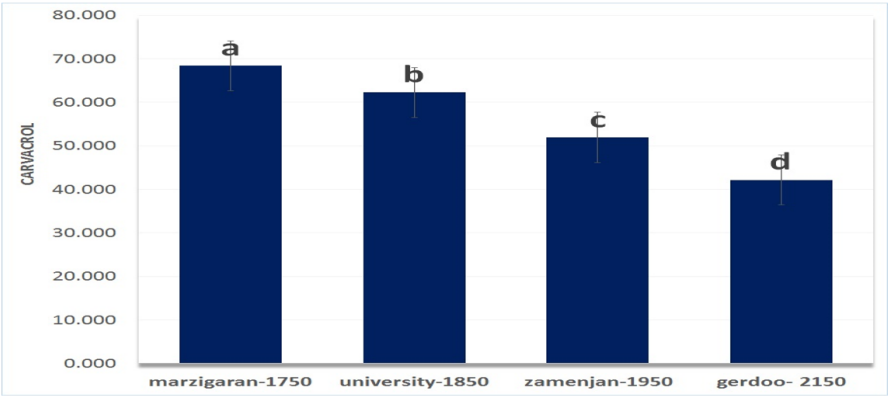

Figure 2: comparison of the composition average of Carvocrol in *Th. kotschyanus* in the regions

Between the height of 1950 m (Zamen jan region) with the height of 1850 meters (University opposite) and 1750 meters (Mrzijan region) significant difference was observed in the percentage of composition Carvocrol in the essential oil. In addition, between the height of 1850 meters (opposite the University) and 1750 meters (Mrzijan region) there was no significant difference. The lowest percentage of Carvocrol of ssential oil was in the height of 2150 m (Jordo region) with 42.19% and the maximum belonged to the height of 1750 m (Mrzijan region) with 68.39% (Table 2 and Figure 2). Studying the mean of percent of Carvocrol combination in essential oil in different altitudes and regions showed that the percent of this combination has increased from the height of 1750 (Mrzijan region) to 2150 m (Gherdoo region) (Figure 2).

Table 3: comparison of mean percentage of essential oils and compounds under the influence of height in *Thymus kotschyanus* at flowering stage

The compounds of essential oil (%)	Marzijeran	University	Zamenjan	Gherdoo	LSD%
	1750	1850	1950	2150	
Essential oil (%)	1.16 d	1.38 c	1.53 b	1.71 a	0.145
Thymol	22.8 c	24.8 c	36.3 b	44.54 a	2.733
Borneol	1.33 d	2.54 a	2.01 c	2.38 b	0.144
γ -Terpinene	0.28 b	1.33 a	1.14 a	1.41 a	0.572
Camphene	0.13 b	0.21 b	0.22 b	0.57 a	0.271
α-pinene	0.14 b	0.28 b	0.22 b	1.18 a	0.499
Linalool	0.91 a	0.48 ab	0.51 ab	0.11 b	0.657
α-terpinene	0.33 a	0.44 a	0.74 a	0.53 a	0.580
Caryophyllene oxide	0.28 b	0.58 b	0.35 b	1.62 a	1.040
β -Pinene	0.09 a	0.07 b	0.04 c	0.04 c	0.006
4-Terpineol	0.38 d	0.68 a	0.59 c	0.63 b	0.035
Carvacrol	68.4 a	62.3 b	52.00 c	42.19 d	3.108
β -Myrcene	0.43 b	0.58 a	0.60 a	0.62 a	0.460
Carvacryl acetate	0.19 a	0.48 a	0.14 a	0.05 a	0.573
α-Humulene	0.14 a	0.11 a	0.06 a	0.04 a	0.283
O-cymene	0.14 b	1.08 a	0.90 a	1.31 a	0.596
Geraniol	1.04 a	0.91 a	0.92 a	0.22 b	0.601
trans-Caryophyllene	0.49 c	1.37 a	1.18 b	1.47 a	0.119
Eucalyptol	0.81 a	0.35 ab	0.62 ab	0.15 b	0.648
3-Octanol	0.05 b	0.08 a	0.05 b	0.05 b	0.004
α-Thujene	0.08 a	0.08 a	0.07 b	0.05 c	0.004
α-Terpineol	0.07 d	0.11 b	0.09 c	0.21 a	0.017
Eugenol	0.04 d	0.12 a	0.08 b	0.07 c	0.009
Camphor	0.09 a	0.07 b	0.05 d	0.06 c	0.005

Groups that have common letters are not significantly different Soil characteristics in four studied regions are described in Table 3. The review Table 3 determined that Lime, Potassium and PH of the soil increased with rises altitude.

Table 4: Compare soil characteristics in four regions

Soil characteristics	Marzijeran	University	Zamenjan	Gherdoo
	1750	1850	1950	2150
Lime	25.09	21.34	24.77	35.89
Potassium	235.26	215.38	424.02	440.7
Phosphorus	6.213	5.318	7.207	4.194
Organic matter	0.432	0.378	0.564	0.308
Nitrogen	0.149	0.169	0.199	0.169
PH	7.336	7.543	7.406	7.535
Electrical conductivity	0.636	0.497	0.695	0.467
Clay (%)	22.54	19.15	16.17	19.14
Silt (%)	33.17	34.43	44.73	34.46
Sand (%)	44.29	46.42	39.10	46.40

Study correlation between soil characteristics and essential oil, Thymol and Carvacrol of *Thymus kotschyanus* showed: essential oil has positive correlation with Lime, Potassium, Nitrogen, sand and PH and Thymol has positive correlation with Lime, Potassium and Nitrogen also Carvacrol has negative correlation with Lime and Potassium.

Table 5: The correlation between soil characteristics and essential oil, Thymol and Carvacrol of *Thymus kotschyanus*

	Lime	Potassium	Phosphorus	Organic matter	Nitrogen	Clay	Silt	Sand	PH	EC
Thymol	0.854**	0.955**	-0.113	0.014	0.585*	-0.121	0.477	0.415	0.411	0.107
Carvacrol	-0.774**	-0.911**	0.263	0.116	-0.49	0.297	-0.362	-0.334	-0.247	0.08
Essential oil	0.765**	0.845**	-0.029	0.077	0.733**	-0.016	0.575	0.645*	0.616*	0.123

This is in agreement with the results of some previous works on the essential oil of different species of the this genus [29,30]. This means that with increasing altitude, plant growth and essential oil production will be limited. The results of this study confirm this subject [31]. The effect of environmental factors on secondary plant metabolites is important and many studies have ever been done about it [32]. Plants active ingredients under the effects of these factors vary in terms of quality and quantity. These results indicate that environmental factors could have an effect on the quality of the oil, which corresponds with the results of previous work on this genus [33-35].

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