Effect of Ecological Factors on Plant Properties of *Thymus daenensis* Celak in Different Regions in Southwest and Central Iran

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Summary

Thymus daenensis Celak. (*Lamiaceae*) is an aromatic medicinal plant endemic to Iran, intensively utilized because of its wide ranging medicinal and culinary properties. The aerial parts of wild populations of *T. daenensis* were collected from different populations in Isfahan and Chaharmahal and Bakhtiari provinces, Southwest and Central Iran. The thymol and carvacrol amounts ranged between 13.89 to 35.89 mg g^{-1} and 6.88 to 23.54 mg g^{-1} respectively, for populations collected from various elevations. Also, our investigation showed that plant height and date of flowering between populations of *T. daenensis* had significant difference. The cluster analysis showed that the amount of thymol and carvacrol, and plant height of *T. daenensis* could be grouped into two distinctive clusters. The results indicate that the amount of thymol and carvacrol of *T. daenensis* are strongly affected by environmental conditions. Since secondary metabolites are the product of a predominantly biological process further studies are needed to evaluate if the reported characteristics of each population are maintained at the level of individual plants and along the breeding and selection program when grown under different soil and climatic conditions.

Key words

Thymus daenensis Celak, thymol, carvacrol, altitude

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Introduction

The genus *Thymus* L. belongs to the family (*Lamiaceae*), subfamily Nepetoideae and consists of about 300-400 species of herbaceous perennials and small shrubs in the world. However, the central area of this genus surrounds the Mediterranean Sea as they are heliophilous and grow well in a climate with moderate to warm temperatures (Golparvar and Hadipanah, 2023). *Thymus*, with the common Persian name of "Avishan" or ''Azorbe," consists of 18 species which are found wild in many regions of Iran, among them 4 species include *T. persicus* (Roniger ex Reach F.) Jalas, *Thymus carmanicus* Jalas, *Thymus trautvetteri* Klokov & Des.-Shost. and *T. daenensis* Celak with two subspecies *T. daenensis* subsp. *daenensis* and *T. daenensis* subsp. *lancifolius* that are endemic to Iran (Jamzad, 2009; Mozaffarian, 2008). *T. daenensis* (Avishan-e-denaee or Denaian thyme) is herbaceous and perennial plant with a maximum height of 30 cm, with short and thick stems that are completely woody at the bottom. The leaves are opposite and small, oval or ovate, pointed, petioleless and rarely linear, the length of the leaf varies from 5.9 to 16 mm and the width varies from 4.2 to 4 mm. The leaves may be overlapped or shorter than the internodes, the flowers are white, purplish or purple, and are complex next to the leaves, the calyx is tubular to a cup, the teeth of which are about 0.5 mm long in the upper part. The calyx is red and the fruit is ovoid, light brown with a smooth surface. Its distribution in Iran is mainly in high altitude places in the high altitudes in Zagros Mountains and Alborz Mountains (Ghasemi Pirbalouti et al., 2015; Golparvar et al., 2012; Pirbalouti et al., 2013).

The aerial parts and volatile constitutes of *T. daenensis* are commonly used as medicinal and aromatic herb. The published results reveal that the major volatile constituents obtained from the aerial parts of *T. daenensis* are oxygenated monoterpenes. The essential oil and extracts isolated from *T. daenensis* have biological properties, including antibacterial, antifungal and antioxidant (Hadipanah and Khorami, 2016; Mumivand et al., 2021; Pirbalouti et al., 2013).

It has been reported that yield and components in thyme species in general are primarily related to their various factors such as genetics, plant species, cultivars, ecotypes, geographical origin, weather conditions, geobotanical conditions, cultivation method, stress type, time of plant collection and ecological factors, including precipitation, temperature, plant competition and nitrogen content in the soil (Golparvar et al., 2015; Golparvar et al., 2014; Ložien and Venskutonis, 2003; Salehi et al., 2014; Thompson et al., 2003).

The result of (Pirbalouti et al., 2011) showed that the altitude should be considered as a major factor influencing the chemical composition of *T. daenensis* Celak. Altitude seems to be affecting essential oil content of only oil rich and oil-intermediate aromatic plants. The main goals of this study were to evaluate the effects of various elevations on thymol and carvacrol amounts of different populations of *T. daenensis* collected from two geographical regions of Iran.

Materials and Methods

Plant Material

In this study, the aerial parts (0.05 to 0.2 kg) of wild populations of *Thymus daenensis* subsp. *daenensis* Celak. (four individuals from each population) were collected from natural habitats at the early flowering stage from April to June 2021 from 15 localities in Isfahan, Chaharmahal and Bakhtiari provinces, Southwest and Central Iran (Table 1). The climate of regions in Chaharmahal and Bakhtiariprovince is cold and semiarid by Emberger's climatology standard and semi humid with temperate summer and very cold winter by Karimi's climatology standard. According to multivariate statistical method Isfahan is considered as cold and arid (Yaghmaei et al., 2009). Each sample was labeled, and its location was recorded using a global positioning system (GPS, Garmin) receiver. These are located between latitude 30º 42' N and 33º 37' N. The elevations range between 1009 and 3889 m above sea level (Fig. 1). 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 temperatures, 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 the center and west of Iran.

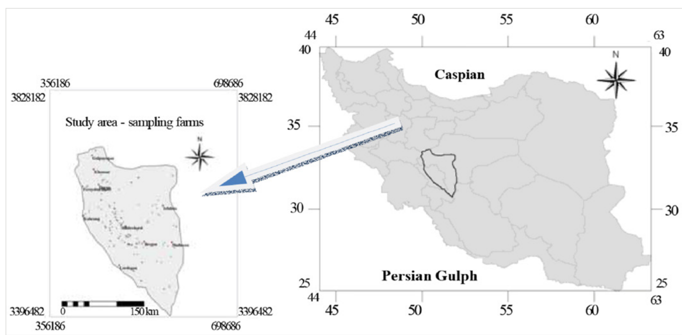

Figure 1. Location of wild populations of *T. daenensis* collected from different populations in Isfahan and Chaharmahal and Bakhtiari provinces, Southwest and Central Iran

The results of some physical and chemical properties of soil samples collected from different populations (15 regions) in Isfahan and Chaharmahal and Bakhtiari provinces were carried out at Islamic Azad University, Khorasgan (Isfahan), Iran. Soil physical and chemical characteristics such as: pH, electrical conductivity (EC), texture, organic carbon (OC%), total neutralizing value (TNV%), contents of nitrogen, N (%); phosphor, P (mg kg^{-1}); and potassium, K (mg kg^{-1}) were also examined (Table 2).

Table 1. Geographical and environmental conditions, date of flowering of wild populations of *T. daenensis* collected from different populations in Isfahan and Chaharmahal and Bakhtiari provinces, Southwest and Central Iran

Accession	Locality	Province	Latitude	Longitude	Altitude (m asl[1])	Date of flowering
Ec01	Semirom	Isfahan	31°40'34.9" N	51°33'13.2" E	2302	4 June
Ec02	Daran	Isfahan	32°56'56.1" N	50°26'44.4" E	2303	5 June
Ec03	Dezak	Chaharmahal va Bakhtiari	32°06'25.4" N	51°03'08.9" E	2298	1 June
Ec04	Sabz-e-koh	Chaharmahal va Bakhtiari	31°49.0'.03" N	50°51'51.3" E	2292	27 May
Ec05	Shahrekord	Chaharmahal va Bakhtiari	32°21'06.1" N	50°53'11.6" E	2045	1 June
Ec06	Larak	Chaharmahal va Bakhtiari	32°34'52.3" N	50°39'35.0" E	2370	3 June
Ec07	Koh-e-sheida	Chaharmahal va Bakhtiari	32°37'22.2" N	50°34'46.9" E	2394	7 June
Ec08	Sheikhshaban	Chaharmahal va Bakhtiari	32°35'1.2" N	50°38'19.2" E	2747	10 June
Ec09	Koohrang	Chaharmahal va Bakhtiari	32°27'59.6" N	50°17'18.0" E	2479	15 June
Ec10	Bardeh	Chaharmahal va Bakhtiari	32°33'59.5" N	50°29'32.6" E	2572	6 June
Ec11	Farsan	Chaharmahal va Bakhtiari	32°19'0.1" N	50°32'11.4" E	2539	31 May
Ec12	Yancheshmeh	Chaharmahal va Bakhtiari	32°37'19.5" N	50°43'15.0" E	2450	29 May
Ec13	Azadegan	Chaharmahal va Bakhtiari	32°40'33.6" N	50°29'06.0" E	2094	5 June
Ec14	Tomanak	Chaharmahal va Bakhtiari	32°31'13.4" N	50°37'23.3" E	2762	5 June
Ec15	Noorjamaloo	Chaharmahal va Bakhtiari	32°39'22" N	50°32'39.3" E	2330	5 June

To determine the contents of thymol and carvacrol, this experiment was carried out at Islamic Azad University, Khorasgan (Isfahan), Iran. Briefly, the aerial parts of each sample were dried at room once a week. The aerial parts containing 100 mg were crushed and homogenized with 30 ml of 80% methanol. The samples were transferred to a tube 250 mL for extraction with 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. Stock standard solutions were prepared by accurately weighing 22.3 mg of thymol and carvacrol reference standard into separate 50 ml volumetric flasks and dissolving in acetonitrile/water (50:50, v/v) with the aid of sonication. Working solutions, 1 to 5 ml, were prepared by dilution from the stock standard solution. To determine the content of thymol and carvacrol, we use the commonest method which is in compliance with HPLC. We pass the abovementioned 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 C18, Loop 20 µL were 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, maintained at 70 to 30% B until 30 min. The flow rate was 1.0 mL min^{-1}. The detection wavelength was 330 nm. The sample injection volume was 20 µl. The chromatographic peaks of thymol were confirmed by comparing their retention times and ultraviolet (UV) spectra with that of their reference standard. Working standard solutions were injected into the HPLC and peak area responses were obtained. Standard graphs were prepared by plotting concentration versus area. The quantification was carried out from integrated peak areas of the samples using the corresponding standard graph.

Statistical Analyses

The slope and elevation information were obtained from the 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. Linear regression analysis was performed to investigate the relationship between elevation and active ingredients using $SPSS_{25}$ software. Cluster analysis was done in order to classify the ecotypes of Denaian thyme using $SPSS_{25}$ software. Graphs were drawn by Excel2016.

Results and Discussion

We investigated morphological traits including plant height and flowering days in different populations of *T. daenensis*. The present data suggest that the morphological status of *T. daenensis* is severely affected by altitude and its effect is different from one region to another. Reproduction is one of the most intrinsic and indispensable characteristics of all living organisms. Indeed,

Table 2. Some physical and chemical properties of soil collected from different populations in Isfahan and Chaharmahal and Bakhtiari provinces, Southwest and Central Iran

Accessions	Clay (%)	Silt (%)	Sand (%)	Total N. (%)	K ava. mg kg^{-1}	P ava. mgkg^{-1}	O.C. (%)	T.N.V. (%)	pH	E.C. dS m^{-1}	Depth (cm)
Ec01	41	35	24	0.106	653	18.7	0.941	15.5	7.78	0.742	0-30
Ec02	48	31	21	0.110	528	56.7	1.095	1.0	7.56	0.662	0-30
Ec03	51	36	13	0.061	456	18.3	0.576	35.0	8.03	0.479	0-30
Ec04	51	38	11	0.288	711	59.1	2.786	19.0	7.60	0.623	0-30
Ec05	39	29	28	0.158	741	15.9	1.133	34.5	8.05	0.760	0-30
Ec06	41	35	24	0.146	998	12.7	1.537	0.5	7.82	0.523	0-30
Ec07	53	41	6	0.166	998	23.3	1.172	2.5	7.66	0.865	0-30
Ec08	41	35	24	0.052	402	11.4	0.641	13.5	7.76	0.493	0-30
Ec09	43	29	28	0.073	567	16.3	0.711	32.5	7.58	0.603	0-30
Ec10	52	37	11	0.124	345	13.2	0.999	40.5	7.82	0.547	0-30
Ec11	49	32	19	0.276	998	10.1	2.843	3.5	7.49	0.739	0-30
Ec12	47	33	20	0.041	287	15.5	0.307	3.5	7.94	0.673	0-30
Ec13	48	35	17	0.245	606	17.6	2.017	41.0	7.38	0.890	0-30
Ec14	49	35	16	0.141	914	39.5	1.402	14.0	7.73	0.687	0-30
Ec15	45	37	18	0.103	871	26.3	1.037	6.5	7.70	0.772	0-30

flowering is an important trait, so our results show the clear variation in date of flowering from wild populations of *T. daenensis* collected from different populations. The results show that the first flowering date was recorded in Sabz-e-koh population at an altitude of 2292 m on May 27 and the last flowering date was recorded in Koohrang population at an altitude of 2479 m on June 15. Thyme can complete its vegetative growth during the months of April and May and start to blossom in May to mid June. Blooming continues until mid July. In July it starts to produce fruits and its seeds mature, which means that when thyme grows in different environmental and geographical regions, it may be a signal for the initial change of plant growth from vegetative to reproductive stage (Table 1).

Determination of Thymol and Carvacrol Contents

The results showed variation in plant height from wild populations of *T. daenensis* collected from different populations. The highest plant height in all investigated samples was recorded in Dezak population (29.2 cm) (Chaharmahal and Bakhtiari province) at an altitude of 2298 m, while the lowest was observed in Farsan population (10.1 cm) (Chaharmahal and Bakhtiari province) at an altitude of 2539 m are shown in (Fig. 2). When plants are subjected to environmental stress, the most important effect is the reduction of plant height. Plant height, severely affected by environmental stress, is closely related to cell enlargement and leaf senescence. The decrease in plant height is mainly due to decreased cell expansion, increased leaf shedding, and impaired mitosis under unsuitable environmental conditions (Misra et al., 2020).

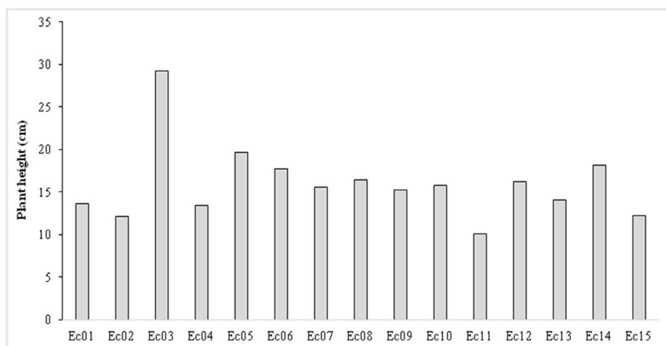

Figure 2. Plant height of wild populations of *T. daenensis* collected from different populations in Isfahan and Chaharmahal and Bakhtiari provinces, Southwest and Central Iran

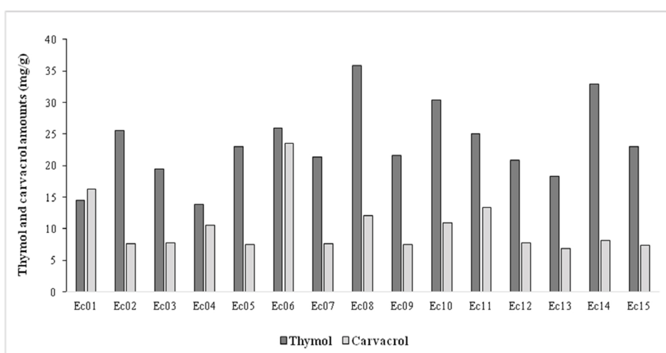

Figure 3. The thymol and carvacrol amounts of wild populations of *T. daenensis* collected from different populations in Isfahan and Chaharmahal and Bakhtiari provinces, Southwest and Central Iran

Our investigation showed very high variations in content of thymol from wild populations of *T. daenensis* collected from different populations. The highest of thymol amount (35.89 mg g^{-1}) in all investigated samples was obtained from the plants growing in the Sheikhshaban population (Chaharmahal and Bakhtiari province) at an altitude of 2747 m, while the lowest was obtained in Sabz-e-koh population (13.89 mg g^{-1}) (Chaharmahal and Bakhtiari province) at an altitude of 2292 m (Figs. 3, 4). Higher elevation and colder temperatures provide a better growing condition resulting in a higher accumulation of thymol amount in the leaves of *T. daenensis*. The highest value of carvacrol (23.54 mg g^{-1}) was related to the plants collected from the Larak population (Chaharmahal and Bakhtiari province) at an altitude 2370 m, while the lowest value of carvacrol (6.88 mg g^{-1}) was found in *T. daenensis* population collected from the Azadegan population (Chaharmahal and Bakhtiari province) at an altitude of 2094 m (Figs. 3, 4). The results of previous studies indicate that altitude is the most important environmental factor influencing secondary metabolites content in *Origanum vulgare* L. ssp. *hirtum* and *Thymbra spicata* var. *spicata* L. (Kizil, 2010).

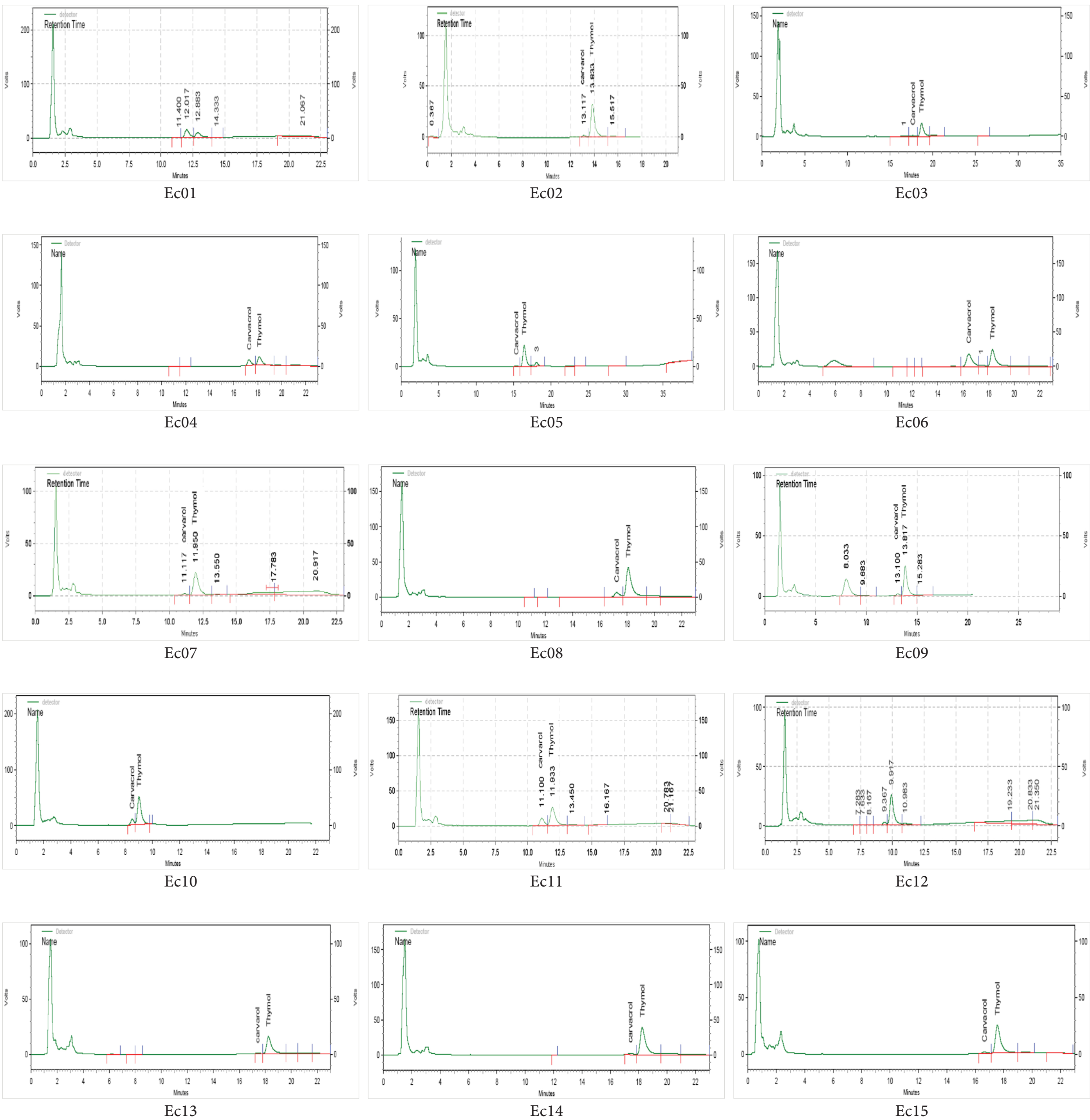

Figure 4. The chromatograms found in thymol and carvacrol amounts of wild populations of *T. daenensis* collected from different populations in Isfahan and Chaharmahal and Bakhtiari provinces, Southwest and Central Iran

The variability of thymol and carvacrol amounts in the methanolic extract obtained from *T. daenensis* wild growing is attributed mainly to environmental conditions and different chemotypes (Ložien and Venskutonis, 2003; Thompson et al., 2003). The physiological situation of plants, time of collection and different ecological conditions have a great effect on both quality and quantity of essential metabolites in medicinal and aromatic plant (Ibañez and Usubillaga, 2006).

The research results of (Talebi Kouyakhi et al., 2008) showed that phytochemical variations were not only found among samples of different regions but also among samples of the same region at different altitudes reflecting the effect of environment on essential oil components.

The results of present study showed a positive and linear relationship (R_2 = 0.61) between content of thymol as major component and elevation (Fig. 5). The results of the correlation showed that some of geographic, climatology and edaphic factors had no significant effects. The cluster analysis of thymol content, carvacrol content and plant height of *T. daenensis* could be grouped into two distinctive clusters (Fig. 6).

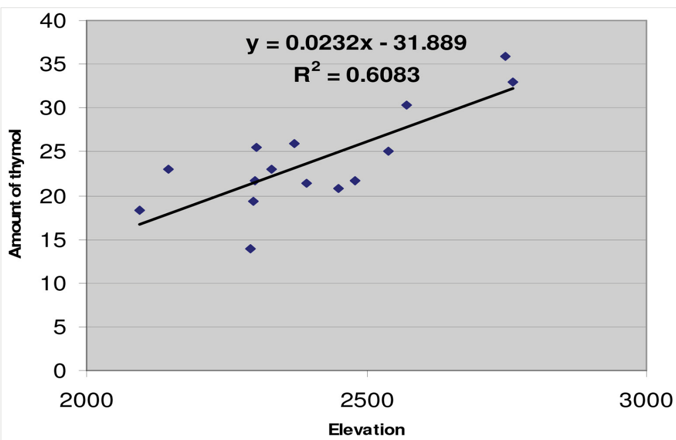

Figure 5. Linear regression between altitude and amount of thymol

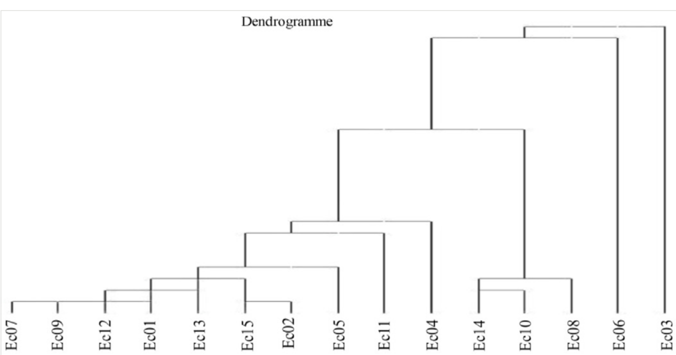

Figure 6. Dendrogram obtained by cluster analysis, based on plant height, amounts of thymol and carvacrol of wild populations of *T. daenensis* collected from different populations in Isfahan and Chaharmahal and Bakhtiari provinces, Southwest and Central Iran

The highest amount of thymol was found in accessions Ec08, Ec14 and Ec10 (35.89, 32.96 and 30.37 mg g^{-1} methanol extract, respectively) and the highest amount of carvacrol was found in accessions Ec06 (23.54 mg g^{-1} methanol extract) (Fig 3, 4). These two quantitative characters may depend on the cultivation and climatic conditions (the soil /water amount, the temperature, the light intensity) or may be genetically determined (Ložien and Venskutonis, 2003). Controlled growth systems also make it feasible to contemplate manipulation of phenotypic variation in the concentration of medicinally important compounds present at harvest (Canter et al., 2005). As discussed by (Madritch and Hunter, 2004) genetically based variation in the secondary compounds present in leaf litter may have significant community and ecosystem level impacts. Understanding facilitation and its ecological role may thus require an appreciation of processes acting at the population level which shape adaptive variation in concert with those affecting species interactions and community dynamics. The shortage of water in arid and semi-arid parts of this region where annual precipitation is less than 300 mm with almost no rainfall during the summer is a prominent limiting factor of *Thymus* production. Unsuitable environmental conditions may limit photosynthesis in plants and alter nutrient uptake and carbon, sugar, amino acid and inorganic ion fluxes (Ghasemi et al., 2013).

Conclusion

In this study, *T. daenensis* subsp. *daenensis* grows naturally in the climatic conditions of Iran at altitude of 2045 to 2762 m above sea level on sandy, loamy and medium clay soils with pH in the range of 7.38 and 8.05. Variable environmental conditions affect thymol content and carvacrol between fifteen populations in Chaharmahal, Bakhtiari and Isfahan provinces. These chemical differences can be most probably explained by the variability of their genetics and the existence of different chemotypes. The amount of thymol in different populations was between 13.89 to 35.89 mg g^{-1}. For the production of thymol from *T. daenensis* in future, the preference should be given to the Sheikhshaban population. The farmers can be encouraged and given incentive to grow the plants with high thymol. Since secondary metabolites are the product of a predominantly biological process, further studies are needed to evaluate if the reported characteristics of each population are maintained at the level of individual plants and along the breeding and selection program when grown under different soil and climatic conditions.

CRediT Authorship Contribution Statement

Ahmad Reza Golparvar: Overall project management, investigation and manuscript writing, project management and editing of the manuscript. **Amin Hadipanah:** Data analysis, manuscript editing, performed some of experiments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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