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Morphological and phytochemical screening of some *Thymus* ecotypes (*Thymus* spp.) native to Iran in order to select elite genotypes

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Summary

Thymus spp. is one of the most important medicinal plants widely used in food, pharmaceutical, and cosmetic industries. In this research, different ecotypes of three *Thymus* species including *T. daenensis*, *T. kotschyanus* and *T. lancifolius* native to Iran were compared to two commercial cultivars of *T. vulgaris* (i.e. 'Varico 3' and 'Deutscher Winter') under identical conditions. Based on the results, there was a remarkable diversity among different ecotypes of *Thymus* species. The highest plant dry weight was found in *T. daenensis* (Malayer 2), *T. kotschyanus* (Azerbaijan gharbi), and *T. lancifolius* (Fars). The highest thymol percentage (>75%) was obtained by *T. daenensis*. The ecotype of Ilam belonging to *T. daenensis* gained highest essential oil percentage (7.83%). In all ecotypes of *T. daenensis*, thymol was the major constituent in their essential oil. Five chemotypes of citral, carvacrol-thymol, thymol-carvacrol, p-cymene-carvacrol, and geranyl acetate-citral were found in *T. kotschyanus* ecotypes, while four chemotypes of thymol, α-terpineol-linalool, carvacrol-thymol and thymol- geraniol were identified for *T. lancifolius*. In addition, in terms of growth, yield, and phytochemical traits, the elite genotypes within ecotypes were selected. Elite ecotypes and genotypes detected during this research could be used in *Thymus* breeding programs.

Keywords: Chemotype, Essential oil, Medicinal plants breeding, Thymol

Introduction

Thymus species, belonging to the family Lamiaceae, includes more than 215 species in the world, in which 14 species are native to Iran, while 4 species are endemic to Iran including *T. persicus*, *T. carmanicus*, *T. daenensis*, and *T. trautvetteri* (Jamzad, 2009; Safaei-Ghomi et al., 2009; Amiri, 2012). Essential oil of *Thymus* species has many applications in different industries such as pharmaceutical, food, sanitary, cosmetic and others, due to its antibacterial, antifungal and antioxidant properties, thereby occupying the top position in world trade of medicinal plants (Alizadeh et al., 2013; Mojab et al., 2008; Rasooli and Mirmostafa, 2002). In *Thymus* species, the major constituents of its essential oil include thymol, carvacrol, and p-cymene (Tohidi et al., 2019). In addition to its essential oil, its aerial parts containing tannin, flavonoids, saponin and bitter materials have been received great attention. Thyme is used as an expectorant for curing cough, sore throat, bronchi, and asthma (Petrović et al., 2017; Edeoga et al., 2005).

Regarding the application of *Thymus* in different industries, the breeding and creating thyme genotypes with high qualitative and quantitative yield is imperative. The main breeding objective of *Thymus* species is to produce plants with higher yield of dry matter, ratio of leaf/shoot, essential oil, thymol content, crop uniformity, freezing tolerance in Winter, upright form of growth, and finally possibility of mechanizing harvesting (Carlen et al., 2010). In this regard, the simplest way for thyme breeding is evaluation of their populations and then selecting the elite genotypes based on their growth and chemotype traits (Hadian et al., 2016; Heydari et al., 2019). Approximately, 60 to 75% of current medicinal crops have been produced through selecting elite genotypes among wild or landraces populations (Bernat, 2000; Nemet, 2000). In terms of dry matter yield, essential oil content, and chemical composition, thyme with higher qualitative traits and uniformity, are highly respected. Progress in plant breeding requires an extended genetic pool (Rahim malek et al., 2009). Awareness of genetic diversity in crops and their wild relatives is an underlying requirement to improve crop yield (Govindaraj et al., 2015). There are basic necessities for achieving high amount of bioactive compounds and their homogeneity in pharmaceutically important plants, such as sufficient genetic diversity in populations for creating favorable traits, appropriate genotypes for different cultivation systems, high harvest index, and low sensitivity to diseases and climate perturbations (Deng et al., 2019; Islam et al., 2019; Deng et al., 2019). Therefore, progress in breeding being in line with the mentioned requirements depends on genetic diversity created by plant breeders (Rahim Malek et al., 2009).

Thyme usually possesses high genetic diversity in terms of morphological and phytochemical traits (Imbrea et al., 2010). According to Loapez-Pujol et al. (2004), higher genetic diversity was found in different ecotypes of *T. loscosil*; this was attributed to its polyploidy, resulted naturally from its heterozygosity as well as its biochemical and genetic diversities. Also, the study of morphological diversity of 5 *T. glabrescens* ecotypes, showed a significant difference in leaf length and width as well as number of leaf secreting trichomes; which are useful traits in thyme breeding programs (Dajic-Stevanovic, 2008).

Hadian et al. (2016) evaluated morphological and essential oil of 6 populations of *T. daenensis* on their natural habitats, and found a remarkable morphological diversity between the populations; the content of essential oil in these ecotypes varied from 1.53 to 4.28%, with the highest essential oil content in Ilam ecotype. However, major components of essential oil in different ecotypes of *T. kotschyanus* ranged from 1 to 1.41%, and the amount of main components in their essential oil was as follows: thymol (31.2%), carvacrol (19.5%), linalool (2.38-20.06%), alpha-terpineol (0.16-11.64%), and geraniol (0.36-11.37%) (Tohidi et al., 2018).

Mostly, breeding thyme (especially those native to Iran) has been focused on selecting naturally ecotypes and elite genotypes on the basis of morphological, phytochemical, and genetic traits. Compared to breeding of agricultural crops, there is limited progress in breeding of medicinal plants. Therefore, the present study aimed to evaluate different ecotypes of three *Thymus* species (i.e. *T. daenensis*,

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T. kotschyanus, and *T. lancifolius*) under field condition and also their comparison with two commercial cultivars of *T. vulgaris* (i.e. 'Varico 3' and 'Deutscher Winter') under identical conditions in order to select elite genotypes and ecotypes qualified with high qualitative and quantitative yield for thyme breeding and cultivation goals.

Materials and methods

Plant materials

The seeds of different ecotypes of *Thymus* spp., provided by different places including Research Institute of Forests and Rangelands (RIFR), Medicinal Plants and Drugs Research Institute of Shahid Beheshti University (MDRI-SUB) and Department of Horticultural Science and Landscape Engineering, University of Tehran (HSD-UT) (Tab. 1), were planted in February 2013 in plant growing trays, containing peat moss, grown in the research greenhouse of HSD-UT and irrigated regularly. In May 2013, the seedlings of studied ecotypes of different *Thymus* spp. such as *T. daenensis* (12 ecotypes), *T. kotschyanus* (5 ecotypes) and *T. lancifolius* (5 ecotypes) along with two commercial cultivars of *T. vulgaris* ('Varico 3' and 'Deutscher Winter') (Tab. 1) were transferred into an experimental field located in Research Station of HSD-UT (located at 35° 46´ N; 50° 55´ E, at an altitude of 1320 m.a.s.l.). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The inter- and intra-planting spaces were 60 and 30 cm, respectively. Each ecotype entailed three blocks containing 16 plants (3×16=48 plants). After transferring transplants into the field, they were irrigated using drip system and weed control was carried out in spring and summer seasons. The average day and night temperatures during growing season were 26.14 °C and 19.92 °C, respectively and precipitation average was 5.47 mm. Physico-chemical characteristics of the soil of field used in this study were: texture: Clay Loam, pH: 8.7, electrical conductivity (EC): 1.3 dS/ m, organic matter: 0.64%, nitrogen (N): 0.07%, Phosphorus (P): 1.03 mg/kg and Potassium (K): 350 mg/kg.

Assessment of growth and yield traits

As thyme is a perennial herbal plant, its growth and yield performance is not considerable in first year of cultivation. Therefore, they were solely maintained during first year and then in the second year, their growth and yield traits were evaluated at full flowering stage (in spring and summer of 2014). In our research, morphological traits consisted of plant height, number of lateral shoot per plant, internode length, internode number, main fluorescence's length, leaf length, leaf width, plant form (from 1, as recumbent to 5, as upright plants), shoot dry weight, leaf dry weight, ratio of leaf/shoot, and percentage of male sterile plants. After measuring growth traits, the plants were harvested at full flowering stage and then dried in shade at room temperature in order to perform phytochemical analysis of essential oil including its content, yield, and constituents.

Essential oil extraction and analysis

After drying, at room temperature, essential oil was extracted using hydrodistillation method for 3 h with a Clevenger type apparatus based on British Pharmacopoeia (1988) and then, was kept in a vial at 4 °C until to be analysed. Analysis of essential oil was carried out using Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography/Flame Ionization Detector (GC/FID) in Phytochemistry Department of Julius Kühn Institut (JKI), Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany. Afterwards, essential oil of different ecotypes was injected to GC/MS (Agilent, MSD 5972/HP) and GC/FID, in which, theGC/MS was equipped with a column of MS-5 HP (30 m × 0.25 mm i.d. and film thickness 0.5 µm). The column temperature was set to start with 60-120 °C at 3 °C/min and then it was held with 220 °C at 8 °C/min and it held for 10 min at this temperature. The temperature of injector was 250 °C. Helium was used as a carrier gas (1.0 mL min^{-1}). Mass Spectrometry operates in electronic ionization mode at 70eV and calculation was carried out using Chemstation software. The detection of Spectrometry was carried out using retention index and resulted data were compared with the indexes found in reference books (Adams, 2017) and information found in computer library. The system of GC/FID (Agilent, N HP6890) was equipped with a column MS-5 HP (30 m × 0.25 mm i.d. and film thickness 0.5 µm). The program of column temperature was set from 60-220 °C at 8 °C/min and then held for 10 min at this temperature. Also, the temperature of injector was 250 °C and helium was used as a carrier gas (1.0 mL min^{-1}).

Data analysis

The ANOVA was made based on RCBD and the means were compared by Duncan's Multiple Range Test (DMRT) at 5% probability level. The analyses were done using SPSS (Ver. 20) and the plotting of graphs was carried out using Excel 2013.

Tab. 1: Plant materials and seed collection origins of thyme samples.

Species	Ecotype name	Origin in Iran (province)	Species	Ecotype name	Origin in Iran (province)
	1. Khan-e-miran	Markazi		1. Tehran	Tehran
	2. Lorestan	Lorestan		2. Zanjan	Zanjan
	3. Isfahan 1	Isfahan	*T. kotschyanus*	3. Ghazvin	Ghazvin
	4. Ilam	Ilam		4. Az. Gharbi	West Azarbaijan
	5. Malayer 1	Hamedan		5. Kordestan	Kordestan
T. daenensis	6. Arak	Markazi		1. Lorestan	Lorestan
	7. Zagheh	Lorestan		2. Kordestan	Kordestan
	8. Malayer 2	Hamedan	*T. lancifolius*	3. Isfahan	Isfahan
	9. Fars	Fars		4. Fars	Fars
	10. Isfahan 2	Isfahan		5. Markazi	Markazi
	11. Markazi 1	Markazi		1. 'Deutscher Winter'	Germany
	12. Markazi 2	Markazi	*T. vulgaris*	2. Varico 3	Switzerland
				3. Isfahan	Isfahan

Results and discussion

Yield and morphological traits

The Analysis of variance (ANOVA) results of morphological traits in thyme species and ecotypes are shown in Tab. 2.

As shown in Tab. 3, the highest plant height was found in *T. vulgaris* (39.4 cm on average) that was significantly higher than those in other species (i.e. *T. daenensis*, *T. kotschyanus*, and *T. lancifolius*). In *T. vulgaris*, 'Varico 3' gained the highest plant height (43.6 cm) compared to 'Deutscher Winter' and Isfahan ecotype. Carlen et al. (2010) substantiated the superiority of 'Varico 3' over 'Deutscher Winter' in terms of plant height. In other studies, it has been confirmed that the height of *T. vulgaris* ecotypes were taller than *T. daenensis* ecotypes (Kaveh et al., 2013; Mondak et al., 2014; Askari et al., 2018; Mohammadi et al., 2019). In *T. daenensis*, the highest plant height (33 cm) was in Markazi 1 which did not show a significant difference with Markazi 2 and Malayer 1. Likewise, the highest plant height in *T. kotschyanus* and *T. lancifolius* was observed in Azerbaijan gharbi (18.6 cm) and Markazi (22.5 cm), respectively (Tab. 4). Our findings are in agreement with those obtained by Kaveh (2013) who concluded that Azerbaijan gharbi is the tallest plant among *T. kotschyanus* ecotypes. The height of plants is important because of its role in mechanizing and facilitating harvest activities. Also, the highest biomass yield was obtained by 'Varico 3' and Azerbaijan gharbi ecotype, possessing highest plant height. According to results of previous studies carried out on *T. pubescens* (Yavari et al., 2010) and different *Thymus* species native to Iran (Mondak et al., 2014), there is a positive relationship between plant height and plant dry weight, and this is in agreement with our findings.

In thyme, number of shoots per plant is attributed effectively in yield performance. Among *Thymus* species evaluated in this research, the highest shoot number (163.2) was observed in *T. vulgaris* that significantly higher than *T. daenensis* (8.5), *T. kotschyanus* (91.2), and *T. lancifolius* (85.6) (Tab. 3). Also, between ecotypes of the three last-mentioned *Thymus* species, no significant difference was found between them in terms of shoot number (Tab 3). The number of shoot in the mentioned species was as follows: *T. daenensis* (Ilam ecotype, 97), *T. kotschyanus* (Azerbaijan gharbi, 120), and *T. lancifolius* (Kurdistan, 95) (Tab. 4).

The highest internode length (2.4 cm) belonged to *T. daenensis* which was significantly higher than that in the other three species. Also, there was no significant difference in terms of internode length between *T. vulgaris*, *T. kotschyanus*, and *T. lancifolius* (Tab. 3). In *T. daenensis*, the highest (2.93 cm) and lowest (1.27 cm) internode length were observed in Markazi 1 and Ilam, respectively (Tab. 4). While in *T. vulgaris*, the highest internode length (2.3 cm) belonged to 'Varico 3' which significantly was higher than 'Deutscher Winter' and Isfahan (with no significant difference between the last two ecotypes); (Tab. 4). Also, the highest internode length in *T. kotschyanus* (2 cm) and *T. lancifolius* (1.83 cm) were belonged to Tehran and Isfahan, respectively (Tab. 4).

T. vulgaris showed the highest internode number (15.9) that significantly differs from that in the other three species, although the difference between these three thyme species was not significant (Tab. 3). The highest internode number (10.7) in *T. daenensis* was allocated to Ilam, being significantly higher than other ecotypes belonged to this species. Likewise, the highest internode number (16.5) was found in 'Deutscher Winter' that significantly longer than 'Varico 3' (14.7), but did not significant difference with Isfahan (Tab. 4). Azerbaijan gharbi (9.6) and Kurdistan (7.6) gained the highest internode number in *T. kotschyanus* and *T. lancifolius* ecotypes, respectively (Tab. 4).

Of all *Thymus* species investigated in this research, the highest florescence length (3.6 cm) was found in *T. daenensis* which higher than *T. kotschyanus* and *T. lancifolius*, whereas it did not have a significant difference with *T. vulgaris* (Tab. 3). In *T. daenensis*, the longest florescence (5.5 cm) was in Markazi 1 which did not have significant difference with Markazi 2 (Tab. 4).

Tab. 2: Analysis of variance (ANOVA) of morphological traits in thyme species and ecotypes.

Sources of variance (S.O.V)	df	ANOVA Mean of the squares (MS)										
Traits		Plant height	Lateral shoot number	Internode length	Internode number	Inflorescence length	Leaf length	Leaf width	Plant form	Shoot dry weight	Leaf to shoot ratio	Male sterile plants
Block	2	12.058*	102.01^{ns}	0.033^{ns}	0.333^{ns}	0.029^{ns}	0.030*	0.0002^{ns}	0.599*	648.6*	8.093^{ns}	46.45^{ns}
Species	3	1331.6**	583.8**	0.411**	179.5**	14.45**	4.61**	0.061**	7.28**	43912.6**	1153.3**	6990.4**
Ecotypes within species	21	36.3**	15535.5**	3.33**	3.74**	0.86**	0.065**	0.0067**	0.585**	1724.6**	52.82**	606.1**
Error	50	172.2	312.4	0.026	0.316	0.114	0.007	0.0009	0.088	42.49	2.87	22.67
Coefficient of variation (CV) (%)		7.6	18.4	8.1	6.5	11.7	6.3	6.7	8.4	8.2	2.9	13.3

* and ** indicate statistical significance at 95% and 99%; ns show statistical insignificance.

Tab. 3: Morphological traits in different *Thymus* species

Species	Plant height (cm)	Lateral shoot number	Internode length (cm)	Internode number	Inflorescence length (cm)	Leaf length (cm)	Leaf width (cm)	Plant form	Shoot dry weight (g)	Leaf to shoot ratio (%)	Male sterile plants (%)
T. daenensis	26.9 ^{b}	85.5 ^{b}	2.4 ^{a}	8.0 ^{b}	3.9 ^{a}	1.8 ^{a}	0.44 ^{a}	3.4 ^{b}	68.4 b	62.1 a	25.7 b
T. vulgaris	39.4 ^{a}	163.2 ^{a}	1.9 ^{b}	15.9 ^{a}	3.8 ^{a}	0.6 ^{c}	0.32 ^{b}	5.0 ^{a}	192.4 a	46.2 b	61.2 a
T. kotschyanus	15.1 ^{c}	91.2 ^{b}	1.6 ^{b}	7.6 ^{b}	2.2 ^{b}	1.0 ^{b}	0.49 ^{a}	3.3 ^{b}	65.9 b	49.0 b	27.3 b
T. lancifolius	18.9 ^{c}	85.6 ^{b}	1.6 ^{b}	7.1 ^{b}	2.4 ^{b}	1.3 ^{b}	0.46 ^{a}	3.3 ^{b}	53.9 b	63.0 a	59.2 a

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, $n = 3$.

Tab. 4: Morphological traits in different *Thymus* ecotypes

Species	Ecotype	Plant height (cm)	Lateral shoot number	Internode length (cm)	Internode number	Inflorescence length (cm)	Leaf length (cm)	Leaf width (cm)	Plant form	Shoot dry weight (g)	Leaf to shoot ratio (%)	Male sterile plants (%)
T. daenensis	Khane miran	28.4 bc	96 a	2.63 ab	8.5 b	3.5 de	1.90 a	0.45 bc	3.5 bcd	83.8 a	63.0 bc	27.7 cd
	Lorestan	26.0 cd	94 a	2.17 c	8.7 b	3.4 de	1.90 a	0.45 bc	2.9 d	62.2 de	61.0 c	0.0 h
	Esfahan1	26.3 cd	95 a	2.27 bc	8.3 b	4.0 bcd	1.87 a	0.36 d	3.1 d	68.3 cd	60.7 c	31.0 bc
	Ilam	23.5 d	97 a	1.27 d	10.7 a	4.4 bc	1.83 a	0.46 bc	4.6 a	48.5 f	66.3 ab	7.7 gh
	Malayer 1	29.4 abc	86 ab	2.63 ab	7.8 bc	3.6 cde	1.83 a	0.42 bcd	4.0 ab	77.1 abc	60.3 c	21.7 def
	Arak	26.8 cd	67 bc	2.67 ab	7.0 cd	3.8 cd	1.80 ab	0.45 bc	3.1 d	59.1 e	62.3 c	38.7 b
	Zagheh	18.2 e	55 c	2.00 c	6.2 d	2.8 e	1.80 ab	0.53 a	3.3 bcd	47.1 f	69.0 a	28.0 cd
	Malayer 2	26.9 cd	69 bc	2.63 ab	6.1 d	3.7 cde	1.77 ab	0.48 ab	3.9 bc	84.6 a	60.0 c	55.3 a
	Fars	28.6 bc	94 a	2.70 a	7.9 bc	3.9 bcd	1.73 ab	0.39 cd	3.2 d	62.6 de	62.7 bc	26.0 cde
	Esfahan 2	23.7 d	84 ab	2.17 c	7.6 bc	3.4 de	1.60 bc	0.43 bcd	2.9 d	70.4 bcd	60.7 c	38.0 gh
	Markazi 1	33.0 a	94 a	2.93 a	8.5 b	5.5 a	1.60 bc	0.42 bcd	3.1 d	78.7 ab	60.7 c	16.0 fg
	Markazi 2	32.2 ab	96 a	2.80 a	8.4 b	4.8 ab	1.43 c	0.46 abc	3.3 cd	79.3 ab	59.3 c	18.3 ef
T. vulgaris	'Deutscher Winter'	37.2 b	155 b	1.8 b	16.5 a	3.73 a	0.57 a	0.31 a	5.0 a	183.1 b	52.3 a	58.7 b
	Esfahan	37.3 b	156 b	1.7 b	16.3 a	3.67 a	0.53 a	0.31 a	5.0 a	182.6 b	52.0 a	57.3 b
	'Varico 3'	43.6 a	179 a	2.3 a	14.7 b	3.87 a	0.57 a	0.33 a	5.0 a	212.6 a	34.0 b	67.7 a
T. kotschyanus	Tehran	14.4 b	88 b	2.0 a	6.4 c	2.4 a	1.1 a	0.49 b	3.2 bc	49.3 bc	48.3 a	18.0 b
	Zanjan	15.4 b	82 b	1.7 ab	8.1 ab	2.3 a	1.0 ab	0.49 b	3.5 ab	33.2 d	48.3 a	28.7 ab
	Ghazvin	13.6 b	89 b	1.7 ab	6.6 bc	2.2 a	1.0 a	0.42 b	2.9 c	54.5 b	50.0 a	41.7 a
	Az. Gharbi	18.6 a	120 a	1.4 b	9.6 a	2.1 a	0.8 c	0.60 a	3.9 a	152.1 a	48.0 a	28.7 ab
	Kordestan	13.7 b	77 b	1.3 b	7.2 bc	2.1 a	0.9 bc	0.46 b	2.9 c	40.4 cd	50.3 a	19.7 b
T. lancifolius	Lorestan	14.6 c	89 a	1.5 a	6.8 a	2.2 a	1.0 c	0.48 a	2.8 b	49.1 bc	56.7 d	33.3 c
	Kordestan	17.6 bc	95 a	1.5 a	7.6 a	2.5 a	1.1 c	0.47 ab	3.1 ab	46.2 c	63.7 bc	48.3 bc
	Esfahan	20.4 ab	89 a	1.8 a	6.7 a	2.7 a	1.3 b	0.39 b	3.4 ab	54.7 abc	67.3 a	46.0 bc
	Fars	19.2 ab	86 a	1.6 a	7.4 a	2.7 a	1.4 b	0.51 a	3.7 a	61.3 a	65.0 b	52.7 c
	Markazi	22.5 a	69 a	1.8 a	6.8 a	2.1 a	1.6 a	0.43 ab	3.5 a	58.1 ab	62.3 c	84.3 a

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, $n = 3$.

According to Tab. 3, the highest and lowest leaf length were found in *T. daenensis* (1.8 cm) and *T. vulgaris* (0.6 cm), respectively. In *T. daenensis*, the highest leaf length was seen in Khane-e-miran and Lorestan (1.9 cm) that, except for Isfahan 2, Markazi 1 and 2, did not have a significant difference with other ecotypes belonging to this species. In terms of leaf length, the ecotypes of *T. vulgaris* (with leaf length of 0.6 cm on average), did not show a significant difference with each other. Also, the highest leaf length in *T. kotschyanus* and *T. lancifolius* was allocated to Tehran and Markazi, respectively (Tab. 4). Among the four investigated *Thymus* species, the highest leaf width (0.49 cm) was seen in *T. kotschyanus* which did not have a significant difference with *T. daenensis* and *T. kotschyanus* species. The lowest leaf width was observed in *T. vulgaris* (0.32 cm) (Tab. 3). The ecotypes of Zagheh (0.53 cm) (*T. daenensis*), Azerbaijan gharbi (0.60 cm) (*T. kotschyanus*), and Fars (0.51 cm) (*T. lancifolius*) were superior in leaf width among ecotypes of the three mentioned species. Moreover, the leaf width in ecotypes of *T. vulgaris* was insignificant (Tab. 4).

In terms of mechanizing harvest, plants' form plays a significant role in breeding program of *Thymus* spp. (Carlen et al., 2010). As stated before, the plants' form is a qualitative trait and ratified by digits in this research as follows: 5 signifies completely upright plant, 3: semi-upright plants and 1: completely recumbent plant. In this regard, *T. vulgaris* significantly ranked 5 and other *thymus* species occupied other positions, although difference between plants' form of other species (i.e. *T. daenensis*. *T. kotschyanus* and *T. lancifolius)* was not statistically significant and they were qualified with semi-upright plants (Tab. 3). In this regard, Ilam (4.8 on average) remarkably demonstrated more upright form in comparison to other ecotypes of *T. daenensis*. In *T. kotschyanus* and *T. lancifolius*, the most upright plants belonged to Azerbaijan gharbi and Fars, respectively (Tab. 4).

The results of our research revealed that shoot dry weight of *T. vulgaris* (192.4 g) was significantly higher than other *Thymus* species. Also, there was no significant difference between shoot dry weight of *T. daenensis* (68.5 g), *T. lancifolius* (65.9 g), and *T. kotschyanus* (53.9 g) (Tab. 3). Our findings are in agreement with those found by Kaveh et al., 2013; Mondak et al., 2014; Askari et al., 2018; Mohammadi et al., 2019 who stated that shoot dry weight of *T. vulgaris* is higher than in the other three mentioned *Thymus* species. The highest shoot dry weight in *T. vulgaris* belonged to 'Varico 3' (213 g) that was significantly higher than in 'Deutscher Winter' and Isfahan (Tab. 4). It is necessary to mention that 'Varico 3' is a hybrid produced from *T. vulgaris* in the Agroscope Research Institute, Switzerland. The results of Carlen et al. (2010) also proved the superiority of 'Varico 3' over 'Deutscher Winter' in terms of shoot dry weight. According to Tab. 4, the highest shoot dry weight was in Malayer 2 (85 g) (*T. daenensis*), Azerbaijan gharbi (152 g) (*T. lancifolius)* and Fars (61 g) (*T. kotschyanus*).

The highest leaf/shoot ratio (63%) was found in *T. lancifolius* which did not have a significant difference with *T. daenensis* (62.2%), and the lowest (42.2%) was obtained by *T. vulgaris* (Tab. 3). In *T. daenensis*, the highest ratio of leaf/shoot was observed in Zagheh (69%) which did not show a significant difference with Ilam (66%), although it had a significant difference with other ecotypes of *T. dae-*

nensis (Tab. 4). In *T. vulgaris*, 'Varico 3' significantly gained the lowest ratio of leaf/shoot (34%) which was lower than 'Deutscher Winter' and Isfahan (Tab. 4). Also, there was no significant difference between ecotypes of *T. kotschyanus* in terms of ratio of leaf/shoot (Tab. 4). In *T. lancifolius*, the highest ratio of leaf/shoot (67%) was shown by Isfahan that significantly was higher than that in other ecotypes belonging to this species (Tab. 4). The ratio of leaf/shoot (called as a harvest index) is of important traits suitable for breeding of *Thymus* species. Regarding higher essential oil content in leaf and flower compared to shoot and also high density of trichrome in leaf and flower secreting essential oil (HADIAN et al. 2016), the yield of essential oil in *Thymus* species with high ratio of leaf/shoot is higher than the other species with low ratio of leaf/shoot. In our research, the ratio of leaf/shoot in *T. vulgaris* was lower than the other species investigated here. Although 'Varico 3' showed highest leaf dry weight (34%), its leaf/shoot ratio was lower than 'Deutscher Winter' (52%). Because of depressing leaf/shoot ratio of 'Varico 3' compared to 'Deutscher Winter', high harvest index (or ratio of leaf/shoot) is one of the most important advantages in *Thymus* species native to Iran such as *T. daenensis*. Ratio of leaf/shoot is directly related to leaf number and essential oil yield; accordingly, it plays a crucial role in cultivation and breeding of *Thymus* species. Compared to *T. vulgaris*, the three-mentioned species also possess higher shoot dry weight and ratio of leaf/shoot, thereby having higher leaf number and essential oil yield.

Percent of male sterile plant

The percent of male sterile plant among different ecotypes of *T. daenensis* is one of most important traits useful in thyme breeding via hybridization. Among the four investigated *Thymus* species, the highest percentage of male sterile plant (61.2%) was seen in *T. vulgaris* that did not show a significant difference with *T. lancifolius* (59.2%) (Tab. 3). The highest percentage of male sterile plant in *T. vulgaris* was found in 'Varico 3' (68%) that showed a significant difference with 'Deutscher Winter' and Isfahan (Tab. 4). In *T. daenensis*, the highest percentage of male sterile plant was obtained by Malayer 2 (55%) that was significantly higher than that in other ecotypes of this species. Also, in Lorestan ecotype, even one male sterile plant was not found (Tab. 4). The highest percentage of male sterile plant (42%) was found in Qazvin ecotype belonged to *T. kotschyanus* which did not show a significant difference with Azerbaijan gharbi and Zanjan ecotypes of this species (Tab. 4). Among ecotypes belonging to *T. lancifolius*, the highest percentage of male sterile plant (84%) was obtained by Markazi which significantly differed to other *T. lancifolius* ecotypes (Tab. 4). As described earlier, male sterility plays an important role in thyme breeding. In general, emasculation of thyme flowers is difficult, due to flower smallness and high density of flower on a fluorescence as well as non-simultaneity in time of opening flowers (REY, 1993; MEWES and PANK, 2006). Accordingly, identifying male sterile plant in thyme populations is important for hybridization activities. The variety 'Varico 3' is produced from *T. vulgaris* via hybridization using male sterile plant (CARLEN et al., 2010). Also, the percentage of male sterile plant in 'Varico 3' was higher than that in 'Deutscher Winter', and this may be due to exploiting male sterile plant in hybridization as maternal parents (CARLEN et al., 2010). Moreover, the type of male sterility in thyme is cytoplasmic; accordingly, male sterility modulation is a complex process and controlled by at least 5 genes (BELHASSEN et al., 1991).

Cluster analysis of *Thymus* ecotypes

In our research, growth and yield traits were subjected to cluster analysis using WARD method. The results showed that all ecotypes were categorized into four groups (Fig. 1). The first group consisted of 11 ecotypes classified into two subclusters. The first sub-cluster consisted of 9 ecotypes of *T. daenensis* (Khan-e-miran, Malayer 1 and 2, Markazi 1 and 2, Lorstan, Isfahan 1, Arak and Isfahan 2). In the second sub-cluster, only Ilam belonging to *T. daenensis* was placed (Fig. 1). In is noteworthy that 11 ecotypes of *T. daenensis* in this group were qualified with high phytochemical attributes (such as thymol) and ordinary growth and yield. In the second group, 10 ecotypes were found in forms of two sub-clusters; at first sub-cluster, 5 ecotypes of *T. lancifolius* including Lorstan, Kurdistan, Isfahan, and Fars and 1 ecotype of *T. daenensis* including Zagheh were observed, and in second sub-cluster, just 4 ecotypes of *T. kotschynanus* including Tehran, Zanjan, Kurdistan, and Qazvin were placed (Fig. 1). This group was qualified with high phytochemical (especially essential oil content and thymol) and low growth and yield. Zagheh, belonging to *T. daenensis*, solely occupied this place, because of its unique characteristics such as low essential oil percentage and low chemical compounds such as thymol, growth, and yield,

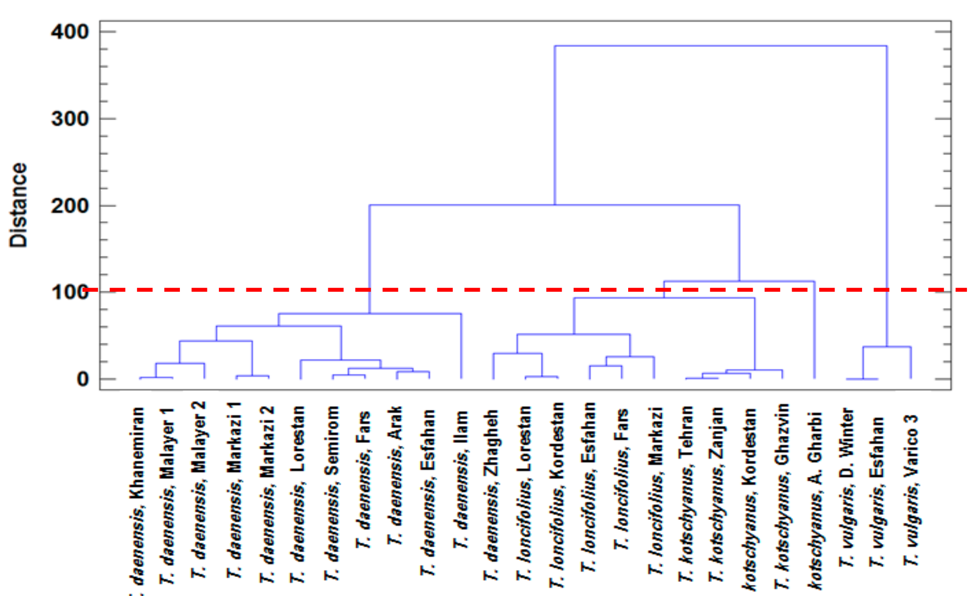

Fig. 1: Clustering different ecotypes and species of *Thymus* based on morphological traits using WARD method

in comparison to the other *T. daenensis*'s ecotypes. In the third group, only Azerbaijan gharbi, belonging *T. kotschyanus* and qualified with relatively high growth and yield, but low phytochemicals (especially essential oil and thymol) was placed. In the fourth group, 3 ecotypes of *T. vulgaris* were placed in forms of two sub-clusters: Isfahan and 'Deutscher Winter' in the first sub-cluster and 'Varico 3' in the second sub-cluster (Fig. 1). This group is characterized by high growth and yield and a moderate rate of essential oil and thymol. In general, it can be concluded that the cluster analysis categorized different ecotypes of *Thymus* species into four groups, in such a way that 11 groups belonged to the first group, 10 ecotypes to the second, one ecotype to the third, and finally three ecotypes to the fourth group (Fig. 1). In a research carried out by Mondak et al. (2014), 22 ecotypes of *Thymus* native to Iran were placed into two main groups in terms of morphologic and essential oil traits. The first group included *T. daenensis* ecotypes and the second group belonged to *T. lancifolius* and *T. kotschynanus* ecotypes.

Phytochemical traits

Essential oil content

Among four *Thymus* species investigated in this research, *T. daenensis* gained the highest essential oil content (5.76%) and its content in other three species was as follows: *T. vulgaris* (3.59%), *T. kotschyanus* (2.43%), and *T. lancifolius* (3.96%) (Fig. 2). In *T. daenensis*, the highest essential oil content was observed in Ilam being significantly higher when compared to other ecotypes of this species (Fig. 2). In *T. vulgaris*, 'Varico 3' showed the highest essential oil content (4.42%) which was significantly higher than in 'Deutscher Winter' (3.21%) and Isfahan (3.16%) (Fig. 2). Azerbaijan gharbi, belonging to *T. kotschyanus*, gained the highest essential oil content (2.79%), although it did not have a significant difference with that in Zanjan and Qazvin belonging to this species (Fig. 2). Among ecotypes belonging to *T. lancifolius*, Fars significantly obtained the highest essential oil (5.38%) in comparison to the other ecotypes belonging to this species (Fig. 2). As presented in Fig. 2, there was a relatively high diversity among ecotypes and species of *Thymus* in this regard. The highest essential oil content (7.83%) was found in *T. daenensis* (Ilam). Also, essential oil in *T. daenensis* ecotypes varied from 2.5 to 5%. Moreover, Khorshidi et al. (2018) reported a remarkable diversity among *T. daenensis* ecotypes (2.5-5%). Based on Rustaiee et al. (2010), the content of essential oil among *T. daenensis* ecotypes, naturally growing in Iran, was 1.8 to 3.83%, and the highest essential oil and thymol content were found in Arak, while the lowest was observed in Daenensis ecotypes. In another study, the content of essential oil in *T. daenensis* ecotypes, gathered from Hamadan regions (Nicaver et al., 2005), was about 2.4%, and its content in ecotypes gathered from 5 regions of Isfahan province, Iran, was about 3.3%. The previous studies showed a higher essential oil content in *T. daenensis* ecotypes, as compared to *T. vulgaris* (Mondak et al., 2014; Askari et al., 2018; Mohammadi et al., 2019). According to our findings, the essential oil content in 'Varico 3' was significantly higher than in 'Deutscher Winter' (Fig. 2), and this is in agreement with that obtained by Carlen et al. (2010), who recorded a superiority of 'Varico 3' over 'Deutscher Winter' in terms of essential oil content. In our research, the lowest essential oil content was observed in *T. kotschyanus* (Fig. 2). Also, Kaveh et al. (2013) reported various levels of essential oil in *T. kotschyanus*, from 0.42 to 2.17%. In other research, the amount of essential oil in this species was 1.1 to 2.5% (Khoshsokhan et al., 2014). Also, in this research, essential oil content in *T. lancifolius*, depending on the individual ecotype, varied from 2.93 to 5.38%. Furthermore, the essential oil content in *T. lancifolius* under greenhouse condition was reported to have 0.93 to 2.32% (Mondak et al., 2014).

Essential oil yield

As shown in Fig. 3, the highest essential oil yield was found in *T. vulgaris* (3.08 mL per plant), although it did not have a significant difference with that in *T. daenensis* (2.47 mL per plant) and conversely had a significant difference with *T. kotschyanus* (0.8 mL per plant) and *T. lancifolius* (1.38 mL per plant) (Fig. 3). The highest yield of essential oil (3.19 mL per plant) was obtained by 'Varico 3', although it did not show a significant difference with 'Deutscher Winter' (3.07 mL per plant) (Fig. 3). Among ecotypes of *T. kotschyanus*, Azerbaijan gharbi gained the highest yield of essential oil (2.04 mL per plant) that was significantly higher than other ecotypes belonging to this species (Fig. 3). In *T. lancifolius*, the highest yield of essential oil was in Fars (2.14 mL per plant) that was significantly higher than other ecotypes of *T. lancifolius* species (Fig. 3). In *Thymus* species, essential oil yield is of the most important traits gained through multiplying essential oil and leaf dry weight. As shown in Fig. 3, the highest yield of essential oil was obtained by *T. vulgaris* which did not show a significant difference with *T. daenensis*. Although dry weight of *T. vulgaris* was higher than *T. daenensis* and, conversely, ratio of leaf/shoot in *T. daenensis* was higher than *T. vulgaris*, but the essential oil yield in *T. vulgaris* and *T. daenensis* was the same. Also, in our research, the yield of essential oil in 'Varico 3' and 'Deutscher Winter' did not show a significant difference (Fig. 3). Although the rate of essential oil and shoot dry weight in 'Varico 3' was higher than

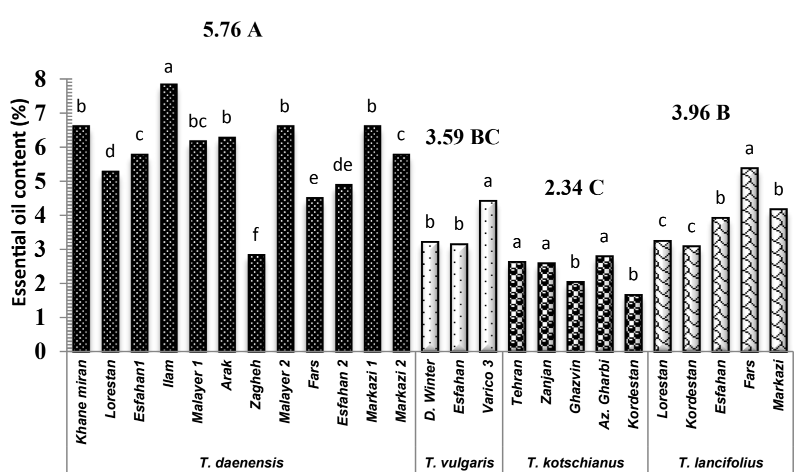

Fig. 2: Mean essential oil content in different *Thymus* ecotypes and species

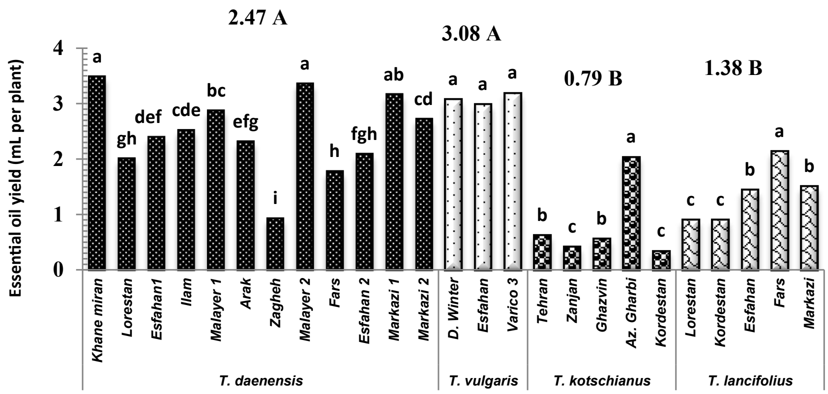

Fig. 3: Mean essential oil yield in different *Thymus* ecotypes and species

'Deutscher Winter', the ratio of leaf/shoot in 'Deutscher Winter' (52%) remarkably was higher than 'Varico 3' (34%); So, leaf dry weight in 'Deutscher Winter' was higher than 'Varico 3', and this leads to insignificant difference in both cultivars in terms of essential oil yield (Fig. 3).

Major essential oil constitutes of *T. daenensis*'s ecotypes

According to Tab. 5, the main components found in different ecotypes of *T. daenensis* include thymol (47.1-82%), carvacrol (0.77-24.39%), p-cymene (2.76-5.37%) and γ-terpinen (1.65-4.07%). Thymol was the major constituent widely found in all ecotypes of *T. daenensis*; all ecotypes, except for ecotype Zagheh (47.08%), contained more than 75% thymol. In contrast, Zagheh showed highest rate of carvacrol (24.39%) in comparison to the other *T. daenensis* ecotypes which contained lower than 5% of carvacrol. In different studies, thymol has been found as a main constituent of *T. daenensis* ecotypes (NICKAVAR et al., 2005; MONDAK et al., 2014; KHORSHIDI et al., 2018, HAYDARI et al., 2019). Also, the rate of carvacrol in Zagheh was 24.4%, while carvacrol in other ecotypes of *T. daenensis* was lower than 5% (Tab. 5). In a study carried out by KHORSHIDI et al. (2018), Zagheh was shown to have higher carvacrol rather than the other ecotypes of *T. daenensis*. Also, among 12 ecotypes of *T. daenensis*, 11 ecotypes were introduced as thymol chemotypes and one of them (i.e. Zagheh) as carvacrol-thymol chemotype.

Major essential oil constitutes of *T. vulgaris*

In *T. vulgaris*, the main components of essential oil included thymol (38.33-42.23%), p-cymene (22.06-24.48%), γ-terpinene (9.97-14.37%), and carvacrol (2.83-2.94%) (Tab. 6). In this respect, 'Varico 3' gained the highest thymol (42.23%). In terms of carvacrol, there was not found a remarkable diversity between ecotypes of *T. vulgaris*, and its rate in this species was reported 2.9% (Tab. 6). CARLEN et al. (2010) recorded high essential oil content in 'Varico 3' compared to 'Deutscher Winter'. In this regard, MONDAK et al. (2014)

Tab. 5: Major constituents in essential oil of different ecotypes of *T. daenensis*

Ecotype	p-Cymene (%)	γ-Terpinene (%)	Thymol (%)	Carvacrol (%)	Chemotype
Khane miran	2.83 b	3.61 a	78.93 a	1.38 b	Thymol
Lorestan	5.37 a	4.07 a	75.98 a	3.52 b	Thymol
Isfahan 1	3.32 b	2.94 a	79.48 a	3.38 b	Thymol
Ilam	3.9 b	3.69 a	78.47 a	3.83 b	Thymol
Malayer 1	3.11 b	3.13 a	80.25 a	2.37 b	Thymol
Arak	4.73 ab	1.065 b	78.15 a	2.67 b	Thymol
Zagheh	5.08 a	3.32 a	47.08 b	24.39 a	Thymol/Carvacrol
Malayer 2	2.76 b	2.33 a	82.01 a	0.77 b	Thymol
Fars	3.57 b	2.36 a	76.26 a	4.01 b	Thymol
Isfahan 2	3.94 b	2.12 ab	77.27 a	3.15 b	Thymol
Markazi 1	3.08 b	2.58 a	78.19 a	3.17 b	Thymol
Markazi 2	3.05 b	2.32 a	76.69 a	2.63 b	Thymol

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, $n = 3$.

Tab. 6: Major constituents in essential oil of *T. vulgaris*

Cultivar	p-Cymene (%)	γ-Terpinene (%)	Thymol (%)	Carvacrol (%)	β-caryophyllene (%)	Chemotype
'Deutscher Winter'	24.68 a	14.37 a	38.33 b	2.94 a	0.85 a	Thymol
Isfahan	24.16 a	14.3 a	39.1 b	2.9 a	0.79 a	Thymol
'Varico 3'	22.06 b	9.97 b	47.23 a	2.83 a	1.0 a	Thymol

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, $n = 3$.

also reported that thymol was the major constituent of essential oil in *T. vulgaris*. Also, it was introduced thymol (51.2%) as a major constitute of monoterpene index in essential oil of *T. vulgaris*; however, with low rate of carvacrol (4%) in this species. In general, thymol was introduced as a major constituent in essential oil of *T. vulgaris* (CARLEN et al., 2010).

Major essential oil constitutes of *T. kotschyanus*'s ecotypes

In *T. kotschyanus*, the main components of its essential oil were different, due to not only type of ecotype but also type and content of main components of essential oil (Tab. 7). The main essential oil components of *T. kotschyanus* were as follows: citral (0.48-42.09%), thymol (7.32-34.07), carvacrol (2.34-19.37%), geranial astat (0.95-24.81%), p-cymene (0.43-18.98%) and gamma terpinene (0.24-11.23%) (Tab. 7). According to type of ecotype, the main essential oil components of *T. kotschyanus*'s ecotypes were consisted of Tehran (citral, carvacrol, thymol, and geranial astat), Zanjan (thymol, carvacrol, geranyol, p-cymene), Qazvin (carvacrol, thymol, p-cymene, and beta-Terpineol), Azerbaijan gharbi (p-cymene, carvacrol, gamma terpinen and thymol), and Kurdistan (geranial astat, citral, thymol, and linalool) (Tab. 7). However, for NICKAVAR et al. (2006), the main components of *T. kotschyanus* were thymol (38.6%) and carvacrol (33.9%). In another study, the main components found in this species were as follows: polygon (18.7%), isomenton (17.8%), thymol (14.3%), Eucalyptol (9%), piperiton (6.3%) and carvacrol (5.5%) (SEMNANI et al., 2006). In this regard, it has also been reported that the major constituents found in essential oil of *T. kotschyanus* ecotypes were different and included thymol (31.2%), carvacrol (19.5%), p-cymene (11.2%), and γ-terpinene (8.4%) (KILIC and ÖZDEMIR, 2017). According to TOHIDI et al. (2018), major constituents of essential oil in different ecotypes of *T. kotschyanus* varied from 1 to 1.41% and main components in its essential oil were consisted of thymol (31.2%), carvacrol (19.5%), linalool (2.38-20.06%), α-terpineol (0.16-11.64%), and geraniol (0.36-11.37%). Also, among 43 chemicals found in essential oil of *T. kotschyanus*, SAJADI and KHATAMSAZ (2003) reported that thymol, carvacrol, and p-cymene were major constituents.

Major essential oil constituents of *T. lancifolius*'s ecotypes

As shown in Tab. 8, the main components of essential oil in *T. lancifolius* were largely different, depending on type of ecotype. The main components of essential oil in ecotypes of *T. lancifolius* were as follows: thymol (13.81-68.67), carvacrol (3.64-48.74), *geraniol* (0.57-25.57), α-terpineol (4.19-14.81), linalool (3.56-12.15), citral (7.88-10.08), geranil astat (1.06-7.33), α-terpinel astat (3.72-6.75), p-cymene (2.10-5.12), and borneol (1.47-4.08) (Tab. 8). Also, the main components of essential oil in all ecotypes of *T. lancifolius* were as follows: Lorstan (carvacrol, thymol and p-cymene), Kurdistan (alpha terpineol, thymol, linalool, carvacrol and citral), Isfahan (thymol, linalool, and alpha terpineol), Fars (thymol, geraniol, citral, and geranil astat), and Markazi (thymol and alpha terpineol astat) (Tab. 8). Furthermore, MONDAK et al. (2014) found thymol, carvacrol, and geraniol as major constituents of essential oil in *T. lancifolius*.

Elite genotypes

By evaluating different ecotypes of *Thymus* species in terms of growth, yield, and phytochemical traits, some genotypes were selected within ecotypes qualified with interesting traits. The preliminary selection of elite genotypes was carried out on the basis of some visual traits such as plant height, growth form, shoot dry weight, and ratio of leaf/shoot; subsequently the qualified genotypes were evaluated in terms of phytochemical traits such as content and yield of essential oil as well as major constituents of essential oil such as thymol, and eventually three genotypes (numbered as 1, 2, and 3), as elite genotypes, were identified. The results showed that all three selected genotypes belong to *T. daenensis* and their characteristics a presented in Tab. 9. These genotypes were qualified with some important traits such as plant height, plant form, number of lateral shoots, dry and fresh weight of shoot, ratio of leaf/shoot, the percent and yield of essential oil, and rate of thymol. The form of plant is important because of its role in mechanizing crop harvest. In our research, the elite genotypes (1, 2, and 3) were in upright form and suitable for harvesting mechanically (Tab. 9). Shoot dry weight in elite genotypes remarkably was higher than that in *T. daenensis* and even in *T. vulgaris*. The ratio of leaf/shoot in elite genotypes was similar to

Tab. 7: Major constituents in essential oil of different ecotypes of *T. kotschyanus*

Ecotype	p-Cymene (%)	γ-Terpinene (%)	beta-terpineol (%)	Linalool (%)	Citral (%)	Geraniol (%)	Thymol (%)	Carvacrol (%)	Geranyl acetate (%)	Chemotype
Tehran	3.37 c	0.81 c	0.17 b	3.5 b	42.09 a	0.46 bc	7.32 c	19.37 a	5.1 b	Citral
Zanjan	8.74 b	2.68 b	0.09 b	1.3 b	0.11 e	9.08 a	34.07 a	18.74 a	0.95 c	Thymol/Carvacrol
Ghazvin	7.00 b	4.44 b	6.79 a	0.25 c	5.49 c	0.12 c	18.62 b	19.35 a	0.16 d	Thymol/Carvacrol
Az. Gharbi	18.98 a	11.23 a	0.21 b	2.27 b	0.48 d	0.18 c	11.09 c	17.31 a	0.12 d	p-Cymene /Carvacrol
Kordestan	0.43 d	0.24 c	0.08 b	8.25 a	22.41 b	2.13 b	14.79 bc	2.34 b	24.81 a	Geranyl acetate/Citral

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, n = 3.

Tab. 8: Major constitutes in essential oil of different ecotype of *T. lancifolius*

Ecotype	Linalool (%)	Borneol (%)	α-terpineol (%)	Citral (%)	Geraniol (%)	Thymol (%)	Carvacrol (%)	α-terpineol acetate (%)	Geranyl acetate (%)	Chemotype
Lorestan	3.56 c	1.84 b	0.09 c	0.22 c	1.12 b	19.46 d	48.74 a	0.20 c	1.06 c	Carvacrol
Kordestan	12.15 a	4.08 a	14.81 a	7.88 b	0.57 b	13.81 e	8.4 b	0.16 c	2.38 b	α-terpineol
Isfahan	6.63 b	0.11 c	4.19 b	0.15 c	0.10 c	60.14 b	4 c	3.72 b	0.12 d	Thymol
Fars	0.21 d	1.47 b	0.13 c	10.08 a	25.57 a	28.69 c	4.5 c	0.11 c	7.33 a	Thymol/Geraniol
Markazi	0.14 d	0.18 c	0.11 c	0.18 c	0.18 c	68.67 a	3.64 c	6.75 a	0.14 d	Thymol

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, n = 3.

Tab. 9: Morphological and phytochemical traits of selected elite genotypes in comparison to different species.

Species	Internode length (cm)	Inter-node number	Inflores-cence length (cm)	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Shoot number	Plant growth form	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf to shoot ratio	Essential oil content (%)	Essential oil yield (ml per plant)	Thymol (%)
T. daenensis	2.4 a	8.0 b	3.9 a	1.8 a	0.44 a	26.9 b	85.5 b	3.4 b	134.3 b	68.5 b	62.1 a	5.76 a	2.47 a	75.73 a
T. vulgaris	1.9 b	15.9 a	3.8 a	0.6 c	0.32 b	39.4 a	163.2 a	5.0 a	405.7 a	192.4 a	46.2 b	3.59 bc	3.18 a	41.55 b
T. kotschyanus	1.6 b	7.6 b	2.2 b	1.0 b	0.49 a	15.1 c	91.2 b	3.3 b	153.4 b	65.9 b	49 b	2.34 c	0.80 b	17.18 c
T. lancifolius	1.6 b	7.1 b	2.4 b	1.3 b	0.46 a	18.9 c	85.6 b	3.3 b	150.8 b	53.9 b	63 a	3.96 b	1.38 b	38.15 b
Genotype 1	1.5	11	10	2	0.5	38	250	5	576.5	226.5	63	8.33	11.89	73.61
Genotype 2	2.2	8	5	2.1	0.5	32	190	4	536.5	219.5	60	7.8	10.27	73.91
Genotype 3	1.7	11	6	2	0.5	32	300	4	765.5	322.5	60	9.07	17.55	75.19

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, n = 3.

that in *T. daenensis*, but larger than *T. vulgaris*. Essential oil content in elite genotypes of 1, 2, and 3 (8.22%, 7.80%, and 9.07%, respectively) was remarkably higher than those in *T. vulgaris* (3.56%) and *T. daenensis* (5.76%). The essential oil yield, as an important traits in *Thymus* species, in elite genotypes of 1, 2, and 3 (11.89, 10.27, and 17.55 mL per plant, respectively), was remarkably higher than essential oil yield in *T. daenensis* (2.47 mL per plant) and *T. vulgaris* (3.18 mL per plant). In terms of thymol, elite genotypes contained 73.61, 73.91 and 75.19%, being similar to those in *T. daenensis* (75.73%), and conversely larger than that in *T. vulgaris* (41.55%) (Tab. 9).

The results of our research revealed a remarkable diversity between and within *Thymus* species in terms of morphological and phytochemical traits. Among four *Thymus* species investigated in this research, the highest shoot dry weight was found in *T. vulgaris* that was significantly higher than that in the other three *Thymus* species. An average yield of dry matter in the mentioned three species, compared to *T. vulgaris*, was relatively low. Depending on the individual ecotype, the rate of dry matter among *Thymus* ecotypes was different and Khan-e-miran and Malayer 2 belonging to *T. daenensis* and Azerbaijan gharbi belonging to *T. kotschyanus* and Fars of *T. lancifolius* had higher dry matter in comparison to the other ecotypes. Moreover, among the four *Thymus* species, the highest essential oil content was found in *T. daenensis* which was remarkably higher than in the others. In *T. daenensis,* Ilam (7.83%) gained highest essential oil content and its essential oil yield did not show a significant difference to *T. vulgaris*; also, the ecotypes of Khan-E-miran, Malayer 2 and Markazi 1 had higher yield in comparison to the other *Thymus* species, especially *T. vulgaris*.

According to the results, the essential oil content and major constituents of essential oil were clearly dependent on the individual species and ecotype. In this regard, our findings exhibited high diversity in the *Thymus* species and their ecotypes. In a nutshell, thymol was found to be a major component in the essential oils of all *T. daenensis* ecotypes, and all the ecotypes contained approximately 75% thymol, except for Zagheh ecotype. Also, thymol was a major constituent in essential oil of *T. vulgaris* (43% on average). In addition to thymol, other main components including p-cymene and γ-terpinene were found in *T. kotschyanus* and *T. lancifolius*. It is necessary to mention that the type and content of essential oil in the *Thymus* species was different, dependent on type of ecotype. In our research, five chemotypes (citral, carvacrol-thymol, thymol-carvacrol, p-cymene-carvacrol and geranial astat) and four chemotypes (thymol, alpha terpineol-linalool, carvacrol-thymol, thymol-geraniol) were identified in different ecotypes of *T. kotschyanus* and *T. lancifolius,* respectively. Therefore, such high diversity in type and content of thyme essential oil is suitable for employing in different industries.

Finally, the three elite genotypes were recognized based on their morphological and phytochemical traits. So, after propagating elite genotypes and examining their yield, they can be used as candidate parents for thyme breeding and cultivation.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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