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Effect of plant density on aerial dry matter and essential oil yield of two native Thyme species (Thymus kotschyanus and T. daenensis) under dryland farming in Lorestan province, Iran

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Abstract:
The thymus is one of the most important medicinal plants in Lorestan province, Iran. In order to study the effect of plant density on the aerial dry matter (DM) yield and oil production, an experiment was conducted using two native Thyme species (Thymus kotschyanus and T. daenensis) in a split plot design based on a completely randomized block with three replications over four years (from 2016 to 2019) in Zagheh, Lorestan province, Iran. Seeds were sown in pots in the greenhouse, then seedling transferred to the field in seven-leaf stage in April 2016. The distance between the rows were 5 cm and between plants within the row were 25, 35, and 50 cm. This distance was corresponded to the density of 4, 6 and 8 plants/m^2. Plant essential oils were extracted using Clevenger apparatus and their compounds were identified in GC-MS analysis. Data were analyzed using split plot in time and years were considered as subplots. Results of ANOVA showed significant differences between years, species and species by year interaction for many traits ($p < 0.05$). The effect of density was significant for canopy diameter and stem number ($p < 0.05$). Higher DM yield (2761.7 and 3332.5 Kg.h^{-1}) and essential oil yield (44.83 and 75.28 Kg.h^{-1}) were obtained in T. kotschyanus and T. daenensis, respectively, in the third year of study. So, essential oil production increased in T. kotschyanus over years, but in T. daenensis, it was increased up to third year, then decreased in the fourth year. In both Thyme species, the higher DM and essential oil yield were obtained in 6 and 8 plants/m² density without significant difference between these two densities. For the main compounds of essential oil, a moderate value of carvacrol (18%) and Thymol (51%) were obtained in T. kotschyanus and a lower value of carvacrol (5%) and higher value of Thymol (78%) were obtained in T. daenensis in the third year of study. Based on the obtained results, the cultivation of both species in density of 6 and 8 plants/m^2 was recommended in the rain fed area and rangeland of Lorestan province, Iran.

Keywords: Dryland farming; Essential oils; Native Thyme species; Stress

1. Introduction

Thyme is of the mint family (Labiate). There are 18 Thyme species that are grown in Iran, They are perennial, have branched stems, and covered with short, dense or broad-hairy hair. The leaves of flowering stems more or less equal to ovate, rounded-tips, with both surfaces covered. Inflorescences are often dense, and the petals are approximately equal in size to the white or pale pink calyx. Flowering time occurs from late spring to mid-summer in mountainous areas where altitude ranges from 650 to 3900 m above sea level [1]. Harvesting medicinal plants directly from their natural habitats has caused a lot of pressure on these plants and their regeneration is threatened by severe declines in their populations and results in the extinction of this valuable plant species [2]. Thymes are used for aroma

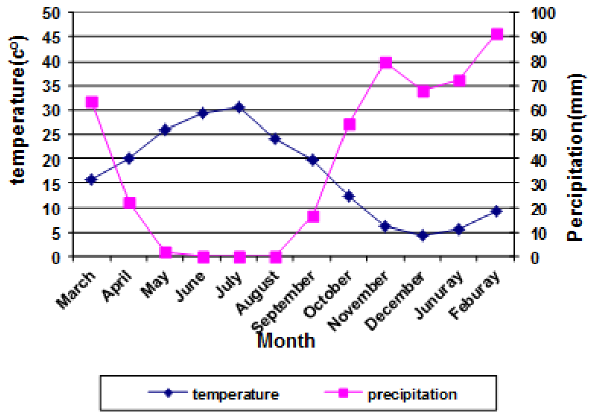

Figure 1. Ambrothermic curve of the Zagheh station.

and medicinal properties in the world and the commercial value of Thyme species is due to their essential oils, antioxidants and antifungal activity [3]. The main chemical compounds in Thyme essential oil are Thymol (2-isopropyl-5-methylphenol) and Carvacrol (5-isopropyl-2-methylphenol), the production of which occur through the hydroxylation of γ-terpinene and p-cymene precursors [4, 5]. Therefore, Thyme is valuable and widely used in medicinal and food industries due to having these two combinations of medicinal plants.

Pourmeidani et al. in a study on Thymus kotschyanus found that the amount of essential oil was not affected by the different years in their experiment [6]. Therefore, they suggested to use this species for cultivation in dryland farming and low-yielding pastures. Azimi et al. in a study on the effects of drought stress on the viability and vegetative growth characteristics of Ziziphora clinopodioides showed that the plant aerial dry weight decreased in response to drought stress [7]. Shaabani et al. using Dracocephalum kotschyi Boiss. showed that the concentration and total content of the phenolic compounds in plants grown under dryland farming are higher than the control plants [8]. In a study on the aerial dry yield of 11 ecotypes of T. daenensis, Aflakian et al. found significant differences in many traits such as flower diameter, leaf area, inflorescence weight, leaf length, inflorescence length, number of flowers in the inflorescence, leaf width, stem height, the largest and smallest canopy diameters, the weight of 1000 seeds, and dry matter yield [9]. They introduced Khorramabad ecotype as superior than other ecotypes in terms of aerial yield components. Omidbaigi et al. in a study on the effects of harvest time on the amount of essential oil in Abyssinian Thyme (Thymus Citriodorus) found that harvest time had a significant effect on the fresh and dry weight of Thyme [10]. The highest fresh and dry weight of the vegetative body was obtained at the stage of complete flowering. Mohammadian et al. in a study on the effect of altitude, as well as soil physical and chemical properties of the active ingredients of Thymus fallax Fischer found that some soil properties in the studied habitats correlated significantly with the existing essential oil compounds [11]. Hasani et al. in a survey of Thymus species adaptation in Kurdistan province, Iran found that Thymus pubescens had the highest essential oil yield with a value of 32.56 kg.h^{-1} [12]. They also found that 8 plants/m^2 was the best density for dryland farming system with an aerial dry matter yield of 2019 kg.h^{-1}. Taheri et al. evaluated the effect of plant density on yield and morphological traits of four Thyme species (Thymus Pubescens, T. kotschyanus, T. vulgaris, T. daenensis) Found the highest aerial DM yield (2206 kg.h^{-1}) and essential oil content (2.1%) in T. kotschyanus, with the planting distance of 25 cm [13].

Zare Zadeh et al. in evaluation of different Thyme species under agricultural conditions of Yazd climatic conditions, Iran found that 8 out of 69 accessions were adapted to the area and average establishment rates were ranged from 58%, to 75% over three years [14]. Kargar Hajiabadi et al. in a study to investigate the effects of bio fertilizers on seedling viability, quantity and quality of Thymus pubescens [15]. Boise. in a farm experiment in Karaj, Iran found that seed inoculation was better than seedling inoculation for plant establishment.

Lorestan province is located in the Iranian Turanian region where there are many endemic plant species. The average annual precipitation of the province is 520 mm, which corresponds with suitability for cultivation perennial crops such as Thyme species. The aim of this study was to find the optimum plant density of two native Thyme species (T. kotschyanus and T. daenensis) in dry land farming system in Lorestan province, Iran.

2. Materials and methods

2.1 Site information

This study was carried out over four cropping years (2016 – 2019) in the research farm of Zagheh station (33° 29′ 56″ N, 48° 42′ 31″ E), with 557 mm average annual rainfall, 45% relative humidity, and 18.4°C as the average annual temperature. According to the Ambeothermic curve, the highest temperature during the study period was in July; also, the rainfall was low from June to August (Fig. 1). There were 119 days of frost per year, and the altitude of the area was 1960 m above sea level in the east of Lorestan province, Iran. The soil samples of the field were taken and

Table 1. Physicochemical properties of soil of the project site.

Soil properties	Depth of soil 0 – 15 (cm)	Depth of soil 15 – 30 (cm)
Soil texture	Silty - clay	Silty - clay
Absorbable potassium (K) (mg. kg^{-1})	580	470
Absorbable phosphorus (P) (mg. kg^{-1})	7.6	11
total Nitrogen (N) (%)	109%	114%
Electrical conductivity (dS.m^{-1})	4.2	5.9
Organic carbon (%)	1.29	1.14
Saturation percentage (%)	56	53

Table 2. Precipitation of the area based on Meteorological statistics of the Khorramabad Agricultural Research Station, growing period (2015 to 2019).

Month	Years Precipitation (mm)			
	2015 – 2016	2016 – 2017	2017 – 2018	2018 – 2019
Oct.	0	0	9	12.4
Nov.	8.6	2.8	139.1	53.9
Dec.	66.2	36.6	150.8	111.9
Jan.	82.2	50.1	127	68.8
Feb.	101	68.7	115.2	36
March	44.3	62.72	96.9	188.8
April	80.8	103.7	309	39.6
May	32.8	151.7	6.1	12.1
June	0	12.1	0	0.1
Total	416.3	488.4	953	523.6

soil physical and chemical properties were determined in the laboratory (Table 1). Meteorological statistics of the Khorramabad Agricultural Research Station for a growing period (2017 to 2019) are shown in Table 2. The highest precipitation value of 953 mm was happening in the third year.

2.2 Research method

For collection of local seeds, the habitats of two native Thyme species (Thymus kotschyanus and T. daenensis) were examined in an appropriate time. Then, the viability of seeds was examined using tetrazolium. The results showed that seed germination was 95%. Then, moist stratification was applied for 10 days at a normal fridge temperature (4°C). The seeds were sown in trays in mid-March and kept in the greenhouse to grow up to the 2-leaf stage. Then, seedlings were moved to 10 cm pots until the seedlings grow to the 7-leaf stage. Finally, seedlings were transferred to the field in April 2016.

Land operation was made using the disc and plowing, immediately after the first rain and the seedlings were spaced as plants in the field at a depth of 15 cm in April 2016. The distance between rows was 50 cm and between plants within a row was 25, 35, and 50 cm. This distance was corresponded to the densities of 4, 6 and 8 plants/m^2. The experimental design plit plot based on a completely randomized blocks with three replications and the plot sizes (experimental unit) were about 3×2.5 m. No irrigation was made after transplanting and seedlings were grown using precipitation according to the dryland farming method.
During the growing period, the weeds were controlled manually in two stages (i.e. 50 and 30 days after seedlings planting in the field). No data were recorded in the first year (Establishment year). In the years 2 , 3 and 4, data were collected for plant height, canopy diameter and stem number. Thyme plants were harvested manually in 50% flowering stage. The harvested plants were dried in the shade and the dry weight of each plant was recorded. From

Table 3. Results of split plot in time analysis of variance of dry matter yield and related traits in two Thyme species in three planting densities over four years (2015 to 2019) in Zagheh rangeland, Khoramabad, Iran.
(ns, * and **, not significant and significant at 5 and 1% probability levels, respectively.)

Source	DF	MS					
		Plant height	Canopy diameter	Flowering stems	DM yield	Essential oils%	Oil yield
Replication	2	2.34	13.05	484.0	11275**	1.86**	7432.8**
Species	1	211.00**	119.1**	3376.0	581	1.73**	2488.5**
Density	2	7.64	19.82*	4223.0*	1260	0.10	136.6
Species *Density	2	2.30	3.94	352.0	206	0.12	148.7
Error1	10	**4.68**	**4.14**	**2534.7**	**2763**	**0.11**	**188.7**
Year	2	144.89**	8.38	18951**	15439**	1.11**	8523.2**
Species *Year	2	18.00**	98.95**	1896*	14143*	2.21**	2697.0**
Density *Year	4	1.03	1.58	436.0	3349	0.03	32.5
Species *Density *Year	4	2.38	0.42	133.0	1304	0.09	68.0
Error2	24	**1.23**	**4.39**	**677.0**	**4642**	**0.11**	**356.1**
Total	53						

Table 4. Mean of aerial DM yield and related traits between two Thyme species average over three planting densities in four years (2017 to 2019) in Zagheh rangeland, Khoramabad, Iran.
(Means followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.)

Species name	Plant height (cm)	Canopy diameter (cm)	Flowering stems No.	DM yield (Kg.h^{-1})	Essential oils%	Oil yield (Kg.h^{-1})
T. kotschyanus	18.29 b	33.75 b	130.2 a	2307.1 a	1.50 b	35.50 b
T. daenensis	22.25 a	36.72 a	114.4 a	2286.3 a	1.86 a	49.08 a

each plot, 100 g of aerial dried plant was grounded and the essential oil was extracted by water distillation (Clevenger). The essential oil was weighed and their phytochemical compound determined only in 8 plants/m^2 treatment in the third year of study, using gas chromatography/ mass spectrometry (GC/MS) in the laboratory of Research Institute Forests and Rangelands, Tehran, Iran.
Data were analyzed using split plot in time ANOVA so that the species and density were considered as the main plots and years as subplots. SAS9.4 software was used for ANOVA and Duncan's multiple range test at $p \leq 0.05$ was used to compare the means of variables.

3. Results

The result of a split plot in time ANOVA is presented in Table 3. There was a significant difference between two species (T. kotschyanus and T. daenensis) for all the traits except stem number and aerial DM yield. There was a significant difference between three planting densities for canopy diameter and flowering stem number ($p < 0.05$). There was a significant difference between years for all traits except canopy diameter ($p < 0.01$). The species by year interaction effect was significant for all traits ($p < 0.01$ and $p < 0.05$) (Table 3).

3.1 Mean of Thyme species

There was a significant difference between the two species for all the traits except flowering stem number and aerial DM yield (Table 4). Result of means comparison between two Thyme species showed that T. daenensis significantly had higher mean values of plant height, canopy diameter, essential oil% and essential oil yield with 22.25 cm, 36.72 cm, 1.86% and 49.08 Kg.h^{-1} than that for T. kotschyanus with values of 18.29, 33.75, 1.50% and 35.50 Kg.h^{-1}, respectively (Table 4).

3.2 Planting densities

There was a significant difference between three planting densities for canopy diameter and stems number ($p < 0.05$) (Table 3). Result of means comparison showed that 6 plants/m^2 density had a significantly higher stem number (134.67) and canopy diameter (36.41 cm) than other densities. For aerial DM yield, the higher values of 2324 and 2363 Kg.h^{-1} were obtained in 6 and 8 plants/m^2 density, respectively (Table 5).

3.3 Mean of years

There was a significant difference between years for all traits except canopy diameter ($p < 0.01$) (Table 3). The mean of plant height, aerial DM yield and essential oil yield increased up to third year and decreased in the fourth year. The higher mean values of plant height (22.7 cm), aerial DM yield (3047 Kg.h^{-1}) and essential oil yield (60.84 Kg.h^{-1}) were obtained in the third year that were significantly higher than other years (Table 6). The higher mean values of many traits in the third year was related to higher precipitation in 2018 (see Table 2).
The flowering stem number was increased by plant growing over four years and the higher stems number (220) was obtained in the fourth year that were significantly higher than other pervious years (Table 6).

3.4 Species by year interaction

The species by year interactions effect were significant for all traits (Table 3). The higher plant height with average values of 20.77 and 24.63 cm were obtained in T. kotschyanus and T. daenensis in the third that were significantly higher than other years (Table 7). The higher canopy diameter with average values of 36.95 cm were obtained In T. kotschyanus in the fourth year that were significantly higher than other years. In T. Daenensis, there were no significant differences

Table 5. Mean of aerial DM yield and related traits between three planting densities average over years and two Thyme species in Zagheh rangeland, Khoramabad, Iran.
(Means followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.)

Density	Plant height (cm)	Canopy diameter (cm)	Flowering stems No.	DM yield (Kg.h^{-1})	Essential oils%	Oil yield (Kg.h^{-1})
D1 (4 plants)	19.52 a	34.39 b	113.05 c	2202.79 a	1.75 a	41.11 a
D2 (6 plants)	20.62 a	36.41 a	134.67 a	2324.41 a	1.61 a	40.33 a
D3 (8 plants)	20.68 a	34.90 b	119.38 b	2363.13 a	1.71 a	45.44 a

Table 6. Mean of aerial DM yield and related traits between three planting years (2017 to 2019) average two Thyme species in Zagheh rangeland, Khoramabad, Iran.
(Means followed by the same letter are not significantly different according to Duncan's multiple range test at p $\leq$ 0.05.)

Year	Plant height (cm)	Canopy diameter (cm)	Flowering stems No.	DM yield (Kg.h^{-1})	Essential oils%	Oil yield (Kg.h^{-1})
Year 2	17.15 c	34.47 a	15.82 c	1261.7 c	1.40 b	18.34 c
Year 3	22.70 a	35.46 a	130.75 b	3047.1 a	1.82 a	60.84 a
Year 4	20.96 b	35.78 a	220.52 a	2581.4 b	1.83 a	47.71 b

between years in canopy diameter (Table 7).
In both species of T. kotschyanus and T. daenensis, the higher stem numbers (239.8 and 201.2), respectively, were obtained in the fourth year which were significantly higher than other years (Table 7). In both species, the higher values of plant height and canopy diameter were obtained in the third year. For aerial DM yield, the higher values of 2761.7 and 3332.5 Kg.h^{-1} were obtained in T. kotschyanus and T. daenensis in the third year, respectively.
However, in T. kotschyanus, there was no significant difference between the year 3 and 4. But in T. daenensis, higher DM yield was obtained in the third year that was significantly higher than other years (Table 8). For essential oil yield, the higher values of 44.83 and 75.28 Kg.h^{-1} were obtained in T. kotschyanus and T. daenensis in the third year, respectively. However, for T. daenensis , there was no significant difference between the essential oil yield of year 3 and 4. But for T. kotschyanus, the obtained values in the third year were significantly higher than the other years (Table 8).
For essential oil percentage, In T. kotschyanus, the higher values of 1.62 and 1.50% were obtained in the second and third years that was significantly higher than that for the fourth year; in contrast, in T. daenensis, the higher essential oil% with average values of 2.18 and 2.24% were obtained in the third and fourth years that was significantly higher than the previous year (Table 8). The higher mean values for most of the traits in the third year was related to higher precipitation in 2018 (see Table 2).

3.5 Thyme phenology

The phenological stages of the T. kotschyanus across the four-year period showed that the flowering stage begins in mid-May and continued up to 10th Jun, and the time of starting and end of the seed ripening and dropping occur from mid to the end of June. Finally, the dormancy stage started from mid-December and continues until mid-May next year.
Also, the study of the phenological stages of the T. daenensis showed that the flowering stage begins in mid-May and ended on 10th Jun, and the time of the start and end of seed ripening and dropping occur in mid-June until 10th Jul. The dormancy stage spans from 20th November to the May next year.

3.6 Phytochemical analysis

The Essential oil compounds of T. kotschyanus and T. daenensis were determined using GC/MS analysis in only 8 plants/m^2 treatment in the third year of study (Table 9). Results showed that in T. kotschyanus, the main compounds of essential oil were moderate values of carvacrol (18%) and Thymol (51%) and for T. daenensis, a lower value of carvacrol (5%) and higher value of Thymol (78%) were obtained in the third year of study (Table 9). Higher Carvacrol (18.5%), p-cymene (5.9%) and γ-terpinene (5.8%) were also detected in T. kotschyanus (Table 6) although the economic value and pharmaceutical applications of Thymol are more important than those of other compounds.

4. Discussion and conclusion

The higher mean value of aerial DM yield in both species was obtained by the planting density of 6 and 8 plants/m^2. Similar to our finding, Al-Ramamneh reported that planting in high densities led to the greatest amount of shoot biomass, which is consistent with the results of our study [16]. Similarly, Lebaschi et al. reported that the cultivation of Thymus vulgaris with higher density can cause higher dry matter yield and percentage of essential

Table 7. Mean of species by year interaction effects for plant height, canopy diameter and number of flowering stems averaged over 3 years and planting densities in Zagheh rangeland, Khoramabad, Iran.
(Means followed by the same letter are not significantly different according to Duncan's multiple range test at p $\leq$ 0.05.)

Year	Plant height (cm)		Canopy diameter (cm)		Flowering stems No.	
	T. kotsch	T. daenen	T.kotsch	T. daenen	T. kotsch	T. daenen
Year 2	16.15 c	18.16 b	31.25 b	37.68 a	20.66 c	11.0 c
Year 3	20.77 a	24.63 a	33.03 b	37.89 a	130.27 b	131.2 b
Year 4	17.95 b	23.96 a	36.95 a	34.59 a	239.87 a	201.2 a

Table 8. Mean of species by year interaction effects for aerial DM yield, essential oils percentage and essential oil yield averaged over 3 years and planting densities in Zagheh rangeland, Khoramabad, Iran.
(Means followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.)

Year	DM yield (Kg.h^{-1})		Essential oils (%)		Oil Yield (Kg.h^{-1})	
	T. kotsch	T. daenen	T.kotsch	T. daenen	T. kotsch	T. daenen
Year 2	1533.7 b	989.67 c	1.62 a	1.17 c	25.24 c	11.43 c
Year 3	2761.7 a	3332.5 a	1.50 ab	2.18 a	44.83 a	75.28 a
Year 4	2626.1 a	2536.9 b	1.43 b	2.24 a	37.10 b	58.30 b

oil under dry land farming conditions in Iran [17]. Higher DM yield (2761.7 and 3332.5 Kg.h^{-1}) was obtained in T. kotschyanus and T. daenensis, respectively, in the third year of study. So, essential oil production increased over the years (Table 7). It seems climate is effective in this parameter. Since, the higher value of DM yield was obtained in the third year that was related to higher precipitation in 2018.

For canopy diameter, there was a significant difference between years, density, as well as density by year interaction ($p < 0.1$) in both T. kotschyanus and T. daenensis species. In comparison, between the different planting densities, the lower canopy diameter of 34.39 cm was obtained in density 4 plants/m^2 that was significantly lower than that 36.41 cm, for 6 plants/m^2 planting densities. It is argued that under optimal growth conditions, by increasing the number of plants per unit area, the general growth of the plant increases and as a result, the number of lateral stems also increases. During the fourth year, the higher canopy diameter with a value of 36.95 cm was obtained in T. kotschyanus (Table 7). Our finding was consistent with previous studies done by Todorovic et al. that obtained the highest vegetative yield of T. kotschyanus in planting distances of 50 cm between rows and 30 cm between plant within rows that was equal to 6 plants/m^2 in the present study [18]. Similar to our study, Todorovic et al. found higher DM production and more establishment rate in T. Kotschyanus in the final year of study that was in agreement with our study [18].

Table 9. Essential oil compounds of T. kotschyanus and T. daenensis by GC/MS analysis in 8 plants/m^2 in the third year.

Essential oil compounds	(RI)	T. daenensis	T. kotschyanus
α-thujene	934	0.4	0.4
α-pinene	946	0.4	0.8
camphene	954	0.1	0.4
β-pinene	980	0.4	0.3
myrcene	993	0.3	0.5
α-phellandrene	1005	0.1	0.1
α-terpinene	1015	0.7	1.1
p-cymene	1024	2.9	**5.9**
limonene	1029	0.2	0.2
1,8-cineole	1031	1.0	1.3
γ-terpinene	1058	2.5	**5.8**
cis-sabinene hydrate	1072	0.1	2.4
terpinolene	1090	0.3	0.1
linalool	1099	0.2	0.0
camphor	1148	0.0	0.3
borneol	1170	0.0	0.6
terpinen-4-ol	1179	0.9	3.9
α-terpineol	1190	0.1	0.4
methyl ether carvacrol	1247	0.3	0.5
Thymol	1292	**78.8**	**51.9**
carvacrol	1302	**5.7**	**18.5**
E-caryophyllene	1424	2.8	1.5
Total		98.4	96.8

In comparison, between Thyme species, the higher canopy diameter with a value of 33.75 cm was obtained from T. daenensis. In agreement with our results, Azizi in a study of four thyme species of: T.daenensis, T. migricus, T. kotschyanus and T. pubescens found the largest canopy diameter in T. daenensis [19]. Ashrafi et al. reported that the reason of resistance to drought stress in Thyme species is due to osmotic regulation of fructose, sucrose and glucose content in the plant organs [20]. Sepahvand et al. went to the same conclusion in T. daenensis [21].

In our study, T. daenensis had higher aerial DM, essential oil yield and essential oil content with average values of 3332.5 kg.h^{-1}, 75.28 kg.h^{-1} and 2.18%, respectively, in the third year. The higher stems number (201.2) and plant height (23.96 cm) were obtained in the fourth year, respectively (Table 8). It seems that higher amount of precipitation in the third year has been effective in these results. Ghasemi Pirbalouti et al. reported that essential oils and oil compositions of Thymus species are strongly affected by climate conditions and agronomic practices [22]. The findings of Taheri et al., Hasani et al., and Omidbaigi et al. confirmed this conclusion that climatic differences can cause such variations in the species where the research is conducted [10, 12, 13].

The production of more DM and essential oil content and oil components were important goals of present research. Thymol is usually the main chemical component of Thyme plants and it is purchased by pharmaceutical companies for making medicinal derivatives, so producers focus on methods that can enhance the capacity of Thymol production [23].

The highest amount of essential oil in T. daenensis was obtained at third and fourth years, which indicated that production of more plants shoot can produce more essential oil. Therefore, one of the most important reasons for the increase in the percentage of essential oil in this species can be related to the climatic conditions of the study area, which had a cold climate and is characterized by mountainous terrain. Therefore, cultivation in the cold climates of Lorestan province can be recommended for T. daenensis which had a large yield of essential oil, as well as a substantial essential oil percentage which was rich in Thymol.

The higher amounts of essential oil yields with average values of 44.83 and 75.28 $Kg.h^{-1}$ were obtained in T. kotschyanus and in T. daenensis, respectively in the third year. The richness of essential oil in T. daenensis can partly result from the plants' genetics and its efforts to adapt to the unstable and stressful environments in which they are cultivated [24].

Phytochemical analysis was made using GC/MS analysis for both species in 8 plants/m^2 treatment in the third year of study. Result of the analysis of T. kotschyanus essential oil resulted in 18% carvacrol and 51% Thymol. In the case of T. daenensis, however, these values were 5% and 78%, respectively. Therefore, the results of this study showed a high level of carvacrol and Thymol compounds compared to other components in the area of study. This finding was consistent with previous findings by Hasani et al. and Taheri et al. in high plant density (8 plants/m^2) [12, 13]. It seems that precipitation in the third year had a positive effect on the performance of many of the studied traits and therefore, the results in the third year compared to the fourth year were significant. The results of Ghasemi Pirbalouti et al. in cultivated of T. daenensis in the Saman region, Iran produced the highest Thymol (70.3%) whereas T. daenensis wild growing produced the highest carvacrol (24.8%) [22].

Ultimately, the best results were obtained from the evaluated traits in the third year, especially in terms of essential oil yield, as the most important parameter. Based on the obtained results, the cultivation of both species in density of 6 and 8 plants/m^2 was recommended in the rain fed area and rangeland of Lorestan province, Iran.

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Conflict of interest statement:
The authors declare that they have no conflict of interest.

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