Journal of Agricultural Sciences
Vol. 64, No. 1, 2019
Pages 37-51

https://doi.org/10.2298/JAS1901037J
UDC: 635.71-155.9
Original scientific paper

THE YIELD AND GROWTH CHARACTERISTICS OF THYMUS (*THYMUS DAENENSIS L.*) TREATED WITH DIFFERENT FERTILIZERS AND PLANT DENSITIES

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Abstract: To study the effect of plant density and different nutritional systems (chemical, biological, and integrated) on the quantitative and qualitative performance of *Thymus daenensis* L. in Mahidasht Agricultural Training Center, Kermanshah/Iran, in 2017, an experiment was conducted in a split-plot design based on a randomized complete block with four replications. The first variable involved two planting distances in the row of 15 and 30 cm (the main factor), and the subsidiary factor comprised four different nutritional systems including control (no-fertilizer), chemical (100 kg superphosphate, 100 kg potassium sulfate, 150 kg urea per ha), biological (8 tons of cattle manure/ha), and integrated (50% cattle manure + 50% chemical) fertilizers. The results showed that the integrated fertilizer treatment produced the highest shoot dry weight (789.1 kg/ha), essential oil content (3.35%) and root length (15.97 cm). The integrated fertilizer treatment along with the 30-cm planting density resulted in the highest essential oil content of 3.35%. The results of this project have suggested that the integrated fertilizer and the 30-cm planting interval is the optimal treatment in thymus production due to the higher chemical fertilizer efficiency and better crop quality.

Key words: *Thymus daenesis L.*, plant density, integrated fertilizer, biological fertilizer, chemical fertilizer.

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Introduction

Optimum planting density in medicinal plants is considered as a method that guarantees the quality and quantity of secondary metabolites in different steps of planting, processing, and harvesting (Griffe et al., 2003). The higher crop and essential oil content in medicinal plants along with the necessity of the lowest chemical residue contamination of the end-product have been the major concern of farmers during production and processing procedures. Therefore, it seems that medicinal plant performance in response to biological and integrated fertilizers (biologic + chemical fertilizers) should be taken into careful consideration.

Production of medicinal plants using natural fertilizers such as animal manure seems promising (Jahan et al., 2007). In a study on thymus by Safaei et al. (2014), the positive effects of manure application on maximizing the shoot and essential oil yield were reported. They declared that chemical fertilizer increased essential oil content of the garden thymus. The reproducible natural fertilizers have shown high performance in promoting the biological activities in soil and plant interactions leading to the modification of soil chemical and physical properties in stressed conditions. Sharifi Ashoorabadi et al. (2001) reported that application of vermicompost of around 20 t/ha in cultivating parsley flower showed a positive and significant effect on growth index and absorption of macro-nutrients. In another research, the results showed that biological fertilizer in Ajwain (*Trachyspermum ammi*) significantly influenced some traits such as biological functions, seed production, and essential oil content (Ghilavizadeh et al., 2013). In research conducted by Naghdi Badi et al. (2003), the significant effect of different planting densities and planting dates on the qualitative and quantitative performance of essential oil ingredients in thymus was reported. The maximum biomass, essential oil content and thymol were obtained at the planting interval of 15 cm in the last harvest. The results from another study by Heydari Zoolah et al. (2004) on cumin in the rainfed farming system in Kermanshah/Iran indicated that seed yield was influenced by date and plant density. El-Gendy et al. (2001) reported that the increment in planting interval increased the shoot number, wet and dry weights as well as essential oil content, while significantly decreased the plant height. The purpose of this study was to determine the optimal density of *Thymus daenensis* using different fertilizing systems to achieve the best qualitative and quantitative yield.

Materials and Methods

This study was conducted in Mahidasht Agricultural Training Center, Kermanshah/Iran, in 2017. The experiment was conducted in a split-plot mode based on a randomized complete block design with four replications. The main treatment comprised planting intervals in rows including distances of 15 and 30 cm (indicating plant density) and subsidiary treatments involved four different fertilizing systems comprising control (no-fertilizer), chemical (100 kg superphosphate, 10 kg potassium sulfate, 150 kg urea 46% per ha based on the soil test), biological (8 t/ha of cattle manure based on the soil test) and integrated (50% cattle manure + 50% chemical fertilizer) fertilizers. The process of seedbed preparations included plowing, disking, softening and plotting which took place on 17 October, 2017. Before cultivation, the soil was sampled at a depth of 30 cm and the chemical and physical characteristics were determined using standard experimental methods (Sharafaldin Shirazi and Fazeli, 2015). After seedbed preparation, a furrow irrigation system was set up in experimental plots of 1.5 (length)×2 (width) m dimensions with 4 planting lines (50 cm apart) in each plot. Uniform natural seedlings (from a rangeland in Kermanshah) were retrieved and planted by hand at a depth of 5 cm in each row within the experimental plots. The planting space in each row was either 15 or 30 cm according to the corresponding treatments. The seedlings were irrigated immediately after planting. Irrigations were repeated at 5-day intervals until the start of the raining season. The agronomic operations during the growing period included weed control by hand with no chemical pesticide or herbicide application (Safaei et al., 2014). The chemical fertilizers including superphosphate, potassium sulfate and one-third of urea as well as cattle manure (in corresponding treatments) were applied before land preparation. The rest of the urea fertilizer was applied in two stages (with the first irrigation and one month after the seedlings were planted) (Safaei et al., 2014). After plant establishment and growth, 6 representative bushes from the middle row from each experimental plot (considering a marginal effect) were randomly selected and marked by red signs to measure the agronomic traits. The studied traits were measured at different growth stages which included the bush height, number of branches per bush, wet and dry weights, aerial organ volume, leaf chlorophyll, and essential oil content.

To measure the essential oil content, 50 g of plant powder was tested using a Clevenger apparatus (the essential oil content was measured in percent). The aerial organ volume of 10 bushes per experimental plot was measured in 1000-ml graduated cylinders and the mean was considered as aerial organ volume (Feiziasl et al., 2014). The chlorophyll was measured using the method of Arancon et al. (2004) by a spectrophotometer apparatus. About 0.1 g of fresh leaves was weighted by a digital balance (with 0.001-gram precision). The samples were rubbed in a crucible using 15 cc acetone 80%. Then the material was passed through filter paper and centrifuged at 4000 rpm for 10 min to remove the solvent. Finally, chlorophylls a and b were measured in 663 and 645nm by a spectrophotometer, respectively.

The test of normality was done before variance analysis using SPSS16. The variance analysis was conducted based on a randomized complete block design and the mean comparison was completed by Duncan's multiple range test at 5% and 1% probability levels using SAS. The figures were prepared in EXCEL and interpreted.

Results and Discussion

Plant height

The results of ANOVA showed that simple effects of planting densities and fertilizing systems were significant on plant height at 5% and 1% levels, respectively (Table 1). The simple effects of fertilizing systems (Figure 1) showed a significant increase in plant height for chemical fertilizer (28.0cm) and integrated fertilizer (25.9cm) compared to the control (21.2cm).

Table 1. ANOVA for *Thymus daenensis* measured characteristics.

		M.S				
S.O.V	df	Plant height	Root length	Chlorophyll a	Chlorophyll b	Dry biomass
Rep	3	6.58ns	1.3ns	0.000012ns	0.000000417ns	0.850ns
A: Planting density	1	38.72*	203.5**	0.000012ns	0.00001ns	0.435ns
E	3	7.82	4.1	0.000012ns	0.000015	0.24
B: Fertilizing system	3	95.01**	13.8**	0.00004ns	0.00000018ns	4.53**
Interaction (AB)	3	13.11ns	9.1ns	0.000012ns	0.00000083ns	0.5ns
E: (Error)	18	3.03	1.48	0.000012ns	0.000012	0.157
CV (%)	-	7.20	8/72	16/16	25	10/58

ns: non-significant; *Significant at p≤0.05; **Significant at p≤0.01.

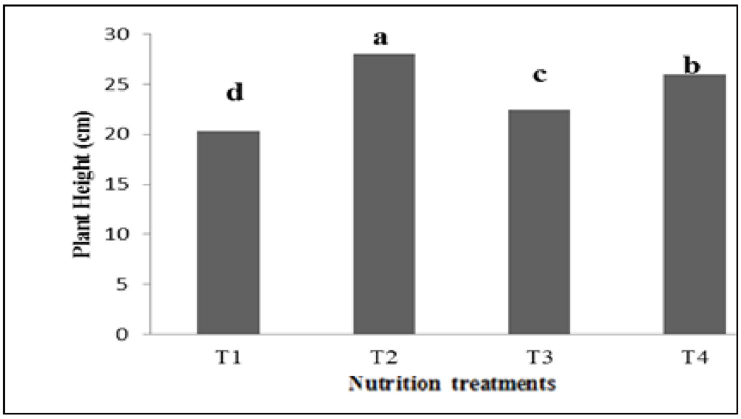

Figure 1. Effects of different fertilizer treatments on plant height.
T1: Control, T2: Chemical fertilizer, T3: Biological fertilizer (manure), and T4: Integrated fertilizer (50% chemical + 50% biological fertilizer).

The planting interval in the row of 30 cm produced significantly taller plants (22.9cm) compared to the 15-cm (26.1 cm) planting space (Figure 2). The biological fertilizer increased the height of plants compared to control, which was due to increased length of internodes (Moghaddam et al., 1997). The integrated fertilizer (cattle manure + chemical fertilizers) showed synergistic effects on plant and soil properties (Sharifi Ashoorabadi et al., 2001; Akbarinia et al., 2010).

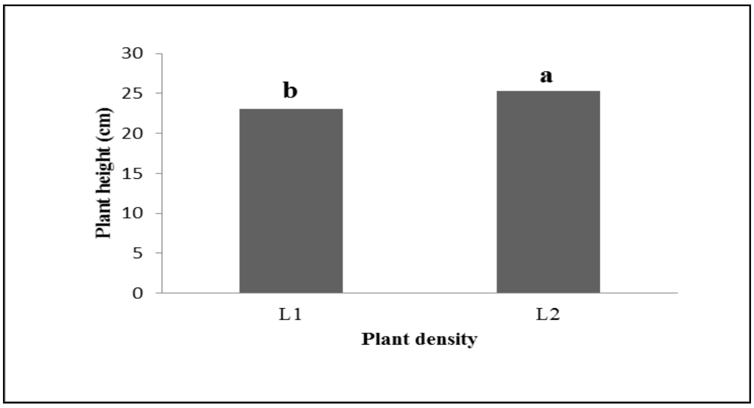

Figure 2. Effects of planting density on plant height.
L1 and L2 (planting intervals of 15 and 30 cm in the row, respectively).

The integrated fertilizer provided the possibility of absorption of essential nutrients in early stages of growth. In the vegetative growth stages, animal manure provided more micro and macro nutrients to support better performance of the plants (Akbarinia et al., 2010; Kandeel et al., 2002). The results of a study on chamomile showed that high planting density could result in higher competition among neighboring plants for light. Higher planting density not only resulted in no beneficial effects on the final size of the plants, but it also decreased the qualitative and quantitative plant characteristics (Franke and Schilcher, 2005; Salamon, 2007).

Root length

Simple effects of planting density and fertilizer treatments were significant on root length ($p<0.01$). The results in Figures 3 and 4 showed that the integrated fertilizer and a planting density of 30 cm with the root lengths of 16 and 16.5 cm, respectively, most positively affected root growth compared to other treatments. Anderson and Impiglia (2002) and Pedersen et al. (2009) reported that the maximum impact of nitrogen application was seen in the root length, while its least impact was noticed in dry root weight among the studied traits. They explained that nitrogen increased the growth of some root traits such as length, number, fresh and dry weights as well as the volume. The results of this study on nitrogen application correspond with the results of Hoad et al. (2004) on wheat.

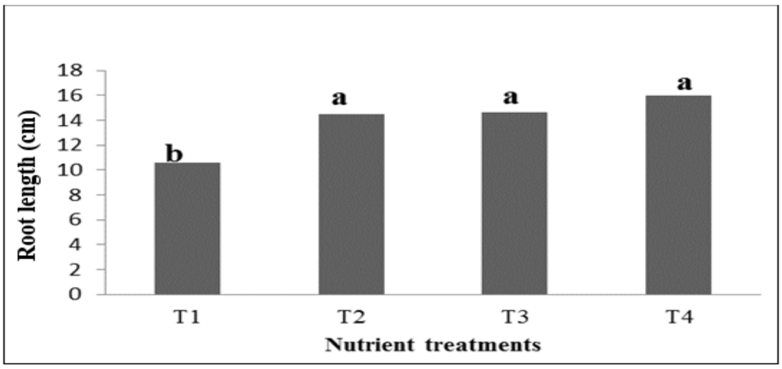

Figure 3. Effects of different fertilizing treatments on root length.
T1: Control, T2: Chemical fertilizer, T3: Biological fertilizer (manure), and T4: Integrated fertilizer (50% chemical + 50% biological fertilizer).

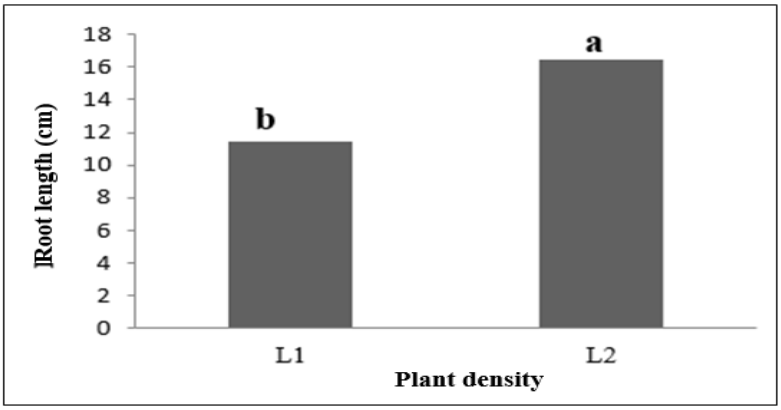

Figure 4. Effects of plant density on root length.
L1 and L2 (plant densities of 15 and 30 cm respectively).

Chlorophylls a and b

No significant effects of planting density, fertilizer treatments or their interactions were observed in chlorophylls a or b. Kafi et al. (1997) indicated that increased plant density decreased the photosynthesis rate because the increased leaf surface area caused more shade on the lower leaves via decreasing light absorption efficiency. They explained that increased plant density caused a kind of internal competition for light, space, water, and fertilizer causing the less chlorophyll content.

Shoot dry weight per plant

The simple effects of planting density and fertilizer treatments were significant on shoot dry weight ($p<0.01$) (Table 1). The integrated fertilizer treatment increased biomass per plant (4.38 g) by 64% compared to control (2.67 g) (Figure 5). Arancon et al. (2004) have explained that the soils treated with cattle manure suggest higher population of micro-organisms, available phosphorus, potassium, calcium, magnesium, and nitrate compared to soils treated with chemical fertilizers. Feiziasl et al. (2014) reported that the fresh and dry weights as well as shoot volume in the 90 kg N_2/ha treatment were higher than in the 30 kg N_2/ha treatment. Solomon (2007) reported that increased density resulted in increasing the dry weight of chamomile to 1200 kg/ha.

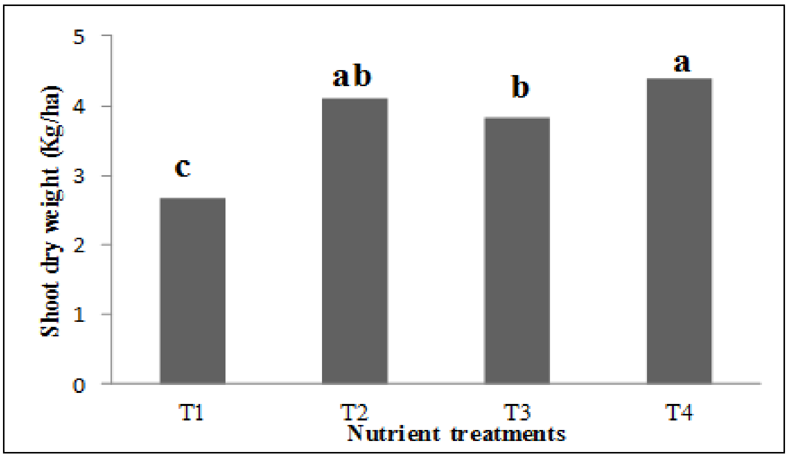

Figure 5. Effects of different fertilizing treatments on shoot dry weight.
T1: Control, T2: Chemical fertilizer, T3: Biological fertilizer (manure), and
T4: Integrated fertilizer (50% chemical + 50% biological fertilizer).

Shoot yield per hectare

The simple effects of planting density and fertilizer treatments were significant on shoot dry weight ($p<0.01$). Integrated fertilizer application produced maximum shoot dry weight in a hectare (78.9 kg/ha), while the least shoot dry matter production was recorded in control (48.1 kg/ha). Arancon et al. (2004), in a research on the pumpkin, showed that animal manure could increase plant biomass compared to control. As the amount of manure application increased, biomass production followed a linearly increasing trend. (Figure 6). These results were supported by other researchers on *Solanum sp.* (Feiziasl et al., 2014). Organic fertilizer made positive changes in physical and chemical characteristics of soil and promoted the availability of the required nutrients for plants during the growing season (Khoramdel et al., 2008). It is reported that the slow releasing characteristic of animal manure prevents nutrients (N in particular) from leaching by excess water (Kolata et al., 1992). The chemical fertilizer had no significant impact on plant performance which could be explained by higher nitrogen leaching to deeper soil layers. Our results correspond to the findings of Khoramdel et al. (2008) working on biologic fertilizer in blackcurrant.

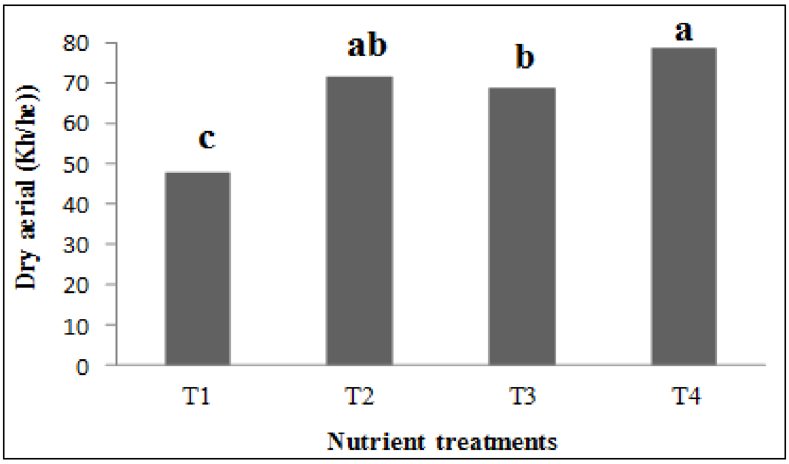

Figure 6. Effects of different fertilizing treatments on shoot dry yield per hectare.
T1: Control, T2: Chemical fertilizer, T3: Biological fertilizer (manure), and
T4: Integrated fertilizer (50% chemical + 50% biological fertilizer).

Essential oil content

The effect of fertilizer treatment was significant on essential oil content ($p<0.01$). Among the fertilizer treatments, the integrated fertilizer treatment produced 2.2 times higher essential oil content (3.35%) compared to control (1.53%) (Figure 7). No significant difference was observed among the plant densities in our project. However, based on a study on thymus, by increasing the planting density, the shoot biomass and essential oil content per unit area significantly increased (Ghilavizadeh et al., 2013). Baranauskiene et al. (2003) studied the effect of chemical fertilizer on thymus and indicated that essential oil content ingredients showed no significant response to different treatments. It seems that, in aromatic plants, the growth and performance of the essential oils are affected by various environmental factors such as temperature, water stress, salinity, nutrient deficiencies, and intra-basal competition.

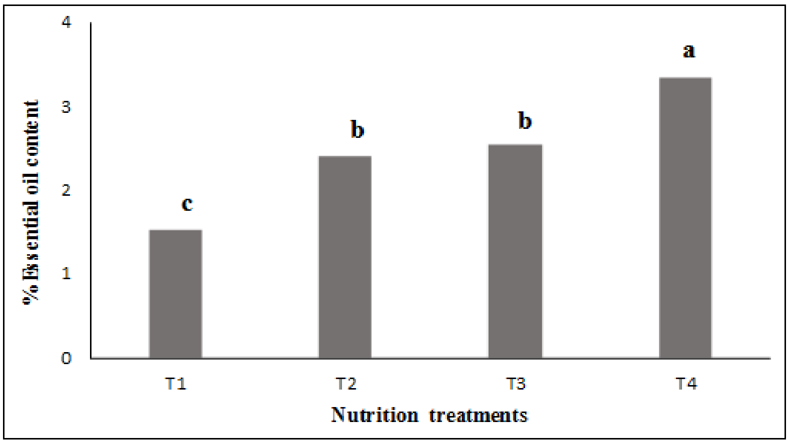

Figure 7. Effects of different fertilizing treatments on essential oil content.
T1: Control, T2: Chemical fertilizer, T3: Biological fertilizer (manure), and
T4: Integrated fertilizer (50% chemical + 50% biological fertilizer).

Root dry weight

The effect of fertilizer treatment was significant on root dry weight ($p<0.01$), while planting density and its interaction with fertilizer treatment showed no significant impact. The results indicated that the lower plant density (30-cm planting interval in the row) and the chemical fertilizer produced the maximum root dry weight (3.0 g and 3.5 g dry root/plant, respectively) (Figures 8 and 9). Feiziasl et al. (2014) indicated that morphological characteristics of root could be changed with soil physical characteristics, soil nitrogen and climatic conditions, therefore, the optimal amount of fertilizers, especially nitrogen, could significantly improve plant growth.

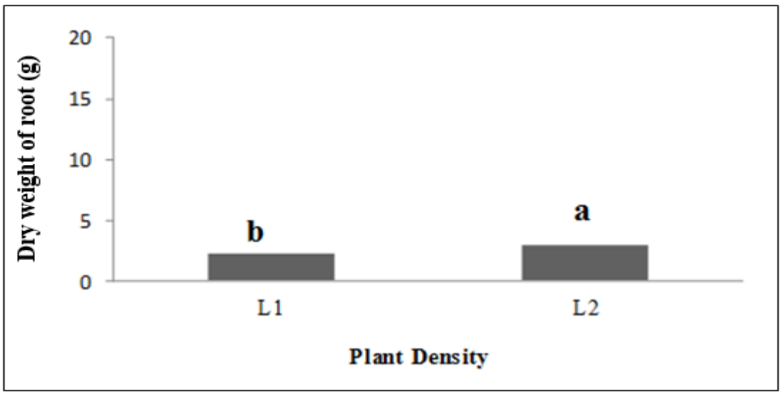

Figure 8. Effects of plant densities on the root dry weight.
L1 and L2 (plant densities of 15 and 30 cm respectively).

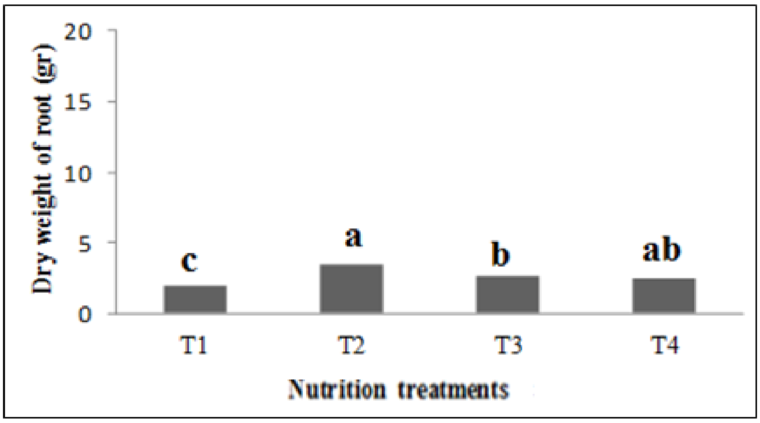

Figure 9. Effects of the different fertilizing treatments on root dry weight.
T1: Control, T2: Chemical fertilizer, T3: Biological fertilizer (manure), and
T4: Integrated fertilizer (50% chemical + 50% biological fertilizer).

Root/shoot ratio

The results indicated that simple effects of planting densities and fertilizer treatments were not significant on root/shoot ratio. Most of the plants responded to water stress by increasing the proportion of photosynthetic materials, which promoted the better root growth (Figure 10). A higher root/shoot ratio warrants more water availability for plants under water stress conditions (Kafi and Mahdavi Damghani, 1997). It seems that the morphological characteristics of the roots changed with soil physical conditions, soil nitrogen, and climate. Therefore, the optimal amount of fertilizers, especially nitrogen, could be critical in plant growth and development.

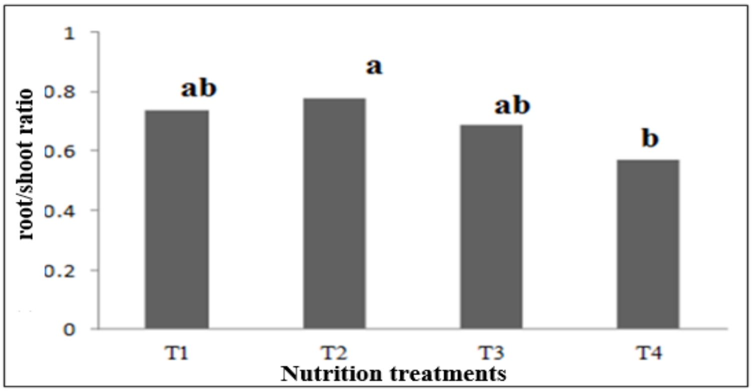

Figure 10. Effects of different fertilizing treatments on root/shoot ratio.
T1: Control, T2: Chemical fertilizer, T3: Biological fertilizer (manure), and
T4: Integrated fertilizer (50% chemical + 50% biological fertilizer).

Aerial organ volume

The results indicated significant ($p<0.01$) simple effects of planting densities and fertilizer treatments on aerial organ volume (Table 2). The results indicated that plant density of 30 cm (13.1 cm^3) and chemical fertilizer (13.7 cm^3) produced maximum aerial organ volume (Figures 11 and 12).

Table 2. Analysis of variance of different levels of nutritional treatments and planting densities on some quantitative and qualitative traits of *Thymus daenensis.*

		M.S				
S.O.V	df	Shoot dry weight	% Essential oil content	Root dry weight	Root/shoot ratio	Aerial organ volume
Replication	3	63.81ns	4862.5ns	0.22ns	0.11ns	1.75ns
Planting density	1	226.5ns	5000ns	5.03**	0.13ns	38.5**
E_a: (Error)	3	87.4	983	0.31	0.64	2.46
Fertilizing systems	3	1398.9**	13379.1**	3.9**	0.12ns	15.62**
Interaction	3	179.33ns	283.3ns	0.19ns	0.04ns	1.25ns
E_b (Error)	18	69.78	745.1	0.19ns	0.042	1.05

ns: non-significant *Significant at p≤0.05 **Significant at p≤0.01.

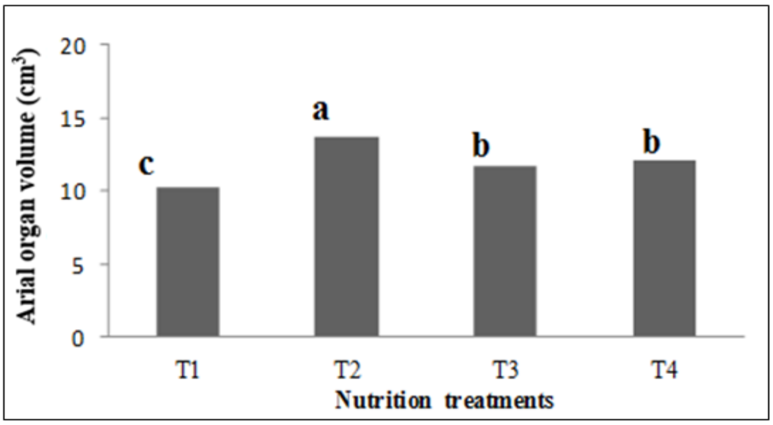

Figure 11. Effects of different fertilizing treatments on aerial organ volume.
T1: Control, T2: Chemical fertilizer, T3: Biological fertilizer (manure), and
T4: Integrated fertilizer (50% chemical + 50% biological fertilizer).

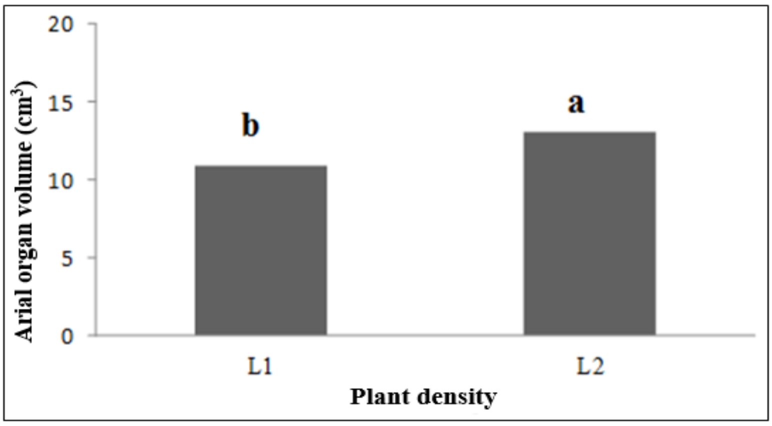

Figure 12. Effects of planting densities on aerial organ volume.
L1 and L2 (plant densities of 15 and 30 cm respectively).

Feiziasl et al. (2014) reported that the effect of nitrogen fertilizer was significant not only on fresh shoot weight but also on the aerial organ volume ($p<0.01$). Biological fertilizer in blackcurrant significantly increased plant height, leaf surface index, aerial organ volume and overall plant performance compared to control. Our results support the findings of El-Gendy et al. (2001) who have reported that by increasing the planting density, a significant increase in shoot yield is achievable. Sharifi Ashour Abadi et al. (2001) have stated that sheep manure contains micro and macro elements necessary for vital activities of the plant, which improves soil texture, increases water absorption capacity and provides a suitable environment for root development.

Conclusion

The results of this study showed that a combination of integrated fertilizer application and lower planting density (30-cm planting intervals in the row) improved the qualitative and quantitative characteristics of the thymus. It seems that the integrated fertilizer method can play an effective role in increasing the quality and quantity of thymus shoot yield. This result could be explained by the slow release of micro and macro nutrients from the manure which increased the nutrient availability and absorption efficiency in this treatment. It could be also suggested that the lower planting density may increase the essential oil content due to better light infiltration in the plant canopy.

Acknowledgements

Special thanks to Dr. Morteza Pourreza from the Razi University for editing the manuscript and useful pieces of advice.

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Received: June 20, 2018
Accepted: November 28, 2018

KARAKTERISTIKE PRINOSA I RASTA MAJČINE DUŠICE (*THYMUS DAENENSIS L.*) U ZAVISNOSTI OD ĐUBRIVA I GUSTINE BILJAKA

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R e z i m e

Kako bi se proučio uticaj gustine biljaka i različitih sistema ishrane (hemijskog, organskog i kombinovanog) na kvantitativne i kvalitativne karakteristike biljke *Thymus daenensis* L. u Poljoprivrednom centru za obuku u Mahidaštu (Kermanšah/Iran) u 2017. godini, sproveden je eksperiment podeljenih parcela zasnovan na potpuno slučajnom blok sistemu sa četiri ponavljanja. Prvi tretman je uključivao dva razmaka biljaka u redu od 15 i 30 cm (glavni faktor), a drugi faktor se sastojao od četiri različita sistema ishrane, uključujući kontrolu (bez đubriva), hemijsko (100 kg superfosfata, 100 kg kalijum sulfata, 150 kg uree po ha), organsko (8 tona goveđeg stajnjaka po ha) i kombinovano (50% goveđeg stajnjaka + 50% hemijskog) đubrivo. Rezultati su pokazali da su u tretmanu sa kombinovanim đubrivom proizvedeni najveća nadzemna suva masa (789,1 kg / ha), sadržaj eteričnih ulja (3,35%) i dužina korena (15,97 cm). U tretmanu sa kombinovanim đubrivom i razmakom u redu od 30 cm utvrđen je najviši sadržaj eteričnih ulja – 3,35%. Rezultati ovog projekta sugerišu da je kombinovano đubrivo i razmak biljaka u redu od 30 cm optimalni tretman u proizvodnji majčine dušice, zbog veće efikasnosti hemijskih đubriva i boljeg kvaliteta useva.

Ključne reči: *Thymus daenesis L.*, gustina biljaka, kombinovano đubrivo, organsko đubrivo, hemijsko đubrivo.

Primljeno: 20. juna 2018.
Odobreno: 28. novembra 2018.

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