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Improving *Thymus daenensis* L. growth and essential oil through integrated nutrient management

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Abstract

Thyme (*Thymus daenensis* L.) is one of the most commonly used medicinal plants with highly valuable essential oil. To evaluate the effect of cut, and chemical and non chemical fertilizers on *T. daenensis*, this experiment was conducted in 2008-2009 at Alborz research station, Research Institute of Forests and Rangelands, Karaj, Iran. Experimental design was split plot in time in the form of a randomized complete block design with three replications. The main factor was 16 combinations of nitrogen (N, in kg/ha), phosphorus (P, in kg/ha), potassium (K, in kg/ha) and manure (M, in t/ha): $N_0P_0K_0M_0$, $N_{40}P_{32}K_{40}M_0$, $N_{80}P_{64}K_{80}M_0$, $N_{120}P_{96}K_{120}M_0$, $N_{160}P_{128}K_{160}M_0$, $N_{140}P_{112}K_{140}M_5$, $N_{120}P_{96}K_{120}M_{10}$, $N_{100}P_{80}K_{100}M_{15}$, $N_{80}P_{64}K_{80}M_{20}$, $N_{60}P_{48}K_{60}M_{25}$, $N_{40}P_{32}K_{40}M_{30}$, $N_{20}P_{16}K_{20}M_{35}$, $N_0P_0K_0M_{40}$, $N_0P_0K_0M_{30}$, $N_0P_0K_0M_{20}$ and $N_0P_0K_0M_{10}$. The sub factor was cut (conducted in late May and early September). Results showed the significant effect of fertilizer, cut, their interaction and year on all measured traits. Mean comparison represented that essential oil percentage and essential oil yield were higher in the treatments containing both chemical fertilizers and manure, though plant height and shoot yield were higher in $N_{160}P_{128}K_{160}M_0$ × the first cut. Essential oil yield was the highest in $N_{60}P_{48}K_{60}M_{25}$ × the first cut (22.14 kg/ha in 2008 and 21.25 kg/ha in 2009). Results of this experiment generally imply that *T. daenensis* reacts well to fertilization, and selection of the best treatment must be conducted based on the objective of production.

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Introduction

Thymus daenensis L. with the common name thyme, is a member of Lamiaceae family, and a medicinal plant native to Iran. Essential oil of this plant shows antispasmodic, antiseptic, carminative, antioxidative, antifungal and antibacterial features (Bolous, 2002; Chiej, 1984; Morales, 2002; Prakash, 1990).

Producing high quality medicinal plants depends on the sustained supply of mineral nutrients. Among different nutrients, nitrogen, phosphorus and potassium, which are the major macronutrients, affect nearly all aspects of plant life cycle. For example, these elements are involved in enzymes, proteins, chlorophyll, cell wall, nucleic acids, energy transfer and storage, and regulation of stomata and turgor pressure (Fageria, 2009; Fageria and Gheyi, 1999; Fageria and Baligar, 2005; Tiessen, 2008; Wiedenhoeft, 2006).

Nutrients may be provided to plants by the application of chemical fertilizers. Verma *et al.* (2010) concluded that applying 100 kg N/ha × 60 kg P/ha had the highest effect on plant growth, essential oil percentage and yield; however, this treatments had no effect on the composition of essential oil. Baranauskiene *et al.* (2003) reported that application of N fertilizer significantly affected *T. vulgaris* yield, but had no significant effect on the essential oil yield. Omidbaigi and Rezaei Nejad (2000) also reported that application of 150 kg N/ha increased *T. vulgaris* yield from 671.88 kg/ha up to 1021 kg/ha; the effect was not significant on essential oil yield. Valmorbida and Boaro (2007) evaluated the influence of different potassium concentrations (6, 3 and 1.5 mmol/L) on the development of *Mentha piperita* in nutrient solution and concluded that shoot growth was higher in 1.5-3 mmol/L K.

Animal manure was another treatment studied in this experiment. Manure, in addition to releasing a wider range of nutrients, improves plant growth and yield by affecting soil biological and physico-chemical properties (Fageria, 2009; Schoenau, 2006). Rahbarian *et al.* (2010) reported that manure application increased leaf relative water content of *Dracocephalum moldavica* under drought stress condition. Tabrizi *et al.* (2011) also reported that adding animal manure to soil significantly affected thyme biomass production; however, had no significant effect on oil production. Considering the proven effects of chemical fertilizers and animal manure on various crops and medicinal plants, this experiment was conducted to assess the effect of different chemical NPK ratios and animal manure on growth and essential oil of *Thymus daenensis* L. in different cuts.

Materials and methods

This experiment was conducted in 2008-2009 at Alborz research station, Research Institute of Forests and Rangelands, Karaj, Iran, to study the effect of chemical and non chemical sources of nutrients, and cut on *Thymus daenensis* L. The experiment was conducted in split plot in time in the form of a randomized complete block design with three replications and two factors:

Fertilizer treatments as the main factor

Included 16 combinations of different ratios of nitrogen (N), phosphorus (P), potassium (K) and animal manure (M) (Table 1). Fertilizers application was repeated in the same way in both years. Manure, P and K were applied in fall; they were incorporated into soil when the field was being prepared in the first year, and were applied in a groove on one side of each row in the second year. Nitrogen was split in two parts in both years. In the first year, 50% was applied at field preparation; the other 50% was applied with the last irrigation before the first cut. In the second year, 50% was applied in early spring with the first irrigation and the other 50% was applied with the last irrigation before the first cut.

Cut as the sub factor

Included two cuts: the first cut was at full flowering stage conducted in late May (2007 and 2008), 3-5 cm above the soil surface. The second cut was conducted in early September (2007 and 2008), when plants reached full flowering stage again, 3-5 cm above the soil surface.

Seed was used to produce seedlings in a frame; seedlings were planted in pots at 6-8 leaf stage. Then, the established seedlings were planted in the prepared field. Planting pattern was a crossed 50 cm (furrows) × 40 cm (plants). During the growth period, field was irrigated, weeded and monitored. At full flowering stage, plant height was recorded and shoots were harvested. Then, samples were dried and weighted.

To measure essential oil percentage and yield, essential oil was produced by hydrodistillation using a Clevenger in 2 hours. Essential oil composition was analyzed using GC and GC-MS to obtain the main compounds in the oil:

The properties and methods of GC analysis

GC analysis was carried out using Shimadzu GC-9A gas chromatograph equipped with DB-5 column (60 m × 0.25 mm × 0.25 µm). The temperature was kept at 50°C for the first 5 min and was programmed to increase up to 250°C at the rate of 4°C/min. Injector and detector temperature was 260°C, the carrier gas was helium with linear velocity of 32 cm/s.

The properties and methods of GC-MS analysis

GC-MS analysis was conducted on a Varian 3400 GC-MS system equipped with a DB-5 column (60 m × 0.25 mm × 0.25 µm). The temperature programming was similar to GC. Carrier gas was helium with linear velocity of 31.5 cm/s; scan time, 1 s; ionization energy, 70 V; and mass range, 40-340 amu.

After recording data, data were tested for their normality and un-normal ones were normalized by radical or logarithmic methods. Then, analysis of variance was conducted using SAS and means were compared by Duncan's multiple range test. A combined analysis was also conducted to compare the effect of years on the measured traits. In combined analysis, two cuts in each year were also combined.

Results and discussion

The effect of fertilizer

Analysis of variance indicated the significant effect of fertilizer on all the measured traits (Table 2). In both years, plant height and shoot yield were the highest in $N_{160}P_{128}K_{160}M_0$, in which the chemical fertilizers were applied at the highest rate and manure was not applied. Nitrogen, phosphorus and potassium are three major macronutrients affecting most of the physiological and biochemical processes in plants. Nitrogen, which is the most frequently found element within plants body, is involved in the structure of amino acids, nucleic acids, chlorophyll, enzymes, proteins and cell walls. Phosphorus is also essential in energy storage and transfer, metabolism, respiration and photosynthesis. Potassium, which is frequently found in most soils, is involved in regulation of stomata, turgor pressure, respiration and photosynthesis, and activation of at least 60 enzymes (Bertrand *et al.*, 2003; Fageria, 2009; Fageria and Baligar, 2005; Fageria and Gheyi, 1999; Tiessen, 2008; Wiedenhoeft, 2006).

Table 1. The combination of fertilizers forming 16 treatments.

Treatments	N (kg/ha)	P (kg/ha)	K (kg/ha)	Manure (t/ha)
Control	0	0	0	0
Chemical	40	32	40	0
	80	64	80	0
	120	96	120	0
	160	128	160	0
Integrated	140	112	140	5
	120	96	120	10
	100	80	100	15
	80	64	80	20
	60	48	60	25
	40	32	40	30
	20	16	20	35
Manure	0	0	0	40
	0	0	0	30
	0	0	0	20
	0	0	0	10

The effect of NPK fertilizers is well studied on different crops and medicinal plants. Shams *et al.* (2012) reported that application of various ratios of nitrogen and phosphorus significantly affected *Thymus daenensis* growth indices such as crop growth rate, relative growth rate, leaf area index and total dry matter content; the best treatment was 150 kg N/ha + 130 kg P/ha, the highest application rate.

Biesiada and Kuś (2010) found that applying 150-250 kg N/ha resulted in the highest basil (*Ocimum basilicum* L.) yield. They reported that increasing nitrogen rate from 50 to 150 kg/ha enhanced yield by 63.90%, while increasing the dose to 250 kg N/ha enhanced yield by only 11.46%; representing that the medium rate was more efficient. In another experiment on basil, it was concluded that application of 60 kg N/ha increased yield; however, higher N rates did not significantly increase yield (Zheljazkov *et al*., 2008). Layegh Haghighi *et al.* (2012) also reported that increasing N application rates from 75 to 225 kg/ha significantly improved growth and yield of *Amaranthus hypochondriacus*; however, they recommended 150 kg N/ha rate based on the overall results. Verma *et al.* (2010) studied the effect of different N and P ratios on clary sage (*Salvia sclarea* L.) and reported that 100 kg N/ha × 60 kg P/ha was the most effective trait on plant growth, essential oil percentage and yield; though the essential oil composition was not affected by the treatments. Anwar *et al.* (2010) also tested the effect of different NPK ratios on mint (*Mentha arvensis* L.) and reported about 12% improvement of dry matter as the result of increasing NPK ratio from 50:20:20 to 200:80:80 kg/ha.

Table 2. Analysis of variance of the effect of treatments on the measured traits.

SOV	df	Mean Squares (MS)													
		2008							2009						
		Plant height	Flowering shoot yield	Essential oil percentage	Essential oil yield	Thymol	P-cymene	γ-terpinene	Plant height	Flowering shoot yield	Essential oil percentage	Essential oil yield	Thymol	P-cymene	γ-terpinene
Block	2	**	**	**	**	**	**	**	ns	**	**	**	**	ns	*
Fertilizer (A)	15	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Error (A)	30	0.16	219.20	5.00	12578.1	0.99	0.05	0.07	0.082	615.65	0.001	3626.10	8.98	2.57	0.32
Cut (B)	1	**	**	**	**	**	**	**	**	**	**	**	**	**	**
A × B	15	**	**	**	**	**	**	**	**	**	**	**	**	**	**
B × Block	2	**	ns	**	**	ns	**	**	**	ns	ns	ns	**	ns	ns
Error	30	0.12	98.21	0.4	11770.3	0.99	0.05	3.07	0.09	615.65	0.001	4396.8	10.43	3.52	0.11
CV (%)	-	11.59	10.22	6.86	8.71	1.44	3.46	5.81	3.317	5.90	2.93	3.99	5.15	18.65	9.07

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Although applying chemical fertilizers at the highest rate without manure application ($N_{160}P_{128}K_{160}M_0$) gave the best plant height and shoot yield in both years; however, essential oil yield which is the main objective in medicinal plant production, was the highest in $N_{80}P_{64}K_{80}M_{20}$ in 2008, and in $N_{60}P_{48}K_{60}M_{25}$ in 2009. In these treatments, which the essential oil percentage is also the highest, animal manure is replaced instead of high doses of chemical fertilizers (Table 3); proving the possibility of using animal manure instead of some parts of chemical fertilizers and lowering the environmental risks of chemical fertilizers. Nitrogen, phosphorus and potassium usually boost vegetative growth and cause a quick growth. On the contrary, manure usually improves the quality of plants because it slowly releases a wider range of nutrients to soil; this may be the reason of essential oil percentage and yield improvement in treatments with manure application in this experiment. Forouzandeh *et al.* (2012) also reached about 12% improvement in basil essential oil percentage when manure was applied instead of chemical fertilizers. The effect of manure on the enhancement of essential oil percentage was also found in experiments of Patra *et al.* (2000) who reported that application of NPK at the rate of 100:20:30 kg/ha + 5 t/ha manure increased essential oil yield by about 18% compared with the treatment in which NPK was applied at the rate of 200:40:60 kg/ha without manure application.

Table 3. Effect of the fertilizers on the measured traits.

Treatments	2008							2009						
	Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)	Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)
$N_0P_0K_0M_0$	16.86m	884.5n	0.64j	5.71m	52.4j	4.78L	3.49i	19.48n	1058.00	0.99j	10.67k	69.38b	7.41gh	2.85gh
$N_{40}P_{32}K_{40}M_0$	20.01i	1061.0k	0.61k	6.56L	70.9e	5.96hi	4.78e	22.23j	1189.0L	1.15i	13.74i	56.80h	13.37b	7.79b
$N_{80}P_{64}K_{80}M_0$	21.16g	1255.4j	0.65j	8.36k	71.5d	5.97h	5.61c	23.68f	1253.83i	1.26gh	15.92g	60.67f	13.04b	4.26d
$N_{120}P_{96}K_{120}M_0$	23.9b	1401.7b	0.81h	11.41h	74.4a	5.53k	4.41g	25.72c	1433.50d	1.26gh	18.23e	53.96i	12.12c	0.28L
$N_{160}P_{128}K_{160}M_0$	24.8a	1499.8a	0.85g	12.98g	69.8f	5.92hi	6.44a	28.27a	1523.33a	1.14i	17.42f	41.17j	24.89a	9.96a
$N_{140}P_{112}K_{140}M_5$	23.98b	1367.1c	0.77i	10.42ij	66.9g	9.16b	5.63c	26.72b	1493.50b	1.26gh	18.87d	65.51de	8.19fg	2.96fg
$N_{120}P_{96}K_{120}M_{10}$	23.15c	1358.0d	0.99f	13.5f	69.9f	7.93d	5.35d	25.68c	1462.0c	1.33e	19.47ab	67.11c	8.75ef	4.32d
$N_{100}P_{80}K_{100}M_{15}$	22.86d	1306.7e	1.21c	15.86d	66.5h	8.89c	3.96h	24.81d	1422.50e	1.34d	19.21bc	60.78f	8.15fg	3.71e
$N_{80}P_{64}K_{80}M_{20}$	23.2c	1285.5f	1.50a	19.37a	73.8b	5.61jk	4.55f	24.06e	1392.50f	1.37c	19.15c	73.18a	5.80j	1.83k
$N_{60}P_{48}K_{60}M_{25}$	22.2e	1250.8g	1.49a	18.75b	67.3g	7.28f	6.27c	23.7f	1350.0g	1.44a	19.56a	61.45f	8.08gh	4.37d
$N_{40}P_{32}K_{40}M_{30}$	22.1f	1226.8h	1.33b	16.4c	59.3i	9.85a	5.71k	23.0g	1310.0h	1.33de	17.57f	69.84b	7.10i	2.13j
$N_{20}P_{16}K_{20}M_{35}$	21.2g	1117.8i	1.32b	14.82e	70.9e	6.95g	3.15e	22.80h	1254.12i	1.39b	17.62f	66.64cd	9.22e	2.48i
$N_0P_0K_0M_{40}$	20.43h	1081.8j	1.15d	12.57g	74.1ab	5.66j	4.71j	22.45i	1232.50j	1.28f	15.99g	58.18g	11.29d	2.83gh
$N_0P_0K_0M_{30}$	19.8j	1083.5j	1.06e	11.71h	73.3c	5.86i	3.35j	21.90k	1211.0k	1.25h	15.29h	66.94c	7.31h	4.70c
$N_0P_0K_0M_{20}$	19.5k	1014.9L	1.05e	10.76i	70.9e	7.77b	4.41g	21.14L	1165.0m	1.16i	13.72i	66.32cd	7.21i	2.81h
$N_0P_0K_0M_{10}$	19.3L	989.6m	1.01f	10.08j	73.1c	5.97h	3.9h	20.23m	1129.0n	1.15i	13.27j	65.01e	9.09e	3.08f

Means in a column followed by the same letter are not significantly different at P≤0.01.

Indices following the letters N, P and K are the application rates of nitrogen, phosphorus and potassium, respectively, in kg/ha.

Indices following the letter M are the application rates of manure in t/ha.

Table 4. Effect of cut on the measured traits.

Treatments	2008							2009						
	Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)	Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)
Cut 1	22.67a	1232.51a	1.19a	14.68a	70.6a	6.38b	4.53b	23.42a	1357.08a	1.33a	18.16a	63.72a	8.20b	6.64a
Cut 2	20.42b	1165.61b	0.87b	10.23b	67.52b	7.25a	4.94a	21.46b	1252.09b	1.18b	15.05b	7.68b	11.93a	0.90b

Means in a column followed by the same letter are not significantly different at P≤0.01.

Results also indicated that fertilizers significantly changed the percentage of different compounds in the essential oil. For example, thymol content in 2008 was the highest in $N_{120}P_{96}K_{120}M_0$ with no significant difference from $N_0P_0K_0M_{40}$; however, p-cymene was the highest in $N_{40}P_{32}K_{40}M_{30}$ and γ-terpinene was the highest in $N_{160}P_{128}K_{160}M_0$. These results represent that treatments must be selected carefully to increase the content of a certain compound for commercial objectives, when a certain compound is more desired than the others; of course further experiments are required to make such decision accurately. The effect of chemical fertilizers and manure on the essential oil composition of medicinal plants was tested in other experiments. Berti *et al.* (2010) found the significant effect of N application on the content of gamma-linolenic acid (GLA), a highly desired acid in borage (*Borago officinalis* L.) seed. Anwar *et al.* (2010) and Alipour Mansoorkhani *et al.* (2012) reported the significant effect of different NPK ratios on the essential oil composition of mint and basil, respectively. Ateia *et al.* (2009) also reported that essential oil composition of thyme was significantly affected by different manure and compost treatments.

Table 5. Effect of interaction of fertilizer × cut on the measured traits.

Fertilizers	Cuts	2008 Plant height (cm)	2008 Flowering shoot yield (kg/ha)	2008 Essential oil percentage (%)	2008 Essential oil yield (kg/ha)	2008 Thymol (%)	2008 P-cymene (%)	2008 γ-terpinene (%)	2009 Plant height (cm)	2009 Flowering shoot yield (kg/ha)	2009 Essential oil percentage (%)	2009 Essential oil yield (kg/ha)	2009 Thymol (%)	2009 P-cymene (%)	2009 γ – terpinene (%)
$N_0P_0K_0M_0$	1	17.73m	926.0s	0.68i-k	6.36ij	64.1k	6.58i-l	3.94k-m	22.50c-i	1174.0L	1.06L	12.61L	71.96b-d	4.13m-o	4.61f-g
	2	16.0n	843.0t	0.60jk	5.06j	40.7n	2.9p	3.04op	17.20i	942.0o	0.92m	8.73n	66.80d-g	10.70g-j	1.1i-k
$N_{40}P_{32}K_{40}M_0$	1	20.8jk	1122.0kL	0.7ij	8.03ij	72.54c	4.7n	4.31i-k	24.60b-g	1200.0kL	1.20ij	14.47jk	46.28m	17.28c	14.29b
	2	19.1L	1000.0pq	0.5k	5.09j	69.35fg	7.1g-j	5.25fg	20.30f-i	1178.0L	1.10kl	13.02L	67.33d-f	9.46h-k	1.30ij
$N_{80}P_{64}K_{80}M_0$	1	22.0h-j	1364.7cd	0.74ij	10.13gh	74.44b	4.7n	4.17j-l	25.0b-g	1310.66h-j	1.32d-g	17.41f-i	54.88j-k	15.21cd	8.4c
	2	20.3k	1146.0k	0.57jk	6.59ij	68.55g-i	7.1g-j	7.06b	22.4c-i	1197.0kL	1.20ij	14.43jk	66.46d-g	10.80g-j	0.13L
$N_{120}P_{96}K_{120}M_0$	1	24.3ab	1425.4b	0.80hi	11.47fg	74.62b	5.1mn	4.74hi	28.0a-c	1467.0cd	1.27gh	18.64de	43.66m	11.95e-i	0.06j-l
	2	23.4b-e	1378.0c	0.82hi	11.36fg	74.21b	5.9k-m	4.09j-l	23.4c-h	1400.0ef	1.26gh	17.83b-f	64.26f-i	12.30d-i	0.50kl
$N_{160}P_{128}K_{160}M_0$	1	25.0a	1560.7a	1.03fg	16.14b-d	71.11c-f	5.05mn	5.36ef	31.0a	1594.66a	1.11kl	17.7e-h	33.55n	29.25a	15.32a
	2	24.6ab	1439.0b	0.68i-k	9.83gh	68.61g-i	6.8h-l	7.53a	25.6a-f	1452.0d	1.18ij	17.14g-i	48.80Lm	20.53b	4.60fg
$N_{140}P_{112}K_{140}M_5$	1	25.0a	1347.2de	1.03fg	13.94d-f	66.73j	10.3c	5.78de	29.4ab	1527.0b	1.23hi	18.98cd	71.15b-e	5.18L-n	4.63ij
	2	22.9ef	1387.0c	0.51k	3.89ij	67.17ij	7.8e-h	5.49ef	24.0b-g	1460.0cd	1.28f-h	18.75de	59.86h-j	11.20f-i	1.3c
$N_{120}P_{96}K_{120}M_{10}$	1	23.4c-e	1338.0e	1.04fg	13.99d-f	71.61c-e	7.5f-i	5.47ef	28.0a-d	1502.0bc	1.36b-e	20.06ab	69.82b-f	4.85m-o	8.48L
	2	22.6e-g	1377.0c	0.94gh	13.0ef	68.12h-j	8.2ef	5.22fg	23.3d-h	1422.d-f	1.29f-h	18.41d-f	64.40f-j	12.66d-h	0.16L
$N_{100}P_{80}K_{100}M_{15}$	1	23.3c-e	1324.4ef	1.11e-g	14.77b-e	71.39c-e	5.9k-m	4.25i-k	26.7a-e	1467.0cd	1.38b-d	20.31ab	68.96c-f	5.31Ln	7.30d
	2	22.3f-h	1289.0gh	1.32cd	16.95bc	61.56L	11.8b	3.68L-n	22.9c-h	1378.0fg	1.31e-g	18.12d-g	52.60kl	11.0g-j	0.13L
$N_{80}P_{64}K_{80}M_{20}$	1	24.6ab	1300.9fg	1.68a	21.92a	77.62a	3.70p	3.73L-n	25.6a-f	1431.0de	1.40bc	20.1b	80.16a	1.67o	1.30ij
	2	21.7h-j	1270.0h	1.32cd	16.81b-d	69.93e-g	7.4f-j	5.38ef	22.5c-h	1354.0gh	1.34c-f	18.21d-g	66.20d-h	9.93h-k	2.36h
$N_{60}P_{48}K_{60}M_{25}$	1	24.0bc	1290.7gh	1.71a	22.14a	62.76k	8.6de	5.49ef	25.4a-g	1400.0ef	1.51i	21.25a	64.20f-i	7.07k-m	8.54c
	2	21.3ij	1211.i	1.27c-e	15.36b-e	71.91cd	5.9Lm	7.05b	22.0d-i	1300.0ij	1.37b-e	17.88d-g	58.70ij	9.10i-k	0.20L
$N_{40}P_{32}K_{40}M_{30}$	1	23.8b-d	1275.0gh	1.37cd	17.54b	48.49m	12.7a	6.57c	25.0b-g	1345.0g-i	1.39b-d	18.76de	75.36ab	3.41no	4.1g
	2	20.3k	1178.0j	1.29cd	15.25b-e	70.05d-g	6.9g-k	4.85gh	21.0e-i	1275.0j	1.28f-h	16.39i	64.33f-i	10.8g-j	0.16L
$N_{20}P_{16}K_{20}M_{35}$	1	23.0d-f	1133.6k	1.46bc	16.62b-d	75.37b	4.3no	2.75p	24.6b-g	1308.0ij	1.5a	19.94bc	74.14bc	4.18m-o	4.69fg
	2	19.3L	1102.0L	1.18d-f	13.03ef	66.46j	9.5cd	3.54mn	21.0e-i	1200.0kl	1.27gh	15.31j	59.13ij	14.26c-f	0.26kl
$N_0P_0K_0M_{40}$	1	22.0h-j	1101.7L	1.56ab	17.25b	77.88a	4.7j-n	3.35no	24.1b-g	1289.0j	1.43b	18.5d-f	59.22ij	8.08j-l	5.54e
	2	18.8L	1062.0m	0.74ij	7.89h-j	70.43d-f	6.5j-l	6.08d	20.8e-i	1176.0L	1.14jk	13.47kl	57.13jk	14.5c-e	0.13L
$N_0P_0K_0M_{30}$	1	21.5h-j	1133.4k	1.39bc	15.85b-e	75.86b	4.9n	4.0k-m	23.6b-h	1270.0j	1.39b-d	17.72e-h	68.88c-f	5.43Ln	8.0cd
	2	18.06m	1033.7no	0.73ij	7.57h-j	70.72c-f	6.8h-l	2.69p	22.2f-i	1152.0L	1.11kl	12.85L	65.0e-i	9.2i-k	1.40i
$N_0P_0K_0M_{20}$	1	21.0jk	1051.9mn	1.3bc	14.62b-e	70.53d-f	7.8e-g	4.55h-j	22.9c-h	1228.0k	1.38b-d	17.01g-i	67.70d-f	3.83m-o	5.29fe
	2	18.0m	978.0qr	0.70ij	6.91i-j	71.26c-e	7.6f-i	4.28i-k	19.4g-i	1102.0m	0.94m	10.42m	64.93e-i	10.6g-j	0.33kl
$N_0P_0K_0M_{10}$	1	20.9jk	1023.1op	1.3cd	14.08c-f	74.67b	4.7n	4.01k-m	22.5c-i	1200.0kl	1.38b-d	16.63hi	69.55b-f	4.32m-o	5.77e
	2	17.6m	956.0r	0.6i-k	7.08ij	71.27c-e	7.1g-j	3.79Ln	18.0hi	1058.0n	0.93m	9.9m	60.46g-j	13.86fd-g	0.40kl

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$.

Indices following the letters N, P and K are the application rates of nitrogen, phosphorus and potassium, respectively, in kg/ha.

Indices following the letter M are the application rates of manure in t/ha.

The effect of cut

Analysis of variance represented the significant effect of cut on all measured traits (Table 2). Length of the growth period, plant regrowth after cut, and climatic factors may be the reasons of variations in the measured traits between the two cuts, because the first cut was conducted in May (27.8°C and 26.7°C mean monthly temperature in 2008 and 2009, respectively) but the second cut was conducted in September (31.4°C and 28.6°C mean monthly temperature in 2008 and 2009, respectively). In this experiment, plant height, shoot yield, essential oil percentage and essential oil yield were all the highest in the first cut (Table 4); the main reason may be the longer growth period before the first cut. Ateia *et al.* (2009) also reported the variation in growth and essential oil of thyme in different cuts

Table 6. Combined analysis of variance of the effect of year and fertilizer on the measured traits.

SOV	df	Mean Squares						
		Plant height	Flowering shoot yield	Essential oil percentage	Essential oil yield	Thymol	P-cymene	γ-terpinene
Year (A)	1	**	**	**	**	**	**	**
Error (A)	4	46.56	149869.66	0.09	642332.15	1008.26	18.75	31.58
Fertilizer (B)	15	**	**	**	**	**	**	**
A × B	15	**	**	**	**	**	**	**
Error	60	0.04	208.72	0.002	4051.07	2.5	0.66	0.10
CV (%)	-	8.88	11.15	13.43	14.38	12.4	9.59	7.42

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

The effect of fertilizer × cut interaction

Results showed the significant effect of fertilizer × cut interaction on most of the measured traits (Table 2). Mean comparison indicated that although plant height and shoot yield were the highest in $N_{160}P_{128}K_{160}M_0$ × the first cut, in both years; however, essential oil percentage and essential oil yield were the highest in treatments containing both chemical fertilizers and manure (Table 5). Essential oil yield was the highest in $N_{60}P_{48}K_{60}M_{25}$ × the first cut, in both years.

Table 7. Effect of year on the measured traits, obtained by combined analysis.

Treatments	Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)
First year	21.54b	1199.05b	1.02	12.45b	69.06a	6.81b	4.73a
Second year	23.49a	1304.97a	1.25a	16.60a	62.68b	10.06a	3.77b

Means in a column followed by the same letter are not significantly different at P≤0.01.

To compare the effect of years on the measured traits, combined analysis was conducted. Results showed that all traits varied significantly in two years (Table 6). Plant height, shoot yield, essential oil percentage and essential oil yield were the highest in the second year (Table 7). Climatic factors such as precipitation and temperature were mostly the same in two years (not shown), so variations in the measured traits may be attributed to plant's aging.

Conclusion

Most of the measured traits were significantly affected by fertilizer, cut, their interaction and year. Among the interactions, essential oil yield was the highest in $N_{60}P_{48}K_{60}M_{25}$ × the first cut (22.14 kg/ha in 2008 and 21.25 kg/ha in 2009). Results also showed that concentration of the three main compounds in essential oil varied greatly between the different treatments. So, treatments must be selected carefully in commercial production in order to increase the concentration of the desired compound.

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