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Original Article

Effect of Nutrition and Harvest Time on Growth and Essential Oil Content of *Thymus vulgaris* L.

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Abstract

Thyme (*Thymus vulgaris* L.) is an important medicinal plant with highly valuable essential oils. In this study, effects of different fertilizer treatments and cuts on *Thymus vulgaris* L. was evaluated in 2008-2009. The research was conducted at Alborz Research Station, Research Institute of Forests and Rangelands, Karaj, Iran. The Experimental design was split plot in time in the form of a randomized complete block design with three replications. The main factor was nutrition in 16 levels consisting of different combinations of nitrogen (N), phosphorus (P), potassium (K) and manure (M): $N_0P_0K_0M_0$, $N_{40}P_{32}K_{40}M_0$, $N_{80}P_{64}K_{80}M_0$, $N_{120}P_{96}K_{120}M_0$, $N_{160}P_{128}K_{160}M_0$, $N_{140}P_{112}K_{140}M_5$, $N_{120}P_{96}K_{120}M_{10}$, $N_{100}P_{80}K_{100}M_{15}$, $N_{80}P_{64}K_{80}M_{20}$, $N_{60}P_{48}K_{60}M_{25}$, $N_{40}P_{32}K_{40}M_{30}$, $N_{20}P_{16}K_{20}M_{35}$, $N_0P_0K_0M_{40}$, $N_0P_0K_0M_{30}$, $N_0P_0K_0M_{20}$ and $N_0P_0K_0M_{10}$ (indices following the letters N, P and K are application rates in kg/ha, indices following the letter M are the application rates of manure in t/ha). The sub factor was harvest (cut) time (late May and early September). Results indicated that fertilizer, harvest date and their interactions significantly affected most of the traits. Mean comparison of the interactions indicated that treatments containing both chemical fertilizers and manure showed positive effects, although the most plant height was achieved with $N_{160}P_{128}K_{160}M_0 \times 1^{st}$ cut, in both years (32.5 cm in 2008 and 32.66 cm in 2009). Essential oil yield was the highest in 2008 in $N_{120}P_{96}K_{120}M_{10} \times 2^{nd}$ cut (26.01 kg/ha), and in 2009 in $N_{100}P_{80}K_{100}M_{15} \times 1^{st}$ cut (26.79 kg/ha). Generally, it can be concluded from the results that *T. vulgaris* responds well to fertilization, and selecting the best treatment depends on the objective of production.

Keywords: Essential oil, P-cymene, Thyme, Thymol, γ-terpinene

Introduction

Thyme (*Thymus vulgaris* L.), belonging to Lamiaceae, is a medicinal plant native to the Mediterranean region and which is cultivated in many parts of the world. Thyme is a highly branched perennial shrub with many applications in food and pharmaceutical industry. The essential oil of this plant has antispasmodic, antiseptic, carminative, antioxidative, antifungal and antibacterial features [1-5].

Meeting the nutritional requirements of medicinal plants is important for sustained economical production. Nitrogen, phosphorus and potassium are the main macronutrients affecting nearly all aspects of plant growth and yield. Nitrogen is one of the most important yield limiting nutrients in crop production. It has roles in amino acids, enzymes, proteins, chlorophyll and cell wall production. Phosphorus is essential for plant growth and reproduction and plays vital role in cell membranes, nucleic acids and energy transfer and storage. Potassium is involved in regulation of stomata and turgor pressure and activation of at least 60 enzymes [6-9].

The mentioned roles reveal the importance of nutrients in crop production. Nutrients may be provided to plants in form of the chemical fertilizers. Omidbaigi and Rezaei Nejad [10] tested the effect of various N fertilizer rates on *Thymus*

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vulgaris and reported that yield increased from 671.88 kg/ha up to 1021.00 kg/ha when 150 kg N/ha was applied; however, essential oil yield was not significantly changed. Baranauskiene *et al*. [11] also found that applying N fertilizer significantly affected *T. vulgaris* yield, but had no significant effect on the essential oil yield. Verma *et al*. [12] studied the effect of different ratios of N and P on clary sage (*Salvia sclarea* L.) and concluded that 100 kg N/ha × 60 kg P/ha had the highest effect on plant growth, essential oil percentage and yield; however, the treatments had no effect on the composition of essential oil. Valmorbida and Boaro [13] evaluated the influence of 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.

As well as the chemical fertilizers, non chemical sources such as biofertilizers and animal manure may have positive effects on plant growth and yield by providing nutrients to plant. Animal manure, in addition to releasing macro and micronutrients, increases plant growth and yield by affecting a wide range of soil biological and physico-chemical properties such as organic matter content, pH and water holding capacity [14]. Tabrizi *et al.* [15] reported that application of animal manure significantly affected thyme biomass; however, had no significant effect on oil production. Rahbarian *et al*. [16] reported that manure application alleviated the effect of drought stress on leaf relative water content in *Dracocephalum moldavica*. Regarding the benefits of chemical fertilizers and animal manure on various crops and medicinal plants, the objective of this experiment was to assess the effect of different chemical fertilizers (NPK) ratios and animal manure on growth and essential oil of *Thymus vulgaris* 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 different sources of nutrients and cuts on *Thymus vulgaris* 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.

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

Harvest (cut) time as the sub factor

Included two cuts. The first cut was at full flowering stage 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 the 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 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

Analysis of variance indicated that fertilizer application significantly affected all the measured traits in both years, including plant height, flowering shoot yield and the main essential oil components (Table 2). In both years, plant height and shoot yield were almost the highest in $N_{160}P_{128}K_{160}M_0$ treatment (Table 3), 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 many physiological and biochemical processes in plants. Nitrogen is the most frequently found element within plants body with greater effect on plants growth and yield than any other nutrient. Nitrogen is involved in the structure of amino acids, nucleic acids, chlorophyll, enzymes, proteins and cell walls. Phosphorus is also a vital nutrient in both vegetative and reproductive stages of plant life cycle and is essential for energy storage and transfer, metabolism, respiration and photosynthesis. Potassium which is an abundant nutrient in most soils, is involved in regulation of stomata, turgor pressure, respiration and photosynthesis, and activation of at least 60 enzymes [6-9,17,18]. So, availability of NPK to plants affects nearly all aspects of plant life including growth, yield and in medicinal plants, essential oil percentage, yield and composition.

The effect of NPK fertilizers is well studied on many plants. Biesiada and Kuś [19] found that the highest basil (*Ocimum basilicum* L.) yield was achieved when 150-250 kg N/ha was applied. They reported that increasing nitrogen dose from 50 to 150 kg N/ha increased yield by 63.90%, while increasing the dose to 250 kg N/ha increased yield by only 11.46%. In another experiment on basil it was concluded that increasing N application rate up to 60 kg/ha increased yield; however, higher N rates did not significantly increase yield [20]. Verma *et al*. [12] studied the effect of different ratios of N and P on clary sage (*Salvia sclarea* L.) and concluded that 100 kg N/ha × 60 kg P/ha had the highest effect on plant growth, essential oil percentage and yield; however, the treatments had no effect on the composition of essential oil. Anwar *et al*. [21] also studied the effect of different NPK ratios on mint (*Mentha arvensis* L.) and reported that increasing NPK ratio from 50:20:20 to 200:80:80 kg/ha increased dry matter from 6.43 to 7.19 t/ha.

Results of this experiment indicated that although the best plant height and shoot yield were achieved in the treatments with the highest fertilizer rates and without manure application; however, essential oil yield which is the main objective in medicinal plant production, was the highest in $N_{120}P_{96}K_{120}M_{10}$ in the first year and in $N_{60}P_{48}K_{60}M_{25}$ in the second year; the treatments that animal manure is replaced instead of high doses of chemical fertilizers (Table 3). The same trend was observed in essential oil percentage which was higher in the treatments with manure application. This proves the possibility of using animal manure instead of some parts of chemical fertilizers; lowering the environmental risks of chemical fertilizers. NPK normally boost vegetative plant growth and cause a quick growth. However, 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. Forouzandeh *et al.* [22] found that applying manure instead of chemical fertilizers increased essential oil percentage from 2.39 to 2.66%. Patra *et al.* [23] also reported the improvement of essential oil yield as the results of manure application. In their experiment, 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.

Results also indicated the variation of essential oil composition between different treatments and years. For example, thymol content in 2008 was the highest in $N_0P_0K_0M_{40}$ however, p-cymene was the highest in $N_{20}P_{16}K_{20}M_{35}$ and γ-terpinene was the highest in $N_{80}P_{64}K_{80}M_{20}$. These results represent that treatments must be selected carefully to improve the content of a certain compound for commercial objectives, when a certain compound is desired; of course further experiments are required to make such decision. The effect of chemical fertilizers and manure on the quality of medicinal plant products was tested in the experiments of Berti *et al.* [24] who found that N application significantly increased gamma-linolenic acid (GLA) content, a highly desired acid in borage (*Borago officinalis* L.) seed. Anwar *et al.* [21] and Alipour Mansoorkhani *et al.* [25] reported the significant effect of different NPK ratios on the essential oil composition of mint and basil, respectively. Ateia *et al.* [26] also reported the effect of different manure and compost treatments on essential oil composition of thyme.

Analysis of variance also indicated the significant effect of cut on all measured traits, except for the essential oil percentage in the first year (Table 2). Differences between the two cuts may be attributed to both plant regrowth and climatic factors, 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 2008, shoot yield was higher in the first cut; however, essential oil percentage was higher in the first cut and essential oil yield was higher in the second cut (Table 4). This proves that essential oil yield is mainly affected by shoot yield rather than by essential oil percentage. In 2009, flowering shoot yield, essential oil percentage and essential oil yield were all the highest in the first cut. The variation of thyme growth and essential oil was also observed in the experiments of Ateia *et al.* [26].

The interaction of fertilizer × cut had also a significant effect on most of the measured traits (Table 2). Mean comparison indicated that most traits were the highest in treatments containing both chemical fertilizers and manure; however, plant height was the highest in $N_{160}P_{128}K_{160}M_0$ in both years (Table 5). Essential oil yield was the highest in 2008 in $N_{120}P_{96}K_{120}M_{10}$ × the second cut, and in 2009 in $N_{100}P_{80}K_{100}M_{15}$ × the first cut.

The combined analysis which was conducted in order to evaluate the effect of year showed the significant effect on all measured traits except for plant height and essential oil yield (Table 6). Although flowering shoot yield was higher in the second year and essential oil percentage was higher in the first year; however, essential oil yield was significantly the same in both years (Table 7). Climatic factors such as precipitation and temperature were mostly the same in two years, so variations in the measured traits may be attributed to plant's aging.

Table 2 Analysis of variance of different traits of thyme in response to nutrition and harvest time factors.

SOV	df	Mean Squares (MS) 2008 Plant height	Flowering shoot yield	Essential oil percentage	Essential oil yield	Thymol	P-cymene	γ-terpinene	2009 Plant height	Flowering shoot yield	Essential oil percentage	Essential oil yield	Thymol	P-cymene	γ-terpinene
Block	2	**	**	ns	**	ns	**	**	**	**	**	**	ns	**	*
Fertilizer (A)	15	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Error (A)	30	2.29	9252.93	0.033	89183.26	0.035	0.14	1.15	0.27	10088.81	0.01	49339.07	5.22	8.85	1.308
Cut (B)	1	**	**	ns	**	**	**	**	**	**	**	**	*	**	**
A × B	15	**	**	ns	ns	**	**	**	**	ns	*	**	**	**	**
B × Block	2	**	**	ns	**	ns	ns	ns	ns	**	**	**	ns	ns	ns
Error	30	1.004	5684.24	15.17	102034.3	3.58	5.207	1.29	0.23	9429.97	0.011	38873.13	5.18	4.57	3.50
CV (%)	-	4.02	4.99	10.37	18.47	3.87	1.85	7.95	96.1	5.64	10.69	14.36	5.98	8.46	19.31

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

Table 3 Mean comparison of different traits of thyme in respond to nutrition factors.

Treatments	2008 Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)	2009 Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)
$N_0P_0K_0M_0$	22.79h	1064.2m	1.1ef	11.73h	34.70j	27.9c	14.6e	19.80o	1255.0g	0.80g	10.15i	34.71gh	32.28bc	12.26c
$N_{40}P_{32}K_{40}M_0$	23.81f	1405.5i	0.83i	11.7h	39.88c	23.10j	15.56d	22.2n	1557.16f	0.81g	13.02h	34.09h	31.56c	14.69a
$N_{80}P_{64}K_{80}M_0$	25.14e	1509.5h	1.18cd	17.95de	40.35c	24.06h	9.31h	22.30L	1582.16ef	0.98de	15.92f	33.91h	33.09b	11.63cd
$N_{120}P_{96}K_{120}M_0$	26.83c	1721.33c	1.18cd	20.78b	35.31i	26.58d	16.01c	26.54d	1801.50c	1.03bc	19.1bcd	37.85f	31.72c	10.83de
$N_{160}P_{128}K_{160}M_0$	28.86a	1868.5a	0.97h	18.18de	28.31m	16.10m	10.11g	29.90a	1863.33ab	0.79g	15.04g	35.45g	32.34bc	10.73e
$N_{140}P_{112}K_{140}M_5$	26.94c	1801.7b	1.01gh	18.19de	36.21g	25.16g	16.65b	28.34b	1847.0b	0.94fe	17.63e	38.40ef	22.51ef	10.93de
$N_{120}P_{96}K_{120}M_{10}$	27.24c	1714.0c	1.3ab	22.42a	36.80f	23.93h	17.58a	27.03c	1863.83ab	1.00cd	18.97cd	38.95de	17.99i	11.02de
$N_{100}P_{80}K_{100}M_{15}$	27.75b	1679.7d	1.13de	18.98cd	38.26e	23.55i	16.10c	26.53cd	1899.33a	1.03cd	20.05a	39.74d	26.49d	4.69i
$N_{80}P_{64}K_{80}M_{20}$	25.74d	1618.16e	1.15cde	18.33de	35.36i	26.33e	17.60a	26.07e	1857.33ab	1.02bc	19.33abcd	41.76c	21.73fg	7.60gh
$N_{60}P_{48}K_{60}M_{25}$	24.9e	1580.0f	1.32a	20.84b	38.81d	25.36f	16.08c	25.45f	1843.83b	1.07ab	20.12a	24.70i	34.76a	11.42de
$N_{40}P_{32}K_{40}M_{30}$	24.0f	1537.5gh	1.30a	20.0bc	33.86k	28.60b	16.15c	24.80h	1790.16c	1.09a	19.79abc	41.41c	22.90e	8.70f
$N_{20}P_{16}K_{20}M_{35}$	24.92e	1556.66fg	1.11def	16.95bf	31.40L	30.18a	13.18f	25.00g	1805.33c	1.11a	19.93ab	49.47a	13.68j	4.39i
$N_0P_0K_0M_{40}$	23.38g	1392.83i	1.30a	18.11de	48.05a	18.33L	5.58i	23.89i	1698.0d	1.11a	19.17bcd	34.77gh	23.40e	8.01fg
$N_0P_0K_0M_{30}$	23.4g	1318.5j	1.22bc	185.99f	33.80k	25.38f	13.20f	23.04j	1671.0d	1.10a	18.75d	37.45f	21.34gh	13.72b
$N_0P_0K_0M_{20}$	22.18i	1273.33k	1.05fg	13.22g	35.76i	28.53b	14.68e	22.77k	1607.50e	0.93f	15.28fg	42.88b	20.65h	7.37gh
$N_0P_0K_0M_{10}$	20.55j	1119.66L	1.18cd	13.28g	43.28b	21.10k	16.63b	21.45n	1563.0f	0.95fe	15.25fg	43.73b	17.82i	7.05h

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 Mean comparison of different traits of thyme in respond to harvest time.

Treatments	2008 Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)	2009 Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)
Cut 1	27.73a	1361.66b	1.17a	15.8b	38.87a	22.72b	13.66b	25.77a	1866.91a	1.19a	22.43a	50.40a	16.77a	12.31a
Cut 2	22.06b	1658.45a	1.12b	18.78a	34.52b	26.56a	14.96a	23.60b	1571.27b	0.78b	12.26b	25.76b	33.76b	7.07b

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

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

Fertilizers	Cuts	2008 Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ-terpinene (%)	2009 Plant height (cm)	Flowering shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Thymol (%)	P-cymene (%)	γ – terpinene (%)
$N_0P_0K_0M_0$	1	24.66g-m	1027.66o	1.14a-g	11.72gh	37.23gh	26.83e	14.10c-f	20.76pq	1285.0kl	1.06b-h	17.76e-l	44.94e	19.94fg	16.33b
	2	20.91p-s	1100.66o	1.06a-g	11.75gh	32.16L	29.13a-c	15.03b-e	18.83r	1225.0L	0.54kl	6.54L	24.48i-k	44.69ab	8.19f-j
$N_{40}P_{32}K_{40}M_0$	1	25.33g-L	1379.0i-n	0.78g	10.2h	45.66c	21.93i	12.10ef	22.50mn	1663.67e-i	1.12a-f	18.85c-h	41.21f	20.78fg	20.45a
	2	22.28L-q	1432.0g-n	0.86e-g	12.49f-h	34.10jk	24.26gh	19.03a	21.54op	1450.67i-k	0.51L	7.18kl	26.97g-j	42.34b	8.93d-g
$N_{80}P_{64}K_{80}M_0$	1	27.16d-h	1451.0f-l	1.17a-f	17.16b-h	48.50b	18.66k	12.93d-f	22.90k-m	1669.33e-i	1.28ab	21.59a-d	45.97de	18.65gh	14.81b
	2	23.12L-q	1568.0c-k	1.18a-f	18.74b-h	20.32L	29.46ab	5.70g	21.70no	1495h-k	0.69i-l	10.28j-l	21.85k	47.54a	8.45e-i
$N_{120}P_{96}K_{120}M_0$	1	30.0a-d	1700.66e-i	1.13a-g	19.87a-f	33.53kl	28.06cd	15.03b-e	27.53c-f	1953.33b-e	1.35a	26.43ab	48.29de	19.79fg	13.99bc
	2	23.66i-p	1742.0c-g	1.23a-e	21.69a-d	37.10gh	25.1fg	17.0a-c	25.56g	1649.66e-i	0.75i-l	11.77i-l	27.42g-i	43.65ab	7.82f-k
$N_{160}P_{128}K_{160}M_0$	1	32.5a	1779.66a-f	0.82f-g	14.49c-h	15.43n	8.16n	5.13g	32.66a	2002.66b-e	1.02a-h	20.49a-f	46.17de	20.67fg	13.49bc
	2	25.22g-m	1957.33ab	1.11a-g	21.88a-d	41.20e	24.03gh	15.10b-e	27.13ef	1724.0dg	0.56j-l	9.59j-l	24.47h-k	12.70ab	7.98f-k
$N_{140}P_{112}K_{140}M_5$	1	29.5b-d	1532.66d-k	1.06a-g	16.26c-h	38.23fg	26.16ef	14.0c-f	28.66b	1999.66b-e	1.17a-d	23.3a-c	53.60c	32.33ij	14.62b
	2	24.38g-m	2070.66a	0.96b-g	20.13a-f	34.2jk	24.16gh	19.30a	28.01b-e	1694.33e-h	0.74h-l	11.99h-l	23.21i-k	11.46cd	7.25g-l
$N_{120}P_{96}K_{120}M_{10}$	1	30.50a-c	1528.0d-k	1.17a-f	17.96b-h	39.56f	20.63j	19.03a-d	28.4bc	2057.66ab	1.22a-c	24.92a-c	55.29bc	24.52j	13.97bc
	2	23.97i-n	1900.0ab	1.4a	26.01a	34.03jk	27.23de	16.16a-d	25.7g	1670.0e-i	0.78e-k	13.02g-l	22.61j-k	18.62ef	8.07f-k
$N_{100}P_{80}K_{100}M_{15}$	1	31.83ab	1487.33e-k	1.19a-e	17.19b-h	43.50d	20.90ij	16.16a-d	28.1b-d	2129.0a	1.27ab	26.79a	55.05bc	34.37gh	5.22j-m
	2	23.66i-p	1872.0abc	1.07b-g	20.17a-f	33.03kl	26.20ef	16.03a-d	25.0gh	1669.66e-i	0.80d-l	13.32f-l	24.44h-k	14.79c	4.15m
$N_{80}P_{64}K_{80}M_{20}$	1	28.5c-f	1395.0i-n	1.22a-e	17.08b-h	35.46ij	26.60e	18.03ab	27.6c-f	2064.0ab	1.22a-c	25.12a-c	19.58b	28.67h-j	7.54g-l
	2	22.97k-q	1841.33a-d	1.09a-g	19.58b-g	35.26ij	26.06ef	17.16a-c	24.56hi	1650.66e-i	0.82b-l	13.54e-l	25.33g-k	34.19de	7.66g-k
$N_{60}P_{48}K_{60}M_{25}$	1	27.63c-g	1366.0i-n	1.24a-d	16.94b-h	39.40f	26.63e	15.10b-e	27.43d-f	2072.66ab	1.26ab	26.01a-c	21.37k	35.33c	15.02b

Table 5 (Continue)

	2	22.16m-q	1794.0a-e	1.4a	24.75ab	38.23gf	24.10gh	17.06a-c	23.47g-l	1615.0f-j	0.88c-k	14.25e-k	28.03gh	14.40c	7.83g-k
$N_{40}P_{32}K_{40}M_{30}$	1	26.33e-i	1349.33j-n	1.32ab	17.75b-h	33.50L	28.13cd	18.16ab	26.8f	1937.0b-e	1.27ab	24.63a-c	53.28c	14.4h-j	11.26c-e
	2	21.66p-r	1725.66b-h	1.28a-c	22.25a-c	30.3L	29.06a-c	14.13c-f	22.8k-m	1643.33f-i	0.91b-j	14.96dj	29.54g	31.40cd	6.15h-m
$N_{20}P_{16}K_{20}M_{35}$	1	29.0b-e	1417.33g-n	1.32ab	18.52b-h	32.50L	30.10a	11.26f	25.5g	1922.66b-e	1.08a-h	20.82a-e	73.24a	5.70k	4.58Lm
	2	20.83p-s	1696.0b-i	0.91b-g	15.39c-h	30.30hi	30.26a	15.10b-e	24.5hi	1688.0e-h	1.13a-f	19.05b-i	25.07g-k	21.66fg	4.21m
$N_0P_0K_0M_{40}$	1	26.0e-j	1137.66L-o	1.39a	15.73c-h	60.0a	11.46m	7.13g	23.93g-i	1851.33b-e	1.27ab	23.65a-c	45.14e	17.37g-i	10.58d-f
	2	20.76p-s	1648.0b-j	1.22a-e	20.49a-e	36.1hi	25.20fg	4.03g	23.9g-i	1544.66g-j	0.95b-h	14.70d-j	24.41h-k	29.42d	5.45j-m
$N_0P_0K_0M_{30}$	1	26.5e-i	1122.33L-o	1.32ab	14.73c-h	34.43jk	23.50h	11.20f	23.53jk	1811.33c-f	1.28ab	23.35a-c	49.27d	14.42h-j	16.03b
	2	20.31q-s	1514.66e-k	1.13a-g	17.26b-h	33.16kl	27.26de	15.20b-e	22.55L-n	1530.66g-j	0.92b-j	14.16e-k	25.62g-k	28.26de	11.40cd
$N_0P_0K_0M_{20}$	1	25.63f-k	1112.66m-o	1.21a-e	13.56e-h	38.46gf	28.86bc	13.13d-f	23.13j-m	1729.66d-g	1.11a-g	19.25b-h	57.16bc	13.41ij	9.67d-g
	2	18.73r-s	1434.0g-n	0.89d-g	12.89f-h	33.06kl	28.20cd	16.23a-d	22.42m-n	1485.33h-k	0.76f-l	11.32j-l	28.60gh	27.89de	5.07k-m
$N_0P_0K_0M_{10}$	1	22.73k-q	1000.33o	1.24a-d	12.4f-h	46.56c	16.96L	16.06a-d	22.8k-m	1751.66d-g	1.15a-e	15.95a-g	58.30b	11.49j	9.45d-g
	2	18.36s	1239.0k-n	1.13a-g	14.15d-h	34.0jk	25.23fg	17.20a-c	20.1q	1404.33j-l	0.75g-l	10.56j-l	29.16g	24.15ef	4.65Lm

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.

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	ns	**	**	ns	**	*	**
Error (A)	4	74	454934.22	0.27	916105.86	1.11	23.0	6.05
Fertilizer (B)	15	**	**	**	**	**	**	**
A × B	15	**	**	*	**	**	**	**
Error	60	0.64	4835.44	0.012	34630.58	1.32	2.25	0.61
CV (%)	-	3.23	4.3	10.05	10.74	3.1	6.01	6.52

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

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	24.90a	1510.06b	1.14a	17.29a	36.69b	24.64b	14.31a
Second year	24.68a	1719.09a	0.99b	17.34a	38.08a	25.27a	9.69b

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

Conclusion

Results of this study indicated the significant effect of fertilizer, cut and year on most of the measured traits. Among the interactions, essential oil yield was the highest in 2008 in $N_{120}P_{96}K_{120}M_{10}$ × the second cut, and in 2009 in $N_{100}P_{80}K_{100}M_{15}$ × the first cut. The concentration of three main compounds in the essential oil varied greatly between the different treatments. So, treatments must be selected carefully in commercial production to increase the concentration of a desired compound.

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