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Influence of Nitrogen Fertilizers on the Yield and Composition of Thyme (*Thymus vulgaris*)

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The influence of nitrogen fertilizers on the yield of crop, as well as on the production and composition of the essential oil and some other chemical characteristics of thyme, was investigated. Different levels of fertilizers ($N = 0, 45, 90$, and 135 kg ha^{-1}) were applied. It was found that fertilizers increase thyme crop, but differences in the yield of essential oil were not remarkable. However, the use of certain amounts of nitrogen fertilizers resulted in higher yields of essential oil obtainable from the cultivation area unit (dm^3 ha^{-1}). Totally, 61 constituents were identified in thyme essential oil by capillary GC and GC-MS. Thymol was the dominating compound in the all analyzed oils (44.4−58.1%), followed by *p*-cymene (9.1−18.5%), γ-terpinene (6.9−18.9%), and carvacrol (2.4−4.2%). Differences in the percentage of these and other compounds in thyme herb cultivated under different fertilization doses were not significant; very slight changes in the percentage composition were detected after drying. Some variations in the amount of individual constituents expressed in arbitrary units per kilogram of herb (which is almost equivalent to mg kg^{-1}) were observed. The highest amounts of sugars and sucrose, in particular, were determined in the second year of thyme cultivation. Differences in the content of dry soluble substances were not meaningful, and there was no effect of nitrogen fertilizers on this chemical characteristic. Some effect of fertilization on the content of vitamin C and carotenes was observed in the first year of thyme cultivation. It was determined that nitrogen fertilizers influence the amount of nitrates, which was highest in the second-year−first-harvest.

KEYWORDS: Thyme; *Thymus vulgaris*; Labiatae; nitrogen fertilizers; chemical composition; essential oil; yield

INTRODUCTION

Thyme (*Thymus vulgaris* L., Labiatae) is an important aromatic plant, and its herb, extracts, and essential oils are used for, for example, the flavoring of sauces, meats, canned foods, and beverages, and the volatile oil is used for the scenting of perfumery products (*1*, *2*). As a valuable medicinal plant, *T. vulgaris* possesses antispasmodic, antiseptic, expectorant, carminative, antitussive, antimicrobial, and antioxidative properties (*3*−*16*).

The content of essential oil and its composition in thyme depend on different factors, particularly its chemo- and biotype and cultivation conditions, such as climate, geographic origin, harvesting time, and use of fertilizers.

The essential oil chemistry and polymorphism in the genus *Thymus* were recently comprehensively reviewed, and especially the phenolic monoterpenes thymol and carvacrol were found to be the most important compounds (*17*−*19*) with a wide range of amount in thyme of different origins (*19*−*26*).

The mineral nutrition of herbs can affect oil production and quality. The addition of fertilizers is dependent on the soil type and existing soil fertility. For instance, U.S. growers broadcast triple-superphosphate fertilizer prior to sowing thyme at a rate of 1360 kg ha^{-1} and, following each harvest, side dress with a nitrogen fertilizer. In Australia most herb crops are treated as leafy vegetables, and fertilizer rates suggested vary between 1200 and 1500 kg ha^{-1} of an NPK fertilizer analysis 5:7:4. Following each harvest a light side dressing of up to 75 kg ha^{-1} ammonium nitrate can be applied (*27*). Experiments conducted in pots showed that dry matter production increased by increasing the nitrogen level to 160 kg ha^{-1}. However, the extra growth resulting from doubling the N rate from 80 to 160 kg ha^{-1} was small, suggesting the lower rate is more economical (*27*).

The higher yield of thyme was achieved by dense cultivation and fertilization; however, the essential oil content was not influenced by either plant spacing or fertilization treatment (*28*). In another work it was concluded that nitrogen rates ranging from 50 to 150 kg ha^{-1} were optimum for thyme production; within this range, the specific rate will vary depending on soil fertility (*29*). The effects of organic and inorganic N fertilizers

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on yield of thyme were compared, and it was found that urea and cow manure were superior to ammonium nitrate in terms of total plant fresh yield. It was concluded that urea and cow manure are the best sources of N fertilizer for thyme production (*30*).

The effect of nitrogen and phosphorus supply was investigated on a 3-year-old garden thyme (*T. vulgaris* L.) population. After the application of six different dosages of nitrogen (0, 100, 150, 200, 250, and 300 kg ha^{-1}) and phosphorus (0, 50, 100, 150, 200, and 250 kg ha^{-1}), it was determined that fertilization had a significant effect on herb yield and essential oil content but did not change the thymol content (*31*).

The above-mentioned studies indicate that there is a need for further and more comprehensive investigations on the effect of fertilizers on thyme yield and quality. This study was aimed to investigate the influence of different levels of nitrogen fertilizers on the thyme crop yield and the production and composition of the essential oil and some other components.

MATERIALS AND METHODS

Plant Material and Isolation of Volatiles. Thyme (*T. vulgaris* L.) was cultivated in the experimental fields of the Lithuanian Institute of Horticulture for two consecutive years, 1997 and 1998. The distance between the plants in the rows was 30 cm; the distance between the rows was 45 cm; the length and width of each testing field were 3 and 3.15 m, respectively (total area = 9.45 m^2). The soil was sod gleyc clay loam on clay loam with the pH 6.1−7.4. The amount of mobile phosphorus was 210−267 mg kg^{-1}; potassium, 163−194 mg kg^{-1}; and humus, 2.1−3.0%. The experimental fields were treated with phosphorus (P_2O_5) at 120 kg ha^{-1} and with potassium (K_2O) at 150 kg ha^{-1}.

In the first year of cultivation on May 20 thyme herb was treated with four different doses of ammonia saltpeter, N = 0, 45, 90, and 135 kg ha^{-1}, and harvested on September 22. After the fertilization, the content of nitrogen in the soil present in the forms of nitrate, ammonia, and mineral was measured; results were similar for all experimental fields; at a 60 cm depth it was almost twice as high as at 30 cm. For instance, the content of mineral nitrogen at the 30 cm depth was 38.0−42.1 kg ha^{-1} and that at the 60 cm depth was 67.9−73.3 kg ha^{-1}. Thyme plants were affected by the 1997/1998 winter frosts; however, shoot growth at the end of April 1998 was successful, and the plants started flowering in time. During the second year of cultivation thyme herb was harvested twice: on June 26, during the intensive flowering phase; and on September 26, after the plants had fully regrown. During the second cultivation year the fields were treated only with ammonia saltpeter. Each experimental field was divided in two equal parts, one of which was not fertilized additionally after first harvesting; the second one was fertilized with 45 kg of nitrogen ha^{-1}. Consequently, eight different levels of nitrogen fertilizer were applied: 0, 0 + 45, 45, 45 + 45, 90, 90 + 45, 135, and 135 + 45 kg ha^{-1}. The first fertilization was performed on May 22 and the second on July 1, 1998.

Harvested herbs were dried at ambient temperature in the dark. The yield of essential oil in fresh and dried thyme was determined by hydrodistillation in a Clevenger-type apparatus (*32*).

Soluble solids were determined by refractometer method using an Abbe refractometer (model I, Carl Zeiss, Jena, Germany) (*33*). Ascorbic acid (vitamin C) was determined by titrimetric method using 2,6-dichloroindophenol (*34*). Sucrose was determined by reducing sugars before and after inversion; sugars (reducing) were determined by inversion method (*35*). Nitrates were determined on a potentiometer pH-150 with an ion selective electrode EM-020604 (NPO Izmeritelnaja technika, Russia) (*36*). Total nitrogen content was determined according to the Kjeldahl method (*37*). Potassium was determined by a flame photometric method (*38*) and phosphorus by a spectrophotometric method with molybdovanadate (*39*). Macro- (calcium and magnesium) and microelements (copper, iron, manganese, and zinc) were determined according to an atomic absorption spectrophotometric method (*40*). Carotenes were determined by a spectrophotometric method measuring extinction at 450 nm in hexane (*41*).

All analyses were replicated four times. Data were statistically handled by one-way analysis of variance (ANOVA, vers. 2.2, 1999). Fisher's test was applied for the calculation of the least significant different (LSD_{05}) among the fertilization treatments.

Gas Chromatography (GC). The oil was diluted in pentane (1% v/v) and analyzed on a Fisons 8000 series (Fisons Instruments Inc., Rodano MI, Italy) gas chromatograph equipped with a flame ionization detector (FID) and a BPX-5 fused silica capillary column (5% phenylpolysiloxane, 50 m length, 0.32 mm i.d., 0.25 μm film thickness, SGE International Pty. Ltd., Ringwood, Australia). The carrier gas was helium at a volumetric flow rate of 2.35 mL min^{-1}; the detector's temperature was 320 °C, and the oven temperature was programmed from 50 °C (2 min) to 280 °C (10 min) at the rate of 4 °C min^{-1}. A split/splitless injector was used at 260 °C in split mode at a ratio of 1:5; the injection volume was 1 μL. The content of eluted compounds was calculated on a DP800 integrator and expressed as a GC peak area percent and in arbitrary units per kilogram of herb (au kg^{-1}). The latter unit would be equal to mg kg^{-1}, in the case of the GC response factor being equal to 1. The mean values were calculated from four injections of the same sample. The coefficient of variation is defined as the ratio of the corresponding standard deviation (%RSD) to the average value from four replicate injections.

Gas Chromatography−Mass Spectrometry (GC-MS). GC-MS analyses were performed on an HP 5890 (II) gas chromatograph coupled to an HP 5971 series mass selective detector (Hewlett-Packard, Avondale, PA) operating in the electron impact ionization mode at 70 eV; the mass range was m/z 30−550. The HP5-MS capillary column (dimethylpolysiloxane, 5% diphenyl, 30 m length, 0.25 mm i.d., 0.25 μm film thickness, Hewlett-Packard) was used at the same temperature program as described above. Helium was used as a carrier gas at a linear flow velocity of 40 cm s^{-1} at 50 °C (1.20 mL min^{-1}).

The components were identified by comparison of their Kovats indices (KI) relative to C_8−$C_{30,32}$ *n*-alkanes (Sigma Chemical Co., St. Louis, MO) on a BPX-5 column with those reported by Adams (*42*) and by comparison of their mass spectra with the data provided by the NIST, NBS 75K, and EPA mass spectra libraries. Additionally, the identity of many compounds was confirmed by co-injection of reference compounds, which were purchased from various companies (Fluka and Sigma-Aldrich).

RESULTS AND DISCUSSION

Thyme Yield and Essential Oil Content. First-year vegetation thyme herb was harvested on September 26. The yields of thyme crop and essential oils are presented in **Table 1**. Although rather high deviations were determined for the total crop measurements, there is a clear tendency showing the increase of thyme herb crop with the increase of nitrogen fertilizers; the highest crop, 6.08 t ha^{-1}, was obtained at a fertilizer dose of 135 kg ha^{-1}. After drying, the weight of thyme herb decreased almost 3 times and varied between 1.83 and 2.23 t ha^{-1}. Dried thyme herb is widely used in flavorings and herbal tea preparations; therefore, it is important to know its yield. The effect of fertilization on the yield of thyme can be described by the second-order polynomial regression equation $y = 2 \times 10^{-5}x^2 + 0.0023x + 5.417$, with a determination coefficient $R^2 = 0.9993$ (fresh herb), or $y = 1.8455 + 0.0049x - 2 \times 10^{-5}x^2$, $R^2 = 0.9433$ (dried herb).

The effect of fertilizers on the essential oil content in herb was not considerable; it was in the range of 0.71−0.82 cm^3 100 g^{-1} (fresh herb) or 2.10−2.27 cm^3 100 g^{-1} (dried herb). However, when calculated on the cultivation area unit basis, the total amount of oil obtained from fertilized fields (43.8−47.5 dm^3 ha^{-1}) was higher as compared with control fields (38.5 dm^3 ha^{-1}); that is, nitrogen fertilizers increased oil output by ∼7 L from 1 ha. It is important to note that in terms of the essential oil yield the doses of 45−90 kg ha^{-1} were the most effective. This is in agreement with the study that reported the

Table 1. Yield of Crop and Content of Essential Oil of Thyme during Two Years of Cultivation

amount of fertilizers, kg ha^{-1}	yield of herb crop, t ha^{-1}		oil content, cm^3 100 g^{-1}		yield of oil, dm^3 ha^{-1}	
	fresh herb	dried herb	fresh herb	dried herb	fresh herb	dried herb
First Year of Cultivation						
N_0	5.42 ± 0.47	1.83 ± 0.08	0.71 ± 0.06	2.16 ± 0.13	38.48 ± 3.37	39.53 ± 1.73
N_{45}	5.55 ± 0.60	2.08 ± 0.11	0.80 ± 0.08	2.27 ± 0.12	44.40 ± 2.40	47.22 ± 2.40
N_{90}	5.79 ± 0.56	2.11 ± 0.10	0.82 ± 0.09	2.14 ± 0.18	47.48 ± 4.73	45.21 ± 2.12
N_{135}	6.08 ± 0.61	2.23 ± 0.20	0.72 ± 0.08	2.10 ± 0.08	43.78 ± 3.35	46.83 ± 4.07
Second Year of Cultivation; First Harvest of Thyme						
N_0	2.14 ± 0.49	0.65 ± 0.04	0.41 ± 0.08	1.21 ± 0.06	8.77 ± 1.76	7.87 ± 0.44
N_{45}	2.31 ± 0.51	0.67 ± 0.05	0.43 ± 0.02	1.68 ± 0.09	9.93 ± 0.38	11.26 ± 0.84
N_{90}	2.24 ± 0.52	0.66 ± 0.03	0.32 ± 0.12	1.33 ± 0.11	7.17 ± 2.83	8.78 ± 0.40
N_{135}	1.95 ± 0.43	0.57 ± 0.07	0.32 ± 0.07	1.40 ± 0.07	6.24 ± 1.39	7.98 ± 0.98
Second Year of Cultivation; Second Harvest of Thyme						
N_0	4.67 ± 0.55	1.42 ± 0.03	0.68 ± 0.10	2.01 ± 0.05	31.76 ± 4.50	28.54 ± 0.67
N_{45}	5.58 ± 0.99	1.71 ± 0.07	0.71 ± 0.01	2.15 ± 0.08	39.62 ± 1.21	36.77 ± 1.33
N_{90}	6.94 ± 1.30	2.15 ± 0.14	0.68 ± 0.05	2.20 ± 0.15	47.19 ± 2.19	47.30 ± 3.15
N_{135}	6.99 ± 0.72	2.31 ± 0.05	0.84 ± 0.10	2.40 ± 0.11	58.72 ± 3.95	55.44 ± 2.45
N_{0+45}	6.74 ± 1.15	1.95 ± 0.12	0.74 ± 0.02	2.49 ± 0.06	49.88 ± 1.10	48.56 ± 2.47
N_{45+45}	7.33 ± 0.97	2.20 ± 0.15	0.68 ± 0.17	2.32 ± 0.13	49.84 ± 4.52	51.04 ± 2.40
N_{90+45}	7.75 ± 1.16	2.33 ± 0.06	0.61 ± 0.02	2.27 ± 0.12	47.28 ± 2.00	52.89 ± 2.76
N_{135+45}	6.44 ± 0.69	1.93 ± 0.09	0.74 ± 0.06	2.45 ± 0.14	47.66 ± 3.79	47.29 ± 2.75

maximum yield of oil in dragonhead was reached with the dose of nitrogen fertilizers of 30 kg ha^{-1}, whereas a further increase to 120 kg ha^{-1} resulted in oil reduction (*43*). The concentration of essential oil in dried herb was ∼3 times higher than in the fresh one; that is, the increase of oil concentration was similar to the decrease of raw material weight after drying. Actually, this shows that the drying procedure did not cause volatile oil losses from thyme herb. The effect of fertilization on the yield of oil per cultivation unit area can be described by the second-order polynomial regression equation $y = 38.283 + 0.2025x - 0.0012x^2$, $R^2 = 0.9815$ (fresh), or $y = 40.196 + 0.1445x - 0.0007x^2$, $R^2 = 0.759$ (dried).

Two crops of herb were harvested during the second year of thyme cultivation (**Table 1**). The first batch was harvested on June 26 during the intensive blooming phase. The first harvest was rather low due to the late start of vegetation of the thyme plants, which were damaged by the winter frosts. It was determined that nitrogen fertilizers almost have no effect on the herb yield, which varied from 1.95 to 2.31 t ha^{-1} (fresh herb) and from 0.57 to 0.67 t ha^{-1} (dried herb). The dependence of the effect of fertilization on the yield of first-harvest thyme can be described by the second-order polynomial $y = 2.141 + 0.0062x - 6 \times 10^{-5}x^2$, $R^2 = 0.9997$ (fresh), or $y = 0.6475 + 0.0013x - 1 \times 10^{-5}x^2$, $R^2 = 0.9801$ (dried).

The content of the essential oil in fresh thyme herb was 0.32−0.43 cm^3 100 g^{-1}, that is, ∼2 times lower as compared with first-year thyme. Due to remarkably lower crops of raw material and essential oil content, the yields of oil per cultivation unit was very low: from 6.24 (N_{135}) to 9.93 (N_{45}) dm^3 ha^{-1} (fresh herb) and from 7.87 (N_0) to 11.26 (N_{45}) dm^3 ha^{-1} (dried herb). The dependence between the amount of nitrogen applied and the yield of oil was not very clear [described as a second-order polynomial, $y = 9.0575 + 0.0118x - 0.0003x^2$, $R^2 = 0.7959$ (fresh), or $y = 8.2475 + 0.0651x - 0.0005x^2$, $R^2 = 0.6185$ (dried)]; however, it was observed that the highest crop of thyme and consequently the yield of essential oil per cultivation area unit were obtained at the dose of 45 kg ha^{-1}.

The second batch of the second-year thyme was harvested on September 26. Climatic conditions were favorable, and the yields of harvested thyme crop were comparable with those obtained during the first year of vegetation, from 4.67 to 7.75 t ha^{-1}. Again, the crops of raw thyme herb in the all cases were higher when nitrogen fertilizers were applied. The highest crop yields, 7.33 and 7.75 t ha^{-1}, were obtained at the doses of N_{45+45} and N_{90+45}, respectively. The second treatment of crops with fertilizers also was quite effective except for the highest dose of nitrogen, N_{135+45}. In general, the positive effect of the second fertilization on the yield decreased with the increase of the amount of nitrogen fertilizers applied during the first fertilization (**Figure 1**).

The total productivity of thyme during the second year of vegetation, when two harvests were collected, was higher compared to the first-year vegetation. For instance, the highest crop harvested during first-year vegetation was 6.08 t ha^{-1} (N_{135}), whereas during the second year it varied from 6.81 (N_0) to 9.99 (N_{90+45}) t ha^{-1}. The dependence of the total thyme crop yield on the amount of applied nitrogen fertilizers in the second year of cultivation is described by a second-order polynomial regression equation: $y = 0.0697x + 13.385 - 0.0004x^2$, $R^2 = 0.905$ (fresh), or $y = 0.0213x + 3.9755 - 0.0001x^2$, $R^2 = 0.9406$ (dried).

The content of essential oil in the second-harvest thyme varied from 0.61 to 0.84 cm^3 100 g^{-1} in fresh herb and from 2.01 to 2.49 cm^3 100 g^{-1} in dried herb. It was ∼2 times higher compared with the first-harvest herb and almost similar to the herb harvested during the first year of cultivation. The yield of oil obtainable from the cultivation area unit varied over a wide range, from 31.8 (N_0) to 58.7 (N_{135}) dm^3 ha^{-1} in fresh herb and from 28.5 (N_0) to 55.4 (N_{135}) dm^3 ha^{-1} in dried herb. Results provided in **Figure 2** clearly show that the use of nitrogen fertilizers can significantly increase the production of essential oil per cultivation area unit.

Chemical Composition. Some chemical characteristics of thyme are provided in **Table 2**. Although similar cultivation conditions were maintained for all experimental fields that were treated with different nitrogen fertilizer levels, standard deviations between replicates in most cases were rather high. Such deviations are quite reasonable for agrotechnological measurements of botanical materials. Therefore, the differences in the concentration for various thyme components determined in the plants treated with different nitrogen levels in most cases were not statistically significant. However, some tendencies showing the effect of fertilization on thyme chemical composition can be observed and will be shortly discussed.

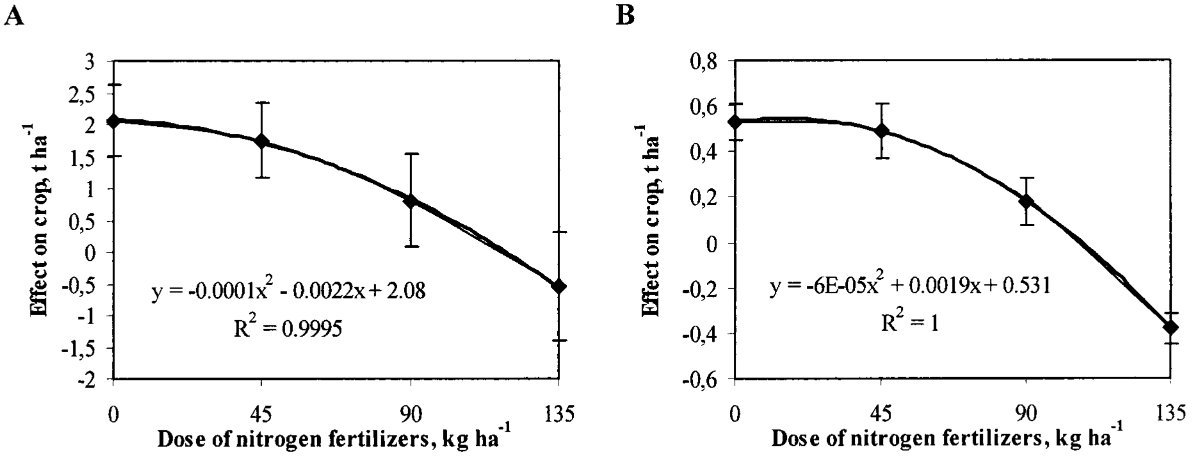

Figure 1. Effect of additional fertilization with N_{45} on the yield changes of second-year vegetation thyme: fresh (A) and dried (B).

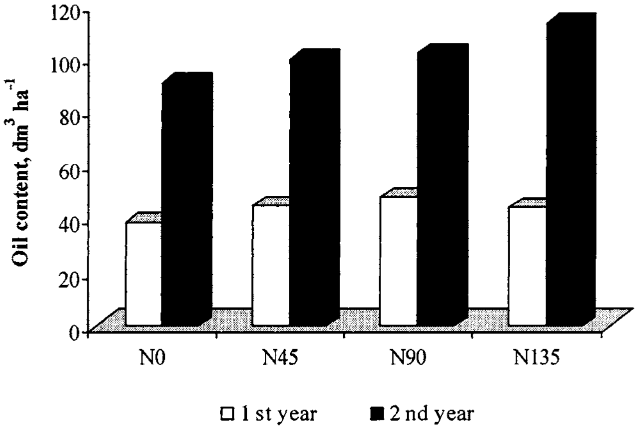

Figure 2. Total essential oil productivity from cultivation area unit.

Table 2. Effect of Nitrogen Fertilizers on Some Chemical Characteristics of Thyme (Fresh Herb)

amount of fertilizers, kg ha^{-1}	vitamin C, mg %	carotenes, mg %	nitrates, mg kg^{-1}
	First Year of Cultivation		
N_0	47.2 ± 9.94	10.18 ± 0.57	406 ± 154
N_{45}	62.4 ± 8.87	10.25 ± 1.09	499 ± 89
N_{90}	64.8 ± 6.45	9.45 ± 0.42	555 ± 192
N_{135}	60.5 ± 6.82	8.62 ± 1.08	649 ± 62
	Second Year of Cultivation; First Harvest of Thyme		
N_0	16.3 ± 2.5	3.51 ± 0.77	1598 ± 72
N_{45}	14.5 ± 1.0	2.93 ± 0.88	1764 ± 122
N_{90}	14.8 ± 1.7	3.75 ± 1.18	2133 ± 306
N_{135}	14.7 ± 0.5	3.17 ± 0.48	1960 ± 188
	Second Year of Cultivation; Second Harvest of Thyme		
N_0	21.0 ± 0.0	6.56 ± 0.52	756 ± 62
N_{45}	25.0 ± 2.83	6.13 ± 0.66	742 ± 191
N_{90}	23.0 ± 0.0	5.49 ± 1.89	674 ± 54
N_{135}	23.0 ± 0.0	5.37 ± 0.54	668 ± 87
N_{0+45}	22.0 ± 1.41	6.18 ± 0.25	715 ± 69
N_{45+45}	23.0 ± 2.83	6.54 ± 1.61	784 ± 76
N_{90+45}	22.0 ± 0.71	4.94 ± 0.15	689 ± 33
N_{135+45}	22.0 ± 1.41	5.90 ± 1.51	734 ± 119

The content of sugars in first-year thyme varied from 1.6 to 2.0% (inverted sugar, 1.1−1.3%; sucrose, 0.5−0.7%); in the second-year thyme, the content varied from 1.8 to 3.0% (inverted sugar, 0.9−1.4%; sucrose, 0.7−1.7%). Higher amounts of sugars and sucrose, in particular, were found in the second-year thyme. A significant increase of total sugars and sucrose was determined in the second-harvest thyme with lower doses of nitrogen (N_{0+45} and N_{45+45}). When the doses were increased to N_{135} and N_{135+45}, some decrease in the content of sugars and sucrose was observed in the second-harvest thyme.

The content of dry soluble substances in the first-year thyme varied between 11.9 and 13.0% and that in the second-year thyme between 9.4 and 12.6%. The differences between thyme treated with different amounts of fertilizers were within the range of standard deviation; however, a lower amount of these substances was found in the second-year−first-harvest thyme.

Some effect of fertilizers on the content of vitamin C was determined in the first-year thyme; it was slightly higher in the plants that were treated with fertilizers. However, more considerable changes in ascorbic acid concentration were found in the second-year thyme. Compared to the first year of cultivation the content of vitamin C in the second year of cultivation herb was lower by approximately 4 and 3 times in the first- and second-harvest plants, respectively. It is possible that the processes of biosynthesis of vitamin C change with plant aging. Also, unfavorable winter conditions, which were mentioned earlier and which severely affected the plants between the first and second years of cultivation, could play some role. It is worth mentioning that the biosynthetic pathway of vitamin C in plants was recently proposed (*44*); however, investigations on the effect of various environmental factors on this pathway in aromatic plants have not been reported. The effect of fertilizers on the concentration of vitamin C during the second year of cultivation was not remarkable. Similar tendencies were observed in the differences of carotene concentrations between the first and second years of cultivation of thyme (**Table 2**). Again, a considerably lower content of carotenes was determined in the second-year−first-harvest thyme. It increased during further growth until the second harvest but did not reach the level found in first-year thyme. The negative effect of high nitrogen doses (>90 kg ha^{-1}) on carotene concentration was determined for the second-harvest thyme.

Due to the use of nitrogen fertilizers it was reasonable to determine the concentration of nitrates in the plants. The content of nitrates varied from 406 (N_0) to 649 mg kg^{-1} (N_{135}) in first-year thyme, and there was a linear dependence (r = 0.9909) between the amount of nitrogen applied to the soil and nitrate concentration in the plants. The highest amounts of nitrates were determined in the first crop of the second year of cultivation: it varied from 1598 to 2133 mg kg^{-1} and was higher in the plants grown in fertilized fields (r = 0.6521). The differences in the amount of nitrates in the second-harvest thyme crop were not significant between various fertilization levels; the concen-

Table 3. Content of Mineral Elements in Thyme Raw Material (Dried Herb)

mineral element	first year	second year, first harvest	second year, second harvest
N, %	1.73	2.26	1.79
P, %	0.22	0.18	0.23
K, %	2.04	1.76	1.86
Ca, %	0.98	0.96	0.97
Mg, %	0.40	0.32	0.30
Fe, mg kg^{-1}	1337	900	1150
Cu, mg kg^{-1}	11.0	16.0	18.0
Mn, mg kg^{-1}	48.8	48.0	29.0
Zn, mg kg^{-1}	17.2	22.5	24.0
B, mg kg^{-1}	17.8	18.5	15.7

tration of nitrates varied in the range of 668−784 mg kg^{-1}. There was a correlation (r = 0.8889) between the nitrogen applied and nitrates in the second-harvest thyme; there was no correlation between the additional fertilization and the amount of nitrates in the raw material. It is interesting to note that the plants harvested in September during both the first and second years of cultivation accumulated comparable amounts of nitrogen fertilizers. It seems that the plants further utilize nitrates accumulated during the early phase of growth until they reach a certain concentration. The contents of most mineral elements were almost similar in both cultivation years of thyme herb (**Table 3**).

Essential Oil Composition. The compositions of thyme essential oil hydrodistilled from the herb harvested during two consecutive years at different doses of nitrogen fertilizers were analyzed by GC and coupled GC-MS. The results of second-year cultivation−second-harvest thyme are presented in **Table 4**. The compounds are listed in order of their elution time on the BPX-5 column, which accounted for >99% of the total peak area of the chromatographic profiles in the examined samples. The major constituents in the volatile oil were characteristic for a widely spread *T. vulgaris* species; however, their percentages were quite different as compared with published results (*21*−*28*).

Phenolic compounds are the aroma principles in this chemotype of thyme. The quantitatively most important compounds are the phenols thymol (44.4−58.1%) and carvacrol (2.4−4.2%), which constituted almost half of the essential oil, followed by monoterpene hydrocarbons *p*-cymene (9.1−18.5%), γ-terpinene (16.1−18.9%), myrcene (1.5−2.2%), and α-thujene (1.0−1.5%). (*E*)-Sabinene hydrate (0.7−1.2%), linalool (1.5−2.8%), and borneol (0.4−1.7%) were dominant among monoterpenic alcohols. Among monoterpene alcohols, (*Z*)- and (*E*)-*p*-menth-2-en-1-ol in the first-year vegetation thyme were present only in traces and were not detected in the second-year harvest thyme. 1,8-Cineole was the unique monoterpene epoxide detected in thyme herb, which constituted from 1.0 to 1.9% of total volatiles. β-Caryophyllene (1.3−2.5%), germacrene D (0.1−0.5%), and δ-cadinene (0.1−0.3%) were the most abundant sesquiterpenes in the oil. Among oxygenated sesquiterpenes, caryophyllene oxide was the most abundant component (0.1−0.7%).

There were some changes in the percentage of these compounds in thyme herb cultivated under different fertilization doses for two years. The differences in the chemical composition of essential oils of first-year thyme treated with different doses of nitrogen fertilizers were not remarkable. Thymol and carvacrol constituted 44.4−49.9 and 3.1−4.2%, respectively; monoterpene hydrocarbons *p*-cymene and γ-terpinene were found from 9.5 to 11.4% and from 16.1 to 18.9%, respectively. The percentage composition of individual constituents of the

Table 4. Percentage Composition of Essential Oil of Second Year of Cultivation, Second Harvest of Thyme (*T. vulgaris* L.) GC Peak Area Percentage[a]

	constituent	KI	fresh herb	dried herb
1	tricyclene^{b}	926	tr^{f}	tr
2	α-thujene^{b,e}	934	1.2–1.4	1.0–1.5
3	α-pinene^{b,e}	942	0.8–0.9	0.8–1.1
4	camphene^{b,e}	949	0.4–0.7	0.5–0.7
5	5sabinene^{b,e}	970	0.3–0.4	0.1–0.3
6	β-pinene^{b,e}	974	0.4–0.6	0.4–0.7
7	oct-1-en-3-ol^{b,e}	976	tr–0.2	tr–0.2
8	myrcene^{b,e}	987	1.7–1.8	1.4–2.0
9	α-phellandrene^{b,e}	1000	0.1–0.2	0.0–0.1
10	δ-3-carene^{b,e}	1007	0.1	tr–0.1
11	α-terpinene^{b,e}	1014	1.3–1.6	0.5–1.4
12	*p*-cymene^{b,e}	1024	9.7–13.7	12.2–15.6
13	1,8-cineole^{b,e}	1031	1.1–1.6	1.1–1.8
14	(*Z*),β-ocimene^{b,e}	1037	0.0–tr	
15	(*E*),β-ocimene^{b,e}	1046	tr–0.1	tr
16	γ-terpinene^{b,e}	1061	12.6–17.8	6.9–12.9
17	(*E*)-sabinene hydrate^{b}	1067	0.8–1.0	0.9–1.2
18	terpinolene^{b,e}	1087	tr–0.1	tr–0.2
19	(*Z*)-sabinene hydrate^{b}	1096	0.0–tr	0.0–tr
20	linalool^{b,e}	1097	1.5–2.5	2.2–2.8
21	camphor^{b,e}	1146	tr–0.2	0.0–0.4
22	isoborneol^{b,e}	1160	0.1–0.3	0.0–0.4
23	borneol^{b,e}	1168	0.7–1.1	1.1–1.7
24	terpinen-4-ol^{b,e}	1174	0.3–0.4	0.4–0.6
25	α-terpineol^{b,e}	1190	0.1–0.2	0.1–0.2
26	*p*-cymen-8-ol^{c}	1193	0.0–tr	0.0–0.1
27	thymol methyl ether^{b}	1233	0.1–0.8	0.0–1.2
28	carvacrol methyl ether^{b}	1243	0.2–0.5	0.2–0.8
29	linalyl acetate^{c}	1253	0.0–0.1	0.0–0.1
30	geraniol^{c,e}	1254	0.0–0.1	0.0–0.1
31	geranial^{c,e}	1266	0.0–tr	tr–0.2
32	bornyl acetate^{c,e}	1281	0.0–0.1	tr–0.1
33	thymol^{b,e}	1302	49.1–56.1	49.0–58.1
34	carvacrol^{b,e}	1307	2.5–3.1	2.4–3.7
35	thymyl acetate^{c}	1362	0.0–0.2	0.0–0.2
36	α-copaene^{b,e}	1382	0.1–0.3	0.1–0.2
37	β-bourbonene^{c}	1391	tr–0.1	tr–0.1
38	β-elemene^{c}	1407	0.0–tr	tr–0.1
39	β-caryophyllene^{b,e}	1428	1.3–2.0	1.5–3.0
40	β-cubebene^{b,e}	1435	tr	tr–0.1
41	β-gurjunene^{b}	1440	0.0–tr	0.0–tr
42	α-humulene^{b,e}	1460	0.1–0.2	0.1
43	*allo*-aromadendrene^{b}	1467	0.0–tr	0.0–tr
44	γ-gurjunene^{b}	1479	0.0–tr	tr–0.1
45	γ-muurolene^{b}	1485	0.0–0.1	0.0–0.1
46	germacrene D^{c}	1496	0.0–0.3	0.1–0.5
47	α-muurolene^{b}	1505	tr–0.1	0.0–0.1
48	β-bisabolene^{c}	1517	0.0–0.1	0.0–0.1
49	γ-cadinene^{b,e}	1524	0.0–0.1	0.1–0.2
50	δ-cadinene^{b}	1530	0.1–0.2	tr–0.1
51	spathulenol^{c}	1585	tr–0.1	0.0–tr
52	caryophyllene oxide^{b,e}	1593	0.1–0.2	0.3–0.7
53	1,10-di-*epi*-cubenol^{c}	1623	0.0–tr	0.0–0.1
54	γ-eudesmol^{c}	1631	tr–0.1	0.0–0.2
55	*epi*-α-cadinol^{c}	1649	0.0–0.2	0.0–0.4
56	α-cadinol^{c}	1663	0.0–0.2	0.0–0.1
57	farnesol^{b,d}	1695	0.0–tr	tr–0.1
	monoterpene hydrocarbons		31.1–37.0	25.6–32.8
	oxygenated monoterpenes		5.2–7.1	7.0–8.8
	p-cymene + γ-terpinene		24.4–29.7	19.2–26.2
	thymol + carvacrol		51.6–58.8	52.7–61.3
	sesquiterpene hydrocarbons		1.9–3.0	2.3–4.3
	oxygenated sesquiterpenes		0.3–0.5	0.7–1.2
	other		tr–0.2	tr–0.2
	total identified		99.0–99.6	99.1–99.5
	%RSD^{g}		14.4–27.4	19.2–32.3

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[a] Average GC peak area percentage of four replicates. [b] Identified on the basis of Kovats index and mass spectrum. [c] Identification based on a very good match of mass spectra. [d] Structure of isomer unknown. [e] Identification confirmed by co-injection of reference compound. [f] tr, peak area percent <0.05%. [g] %RSD, average coefficient of variance of individual compounds.

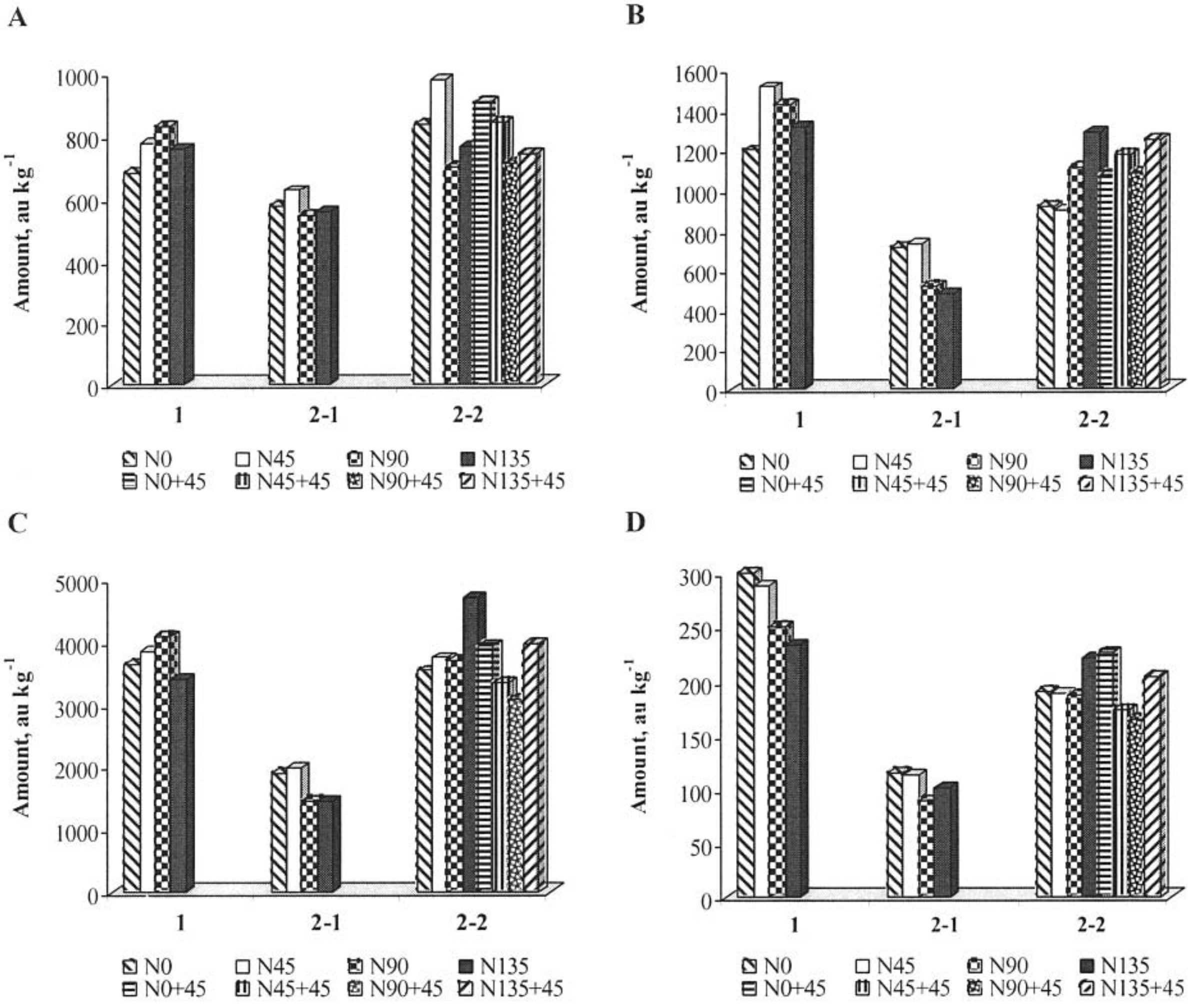

Figure 3. Variation in absolute amount (in arbitrary units, au) of major components in fresh thyme herb treated with different amounts of nitrogen fertilizers: *p*-cymene (A), γ-terpinene (B), thymol (C) and carvacrol (D); 1, first year of cultivation; 2-1, second year, first harvest; 2-2, second year, second harvest [(au = essential oil yield (%) × percentage content of individual component × 1000/100)].

second-year thyme harvested under different nitrogen fertilization doses varied over wider ranges compared to first-year thyme: the content of *p*-cymene was higher by almost 2 times and varied from 9.1 to 18.5%, whereas the percentage of γ-terpinene decreased, especially in the second-year cultivation–second-harvest thyme (6.9–17.8%); the content of thymol increased in the second-year thyme herb and was detected from 44.9 to 58.1%. Some reduction in the percentage of volatile monoterpene hydrocarbons was observed after drying.

More significant changes were observed in the variation of individual constituents expressed in arbitrary units, au kg^{-1} (**Figure 3**), which are equivalent to the absolute amount of the component. Thymol constituted almost half of total volatiles and was from 3630.6 (N_0) to 4079.4 (N_{90}) au kg^{-1} (**Figure 3C**). The amount of monoterpene hydrocarbons ranged from 2438.2 (N_0) to 2921.6 (N_{45}) au kg^{-1}. The major components of this group, *p*-cymene (**Figure 3A**) and γ-terpinene (**Figure 3B**), constituted 678.0 (N_0)–829.5 (N_{90}) and 1199.5 (N_0) – 1514.6 (N_{45}) au kg^{-1}, respectively. The concentration of oxygenated monoterpenes was from 452.0 (N_0) to 522.0 (N_{90}) au kg^{-1}. The amount of 1,8-cineole varied from 78.2 (N_{135}) to 98.5 (N_{45}) au kg^{-1}. The amount of sesquiterpene hydrocarbons was 166.6 (N_0)–200.2 (N_{90}) au kg^{-1}, and the amount of the major compound of this group, β-caryophyllene, varied from 99.6 (N_0) to 119.3 (N_{90}) au kg^{-1}. Oxygenated sesquiterpenes constituted only 24.7 (N_0)–30.7 (N_{90}) au kg^{-1}.

The amount of thymol in the second-year–first-harvest thyme was considerably lower, 1437.9 (N_{90})–1975.9 (N_{45}) au kg^{-1} (**Figure 3C**). The amount of total volatiles in second-harvest thyme and consequently the amount of individual components present in herb considerably increased.

Quite similar results were determined in dried thyme herb. The amount of individual constituents present in kilogram of dried herb was almost 2–3 times higher as compared to fresh herb. The percentage composition of volatile compounds after drying was almost similar to that before drying. However, a slight decrease in the content of monoterpene hydrocarbons (most volatile compounds) and an increase of oxygenated terpenes (possibly due to oxidation) can be observed. Some variations in the amounts of each individual component in dried herbs expressed in arbitrary units were observed; however, these variations did not reveal any clear dependencies between fertilization doses and the amount of individual constituents.

ABBREVIATIONS USED

EO, essential oil; KI, Kovats retention indices; RSD, relative standard deviation; LSD_{05}, least significant different, $P = 0.05$; au, arbitrary units.

LITERATURE CITED

(1) Senatore, F. Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L.) growing wild in Campania (southern Italy). *J. Agric. Food Chem.* **1996**, *44*, 1327−1332.

(2) Guillen, M. D.; Manzanos, M. J. Composition of the extract in dichlormethane of the aerial parts of a Spanish wild growing plant *Thymus vulgaris* L. *Flavour Fragrance J.* **1998**, *13*, 259−262.

(3) Van Den Broucke, C. O.; Lemli, J. A. Pharmacological and chemical investigation of thyme liquid extracts. *Planta Med.* **1981**, *41*, 129−135.

(4) Paster, N.; Menasherov, M.; Ravid, U.; Juven, B. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. *J. Food Prot.* **1995**, *58*, 81−85.

(5) Hammer, K. A.; Carson, C. F.; Riley, T. V. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.* **1999**, *86*, 985−990.

(6) Juven, B. J.; Kanner, J.; Schved, F.; Weisslowicz, H. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *J. Appl. Bacteriol.* **1994**, *76*, 626−631.

(7) Manou, I.; Bouillard, L.; Devleeschouwer, M. J.; Barel, A. O. Evaluation of the preservative properties of *Thymus vulgaris* essential oil in topically applied formulations under a challenge test. *J. Appl. Microbiol.* **1998**, *84*, 368−376.

(8) Dorman, H. J. D.; Deans, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol*. **2000**, *88*, 308−316.

(9) Dorman, H. J. D.; Deans, S. G.; Woble, R. C.; Surai, P. Evaluation *in vitro* of plant essential oils as natural antioxidants. *J. Essent. Oil. Res.* **1995**, *7*, 645−651.

(10) Miura, K.; Nakatan, N. Antioxidative activity of flavonoids from thyme (*Thymus vulgaris* L.). *Agric. Biol. Chem.* **1989**, *53*, 3043−3045.

(11) Youdim, K. A.; Dorman, H. J. D.; Deans, S. G. The antioxidant effectiveness of thyme oil alpha-tocopherol and ascorbyl palmitate on evening primrose oil oxidation. *J. Essent. Oil Res.* **1999**, *11*, 643−648.

(12) Lacroix, M.; Smoragiewicz, W.; Pazdernik, L.; Kone, M. I. Prevention of lipid radiolysis by natural antioxidants from rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.). *Food Res. Int.* **1997**, *30*, 457−462.

(13) Dapkevicius, A.; Venskutonis, R.; van Beek, T. A.; Linssen, J. P. H. Antioxidant activities of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agric*. **1998**, *77*, 140−146.

(14) Dorman, H. J. D.; Figueiredo, A. C.; Barroso, J. G.; Deans, S. G. *In vitro* evaluation of antioxidant activity of essential oils and their components. *Flavour Fragrance J.* **2000**, *15*, 12−16.

(15) Nguyen, D. V.; Takascova, M.; Jakubik, T.; Dang, M. N. Antioxidant effect of thyme in rape-seed oil. *Biologia* **2000**, *55*, 277−281.

(16) Dapkevicius, A.; van Beek, T. A.; Lelyveld, G. P.; van Veldhuizen, A.; de Groot, Ae.; Linssen, J. P. H.; Venskutonis, R. Isolation and structure elucidation of radical scavengers from *Thymus vulgaris* leaves. *J. Nat. Prod.* **2002**, *65*, 892−896.

(17) Stahl-Biskup, E. Essential oil chemistry of the genus *Thymus*—a global view. In *Thyme. The Genus Thymus*; Stahl-Biskup, E., Saez, F., Eds.; Taylor and Francis: London, U.K., 2002; pp 75−124.

(18) Stahl-Biskup E.; Sáez F. Essential oil polymorphism in the genus *Thymus*. In *Thyme. The Genus Thymus*; Stahl-Biskup, E., Saez, F., Eds.; Taylor and Francis: London, U.K., 2002; pp 125−143.

(19) Stahl-Biskup, E. The chemical composition *Thymus* oils: a review of the literature 1960−1989. *J. Essent. Oil Res.* **1991**, *3*, 61−82.

(20) Granger, R.; Passet, J. *Thymus vulgaris* native of France. Chemical varieties and chemotaxonomy. *Phytochemistry* **1973**, *12*, 1683−1691.

(21) Adzet, T.; Granger, R.; Passet, J.; San Martin, R. Chemical polymorphism in the genus *Thymus*: taxonomic importance. *Biochem. Syst. Ecol.* **1977**, *5*, 269−272.

(22) Passet, J. Chemical variability within thyme, its manifestations and its significance. *Parfums, Cosmet., Aromes* **1979**, *28*, 39−42.

(23) Piccagalia, R.; Marotti, M. Composition of the essential oil of an Italian *Thymus vulgaris* L. ecotype. *Flavour Fragrance J.* **1991**, *6*, 241−244.

(24) Venskutonis, P. R.; Poll, L.; Larsen, M. Influence of drying and irradiation on the composition of volatile compounds of thyme (*Thymus vulgaris* L.). *Flavour Fragrance J.* **1996**, *11*, 123−128.

(25) Venskutonis, P. R. Effect of drying on the volatile constituents of thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.). *Food Chem.* **1997**, *59*, 219−227.

(26) Letchamo, W.; Gosselin, A.; Hoelzi, J.; Marquard, R. The selection of *Thymus vulgaris* cultivars to grow in Canada. *J. Essent. Oil Res.* **1999**, *11*, 337−342.

(27) Fraser, S.; Whish, J. P. M. A Commercial Herb Industry for NSW—an Infant Enterprise. *RIRDC Res. Paper Ser.* **1997**, *No. 97/18*.

(28) Shalaby, A. S.; Razin, A. M. Dense cultivation and fertilization for higher yield of thyme (*Thymus vulgaris* L.). *J. Agron. Crop Sci.* **1992**, *168*, 243−248.

(29) Palada, M. C.; Crossman, S. M. A.; Kowalski, J. A. Evaluation of Improved Crop Management Practices For Culinary Herb Production in the Virgin Islands. *AES Tech. Bull.* **1998**, *No. 6*.

(30) Palada, M. C.; Crossman, S. M. A.; Kowalski, J. A. Growth and yield response of thyme (*Thymus vulgaris* L.) to sources of nitrogen fertilizer. In *Proceedings of the Caribbean Food Crops Society, "Caribbean Economic Stabilization through Agricultural Development Strategies"*; Palada, C., Clarke, C. C., Eds.; The University of the Virgin Islands Cooperative Extension Service and The Carribbean Food Crops Society: Virgin Islands, 1995; pp 58−64.

(31) Omidbaigi, R.; Arjmandi, A. Effects of NP supply on growth, development, yield and active substances of garden thyme (*Thymus vulgaris* L.). *Acta Hortic. (ISHS)* **2002**, *576*, 263−265.

(32) AOAC. Volatile oil in spices. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC, Inc: Arlington, VA, 1990; p 1001.

(33) AOAC. Solids (soluble) in fruits and fruit products. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC, Inc: Arlington, VA, 1990; p 915.

(34) AOAC. Vitamin C (ascorbic acid) in vitamin preparations and juices. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC, Inc: Arlington, VA, 1990; p 1058.

(35) AOAC. Sucrose in fruits and fruit products. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC, Inc: Arlington, VA, 1990; p 922.

(36) Methodology directions for determination of nitrates in the plant production. Confirmed by Lithuanian Ministry of Agriculture and Ministry of Health, order 160/3-2841; Ministry of Agriculture: Vilnius, Lithuania, 1990; p 41.

(37) AOAC. Nitrogen (total) (crude proteins) in plants. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC, Inc: Arlington, VA, 1990; p 59.

(38) AOAC. Potassium and/or sodium in plants. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC, Inc: Arlington, VA, 1990; p 47.

(39) AOAC. Phosphorus in fruits. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC, Inc: Arlington, VA, 1990; p 916.

(40) AOAC. Metals in plants. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC, Inc: Arlington, VA, 1990; p 42.

(41) Davies, B. H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: London, U.K., 1976; p 365.

(42) Adams, R. P. Quadrupole Mass Spectra of Compounds Listed in Order of Their Retention Time on DB-5. In *Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectroscopy*; Allured Publishing: Carol Stream, IL, 2001.

(43) Galambosi, B.; Holm, Y.; Hiltunen, R. The effect of some agrotechnical factors on the herb yield and volatile oil of dragonhead. *J. Essent. Oil Res.* **1989**, *1*, 287−292.

(44) Conklin, P. L. Recent advances in the role and biosynthesis of ascorbic acid in plants. *Plant Cell Environ.* **2001**, *24*, 383−394.

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