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Essential oil composition of sweet basil cultivars as affected by nitrogen and potassium fertilization

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Abstract: This study was designed to find correlations between the rate of nitrogen and potassium and basil herb quality resulting from essential oil content and composition. An increase in the amount of nitrogen in the nutritional environment of the plants resulted in the enhanced accumulation of essential oil, as well as in a rise in linalool and germacrene D concentration. The present study also showed increased content of essential oil in the herb, as well as an increase of 1,8-cineole in the oil under the influence of the increased rate of potassium. The studied basil cultivars Kasia and Wala were characterized by a high content of essential oil, whose dominant component was linalool (64.7%). In addition, the components that occurred in larger amounts were as follows: geraniol (12.6%), 1,8-cineole (4.1%), and *epi-α*-cadinol (3.8%).

Key words: Essential oil content, geraniol, linalool, *Ocimum basilicum* L.

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is grown in many countries of the world as a spice, medicinal, and aromatic plant. The medicinal and aromatic properties of basil are associated with the presence of an essential oil that accumulates in the largest amount in its leaves and flowers. The fresh and dried basil herb is used as an aromatic spice and a source of essential oil, and its main components are also used as plant drugs, since it has antimicrobial (Koba et al. 2009) and fungistatic activity (Dambolena et al. 2010); moreover, basil oil and its pure components have antimutagenic activity (Stajković et al. 2007). Basil oil is a mixture of numerous compounds and its composition is extremely rich and varied. Some constituents of the volatile oil distilled from the basil herb, such as linalool, 1,8-cineole, eugenol, or camphor, show documented biological activity. Linalool, which is the dominant compound of the oil derived from European basil varieties (Marotti et al. 1996; Sifola and Barbieri 2006; Seidler-Łożykowska and Król 2008; Dzida 2010), has antiinflammatory, antibacterial, antiviral, antifungal, and relaxant properties (Peana et al. 2004; Özek et al. 2010). Methyl chavicol, a compound showing a probable carcinogenic effect (De Vincenzi et al. 2000; Kaledin et al. 2009), which is found in smaller amounts in European basil varieties (Nurzyńska-Wierdak 2007a), has a relaxant and anticonvulsive effect as well as fungistatic and antifungal activity (Leal-Cardoso et al. 2004; Młodnicki and Matławska 2006). Eucalyptol (1,8-cineole) has an antiseptic and anesthetic effect, which is utilized in dentistry; furthermore, this compound has been found to have possible anticarcinogenic (Pisano et al. 2007), antioxidant (Ogata et al. 2000), and antiinflammatory activity (Magalhães et al. 2010). Eugenol, as one of the phenolic compounds characteristic of basil volatile oil, shows antioxidant (Juliani and Simon 2002) and antimicrobial activity (Ali et al. 2005). Germacrene C and D, components from the group of sesquiterpene hydrocarbons, exhibit antimicrobial and insecticidal activity (Røstelien et al. 2000; Stranden et al. 2002).

The chemical composition of sweet basil essential oil is dependent on genetic, ontogenetic, and environmental factors, similarly as in other oil plants (İpek et al. 2012). The morphological and chemical variability of *Ocimum basilicum* L. creates great possibilities for growing different cultivars of this valuable herbal plant. Numerous basil cultivars and forms are currently cultivated in Europe and in the world; they differ in plant size and habit; the color, shape, and size of leaves and flowers (Nurzyńska-Wierdak

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2007b); and the content and chemical composition of essential oil (Sifola and Barbieri 2006; Nurzyńska-Wierdak 2007a; Dzida 2010) as well as other biologically active substances (Golcz et al. 2006; Nguyen et al. 2010). Environmental and agrotechnical factors are another group of factors that affect the amount and quality of basil yield. Optimal plant fertilization, in particular nitrogen and potassium fertilization, is of great importance in the cultivation of basil (Kandil et al. 2009; Zheljazkov et al. 2008; Daneshian et al. 2008; Nurzyńska-Wierdak et al. 2011).

Nitrogen and potassium, 2 important macronutrients necessary for the proper functioning of the plant organism, have a significant effect on yield amount and quality of oil plants. Arabaci and Bayram (2004) proved an increase in fresh and dry basil herb yield, as well as in essential oil content and yield, under the conditions of nitrogen fertilization application. These authors also showed the effect of applied nitrogen on basil essential oil composition. Sifola and Barbieri (2006) report that an increase in the rate of applied nitrogen up to 300 kg ha^{-1} contributed to a rise in above-ground yield and fresh leaf biomass, as well as in essential oil yield in basil. The above-mentioned authors also found that the cultivar had an impact on the effectiveness of nitrogen application in terms of an increase in the amount of essential oil in the basil herb. Likewise, Golcz et al. (2006) found an increase in leaf weight, essential oil yield, and chloroplast pigment content in basil under the influence of increased nitrogen fertilization. Differently, Chatzopoulou et al. (2006) found that essential oil composition and yield in fennel were dependent on the cultivar, and not on the rate of applied nitrogen. In turn, Omer (1999) confirmed a positive correlation of the interaction between the nitrogen rate and oil content in Egyptian oregano. In addition, this author indicated a probable increase in thymol and carvacrol biosynthesis under the influence of nitrogen fertilization, conversely than in the case of α-terpinene and *p*-cymene. Nitrogen applied in the cultivation of herbal plants also stimulates the synthesis of other biologically active substances. Nguyen and Niemeyer (2008) proved that changes in the level of nitrogen fertilization during the growing period of basil had a significant impact on the production of phenolic compounds, in particular rosmarinic acid. Similarly, as nitrogen fertilization affects the growth and yield of basil and other oil plants, potassium fertilization may also contribute to herb yield and oil quality. Nguyen et al. (2010) reported that the rate of potassium has an effect on antioxidant activity and the concentration of phenolic compounds in basil leaves. Ez-Ell Din et al. (2010) found that nitrogen and potassium fertilization had an influence on essential oil yield and quality in caraway and, simultaneously, that the applied rates of nitrogen and potassium had no effect on oil concentration in the investigated raw material. The aim of the present study was to determine the effect of the rate of nitrogen and rate of potassium on essential oil content and composition in 2 Polish basil cultivars: Kasia and Wala. In addition to nitrogen, which is the main yield-enhancing component also affecting plant metabolism, we also selected potassium for our study, since it plays a significant role in various physiological processes, including those relating to the metabolism of biologically active substances (Nguyen et al. 2010). It seemed interesting to investigate the impact of this component and of the interaction of nitrogen and potassium on the accumulation and composition of basil essential oil.

2. Materials and methods

A plant growth experiment was conducted in a heated greenhouse of the Department of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin, during the period from February to May 2010. The detached greenhouse is situated in the north-south direction. Temperature in the greenhouse was maintained in the range of 18–25 °C during the day and 12–15 °C at night. Plants of 2 Polish basil cultivars, Kasia and Wala, were the object of the present study. The study was designed to find correlations between, on the one hand, the rate of nitrogen and rate of potassium and, on the other hand, basil herb quality resulting from essential oil content and composition. Basil was grown from seedlings in 4-dm^3 pots filled with sphagnum peat (pH of 5.5–6.0). The seedlings were produced in the heated greenhouse, while the seed material was supplied by a seed breeder (the Institute of Natural Fibres and Medicinal Plants in Poznań). Basil seeds were sown at the end of February, whereas the seedlings were planted in pots in the middle of March. The experiment was conducted using complete randomized design. One basil plant, being an experimental unit, grew in 1 pot, with each experimental series comprising 8 replicates. The following amounts of nutrients (in g dm^{-3} of growing medium) were applied: 0.2, 0.4, 0.6, 0.9 N in the form of ammonium nitrate; 0.4, 0.8 K in the form of potassium sulfate; 0.4 P as superphosphate (20% P); 0.3 Mg in the form of magnesium sulfate monohydrate; and the following micronutrients (in g dm^{-3} of growing medium): 8.0 Fe (EDTA), 5.1 Mn ($MnSO_4 \cdot H_2O$), 13.3 Cu ($CuSO_4 \cdot 5H_2O$), 0.74 Zn ($ZnSO_4 \cdot 7H_2O$), 1.6 B (H_3BO_3), and 3.7 Mo ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$). During the experiment, the plants were watered with the same amount of water as necessary every 1–2 days, and the greenhouse was successively aired. The conditions inside the greenhouse were optimal for the plants; this was confirmed by their quick and luxuriant growth, as well as proper development. The experiment was carried out under strictly controlled conditions; no presence of diseases or pests was found, and hence no chemical protection was used. The plants were harvested at the beginning of flowering by cutting off the above-ground portion of the stem above its lignified parts. The herb was dried in a drying oven at a temperature of 35 °C.

The essential oil was extracted from air-dried powdered material (30 g) in a glass Clevenger-type distillation apparatus following Polish Pharmacopoeia VIII guidelines (2008) and subjecting the material to hydrodistillation for 3 h. The assays were conducted in triplicate. The extracted essential oil was stored in a dark glass container at a temperature of –10 °C until the time of chromatographic separation. Qualitative and quantitative analysis of the basil essential oil was performed using a Varian Chrompack CP-3800 gas chromatograph with a mass detector (4000 GC-MS/MS) and a flame ionization detector (FID). A temperature of 50 °C was applied for 1 min, and then the temperature was incremented to 250 °C at a rate of 4 °C min^{-1}; 250 °C was applied for 10 min. A VF-5ms column was used (the equivalent of a DB-5). Helium was the carrier gas, with a constant flow of 0.5 mL min^{-1}, injector 250 °C, split 1:100. One microliter of the solution was injected (10 µL of the sample in 1000 µL of hexane). A Varian 4000 MS/MS detector was used with recorded range of 40–1000 m/z and scan rate of 0.8 s $scan^{-1}$. The retention indices were determined based on the alkane series C_{10}–C_{40}. A Varian 3800 Series instrument with a DB-5 column (J&W, USA) was used, operated under the same conditions as the GC-MS, FID 260 °C, split ratio 1:50. The qualitative analysis was carried out on the basis of MS spectra, which were compared with the spectra in the NIST Mass Spectral Library (NIST 2002) and with data available in the literature (Joulain and König 1998; Adams 2001). The identity of the compounds was confirmed by their retention indices, taken from the literature (Joulain and König 1998; Adams 2001), and our own data. The obtained results of the assays were statistically analyzed using analysis of variance for 2-way cross-classification, evaluating the significance of differences with Tukey's confidence intervals and performing least significant difference (LSD) calculations at the level of significance α = 0.05.

3. Results

The studied basil cultivars were characterized by a high concentration of essential oil, whose average content in the air-dried herb was 1.55% and was not significantly dependent on the cultivar (Table 1). The highest oil content was found in the herb of the plants fertilized with the medium rate of nitrogen compared to the other plants. The rate of potassium significantly differentiated oil content in the basil herb. The plants fed with a higher rate of potassium were marked by a higher amount of oil compared to the plants provided with a lower dose of potassium. The basil cultivars were found to be characterized by a rich and varied chemical composition of their essential oil, the composition of which was significantly affected by the nitrogen and potassium fertilization applied (Tables 2–4). The presence of 67 components was found in the essential oil extracted from the basil herb in the cultivar Kasia, whereas in the oil obtained from the cultivar Wala, 70 compounds were identified. Linalool was the dominant

Table 1. Essential oil content in dependence on the basil cultivar, nitrogen, and potassium fertilization.

Cultivar	N dose	K dose	Essential oil content
	g dm^{-3}		(%)
Kasia	0.2	0.4	1.39
		0.8	1.39
	0.4	0.4	1.44
		0.8	1.44
	0.6	0.4	1.59
		0.8	1.64
	0.9	0.4	1.64
		0.8	1.79
Wala	0.2	0.4	1.33
		0.8	1.33
	0.4	0.4	1.59
		0.8	1.74
	0.6	0.4	1.64
		0.8	1.69
	0.9	0.4	1.39
		0.8	1.69
Mean for cultivar	Kasia		1.54
	Wala		1.55
Mean for N dose	0.2		1.36
	0.4		1.55
	0.6		1.64
	0.8		1.63
Mean for K dose		0.4	1.50
		0.8	1.59
mean			1.55
LSD $_{0.05\ for}$ A $_{cultivar}$			n.s.
B $_{N\ dose}$			0.01
C $_{K\ dose}$			0.01
A × B			0.01
A × C			0.01
B × C			0.01

Table 2. Essential oil composition (%) of sweet basil Kasia plants in dependence on nitrogen and potassium fertilization.

Compound	RI	N dose (g dm^{-3}) 0.2		0.4		0.6		0.9	
		K dose (g dm^{-3}) 0.4	0.8	0.4	0.8	0.4	0.8	0.4	0.8
α-Pinene	941	0.1 ± 0.2	tr.	0.3 ± 0.0	0.2 ± 0.0	tr.	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
Sabinene	979	0.2 ± 0.0	tr.	0.3 ± 0.0	0.2 ± 0.0	tr.	0.2 ± 0.0	tr.	0.2 ± 0.0
β-Pinene	984	0.5 ± 0.0	0.2 ± 0.0	tr.	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Myrcene	994	0.5 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.1
Limonene	1033	0.3 ± 0.0	tr.	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
1,8-Cineole	1037	4.6 ± 0.1	2.4 ± 0.1	3.1 ± 0.0	4.6 ± 0.0	4.3 ± 0.0	4.6 ± 0.1	3.7 ± 0.0	4.8 ± 0.2
(*E*)-*β*-Ocimene	1049	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.4 ± 0.0
Fenchone	1094	0.2 ± 0.0	0.3 ± 0.0	tr.	0.2 ± 0.0	tr.	tr.	0.3 ± 0.0	0.3 ± 0.0
Linalool	1105	64.2 ± 2.2	67.4 ± 2.8	74.0 ± 0.8	58.6 ± 0.4	66.7 ± 0.3	63.8 ± 0.6	63.4 ± 0.2	70.3 ± 0.9
Camphor	1157	0.6 ± 0.1	tr.	tr.	tr.	tr.	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
Terpinen-4-ol	1192	tr.	tr.	tr.	0.2 ± 0.0	tr.	tr.	0.5 ± 0.0	tr.
α-Terpineol	1208	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.1
Methyl chavicol	1213	tr.	tr.	tr.	2.1 ± 0.0	tr.	tr.	tr.	tr.
Geraniol	1261	14.5 ± 0.5	14.8 ± 1.5	10.0 ± 0.6	14.8 ± 0.1	11.9 ± 0.1	14.6 ± 0.1	12.3 ± 0.1	10.1 ± 0.1
α-Copaene	1382	tr.	0.2 ± 0.0	0.3 ± 0.0	tr.	0.3 ± 0.0	tr.	0.2 ± 0.0	0.3 ± 0.0
Geranyl acetate	1388	0.3 ± 0.1	tr.	tr.	0.5 ± 0.0	tr.	tr.	tr.	tr.
β-Elemene	1395	1.0 ± 0.1	1.1 ± 0.2	0.9 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.2 ± 0.0	1.0 ± 0.1
Methyl eugenol	1418	0.4 ± 0.2	0.3 ± 0.1	tr.	tr.	tr.	0.3 ± 0.0	tr.	tr.
(*E*)-Caryophyllene	1430	0.4 ± 0.1	0.6 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
α -trans-Bergamotene	1441	1.1 ± 0.1	1.9 ± 0.2	1.2 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	0.7 ± 0.0	1.0 ± 0.0	1.2 ± 0.1
α-Guaiene	1445	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	tr.	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
cis-Muurola-3,5-diene	1458	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	tr.
α-Humulene	1470	tr.	0.2 ± 0.0	tr.	0.2 ± 0.0	tr.	tr.	0.2 ± 0.0	tr.
cis-Muurola-4(14),5-diene	1476	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.4 ± 0.1
Germacrene D	1497	1.5 ± 0.2	1.3 ± 0.2	1.1 ± 0.0	1.7 ± 0.0	1.8 ± 0.1	1.5 ± 0.1	1.7 ± 0.0	1.4 ± 0.2
Bicyclogermacrene	1511	0.7 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.5 ± 0.1
α-Bulnesene	1516	0.7 ± 0.1	0.7 ± 0.0	0.5 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	0.8 ± 0.0	0.6 ± 0.1
γ-Cadinene	1528	1.4 ± 0.1	1.5 ± 0.1	1.3 ± 0.0	2.0 ± 0.0	1.9 ± 0.0	1.8 ± 0.1	1.9 ± 0.0	1.4 ± 0.1
trans-Calamenene	1536	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
1,10-di-epi-Cubenol	1630	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.9 ± 0.0	0.5 ± 0.0
epi-*α*-Cadinol	1658	4.0 ± 0.7	3.3 ± 0.6	2.8 ± 0.1	5.1 ± 0.2	4.3 ± 0.1	4.1 ± 0.4	5.7 ± 0.1	3.1 ± 0.4
α-Cadinol	1675	0.3 ± 0.0	tr.	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.0	0.2 ± 0.0
Total: 100%									

The following compounds were found in trace (tr.) amounts: *α*-thujene, camphene, *α*-terpinene, *γ*-terpinene, terpinolene, *cis*-linalool oxide, *Z*-miroxide, pinocarvone, *δ*-terpinolene, fenchyl acetate, nerol, neral, bornyl acetate, *δ*-elemene, *α*-cubebene, eugenol, *β*-cubebene, *β*-cedrene, *β*-acoradiene, *γ*-muurolene, *β*-selinene, germacrene A, *δ*-amorphene, 10-epi-cubebol, *α*-cadinene, longipinanol, spathulenol, caryophyllene oxide, globulol, viridiflorol, 1-epi-cubenol, and *α*-bisabolol.

Table 3. Essential oil composition (%) of sweet basil Wala plants in dependence on nitrogen and potassium fertilization.

Compound	RI	N dose (g dm^{-3}) 0.2		0.4		0.6		0.9	
		K dose (g dm^{-3}) 0.4	0.8	0.4	0.8	0.4	0.8	0.4	0.8
α-Pinene	941	0.2 ± 0.0	0.3 ± 0.0	tr.	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Sabinene	979	0.2 ± 0.0	0.3 ± 0.0	tr.	tr.	tr.	tr.	0.2 ± 0.0	tr.
β-Pinene	984	0.4 ± 0.0	0.6 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
Myrcene	994	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
Limonene	1033	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	tr.	tr.	0.3 ± 0.0	0.3 ± 0.0
1,8-Cineole	1037	4.6 ± 0.1	5.6 ± 0.2	3.0 ± 0.3	4.2 ± 0.1	3.8 ± 0.0	3.7 ± 0.1	4.5 ± 0.2	4.2 ± 0.0
(*E*)-*β*-Ocimene	1049	0.5 ± 0.1	0.6 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.2	0.4 ± 0.0	0.4 ± 0.0
cis-Sabinene hydrate	1076	0.2 ± 0.0	0.3 ± 0.0	tr.	0.3 ± 0.1	tr.	tr.	0.3 ± 0.0	0.2 ± 0.0
Fenchone	1094	0.2 ± 0.0	0.5 ± 0.0	tr.	0.3 ± 0.0	0.3 ± 0.0	tr.	0.3 ± 0.0	0.3 ± 0.0
Linalool	1105	57.2 ± 1.4	59.4 ± 2.0	65.5 ± 2.3	59.8 ± 2.5	67.0 ± 0.1	67.6 ± 0.2	61.8 ± 2.5	69.4 ± 0.2
Camphor	1157	tr.	0.4 ± 0.0	tr.	tr.	0.4 ± 0.0	tr.	tr.	tr.
Terpinen-4-ol	1192	0.5 ± 0.0	0.5 ± 0.0	0.8 ± 0.1	1.1 ± 0.1	tr.	tr.	0.8 ± 0.0	0.7 ± 0.0
α-Terpineol	1208	0.6 ± 0.1	0.6 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	-	0.4 ± 0.0
Methyl chavicol	1213	5.2 ± 0.2	11.3 ± 0.2	0.5 ± 0.1	0.3 ± 0.0	7.3 ± 0.1	0.5 ± 0.1	3.3 ± 0.2	0.5 ± 0.0
Geraniol	1261	13.5 ± 0.5	7.4 ± 0.4	15.4 ± 0.9	16.5 ± 0.4	10.3 ± 0.1	12.6 ± 0.4	14.6 ± 0.4	11.2 ± 0.5
Geranyl acetate	1388	1.6 ± 0.2	0.9 ± 0.0	1.2 ± 0.0	0.9 ± 0.2	0.6 ± 0.1	1.1 ± 0.1	1.7 ± 0.5	1.0 ± 0.1
β-Elemene	1395	0.9 ± 0.1	0.7 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	1.1 ± 0.1	1.1 ± 0.2	0.8 ± 0.1
(*E*)-Caryophyllene	1430	tr.	tr.	0.2 ± 0.0	tr.	tr.	tr.	0.3 ± 0.0	tr.
α-trans-Bergamotene	1441	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.3 ± 0.0
α-Guaiene	1445	0.2 ± 0.0	tr.	0.3 ± 0.0	tr.	tr.	0.4 ± 0.1	0.3 ± 0.1	tr.
cis-Muurola-3,5-diene	1458	0.2 ± 0.0	tr.	0.2 ± 0.0	tr.	tr.	0.2 ± 0.2	0.3 ± 0.0	0.2 ± 0.0
α-Humulene	1470	0.2 ± 0.0	tr.	tr.	tr.	tr.	tr.	0.2 ± 0.0	tr.
Cis-Muurola-4(14),5-diene	1476	0.5 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
Germacrene D	1497	2.2 ± 0.0	1.9 ± 0.2	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.0	2.3 ± 0.0	1.8 ± 0.3	1.9 ± 0.1
β-selinene	1506	0.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	tr.	0.3 ± 0.0
Bicyclogermacrene	1511	0.7 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
α-Bulnesene	1516	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	tr.	0.6 ± 0.0	0.5 ± 0.1	0.4 ± 0.1
γ-Cadinene	1528	1.7 ± 0.0	1.4 ± 0.2	1.5 ± 0.3	1.4 ± 0.1	1.4 ± 0.0	1.6 ± 0.1	1.4 ± 0.2	1.5 ± 0.0
δ-Amorphene	1531	0.3 ± 0.0	0.2 ± 0.0	tr.	tr.	tr.	0.3 ± 0.0	tr.	tr.
1,10-di-epi-cubenol	1630	0.7 ± 0.0	0.5 ± 0.1	0.6 ± 0.2	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	0.5 ± 0.0
epi-*α*-Cadinol	1658	5.1 ± 0.3	3.6 ± 1.3	4.4 ± 1.3	2.5 ± 0.3	2.6 ± 0.1	3.9 ± 0.8	3.0 ± 0. 6	3.3 ± 0.1
α-Cadinol	1675	0.4 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	tr.	tr.	0.2 ± 0.2	tr.	tr.
Total: 100%									

The following compounds were found in trace amounts: *α*-thujene, camphene, *α*-phellandrene, *α*-terpinene, *γ*-terpinene, terpinolene, *cis*-linalool oxide, endo-fenchole, pinocarvone, *δ*-terpineol, fenchyl acetate, nerol, neral, bornyl acetate, *δ*-elemene, *α*-cubebene, *α*-copaene, *β*-cubebene, methyl eugenol, *β*-cedrene, aromadendrene, *β*-(*E*)–farnesene, *β*-acoradiene, *γ*-muurolene, germacrene A, 10-epi-cubebol, *α*-cadinene, spathulenol, caryophyllene oxide, globulol, viridiflorol, 1-epi-cubenol, neo-intermedeol, intermedeol, and *α*-bisabolol.

Table 4. Main compounds of basil essential oil in dependence on the cultivar, nitrogen, and potassium fertilization.

Factor		Linalool	Methyl chavicol	Geraniol
			(%)	
Cultivar	Kasia	66.1	0.3	12.6
	Wala	63.4	3.6	12.7
N dose ($g\ dm^{-3}$)	0.2	62.0	4.1	12.6
	0.4	64.5	0.7	13.5
	0.6	66.3	2.0	12.4
	0.8	66.2	0.9	12.0
K dose ($g\ dm^{-3}$)	0.4	65.0	2.0	12.5
	0.8	64.5	1.8	12.8
$LSD_{0.05\ for}$	$A_{cultivar}$	1.1	1.3	n.s.
	$B_{N\ dose}$	0.8	0.3	1.3
	$C_{K\ dose}$	0.5	0.7	n.s.
	A × B	1.4	0.5	2.2
	A × C	1.5	1.1	1.3
	B × C	1.5	1.4	2.1

compound in the essential oils under investigation; its concentration averaged 63.44% in the cultivar Wala and 66.05% in the cultivar Kasia. In addition to linalool, the following compounds were found to occur in larger amounts in the basil oil of the investigated varieties: 1,8-cineole, geraniol, germacrene D, γ-cadinene, epi-α-cadinol, and methyl chavicol (only in the cultivar Wala). The linalool concentration in the studied oils increased after the application of the medium rate of nitrogen and subsequently slightly decreased after the highest rate was applied (Table 4). On the other hand, an increase in the rate of potassium resulted in a decrease in the proportion of linalool in the oil of the basil cultivars under study. The cultivar and nitrogen rate, the cultivar and potassium rate, and the nitrogen rate and potassium rate were all shown to have a significant interaction effect on the average linalool concentration in the basil essential oil. The investigated oils were characterized by a significant percentage of geraniol, whose average content was 12.62%. No significant differences were found in the average concentration of this component in the oils obtained from the basil herb of the studied cultivars (Table 4). The significantly highest amount of geraniol in the investigated oils was demonstrated in the case of the medium rate of nitrogen. The rate of potassium was not found to have a significant effect on the proportion of this compound in the studied oils, but there could be observed a significant interaction of the investigated factors on the content of the component in question. 1,8-Cineole and epi-α-cadinol were other constituents of the oil of the studied basil cultivars that occurred in larger amounts. The applied rates of nitrogen and potassium significantly differentiated the proportions of the above-mentioned constituents of the oils under study. Likewise, the interaction of the selected factors significantly affected the concentration of 1,8-cineole and epi-α-cadinol in the basil oil. Germacrene D and γ-cadinene were the next constituents of the oil of the investigated basil cultivars that could be described as the main components. Their proportions were not significantly dependent on the cultivar and the rate of applied potassium. Different rates of nitrogen significantly differentiated the concentration of the above-mentioned oil constituents; their concentration was the highest at the medium rate of nitrogen. The concentration of methyl chavicol in the investigated oils was significantly dependent on the cultivar. The oil of the cultivar Kasia was characterized by a low content of methyl chavicol, which was on average 0.26% (Table 4). Nitrogen and potassium fertilization was shown to have a significant effect on the average content of this component in the oil. The presence of methyl chavicol was found only after the application of the medium rate of nitrogen and the highest rate of potassium (Table 2). An increase in the rate of nitrogen caused an initial decrease in the proportion of methyl chavicol in the studied oil and then a rise in the concentration of this compound, while at the highest level, the proportion declined. An increase in the amount of applied potassium resulted in a reduction in the concentration of methyl chavicol in the basil oil. The effect of the interaction of the investigated factors on the methyl chavicol content in the basil oil was also proven. The proportions of the remaining components of the essential oil of the studied basil cultivars were much lower than those of the above discussed oil constituents; they ranged from trace amounts (<0.05%) to amounts of <1.50% and were also influenced by the factors under study: cultivar, nitrogen rate, and potassium rate (Tables 2 and 3). Only the percentage of geranyl acetate in the oil extracted from the herb of the Wala cultivar plants was higher, ranging from 0.62% to 1.73%, but in turn this compound occurred in a small amount (0.25%–0.51%) in the other studied varieties.

4. Discussion

The studied basil cultivars Kasia and Wala were characterized by a high content of essential oil in the air-dried herb, but the cultivar did not have a significant effect on the amount of this substance. The essential oil concentration in the basil herb is clearly influenced by genetic, ontogenetic, and environmental variability (Özcan and Chalchat 2002; Rakic and Johnson 2002; Nurzyńska-Wierdak 2007a; Chang et al. 2008). Vieira and Simon (2006) report that the essential oil content in 15 studied varieties of *Ocimum basilicum* ranged from 0.58% to 1.64%, and the composition of the analyzed oils varied substantially. An increase in the rate of nitrogen and in the rate of potassium resulted in a rise in the concentration of essential oil in the investigated basil plants and it also affected the composition of this substance. The highest essential oil content was characteristic for the medium rate of nitrogen (0.6 g N dm^{-3}), but the differences were not significant statistically at the medium and highest rates. Similarly, Zheljazkov et al. (2008) showed maximum basil oil yield when the medium rate of nitrogen was applied. Sifola and Barbieri (2006) found that nitrogen fertilization increased above-ground yield, fresh leaf biomass, and oil concentration and yield in basil. Likewise, Sarab et al. (2008) obtained the highest dry basil herb yield and the highest essential oil concentration in the herb in the case of the application of the highest rate of nitrogen. Kandil et al. (2009) obtained the highest fresh basil herb yield and the highest basil essential oil yield when the highest NPK rates were applied. The enhanced accumulation of essential oil under the conditions when plants are well supplied with nitrogen results from the increased production of biomass as well as from the direct impact on the biosynthesis of this substance (Sangwan et al. 2001). The significant effect of the interaction of the factors under investigation on the essential oil content in the basil herb, demonstrated in the present study, shows the need to apply appropriate and balanced rates of nitrogen and potassium in growing basil. Zheljazkov et al. (2008) suggest that the rates of nitrogen and sulfur, as well as their probable interaction, can have a significant influence on the productivity of sweet basil as well as the essential oil content and composition. Moreover, Yamamoto and Takano (1996) reported that, in order to obtain high essential oil yield, the optimal ratio of NO_3^-: $H_2PO_4^-$: SO_4^- in the nutrient supplement should be 47%:32%:21% of total anion concentration, and the results obtained by the above-mentioned authors suggest that in basil plants there is a substantial demand for phosphorus and sulfur in terpene synthesis.

The chemical composition of the essential oil extracted from the herb of the studied basil cultivars Kasia and Wala varied and was dependent on the applied rates of nitrogen and potassium. The dominant component in the oil obtained from both varieties was linalool, a compound characteristic of the European type of sweet basil oil (Labra et al. 2004; Seidler-Łożykowska and Król 2008; Dzida 2010). The linalool concentration increased under the influence of the increasing rate of nitrogen. Zheljazkov et al. (2008) obtained the highest linalool yield and concentration at the medium rate, whereas the yield of the above-mentioned component decreased if the rate of nitrogen was further increased. The study of Yamamoto and Takano (1996) shows that the yield of sweet basil essential oil with an increased proportion of eugenol and linalool was high at a relatively high level of NO_3^-. Differently, Daneshian et al. (2009) found a decrease in linalool concentration in the basil oil obtained from the first harvest under the influence of an increased rate of nitrogen. Subsequently, these authors showed a certain tendency towards an increase in the content of this component in the oil with an increasing rate of nitrogen during the second harvest of basil herb. Rao et al. (2007) demonstrated a reverse correlation: the application of nitrogen significantly reduced the linalool concentration in the basil oil. The above cited studies and the present study prove the significant effect of an increased amount of nitrogen on the concentration of linalool in basil essential oil, but the differences may result from genetic, ontogenetic, and environmental variations in the chemical composition of the essential oil obtained from the basil herb (Özcan and Chalchat 2002; Nurzyńska-Wierdak 2007a; Chang et al. 2008).

The proportions of the remaining main components of the investigated oils changed in an undirected way, though they were affected by the rate of nitrogen. Similar correlations were shown by Zheljazkov et al. (2008) and Daneshian et al. (2009), which would indicate the probable connection between nitrogen and the biosynthesis of geraniol, 1,8-cineole, epi-α-cadinol, germacrene D, and γ-cadinene, but also of other components. In addition, Adler et al. (1989) suggested that nitrogen forms change the growth of basil as well as essential oil content and composition. One of the constituents of the studied oils was methyl chavicol, a compound that is described as a probable carcinogen (De Vincenzi et al. 2000; Kaledin et al. 2009), which limits the application of basil oil in therapy. The analyzed oils contained on average from 0.26% to 3.62% of this constituent, which can be considered to be a small amount (in particular in the cultivar Kasia) that does not pose a major risk to human health. Given these results, the studied oils can be regarded as safe and the cultivars Kasia and Wala as useful in cosmetic, food, and pharmaceutical production. In analyzing the chemical composition of the studied oils, one should also take into account interrelationships between the individual oil constituents. Linalool and estragole show a negative correlation (Vieira and Simon 2006; Rao et al. 2007), while the concentration of eucalyptol in the basil oil was positively correlated with the linalool concentration (Zheljazkov et al. 2008). On the one hand, changes in the content of the compounds in question can be caused by the amount of nutrients in the nutritional environment of the plants, while on the other hand, they can be associated with biosynthetic transformation of the oil constituents under discussion.

Potassium, applied at an increased rate, significantly differentiated the proportions of the particular components in the investigated sweet basil oils. In analyzing the concentration of the main components of the volatile substances in question, it was proven that the increased rate of potassium had a positive impact on the content of 1,8-cineole and geraniol and that the increased rate of this macronutrient had a negative effect on the concentration of linalool, methyl chavicol, and epi-α-cadinol. The proportions of germacrene D and γ-cadinene were not affected by the increased rate of potassium. Furthermore, the established significance of the interaction of the nitrogen rate and potassium rate as well as of the cultivar and potassium rate on the proportions of the above-mentioned components indicates the important impact of the appropriate ratio of nitrogen and potassium in the nutritional environment of plants, in particular in relation to cultivated basil varieties. The results of the studies of other authors show the important effect of potassium on the biological value of basil raw material and that of other oil plants (Chatzopoulou et al. 2006; Said-Al Ahl et al. 2009; Ez-Ell Din et al. 2010; Nguyen et al. 2010), though Rao et al. (2008) did not show any effect of the application of an increased rate of potassium on the essential oil content as well as the linalool and methyl chavicol concentration in basil. As an activator of enzymes participating in starch and protein photosynthesis and biosynthesis (Amtmann et al. 2008), potassium is, as a consequence, responsible for plant growth. Hence, increased rates of potassium result in increased growth, but they also affect the biosynthesis of different plant substances. The changes in the concentration of the components of the basil essential oils under the influence of the increased rate of potassium could therefore have been caused by the active role of this macronutrient in the metabolic processes taking place in the substances under investigation.

The study results presented in this paper prove the significant effect of the increased rates of nitrogen and potassium on the content and chemical composition of sweet basil essential oil. An increase in the amount of nitrogen in the nutritional environment of the plants resulted in the enhanced accumulation of essential oil as well as in a rise in linalool and germacrene D concentration. The present study also showed an increased content of essential oil in the herb as well as of 1,8-cineole in the oil under the influence of the increased rate of potassium. In addition, in our opinion, this is the first study to quantify the interaction effect of nitrogen and potassium application, as well as of nitrogen and potassium dose and cultivar, on the 1,8-cineole, linalool, methyl chavicol, geraniol, germacrene D, γ-cadinene and epi-α-cadinol content in sweet basil oil. We have demonstrated that the content of monoterpenoids (1,8-cineole, linalool, and geraniol) and sesquiterpenes (germacrene D, γ-cadinene, and epi-α-cadinol) in sweet basil oil was altered by the nitrogen application, while the potassium dose influenced only 1,8-cineole, linalool and epi-α-cadinol content. However, the methyl chavicol concentration was affected both by nitrogen and potassium dose. The significant impact of the interaction of the experimental factors under study on essential oil accumulation and composition suggests the need for optimal application of nitrogen and potassium in the cultivation of different sweet basil varieties. We may indicate that sweet basil should be cultivated in greenhouse conditions in peat medium containing 0.6–0.8 g dm^{-3} mineral nitrogen and 0.8 g dm^{-3} potassium. From a practical point of view, the increase in oil production and 1,8-cineole and linalool content and the decrease in methyl chavicol content induced by increasing N and K doses has a positive effect, particularly considering the quality and medicinal properties of basil oil.

The studied basil cultivars Kasia and Wala were characterized by a high content of essential oil, whose dominant component was linalool (64.7%). In addition, the components that occurred in larger amounts were as follows: geraniol (12.6%), 1,8-cineole (4.1%), and epi-α-cadinol (3.8%). The investigated oils were marked by a low proportion of methyl chavicol, which was 0.26% in the cultivar Kasia and 3.6% in the cultivar Wala. Due to the high proportion of linalool and the presence of eucalyptol, eugenol, and geraniol, as well as the low concentration of methyl chavicol, the characterized essential oils can be considered to be valuable and safe for use as food essences and for cosmetic or medicinal applications. Furthermore, the studied basil cultivars Kasia and Wala should be regarded to be valuable on account of their essential oil content and composition, in particular when they are grown using appropriate rates of nitrogen and potassium.

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