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Changes in the content and chemical composition of sweet basil essential oil under the influence of fertilization of plants with nitrogen and potassium

Zmiany zawartości oraz składu chemicznego olejku eterycznego bazylii pospolitej pod wpływem żywienia roślin azotem i potasem

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) of the family *Lamiaceae* belongs to valuable and extremely popular oil plants that are widely used in the pharmaceutical, cosmetic, perfume, and food industries. The essential oil extracted from the basil herb is characterized by a rich and varied chemical composition [19, 36, 39]. The biological activity of this substance is associated with the presence of the following dominant compounds: linalool, geraniol, eugenol, methyl chavicol, germacrene D, camphor, 1,8-cineole; their proportions are largely affected by the genotype [20]. Basil essential oil and its main components show antimicrobial [38], antifungal and fungistatic [29], anti-inflammatory [23], relaxant and antioxidant properties [13, 15].

In addition to the genetic and ontogenetic factors, which modify the composition and activity of basil essential oil, one should also take into account environmental factors and agricultural practices affecting yield [18, 35] and quality of raw material and oil [41]. Temperature, insolation, soil and air moisture content as well as macro- and micronutrient concentration and availability distinctly contribute to variations in the amount and quality of essential oil in various herbal plant species [7, 8, 14]. Nitrogen and potassium, nutritional components that play a key role in plant metabolism, also have an effect on the synthesis and accumulation of nutrients and secondary metabolites. Nitrogen, as a nutritional component, is considered to be the most important yield-enhancing macronutrient. This results from the essential function of this element that is necessary for building amino acids, structural elements of proteins as well as pyrimidine and purine bases, nucleotides, and nucleic acids [9]. Potassium, as a strongly alkaline cation, neutralizes organic and inorganic anions, especially in the vacuoles. It activates more than 50 different enzymes that participate primarily in photosynthesis and respiration [6].

Basil, showing quite a high nutrient requirement, responds to nitrogen application by increasing herb yield and essential oil yield [5, 32, 37]. Moreover, certain variations in the content of particular compounds in essential oil were found when different levels of nitrogen fertilization of basil plants were used [10, 32, 41]. The aim of the present study was to investigate the effect of different nitrogen rates (0.2, 0.4, 0.6, and 0.8 g dm⁻³) and potassium rates (0.4 and 0.8 g dm⁻³) on the content and chemical composition of essential oil obtained from the sweet basil herb.

MATERIALS AND METHODS

P l a n t m a t e r i a l. The present experiment was conducted in a heated greenhouse of the Department of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin, from February to May 2010. The detached greenhouse is situated in the north-south direction. Temperature in the greenhouse was maintained in the range 18-250C during the day and 12-150C at night. Plants of the green-leaf form of sweet basil, popular in the domestic fresh herbs market, were the object of the study. Basil was grown from seedlings, produced in the greenhouse, in 4 dm³ pots filled with sphagnum peat deacidified to a pH of 5.5-6.0. The seed material was supplied by a domestic seed supplier, the company PNOS Ożarów Mazowiecki. Basil seeds were sown at the end of February, while 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 one pot, with each experimental series comprising 8 replicates. The following amounts of nutrients (in g per 1 dm³ 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 sulphate; 0.4 P as superphosphate (20% P); 0.3 Mg in the form of magnesium sulphate monohydrate; as well as the following micronutrients (in g per 1 dm³ of growing medium): 8.0 Fe; 5.1 Mn; 13.3 Cu; 0.74 Zn; 1.6 B; and 3.7 Mo. During the experiment, the plants were watered with the same amount of water (400 ml) 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, 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 350 C.

Is o l a t i o n o f e s s e n t i a l o i l. The essential oil was extracted from air-dried powdered material (20 g) in a glass Clevenger-type distillation apparatus by using a method following Polish Pharmacopoeia VIII guidelines [31] and subjecting the material to hydrodistillation for three hours. The extracted essential oil was stored in a dark glass container at a temperature of -100C, until the time of chromatographic separation.

G C/M S a n d G C/F I D. 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 500 C was applied for 1 min., then it was incremented to 2500 C at a rate of 40 C/min; 2500 C was applied for 10 min. A VF-5ms column was used (an equivalent of DB-5). Helium was the carrier gas, with a constant flow

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of 0.5 ml/min. Injector: 2500 C; split 1:100. 1 μ l of the solution was injected (10 μ l of the sample in 1000 μ l of hexane). A Varian 4000 MS/MS detector was used, recorded range: 40-1000 m/z, scan rate 0.8 sec/scan. The retention indices were determined based on the alkane series C¹⁰-C⁴⁰The qualitative analysis was carried out on the basis of MS spectra, which were compared with the spectra of the NIST library [26] and with data available in the literature [1, 15]. The identity of the compounds was confirmed by their retention indices, taken from the literature [1, 15] and own data. The obtained results on the essential oil content and the concentrations of the dominant components were statistically analysed using analysis of variance for double-way cross-classification, evaluating the significance of differences with Tukey's confidence intervals and performing LSD calculations at the level of significance α =0.05.

RESULTS

The essential oil content in the investigated basil herb averaged 0.64% and it varied depending on fertilization applied (Table 1). An increase in the rate of nitrogen caused a significant rise in the concentration of essential oil in the air-dried basil herb. The largest amount of essential oil (0.96%) was found in the herb of the plants fertilized with the highest rate of nitrogen, whereas the lowest amount (0.49%) was found when the smallest dose of this component was applied (Table 1). The oil content in basil was shown to rise under the influence of an increased rate of potassium (respectively: 0.62 and 0.66%). Moreover, the study found a significant interaction between the nitrogen rate and potassium rate on the average essential oil concentration in the basil herb. Table 2 shows the full qualitative composition of basil essential oil in which the presence of 77 compounds was identified; they represent 100% of the oil components, but not all components were identified in the particular oil samples. The dominant compound was linalool, the proportion of which was from 47.16% to 60.51% of all oil constituents, depending on the applied rates of nitrogen and potassium. The linalool content was dependent on the applied rate of nitrogen (Table 1). The oil of the plants fertilized with the lowest nitrogen rate was characterized by the highest (59.93%) linalool content. An increase in the rate up to 0.6 g N \cdot dm-3 of growing medium resulted in a reduction in the concentration of linalool, on average down to 50.46%. A further increase in the amount of nitrogen contributed to a rise in the percentage of this compound. An increase in the amount of potassium was shown to result in a rise in the content of the dominant compound, i.e. linalool, on average from 52.99% to 54.33%. The effect of the interaction of the factors in question on the proportion of this compound in the oil was also found to be significant (Table 1).

Variation source	Essential oil	1,8-Ci- neol	Linalool	Methyl chavicol	Eugenol	Germa- crene D	γ- Cadinene	Epi-α- cadinol
A1	0.49	9.70	59.93	0.14	6.69	1.58	1.29	2.53
A2	0.54	12.40	51.23	9.59	5.66	1.56	1.09	1.82
A3	0.57	12.58	50.46	0.00	6.71	1.94	1.57	3.13
A4	0.96	12.21	53.02	0.00	5.71	1.82	1.54	3.48
B1	0.62	11.32	52.99	4.80	6.01	1.71	1.28	2.44
B2	0.66	12.12	54.33	0.07	6.37	1.73	1.46	3.03
mean	0.64	11.72	53.66	2.43	6.19	1.72	1.37	2.74
A (N dose)	0,01	0.06	0.06	0.06	0.06	0.06	0.06	0.06
B (K dose)	0,01	0.03	0.03	0.03	0.03	ns	0.03	0.03
AxB	0,01	0.11	0.11	0.10	0.11	0.11	0.11	0.10

Table 1. Analysis of variance results related to major compounds of sweet basil essential oil

 Table 2. Essential oil content and composition of sweet basil plants in dependence on nitrogen and potassium fertilization

	Compound	RI	N dose (g·dm ⁻³)								
No			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	
1	α-tujene	937	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
2	α-pinene	941	0.49	0.37	0.58	0.46	0.38	0.49	0.30	0.58	
3	camfene	957	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
4	sabinene	979	0.57	0.37	0.58	0.54	0.49	0.60	0.40	0.77	
5	β-pinene	984	1.16	0.79	1.29	1.20	1.08	1.37	0.84	1.57	
6	myrcene	994	1.44	0.82	1.65	1.37	1.36	1.37	0.86	1.56	
7	α -phellandrene	1012	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
8	α- terpinene	1022	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
9	limonene	1033	0.54	0.37	0.54	0.56	0.52	0.64	0.44	0.42	
10	1,8-cineole	1037	11.95	7.46	12.17	12.63	11.50	13.67	9.68	14.74	
11	(E)-β-ocimene	1049	0.33	0.35	tr.	0.71	0.39	0.49	tr.	0.44	
12	γ-terpinene	1061	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
13	cis-sabinene hydrate	1076	tr.	0.28	tr.	0.31	tr.	0.39	0.30	0.48	
14	terpinolene	1087	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
15	cis-linalool oxide	1091	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
16	fenchone	1094	tr.	0.25	tr.	tr.	tr.	tr.	tr.	tr.	
17	linalool	1105	59.36	60.51	47.16	55.30	50.62	50.30	54.84	51.21	

18	Z-myrokside	1151	fr	fr	fr	0.28	tr	tr	tr	tr
19	camphor	1157	1.32	0.58	0.84	0.32	1.45	0.45	0.79	0.47
20	ninokaryone	1175	1.52	tr	tr	0.52	tr	tr	tr	tr
20	δ-ternineol	1182	fr	tr	tr	tr	tr	tr	tr.	tr
22	borneol	1184	0.64	0.34	0.56	0.81	0.45	0.79	0.71	0.57
23	terninen-4-ol	1192	tr	0.36	tr	tr	tr	tr	tr	tr
24	a-terpineol	1208	1.31	0.81	-	1.35	1.44	1.47	1.25	1.68
25	methyl chavicol	1213	-	0.28	19.18	-	-	-	-	-
26	octanol acetate	1222	-	-	-	-	0.41	tr.	tr.	tr.
27	fenchyl acetate	1226	tr.	tr.	tr.	0.29	-	-	-	-
28	nerol	1239	tr.	tr.	tr.	tr.	-	-	-	-
29	neral	1252	tr.	tr.	tr.	tr	-	-	-	-
30	geraniol	1261	tr.	5.75	tr.	tr.	-	-	-	-
31	bornyl acetate	1295	1.17	0.66	1.04	1.70	1.22	1.82	2.38	0.99
32	<i>trans</i> -pinocarvyl acetate	1308	-	-	-	-	tr.	tr.	tr.	tr.
33	carvacrol	1319	-	-	-	-	tr.	tr.	tr.	tr.
34	myrtenyl acetate	1336	-	-	-	-	tr.	tr.	tr.	tr.
35	δ-elemene	1339	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
36	α-cubebene	1354	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
37	α-terpinyl acetate	1357	-	-	-	-	-	tr.	tr.	tr.
38	eugenol	1365	8.20	5.19	4.06	7.26	6.69	6.73	5.11	6.32
39	α-copaene	1382	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
40	geranyl acetate	1388	tr.	0.60	tr.	tr.	tr.	tr.	tr.	tr.
41	β-elemene	1395	0.80	0.96	0.94	1.13	1.21	1.31	1.48	1.53
42	β-cubebene	1400	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
43	methyl eugenol	1418	0.32	0.31	tr.	0.92	1.84	0.59	3.58	1.01
44	(E)-caryophyl- lene	1430	tr.	tr.	tr.	tr.	tr.	tr.	0.31	tr.
45	β-cedrene	1436	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
46	α- <i>trans</i> - bergamotene	1441	3.92	2.41	3.49	4.26	6.71	7.03	5.64	3.89
47	α-guaiene	1445	0.36	0.36	0.50	0.57	0.59	tr.	0.71	0.85
48	aromadendrene	1451	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
49	cis-muurola-3,5- -diene	1458	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
50	β-(E)-farnesene	1462	0.32	0.37	0.34	0.54	0.72	0.62	0.72	0.64
51	α-humulene	1470	0.45	0.27	tr.	0.35	0.47	0.57	0.51	0.49
52	cis-muurola- -4(14),5-diene	1476	0.37	0.44	tr.	0.32	0.39	0.45	tr.	0.37
53	β-acoradiene	1481	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.

γ-muurolene	1489	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
germacrene D	1497	1.26	1.90	1.47	1.66	1.95	1.94	2.19	1.45
β-selinene	1506	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
bicyclogerma- crene	1511	0.36	0.47	0.36	0.32	0.46	0.41	0.41	0.46
α-bulnesene	1516	0.63	0.63	0.72	0.87	1.06	1.11	1.01	1.18
germacrene A	1523	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
γ-cadinene	1528	1.05	1.54	1.03	1.15	1.62	1.52	1.44	1.64
δ-amorphene	1531	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
trans-calamene	1536	tr.	0.30	tr.	0.29	0.28	tr.	tr.	tr.
β- sesquiphellan- drene	1539	tr.	tr.	tr.	-	0.29	tr.	tr.	tr.
10-epi-cubebol	1546	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
α-cadinene	1550	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
longipinanol	1586	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
spathulenol	1592	tr.	tr.	tr.	tr.	0.33	0.47	0.34	0.55
caryophyllene oxide	1597	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
globulol	1601	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
viridiflorol	1609	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
1,10-di-epi-cu- benol	1630	tr.	0.52	tr.	0.41	0.44	0.45	0.47	tr.
1-epi-cubenol	1644	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
epi-α-cadinol	1658	1.67	3.39	1.53	2.12	3.32	2.93	3.26	3.70
α-cadinol	1675	tr.	tr.	tr.	tr.	0.33	tr.	tr.	tr.
neo-intermedeol	1679	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
intermedeol	1686	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
α-bisabolol	1711	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
-		100%							
Essential oil content (%)			0.49	0.54	0.54	0.55	0.59	0.89	1.04
	γ -muurolene germacrene D β -selinene bicyclogermacrene A α -bulnesene germacrene A γ -cadinene δ -amorphene <i>trans</i> -calamene β - sesquiphellan- drene 10-epi-cubebol α -cadinene longipinanol spathulenol caryophyllene oxide globulol viridiflorol 1,10-di-epi-cu- benol 1-epi-cubenol epi- α -cadinol α -cadinol α -cadinol α -cadinol cachiol neo-intermedeol intermedeol α -bisabolol : Essential oil content	γ-muurolene 1489 germacrene D 1497 β-selinene 1506 bicyclogermacrene 1511 α-bulnesene 1516 germacrene A 1523 γ-cadinene 1531 trans-calamene 1536 β- sesquiphellan- drene 1550 longipinanol 1586 spathulenol 1592 caryophyllene 1597 oxide 1609 1,10-di-epi-cu- 1630 1-epi-cubenol 1644 epi-α-cadinol 1658 α-cadinol 1675 neo-intermedeol 1679 intermedeol 1679 intermedeol 1711	γ-muurolene 1489 tr. germacrene D 1497 1.26 β-selinene 1506 tr. bicyclogermacrene 1511 0.36 α-bulnesene 1516 0.63 germacrene A 1523 tr. γ-cadinene 1528 1.05 δ-amorphene 1531 tr. trans-calamene 1536 tr. β- sesquiphellandrene 1550 tr. 10-epi-cubebol 1546 tr. tr. a-cadinene 1550 tr. tr. longipinanol 1586 tr. tr. globulol 1601 tr. tr. viridiflorol 1609 tr. tr. 1,10-di-epi-cubenol 1644 tr. tr. intermedeol 1675 tr. tr. intermedeol 1679 tr. tr.	γ-muurolene 1489 tr. tr. germacrene D 1497 1.26 1.90 β-selinene 1506 tr. tr. bicyclogerma- crene 1511 0.36 0.47 α-bulnesene 1516 0.63 0.63 germacrene A 1523 tr. tr. γ-cadinene 1528 1.05 1.54 δ-amorphene 1531 tr. tr. trans-calamene 1536 tr. 0.30 β- sesquiphellan- drene 1530 tr. tr. 10-epi-cubebol 1546 tr. tr. tr. iongipinanol 1586 tr. tr. tr. iongipinanol 1592 tr. tr. tr. globulol 1601 tr. tr. tr. yridiflorol 1609 tr. tr. tr. jlobulol 16101 tr. tr. tr. intoriflorol 1609 tr	γ-muurolene 1489 tr. tr. tr. tr. germacrene D 1497 1.26 1.90 1.47 β-selinene 1506 tr. tr. tr. tr. bicyclogerma- crene 1511 0.36 0.47 0.36 α-bulnesene 1516 0.63 0.63 0.72 germacrene A 1523 tr. tr. tr. γ-cadinene 1528 1.05 1.54 1.03 δ-amorphene 1531 tr. tr. tr. tr. trans-calamene 1536 tr. 0.30 tr. β- sesquiphellan- drene 1539 tr. tr. tr. 10-epi-cubebol 1546 tr. tr. tr. tr. longipinanol 1586 tr. tr. tr. globulol 1601 tr. tr. tr. yridiflorol 1609 tr. tr. tr. globulol	γ-muurolene 1489 tr. tr.	γ-muurolene 1489 tr. tr.	γ-muurolene1489tr.tr.tr.tr.tr.tr.tr.tr.germacrene D14971.261.901.471.661.951.94β-selinene1506tr.tr.tr.tr.tr.tr.tr.bicyclogermacrene15110.360.470.360.320.460.41α-bulnesene15160.630.630.720.871.061.11germacrene A1523tr.tr.tr.tr.tr.tr.tr.γ-cadinene1531tr.tr.tr.tr.tr.tr.tr.β- sesquiphellan- drene1539tr.0.30tr.0.290.28tr.10-epi-cubebol1546tr.tr.tr.tr.tr.tr.tr.10-gipinanol1586tr.tr.tr.tr.tr.tr.tr.globulol1601tr.tr.tr.tr.tr.tr.tr.jobenol1586tr.tr.tr.tr.tr.tr.tr.globulol1601tr.tr.tr.tr.tr.tr.tr.jobenol1644tr.tr.tr.tr.tr.tr.tr.idrene1597tr.tr.tr.tr.tr.tr.tr.jobenol1609tr.tr.tr.tr.tr.tr.tr.jobenol1609	γ -muurolene1489tr.tr.tr.tr.tr.tr.tr.tr.tr.tr.germacrene D14971.261.901.471.661.951.942.19 β -selinene1506tr.tr.tr.tr.tr.tr.tr.tr.tr.bicyclogermacrene15110.360.470.360.320.460.410.41 α -bulnesene15160.630.630.720.871.061.111.01germacrene A1523tr.tr.tr.tr.tr.tr.tr.tr. γ -cadinene1531tr.tr.tr.tr.tr.tr.tr.tr. γ -cadinene1536tr.0.30tr.0.290.28tr.tr. β -sesquiphellan- drene1539tr.tr.tr.tr.tr.tr. β -sesquiphellan- drene1550tr.tr.tr.tr.tr.tr.tr. α -cadinene1550tr.tr.tr.tr.tr.tr.tr.tr. α -cadinene1597tr.tr.tr.tr.tr.tr.tr.tr. α -pholol1609tr.tr.tr.tr.tr.tr.tr.tr. α -pholol1630tr.tr.tr.tr.tr.tr.tr.tr. β -sesquiphellan- drene1597tr.<

The following oil constituents occurred in significant amounts: methyl chavicol (0.28-19.18%) and 1,8-cineole (7.46-14.74%), whose proportions were also dependent on fertilization applied (Table 2). The average content of methyl chavicol in the studied oil was low (2.41%), which was associated with the fact that this component had been identified only in two oil samples (the series with N¹K² and N²K¹). Increased potassium rates caused a significant decrease in the methyl chavicol content in the oil. The content of 1,8-cineole was dependent on the applied rates of nitrogen and potassium as well as on the interaction between these factors (Table 1). An increase in the nitrogen rate up to 0.6 g N \cdot dm⁻³ contributed to a rise in the content of 1,8-cineole in the investigated oil, on average from 9.70% to 12.58%. A further increase in the amount of nitrogen resulted in a decrease in

the concentration of 1,8-cineole. Furthermore, the percentage of 1,8-cineole rose under the influence of the increased potassium rate (respectively: 11.32% and 12.12%).

The next components of the basil oil, with much lower percentages, were as follows: eugenol (4.06-8.20%), α -trans-bergamoten (2.41-7.03%), epi- α -cadinol (1.53-3.70%), methyl eugenol (<0.05 - 3.58%), germacrene D (1.26-2.19%), γ-cadinene (1.03-1.64%), bornyl acetate (0.66-2.38%), α-terpineol (0.81-1.68%), β-pinen (0.79-1.57%), myrcene (0.82-1.65%), β-elemene (0.80-1.53%), and camphor (0.32-1.45%) (Table 2). The proportions of these compounds were clearly influenced by increased nitrogen and potassium fertilization. The eugenol concentration in the oil changed in an undirected way under the influence of an increased dose of nitrogen and it increased with an increase in the potassium rate. The nitrogen rate and potassium rate were shown to have an effect on the average content of epi- α -cadinol in the oil of the studied form of basil (Table 1). The oil of the plants fertilized with the highest rate of nitrogen was characterized by the highest (3.48%) concentration of epi- α -cadinol. An increase in the rate of potassium contributed to a rise in the percentage of this compound in the investigated oil. The effect of the interaction of the factors under study on the average epi- α -cadinol content was also proved to be significant. The rate of nitrogen was found to have an impact on the content of germacrene D in the investigated basil oil. The highest (1.94%) concentration of this compound was characteristic for the plants fertilized with the medium rate of nitrogen, compared to the other plants. It was shown that the rate of potassium had no influence on the proportion of germacrene D in the investigated oil, but the interaction between the nitrogen rate and potassium rate was found to have an effect on the content of germacrene D in the oil. The average γ -cadinene content was 1.37% and it was affected by the factors in question and their interaction. The oil of the plants fed with the medium dose of nitrogen was characterized by the lowest (1.09%) concentration of γ -cadinene. An increase in the rate of potassium resulted in an increased proportion of this compound in the studied oil. The concentrations of the other constituents of the investigated basil oil were lower than 1% and were also dependent on the applied amounts of nitrogen and potassium (Table 2).

DISCUSSION

The essential oil content in the studied sweet basil herb was quite high and comparable to the results obtained for similar basil forms and varieties by other authors [4, 39, 41]. The increased concentration of oil in the basil herb under the influence of an increased dose of nitrogen and potassium shows the great importance of agricultural treatments in the cultivation of this plant as far as the quality of raw material obtained is concerned. In addition, a significant interaction of the rates of nitrogen and potassium shows the need to apply the above-mentioned nutrients in appropriate proportions if they are applied in combination. It can be concluded on the basis of the obtained results that an increase in the rate of potassium produces a measurable effect in the form of an increased concentration of essential oil only if higher rates of nitrogen are applied. Similar correlations between the increased rate of nitrogen and the essential oil content in the basil herb were demonstrated by Sifola and Barbieri [37], Golcz et al. [11], Sarab et al. [35]. Moreover, Kandil et al. [18] proved that 'Genovese' basil oil yield increased under the influence of increased NPK fertilization, while Rao et al. [32] additionally showed

the significant effect of the interaction of the rates of nitrogen and potassium on basil oil yield. This correlation can be explained by an increase in plant biomass yield, leaf surface area, and photosynthesis rate, as the amount of nutrients in the nutritional environment of plants increases [17, 27, 32], though, on the other hand, such effects are not always unambiguous [10].

The investigated basil essential oil was characterized by a rich chemical composition, and linalool was its dominant component. The dominant nature of linalool in the oil shows the European type of oil, which is valued in the pharmaceutical and perfume industries. This type is predominant among European varieties of sweet basil [4, 19, 20, 36]. The composition of the investigated essential oil was dependent on the varied rates of nitrogen and potassium. The linalool concentration initially decreased under the influence of the increasing rate of nitrogen, and then it increased after the highest dose was applied. An increase in the rate of potassium resulted in a rise in the concentration of linalool in the oil; the demonstrated significant interaction between the nitrogen rate and potassium rate shows the possibility of producing basil with a specific oil profile through the application of these nutrients in appropriate proportions. The correlation between nitrogen fertilization and the amount of linalool is confirmed by the results obtained by other authors. Zheljazkov et al. [41] showed a significant effect of nitrogen fertilization on linalool yield in basil oil, with the highest concentration of this compound found at medium rates of nitrogen. In turn, Arabaci and Bavram [3] found no effect of nitrogen fertilization on the composition of basil oil, but they showed an increase in the content of this compound under the influence of nitrogen application in the first year of the study. Increasing the linalool concentration in basil essential oil by increasing the rates of nitrogen and potassium can prove to be an effective method of obtaining larger amounts of this valuable component that is widely used in the cosmetics and perfume industry as well as for the production of biopesticides, vitamin E. and linalyl esters of different aroma [41].

The percentage of eucalyptol (1,8-cineole) in the investigated oil, the second main component, was clearly influenced by the applied rates of nitrogen and potassium. The concentration of eucalyptol initially increased and then decreased with the increase in the rate of nitrogen. Such a correlation was also shown by Zheljazkov et al. [41], who additionally found a positive correlation between eucalyptol concentration and linalool concentration. The content of methyl chavicol in the basil oil discussed in the present paper was low, which is characteristic for high-linalool chemotypes of basil [12, 40], and it was confirmed by a high negative correlation of these compounds [39]. The presence of methyl chavicol in the studied basil oil was found only in the series with the lowest and medium rate of nitrogen and at different levels of potassium fertilization. This compound, with a probable carcinogenic effect [16], limits the application of basil essential oil, in particular in the pharmaceutical industry. The positive effect of the application of higher doses of nitrogen and potassium on the decrease in the percentage of this compound, which was shown in the present study, may prove to be the right way to obtain valuable natural oil that is safe to use. The third main compound in the investigated oil was eugenol, a phenolic compound found in the largest amounts in clove oil. The concentration of this oil constituent, likewise germacrene D, changed irregularly under the influence of nitrogen fertilization and it was the highest when the third rate of nitrogen was applied. At the same time, the proportions of the abovementioned components increased with the increase in the rate of potassium and were clearly influenced by the interaction of the aforementioned factors. Given the valuable anti-inflammatory [23], antioxidant [28], and antibacterial properties of eugenol [2], as well as the properties of germacrene D [33, 34], the possibility of increasing of proportions of these compounds in basil oil, through the application of appropriate amounts of nitrogen and potassium, seems to be interesting. In addition to eugenol, a product of its methylation was found – methyl eugenol. The concentration of methyl eugenol increased as an effect of an increased rate of nitrogen; a tendency towards a decrease in the percentage of this component with the increase in the nitrogen rate was also shown. These correlations were rather different than in the case of eugenol. Methyl eugenol, in the opinion of some researchers similarly to methyl chavicol, shows a structural similarity to carcinogenic phenylpropanoids, and therefore chemotypes and varieties of oil plants grown for industrial purposes should be characterized by the lowest possible proportion of the above-mentioned compounds. Methyl eugenol is considered to be a moderately toxic compound with an activity similar to that of estragol; however, it is taken into consideration as a multi-site, multi-species carcinogen [29].

Ocimum basilicum L. is a species which is characterized by high morphological and chemical variation and which forms numerous chemotypes. Two biosynthetic pathways produce completely different types of compounds; the shikimic acid pathway produces aromatic compounds and their derivates, whereas the mevalonic acid pathway participates only in the production of monoterpenes [21]. The present study shows that the proportions of the compounds produced by the abovementioned metabolic pathways were different under the influence of the increased rates of nitrogen and potassium, which may indicate the participation of the aforementioned macronutrients in the above transformations. An increased concentration of methyl eugenol and a decreasing trend in the content of 1,8-cineole, eugenol and methyl chavicol were found under the effect of an increasing rate of nitrogen. In turn, an increase in the rate of potassium caused a rise in the content of linalool, 1.8-cineole and eugenol as well as a decrease in the concentration of methyl chavicol and methyl eugenol. Hence, it can be noticed that nitrogen is used more in the biosynthetic shikimic acid pathway. whereas potassium is used rather in the mevalonic acid pathway, but these relations are not clear. An increased amount of nitrogen resulted in an increase in the amount of methyl eugenol, whereas a reduction in the dose of this macronutrient usually resulted in a decrease in the concentration of eugenol, which can be linked to the role of methionine as the methyl radical donor and of methyltransferase that catalyzes methylation [22]. In addition, numerous experiments show that derivatives of chavicol and eugenol are formed from phenylalanine and cinnamic acid in different plant species, also including basil [24, 25].

CONCLUSIONS

The investigated essential oil of sweet basil (*Ocimum basilicum* L.) was characterized by a rich chemical composition and its dominant compound was linalool. Varied nitrogen and potassium fertilization of the plants contributed to an increase in the quality of the oil extracted from the dried herb. Increasing nitrogen and potassium rates resulted in an increased accumulation of essential oil in the basil herb as well as in a rise in the concentration of methyl eugenol (nitrogen), linalool (partly nitrogen and potassium), 1,8-cineole, and eugenol (potassium). In turn, a reduction in the amount of nitrogen contributed to a decrease in the content of 1,8-cineole, eugenol, and methyl chavicol, while a reduction in the rate of potassium resulted in a clear decline in the concentration of methyl chavicol

and methyl eugenol. The results of the present study show the possibility of obtaining a larger amount of basil essential oil and producing certain changes in the composition of this substance under the influence of different rates of nitrogen and potassium.

REFERENCES

- 1. Adams R.P.: Identification of essential oil compounds by gas chromatography/Quadrupole mass spectroscopy. Allured: Carol Stream, IL 2001.
- Ali S.M., Khan A.A., Ahmed I., Musaddiq M., Ahmed K.S., Polasa H., Rao L.V., Habibullah C.M., Sechi L.A., Ahmed N.: Antimicrobial activities of eugenol and cinnamaldehyde against the human gastric pathogen *Helicobacter pylori. Ann. Clinic Microb. Antimicrob.*, 2005, 4, 20, doi: 10.1186/1476-0711-4-20.
- Arabaci O., Bayram E.: The effect of nitrogen fertilization and different plant densities on some agronomic and technologic characteristic of *Ocimum basilicum* L. (Basil). J. Agron., 3 (4), 255, 2004.
- 4. Benedec D., Oniga I., Oprean R., Tamas M.: Chemical composition of the essential oil from *Ocimum basilicum* L. cultivated in Romania. Farmacia, 57 (5), 625, 2009.
- Biesiada A., Kuś A.: The effect of nitrogen fertilization and irrigation on yielding and nutritional status of sweet basil (*Ocimum basilicum* L.). Acta Sci. Pol. Hortorum Cultus, 9 (2), 3, 2010
- Britto D.T., Kronzucker H.J.: Celluar mechanisms of potassium *trans*port in plants. Physiol. Plant., 133 (4), 637, 2008.
- Castro L.W.P., Deschamps C., Biasi L.A. et al.: Development and essential oil yield and composition of mint chemotypes under nitrogen fertilization and radiation levels. 19th World Congress of Soil Science, Soil Solutions for a Changing World, 1-6 August 2010, Brisbane, Australia, 13-15, Pub. On DVD.
- 8. Chatzopoulou P.S., Koutsos T.V., Katsiotis S.T.: Study of nitrogen fertilization rate on fennel cultivars for essential oil yield and composition. J. Vegetable Sci., 12, 2, 85, 2006.
- Crawford N.M., Glass D.M.: Molecular and physiological aspects of nitrate uptake in plants. Trends in Plant Sci., 3 (10), 389, 1998.
- Daneshian A., Gurbuz B., Cosge B., Ipek A.: Chemical components of essential oils from basil (*Ocimum basilicum* L.) grown at different nitrogen levels. Internat. J. Nat. Engin. Sci., 3 (3), 8, 2009.
- Golcz A., Politycka B., Seidler-Łożykowska K.: The effect of nitrogen fertilization and stage of plant development on the mass and quality of sweet basil leaves (*Ocimum basilicum* L.). Herba Pol., 52 (1/2), 22, 2006.
- 12. Grayer R.J., Kite G.C., Goldstone F.J. et al.: Infraspecific taxonomy and essential oil chemotypes in sweet basil (*Ocimum basilicum*). Phytochem., 43(5), 1033, 1996.
- Ismail M.: Central Properties and Chemical Composition of *Ocimum basilicum* essential oil. Pharm. Biol., 44(8), 619, 2006.
- 14. Jabbari R., Dehaghi M.A., Sanavi M.M., Agahi K.: Nitrogen and iron fertilization methods

affecting essential oil and chemical composition of thyme (*Thymus vulgaris* L.) medical plant. Advances in Environ. Biol., 5 (2), 433, 2011.

- Juliani H.R., Simon J.E.: Antioxidant activity of basil. In: Janick J., Whipkey A. (eds.), Trends in new crops and new uses. ASHS Press, Alexandria, VA, 2002.
- Kaledin V.I., Pakharukova M.Y., Pivovarova E.N. et al.: Correlation between hepatocarcinogenic effect of etragole and its influence on glucocorticoid induction of liverspecific enzymes and activities of FOXA and HNF4 *trans*cription factors in mouse and rat liver. Biochem. (Moscow), 74, 4, 377, 2009.
- Kandeel A.M., Naglaa S.A.T., Sadek A.A.: Effect of biofertilizers on the growth, volatile oil yield and chemical composition of *Ocimum basilicum* L. plant. Ann. Agr. Sci. Cairo, 1, 351, 2004.
- Kandil M.A.M., Khatab M.E., Ahmed S.S., Schnug E.: Herbal and essential oil yield of Genovese basil (*Ocimum basilicum* L.) grown with mineral and organic fertilizer sources in Egypt. J. Kulturpflanz., 61 (12), 443, 2009.
- Krüger H., Wetzel S.B., Zeiger B.: The chemical variability of Ocimum species. J. Herbs Spices & Med. Plants, 9 (1), 335, 2002.
- Labra M., Miele M., Ledda B., Grassi F., Mazzei M., Sala F.: Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. Plant Sci., 167, 725, 2004.
- Lawrence B.M.: Essential oils: from agriculture to chemistry. Internat. J. Aromather., 10, 3/4, 2001.
- Lewinsohn E., Ziv-Raz I., Dudai N. et al.: Biosynthesis of estragole and methyl-eugenol in sweet basil (*Ocimum basilicum* L.). developmental and chemotypic association of allylphenol O-methyl*trans*ferase activities. Plant Sci., 160, 27, 2000.
- Magalhães C.B., Riva D.R., DePaula L.J. et al.: In vivo anti-inflammatory action of eugenol on lipopolysaccaride-induced lung injury. J. Appl. Physiol., 108, 845, 2010.
- Manitto P., Gramatica P., Monti D.: Biosynthesis of phenylopropanoid compounds. Part II. Incorporation of specifically labelled cinnamic acids into eugenol. J. Chem. Soc. Perkin I, 1548, 1975.
- Manitto P., Monti D., Gramatica P.: Biosynthesis of phenylopropanoid compounds. Part. I. Biosynthesis of eugenol in *Ocimum basilicum*. J. Chem. Soc. Perkin I, 1727, 1974.
- 26. Mass Spectral Library, NIST/EPA/NIH:USA, 2008.
- Meneghini A., Pocceschi N., Venanzi G., Tomaselli P.B.: Effect of nitrogen fertilization on photosynthetic rate, nitrogenous metabolites and β-asarone accumulation in triploid *Acorus calamus* L. leaves. Flavour Fragr. J., 13, 319, 1998.
- Ogata M., Hoshi M., Urano S., Endo T.: Antioxidant activity of eugenol and related monomeric and dimeric compounds. Chem. Pharm. Bull., 48, 10, 1467, 2000.
- Opinion of the Scientific Committee on Food on Methyleugenol (4-Allyl-1,2dimethoxybenzene). European Commission Health & Consumer Protection Directorate-General, Scientific Committee on Food, SCF/CS/FLAVOUR/4 ADD1 FINAL, 2001.
- 30. Özek T., Tabanca N., Demirci F. et al.: Enantiomeric distribution of some linalool containing

essential oils and their biological activities. Rec. Nat. Prod., 4 (4), 180, 2010.

- 31. Polish Pharmacopoeia VIII, Warsaw, 2008.
- Rao E.V.S.P., Puttana K., Ganesha R.S., Ramesh S.: Nitrogen and potassium nutrition of French basil (*Ocimum basilicum* Linn.). J. Spices Aromat. Crops, 16 (2), 99, 2007.
- Røstelien T., Borg-Karlson A.K., Fäldt J. et al.: The plant sesquiterpene germacrene D specifically activates a major type of antennal receptor neuron of the tobacco budworm moth *Heliothis virescens*. Chem. Senses, 25, 141, 2000.
- 34. Sala A., Racio M.C., Giner R.M. et al.: Anti-phospholipase A2 and anti-inflammatory activity of *Santolina chamaecyparissus*. Life Sci., 66 (2), 35, 2000.
- Sarab D., Naghdi Badi H., Nasri M., Makkizadeh M., Midi H.: Changes in essential oil content and yield of basil in response to different levels of nitrogen and plant density. J. Med. Plants, 7 (27), 60, 2008.
- Seidler-Łożykowska K., Król D.: The content of essential oil in ten sweet basil (*Ocimum basilicum* L.) cultivars and its composition. Herba Pol., 54 (3), 7, 2008.
- 37. Sifola M.I., Barbieri G.: Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Sci. Hort., 108, 408, 2006.
- Tomar U.S., Daniel V., Shrivastava K. et al.: Comparative evaluation and antimicrobial activity of *Ocimum basilicum* Linn. (*Labiatae*). J. Global Pharma. Technol., 2 (5), 49, 2010.
- Vieira R.F., Simon J.E.: Chemical characterization of basil (*Ocimum* spp.) based on volatile oils. Flavour Fragr. J., 21, 214, 2006.
- 40. Viña A., Murillo E.: Essential oil composition from twelve varieties of basil (*Ocimum* spp.) grown in Colombia. J. Braz. Chem. Soc., 14, 5, 744, 2003.
- Zheljazkov V.D., Cantrell C.L., Ebelhar M.W. et al.: Productivity, oil content, and oil composition of sweet basil as a function of nitrogen and sulfur fertilization. HortSci., 43 (5), 1415, 2008.

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SUMMARY

Sweet basil (*Ocimum basilicum* L.) belongs to valuable and extremely popular oil plants that are widely used in the pharmaceutical, cosmetics, perfume, and food industries. The essential oil extracted from the basil herb is characterized by a rich and varied chemical composition. The aim of the present study was to investigate the effect of different nitrogen rates (0.2, 0.4, 0.6, and 0.8g dm-3) and potassium rates (0.4 and 0.8g dm-3) on the content and chemical composition of essential oil obtained from the sweet basil herb. The investigated essential oil of sweet basil was characterized by a rich chemical composition and its dominant compound was linalool. Varied nitrogen and potassium fertilization of the plants contributed to an increase in the quality of the oil extracted from the dried herb. Increasing nitrogen and potassium rates resulted in an increased accumulation of essential oil in the basil herb as well as in a rise in the concentration of methyl eugenol (nitrogen), linalool (partly

nitrogen and potassium), 1,8-cineole, and eugenol (potassium). In turn, a reduction in the amount of nitrogen contributed to a decrease in the content of 1,8-cineole, eugenol, and methyl chavicol, while a reduction in the rate of potassium resulted in a clear decline in the concentration of methyl chavicol and methyl eugenol.

Keywords: Ocimum basilicum L., rate of nitrogen and potassium, linalool, eugenol, 1,8-cineole, streszczenie

Bazylia pospolita (*Ocimum basilicum* L.) należy do cennych i niezwykle popularnych roślin olejkowych, szeroko wykorzystywanych w przemyśle farmaceutycznym, kosmetycznym, perfumeryjnym i spożywczym. Olejek eteryczny ekstrahowany z ziela bazylii charakteryzuje się bogatym i zróżnicowanym składem chemicznym. Celem przedstawionych badań było zbadanie wpływu dawki azotu (0,2; 0,4; 0,6 i 0,8g dm-3) oraz dawki potasu (0,4 i 0,8g dm-3) na zawartość i skład chemiczny olejku eterycznego otrzymanego z ziela bazylii pospolitej. Badany olejek eteryczny bazylii pospolitej charakteryzował się bogatym składem chemicznym, a jego związkiem dominującym był linalol. Zróżnicowane nawożenie azotowe i potasowe roślin przyczyniło się do podniesienia jakości olejku ekstrahowanego z wysuszonego ziela. Wzrastające dawki azotu i potasu powodowały zwiększoną kumulację olejku eterycznego w zielu bazylii, jak również podwyższenie koncentracji metyloeugenolu (azot), linalolu (częściowo azot oraz potas), 1,8-cyneolu i eugenolu (potas). Z kolei zmniejszenie ilości azotu przyczyniło się do obniżenia zawartości 1,8-cyneolu, eugenolu i metylochawikolu, a zmniejszenie dawki potasu powodowało wyraźny spadek koncentracji metylochawikolu.

Słowa kluczowe: Ocimum basilicum L., dawka azotu i potasu, linalol, eugenol, 1,8-cyneol,