Journal of Applied Botany and Food Quality 90, 25 - 30 (2017), DOI:10.5073/JABFQ.2017.090.005

Department of Medicinal and Aromatic Plants, Szent István University, Budapest, Hungary

Effect of genotype and age on essential oil and total phenolics in hyssop (*Hyssopus officinalis* L.)

Éva Németh-Zámbori*, Péter Rajhárt, Katalin Inotai

(Received October 22, 2016)

Summary

Five *Hyssopus officinalis* L. accessions (German, Hungarian and Polish ones) were compared over three years with regard to their development and secondary metabolite production, in an open field experiment. The Hungarian variety 'Sophie' produced the highest essential oil (EO) yield (up to 2.037 ml/100 g). In general, one-year old individuals accumulated the most volatile organic compounds (VOCs) and the accumulation was influenced significantly by genotype and year.

A total of 47 components were identified in all of the oils. In all accessions *cis*- and *trans*-pinocamphones were most frequently the major compounds, but there were quantitative differences among genotypes. Highest proportions of these two components together appeared in 'Erfurter Ysop' (70.7 %). The third main compound was β-pinene that accumulated in the Hungarian accessions in the highest proportions (11-19 %). The cultivation year did not have a considerable influence on the EO composition.

Significant difference in the total phenolic content was evident among genotypes, and ranged from 443.64 mg/g DW ('Erfurter Ysop') and 329.32 mg/g DW ('Hyzop lekarsky') calculated as gallic acid. The effect of the year was not significant, although we detected a significant variety × year interaction.

In general, the selected hyssop cultivars showed an advance to commercial batches.

Keywords: intraspecific variability, effect of year, flowering time, essential oil, pinocamphone, total phenolics, plant age

Introduction

Hyssop (*Hyssopus officinalis* L.) herb has been traditionally used as a spice, a preservative, antiseptic and flavoring agent in the food industry, in cosmetics and in pharmacognosy in preparations against cough, asthma and spasms of the digestive system. Although the shrub is native to the Mediterranean Sea and the Middle East, it is cultivated in several temperate regions of the world. According to the official list of the Community Plant Variety Office (CPVO), 28 hyssop varieties have been announced since 1972, among which only five are currently officially registered. The huge majority of the selected genotypes are accessions of different petal colour, selected supposedly for ornamental purposes, while cultivars for production of high quality drug are hardly available.

The plant is aromatic and an essential oil (EO), characterised mainly by monoterpenes, is obtained from the aerial parts. The majority of references describe pinocamphone (*cis* and *trans*) as main compounds while β-pinene and pinocarvone occur frequently in high concentrations (Chialva et al., 1983; Galambosi et al., 1993; Koller and Range, 1997). Sesquiterpenes are less abundant, among which germacrene-D seems to be the most prevalent constituent (Németh-Zámbori, 2015).

The EO yield of the herb varies from 0.17 % (Hodzsimatov and Ramazanova, 1974) to 1.77 % (Zawislak, 2011). There are considerable differences in the EO yields of different genotypes, and the accumulation seems to be influenced by the climate. A strong negative correlation between the amount of precipitation and the accumulation level of volatile organic compounds (VOCs) in the flowering shoots has been demonstrated: rain during the development of flowers seems to have negative effect on the yield (Németh et al., 2001). The peak accumulation of VOCs could be measured at the time of full flowering, while it was significantly lower before and after flowering (Németh et al., 2001; Zawislak, 2011).

Although hyssop is known as a species rich in polyphenols (Veres et al., 1995; Chrpová et al., 2010), the number of adequate references on their levels and composition in this plant is limited. Ferulic acid, isoquercitrin and rutin have been reported as main components of the phenolic fraction (Vlase et al., 2014). In addition to flavonoids and tannins (Zawislak, 2011), the herb contains rosmarinic acid at concentrations up to 0,24 % (Veres et al., 1995). However, variability in the concentrations of phenolic compounds in hyssop has hardly been studied until now and the influencing factors are practically unknown. The goal of this study was to widen the knowledge on factors influencing the variability of active ingredients in hyssop and thus, influencing the quality of the drug. For this reason we followed the growth, EO and phenolics of five hyssop accessions of known origin over three consecutive years.

Materials and methods

Plant material: Five accessions of *Hyssopus officinalis* L. of different origin were investigated throughout 2012-2014 (Tab. 1). Seeds were sown in propagation trays on 22 March, 2012 and grown in a greenhouse. The seedlings of appr. 15 cm height were planted into open field plots on 15 May of the same year. Each plot consisted of 25 plants and the layout of the plots followed was a random block design with two replications. During the experiment the same plots were investigated over three years with first, second- and three-year old plant individuals, respectively.

Growing conditions and open field measurements: The soil is slightly calcareous (pH 7.78), loamy-sand (clay content below <15 %) with low humus content (1.14 %). Content of the main nutrients in the 0-30 cm soil layer are as follows, NO_3-N: 10.02 mg/kg, P_2O_5: 322 mg/kg, K_2O: 180 mg/kg, Mg: 82.5 mg/kg. No additional fertilization was applied except for top-dressing with nitrogen (NH_4NO_3, 40 kg/ha) after the harvest each year. Weather conditions of the experimental periods are presented in Tab. 2. Before sampling the experimental site received only moderate amounts of precipitation and the weather was relatively warm. In 2014, the temperatures rose somewhat higher during this period than in former years. The stands received natural precipitation and irrigation was applied only in the very dry periods, however, this additional watering occurred only after sampling had taken place each year (Tab. 1). Rainfall in the period before harvest may significantly influence the accumulation of volatile compounds

* Corresponding author

Tab. 1: The examined accessions of hyssop and their harvest times

Nr.	Denomination	Origin	Date of harvest		
			2012	2013	2014
1.	'Sophie'	Registered variety, breeder: Dept. of Medicinal and Aromatic Plants, Corvinus University	9August	21 June	22 June
2.	'Erfurter Ysop'	Commercial item from N. L. Chrestensen GmbH (Erfurt, Germany)	9August	21 June	5 June
3.	'Blankyt'	Commercial item from Pharmasaat GmbH (Artern, Germany)	9August	1 July	5 June
4.	'Hyzop lekarsky'	Commercial item from PNOS Ożarów Mazowiecki (Poland)	30 July	21 June	5 June
5.	'Cyrano'	Selected strain from genebank stock material, breeder as for 1.	9August	1 July	22 June

Tab. 2: Average temperatures and sum of precipitation during the experimental years presented by decades (data from sprouting till the last harvest)

	Month	2012				2013				2014			
		Decades			Mean		Decades		Mean		Decades		Mean
		I.	II.	III.*		I.	II.	III.*		I.	II.	III.*	
Temperature	April	8.0	9.0	13.8	14	3.9	10.9	16.1	10.3	11.8	10.7	14.6	12.4
	May	16.1	12.7	16.0	19	16.7	14.5	12.1	14.4	13.3	13.1	18.2	14.9
	June	17.2	19.3	20.2	22	14.7	**21.0**	**17.1**	17.6	**19.2**	**20.0**	18.4	19.2
	July	25.3	18.7	**20.1**	23	20.1	18.9	21.8	20.3	20.6	22.1	21.9	21.5
	Aug	**21.9**	-	-		-	-	-		-	-	-	
Precipitation					Sum				Sum				Sum
	April	12.6	2.6	6.2	20	24.8	5.4	0.6	30.8	9.2	4.4	25.4	39.0
	May	14.8	13.8	30.0	45	34.6	15.6	28.0	78.2	40.0	74.8	10.0	124.8
	June	22.8	9.0	27.6	60	29.2	**0.0**	**15.8**	45.0	**12.2**	**0.0**	15.2	27.4
	July	6.0	29.2	**29.6**	75	0.0	0.0	1.4	1.4	24.0	54.6	82.0	160.6
	Aug	**0.0**	-	-		-	-	-		-	-	-	

*In months with 31 days it indicates 11 days.

(Németh et al., 2001), however, the conditions of the experimental years were practically similar to each other. Weed control was assured mechanically and no other plant protection measures were needed.

In the experimental years, the time of flowering for each accession was determined by counting the number of individuals with opened flowers on each plot, at three different times, and expressing the counts as a percentage of the total number of individuals for each period. In the first year before harvesting time, we observed also the colour of the petals for each individual, to evaluate the homogeneity of the populations. The number of plants with flower colours other than blue, was noted for each plot.

Sampling*:* The harvests (samples) were adjusted to the phenological phase of the plants: each sample was taken at full flowering stage, which occurred in slightly different dates (Tab. 1). The flowering shoots above the woodened part of the stem were cut from randomly selected individuals and bulk samples to form four replications (two replication/plot) were prepared to assure representativeness. The samples were dried at room temperature and processed for determination of EO yield and composition, and total phenolic content.

Isolation of the essential oil: Each sample (50 g) was hydrodistilled for three hours in a Clevenger-type apparatus as recommended by the VII. Hungarian Pharmacopoeia [Pharmacopoeia Hungarica, 1986]. The EO content was calculated as volume (mL) of essential oil per 100 g of dried weight (three hours, at 105 °C). The collected oil was dried over anhydrous sodium sulfate and stored in glass vials at +4 °C in the dark prior to gas chromatography (GC) analysis.

Analysis of the essential oil: GC-flame ionisation detection (GC-FID) analysis was carried out using an Agilent Technologies 6890N GC System instrument, equipped with a HP-5 (5% phenyl methyl siloxane) capillary column (length: 30 m, d = 350 μm, film thickness: 0.25 μm), programmed as follows: initial temperature 50 °C (10 min), from 50 to 150 °C at a rate of 4 °C min^{-1}; from 150 to 220 °C at a rate of 12 °C min^{-1} and 220 °C (10 min). Carrier gas: helium (constant flow rate 0.5 ml min^{-1}), injector and detector temperatures: 250 °C, split ratio: 22.6:1, injected quantity: 0.2 μL. The percentage composition of the essential oil was computed from the GC peak areas.

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out from the EOs using the instrument mentioned above, equipped with an Agilent Technologies MS 5975 inert mass selective detector. The temperature programme was as follows: initial temperature 60 °C (10 min), from 60 to 240 °C at a rate of 3 °C min^{-1}, held for 5 min. Carrier gas: helium (constant flow rate: 1 mL min^{-1}); injector: 230 °C, split ratio: 30:1, transfer line: 240 °C. Ionization energy was 70 eV. Injected quantity: 0.2 μL. EO compounds were identified by matching their recorded mass spectra with those in mass spectral library references (NIST and Wiley) and by comparing their linear retention indices (LRI) relative to the elution ranking of *n*-alkanes (C9-C20) with those of authentic compounds under the same conditions. In case of EO composition the average values of the two replicates are presented and discussed.

Total phenolic content: For the determination of the total phenolic (TPC) content, 1 g dried and powdered plant material was extracted with 100 mL boiling distilled water, which was allowed to stand for 24 h. Then the extracts were filtered and stored in a freezer until the assay was conducted.

The total phenolic content was determined by using the method of SINGLETON and ROSSI (1965) with slight modifications. Sample solution (0.5 mL) was transferred to a test tube and then 2.5 mL Folin-Ciocalteau's reagent (Sigma Aldrich Kft., Budapest) in 10% v/v was added. After 1 min of incubation, 2 mL of sodium carbonate (0.7 M) was added. The absorbance was measured at 760 nm in a Thermo Evolution 201 spectrophotometer after a 5 min incubation period in hot water (50 °C). Gallic acid (0.3 M) was used as chemical standard for calibration. The total phenolic content of the sample was expressed as mg of gallic acid equivalents per g of dry weight of extract (GAE mg/g DW). A blank, containing distilled water instead of extract, was prepared. Triplicate measurements were made for each sample.

Statistical analysis: The results were analysed with the IBM SPSS Statistics 22 software using the MANOVA method to evaluate statistical differences among treatments. Wilk's lambda as unexplained variance rate was tested. Homogeneity of variances was tested by Levene's method and genotypes/years were separated by Tukey's post hoc test.

Results

During the experiment, each accession developed flowers already in the year of propagation. Concerning the colour of the petals, blue flowers appeared on the majority of the individuals in each accession. In 'Erfurter Ysop' 4% of individuals displayed a pink petal colour and in 'Cyrano' we found a single plant with a white flower. The Hungarian 'Sophie', the German 'Blankyt', and the Polish material consisted of homogenous blue flowering individuals.

The examined accessions displayed considerable differences in flowering time (Fig. 1). The earliest one was the Polish commercial material (accession 4), which reached full flowering 10-15 days before the others. The latest one each year was 'Cyrano', which never flowered before mid-June. The other three accessions flowered intermediate to the two mentioned. It was, however, characteristic for each of the genotypes, that flowering happened earlier as the plants became older (Tab. 1).

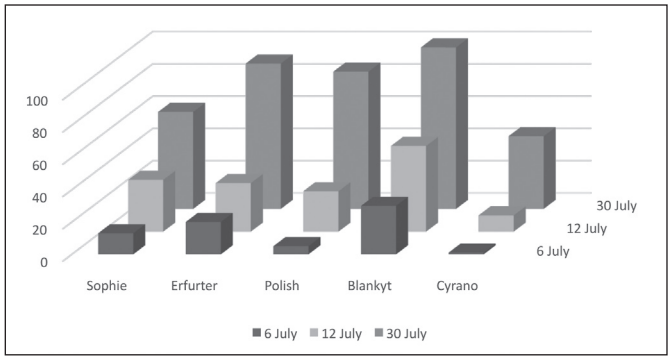

Fig. 1: Proportion of flowering individuals of one-year old hyssop accessions at different observation times (2012)

For EO yield, the multivariate test was significant (p=0.000), both for variety and year, as well as for their interaction (Tab. 3). Evaluating the effect of the variety (genotype), the yield of EO was outstanding in the case of the variety 'Sophie' (up to 2.037 ml/100 g). This indicates that this variety has been selected specifically for its high EO yield. Comparably high values (up to 2.250%) were reported formerly only as a result of treatment with gamma-rays (GILLE and FLORIA, 2000). The lowest EO yield (0.565%-0.987 ml/100 g) was determined in the German varieties (Tab. 4). The statistical analysis showed significant differences between 'Sophie' and each 'Erfurter Ysop', 'Blankyt' and 'Hyzop lekarsky'. Similarly, 'Cyrano' proved to be significantly different from these three varieties, but not from 'Sophie'.

During the three year experimental period, the vegetation year had a significant effect on the EO yield (Tab. 3). Interaction between genotype and year was also found to be significant. In general, the accumulation levels of the VOCs showed a decreasing tendency throughout 2012-14 (Tab. 4). The mean value of the five accessions in the third year of their cultivation represented only 62% of the value measured in the first year. This tendency was obvious for three of the studied genotypes. 'Hyzop lekarsky' exhibited a sudden drop of EO content in the second year but no further decrease in the third one. A totally opposite tendency was observable only in case of 'Blankyt': the samples of the third year showed the highest values. Nevertheless, this increase (0.2 ml/100 g DW) did not reach the size of the decrease observed in the other genotypes.

Altogether 47 components were identified in all the EO samples, and among them, 23 were present in all of the oils. Among these compounds, only 13 compounds were present at concentrations above 1% of the total and the variation of these compounds was further investigated (Tab. 5). When evaluating these compounds, no characteristic qualitative differences could be established between the accessions. Highest proportions of *cis*-pinocamphone, *trans*-pinocamphone and β-pinene were present in all the accessions. The characteristic differences among the accessions are quantitative ones. In 'Erfurter Ysop' and 'Hyzop lekarsky', *cis*-pinocamphone was present in high concentrations (up to 73.6 and 64.4%, respectively), therefore these varieties can be described as *cis*-pinocamphone chemotypes. A similar profile was reported by other authors (KOLLER and RANGE, 1997; BERNOTIENĖ and BUTKIENĖ, 2010). *Cis*-pinocamphone is also the major compound of 'Sophie' (51.8-55.9%) an 'Blankyt' (52.2-54.8%), and in 'Sophie', relatively high levels (6.9-8.9%) of pinocarvone are also present. In 'Cyrano', almost equal amounts of *cis*- and *trans*-pinocamphones were present, which is different from all of the other materials (Tab. 5) and similar to the majority of accessions studied by GALAMBOSI et al. (1993) and CHALCHAT et al. (2001). The highest proportions of the pinocamphone isomers together were found in the genotype 'Erfurter Ysop' (70.7%), while the lowest percentages were measured in 'Blankyt' (59.3%). These two terpenic ketons especially *cis*-pinocamphone were reported to have strong antifungal activity (FRATERNALE et al., 2004). The lower limit for the two ketons (33%) fixed in the international standard on hyssop oil (ISO 9841:2013) was reached by each accession while the upper limit (70%) was slightly exceeded in some cases by the samples of 'Erfurter Ysop'.

Tab. 3: Results of the multivariate test for EO yield and total phenolics content data

	Wilks' Lambda	Essential oil			Total phenolics		
		F	df	sign. level	F	df	sign.
Variety	0.039	87.725	4	0.000	25.082	4	0.000
Year	0.169	98.853	2	0.000	1.472	2	0.240
Variety × year	0.147	16.737	8	0.000	4.202	8	0.001

Tab. 4: Yield of essential oil and total phenolic content for the examined accessions during three vegetation years (mean values)

Accession	Essential oil (ml/100g DW)				Total phenolics (GAE mg/g DW)			
	2012	2013	2014	Mean	2012	2013	2014	Mean
Sophie	2.037a	1.457a	1.020a	1.507a	309.33c	406.63b	410.77a	375.58b
Erfurter Ysop	0.933c	0.893 b	0.565c	0.797b	440.31a	445.10a	431.66a	443.64a
Blankyt	0.781c	0.862b	0.987a	0.847b	436.02a	406.61b	385.53b	409.39ab
Hyzop lekarsky	1.317b	0.674c	0.759b	0.916b	342.63b	337.11c	308.23c	329.32c
Cyrano	1.940a	1.224a	1.066a	1.411a	358.23b	353.27c	372.69b	361.74b
Mean	1.401	1.022	0.880	-	377.30	401.82	381.77	-

Different letters indicate significant differences in the coloumns

Tab. 5: Proportions (as GC peak area %) of the main components (present in >1%) of the studied hyssop essential oils during the three years

Compound	LRI[a]	KI[b]	'Sophie'	'Erfurter Ysop'	'Blankyt'	'Hyzop lekarsky'	'Cyrano'
sabinene	976	975	1.5-2.0	tr-1.0	1.1-1.2	tr-0.1	1.4-1.5
β-pinene	981	979	16.0-19.1	8.2-8.5	8.0-8.2	8.6-8.9	11.7-14.2
β-myrcene	995	990	0.9-1.5	tr-1.2	1.1-1.3	1.0-1.2	1.4-1.6
β-phellandrene	1029	1029	1.2-2.3	3.0-6.6	2.6-3.0	4.2-4.9	3.3-5.0
unidentified			3.0-3.5	tr-3.5	3.8-5.0	1.6-2.0	tr-0.8
trans-pinocamphone	1163	1162	1.3-5.0	5.2-11.6	4.4-8.3	0.3-2.5	27.1-35.0
pinocarvone	1166	1164	6.9-8.9	tr-0.8	2.9-5.7	2.9-4.5	3.3-4.8
cis-pinocamphone	1170	1175	51.8-55.9	65.5-73.6	52.2-54.8	58.1-64.4	27.7-38.6
myrtenol	1194	1195	1.0-1.7	tr-1.1	0.6-0.8	1.2-2.2	2.1-2.7
germacrene-D	1482	1481	0.6-1.0	0.5-1.2	1.3-1.8	0.3-0.9	0.3-1.1
bicyclogermacrene	1497	1500	0.5-0.9	0.9-2.2	2.3-2.6	0.9-1.8	0.3-1.5
elemol	1553	1549	0.2-0.8	0.5-1.8	1.0-1.8	0.4-1.2	0.2-1.2
β-bisabolol	1671	1675	0.3-0.5	0.2-1.4	1.8-2.4	1.8-2.9	0.5-1.0

[a]Linear retention indices (LRI) calculated relative to the elution ranking of *n*-alkanes on HP-5 column, [b]Kovats retention index according to ADAMS (2007)

Another characteristic difference among our populations was the concentration of β-pinene which ranged from 11-19% in the Hungarian accessions, while the others contained 8-9% of the compound. According to most references, all the other monoterpenes present in the investigated oils are common components of hyssop (CHIALVA et al., 1983; LAWRENCE, 1992; PICCAGLIA et al., 1999; CHALCAT et al., 2001; ZAWISLAK, 2013b). The concentrations of some of them might vary on a large scale as reported e.g. for 1.8-cineole up to 53% (VALLEJO et al., 1995). Camphor, which was present at a concentration of more than 20% in the samples of SCHULZ and STAHL-BISKUP (1991) and limonene and methyl eugenol reported previously at 16% and 44%, respectively, by PICCAGLIA et al., (1999), were not detected in the investigated oils. Sesquiterpenes were in the minority in the EO from each genotype; their levels do not exceed 10% in any of them. According to available data, primarily camphor and limonene might contribute significantly to the antifungal activities of the EO (FRATERNALE et al., 2004).

The vegetation year did not have a significant effect on the composition as reflected by the marginal values in Tab. 5.

For the TPC, the multivariate test was significant (p=0.000) for variety and variety × year interaction, but not for year (Tab. 3). In contrast to the EO yield, the content of polyphenols reached higher values in the German genotypes, than in the Hungarian and Polish ones. The highest value was measured in 'Erfurter Ysop' (443.64 GAE mg/g DW) and it differed significantly from each of the other accessions, with the exception of 'Blankyt' (Tab. 4). In contrast, the lowest accumulation level (329.32 GAE mg/g DW) was established in samples of 'Hyzop lekarsky', which differed significantly from the other four accessions.

The vegetation year had no significant influence on the accumulation of phenolics in hyssop. The mean values of the five accessions indicate, that in the second year of their cultivation (2013), the plants produced slightly (by 6.4% and 5.2%) higher contents compared to 2012 and 2014, respectively. However, not each of the studied genotypes followed this tendency, what is reflected also in the significant variety × year interaction (Tab. 3).

Discussion

Differences in petal colour of hyssop are well known (GALAMBOSI et al., 1993), heterogeneity in this respect may be considered as low in our accessions. The difference in flowering time has never been demonstrated in a comparison trial until now and reveals an additional feature of intraspecific variability in hyssop.

The measured yield values of the EOs in the experimental populations can be evaluated as advantageous compared to the majority of references (HODZSIMATOV and RAMAZANOVA, 1974; KOLLER and RANGE, 1997; PICCAGLIA et al., 1999; BERNOTIENĖ and BUTKIENĖ, 2010). The statistical similarity of 'Cyrano' and 'Sophie' and their differences from the other three varieties may be a reflection of the fact that the Hungarian variety 'Sophie' is the result of long year selection from the stock material of 'Cyrano', thus, these two ones are related taxa. No such relationship is known for the other three accessions, although it cannot be excluded. The EO yield of 'Blankyt' according to the homepage of the firm *Pharmasaat*, is between 0.4

and 0.7 ml/100 g, thus the data measured at our experimental station represents better values than this. The Polish material has previously been investigated by ZAWISLAK (2011) who found similar high values (up to 1.7%) in one-year old populations.

The age of the plants might have an important effect on VOC content of the flowers, with decreasing tendency in the older plantations. There are very few former reports concerning this aspect. Our data seem to be in accordance with the findings of HODZSIMATOV and RAMAZANOVA (1974). On the other hand, GILLE and FLORIA (2000) demonstrated a significant elevation in EO yield in a local pink flowering population called 'De Ciorani' from the second to the third year. The authors concluded on the most advantageous meteorological conditions as a reason for this finding. GALAMBOSI et al. (1993) reported interaction between the effects of age and genotype, however, not each accession has been investigated in each year in that study. We could systematically ascertain this statement calculating the significant interaction between genotype and year. It shows, that the degree of influence of the year on the EO yield is dependent on the variety.

Obviously, vegetation year and weather conditions are associated closely with each other. Previously we demonstrated that in years of higher precipitation during the development of the flowers, the EO yield tends to decrease (NÉMETH et al., 2001). In the recent experiment, there was only moderate rainfall in the flowering period, and in none of the years was more than 30 mm precipitation registered in the decades before harvests (Tab. 2). Thus, a large influence of the weather may not be concluded.

It was established, that each of the experimental accessions produced *cis*- and *trans*-pinocamphones as main constituents of their VOCs (LAWRENCE, 1992; GALAMBOSI et al., 1993; KOLLER and RANGE, 1997; FRATERNALE et al., 2004; ZAWISLAK, 2013a). The composition of the EO proved to be qualitatively similar for each accessions. The main quantitative differences were found in the proportion of the pinocamphone isomers. ZAWISLAK (2013a) reported a significant change in the proportions *cis*- and *trans*-pinocamphones from vegetative to full flowering phase. Nevertheless, all of our samples were harvested when in full flower, therefore the detected chemical variability is probably a genetic characteristic.

The very low proportions of sesquiterpenes described in our experimental samples seems to be a common feature of *Hyssopus officinalis* (CHIALVA et al., 1983; VALLEJO et al., 1995; PICCAGLIA et al., 1999; ZAWISLAK, 2013b).

In contrast to our findings regarding the EO yield, the data demonstrated that the year did not have a considerable influence on the EO composition. This coincides with several former findings for other species (NÉMETH-ZÁMBORI, 2015).

There are only few reports on the polyphenolic content of hyssop and no comparisons of different accessions of the species. ZAWISLAK (2011) reported 0.32-0.55% flavonoids and 0.34-0.75% tannins in the herb. Although the genotype was identical to the one examined by us ('Hyzop lekarsky' from firm PNOS), the analytical methods based on Polish Pharmacopoeia VII are different, thus, data can not be directly compared. Similarly to the EO composition, the vegetation year seems to have less influence on this phytochemical characteristics than does the genotype.

The three year-long study on five hyssop accessions demonstrated that selected materials exhibit a better quality and morphological homogeneity compared to trade items of genetically uncertain origin, thus, a more intensive breeding of hyssop would be necessary to support cultivation. However, to optimize VOC and phenolic production in parallel, a well oriented selection is needed for both traits.

Conflict of interest: The authors declare that they have no conflict of interest.

References

ADAMS, R.P., 2007: Identification of essential oil components by gas chromatography/mass spectrometry. 4th edn. Allured Publ. Corp, Carol Stream, IL USA.

BERNOTIENĖ, G., BUTKIENĖ, R., 2010: Essential oils of *Hyssopus officinalis* L. cultivated in East Lithuania. Chemija, 21, 135-138.

CHALCHAT, J., ADAMOVIC, D., GORUNOVIC, M.S., 2001: Composition of oils of three cultivated forms of *Hyssopus officinalis* endemic in Yugoslavia: f. *albus*, f. *cyaneus* and f. *ruber*. J. Essent. Oil Res. 13, 419-421. doi:10.1080/10412905.2001.9699712.

CHIALVA, F., LIDDLE, P.A.P., DOGLIA, G., 1983: Chemotaxonomy of wormwood (*Artemisia absinthium* L.). Z. Lebensmittel-Untersuchung und -Forschung, 176, 363-366. doi:10.1007/BF01057728.

CHRPOVÁ, D., KOUŘIMSKÁ, L., GORDON, M.H., HEŘMANOVÁ, V., ROUBÍČKOVÁ, I., PÁNEK, J., 2010: Antioxidant activity of selected phenols and herbs used in diets for medical conditions. Czech J. Food Sci. 28, 317-325.

FRATERNALE, D., RICCI, D., EPIFANO, F., CURINI, M., 2004: Composition and antifungal activity of two essential oils of hyssop. J. Essent. Oil Res. 16, 617-622. doi: 10.1080/10412905.2004.9698810.

GALAMBOSI, B., SVOBODA, K,P., DEANS, A.G., HÉTHELYI, É., 1993: Agronomical and phytochemical investigation of *Hyssopus officinalis*. Agric. Sci. Finl. 2, 293-302.

GILLE, E., FLORIA, E., 2000: A comparative morphological study of some varieties of *Hyssopus officinalis* L. treated with gamma irradiation. Proc. of the First Conference on Medicinal and Aromatic Plants of Southeast European Countries, May 29 - June 3, Arandelovac, Jugoslavia, 387-393.

HODZSIMATOV, K.H., RAMAZANOVA, N., 1974: Some biological characteristics of essential oil accumulation and composition in hyssop cultivated in Taskent region. (In Russian). Rastitelnije Resursi, 11, 238-242.

KOLLER, W.D., RANGE, P., 1997: Geruchsprägende Inhaltsstoffe von Fenchel und Ysop, Z. Arzn. Gew. Pfl. 2, 73-80.

LAWRENCE, B.M., 1992: Progress in essential oils. Perfum. Flav. 17, 54-55.

NÉMETH, É., VARGA, E., FRANKE, R., 2001: Variabilität des ätherischen Ölgehaltes und deren Ursachen in *Hyssopus officinalis* L. Z. Arzn. Gew. Pfl. 6, 29-34.

NÉMETH-ZÁMBORI, É., 2015: Natural variability of essential oil components. In: Baser, K.H.C, Buchbauer, G. (eds.), Handbook of Essential Oils, 87-126. CRC Press - Taylor and Francis Group LLC, Boca-Raton U.S.

PICCAGLIA, R., PACE, L., TAMMARO, E., 1999: Characterisation of essential oils from three Italian ecotypes of hyssop (*Hyssopus officinalis* ssp. *artistatus* Briq.). J. Essent. Oil Res. 11, 693-699. doi: 10.1080/10412905.1999.9711998.

SCHULZ, G., STAHL-BISKUP, E., 1991: Essential oils and glycosidic bound volatile from leaves, stems, flowers and roots of *Hyssopus officinalis*. Flavour Frag. J. 6, 69-73. doi: 10.1002/ffj.2730060109.

VALLEJO, M.C.G., HERRAIZ, J.G., PÉREZ-ALONSO, M.J., VELASCO-NEGUERUELA, A., 1995: Volatile oil of *Hyssopus officinalis* L. from Spain. J. Essent. Oil Res. 7, 567-568. doi: 10.1080/10412905.1995.9698590.

VERES, K., VARGA, E., HAJDÚ, ZS., MÁTHÉ, I., JANICSÁK, G., DOBOS, Á., TARJÁN, G., 1995: Changes of chemical composition of *Hyssopus officinalis* L. during vegetation period. Summaries of the 9th Hungarian Medicinal Plant Conference and 4th Conference on Phytotherapy, (October, 18-20. Szeged, Hungary), 51.

VLASE, L., BENEDEC, D., HANGANU, D., DAMIAN, G., CSILLAG, I., SEVASTRE, B., MOT, A.C., SILAGHI-DUMITRESCU, R., TILEA, I., 2014: Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis*, *Ocimum basilicum* and *Teucrium chamaedrys*. Molecules, 19, 5490-5507. doi: 10.3390/molecules19055490.

ZAWISLAK, G., 2011: Hyssop herb yield and quality depending on harvest term and plant spacing. Acta Sci. Pol. Hortorum Cultus, 10, 331-342.

ZAWISLAK, G., 2013a: The chemical composition of essential hyssop oil depending on plant growth stage. Acta Sci. Pol. Hortorum Cultus, 12, 161-170.

ZAWISLAK, G., 2013b: Morphological characters of *Hyssopus officinalis* L. and chemical composition of its essential oil. Modern Phytomorhology, 4, 93-95.

Address of the corresponding author:
Dr. Éva Németh-Zámbori· Department of Medicinal and Aromatic Plants, Szent István University, 1518 Budapest, P.O. Box 53, Hungary
E-mail: zamborine.nemeth.eva@kertk.szie.hu