Evaluation of caraway essential oil from different production areas of Serbia

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Abstract

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Caraway fruits contain essential oils that give caraway its characteristic aroma. This paper evaluates quantity and composition of the essential oil from the annual caraway grown in different production areas of Serbia. Experiments were conducted in three production areas: Banat, Bačka and Srem, during two years. The fruits contained 3.95% of oil on average. The highest content of essential oil was obtained in the production area of Banat, a part of Serbia where environmental conditions are very favourable for cultivation of medicinal plants. Weather conditions have not a significant influence on the content of essential oil in caraway fruits. However, weather conditions as well as production region had a great influence on seed yield and essential oil yield. There were 22 compounds determined in caraway essential oil by GC/MS. The main components were carvone and limonene, whose mixture constituted from 97.69 to 98.62% of total oil composition. Carvone/limonene ratio was 0.58% on average, therefore, it can be concluded that this annual caraway belongs to limonene chemotype.

Keywords: *Carum carvi* var. *annuum*; essential oil content; essential oil composition; carvone/limonene ratio

Caraway (*Carum carvi* L.) fruits contain from 1 to 6% of essential oils that give caraway its characteristic aroma (Sedláková et al. 2003a). There are approximately 30 compounds in caraway essential oil, among which carvone and limonene account compose about 95%. Other components are present in small proportions (Bouwmeester et al. 1995). Based on experiments, these two components from caraway essential oil have a wide range of application. Carvone can be used as sprouting inhibitor of potatoes, bacteriostatic, as well as a fungistatic agent. This component also possesses repellent and insecticidal properties. Limonene was found to possess antifungal, antibacterial, antioxidantand anticancer properties. Limonene is used as insecticide to control ectoparasites in pet animals, but it has effect on many insects.

The content of essential oils, amount of carvone and limonene and ratio of both substances are the main quality criteria in production of caraway (Sedláková et al. 2003b). It is known that the amount and composition of essential oil are genetically conditioned, but also depend on climatic conditions during stages of fruit formation and ripening (Sedláková et al. 2003a). Weather conditions during the growing period determine plant development and distribution of assimilates, which causes variations in the amount of accumulated essential oil and its composition.

Biannual variety of caraway "Domaći rani" and annual variety "Annualis" (Adamović 1990) were registered in Serbia. However, farmers usually use their own seed for sowing, therefore, we can say that local ecotypes are usually grown (Popović et al. 1997; Dražić et al. 1998). However, this plant is grown in very small areas, mainly in the gardens and yards, and it is therefore difficult to estimate the exact production area. In Serbia, according to Kišgeci and Sekulović (2000), caraway is grown approximately on 50 ha, and the average yield is from 600 to 1,200 kg/ha (Kišgeci 2002), whereas in dry weather conditions the yield is significantly reduced, even less than 200 kg/ha (Dražić et al. 1998).

This paper presents the results of a study about the seed yield and essential oil yield, as well as quantity and composition of essential oil and ratio between two main components in essential oil of caraway plants grown in different production areas of the Vojvodina Province, Serbia. The study was carried out at three localities during two harvest years (2011 and 2012), using annual caraway type which is widely grown in the mentioned region of Serbia. The aim of this study was to evaluate the effect of weather conditions based on the mentioned parameters during the year. Also, based on the results, the most favourable microclimatic and soil conditions for growing caraway were determined.

MATERIAL AND METHODS

Plant material and experiment location. The seed used in this study was annual local ecotype of caraway seed (*Carum carvi* L. var. *annuum*) obtained from a farmer from Kulpin (Bačka region: latitude 45°24'2''N; longitude 19°35'8''E; altitude 83 m a.s.l.). The field experiment was carried out during 2011 and 2012 at three production areas in Vojvodina Province: (1) Banat region (Ostojićevo: latitude 45°53'16''N; longitude 20°09'31''E; altitude 88 m a.s.l.), (2) Bačka region (Mošorin: latitude 45°18'5''N; longitude 20°09'32''E; altitude 111 m a.s.l.) and (3) Srem region (Veliki Radinci: latitude 45°02'26''E; longitude 19°40'15''E; altitude 111 m a.s.l.). The experiment was carried out under field conditions, in different microclimatic and soil environments.

The climate in Vojvodina Province is moderate continental with some tendencies towards continental. The whole region is located in semi-arid area where variations in the amount of precipitation, air temperature and other important climatic elements are substantial over the years. The weather conditions in the analysed years indicate that they were moderately (2011) to severely dry (2012) in the period of active growing season (April–September), in relation to long-term average values (1965–2013, Republic Hydrometeorological Service of Serbia). Temperature conditions during both years, at all three localities, were similar, but there were significant variations in the amount and distribution of rainfall between the locations and years. Meteorological data were obtained from the meteorological stations nearest to the experimental fields (< 30 km) and are shown in Fig. 1.

In order to determine basic agrochemical properties of soil types in each analysed locality, individual soil samples were taken from top soil layer (0–30 cm) and were analysed in the Soil testing laboratory of Agricultural Extension Service Sremska Mitrovica, according to standardized methods adopted in Serbia. Soil pH value was determined potentiometrically in KCl suspensions (10 g/25 cm^3 soil) using an electronic pH meter (PHS-38w; Bante Instrument, Shanghai, China). Content of $CaCO_3$ was determined by molar volume of carbon dioxide using Scheibler calcimeter, and content of humus according to the Tjurin method by oxidation of organic matter by $K_2C_2O_7$ in acid medium (H_2SO_4) and phenyl anthranilyc acid as indicator. The total nitrogen content was determined according to the Kjeldahl method (oxidation of soil organic matter using sulphuric acid and digestion catalyst and conversion of organic nitrogen to ammonium nitrogen), and it was measured by Jenway 6715 UV–Vis spectrophotometer (Bibby Scientific Ltd., Stone, UK). Available phosphorous and potassium was determined by Al-method (Egner-Riehem). Extraction was done with ammonium-lactate and content of phosphorus was determined by spectrophotometer (6715 UV/Vis), and content of potassium was determined by flame photometer (both Jenway PFP-7; Bibbiy Scientific Ltd., Stone, UK). Results of agrochemical analyses are shown in Table 1.

At all three production areas, soils had a neutral reaction to soil solution (pH 7.1–7.3) and

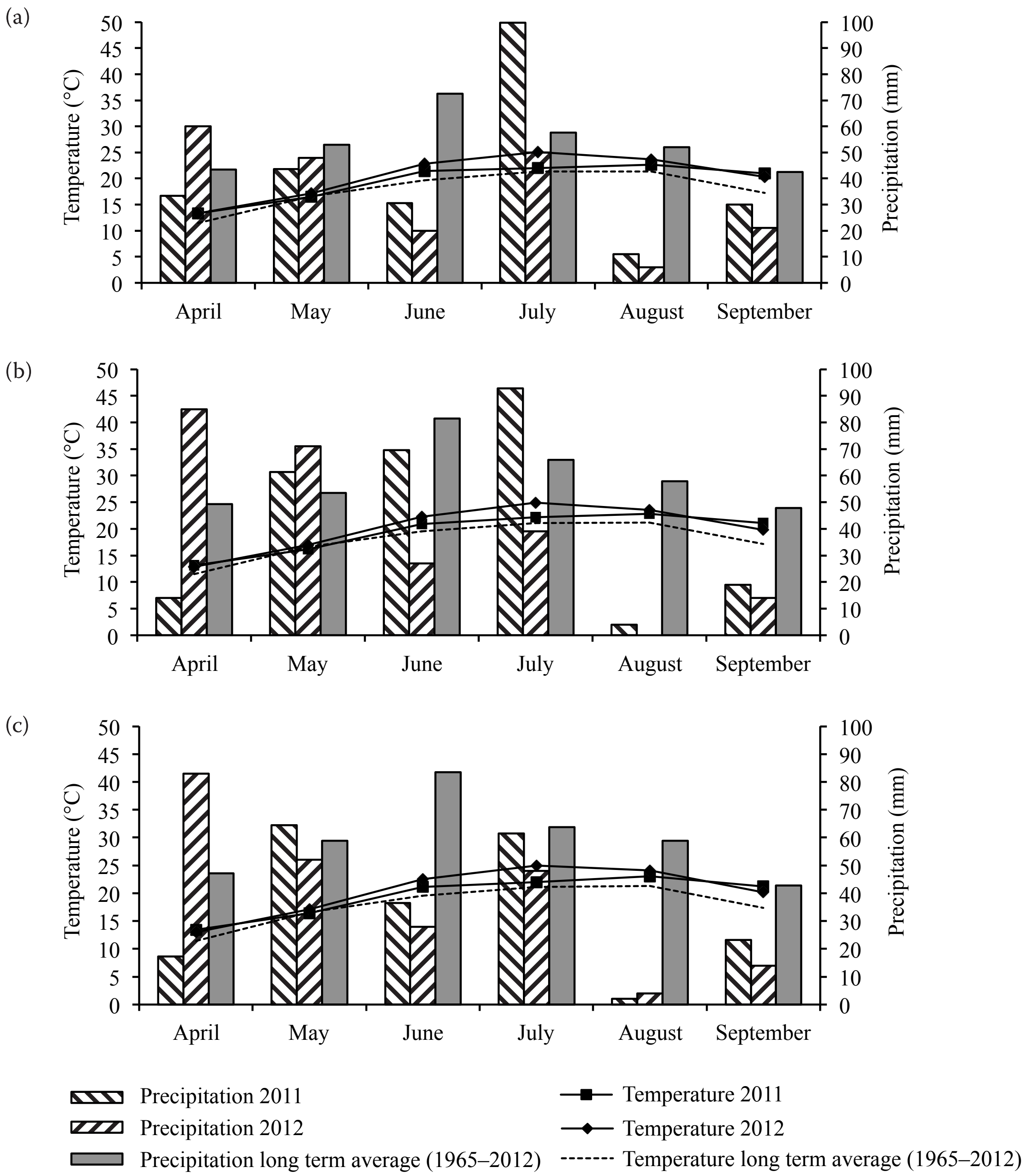

Fig. 1. Average daily temperatures and precipitations in field experiments with caraway; average values for two investigated years 2011 and 2012, and long term average (1965–2012) for each locality
(a) Banat region (meteorological station Kikinda), (b) Srem region (meteorological station Sremska Mitrovica), (c) Bačka region (meteorological station Novi Sad)

were slightly to moderately supplied with humus (2.2–2.7%). The soil at experimental fields in Bačka (locality Mošorin) and Srem (locality Veliki Radinci) production areas was a highly calcareous loamy chernozem, whereas the content of $CaCO_3$ in Banat production area (locality Ostojićevo) was significantly lower. The content of readily available phosphorus and potassium was the highest in locality Mošorin – Bačka region (81.6 and 75.1 mg/100 g of soil, respectively), while in Ostojićevo and Veliki Radinci (Banat and Srem regions) the content of these two nutrients was moderate to optimal (Table 1).

Growing practices. Caraway seeds were sown in the first decade of April (optimal time in Serbi-

Table 1. Basic chemical characteristics of soils in all three production regions in the Vojvodina Province

Production areas	pH (in KCl)	$CaCO_3$ %)	Humus (%)	Total nitrogen (%)	Al P_2O_5	Al K_2O
					(mg/100 g of soil)	
Banat region	7.1	2.0	2.5	0.168	22.4	21.7
Bačka region	7.3	8.4	2.7	0.185	81.6	75.1
Srem region	7.3	8.8	2.2	0.142	17.6	30.3

an agroecological conditions), with row spacing of 0.35 m, respecting density of 200 plants/m^2. The size of the experimental plots was 5 m^2. Weeds were controlled by hoeing and weeding when needed. Measures of protection from diseases and insects were not used. Harvest was carried out by hand in phase of full maturity. In 2011, the harvest was performed in the middle of September, and in 2012 because of water deficit and high temperatures the vegetation period was shortened and the harvest was performed in the last decade of August. Six samples (different fertilizer types, results were not shown in this paper) were taken from each locality in both years in order to determine the seed yield per hectare, essential oil content, composition and yield per hectare.

Extraction of essential oil. The dried samples of Caraway were subjected to hydro-distillation using an all glass Clevenger-type apparatus (ATICO Medical Pvt., Ambala, India) to extract essential oils according to the method outlined by the European Pharmacopoeia (2004). The samples were ground, homogenized and made into a fine powder. In order to extract the essential oils, 100 g of the powder was placed in 1 l conical flask and connected to the Clevenger apparatus. 500 ml of distilled water was added to the flask and heated to the boiling point. The steam in combination with the essential oils was distilled into a graduated cylinder for 4 h and then separated from aqueous layer. The oil was kept refrigerated until required for further analysis.

GC/MS analysis. Gas chromatographic-mass spectrometric analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD) (both Agilent, Santa Clara, USA), in positive ion electron impact (EI) mode. The separation was effected using Agilent 19091S-433 HP-5MS fused silica capillary column with 30 m × 0.25 mm i.d., 0.25 μm film thickness. The GC oven temperature was programmed from 60°C to 285°C at a rate of 3°C/min. Helium was used as carrier gas; inlet pressure was 20.3 kPa; linear velocity was 1 ml/min at 210°C. Injector temperature: 250°C; injection mode: splitless. MS scan conditions: MS source temperature, 230°C; MS Quad temperature, 150°C; energy, 70 eV; mass scan range, 40–550 amu. The identification of components was carried out on the basis of retention index and by comparison with reference spectra (Wiley and NIST databases).

Statistical analysis. Data was subject to statistical analysis using the program package Statistica 10 (StatSoft Inc., 2011, University of Novi Sad licence) and were expressed as mean ± standard deviation (SD). To measure dispersion, coefficient of variation (CV) was used; mean values were compared using analysis of variance (ANOVA) followed by the Fisher's test. Differences between individual means were deemed to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Essential oil content

The average yield of oil from fruit of annual caraway grown in three production areas of Serbia during two seasons was 3.95%. From Table 2 it can be seen that in dry and hotter year it accumulated more essential oil. However, weather conditions have not a significant influence on the content of essential oil in caraway fruits. The highest content of essential oil was obtained in Banat production area, 4.38% on average. Significantly less essential oil was obtained in other two production areas: Srem around 3.60% and Bačka around 3.87%.

It can be said that the Banat region is known as the driest area in the Vojvodina Province, with about 320 mm of rainfall during the growing season (IV–IX), while in the region of Bačka and Srem long-term average rainfall in the same period is about 30 mm higher. The assumption is that the lack of rainfall in the region of Banat caused a statistical increase in the essential oil content in caraway fruits. Laribi et al. (2009) established that

Table 2. Essential oil content, seed yield per hectare, essential oil yield per hectare, carvone (C) and limonene (L) content and C/L ratio

	Essential oil content (%)	Seed yield (kg/ha)	Essential oil yield (kg/ha)	Carvone content (%)	Limonene content (%)	C/L ratio
Average	3.95 ± 0.59	521 ± 387	20.2 ± 14.8	35.71 ± 6.40	62.52 ± 6.08	0.58 ± 0.15
CV	15.05	74.26	73.53	17.92	9.72	25.98
Influence of the weather condition during year (Y)						
2011	3.78^a ± 0.78	850^a ± 205	32.1^a ± 9.9	40.49^b ± 0.92	57.88^a ± 0.73	0.70^a ± 0.02
2012	4.12^a ± 0.44	193^b ± 95	8.2^b ± 4.7	30.93^a ± 5.75	67.16^b ± 5.21	0.47^b ± 0.13
Influence of the production area (A)						
Banat	4.38^a ± 0.40	551^b ± 487	25.1^a ± 23.5	38.70^a ± 1.60	59.78^a ± 1.94	0.65^a ± 0.05
Bačka	3.87^b ± 0.98	654^a ± 529	22.7^a ± 14.8	34.69^a ± 9.68	63.54^a ± 9.19	0.56^a ± 0.23
Srem	3.60^b ± 0.13	359^c ± 378	12.7^b ± 13.1	33.75^a ± 8.99	64.24^a ± 8.57	0.54^a ± 0.21
***F*-test**						
Y	ns	*	*	*	*	*
A	*	*	*	ns	ns	ns
Y × A	ns	ns	*	ns	ns	ns

CV – coefficient of variation; mean values in a row with different letters are significantly different at $P < 0.05$ (Fisher's test); ns – not significant

the water deficit increased the essential oil yield in caraway seed, as confirmed by our research.

As it can be seen, the yield of essential oil from caraway seeds in our experiments is much higher than the yield from Tunisian seeds (0.86–1.20%) (Laribi et al. 2010), or from annual caraway grown in Austria (2.8–3.3%) (Bailer et al. 2001), and it is higher even than yield of essential oil from caraway seeds from Canada (3.2–3.5%) (Arganosa et al. 1998). It was found that the content of essential oil in fruits of annual caraway from Serbia ranges from 2.62–4.51%, depending on altitude (Popović et al. 1997). The results of research show that caraway fruits cultivated at higher altitude have greater content of essential oil. The expression of genetic material from caraway is better in ecological environment at higher altitude.

Seed yield

Average yield of caraway seed was 521 kg/ha, and weather conditions as well as production region had a great influence on this. 2011 was more favourable, so the highest yield was achieved in comparison to dry and hot 2012, when the yield was very poor. High daily temperatures during July and August, and drought in August had a negative influence on filling grain; as a result many seeds were empty, especially in high order umbels. In Bačka locality, the highest yield was recorded, and this can be the consequence of high content of phosphorus in the soil (81.6 mg/100g). According to Farahani et al. (2008) phosphorus had significant effects on water use efficiency in coriander plants.

Essential oil yield

The yield of essential oil per hectare depends directly on the seed yield per hectare and essential oil content in seeds. Average yield of caraway essential oil is around 20.2 kg/ha, but weather conditions and production area have a significant influence on this parameter, as well as its interaction. Due to the very low yields in the dry year 2012, a small amount of the essential oil per hectare was obtained (8.2 kg/ha), while in the favourable year higher yield was achieved (32.1 kg/ha). In Srem locality, the smallest yield of essential oil per hectare was recorded; as a result of lowest seed yield per hectare there was a min. amount of essential oil in fruits. In Bačka and Banat localities essential oil yield was significantly higher, from 22.7 to 25.1 kg/ha. In the neighbouring Croatia, the yield of essential oil per hectare is around 10–12 kg (Šilješ et al. 1992), and in Austria 35–40 kg/ha (Bailer et al. 2001). In Egypt the yield of essential oil per hectare had great variations from 25 to 52 l/ha depending on the fertilization (El-Din et al. 2010).

The researches carried out with coriander in 2009 and 2010 (Acimovic et al. 2011) showed that the Banat region has a high potential for growing medicinal plants of good quality, and the fact that majority of companies dedicated to growing and processing of medicinal, aromatic and spice herbs in Serbia are concentrated in this area only goes to prove the abovementioned conclusion. Sedláková et al. (2001) has also determined that production area has important effect on quantity of essential oil in fruits of caraway.

Essential oil constituents

There were 22 compounds determined in caraway essential oil, among them there were two unidentified compounds (Table 3). Some authors (Kallio et al. 1994; Sedláková et al. 2003a; Laribi et al. 2010) postulate that there are more components in caraway essential oil.

It was established that the essential oil of caraway is constituted mainly of monoterpenes. In our experiment, the main components were carvone and limonene, whose mixture constituted from 97.69 to 98.62% of total oil composition. According to other researchers (Kallio et al. 1994; Ahroet al. 2001; Sedláková et al. 2003a; Seidler-Lozykowska et al. 2010), the percentage of carvone and limonene in essential oil ranges from 93.2 to 99.8%. Apart from monoterpenes, the essential oil contains sesquiterpenes (β-bourbonene, β-elemene, *trans*-caryophyllene, *trans*-muurola-4(14), 5-diene and caryophyllene oxide). In our research, these components were present in small amounts.

As it can be seen from Table 3, the dominant compound in caraway essential oil is limonene (54.04–70.30%). It was determined by Jiang et al. (2011) that limonene is the main component in essential oil of Chinese caraway. Weather conditions have a significant influence on its content in essential oil, as well as in case of carvone (Table 2), whose content in essential oil ranged from 27.39 to 44.51% (Table 3).

Interestingly, there is evidence that biosynthesis of carvone and limonene occurs via the same pathway. This implies that limonene is both an intermediate in the biosynthesis of carvone as well as the end product (Bouwmeester et al. 1995). The biosynthesis of monoterpenes limonene and carvone in the fruits of caraway proceeds from geranyl diphosphate via a three-step pathway. First, geranyl diphosphate is cyclized to limonene by a monoterpene synthase. Second, this intermediate is either stored in the essential oil ductus without further metabolism or is converted by limonene-6-hydroxylase to *trans*-carveole. Third, *trans*-carveole is oxidized by a dehydrogenase to *trans*-carvone (Bouwmeester et al. 1998). At the enzyme level, the hydroxylation of limonene to *trans*-carveole seems to be a critical, rate limiting step in carvone biosynthesis.

Embong et al. (1997) postulated that carvone content is increased by dry and sunny weather; either by the stimulation of photosynthesis which would in turn favour formation of carvone to formation limonene, or increased volatilization of the more volatile limonene leading thus to an altered ratio. However, water deficit can change plant behaviour regarding the biosynthesis of bioactive compounds (Laribi et al. 2009). Water deficit induced an increase in the limonene proportion and decrease in carvone proportion, but did not affect the chemotype of caraway essential oils.

The analyses show that the highest content of carvone was obtained in Banat production area. However, differences in quantity of oil obtained in other two production areas were not considerable. In this locality, as it is mentioned before, the smallest amount of precipitations in both experimental years was recorded. The highest precipitations were in August, in comparison to the other two localities, during fruit formation and ripening of caraway. Precipitations in this time are favourable for the accumulation of carvone.

Carvone/limonene ratio

The overall quality of fruits is considered to correlate with the content of essential oil and its carvone/limonene (C/L) ratio: the higher the ratio, the better the quality (Kallio et al. 1994). As it can be seen from our findings, the C/L ratio was 0.58 on average (Table 2). Therefore, it can be concluded that *Carum carvi* var. *annuum* tested in our research belongs to limonene chemotype.

From the analysis of year it can be concluded that in 2011 a higher content of carvone in essential oil was achieved (40.49%) compared to 2012 (30.93%).

Table 3. Components of EO (%) and C/L ratio in three production regions in the Vojvodina Province

Compound	R.T. (min)	R.I.	Banat		Bačka		Srem	
			2011	2012	2011	2012	2011	2012
α-Pinene	5.838	921	0.11	trace	0.00	trace	trace	trace
Sabinene	6.947	968	trace	0.00	0.00	0.00	trace	0.00
Myrcene	7.447	981	0.22	0.13	0.29	0.16	0.32	0.16
p-Cymene	8.581	1,017	trace	0.26	0.00	0.26	0.10	0.12
Limonene	8.780	1,021	54.04	70.30	57.05	70.04	56.19	61.15
γ-Terpinene	9.853	1,048	0.10	trace	0.00	trace	0.18	trace
Linalool	11.483	1,089	trace	0.00	0.00	0.00	0.00	0.00
cis-Limonene oxide	12.780	1,125	0.11	trace	0.28	trace	0.14	0.00
Mentha-2,8-dien-1-ol cis-para	12.821	1,128	0.00	0.00	0.00	0.00	trace	0.00
trans-Limonene oxide	12.970	1,131	0.12	0.22	0.26	0.21	0.14	0.00
trans-Dihydrocarvone	15.869	1,198	0.16	0.22	0.15	0.19	0.32	0.11
trans-Carveole	16.553	1,213	0.15	0.34	0.29	0.31	0.15	0.23
Unidentified	16.664	1,214	0.00	0.16	0.00	0.19	0.00	0.00
Dihydrocarveol neo iso	17.038	1,222	0.00	0.30	0.00	0.25	0.00	0.17
Unidentified	17.160	1,224	0.00	0.19	0.00	0.17	0.00	trace
Carvone	17.738	1,239	44.51	27.39	41.53	27.84	41.56	37.57
Perilla aldehyde	18.979	1,260	0.11	0.00	trace	0.00	trace	0.00
β-Bourbonene	23.895	1,369	0.00	0.00	0.00	0.00	0.02	0.00
β-Elemene	24.214	1,382	trace	0.00	0.00	0.00	trace	0.00
trans-Caryophyllene	25.399	1,461	0.13	0.19	trace	0.20	0.24	0.23
trans-Muurola-4(14),5-diene	28.043	1,485	trace	0.00	0.00	0.00	0.24	0.00
Caryophyllene oxide	32.245	1,581	trace	0.22	0.00	0.10	trace	0.19
None of identified components			17	15	9	15	18	12
None of components presented in traces			6	3	2	3	6	3
Other minor components (%)			1.47	2.38	1.45	2.21	2.17	1.37
Carvone + limonene mixture (%)			98.54	97.69	98.58	97.87	97.75	98.72
C/L ratio			0.82	0.39	0.73	0.40	0.74	0.61

EO – essential oil; R.T. – retention time; R.I. – retention index; C/L – carvone/limonene; values are mean of six replicates (different fertilizers); trace – component present less than 0.1%

These data show that in dry and hot year C/L ratio was lower, i.e. that quality of essential oil was poor (0.47). The C/L ratio did not vary significantly between localities, i.e. limonene and carvone were accumulated in a relatively fixed ratio. It seems that there is a correlation between assimilate availability and essential oil formation. According to other researchers (Puschmann et al. 1992; Kallio et al. 1994; Sedláková et al. 2003a) C/L ratio varies from 0.90 to 2.74.

CONCLUSION

Seed yield and essential oil yield were strongly influenced by weather conditions. Drought had a negative influence on yield of caraway seed, which could be significantly reduced in high temperature stress conditions. The highest content of essential oil was obtained in the Banat production area, as a consequence of dry conditions during vegetation period. It is known that plants in stress conditions accumulate more secondary metabolites, among which are the essential oils. Apart from this, the highest content of carvone and the highest C/L ratio were established on this locality. This is perhaps the effect of optimal availability of phosphorus and potassium in the soil and precipitation in periods of fruit formation and ripening. This region could be used successfully for the production of caraway, which should be further investigated on other varieties. Also, further investigation could determine if the transformation process of limonene to carvone is genetically fixed or it is influenced by specific climate. In the variety that we used in our investigation, the content of limonene is a main component in essential oil; therefore, it can be concluded that this annual caraway belongs to limonene chemotype. It is known that limonene is used in many organic products as an active ingredient of commercially available flea shampoos, mosquito repellents and different agrochemicals. Due to the fact that caraway has good conditions for growth in Europe, this plant can be a significant source of natural limonene.

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