Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 128, 67—75, 2015

UDC 635.75:665.52(497.113)
DOI: 10.2298/ZMSPN1528067A

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ESSENTIAL OIL CONTENT AND COMPOSITION OF ANISEED

ABSTRACT: The field experiments were carried out during 2011 and 2012 in three localities in Vojvodina (Serbia) with the application of six different fertilizer regimes aimed at determining the content and composition of the aniseed essential oil. It was found that the average essential oil content of aniseed, obtained by hydrodistillation, was 3.72%. The weather conditions during the year and the locality had a statistically significant effect on the essential oil content, while different source of fertilizers was not statistically significant for the essential oil content and its composition. Essential oil composition was determined using GC–MS technique, and a total of 15 compounds were identified. It was found that the major component was *trans*-anethole, 94.78% on the average, and the coefficient of variation was 2%. The second most abundant component was γ-himachalene with 2.53% (CV 28%). All other components were present in less than 1%.

KEYWORDS: fertilization, location, *Pimpinella anisum* L., *trans*-anethole, weather conditions

INTRODUCTION

Aniseed (*Pimpinella anisum* L.) belongs to the *Apiaceae* family. This family is well known for its distinctive flavors, which come from essential oils and are a mix of volatile fragrant compounds that make the essence of the plant. In aniseed there is around 1.5–5.0% of essential oil, and the main component

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of the aniseed essential oil is *trans*-anethole that gives sweet herbaceous odor and taste to this plant [Ullah and Honermeier 2013].

It is known from the literature that aniseed has an effect on the digestive system by increasing secretion of the salivary glands, reducing the acidity of gastric juice. It also has a laxative effect. Apart from this, it has been found that anise has a positive effect on the liver function. Anise has an effect on the nervous system as antiepileptics and analgesics. It also possesses anti-inflammatory and anticancer activity. The effect of anise on the respiratory system is particularly important for the treatment of bronchial asthma. Anise is known for its estrogenic action, thereby its application helps the reduction of bleeding duration and menstrual pains and the reduction of hot flashes in postmenopausal women. Numerous studies have found that anise has an impact on microorganisms that cause many diseases. Aniseed has a wide range of effects on many bacteria, fungi, viruses and amoebas [Shojaii and Fard 2012].

The content and the composition of essential oils of aniseed are affected by ecological conditions, growing techniques and fruit maturity level [Özel 2009]. In case of coriander, which also belongs to Apiaceae family, it was concluded that dependence among temperature, radiance during fruit development and water supply of crops influence essential oil contents and its composition [Gil *et al.*, 2002]. The investigation of the composition of essential oil from anise fruits cultivated in different countries show that some significant differences in concentration of some compounds in oil are present [Orav *et al.*, 2008]. But in different countries, apart from different climatic and soil characteristics, different ecotypes are cultivated, so differences in essential oil content and composition are huge. Different populations grown in one country also show great variations of chemical compounds in essential oil [Arslan *et al.*, 2004; Ipek *et al.*, 2004; Naher *et al.*, 2012].

For crops such as cereals, pulses, fodder crops, and vegetables, fertilization represents one of the most studied aspects of agronomic techniques, but there are less information about medicinal and aromatic plants [Carrubba 2009]. There are a few papers written about the application of different fertilizers in aniseed crop [Jevđović and Maletić 2006; Darzi *et al.*, 2012; Nabizadeh *et al.*, 2012; Jevđović *et al.*, 2012]. However, there are no information about the influence of fertilizers on chemical composition of essential oil.

The aim of this study was to determine the influence of weather conditions, locations and fertilizers on the composition of anise essential oil. In order to determine this, the field experiments were carried out during 2011 and 2012 in three localities in Serbia with the application of six different fertilizer regimes.

MATERIAL AND METHOD

The field experiments were carried out during 2011 and 2012 in three localities in the Province of Vojvodina: Mošorin, Ostojićevo and Veliki Radinci. The field experiments were set up as a randomized block design with four replications. An experimental plot size was 5 m^2. Sowing of aniseed was carried out at optimum time for agroecological conditions of Serbia (during April) with a hand seeder. Seeds were sown at row spacing of 0.35 m respecting the density of 200 plants per square meter. Weeds were controlled by hoeing and weeding when needed. The harvest was performed by hand at a full ripening stage.

The influence of six treatments: Slavol, Bactofil B-10, Royal Ofert, vermicompost, NPK (15:15:15), and control (without fertilization) were examined. Slavol and Bactofil B-10 are microbiological fertilizers containing products of bacterial fermentation, natural vitamins, enzymes and growth stimulators. Royal Ofert is a specific poultry manure inoculated with domestic fly larvae, dehydrated and pelleted, whereas vermicompost is cattle manure modified with *Lumbricus terrestris*.

The requested quantities of fertilizers, excluding Slavol, were applied by incorporation to the 5 cm layer of soil before the sowing of seeds. Slavol was applied two times during vegetation period – first time when plants had 3–4 leaves, and second time after 7 days. The dosage of application of these fertilizers is as follows: Slavol (7 l/ha by watering), Bactofil B-10 (1.5 l/ha), Royal Ofert granules (3 t/ha), vermicompost (5 t/ha), and chemical ferilizer NPK (400 kg/ha in formulation 15:15:15, i.e. 60 kg per hectare N, P_2O_5 and K_2O).

Climate in the region of Vojvodina is moderate continental with some continental tendencies. Both experimental years, in the period of active growing season, were warmer in relation to long-term average values and thus were moderately (2011) to severely dry (2012). Chernozem was a type of soil present in all three locations, with neutral reaction to the soil solution and moderate humus content (2.2–2.7%).

Essential oil was obtained from crushed mature fruits by hydrodistillation using Clevenger-type apparatus. The content of essential oil in anise fruits was 3.91% on the average. The chemical composition of essential oil was analyzed using GC/MS (gas chromatography- mass spectrometry) technique. GC-MS analysis was performed using an Agilent 6890 gas chromatograph in conjunction with an Agilent 5973 Network mass selective detector (MSD) in positive ion electron impact (EI) mode. The separation was achieved using Agilent 19091S-433 HP-5MS fused with silica capillary column, 30m × 0.25 mm, i.e. 0.25 µm film thickness. The GC oven temperature was programmed from 60 °C to 285 °C at a rate of 4.3 °C/min. Helium was used as carrier gas; inlet pressure was 25 kPa; linear velocity was 1ml/min at 210 °C. Injector temperature was 250 °C and the injection mode was splitless. MS scan conditions: source temperature – 200 °C; interface temperature – 250 °C; energy – 70 eV; mass scan range was 40–350 amu. Identification of components was done on the basis of retention index and by comparison with reference spectra (Wiley and NIST databases).

All the data were subjected to statistical analysis (one way ANOVA) using STATISTICA software. Differences between the treatments were performed by LSD range test at 0.05 level. Coefficient of variation (CV) was calculated as standard deviation/mean x 100.

RESULTS

In this study, the average content of essential oil in aniseed obtained by hydrodistillation was 3.91%. It has been found that essential oil content in aniseed depends on weather conditions and locality, while the application of different kinds of fertilizers does not have a significant effect on essential oil content. In the dry year (2012), a significantly lower essential oil amount was noted, and plants grown in Ostojićevo locality contained less essential oil than plants from other two localities.

A total of 15 compounds were identified from aniseed essential oil. It was found that the major component is *trans*-anethole, 94.78% on the average, and coefficient of variation was 2%. This component of aniseed essential oil was influenced only by weather conditions, while the effect of different localities and applied fertilizers was not statistically significant. As it can be seen from Table 1, in the year with average weather conditions (2011), there was less *trans*-anethole in essential oil when compared with dry year (2012).

The second most abundant component of the essential oil is *γ*-himachalene with 2.53% and with coefficient of variation of 28%. The quantity of this component of the essential oil, like in the previous case, was influenced only by weather conditions. In contrast to the *trans*-anethole, the content of this component was lower in the dry year.

All other components of aniseed essential oil were present in less than 1%, and their coefficients of variation were significantly greater. The impact of weather conditions during the year was significant for almost all components, except for *trans*-2-pseudoeugenyl metylbutirate, the presence of which in the essential oil was not affected by any of the factors.

Compounds as *β*-bisabolene, epoxy-2-pseudoeugenyl metylbutirate and *β*-farnesane were present only in the first experimental year, while *cis*-dihydrocarvone and *β*-elemene were present only in the second. These compounds have coefficient of variation above 100, while in the case of *β*-farnesane CV=201, which indicates that this is not a typical component of the aniseed essential oil.

In addition to the weather conditions, locality had a statistically significant impact on the presence of methylchavicol, *α*-zingiberene, *α*-himachalene, *β*-bisabolene, *cis*-anethole, and epoxy-2-pseudoeugenyl metylbutirate.

DISCUSSION

Drought stress significantly affected the essential oil percentage [Aloghareh *et al.*, 2013]. Water deficit during stem elongation and umbel appearance reduced oil production in aniseed [Zehtab-Salmasi *et al.*, 2001]. Dry conditions during the second experimental year caused significant decrease in essential oil content in our research, similarly to previous studies. Chemical fertilizers were effective in increasing oil percentage compared with unfertilized treatment

Table 1: Content and components of aniseed essential oil (%)

Source of variation		*Essential oil content*	*trans*-anethole	γ-himachalene	*trans*-pseudoisoeugenyl 2-methylbutyrate	methyl chavicol	*trans*-muurola-4(14),5-diene	α-zingiberene	α-himachalene	NI	β-himachalene	*cis*-dihydro carvone	β-bisabolene	*cis*-anethole	epoksy-psdoisoeugenyl 2-methylbutyrate	β-elemene	β-farnesene
Year	2011	3.93	93,20	3,13	0,95	0,79	0,46	0,36	0,31	0,23	0,19	0,00	0,19	0,08	0,11	0,00	0,02
	2012	3.52	96,35	1,93	0,66	0,19	0,07	0,10	0,12	0,09	0,11	0,28	0,00	0,04	0,00	0,07	0,00
Locality	Mošorin	3.73	94,58	2,59	0,79	0,54	0,25	0,28	0,23	0,16	0,16	0,13	0,13	0,03	0,10	0,03	0,02
	V. Radinci	3.93	95,02	2,44	0,75	0,54	0,24	0,19	0,20	0,16	0,15	0,12	0,07	0,07	0,02	0,03	0,01
	Ostojićevo	3.50	94,73	2,56	0,86	0,38	0,31	0,21	0,22	0,16	0,15	0,16	0,09	0,07	0,05	0,04	0,00
Fertilizer	Control	3.64	94,96	2,35	1,03	0,41	0,23	0,19	0,20	0,15	0,15	0,12	0,08	0,04	0,06	0,02	0,01
	Slavol	3.66	94,35	2,80	0,86	0,51	0,27	0,24	0,24	0,18	0,16	0,18	0,09	0,07	0,05	0,04	0,01
	Bactofil	3.66	94,68	2,60	0,96	0,46	0,24	0,21	0,22	0,16	0,16	0,13	0,06	0,03	0,04	0,04	0,00
	Royal Ofert	3.90	94,89	2,40	0,66	0,53	0,37	0,25	0,21	0,16	0,16	0,15	0,12	0,07	0,07	0,03	0,02
	Vermicompost	3.63	94,98	2,53	0,60	0,51	0,24	0,23	0,22	0,16	0,14	0,13	0,11	0,07	0,06	0,03	0,02
	NPK	3.85	94,80	2,51	0,71	0,50	0,25	0,25	0,21	0,15	0,14	0,13	0,11	0,07	0,07	0,05	0,01
LSD 0.05																	
Year		*	*	*	ns	*	*	*	*	*	*	*	*	*	*	*	*
Locality		*	ns	ns	ns	*	ns	*	*	ns	ns	ns	*	*	*	ns	ns
Fertilizer		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Trial average		**3.72**	94,78	2,53	0,80	0,49	0,27	0,23	0,22	0,16	0,15	0,14	0,09	0,06	0,06	0,03	0,01
CV(%)		9	2	28	63	69	88	70	47	47	34	108	120	75	140	129	201

*significant at level 0.05 according to Fisher's Least Significant Difference Test, ns – not significant, CV – Coefficient of Variation, Ni – Compound is Not Identified

[Yassen *et al*., 2010]. The treatment with phosphate solubilizing microorganisms had a positive effects on essential oil content [Zand *et al*., 2013]. In our experiments, application of different types of fertilizers increased essential oil content of aniseed, but it was not statistically significant.

In a study conducted in Bangladesh [Naher *et al*., 2012] it was concluded that a slight variation of essential oil content and composition of anise depends on several factors such as genotype, stage of maturity, cultivation practices, soil composition and climate differences in various geographical locations. However, there is no data on how weather conditions influence the quality of anise essential oil, but it was known that high temperature and intensive insolation had a strong positive effect on *α*-bisabolol content, the main component of chamomile essential oil [Seidler-Lozykowska 2010]. This is similar to our investigation, where the content of *trans*-anethole was higher (96.35%) in the year with higher temperatures during vegetation period (2012).

In a study conducted in Turkey, *trans*-anethole ratio varied among localities from 95.57 to 97.24%, and the content of methyl chavicol varied between 1.89 and 3.18% [Tort and Honermeier 2005]. In this study, there was a significantly smaller variation between localities in case of *trans*-anethole (94.58–95.02%), but for methyl chavicol variations between 0.38 (locality Ostojićevo) and 0.54 (localities Mošorin and Veliki Radinci) were registered. Therefore, it can be concluded that in the case of methyl chavicol the influence of locality was significant.

Apart from isomers *trans*-anethole and methyl chavicol, *cis*-anethole was also present in aniseed, but in small proportion (0.06% in trial average). Because *cis*-anethole has toxic properties, international laws limit the concentration of this compound in isolated natural anethole to 0.2% [Orav *et al*., 2008].

Phenylpropanoids such as pseudoeugenyl 2-metylbutirate and *epoxy*-pseudoeugenyl 2-metylbutirate are characteristic of the genus *Pimpinella* and are phytochemical markers for it [Orav *et al*., 2008]. In this study, the pseudoeugenyl 2-metylbutirate was present in all samples, but *epoxy*-pseudoeugenyl 2-metylbutirate was not evident in the dry year (2012). Also, this component was noted in traces in localities Veliki Radinci and Ostojićevo, and in locality Mošorin it was present in the amount of 0.1%.

In essential oils of anise from different European countries [Ullah *et al*., 2013] *α*, *β* and *γ*-himachalene were noted in different concentrations depending on the country origin, as well as *α*-zingiberene, *β*-bisabolene and *β*-elemene. *Cis*–dihydrocarvone in this study was present with 0.26%. This component is usually present in other plants from Apiaceae family like caraway [Raal *et al*., 2012] and dill [Charles *et al*., 1995; Krüger and Hammer 1996]. However, it has not been detected in aniseed essential oil obtained by hydrodistillation. Using supercritical CO_2 extraction [Vîlcu *et al*., 2003], there was obtained 0.16 to 0.24% of this component of anise essential oil, while other authors, using the same method, did not detect this compound [Yamini *et al*., 2008; Rodrigues *et al*., 2003].

CONCLUSION

Essential oil content of aniseed depends on weather conditions and locality, while the application of different types of fertilizers does not have a significant effect on essential oil content and compounds in the oil. Weather conditions during the year had an influence on the content of all compounds in essential oil excluding *trans*-pseudoisoeugenyl 2-methylbutirate. This compound was not influenced by any factor, which asserts the fact that it is a phytochemical marker for genus *Pimpinella*.

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САДРЖАЈ И ХЕМИЈСКИ САСТАВ ЕТАРСКОГ УЉА АНИСА

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РЕЗИМЕ: У раду су приказани резултати испитивања количине и хемијског састава етарског уља аниса гајеног током 2011. и 2012. године на три локалитета у Војводини (Србија), при примени шест различитих врста ђубрива. Утврђено је да је просечна количина етарског уља у плодовима аниса добијена методом дестилације воденом паром 3.72%. Временски услови током године и локалитет имају статистички значајан ефекат на садржај етарског уља и његов хемијски састав, док примена различитих врста ђубрива не утиче на ове параметре. Хемијски састав етарског уља одређен је применом GC–MS анализе, при чему је идентификовано укупно 15 компоненти. Установљено је да је главна компонента *trans*-анетол, се просечно 94,78%, и коефицијентом варијације 2%. Друга најзаступљенија компонента била је γ-химахален са 2,53% (CV 28%). Све остале компоненте биле су присутне са мање од 1%.

КЉУЧНЕ РЕЧИ: ђубрење, локалитет, *Pimpinella anisum* L., *trans*-анетол, услови године