

Influence of Ecological Conditions on Seeds Traits and Essential Oil Contents in Anise (*Pimpinella anisum* L.)

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Abstract

Anise (*Pimpinella anisum* L.) is an annual plant of the Apiaceae family, widely cultivated for the seed and essential oil. Under field condition, anise is cultivated in a vast number of countries including Serbia. Field experiments were carried out during two growing seasons, at three localities, in order to determine the effect of different soil and climatic conditions on the quality of anise seed principal traits (thousand seed weight, germination energy and total germination) as well as on the content and quality of its essential oil. During the experiment it was found that the value of aniseed principal traits was significantly lower in hotter and drier year in comparison to the year with moderate conditions, because of more favourable weather conditions for seed development during this year. A significantly higher concentration of essential oil was also accumulated in the moderate year in comparison to the dry and hot one. This can be attributed to a longer period of fruit formation and synthesis of essential oils and better climatic conditions. It can be concluded that drought caused a significant decrease in thousand seed weight, germination energy and total germination as well as essential oil content in anise. Contrary to this, the content of *trans*-anethole was significantly higher in the dry year. It can be assumed that under stress conditions the amount of *trans*-anethole in the essential oil increases, because in such conditions the plants produce more secondary metabolites, substances that prevent oxidation processes in the cells.

Keywords: essential oil content, germination, *Pimpinella anisum*, seed weight, *trans*-anethole

Introduction

Anise (*Pimpinella anisum* L.) is an annual plant of the Apiaceae family, widely cultivated for fruit and essential oil. This plant, originating from the Mediterranean region, is cultivated under field conditions in a vast number of countries, especially in Southern Europe and Southeast Asia, but also in the United States, China and Chile (Embong *et al.*, 1977). Since anise favors warm climatic conditions throughout the growing season, it is particularly cultivated in subtropical regions (Ullah *et al.*, 2013).

Anise is an important raw natural material mostly used in medicine, pharmaceuticals, perfumery and cosmetic industries. It is also used as functional food; it is added to confectionary products (honey cookies, candies) and alcoholic beverages (liqueurs and sweet flavoured wines). Anise fruits, also known as aniseed, contain essential oil (*Anisi aetheroleum*). Recent research found that this essential oil has an antimicrobial and antifungal properties (Kubo and Himejima, 1991; Kosalec *et al.*, 2005; Ozcan and Chalchat 2006; Yazdani *et al.*, 2009) and antioxidant

effect on the human health (Gülcin *et al.*, 2003; Tepe *et al.*, 2006; Rajeshwari *et al.*, 2011). The primary constituent of anise essential oil is *trans*-anethole, a volatile phenylpropanoid, which comprises 80 to 90% of the oil (Tabanca *et al.*, 2006; Orav *et al.*, 2008). As a predominant component, it gives off the characteristic odour and has sweet aromatic flavour (Koeduka *et al.*, 2009).

The quality of anise is determined mainly on the basis of the essential oil content and its composition. Both of these parameters are significantly affected by environmental factors, i.e. soil type and weather conditions during the year (temperature, precipitation, etc.) especially during the development of anise fruit (stages of plant maturity) and by the applied agronomic practices (Zehtab-Salmasi *et al.*, 2001; Omidbaigi *et al.*, 2003; Tuncurk and Yildirim, 2006; Özel, 2009; Jevdžović *et al.*, 2012). Also, the concentration of essential oil may significantly vary among anise fruits of different origin (Tabanca *et al.*, 2006; Orav *et al.*, 2008).

The aim of this study was to determine the effect of different localities, with different soil and microclimatic

conditions during two growing seasons, on the quality of anise seed principal traits (thousand seed weight, germination energy and total germination), on its essential oil content, as well as on the content of *trans*-anethole, the main constituent of the essential oil.

Materials and Methods

Experiment locations

Field experiments were carried out during two years (Y), at three localities (L) in the Vojvodina Province, Republic of Serbia: L1 – Mošorin (45°18' N, 20°09' E, 111 m above sea level), L2 – Veliki Radinci (45°02' N, 19°40' E, 110 m above sea level) and location L3 – Ostojićevo (45°54' N, 20°09' E, 88 m above sea level). All sites are located in Vojvodina region (northern Serbia), which is mostly a flat area located in the southern part of the Pannonian lowland.

Soil conditions

In Vojvodina region, the dominant soil type is calcareous chernozem on loess terrace. Individual soil samples were taken from the top soil layer (0-30 cm) and analyzed according to standardized methods adopted in Serbia and

EU countries for the purpose of determining agrochemical properties of soil types on each locality. At all three locations, all soil had a neutral reaction to the soil solution and had moderately humus content (2.2-2.7%). The soil at localities L1 and L2 were highly calcareous chernozem, whereas the content of CaCO₃ at L3 was significantly lower. The content of available phosphorus and potassium was the highest on locality L1 (81.6 mg 100g⁻¹ of soil), while at L2 and L3 the content of these nutrients was moderate to optimal (results of soil analyses are not shown in this paper).

Climate conditions

Climate in Vojvodina region is moderate continental with some tendencies towards continental. The region is located in a semi-arid area where the variations as amount of precipitation, air temperature and other important climatic elements among years are substantial. Data for basic meteorological elements per month - precipitation (P, mm) and average air temperatures per month (T, °C) for both analyzed years and for all three localities is shown in Tab. 1. Weather conditions in the analysed years indicate that, in the period of active growing season (IV-IX), both years were hotter in relation to long-term average values (LTA) and were moderately to severely dry.

Tab. 1. Precipitation and temperature conditions at analyzed locations

| Year | Month | | | | | | | | | | | | GS ¹ | WP ² | Yearly (1.X-30.IX) | |
|---------------------|------------------|----|-----|----|----|----|-----|------|----|----|----|-----|-----------------|-----------------|-----------------------|-----|
| | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | | | | |
| L1 – Mošorin | | | | | | | | | | | | | | | | |
| T (°C) | 2011 | 0 | 0 | 6 | 13 | 17 | 21 | 22 | 23 | 20 | 11 | 3 | 4 | 19 | - | 12 |
| | 2012 | 2 | -5 | 8 | 13 | 17 | 23 | 25 | 24 | 21 | 14 | 10 | 3 | 20 | - | 13 |
| | LTA ³ | 0 | 2 | 6 | 12 | 17 | 20 | 22 | 21 | 17 | 12 | 6 | 1 | 18 | - | 11 |
| P (mm) | 2011 | 25 | 37 | 26 | 23 | 63 | 37 | 62 | 2 | 25 | 35 | 2 | 49 | 212 | 266 | 478 |
| | 2012 | 43 | 67 | 4 | 83 | 51 | 31 | 48 | 4 | 14 | 48 | 36 | 55 | 230 | 200 | 430 |
| | LTA | 39 | 34 | 38 | 48 | 60 | 87 | 68 | 58 | 47 | 47 | 50 | 50 | 360 | 255 | 617 |
| L2 – Veliki Radinci | | | | | | | | | | | | | | | | |
| T (°C) | 2011 | 0 | 0 | 7 | 13 | 17 | 21 | 22 | 24 | 21 | 11 | 4 | 4 | 20 | - | 12 |
| | 2012 | 2 | -4 | 8 | 13 | 17 | 22 | 25 | 24 | 20 | 14 | 10 | 1 | 20 | - | 12 |
| | LTA | 1 | 2 | 7 | 11 | 17 | 20 | 21 | 21 | 17 | 11 | 6 | 1 | 18 | - | 11 |
| P (mm) | 2011 | 31 | 31 | 15 | 20 | 46 | 69 | 93 | 0 | 19 | 25 | 5 | 46 | 247 | 246 | 493 |
| | 2012 | 40 | 52 | 4 | 85 | 72 | 31 | 40 | 0 | 14 | 47 | 31 | 41 | 242 | 172 | 414 |
| | LTA | 37 | 36 | 38 | 52 | 56 | 86 | 65 | 63 | 53 | 54 | 54 | 42 | 375 | 261 | 636 |
| L3 – Ostojićevo | | | | | | | | | | | | | | | | |
| T (°C) | 2011 | 0 | 0 | 7 | 13 | 17 | 21 | 22 | 24 | 21 | 11 | 3 | 4 | 20 | - | 12 |
| | 2012 | 1 | -5 | 8 | 13 | 17 | 23 | 25 | 24 | 20 | 14 | 9 | 0 | 20 | - | 12 |
| | LTA | 0 | 1 | 6 | 11 | 17 | 20 | 22 | 21 | 17 | 11 | 5 | 1 | 18 | - | 11 |
| P (mm) | 2011 | 16 | 18 | 36 | 10 | 47 | 31 | 101 | 0 | 30 | 37 | 0 | 38 | 219 | 235 | 454 |
| | 2012 | 37 | 57 | 4 | 60 | 52 | 24 | 55 | 7 | 21 | 68 | 27 | 46 | 219 | 173 | 392 |
| | LTA | 31 | 31 | 33 | 48 | 53 | 74 | 59 | 54 | 49 | 43 | 45 | 41 | 337 | 224 | 561 |

¹GS – Growing Season (IV-IX); ²WP – Winter Precipitation (X-III); ³LTA – Long-Term Averages (1965-2012)

Conducting the experiment

The anise seed used in this study was a widely grown early ripening cultivar, named N-210. In both years, at all three localities, anise was sown by hand at the end of March and during the first decade of April (depending on the year; Tab. 2), which is optimal time for sowing anise in Serbian climate. It was done directly into the soil with seedling depth of 2-3 cm, in continuous rows, 35 cm apart. One experimental plot contained five rows, but only the middle

row from each plot was harvested to eliminate the marginal effect.

After germination of the plants in the field, they were thinned by hand in order to get 70 plants per meter. The plots were kept weed-free by hand weeding and hoeing, without the application of herbicides. The plants were harvested manually in August (2011) and at the end of July (2012) at the stage of their full maturity.

The seed materials were analyzed 3 months after harvesting in order to determinate the following: thousand

seed weight (TSW), germination energy (GE), total germination (TG), as well as quantity and quality of anise essential oil (essential oil content – EOC and *trans*-anethole content – TAC).

Thousand seed weight was determined and the germination test was carried out in Seed testing laboratory of the Agricultural Extension Service in Sremska Mitrovica. Eight samples from every locality, containing 100 seeds each, were taken and weighed. Their average value was multiplied by 10 to calculate the thousand seed weight. Standard germination was carried out in a plastic box (10x15x6 cm) containing moist plated paper. There were six replications from each locality. A replicate consisted of a single box with 100 seeds. The plastic boxes were incubated at alternating temperatures of 20/30 °C with 8/16 h light/darkness regime and illumination was provided by white fluorescent tubes.

The seedlings were evaluated 7 and 21 days after sowing. The first count test was performed with a standard germination test. The percentage of normal seedlings was

recorded 7 days after sowing, according to the International rules for seed testing (ISTA).

Essential oil content was determined at the Faculty of Chemistry, Belgrade, by hydrodistillation using Clevenger-type apparatus. The seed from each locality (10 g, in six replications) was crushed in an electric mill. The oil quality was assessed by applying combined gas chromatography-mass spectrometry. GC-MS analysis was performed by using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD) in positive ion electron impact (EI) mode. The separation was achieved by using Agilent 19091S-433 HP-5MS fused silica capillary column, 30 m x 0.25 mm i.d. and 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 the carrier gas; inlet pressure was 25 kPa; linear velocity was 1ml/min at 210 °C. Injector temperature: 250 °C; injection mode: splitless. MS scan conditions: source temperature, 200 °C; interface temperature, 250 °C; energy, 70 eV; mass scan range, 40-350 amu. Identification

Tab. 2. Sowing and harvest dates and phenological observations - developmental stages of anise and their duration (days) during two years at three analyzed localities

| Developmental Stages / Year | 2011 | | | | | | 2012 | | | | | |
|--------------------------------|--------|------|--------|------|--------|------|--------|------|--------|------|--------|------|
| | L1 | days | L2 | days | L3 | days | L1 | days | L2 | days | L3 | days |
| Sowing date | 26.03. | - | 01.04. | - | 06.04. | - | 01.04. | - | 04.04. | - | 14.04. | - |
| Germination | 11.04. | 16 | 26.04. | 25 | 12.05. | 36 | 19.04. | 18 | 22.04. | 18 | 28.04. | 14 |
| Stem elongation | 17.05. | 36 | 28.05. | 32 | 06.06. | 25 | 05.06. | 47 | 08.06. | 47 | 10.06. | 43 |
| Flowering | 18.06. | 32 | 29.06. | 30 | 09.07. | 33 | 18.06. | 13 | 20.06. | 12 | 01.07. | 21 |
| Fruit formation | 10.07. | 22 | 18.07. | 19 | 02.08. | 24 | 01.07. | 13 | 13.07. | 23 | 16.07. | 15 |
| Maturation (harvest) | 06.08. | 27 | 16.08. | 29 | 29.08. | 27 | 28.07. | 27 | 31.07. | 18 | 04.08. | 19 |
| Total length of growing season | - | 133 | - | 135 | - | 145 | - | 118 | - | 118 | - | 112 |

of components was done on the basis of retention index and by comparison with reference spectra (Wiley and NIST databases).

Statistical analyses

The experiments were conducted in six replications at each locality. The results were processed as randomized complete block design combined over years by analysis of variance (ANOVA, GenStat Release v.9.1., Rothamsted Experimental Station, trial version), whereby in the sums of squares the year appears as an additional component. The assumed mathematical model of the experiment in a completely randomized design was:

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where α_i and β_j represent effects of localities and years, $(\alpha\beta)_{ij}$ is their interaction, and ε_{ijk} is error, i.e. random variable with distribution $N(0, \sigma^2)$. The significance of the influence of localities, conditions of the years, as well as their interaction were tested using the F-test of ANOVA. Duncan's Multiple Range Test at 5% probability level ($p=0.05$) was used to test the significance of differences between mean values of analysed localities and the years (Statistica v.9. software; StatSoft, Inc., 2007). Correlation

analysis was performed by using GenStat Release v.9.1. software according to Pearson's methods to determine the relationship among the analysed traits.

Results and discussion

The F-test used for the analysis of trait variance (Tab. 3) indicates that the thousand seed weight, germination energy and total germination were affected by weather conditions – with statistically significant effect ($p<.001$), localities (L) and their interaction (YxL) during the years (Y). In the case of thousand seed weight, this interaction was significant only for probability level $p<0.05$. Annual conditions and locations had a significant effect on essential oil content, while the effect of their interaction was not significant. The effect of weather conditions during the years was also highly significant for the *trans*-anethole content. Although in this case the effect of localities was absent, the effect of YxL interaction was statistically significant. However, based on the percentage of individual sources of variation in the total sum of squares, it can be concluded that the localities had a dominant influence (48.6%) on the overall variability of thousand seed weight in the experiment, whereas the annual conditions had a dominant influence on germination energy, total germination and essential oil content (37.6; 44.5 and 36.7%, respectively). The strongest influence of the

Tab. 3. Selected indicators of ANOVA for weight of 1000 seeds (WS), germination energy (GE), total germination (TE), quantity and quality of aniseed essential oil (EO and TA)

| Source of variation | d.f. | WS (g) | | GE (%) | | TG (%) | | EO (%) | | TA (% in EO) | |
|---------------------|------|----------|---------------------|----------|---------------------|----------|---------------------|----------|---------------------|--------------|---------------------|
| | | s.s. (%) | F-pr. | s.s. (%) | F-pr. | s.s. (%) | F-pr. | s.s. (%) | F-pr. | s.s. (%) | F-pr. |
| Year (Y) | 1 | 37,0 | <,001** | 37,6 | <,001** | 44,5 | <,001** | 36,7 | <,001** | 74,1 | <,001** |
| Locality (L) | 2 | 48,6 | <,001** | 26,4 | <,001** | 27,8 | <,001** | 27,2 | <,001** | 1,0 | 0,542 ^{ns} |
| Y x L | 2 | 3,7 | 0,039* | 24,9 | <,001** | 17,6 | <,001** | 2,5 | 0,333 ^{ns} | 6,2 | 0,034* |
| Repetition | 5 | 0,4 | 0,966 ^{ns} | 2,3 | 0,294 ^{ns} | 2,7 | 0,120 ^{ns} | 10,4 | 0,133 ^{ns} | 1,4 | 0,873 ^{ns} |
| Y x Repetition | 5 | 0,7 | 0,913 ^{ns} | 2,0 | 0,359 ^{ns} | 2,1 | 0,216 ^{ns} | 1,7 | 0,894 ^{ns} | 1,9 | 0,768 ^{ns} |
| Residual | 20 | 9,6 | | 6,9 | | 5,3 | | 21,4 | | 15,4 | |
| Total | 35 | 100 | | 100 | | 100 | | 100 | | 100 | |

d.f. – degrees of freedom; s.s. – sum of square (as % of total s.s.); F-pr. – probability of the F-test of ANOVA
 ** Statistically significant at the level $p < 0.01$; * significant at the level $p < 0.05$; ^{ns} non significant

years was evident on the variability of *trans*-anethole content (74.1%), while the impact of the localities and YxL interaction was significantly lower.

Thousand seed weight is an important yield parameter which has direct impact on the crop's final yield and quality of the seed. By observing the average thousand seed weight value for both analyzed years, it can be concluded that there were significant differences between localities, i.e. the highest values were obtained in locality L3 and they were significantly higher than those in L1 and L2 (Fig. 1). On average, the thousand seed weight values obtained in all three localities in 2012 were significantly lower than the values from the previous year. This can be explained by the fact that the temperatures in June 2012, and especially in July 2012 (critical periods for the developmental stages of anise: flowering and seed formation; shown in Tab. 2) were much higher (for 1-3 °C) than in the previous year. In addition, the total length of growing season in 2011 was longer (133-145 days) than in 2012 (112-118 days), which resulted in a longer period of seed formation, also shown in Tab. 2.

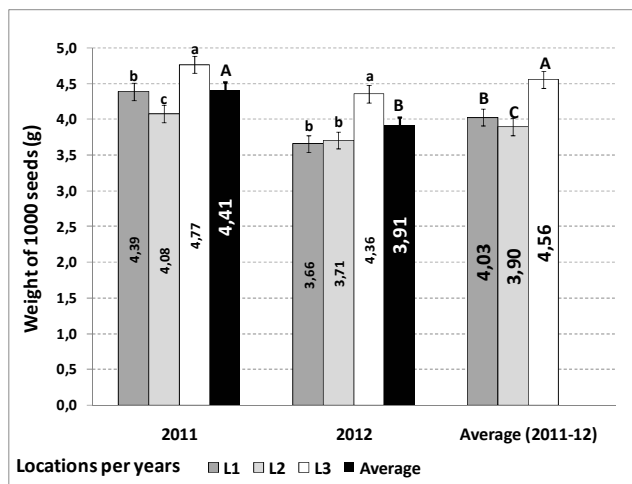


Fig. 1. The effects of years and localities on weight of 1000 seeds.

Bars represent differences between individual means which were deemed to be significant at $p < 0.05$. Values followed by different small letter (a-c) on the bars indicate differences between localities in each year at the same significance level. Values followed by capital superscripts (A-C) in the same way relate to the average values per years (2011 and 2012) and localities (in average for both years)

More severe drought in 2012 caused a decrease in the 1000 seed weight. The average weight of 1000 seeds in 2012 was 3.91 g, which was lower by 11.3% than in 2011. According to Ipek *et al.* (2004); Jevdović and Maletić, (2006); Tunçturk and Yildirim (2006) and Darzi *et al.* (2012), the mass of 1000 aniseed ranges from 2.08-5.46 g. These values are in accordance with our experiment, where the mass of 1000 seeds ranged from 3.66 to 4.77 g depending on the climatic conditions during the year and localities. Diederichsen (1996) reported that in the years with unfavourable weather conditions, coriander plants had a minimum weight of 1000 seed, which was confirmed by our results. The differences in the seed weight can be attributed to temporary variability of weather conditions (Hendrix and Trapp, 1992). The authors also reported that larger parsnip seed has greater germination than smaller seed, while Hasanah and Setyaningsih (1993) reported that seed size has not affected the germination of coriander.

Germination energy throughout the experiment had an average of 79.76% (Tab. 4) and was statistically significantly higher in the first year of the study (85.64%) than in the second (73.88%). As previously mentioned, during the first investigated year, climatic conditions were more favourable for seed formation, i.e. the higher thousand seed weight was obtained, which probably resulted in higher values of germination energy and total germination.

On average, there were statistically significant differences between localities in both years, i.e. the highest germination energy was obtained in L3 (86.27%), followed by L2 and the lowest germination energy was obtained in L1 (74.36%). Generally, in our research, anise had rather high germination energy (78.46-91.04% in 2011 and 62.13-81.50% in 2012) and total germination (87.38-93.71% in 2011 and 68.42-84.67% in 2012). Jevdović and Maletić, (2006), found that germination energy ranged from 42.50 to 73.00% and total germination from 45.25 to 83.00% and that these values greatly depended on fertilization rate and year conditions. Similarly to germination energy, our results indicate that total germination was higher in the first investigated year, when it was up to 90.26%, in relation to 79.13% in year 2012. On average, there were significant differences between localities in both years, i.e. the highest total germination values were obtained in L3 and the lowest in L1. Other comparison and further comments are very similar to the relations obtained for germination energy.

A significantly higher concentration of essential oil was synthesized from plants in 2011 (3.93%) compared to the second investigated year (3.52%) (Fig. 2a). This might be due to suitable environmental conditions during essential oil accumulation. Higher essential oil content in 2011 can be attributed to better climatic conditions, to a longer period of seed formation and synthesis of essential oils (27-29 days in 2011 in relation to 18-27 days in 2012). The period of seed formation and filling in 2011 lasted longer and was prolonged by two months - July and August, when the temperatures in July were at the LTA level, and in August they were even higher (+2-3 °C in relation to LTA). Precipitation in July was at the LTA level at locality L1, which is significantly higher than the LTA values at L2 and L3 (+28 and +42 mm, respectively), whereas in August there was no rainfall. It can be concluded that the first part of this critical period provided favourable conditions for seed formation and filling, while the second, warmer and drier period favoured the synthesis of essential oil and fruit ripening.

Tab. 4. Germination energy and total germination (%) of aniseed depending on years and localities

| Locality / Year | Germination energy (%) | | | Total germination (%) | | |
|-----------------|------------------------|--------------------|--------------------|-----------------------|--------------------|--------------------|
| | 2011 | 2012 | Average | 2011 | 2012 | Average |
| L1 | 86,58 ^b | 62,13 ^d | 74,36 ^C | 88,88 ^b | 68,42 ^d | 78,68 ^C |
| L2 | 78,46 ^c | 78,00 ^c | 78,23 ^B | 87,38 ^b | 84,67 ^c | 86,03 ^B |
| L3 | 91,04 ^a | 81,50 ^c | 86,27 ^A | 93,71 ^a | 84,29 ^c | 89,00 ^A |
| Average | 85,64 ^A | 73,88 ^B | 79,76 | 90,26 ^A | 79,13 ^B | 84,69 |

Values followed by small superscript letter (a-d) across the years indicate differences between localities in each year and between years at the significance level $p < 0.05$. Values followed by capital superscripts (A-C) in the same way relate to the averaged values per years (2011, 2012) and localities (average for both years).

Unlike the previous year, in 2012, the period of seed formation and filling was shorter and occurred only in July, with significantly higher temperatures in relation to LTA (+3-4 °C) and lower amounts of rainfall (from -4 mm in the locality L3 to -25 mm in L2; comparing to the LTA values). Thus, drought in 2012 caused lower content of essential oil in anise. Higher temperatures and precipitation deficiency during this year led to a decrease of 10.4% in essential oil content compared to 2011.

There were significant differences regarding the essential oil concentration among localities. In both years, the highest essential oil content was obtained in the locality L2 (4.08 and 3.79%, respectively) and the lowest in L3. Higher amount of precipitation in the period of aniseed filling might have influenced lower concentration of essential oil in anise (3.78 and 3.23%) in locality L3, in comparison to other localities. The fact that the locality has a significant role in the essential oil formation was indicated by Dražić *et al.*, (2007). In their research, the content of essential oil of anise grown in five localities in Serbia ranged from 0.5-1.2%. According to Orav *et al.* (2008), essential oil content from anise seed originating from different European countries ranged from 1 to 5%, which is more consistent with our results. Essential oil content of anise obtained from experiments carried out in Iran ranged from 2.21 to 2.75%

(Zehtab-Salmasi *et al.*, 2001) and in investigations carried out in Turkey from 2.66 to 2.74% (Tuncturk and Yildirim, 2006). However, a higher level of anise essential oil, 3.5 to 5.5%, was observed in another experiment carried out in Iran (Omidbaigi *et al.*, 2003).

Contrary to the content of essential oil, the content of *trans*-anethole in essential oil was significantly higher in 2012 (Fig. 2b) in comparison to 2011. In both years, the differences between localities were minor when compared to the other investigated traits. This observation is in accordance with the F-test of ANOVA analysis (Tab. 3), where it was found that the year had an important influence on the *trans*-anethole content, while the effect of the locality was absent. Also, on average, there were no significant differences between localities in both years.

Throughout our experiment, the average content of *trans*-anethole was 94.78%, which is similar to an earlier study conducted by Orav *et al.* (2008). The content of *trans*-anethole was highly influenced by the year, while the effect of the locality was absent. In contrast to the content of the essential oil, the content of *trans*-anethole in the essential oil was significantly higher in the dry 2012 (96.35%) in comparison with 2011 (93.20%). On average, there were no significant differences between localities in both years.

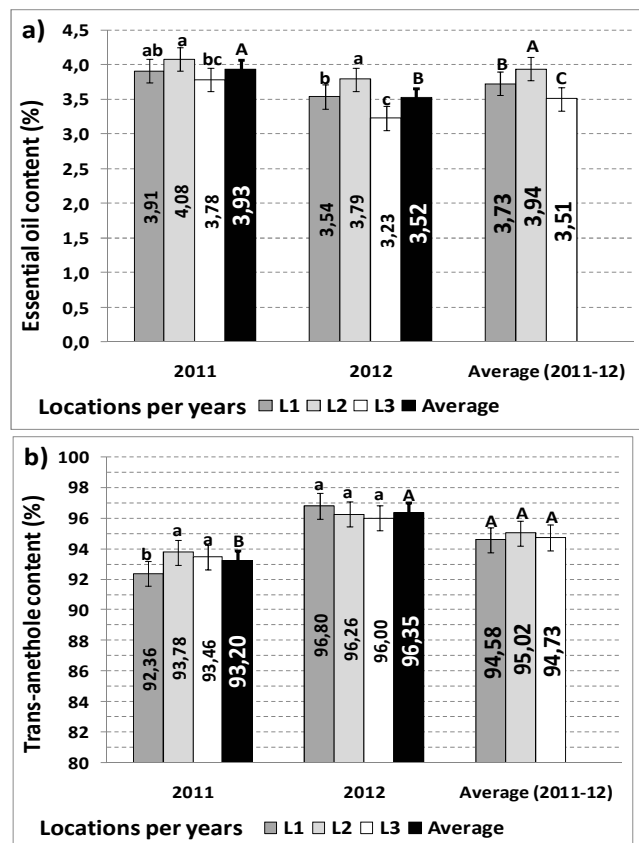


Fig. 2. The effects of years and localities on Essential oil content (a) and Trans-anethole content in EO (b)

Bars represent differences between individual means which were deemed to be significant at $p < 0.05$. Values followed by different small letters (a-c) on the bars indicate differences between localities in each year at the $p < 0.05$. Values followed by capital superscripts (A-C) in the same way relate to the average values per years (2011 and 2012) and localities (in average for both years)

It can be assumed that under stress conditions the amount of *trans*-anethole in the essential oil increases, because in such conditions the plants produce more secondary metabolites, the substances that prevent oxidation processes in the cells.

Correlation analysis (Tab. 5) shows that germination energy was in strong positive correlation with thousand seed weight ($r=0.799^*$) and in negative correlation with *trans*-anethole content ($r=-0.641^*$). Total germination was in the higher positive correlation with germination energy ($r=0.952^*$) than with thousand seed weight ($r=0.694^*$) and it was in moderate correlation with essential oil content ($r=0.369^*$) as well as in strong negative correlation with *trans*-anethole content ($r=-0.648$). Also, *trans*-anethole content was in moderate negative correlations with thousand seed weight and the essential oil content ($r=-0.604^*$ and -0.543^* , respectively).

Tab. 5. Simple correlation coefficients (r) between investigated parameters

| Parameters | Weight of 1000 seeds (g) | Essential oil content (%) | <i>Trans</i> -anethole content (%) | Germination energy (%) |
|------------------------------------|--------------------------|---------------------------|------------------------------------|------------------------|
| Essential oil content (%) | 0.052 | - | - | - |
| <i>Trans</i> -anethole content (%) | -0.604* | -0.543* | - | - |
| Germination energy (%) | 0.799* | 0.234 | -0.641* | - |
| Total germination (%) | 0.694* | 0.369* | -0.648* | 0.952* |

Zehtab-Salmasi *et al.* (2001) show that the percentage of essential oil significantly decreases due to delayed sowing, while water deficit resulted in the increase of the essential oil content. A recent research confirms that the amount of the essential oil obtained from different species produced under drought conditions was either maintained or enhanced, depending on the extent of stress (Zehtab-Salmasi *et al.*, 2001). Water deficit during stem elongation and umbel formation reduces anise growth, grain yield and oil production (Zehtab-Salmasi *et al.*, 2001). In a two-year experiment in Pančevo, Serbia, Jevdjović *et al.* (2012) analyzed the effect of NPK fertilization on seed yield, quality and content of anise essential oil.

The results showed that, in comparison to the control plot, significantly higher mean values were obtained for all examined traits in the fields where NPK fertilizer was applied. Anise oil content was higher in the dry 2011 compared to the extremely wet 2010, while the overall seed germination rate of anise did not significantly differ between the years.

The correlation analysis shows that total germination is in positive correlation with germination energy, thousand seed weight and essential oil content, but in strong negative correlation with *trans*-anethole content. Also, *trans*-anethole was in moderate negative correlation with thousand seed weight and essential oil content. Kiralan *et al.* (2009) found that there is a correlation between essential oil yield and fruit size in coriander. Small fruits have a higher percentage of essential oil than large fruits.

Conclusions

Drought causes a significant decrease in thousand seed weight, germination energy and total germination as well as content of essential oil in anise. Contrary to this, *trans*-anethole content was significantly higher in the dry year. It can be assumed that under stress conditions the amount of *trans*-anethole in the essential oil increases, because in such conditions the plants produce more secondary metabolites, the substances that prevent oxidation processes in the cells.

Acknowledgements

This work was supported by the Ministry of Science and Technological Development, Republic of Serbia (Project TR-31051).

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