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Seasons influence on content, yield and chemical composition of *Origanum majorana* L. essential oil

Jéssica Maronez de Souza*, Alberto Cargnelutti Filho, Fernanda Carini, Samanta Luiza da Costa, Berta Maria Heinzmann, Patrícia Rodrigues

Federal University of Santa Maria, Santa Maria, Brazil
*Corresponding author, e-mail: jessica_maronez@hotmail.com

Abstract

The aim of this study was to determine the season that promotes the highest production and best quality of marjoram (*Origanum majorana* L.) essential oil in summer and winter transplantations. Two experiments were performed in a protected cultivation, the first with transplanting in summer and the second with transplanting in winter, with a completely randomized design with four treatments, corresponding to the seasons of the year. Content, yield, and chemical composition of the essential oil were determined. The essential oil was extracted by hydrodistillation and analyzed by gas chromatography. In the summer transplanting, the essential oil content and yield were higher in the summer and spring seasons. In the winter transplanting, the essential oil content was higher in the summer season and the yield in summer and spring. Carvacrol was the major component of marjoram essential oil regardless of harvest and transplanting season. Of the other main compounds, only terpinolene was detected in all samples analyzed, with the other essential oil components varying between transplanting and harvest seasons. The highest production of marjoram essential oil is obtained in summer and spring harvests from plants transplanted in summer and winter. Carvacrol was the major component of marjoram essential oil and the other main components vary among seasons.

Keywords: Carvacrol, Protected Cultivation, Terpinolene

Introduction

Marjoram (*Origanum majorana* L.), belonging to the Lamiaceae family, is an aromatic species used in cooking, meat seasoning, salads, and flavoring of beverages (Clemente & Haber, 2013). In addition, it is considered a medicinal plant, with antimicrobial activity against various pathogens, due to the presence of phenolic compounds in its essential oil (Guerra-Boone et al., 2015; Walker et al., 2016). It also has antioxidant activity, being considered promising as a natural agent in several applications, such as food preservation and preparation of cosmetic and pharmaceutical products (Hajlaoui et al., 2016). Other medicinal properties of this species include the antispasmodic and myorelaxant effects (Makrane et al., 2018), as well as its hepatoprotective potential (Mossa et al., 2015).

The medicinal properties of plants come from the chemical components produced in secondary metabolism, which originate, among others, essential oils (Kabera et al., 2014). Essential oils are colorless, aromatic liquids with high refraction index, present in various parts of plants, such as glandular trichomes, specialized cells, pockets and reservoirs, and even in intercellular spaces (Ali et al., 2015). In general, the main components of marjoram essential oil are carvacrol, thymol, trans-caryophyllene, gamma-terpinene, terpinen-4-ol, trans-sabinene hydrate, alpha-terpineol, alpha-terpinene, sabinene, and p-cymene (Hajlaoui et al., 2016; Radaelli et al., 2016; Partovi et al., 2018).

However, the composition can be influenced by the season of the year in which the plant is cultivated (Soliman et al., 2009). The harvest period also alters the yield and content of marjoram essential oil, being related to weather conditions (Zawiślak & Dzida, 2010). Temperature variations trigger adaptive responses in plants, altering the production of secondary metabolites,

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such as the components of essential oils. In addition, light intensity can influence the yield and composition of plant essential oils (Morais, 2009).

The time of transplanting the seedlings has an influence on the production of essential oil, as verified by Luz et al. (2014) in basil (*Ocimum basilicum* L.) plants. Omer et al. (2016) found a higher content of basil essential oil for sowing performed in April, in Egypt, compared to that obtained with sowing in May, both in the spring season in the region. Thus, the objective of this study was to determine the season that promotes the highest production and best quality of marjoram essential oil in transplantations performed in summer and winter.

Material and Methods

Two experiments with the marjoram crop (*Origanum majorana* L.) were conducted in a soilless cultivation system, in a shelter-type protected environment of 115 m² (5×23 m), covered with 150-µm-thick anti-UV polyethylene, located at coordinates 29°42' S and 53°49' W at 95 m of altitude. The first experiment was installed on December 28, 2017 (transplanting in summer) and conducted until December 23, 2018 (12 months), and the second was installed on June 28, 2018 (transplanting in winter) and conducted until June 23, 2019 (12 months).

During the growing period, the air temperature inside the shelter was recorded by a digital data logger (resolution of 0.1 °C and accuracy of 0.5 °C), installed on site. Solar radiation was recorded at the automatic weather station, belonging to the 8th District of Meteorology - National Institute of Meteorology (INMET), located 300 m from the cultivation environment.

Fertigation was performed by means of drip tapes, positioned at the top of the pots, with one dripper per plant. The nutrient solution was prepared and stored in 500L polypropylene tanks and supplied to plants by means of a motor pump controlled by a timer. Nutrient solution with the following composition was used: 8.69 of NO_3^-; 1.86 of NH_4^+; 4 of $H_2PO_4^-$; 6 of K^+; 4 of Ca^{2+}; 2 of Mg^{2+}; and 2 of SO_4^- (in mmol L^{-1}). Micronutrients were supplied at the concentrations (in mg L^{-1}) of 0.03 of Mo; 0.26 of B; 0.06 of Cu; 0.50 of Mn; 0.22 of Zn; and 1.0 of Fe. The electrical conductivity (EC) of the nutrient solution was monitored weekly and corrected with the addition of aliquots of new solution whenever necessary, keeping the value at 1.84 dS m^{-1}.

The marjoram seedlings used in experiment 1 (transplanting in the summer) were obtained in an Agricultural shop of Santa Maria, State of Rio Grande do Sul, with approximately 14 cm of height. The marjoram seedlings used in experiment 2 (transplanting in winter) were produced using adult parent plants, remaining from the first experiment, grown in a greenhouse, in soilless cultivation. For this, 4-cm-long cuttings from the apex of the branches, leaving two expanded leaves at the end, were used. These were placed in contact with the powdered hormone indole butyric acid (IBA) (concentration of 0.1%), and then placed in trays of expanded polystyrene (128 cells) containing commercial substrate (*MecPlant*). The trays were placed on a bench, inside the greenhouse, under sprinkler irrigation. The transplanting of seedlings was performed after 45 days, when the root system was well formed, that is, with rooted seedlings.

The seedlings were transplanted to 3dm³ white polyethylene pots, filled with the commercial substrate *MecPlant* (composed of Pine bark, vermiculite, acidity corrective, and macronutrients). The pots were arranged on benches with 1.10 m width, 4 m length, and 80 cm height. Two benches were used in each experiment. Each bench had 44 pots, resulting in a total of 88 pots and, consequently, 88 plants in each experiment.

For experiment 1, four plants located on the sides of the benches were marked, because they had a large amount of leaves since the first 30 days after transplanting. For experiment 2, in which the plants showed a slow growth after transplanting, more than four plants were used in order to obtain an adequate amount of leaves (above 300 g of leaves) for oil extraction. The plants used were border plants present on the benches and were also harvested every 30 days, to maintain the standardization of the experiments.

A completely randomized design was used, with four treatments, corresponding to the seasons (summer, spring, winter, and autumn) in which the samples were collected.

To determine the content and composition of marjoram essential oil, in each experiment, plants were collected every 30 days, resulting in 12 harvests in each experiment. The harvests were carried out at a 7 cm height from the base of the plant, in order to allow the regrowth of the branches, so the plants were always harvested during vegetative growth. The collected material was separated into leaves and branches; the leaves were placed in plastic bags and stored at -15 °C for subsequent extraction and analysis, whereas the branches were discarded. To verify the production of oil by season of the year, leaf samples from three consecutive harvests, for the same season, were combined. For example, the samples collected in January, February, and March corresponded to the summer season.

The extraction of essential oil from marjoram leaves was performed by the hydrodistillation method in a Clevenger apparatus, for two hours, in triplicates, that is, three extractions per season. 100g samples of fresh leaves were placed in a 2L flask containing 1.25 L of distilled water. The essential oil content (%), oil yield (g plant^{-1}), and oil yield (ml plant^{-1}) were determined by equations Eq. 1, Eq. 2, and Eq. 3:

$$C\ (\%) = \frac{W}{SM} \times 100 \quad (1)$$

$$Y\ (g\ plant^{-1}) = \frac{(FML\ plant^{-1} \times W)}{SM} \quad (2)$$

$$Y\ (ml\ plant^{-1}) = \frac{(FML\ plant^{-1} \times Q)}{SM} \quad (3)$$

Where: C (%) = oil content in percentage; W = weight of oil in grams; SM = sample mass in grams; Y = oil yield; FML plant^{-1} = average value of fresh mass of leaves per plant; Q = quantity of oil in milliliters. The values of content, in %, and yield, in g plant^{-1} and in ml plant^{-1}, of each repetition (triplicate) were used in the analysis of variance and means comparison tests.

The chemical composition of the essential oil was analyzed in the Laboratory of Plant Extractives (LABEVE), using a gas chromatograph. The identification of the compounds was performed by gas chromatography coupled to mass spectrometry (GC-MS) in triplicates, using an Agilent 7890A hyphenated system, equipped with a 5975C series selective mass detector. Analysis parameters: Split injection mode (1:50, v/v); carrier gas: He (flow rate of 1.0 ml/min); DBS-MS fused silica capillary column (5% phenylmethyl siloxane, 30 m × 0.25 mm, film thickness: 0.25 µm); and ionization energy: 70 eV. Helium was used as drag gas at a flow rate of 1.0 ml/min, with temperature of injector, detector and interface set at 250 °C and auxiliary temperature set at 280 °C. The oven temperature was maintained at 40 °C for 4 min and increased to 320 °C at a rate of 4 °C/min.

The essential oil components were identified based on Kovats retention indices (KI), determined by using a calibration curve of a homologous series of n-alkanes (C8-C40), and had their fragmentation patterns compared to the mass spectra data found in the literature and to the database of the equipment's spectrometer (Adams, 2001; Nist, 2008). The components were quantified by gas chromatography with flame ionization detector (CG-FID), performed in an Agilent 7890A chromatograph, and the analysis parameters are equivalent to those mentioned above, by GC-MS analysis, except for injection, by the splitless mode, and the injector and detector temperature, equal to 300 °C.

For each variable (essential oil content and yields), the normality of errors was verified by the Shapiro-Wilk test and homogeneity of residual variances was verified by Bartlett test. Analysis of variance was performed, followed by the Scott-Knott test for grouping the means. Statistical analyses were performed using the programs Action (Estatcamp, 2014) and Sisvar 5.7 (Ferreira, 2014).

Results and Discussion

The content and yield of marjoram essential oil in the transplanting performed in the summer showed significant differences between the seasons (Table 1). The essential oil content was higher for harvests carried out in the summer (0.1727%) and in the spring (0.1030%), as well as the oil yield in g plant^{-1} (0.1361 and 0.0881 g plant^{-1}, respectively) and the oil yield in ml plant^{-1} (0.2102 and 0.1710 ml plant^{-1}, respectively). These results show higher production of essential oil in periods of higher temperature and solar radiation (Figure 1), highlighting the importance of climatic conditions in the cultivation of aromatic plants.

Table 1. Content and yield of *Origanum majorana* L. essential oil in the seasons for transplanting carried out in summer and winter.

Transplanting in summer			
Season	Oil content (%)	Oil yield (g plant^{-1})	Oil yield (ml plant^{-1})
Summer	0.1727 a	0.1361 a	0.2102 a
Autumn	0.0323 b	0.0418 b	0.1291 b
Winter	0.0050 b	0.0044 b	0.0501 c
Spring	0.1030 a	0.0881 a	0.1710 a
CV(%)	59.64	55.03	16.61
Transplanting in winter			
Season	Oil content (%)	Oil yield (g plant^{-1})	Oil yield (ml plant^{-1})
Summer	0.2133 a	0.1745 a	0.2318 a
Autumn	0.1443 b	0.0594 b	0.0823 b
Winter	0.0463 c	0.0158 b	0.0170 c
Spring	0.1473 b	0.1430 a	0.2265 a
CV(%)	21.84	27.35	13.14

Means not followed by the same letter in the column differ by the Scott-Knott test at 5% probability level.

For the transplanting performed in winter, the contents and yield of marjoram essential oil also showed significant differences between the seasons (Table 1). The summer season promoted the highest contents of essential oil (0.2133%). For the autumn and spring seasons, the values were similar (0.1443 and 0.1473%, respectively). In the winter season, lower values of essential oil content (0.0463) were verified. The oil yields in g plant^{-1} and in ml plant^{-1} were higher in the summer (0.1745 g plant^{-1} and 0.2318 ml plant^{-1}) and spring (0.1430 g plant^{-1} and 0.2265 ml plant^{-1}). In this transplanting season, higher oil production in warmer periods and lower production in colder periods were also observed.

Unlike the oil content, which considers only

the weight of the oil obtained, the yield of essential oil considers the fresh mass of leaves. If this variable is higher, consequently, the value of oil yield will also be higher, which occurred in the summer and spring seasons.

Results observed in the literature show different responses of the essential oil content and yield in relation to the seasons. Soliman et al. (2009) verified the influence of harvest season on the content of essential oil of marjoram, grown in Egypt, and obtained values of 3.0% in spring (in full flowering), 2.8% in winter and 2.5% in summer and autumn. In a study conducted in Poland by Nurzyñska-Wierdak et al. (2015), there was a higher yield of essential oil, in kg m^{-2}, in the harvest carried out in the summer (3.54) compared to the harvest performed in autumn (1.96); however, there were no differences in the essential oil content, being equal to 1.15 and 1.2% in summer and autumn, respectively. In another study conducted in Poland, by Zawiślak and Dzida (2010), higher contents of marjoram essential oil were verified in the second harvest (2.39%), carried out in autumn, compared to those obtained in the first harvest (1.95%), in summer. According to the authors, the influence of the harvest time on the content of marjoram essential oil is highly related to the meteorological conditions of the period.

For another medicinal species, lemon balm (*Melissa officinalis* L.), Said-al Ahl et al. (2018) verified the influence of harvest time on the essential oil content, with variations from 0.013 to 0.115%, being higher in the months of higher temperatures, similar to that observed in the present study for marjoram.

In the first transplanting season, marjoram harvest in the summer favored the production of essential oil, since the highest contents were verified in this season. The same occurred for the summer and spring seasons in the second transplanting season. During this period, the highest temperatures and solar radiation occurred inside the greenhouse where the plants were grown, in the years 2018 and 2019 (Figures 1 and 2). Thus, one can understand the influence of these climatic factors on the production of marjoram essential oil. The content of marjoram essential oil in the summer was higher for the transplanting performed in the winter, compared to the transplanting performed in the summer.

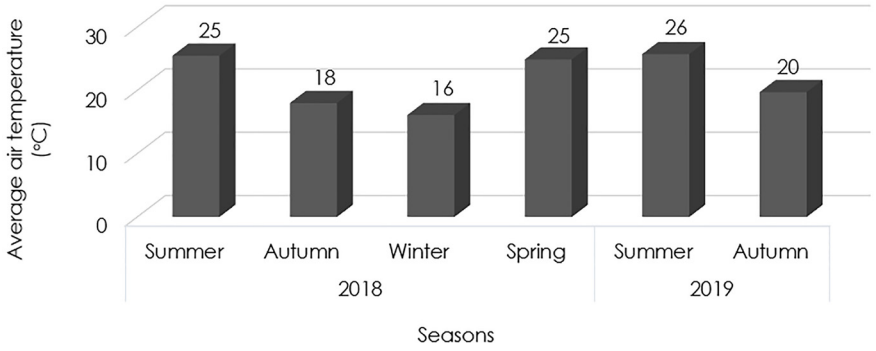

Figure 1. Average air temperature (°C) inside the greenhouse in the seasons, from December 2017 to June 2019.

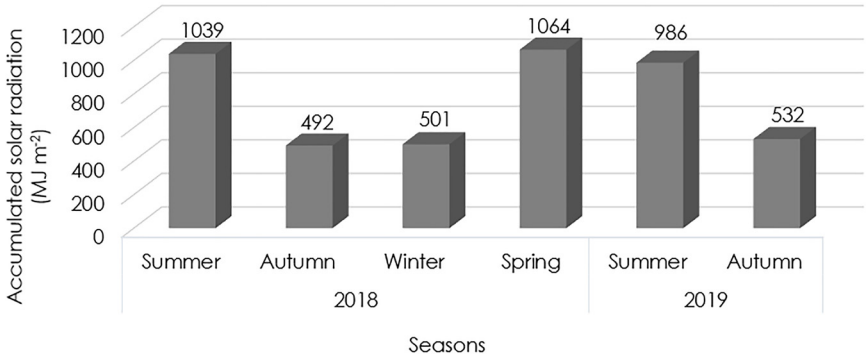

Figure 2. Accumulated solar radiation (MJ m^{-2}) inside the greenhouse in the seasons, from December 2017 to June 2019.

The influence of transplanting season on the production of essential oil was also verified by Luz et al. (2014) in basil plants (*Ocimum basilicum* L.), in Uberlândia, MG (Brazil), in crops lasting two months (in spring) and three months (in summer), in which summer transplanting resulted in higher essential oil yield (g plant^{-1}) in fresh leaves compared to transplanting in spring. Omer et al. (2016) found higher content of basil essential oil in Egypt for sowing carried out in April (0.630 and 0.640%) in comparison to that obtained with sowing in May (0.560 and 0.550%), both during spring season in the region.

The components of the marjoram essential oil, for the transplanting performed in the summer, showed differences between the seasons (Table 2).

For the transplanting performed in the winter, a greater number of main components were verified (totaling at least 75%), and these had a lower content in comparison to the total of the essential oil (Table 3). Greater differences in the composition of the essential oil were observed between the seasons, in relation to the transplanting in the summer.

Table 2. Chemical composition (%) of *Origanum majorana* L. essential oil in the seasons for transplanting carried out in summer.

KI*	Components	Season			
		Summer	Autumn	Winter	Spring
961	Sabinene	1.50 ± 0.01**	-	1.00 ± 0.01	1.19 ± 0.01
979	β-Pinene	0.29 ± 0.00	-	-	0.64 ± 0.00
1005	α-Phellandrene	0.46 ± 0.00	-	0.47 ± 0.00	0.31 ± 0.00
1013	p-Cymene	1.16 ± 0.01	1.09 ± 0.01	0.81 ± 0.00	1.31 ± 0.01
1017	α-Terpinene	1.09 ± 0.01	-	-	2.09 ± 0.02
1026	α-Ocimene	-	1.24 ± 0.01	1.04 ± 0.01	0.51 ± 0.00
1036	cis-β-Ocimene	0.25 ± 0.00	-	-	0.36 ± 0.00
1047	Trans-sabinene hydrate	1.49 ± 0.01	0.13 ± 0.00	2.26 ± 0.01	4.28 ± 0.03
1058	trans-β-Ocimene	1.40 ± 0.01	1.71 ± 0.01	1.02 ± 0.01	0.97 ± 0.01
1074	γ-Terpinene	-	-	-	0.84 ± 0.01
1088	Terpinolene	15.86 ± 0.11	14.15 ± 0.08	11.95 ± 0.07	12.24 ± 0.09
1112	Linalool	2.37 ± 0.01	1.00 ± 0.01	1.10 ± 0.01	1.60 ± 0.01
1144	trans-p-Menthone	2.23 ± 0.01	2.84 ± 0.02	1.07 ± 0.01	2.17 ± 0.02
1168	Terpinen-4-ol	15.20 ± 0.11	20.14 ± 0.012	10.64 ± 0.06	9.31 ± 0.07
1182	α-Terpineol	4.17 ± 0.03	4.64 ± 0.03	2.98 ± 0.02	2.71 ± 0.02
1216	cis-Geraniol	2.95 ± 0.2	3.64 ± 0.02	5.60 ± 0.03	5.91 ± 0.04
1226	Thymol methyl ether	1.19 ± 0.01	1.41 ± 0.01	1.66 ± 0.01	1.37 ± 0.01
1237	Bergamiol	0.56 ± 0.00	0.63 ± 0.00	0.94 ± 0.01	0.89 ± 0.01
1276	Carvacrol	30.37 ± 0.21	26.47 ± 0.15	33.70 ± 0.19	27.74 ± 0.20
1364	Geranyl acetate	-	-	-	0.55 ± 0.00
1403	β-Caryophyllene	0.32 ± 0.00	2.21 ± 0.01	1.19 ± 0.01	1.12 ± 0.01
1439	α-Caryophyllene	9.52 ± 0.07	10.29 ± 0.06	11.67 ± 0.07	11.97 ± 0.08
1465	Germacrene-D	1.58 ± 0.01	4.48 ± 0.03	0.58 ± 0.00	0.56 ± 0.00
1479	Bicyclogermacrene	3.67 ± 0.02	1.51 ± 0.01	5.67 ± 0.03	5.49 ± 0.04
1492	δ-Cadinene	1.04 ± 0.01	-	-	2.18 ± 0.02
1502	γ-Cadinene	0.79 ± 0.00	1.20 ± 0.01	3.85 ± 0.02	1.21 ± 0.01
1559	Spathulenol	0.54 ± 0.00	0.79 ± 0.00	-	0.48 ± 0.00
% total (N° of compounds)	27	100.00 (24)	99.57 (19)	99.20 (20)	100.00 (27)

* KI = Kovats index. ** Average of three injections ± standard deviation. Symbol "-" indicates that the component was not observed in that season.

Table 3. Chemical composition (%) of *Origanum majorana* L. essential oil in the seasons for transplanting carried out in winter.

KI*	Components	Season			
		Summer	Autumn	Winter	Spring
915	α-Thujene	0.20 ± 0.00**	0.18 ± 0.00	0.23 ± 0.00	0.22 ± 0.00
922	α-Pinene	1.89 ± 0.01	1.68 ± 0.01	1.31 ± 0.01	1.85 ± 0.01
961	Sabinene	4.67 ± 0.03	4.43 ± 0.02	3.44 ± 0.02	4.81 ± 0.02
965	β-Myrcene	2.37 ± 0.01	2.33 ± 0.01	-	2.36 ± 0.01
980	β-Pinene	0.70 ± 0.00	0.55 ± 0.00	2.25 ± 0.01	0.64 ± 0.00
1005	α-Phellandrene	5.37 ± 0.03	4.20 ± 0.02	0.58 ± 0.00	5.70 ± 0.04
1013	p-Cymene	2.31 ± 0.01	2.10 ± 0.01	4.79 ± 0.03	3.44 ± 0.02
1018	α-Terpinene	3.25 ± 0.02	2.76 ± 0.02	3.16 ± 0.02	3.64 ± 0.03
1026	α-Ocimene	0.32 ± 0.00	0.33 ± 0.00	3.28 ± 0.02	0.39 ± 0.00
1036	cis-β-Ocimene	0.71 ± 0.00	0.82 ± 0.00	0.79 ± 0.00	0.60 ± 0.00
1047	Trans-sabinene hydrate	14.07 ± 0.00	12.16 ± 0.07	14.50 ± 0.08	14.58 ± 0.00
1059	trans-β-Ocimene	0.94 ± 0.01	1.12 ± 0.01	1.77 ± 0.01	1.00 ± 0.00
1074	γ-Terpinene	1.77 ± 0.01	1.52 ± 0.00	0.45 ± 0.00	1.86 ± 0.01
1089	Terpinolene	7.45 ± 0.04	11.48 ± 0.07	5.10 ± 0.03	5.48 ± 0.04
1112	Linalool	0.37 ± 0.00	0.23 ± 0.00	0.40 ± 0.00	2.15 ± 0.01
1130	β-Phellandrene	-	-	-	0.30 ± 0.00
1130	trans-p-Menthone	5.56 ± 0.03	0.49 ± 0.00	6.47 ± 0.04	6.58 ± 0.04
1168	Terpinen-4-ol	12.86 ± 0.07	5.61 ± 0.03	9.94 ± 0.06	0.64 ± 0.00
1182	α-Terpineol	2.40 ± 0.01	9.62 ± 0.05	2.26 ± 0.01	11.85 ± 0.07
1196	NI	0.17 ± 0.00	-	-	-
1217	Cis-Geraniol	2.01 ± 0.01	2.23 ± 0.01	4.07 ± 0.02	2.27 ± 0.01
1226	Thymol methyl ether	1.10 ± 0.01	3.14 ± 0.01	1.08 ± 0.01	2.32 ± 0.01
1237	Bergamiol	0.34 ± 0.00	1.24 ± 0.00	0.69 ± 0.00	1.07 ± 0.01
1277	Carvacrol	19.64 ± 0.11	19.84 ± 0.11	15.06 ± 0.09	16.05 ± 0.09
1284	Thymol	1.32 ± 0.01	1.94 ± 0.01	-	1.49 ± 0.02
1345	NI	-	0.36 ± 0.00	2.25 ± 0.01	-
1364	Geranyl acetate	-	0.20 ± 0.00	0.71 ± 0.00	-
1404	β-Caryophyllene	3.78 ± 0.02	0.66 ± 0.00	6.99 ± 0.04	4.25 ± 0.02
1439	α-Caryophyllene	1.05 ± 0.01	0.38 ± 0.00	1.75 ± 0.01	1.12 ± 0.01
1465	Germacrene-D	0.84 ± 0.00	4.89 ± 0.02	1.33 ± 0.01	0.85 ± 0.00
1479	Bicyclogermacrene	1.00 ± 0.01	1.57 ± 0.01	2.15 ± 0.01	1.33 ± 0.01
1488	α-Farnesene	1.11 ± 0.01	1.37 ± 0.01	-	-
1501	γ-Cadinene	0.43 ± 0.01	0.54 ± 0.00	1.84 ± 0.01	1.16 ± 0.01
% total (Nº of compounds)	33	100.00 (30)	99.97 (31)	98.64 (28)	100.00 (29)

* KI = Kovats index. ** Average of three injections ± standard deviation. NI: Not identified. Symbol "-" indicates that the component was not observed in that season.

In the comparison, it is possible to observe differences for each season of the year in the two periods of transplanting. The components carvacrol and terpinolene were found in all seasons, with carvacrol having the highest content for the transplanting performed in the summer compared to that in the winter. In the summer season, the components carvacrol, terpinolene, and 4-terpineol were found in both transplanting seasons; however, alpha-caryophyllene and alpha-terpineol were also found in the first period and were found six more components were found in the second period, namely: trans-sabinene hydrate, trans-p-menthone, alpha-phellandrene, sabinene, beta-caryophyllene, and alpha-terpinene. In the autumn, the components carvacrol, terpinen-4-ol, terpinolene, and alpha-terpineol were obtained in both seasons, in addition to alpha-caryophyllene in the first season and five other compounds in the second period, namely: trans-sabinene hydrate, germacrene-D, sabinene, alpha-phellandrene, and thymol methyl ether. In the winter season, the components found in common in the two transplanting seasons were carvacrol, terpinolene, terpinen-4-ol, and cis-geraniol.

For the transplanting performed in the

summer, the compounds alpha-caryophyllene and bicyclogermacrene were also obtained. For the transplanting performed in winter, seven other main components were verified, namely: trans-sabinene hydrate, beta-caryophyllene, trans-p-menthone, p-cymene, sabinene and alpha-terpinene. In the spring, the compounds carvacrol, terpinolene, and trans-sabinene hydrate were obtained in both seasons. In addition, in the first period (transplanting in summer), the components alpha-caryophyllene, terpinen-4-ol, cis-geraniol, and bicyclogermacrene were also verified. In the second period (transplanting in winter), six other compounds were obtained: alpha-terpineol, trans-p-menthone, alpha-phellandrene, sabinene, beta-caryophyllene, and alpha-terpinene.

Thus, the differences in the composition of marjoram essential oil between transplanting seasons may be related to differences in temperature conditions and duration of the day to which the plants were exposed, since these factors influence the presence of certain enzymes, responsible for the increase or decrease of certain components. According to Novak et al. (2010), the highest concentration of carvacrol for transplanting performed in summer may be due to the higher temperatures, which favor production.

In addition, the influence of the season on the composition of marjoram essential oil was also verified by Soliman et al. (2009), who observed smaller amounts of most components at harvests carried out in the winter. The main components found in the essential oil of marjoram were thymol (38.4% in spring), terpinen-4-ol (7.7 to 37.4%), cis-sabinene hydrate (7.4 to 54.4%), gamma-terpinene (5.3 to 18.3%), p-cymene (2.3 to 13.9%), and alpha-terpinene (0.4 to 13.3%). Nurzyñska-Wierdak et al. (2015) verified the same main compounds in marjoram essential oil in Poland in summer and autumn, with small differences in content: linalool + trans-sabinene hydrate (50.43 and 51.55%), terpinen-4-ol (8.06 and 6.35%), linalyl acetate + trans-sabinene hydrate acetate (4.38 and 8.87%), sabinene (5.41 and 5.36%), gamma-terpinene (5.44 and 4.3 1%), trans-caryophyllene (4.06 and 4.23%), cis-sabinene hydrate (4.26 and 3.91%), alpha-terpineol (3.94 and 3.53%), limonene + beta-phellandrene (3.46 and 3.47%), and alpha-terpinene (3.41 and 2.71%).

The results observed in the present study are similar to those reported in other studies with this species. Guerra-Boone et al. (2015) found as main components of the essential oil of marjoram terpinen-4-ol (23.1%), trans-sabinene hydrate (15.3%), γ-terpinene (11.5%), and thymol (16.3%). Hajlaoui et al. (2016) verified as main components terpinen-4-ol (23.2%), cis-sabinene hydrate (17.5%), gamma-terpinene (10.5%), p-cymene (9%), sabinene (7.5%), and alpha-terpinene (5.6%).

Despite some similarities in the main components obtained in the cited studies, carvacrol was found by some authors, but did not appear among the major components of marjoram essential oil, contrary to what was observed in the present study, in which carvacrol was the component with the highest content for all transplanting periods and seasons. However, this compound is isomer of thymol, and its production is influenced by temperature; higher temperatures favor the production of carvacrol to the detriment of thymol, while lower temperatures favor the production of thymol to the detriment of carvacrol (Novak et al., 2010).

Carvacrol is a colorless substance with a distinct odor, responsible for the slightly spicy flavor of marjoram. This compound is used at low concentrations as a flavoring ingredient and preservative in food and has antifungal, antibacterial, antioxidant, hepatoprotective, vasorelaxant, and spasmolytic properties (Suntres et al., 2015). In addition to the antimicrobial and antioxidant activities attributed to this substance, its antiproliferative activity has been reported, mainly in in vitro studies (Sharifi-Rad et al., 2018). This phytochemical component also has potential for use in agriculture due to its pesticide activity against a large number of pest insects and from various origins (Bendre et al., 2018). In addition, its potential for use against various plant diseases has recently been verified, mainly in fruit and horticultural species, so its use is promising in this field (Liu et al., 2019).

Another main component found in marjoram essential oil in all seasons and transplanting periods was terpinolene. This compound has antiproliferative activity in brain tumor cells, which also highlights its potential as an anticancer agent (Aydin et al., 2013). In addition, terpinolene and alpha-phellandrene, another main component of marjoram essential oil, have similar chemical characteristics, with healing properties, showing potential for the treatment of superficial skin lesions, attenuating skin inflammations (Scherer et al., 2019).

Another main component of interest in different areas is terpinen-4-ol, due to the biological activities already proven in vivo, such as the antibacterial, antifungal, and antitumor effects. This is also the major component of *Melaleuca alternifolia* essential oil, which is of great industrial interest for obtaining cleaning and personal hygiene products, being also of interest in the fields of veterinary and agriculture (Padovan et al., 2017).

Thus, the influence of climatic conditions on the

production of marjoram essential oil can be verified, which should be taken into account in the management and scaling of production, considering mainly the time for harvesting the plants.

Conclusions

The highest production of marjoram essential oil is obtained in harvests carried out in summer and spring in plants transplanted in summer and winter. Carvacrol was the major component of marjoram essential oil, regardless of the season and transplanting period. The other main components of marjoram essential oil vary between seasons.

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