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Production and composition of peppermint essential oil in seasons after summer and winter transplantations

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Abstract

The aim of this study was to determine the season that provides the highest production and best quality of peppermint (*Mentha x piperita* L.) essential oil in summer and winter transplantations. Two experiments were performed in a protected cultivation, the first with transplantation in summer and the second with transplantation in winter, with a completely randomized design with four treatments, being 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. Essential oil content in summer and winter transplantations was higher in summer and essential oil yield was higher in spring. In the summer transplanting the major components were menthone, isomenthone and pulegone. In the winter transplanting menthone, menthofuran, isopulegone, pulegone and menthol were detected as major components. Menthofuran, a compound which reduces the essential oil quality, were found in high amounts in winter transplanted plants. The higher production of peppermint essential oil is obtained in summer and spring harvests and the main compound is menthone in all seasons. The best quality of essential oil is obtained in the summer transplanting.

Keywords: *Mentha x piperita L.*, menthone, menthofuran, protected cultivation, transplantation

Introduction

Peppermint (*Mentha x piperita* L.) is a medicinal and aromatic species, of the Lamiaceae family, commonly used in the industry for preparing food, medicines and cosmetics (Mahboubi & Kazempour, 2014). Among its pharmacological activities stand out the decongestant, anti-inflammatory, analgesic, antimicrobial, digestive, antispasmodic, astringent, carminative, fungicide and vasoconstrictor effects, which are due to the presence of different components in essential oils (Ali *et al*., 2015).

The essential oils of *M. X piperita* L. have menthol, carvacrol, carvone, methyl acetate, limonene, and menthone as the most common components (Ali *et al*., 2015). However, both the content and composition of essential oils of plants of the genus *Mentha* are influenced by some factors, as the species, cultivar, seasons and harvest period (Deschamps *et al*., 2008; Oliveira *et al*., 2012; Machiani *et al*., 2018). Highlighting the need to evaluate the chemical composition and content of essential oils producing plants at different times of the year.

Thus, aiming at a greater quantity of components of interest in the essential oil of aromatic plants, the ideal moment of harvest must be determined, which can be influenced by temperature, photoperiod and solar radiation (Pinto *et al*., 2007). Temperature variations alter the production of secondary metabolites, such as essential oil components, through adaptive responses from plants. The luminous intensity can also influence the content and quality of the essential oils of aromatic plants (Morais, 2009).

The influence of the harvest period was verified in essential oil yield of *Mentha* plants, in which higher values of essential oil yield were observed in the summer (Deschamps *et al*., 2008). Thus, it is important to have a good schedule for transplanting and harvesting medicinal plants, in order to obtain higher yields and quality of

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essential oil. Consequently, it is necessary to know the response of these plants to environmental conditions and choose the period of cultivation that provides the best results.

Therefore, the objective of this work was to determine the season that provides the highest production and best quality of peppermint essential oil in transplantations performed in summer and winter.

Material and Methods

Two experiments with the peppermint crop (*M. x piperita* L.) were conducted in a soilless cultivation system(in pots filled with substrate), in a shelter-type protected environment of 115 m² (5×23 m), covered with 150-µm-thick anti-UV polyethylene, located at Santa Maria (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 cultivation period, air temperature inside the shelter was recorded by a digital Data Logger (0.1 °C resolution and 0.5 °C accuracy), installed one meter above the plants. Solar radiation was recorded at the automatic weather station, belonging to the 8th District of Meteorology – National Institute of Meteorology (INMET), located 300 m away from the cultivation environment.

Fertigation was performed through drip tapes, positioned at the top of the pots, with one dripper per plant. The nutrient solution was prepared and stored in 500-L polypropylene boxes and supplied to plants by means of a motor pump controlled by a timer. The nutrient solution used had the following composition: 9.68 of NO_3^-, 0.88 of NH^{4+}, 1.36 of $H_2PO_4^-$, 5 of K^+, 2.58 of Ca^{2+}, 2 of Mg^{2+}, and 2 of SO_4^- (in mmol L^{-1}). Micronutrients were supplied at 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 in the chelated form. The nutrient solution was adapted for the crop (Mambri et al., 2018), and the macronutrients were supplied through potassium nitrate (KNO_3), monobasic ammonium phosphate ($NH_4H_2PO_4$), calcium nitrate ($Ca(NO_3)_2$) (commercial product Calcinit®), and magnesium sulfate ($MgSO_4$). The electrical conductivity (EC) of the nutrient solution used was 1.84 Ds m^{-1}, being monitored weekly and corrected with the addition of aliquots of a new solution whenever necessary, in order to maintain the original value.

The peppermint seedlings used in experiments 1 (transplantation in summer) and 2 (transplantation in winter) were produced at the same site of the experiment, using parent plants grown in greenhouse, in soilless cultivation. For this, cuttings of 4 centimeters from the apex of the branches, leaving two expanded leaves at the end, were placed in polystyrene trays containing commercial substrate. The trays with the seedlings produced were placed on a bench, inside the greenhouse, under sprinkler irrigation, where they remained until the formation of the root system. Seedling transplantation was performed when the root system was well formed, which occurred after 30 days in experiment 1 (transplantation in summer) and after 45 days in experiment 2 (transplantation in winter).

In both experiments, the seedlings were transplanted to 3 dm^3 white polyethylene pots, filled with 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 from the concrete floor. Two benches were used in each experiment. Each bench had 44 pots, resulting in 88 pots and, consequently, 88 plants in each experiment.

For experiment 1, four plants were marked. In these plants, 12 evaluations were carried out (in 30 days intervals). These plants were located on the sides of the benches and had a high volume of leaves since the first 30 days after transplantation. In experiment 2, the plants showed a slow growth after transplantation, presenting a low volume of leaves at the first harvest period. Thus, more than four plants were used in order to obtain a sufficient amount of leaves for the extraction of essential oil, requiring a volume greater than 100 g of fresh leaves per harvest. All plants used were harvested at the same time and on the same date, every 30 days, for 12 months, resulting in 12 harvests in each experiment. With this standardization, the plants had the same conditions of cultivation and growth, that is, in each harvest date the plants were in vegetative growth, with similar size and volume.

The harvests were carried out at a height of 7 cm from the base of the plant, in order to allow the sprouting of the branches, thus, the plants were always harvested during vegetative growth. The collected material was separated into leaves and branches, being the leaves packed in plastic bags and stored at -15 °C for later extraction and analysis, and the branches were discarded.

At the end of the experiment, leaf samples collected from three consecutive harvests, obtained in the same season, were united. For example, the samples collected in the months of January, February and March corresponded to the summer season, as the plants grew in that season, and so on, obtaining a total of 4 samples per experiment,

equivalent to the 4 seasons (treatments). Thus, a completely randomized design was used, with four treatments, which are the seasons (summer, spring, winter and autumn) in which the samples were collected.

Leaf samples from each treatment (season) were divided into three leaf subsamples, with 100 g each. Each subsample is equivalent to one repetition, forming a total of three repetitions per treatment (seasons), that is, three oil extractions were performed per season. The extraction of essential oil from the peppermint leaves was carried out by the method of hydrodistillation in a Clevenger apparatus, for two hours. For this, in each extraction, the subsamples of 100 g of fresh leaves were used, in a 2 L flask containing 1.25 L of distilled water. Essential oil content (%) and oil yield (in g plant^{-1} and ml plant-^{1}) were determined using the equations 1, 2 and 3:

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

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

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

Where: C = oil content in percentage; W = oil weight in grams; SM = sample mass in grams; Y = oil yield in g plant^{-1}; FLW plant^{-1} = average value of fresh leaf weight per plant; Q = quantity of oil in milliliters.

The chemical itrateionn of the essential oil was analyzed at the Plant Extractive Laboratory (LABEVE), at Santa Maria Federal University, 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 of 1.0 ml min^{-1}); capillary column of fused silica DB5-MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness: 0.25 µm); and ionization energy: 70 Ev. Helium was used as carrier gas at a flow rate of 1.0 ml min^{-1}, with the temperature of theitrattor, detector and interface adjusted to 250 °C and the auxiliary temperature adjusted to 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^{-1}.

The essential oil components were identified based on the Kovats retention indices (IK), determined using a calibration curve of a homologous series of *n*-alkanes (C8-C40), compared to mass spectra data literature and the equipment database (Adams, 2017; Nist, 2008). The quantification of the components was obtained by gas chromatography with a flame ionization detector (CG-FID), performed on an Agilent 7890A chromatograph. The analysis parameters are equivalent to those mentioned above, for the analysis by GC-MS, except for injection by the splitless mode and the temperature of the injector and detector: 300 °C.

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

Results and Discussion

The peppermint essential oil content and yield, in the summer transplant, showed significant differences between the seasons (**Table 1**). The essential oil content was higher in the harvest carried out in summer (0.4780%), followed by spring (0.4053%) and the essential oil yield was higher in spring (0.9373 g plant^{-1} and 1.1563 ml plant^{-1}), followed by summer (0.7530 g plant^{-1} and 0.9189 ml plant^{-1}), with lower oil content and yield at autumn and winter.

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

Summer transplant				
Season	Oil content (%)	Oil yield (g plant^{-1})	Oil yield (ml plant^{-1})	Fresh leaf weight (g plant^{-1})
Summer	0.4780 a	0.7530 b	0.9189 b	157.5275
Autumn	0.3447 c	0.2905 c	0.3372 c	84.2950
Winter	0.1810 d	0.1880 d	0.2424 d	103.8725
Spring	0.4053 b	0.9373 a	1.1563 a	231.2500
CV (%)	7.78	7.20	9.35	-
Winter transplant				
Season	Oil content (%)	Oil yield (g plant^{-1})	Oil yield (ml plant^{-1})	Fresh leaf weight (g plant^{-1})
Summer	0.4943 a	0.3228 b	0.3701 b	65.3150
Autumn	0.2267 c	0.0947 c	0.1254 c	41.7850
Winter	0.1687 c	0.0869 c	0.1031 c	51.5500
Spring	0.4137 b	0.5083 a	0.6349 a	122.8800
CV (%)	10.20	11.44	6.52	-

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

For winter transplantation, the peppermint essential oil content and yield also showed significant differences between the seasons (Table 1), with the highest content of essential oil found in summer (0.4943%), followed by spring (0.4137%) and lower content in autumn and winter. The yield of essential oil was higher in spring (0.5083 g plant^{-1} and 0.6349 ml plant^{-1}), which is due to the high production of fresh matter of leaves per plant in this season.

These results demonstrate greater essential oil production in periods of higher temperature and solar radiation, spring and summer, at both transplants periods (**Figure 1**), showing the importance of climatic conditions in the cultivation of aromatic plants. Furthermore, the essential oil yield calculation considers the leaves fresh matter mass of the plants, not just the weight of the sample and the weight of the extracted essential oil, as occurs with the oil content. In addition, the oil yield is directly proportional to the plants fresh matter mass, thus, it can be seen that the greater mass verified in the spring compared to the summer, influences the final value, although the oil content was lower.

In a study carried out with mint species, it was found higher average yields in the summer harvest (0.348%), compared to the yields obtained in the winter harvest (0.177%), and attribute these results to the different climatic conditions of the periods, mainly the air temperature and solar radiation, which were higher in summer (Deschamps *et al*., 2008). In addition, according to the authors, the reduction in essential oil biosynthesis in winter is due to the possible deviation of the metabolic routes, prioritizing the maintenance and survival of plants in adverse conditions at the expense of oil production.

Another study with *M. piperita* L. at two harvest times, July and October, in Iran, showed a higher essential oil yield in the harvest carried out in July, a period of higher temperatures and less rainfall (Machiani *et al*., 2018). Thus, the authors also observed the influence of climatic conditions during the harvest period. The influence of temperature and photosynthetically active radiation on the production of essential oil was verified at a study conducted with the species *M. x piperita* var. *itrate*, in which the highest content (1.33%) was obtained at the time of highest temperature and solar radiation (Oliveira *et al*., 2012).

The components of peppermint essential oil, in the summer transplant, were different between the seasons (**Table 2**). In spring were identified compounds that were not verified in other seasons. The major components (more than 75%) identified in the summer season were menthol (38.25%), isomenthone (31%) and pulegone (14.10%). In the autumn season, isomenthone (24.90%), menthol (24.20%), menthone (23.03%) and pulegone (18.93%). In winter, the components identified were menthone (38.25%), isomenthone (31.00%) and menthol (14.10%). In the spring, the major components were menthone (51.22%), menthol (17.20%) and isomenthone (14.98%).

In the summer transplant the major components

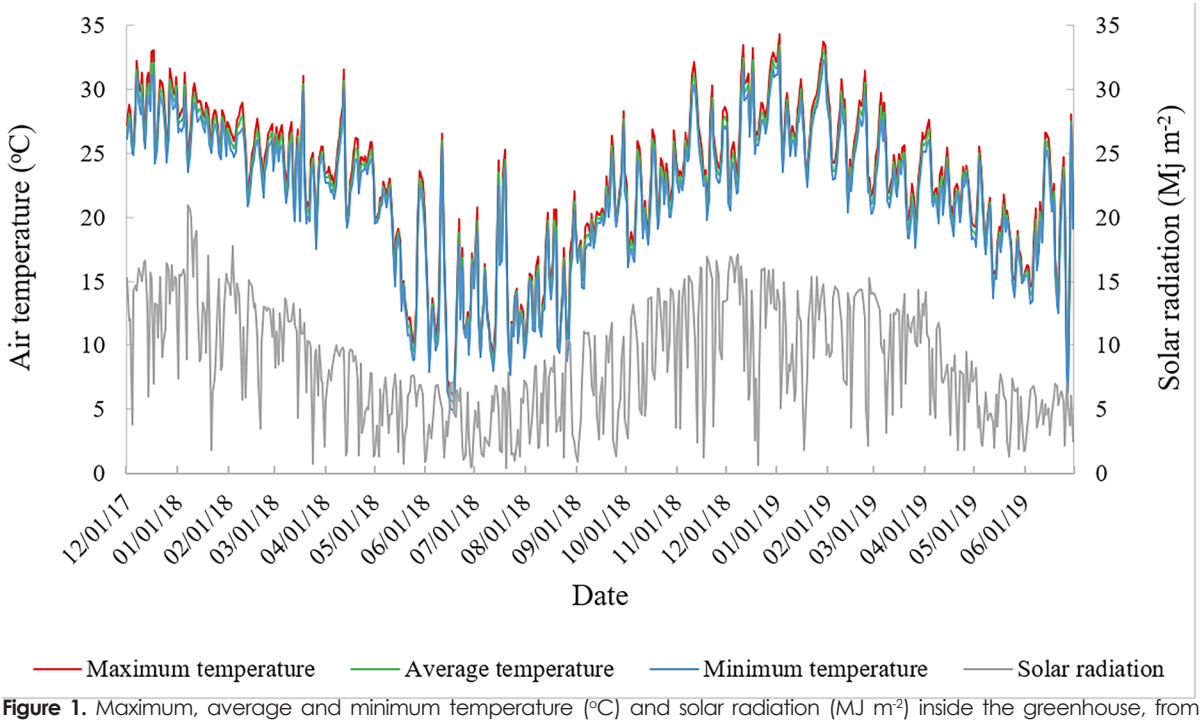

Figure 1. Maximum, average and minimum temperature (ºC) and solar radiation (MJ m^{-2}) inside the greenhouse, from December 2017 to June 2019, at Santa Maria, RS. Santa Maria, UFSM, 2020.

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

IK*	Components	Season			
		Summer	Autumn	Winter	Spring
965	β-Pinene	-	-	-	0.11 ± 0.00**
979	Sabinene	-	-	-	0.17 ± 0.00
987	n-Octan-3-ol	-	-	-	0.30 ± 0.00
1005	α-Phellandrene	-	-	-	0.20 ± 0.00
1017	Limonene	0.49 ± 0.00	-	0.49 ± 0.00	0.21 ± 0.00
1019	1-8 Cineole	4.09 ± 0.02	-	4.09 ± 0.02	1.25 ± 0.00
1047	γ-Terpinene	-	-	-	0.41 ± 0.00
1058	cis-Sabinene hydroxide	0.87 ± 0.01	0.94 ± 0.01	0.87 ± 0.01	0.92 ± 0.00
1088	α-Terpinolene	0.28 ± 0.00	0.54 ± 0.00	-	0.57 ± 0.00
1144	Menthone	38.25 ± 0.22	23.03 ± 0.13	38.25 ± 0.22	51.22 ± 0.29
1150	Isomenthone	31.00 ± 0.18	24.90 ± 0.14	31.00 ± 0.18	14.98 ± 0.10
1157	Menthofuran	2.03 ± 0.01	3.63 ± 0.02	2.03 ± 0.01	2.23 ± 0.01
1165	Menthol	2.03 ± 0.08	24.20 ± 0.14	14.10 ± 0.08	17.20 ± 0.10
1224	Pulegone	14.10 ± 0.04	18.93 ± 0.11	7.47 ± 0.04	5.97 ± 0.04
1240	Piperitone	-	-	-	0.73 ± 0.00
1276	Menthyl acetate	1.35 ± 0.01	3.08 ± 0.02	1.41 ± 0.01	2.02 ± 0.01
1404	Caryophyllene	-	-	-	0.82 ± 0.00
1465	Germacrene D	-	-	-	0.35 ± 0.00

*IK = Kovat Index. **Average of three injections ± standard deviation. Symbol "-" indicates that the component has not been verified at that season.

identified in summer and autumn were similar, with small differences in the percentage of each, being menthone, isomenthone and pulegone, in addition to menthol wich was a major component in autumn. In the winter and spring seasons, the major components were the same, menthone, isomenthone and menthol, but with differences in the content. With the exception of autumn, in which the major compound was isomenthone, the other seasons presented menthone as the main component. Also, the concentration of menthol was high in autumn, winter and spring and not significant in summer (Table 2).

In the winter transplant, differences were found in the major components in relation to the transplant performed in the summer (**Table 3**). In the summer season, the major components were menthone (31.85%), menthofuran (25.83%), isopulegone (15.60%) and menthol (13.32%). In autumn, the components were menthofuran (48.01%), pulegone (23.60%) and menthone (14.63%). In winter there were menthone (42.67%), menthofuran (22.95%) and menthol (18.77%). In the spring, the major components were menthone (44.51%), isopulegone (22.79%) and menthofuran (13.91%). The major components were similar in summer and spring, with the exception of menthol in summer. The pulegone compound had a high concentration in autumn and the menthol in winter. All seasons presented menthone and menthofuran as major components of peppermint essential oil in this transplant period.

In the comparison, it is possible to observe differences for each season of the year in the two periods of transplanting. Menthone was the only compound found to be the majority in all treatments. In autumn, pulegone was also found to be a major component of both transplant periods, as well as menthol in the winter season. In summer and spring, the other compounds were different between transplant periods, demonstrating their influence on the composition of peppermint essential oil.

In a study carried out with mint species, the seasonal variation in the leaves essential oil content and chemical was found, with *M. piperita* being the species that presented the highest essential oil content in the summer (12.2 g kg^{-1}) in relation to the winter (10.5 g kg^{-1}). The major components in the summer were menthone, menthyl acetate, limonene and neoisomentho and the major components in winter were menthone, menthyl acetate, limonene and isomenthone, showing some similarities with the present study (Hussain *et al*., 2010).

The seasonal variation in the yield and composition of essential oil from mint genotypes was verified at two seasons of harvest, summer, in February summer, and autumn, in May (Santos *et al*., 2012). The essential oil content and yield of the genotype for the species *M. piperita* was higher in February (2.9%) compared to May (2.2%). The components were similar, with some differences in quantity, being menthol, menthone, neomenthol and 1.8-cineole in February and menthol, menthone, neomenthol and menthyl acetate in May.

Compounds such as menthol and menthone as major components of peppermint essential oil, exhibit antimicrobial characteristics against bacteria, yeasts and fungi, having the potential to be used as a natural antimicrobial, allowing the reduction of antibiotic doses (Mahboubi & Kazempour, 2014). In addition to

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

IK*	Components	Season			
		Summer	Autumn	Winter	Spring
922	α-Pinene	0.85 ± 0.00**	0.26 ± 0.00	0.43 ± 0.00	0.27 ± 0.00
961	β-Thujene	1.80 ± 0.01	0.29 ± 0.00	0.99 ± 0.00	0.71 ± 0.01
965	β-Pinene	0.48 ± 0.00	0.17 ± 0.00	0.44 ± 0.00	0.29 ± 0.00
971	Sabinene	-	-	0.22 ± 0.00	-
979	n-Octan-3-ol	6.19 ± 0.04	1.67 ± 0.01	0.19 ± 0.00	0.23 ± 0.00
1005	α-Phellandrene	0.36 ± 0.00	-	-	3.53 ± 0.02
1018	Limonene	0.74 ± 0.00	0.14 ± 0.00	4.29 ± 0.02	0.30 ± 0.00
1020	1-8 cineole	0.14 ± 0.00	0.72 ± 0.00	0.36 ± 0.00	0.70 ± 0.00
1047	γ-Terpinene	0.24 ± 0.00	-	0.45 ± 0.00	0.13 ± 0.00
1059	cis-Sabinene hydroxide	0.18 ± 0.00	0.12 ± 0.00	0.15 ± 0.00	0.36 ± 0.00
1088	α-Terpinolene	-	-	-	0.13 ± 0.00
1138	trans-2-Menthenol	-	0.16 ± 0.00	-	-
1144	Menthone	31.85 ± 0.23	14.63 ± 0.08	42.67 ± 0.25	44.51 ± 0.31
1151	Menthofuran	25.83 ± 0.18	48.01 ± 0.28	22.95 ± 0.13	13.91 ± 0.08
1158	Menthol	13.32 ± 0.09	8.51 ± 0.05	18.77 ± 0.01	7.51 ± 0.01
1163	Isopulegone	15.60 ± 0.11	-	0.49 ± 0.00	22.79 ± 0.16
1176	Neoisomenthol	1.63 ± 0.01	-	0.27 ± 0.00	0.75 ± 0.01
1224	Pulegone	1.00 ± 0.01	23.60 ± 0.14	5.54 ± 0.03	1.66 ± 0.01
1240	Piperitone	-	-	-	0.80 ± 0.00
1276	Menthyl acetate	0.39 ± 0.00	0.96 ± 0.01	1.02 ± 0.00	0.41 ± 0.00
1404	Caryophyllene	0.31 ± 0.00	0.46 ± 0.00	0.49 ± 0.00	0.59 ± 0.00
1465	Germacrene D	0.31 ± 0.00	0.43 ± 0.00	0.27 ± 0.00	0.55 ± 0.00
1576	Germacrene B	-	-	-	0.73 ± 0.00

*IK = Kovat Index. **Average of three injections ± standard deviation. Symbol "-" indicates that the component has not been verified at that season.

these properties, menthol has an analgesic effect. This compound exists in several forms, such as L-menthol, which allows increased penetration of other compounds into the skin, and is normally associated with another anti-inflammatory analgesic (Lhez *et al*., 2010; Kamatou *et al*., 2013).

In the analysis of the chemical composition of mint species essential oil, the major components of *M. piperita* ("chocolate mint") were menthofuran, menthone, p-neoisomenthol, pulegone and iso-methyl acetate. For the species *M. piperita* ("grapefruit mint") were linalyl acetate and L-linalool. For the species *M. piperita* ("peppermint") were D-carvone and limonene (Barros *et al*., 2015). According to the authors, the presence of pulegone is directly related to the antifungal properties of mint. Another major component found in mint essential oil was isopulegone, which demonstrated good antioxidant potential in a study carried out *in vitro*, reducing free radicals (Silva *et al*., 2012).

In another study, evident antimicrobial activity was found in the essential oil of *Mentha* species, allowing its use in natural antimicrobial therapies for the treatment of infectious diseases in humans and plants, in addition to the use for preserving processed foods (Hussain *et al*., 2010). Compounds as menthol and isomenthone, which is an isomer of menthone, have the ability to increase bile secretion and menthol also decreases the level of total cholesterol and increases the total level of bile acids (Hu *et al*., 2015).

Menthofuran, a major compound with a high content of peppermint essential oil in winter transplant, is considered a hepatotoxic substance (Khojasteh *et al*., 2010), which reduces the quality of the essential oil. This compound is produced in conditions of low solar radiation (Behn *et al*., 2010). Therefore, it is not recommended to transplant the seedlings in winter.

Conclusions

The highest production of peppermint essential oil is obtained in harvests carried out in summer and spring in plants transplanted in summer and winter. The main compound of peppermint essential oil is menthone in all seasons and the best quality of essential oil is obtained in the summer transplanting, as the winter transplanting favors the production of the menthofuran compound, which reduces the quality of the essential oil.

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