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Assessment of essential oil yield in three mint species in the climatic conditions of Central Russia

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Abstract. The aim of the study was to study the harvest time for the essential oil yield and its qualitative composition in three species of mint *Mentha piperita* L. (Peppermint), *M. spicata* L. (Spearmint) and *M. arvensis var. piperascens* Malinv. ex. Holmes (Sakhalinmint). In 2018, the research was performed with plants of second year of vegetation in the Laboratory of Plant Physiology and Immunity of the NV Tsitsin Main Botanical Garden of the RAS. As a result, it was found that the optimal harvest period for Sakhalinmint and Peppermint should be recommended in a phase of mass flowering: the yield of fresh raw materials was 509–479 g m^{-2}, air-dry raw materials - 110–107 g m^{-2}; the content of essential oil in the aboveground part (a mixture of leaves and inflorescences) of plants - 3.24–4.01%; the proportion of the main component of essential oil (menthol) - 57.3–50.2%. In Spearmint, the optimum time for harvesting is the phase of budding. The content of the main component of essential oil (carvon) was maximum - 67.9%, and the yield of essential oil was 2.6%, while the yield was 381 g m^{-2} of fresh raw materials (81.9 g m^{-2} of air-dry raw materials) at harvesting in this ontogenesis stage. Analysis of the secretory apparatus parameters on a surface of some green tissues in three mint species showed that the maximum density of secretory glands on both sides of the leaf is characteristic of peppermint, which provides a higher yield of essential oil in this type of mint. The study allowed determining the optimal harvesting time for highly productive mint species when they are grown in the conditions of Central Russia. The raw materials of these mint species can be used for the production of essential oils and are of interest for pharmacology and the perfume and cosmetics industry.

Key words: *Mentha piperita* L. (peppermint), *Mentha spicata* L.(spearmint) and *Mentha arvensis var. piperascens* Malinv. ex. Holmes (Sakhalinmint), harvest time, essential oil, secretory glands, chemical composition.

INTRODUCTION

Mint (*Mentha* L.) is one of the most important essential oil crops, widely used in the perfumes, cosmetics, pharmaceuticals, food industries, and medicine (Tucker, 2007; Taneja & Chandra, 2012; Anwar et al., 2019). Mint essential oil has antibacterial, fungicidal, analgesic, neurospasmodic, stimulating and other effects (Sujana et al., 2013; Brahmi et al., 2017; Kalemba & Synowiec, 2020). *Mentha piperita* L. (peppermint), *M. spicata* L. (spearmint) and *M. arvensis* var. *piperascens* Malinv. ex. Holmes (Sakhalinmint) are the most significant members of the *Lamiaceae* family (Lawrence, 2006).

M. piperita is an aromatic herb that is widely used in the cosmetic, confectionery, food and pharmaceutical industries and for the production of essential oils (Mahendran & Rahman, 2020). Peppermint leaves are commonly used in herbal teas and in cooking to add flavor and aroma. The resulting essential oil has astringent, antiseptic, antipyretic, antispasmodic, antimicrobial properties (Sujana et al., 2013; Kalemba & Synowiec, 2020). *M. arvensis* var. *piperascens* (Sakhalinmint) is a valuable medicinal plant that is cultivated in tropical and subtropical regions under irrigation (Singh & Saini, 2008). The characteristic odor and taste of this mint is due to its high menthol content (up to 75–80%) (Singh & Lal, 2020). The essential oil of these mint two species contains the same main components - menthol and menthone. They are among the most produced and sold essential oils in the world. The main producers of peppermint oil are India, United States and China, while the Sakhalinmint is grown in China, India, Brazil and Japan (Jeyakumar et al., 2011; Balakrishnan, 2015). Spearmint (*M. spicata*) is a valuable herb that is used fresh and dried in traditional medicine as a stimulant and adaptogenic agent. It is also widely used for the essential oil production, as the oil has antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties (Singh & Agarwal, 2013; Lukošiūtė et al., 2020). The distinguishing odor of spearmint oil is due to the main major component of the essential oil, carvone. In addition, this herb contains significant amounts of limonene, dihydrocarvone and 1,8-cineole (Hussain et al., 2010).

The production of essential oil by mint plants is mainly due to species characteristics, but it changes significantly under the influence of external environmental factors. To use the mint as an essential oil producer, the sum of the effective temperatures it is believed should be 3,200–3,400 °C for the growing season (Kirichenko, 2008). For the Central Russia region, in particular, for the Moscow region, according to long-term observations this indicator is 2,400–2,600 °C. So, the mint in this region is grown to obtain a pharmacy leaf. But the more frequent weather anomalies in recent years have led to a significant warming of the climate and an increase in the sum of effective temperatures in Central Russia (Pozdnyakov et al., 2019). Thus, if this indicator in the Moscow region exceeded the long-term average value by 1,144 °C in the abnormally hot summer of 2010, while in ordinary years (2015–2019) by 204.8–605.4 °C (Meteorological archive, 2020). Changes in climatic conditions can contribute to an increase in the productivity of mint biomass, and an increase in the yield and quality of essential oil, since air temperature is one of the main regulating factors during the period of intensive biosynthesis of essential oil (Clark & Menary, 1980). In recent years, the growing market demand for essential oil containing natural menthol for various industrial needs has led to an increase in the necessity for mint oil obtaining (Jain, 2017). The production can be increased through the development of high-yielding varieties and by expanding the growing area outside traditional cultivation areas through the development of new cultivation technologies (Rohloff et al., 2005; Aflatuni et al., 2006; Kapp et al., 2020). It is these factors that determine the expansion of the boundaries of the use of highly productive species and hybrids of mint of southern origin to obtain high-quality essential oil in Central Russia (Shelepova et al., 2011).

When grown in warm climatic conditions, a double harvest of green mass is possible, which significantly increases the yield of essential oil. Mint growing in Central Russia allows only one harvest (Shelepova et al., 2016). There is a lot of data on the effect of harvest time on the quality and quantity of mint oil when grown in warm climatic conditions (Singh & Singh, 1997; Santos et al., 2012). There is no information about the effect of the dates of harvesting the green mass of three mint species (Sakhalinmint, Peppermint and Spearmint) on the yield of essential oil in the conditions of the Central Russia in various studies performed. The aim of the study was to determine the dates of the phenological cycle, allowing obtain the maximum biomass (yield) of Sakhalin mint, peppermint and spearmint, as well as a high content of essential oil, to determine the change in the qualitative composition of plant essential oil.

MATERIAL AND METHODS

The three species of mint, mainly grown for the commercial production of mint essential oil: *M. arvensis* var. *piperascens* Malinv. ex. Holmes (Sakhalinmint), *M. piperita* L. (peppermint) and *M. spicata* L. (spearmint) were selected as objects of research. The source of origin and introduction of these species was discussed in detail (Shelepova et al., 2011; Shelepova et al., 2016). The taxonomic affiliation of the samples was carried out by the staff of the Herbarium of the Main Botanical Garden of Russian Academy of Sciences (MHA), later it was clarified with the involvement of herbarium samples of this variety from the SEINet and JSTOR database. The herbarium specimens are stored at the herbarium of the MHA.

The studies were carried out at the experimental site of the Laboratory of Plant Physiology and Immunity of the Main Botanical Garden of Russian Academy of Sciences (55°837′ N – 37°588′ E). Mint plants were propagated by means of runners and rhizomes. Plants were planted at the end of May in furrows at a distance of 10–15 cm from each other with a row distance of 40 cm. Cultivation was performed with additional irrigation during the growing season. The studies were carried out in 2018 on second year plants. Essential oil was obtained from an average sample of aerial parts (a mixture of inflorescences and leaves) of plants by hydrodistillation. A sample (up to 20 g) of air dried aerial parts was ground and then subjected to hydrodistillation for 2 h, according to the standard procedure described in the European Pharmacopoeia V (2004). The essential oil content is in% (v/w). The oils were stored in the dark until analyzed.

Qualitative composition of the oil was determined by gas chromatography at the Center for Collective Use of the Federal Research Center 'Fundamentals of Biotechnology', Russian Academy of Sciences, Moscow (RFMEFI62114X0002).

The identification of essential oil components was obtained by GC/MS. Analyses were carried out on a Shimadzu GS 2010 gas chromatograph with a GCMS-QP 2010 mass detector. The gas chromatography column was a using a SPB-1 nonpolar column (solid-stage-bound methyl silicone) (Supelco, Sigma-Aldrich) (30 m × 0.25 mm ID, 0.25 µm film thickness). The carrier gas was helium. The column oven temperature was set at 60 °C for 3 min, increased to 100 °C at a rate of 1.5 °C min^{-1}, increased to 180 °C at a rate of 4 °C min^{-1}, and held at 180 °C for 1 min. Next, it was increased to 200 °C at a rate of 10 °C min^{-1}, increased to 250 °C at a rate of 2.5 °C min^{-1}, and held at 250 °C for 5 min. Injector, interface and detector temperatures were 180 °C, 205 °C and 250 °C, respectively.

The gas chromatography mass analysis was carried out with the same characteristics as used in gas chromatography. A 1.0 μL sample was injected in the split mode, with a split ratio of 1:150. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Column oven temperature program was the same as in GC analysis. Helium was used as a carrier gas at a flow rate of 1.5 mL min^{-1}. Mass range was 30–400 m z^{-1}, while the injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The percentage composition of the oils was computed by the normalization method from the GC peak areas, which were calculated as mean values of two injections of each oil sample, without using response factors. The identity of the components was assigned by comparison of their retention indices, relative to was assigned by comparison of their retention indices, relative to a mixture of linear paraffins consisting of nonane, decane, tridecane, and pentadecane.

Biometric analysis of glandular trichomes on mint leaves and flowers was carried out on fresh preparations using a Keyence VHX-1000E digital microscope (Itasca, IL, USA). There are mainly two types of secretory glands represented by peltate and capitate glandular trichomes on the surface of leaves and flowers. Identification of secretory glands using a digital microscope does not allow separating these two types of glands, so in our experiment we recorded the sum of peltate and capitate glandular trichomes (Fig. 1).

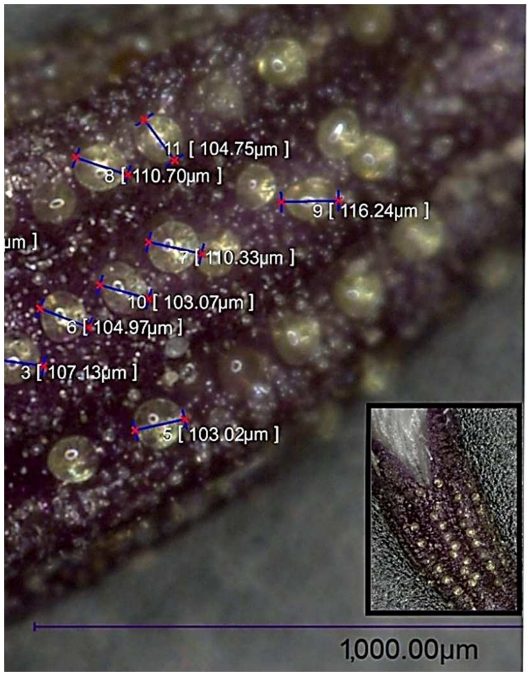

Figure 1. Light microscopy micrographs showing a method for measuring the diameter of the secretory glands of mint plants (bar = 1,000 μm).

Processing of experimental data was done by dispersion analysis using Microsoft Excel, with significance level $p \leq 0.05$.

RESULTS AND DISCUSSION

At the beginning of the plant growing season from late May to early July, all three species developed well-leafy bushes with 2–3 stems. The budding stage was beginning in the last decade of July; the mass flowering of spearmint and peppermint was in the last decade of August, and Sakhalinmint, in the first decade of September.

Studying productivity parameters revealed the lowest yield of raw materials was registered in the budding stage. At the same time, the green mass yield was maximum in Sakhalinmint both in the budding stage and in the mass flowering stage and amounted to 414.9–508.5 g m^{-2}. Respectively, in peppermint the yield was 5–6% lower, and in spearmint - by 8–21% lower than that of Sakhalinmint (Table 1). The productivity of fresh raw materials of Sakhalinmint significantly exceeded the yield of peppermint and spearmint only is due to a high proportion of stems in the total weight.

Table 1. Yield and essential oil content of three *Mentha* species at different stage of ontogenesis

Species	Green crops, g m^{-2}				Essential oil content,% (v/w)		Essential oil production, g m^{-2}	
	Fresh raw materials		Air-dried raw materials					
	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage
Sakhalinmint	414.9	508.5	88.5*	110.3*	2.97	3.24	12.32*	16.48
Peppermint	392.9*	478.9	87.1*	106.5*	3.39	4.01	13.32*	19.20
Spearmint	380.7*	399.5	81.9	86.4	2.58	2.71	9.82	10.83
LSD (0.05)	2.9	3.0	0.9	1.0	0.11	0.12	1.10	1.17

Note: means bearing same * in each column are statistically similar at $p \leq 0.05$.

It should be noted that the yield of raw materials of spearmint did not differ in the budding stage and the mass flowering stage while that of Sakhalinmint and peppermint were the highest in the mass flowering stage. The increase in the yield of green biomass during harvesting in the mass flowering stage in Sakhalinmint and peppermint was by 22.6–21.9%, and in spearmint was by only 4.9%, respectively.

At the same time, there revealed the presence of significant differences between Sakhalinmint and peppermint in terms of yield of fresh materials in both phases of ontogenesis. The samples did not differ in the yield of air-dried raw materials. The total yield of fresh raw materials of spearmint in budding stage did not significantly differ from that of peppermint and was lower than the yield of Sakhalinmint. The productivity of fresh and air-dry raw material of spearmint in the mass flowering stage are significantly lower than those of peppermint and Sakhalinmint.

According to the data obtained and the results of other researchers, the total yield of fresh and air-dry raw material of Sakhalinmint and peppermint increased from budding stage to mass flowering stage. Cutting during mass flowering stage gave the highest biomass and essential oil yield (Rohloff et al., 2005; Zheljazkov et al., 2010). A similar result was also observed in spearmint grown in Northern Finland (Aflatuni et al., 2006). The main reserve of essential oil is formed exactly in the budding and mass flowering stage, so, the maximum yield of essential oil is observed in plants of almost all species of the genus *Mentha* L. in these stages of ontogenesis (Singh & Agarwal, 2013; Straumite et al., 2015; Shelepova et al., 2016; Kapp et al., 2020).

The content of essential oil in the air-dry mass of the above-ground parts of plants of three species of mint was from 2.58 to 3.39% in the budding stage and from 2.71 to 4.04% in the stage of mass flowering (Table 1). The maximum content of essential oil was recorded in the leaves and inflorescences of peppermint, and was 12.4–19.2% lower in Sakhalinmint. Spearmint had the minimum essential oil content, by 23.9–32.4% lower than, that of peppermint.

The yield of essential oil from the aboveground mass of plants in the mass flowering stage due to the higher yield and the maximum content of essential oil was the highest in peppermint (19.20 g m^{-2}), and 16.48 g m^{-2} in Sakhalinmint. Overall, in the mass flowering stage the essential oil yield was increased by 1.44 times in peppermint and by 1.34 times in Sakhalinmint in compared with the budding stage. And only spearmint had the same level of essential oil yield both in the budding stage and in the mass flowering stage - 9.82 and 10.83 g m^{-2}, respectively.

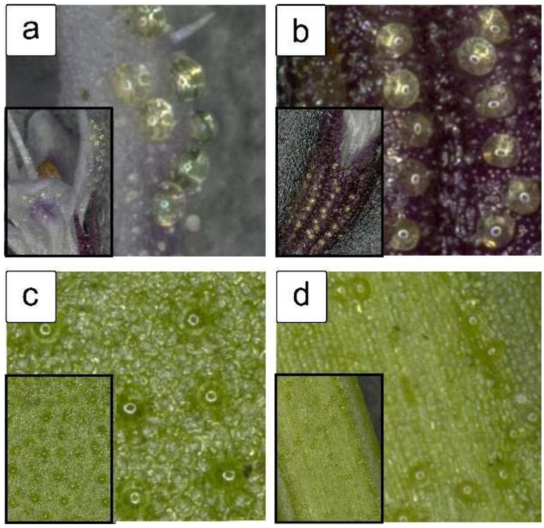

Figure 2. Microscopy micrographs showing the location of glands on the corolla (a), calyx (b), leaf (c) and stem (d) of peppermint plants.

It is known that essential oil, which is a complex mixture of secondary metabolites, is generated and stored in the secretory glands on all organs of the aerial parts of mint plants (stems, leaves, calyx, corolla) (Fig. 2). As the leaves and flowers grow and increase in size, new glands are formed. Moreover, their maximum number is located on flowers, leaves of the middle and upper tiers, and the minimum number is situated on plant stems. Direct observation of glands showed that the gland development is a fairly rapid process, accompanied by the biosynthesis of mono- and sesquiterpenes of essential oil (Tiwari, 2016; Yu et al., 2018).

The glands of the maximum size are recorded on the corolla and calyx of the Sakhalinmint flower - 90.3 ± 7.8 and 93.5 ± 8.8 µm, respectively (Table 2, Fig. 3). Glands of slightly smaller sizes were formed on the leaves, while in all studied species they were generally of the same diameter - 86.8 ± 3.2 - 87.1 ± 2.8 µm on the abaxial side and 86.4 ± 3.5 - 87.0 ± 2.9 µm on the adaxial side (Table 2, Fig. 4).

Table 2. Parameters of the secretory glands of the three *Mentha* species

Species	Diameter of glands on the corolla of a flower, µm	Diameter of glands on the calyx, µm	Diameter of glands on mint leaves, µm		Density of glands on mint leaves, pcs cm^{-2}	
			Abaxial side	Adaxial side	Abaxial side	Adaxial side
Sakhalinmint	90.3 ± 7.8	93.5 ± 8.8	87.1 ± 2.8	87.0 ± 2.9	1,381 ± 72	931 ± 66
Peppermint	87.4 ± 5.5	86.2 ± 6.7	87.0 ± 3.9	86.9 ± 3.2	1,485 ± 54	1,047 ± 65
Spearmint	85.4 ± 3.9	85.0 ± 4.5	86.8 ± 3.2	86.4 ± 3.5	1,032 ± 82	854 ± 61

The density of glands on the abaxial side of the leaves of the middle and upper layers during the mass flowering stage is by 1.21–1.48 times higher than on the adaxial side. The findings are consistent with those of Yu et al. (2018), which demonstrated higher density on the abaximal leaf surface in seven *Mentha* species.

The maximum density of secretory glands on surface with larger size glands recorded in peppermint resulted in a higher yield of essential oil in this species of mint.

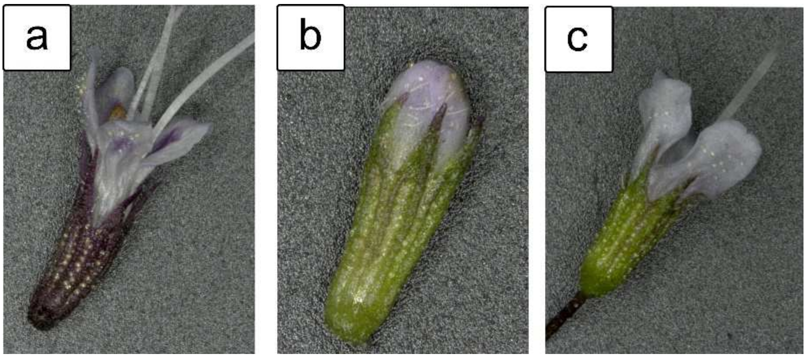

Figure 3. Micrographs showing glands on peppermint flower (a), Sakhalinmint flower (c) and spearmint bud (b).

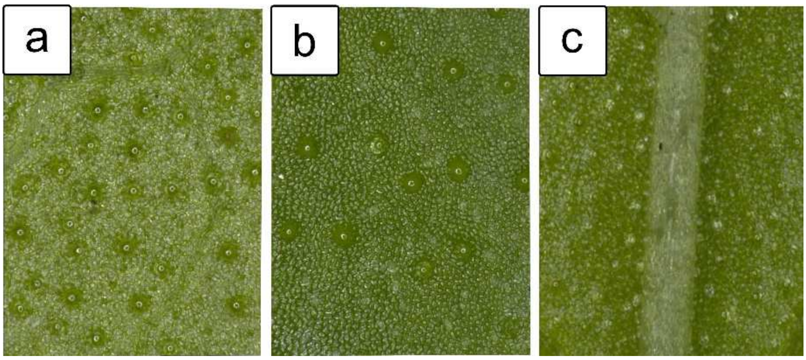

Figure 4. Microscopy micrographs showing glands on the abaxial side of a mature leaf from three species of mint: peppermint (a), spearmint (b), Sakhalinmint (c).

Up to 65 components were found in the composition of the essential oil of three mint species. All components with a content of more than 0.1% of the total amount were easily identified by retention time and mass spectra. According to European Pharmacopoeia (2005), the limits of 10 main components are established in the oil of both species. So, in peppermint oil, determined by gas chromatography, the limits of the content of various compounds were: menthol (30.0–55.0%), menthone (14.0–32.0%), isomenthone (1.5–10.0%), menthyl acetate (2.8–10.0%), menthofuran (1.0–9.0%), 1.8-cineole (3.5–4.0%), limonene (1.0–5.0%), isopulegol (max 0.2%), pulegon (max 4.0%) and carvone (max 1.0%). The limits of the content of these compounds in sakhalinmint oil were similar: menthol (30–50%), menthone (17–35%), isomenthon (5.0–13.0%), menthyl acetate (1.5–7.0%), 1,8-cineole (max. 1.5%), limonene (1.5–7.0%), isopulegol (1–3%), pulegone (max. 2.0%) and carvone (max. 2.0%).

The peppermint and Sakhalinmint essential oils obtained in field trials comply with the recommendations of European Pharmacopoeia (EP 5.0, 2005). It is maximal in the stage of mass flowering in both Sakhalinmint (up to 57%) and peppermint (up to 50%) (Table 3).

Table 3. Composition of essential oil of three *Mentha* species at different stages of ontogenesis

	Sakhalinmint		Peppermint		Spearmint	
	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage	Budding stage	Mass flowering stage
α-pinene	0.20	0.06	0.33	0.29	0.42	0.23
β-pinene	0.46	0.10	0.27	0.32	0.69	0.38
sabinen	0.16	0.08	0.23	0.18	0.20	0.14
β-myrcene	0.64	0.19	0.39	0.25	0.85	0.56
D-limonene	1.83	0.62	1.79	0.95	9.21	6.23
1,8-cineole	0.38	0.29	0.24	0.22	2.37	2.27
menthon	7.80	6.56	10.21	8.97	0.15	0.19
isomenthon	18.92	19.75	19.17	18.51	0.24	0.28
menthyl acetate	8.11	13.20	8.27	18.89	0.70	0.72
isomenthol	1.11	1.17	1.08	1.17	t	t
menthol	52.82	57.30	48.37	50.18	2.15	2.26
pulegone	0.42	0.15	0.16	0.14	t	t
carvone	t	t	t	t	67.89	66.98
dihydrocarvone	t	t	t	t	7.17	10.38
dihydrocarvil	t	t	t	t	3.58	4.51

t – the compound is absent in the oil.

The content of major monoterpenes (menthol, menthone, isomenthone, methyl acetate) in the composition of Sakhalinmint and peppermint essential oils was 90%. But the composition of the essential oil of both mint species was slightly different from each other. Thus, the menthol content was 1.3 times higher in Sakhalinmint than in peppermint. At the same time, the content of menthol and its precursor menthyl acetate in Sakhalinmint and peppermint was higher during the period of mass flowering compared to the budding stage: in Sakhalinmint by 1.1 and 1.6 times, and in peppermint by 1.03 and 2.3 times, respectively. Most studies suggest that the content of menthol and menthyl acetate increases as the Sakhalinmint and peppermint plants develop to the mass flowering stage (Tiwari, 2016; Ostadi et al., 2020). The menthol / menthone ratio in the mass flowering stage increased in both species compared to the budding stage, while in Sakhalinmint it increased more significantly (from 8.05 to 10.26). According to Kalemba & Synowiec (2020), the menthone content of both mint species decreases with increasing menthol content. In peppermint, the maximum menthol content was reached after about 85 days from the start of the growing season to mass flowering. The menthol content in Sakhalinmint was increasing when the harvest was delayed up to 110 days after the start of the growing season.

It should be noted that Sakhalinmint and peppermint have a decrease in the amount of minor monoterpenes and terpenoids (α- and β-pinene, sabinene, D-limonene, 1.8-cineole, and a number of others), which have antifungal and insecticidal properties, during the stage of mass flowering.

Spearmint's major monoterpene are carvone and its derivatives - dihydrocarvone, carviol, dihydrocarvil, which account for up to 80% of the composition. In spearmint, the content of carvone slightly decreases, but the proportion of its derivatives, dihydrocarvone and dihydrocarvine, increases in the mass flowering stage. At the same time, the percentage of minor monoterpenes and terpenoids (α- and β-pinene, sabinene, β-mercene, D-limonene) was also decreased significantly.

CONCLUSION

Thus, the results show that the yield of green mass and the essential oil content depend on the mint species. It should be noted that the amount of oil in the leaves and inflorescences of all mint species studied in late August - early September was significantly higher compared to the beginning of August in the climatic conditions of Central Russia. The optimal harvest period for Sakhalinmint and Peppermint should be recommended in a phase of mass flowering: the yield of fresh raw materials was 509–479 g m^{-2}, air-dry raw materials - 110–107 g m^{-2}; the content of essential oil in the aboveground part (a mixture of leaves and inflorescences) of plants - 3.24–4.01%; the proportion of the main component of essential oil (menthol) - 57.3–50.2%. The yield of fresh raw materials was 509–479 g m^{-2}, air-dry raw materials - 110–107 g m^{-2}; the content of essential oil in the aboveground part (a mixture of leaves and inflorescences) of plants - 3.24–4.01%; the proportion of the main component of essential oil (menthol) - 57.3–50.2%. As for spearmint, carvone content was higher (67.9%) when harvested in the budding stage, while the fresh / air-dry raw materials (381/81.9 g m^{-2}) and essential oil yield (2.6%) were slightly lower than in the mass flowering phase - 400/86.4 g m^{-2} and 2.7%, respectively. Therefore, the optimal harvest time for this mint species should be the end of July - early August.

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