



## Estimating peppermint essential oil levels: water-stream and classical hydroponic systems

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### ABSTRACT:

The leaves of peppermint and the essential oil received from them are used in medicine. The chemical content of essential oil is quite complex. It contains about 30 terpene compounds. The content of essential oil in flowers is 4-6%; in leaves – 2.4-2.8%, and in stems 0.3%. The main components that define the quality of essential oils are menthol, menthone, limonene, menthofuran, and isomenthone. Peppermint is used in the form of tea, tincture, extract, and salves. It has a regulating influence on the heart and circulatory system. It calms the heartbeat and supports the decrease of blood pressure, and the oil stream is used for dyspnea in the form of inhalation. Plant cream is used against insect bites, eczema, hemorrhoids, muscle pains, and some chronic diseases.

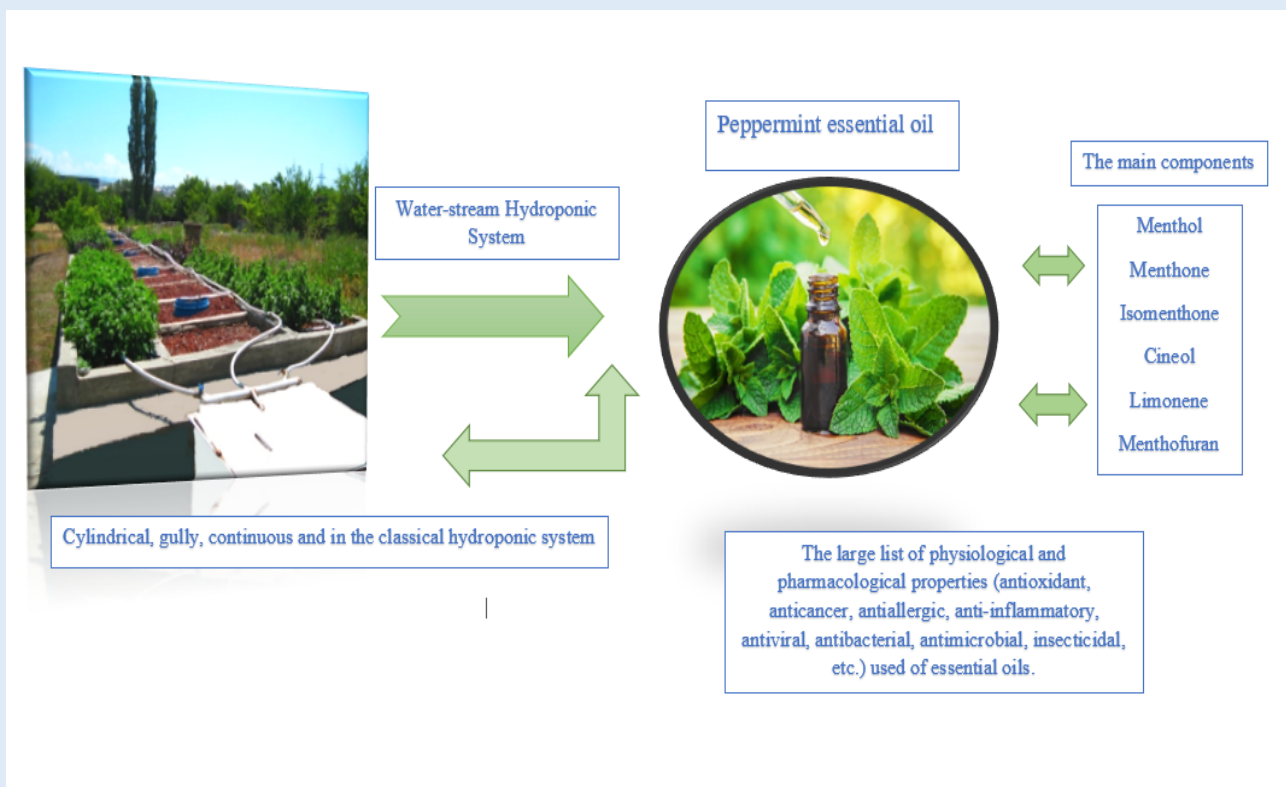
**Context and purpose of this study:** The work aimed to study the content of the main components from the quality indicators of the essential oil of peppermint grown in different water-stream and classical hydroponic systems for the first time in Armenia.

**Results:** From the analyses, it was revealed that the strengthening of the essential oil of peppermint grown in different systems of the water-stream hydroponics (cylindrical, gully, continuous) and classical hydroponics was observed in August. At the same time, high-yield plants of cylindrical and classical hydroponic systems exceeded other variants by 1.3-1.5 times with the essential oil output. In hydroponic systems, the specificities of the physical-chemical indices of peppermint essential oil were also studied. In August, the refractive indicator of essential oil increased to some extent

in all variants, except for continuous hydroponics. The lack of increase in continuous hydroponics can likely be explained by the change in the ratio of essential oil to different compounds. At the end of vegetation, in September, some increase of essential oils' specific gravity was observed in the gully (2.1-3.5%) and continuous (4.5-4.7%) modules, while in cylindrical and classical hydroponics the change was minor. During vegetation, the essential oil of plant leaves was subject to not only quantitative but also qualitative changes. The cylindrical system exceeded other water-stream hydroponics variants with the main essential oil menthol component by 1.1-1.4 times and classical hydroponics by 1.1 times. The Gully system exceeded other water-stream systems with the content of D-Limonene and Menthyl acetate 3.6-9.7 and 1.3-3.0 times, respectively. And classical hydroponics 1.4 and 1.7 times, respectively. In the continuous system, the content of Isomentone, Pulegone, and Mint furanone was 1.3-2.0, 3.3-3.5, and 5.7-6.2 times higher, respectively, compared with the other water-stream hydroponic systems, and 1.6, 2.6, and 1.7 times, compared with classical hydroponics. Classical hydroponics exceeded all variants of water-stream hydroponics in the content of Piperidone by 3.3-4.1 times.

**Conclusion:** Although the strengthening of essential oil biosynthesis was observed in all hydroponic variants in August, the cylindrical hydroponic system excelled in the content of essential oils, the most important qualitative indicator of menthol. High output of essential oil in plant raw material was observed in classical hydroponics and cylindrical system of water-stream hydroponics, which provides optimal regimen for the normal growth and development of plants.

**Keywords:** cylindrical, medicinal plant, bioactive compounds, menthol



## INTRODUCTION

The cultivation of essential oil plants has a relatively small share of agricultural production. However, they are very valuable: they are widely used mainly in the pharmaceutical industry, perfumery and cosmetics, alcoholic beverages, and food industry. Almost all essential oil plants are remedies and are used in traditional medicine and the production of various medicinal preparations [1,2]. Among such crops is a valuable essential oil herb peppermint (*Mentha piperita* L.), belonging to the Lamiaceae family. Peppermint essential oil is a yellowish, viscous liquid. The chemical composition is quite compound, it contains about 350 terpene compounds [3]. The main components that determine the quality of essential oil are menthol, menthone, isomenthone, cineol, limonene, and menthofuran [4]. The above-ground part of peppermint contains essential oil, 4-6% in flowers, 2.4-2.8% in leaves, and up to 0.3% in stems. For therapeutic purposes, the leaves of the plant and the essential oil obtained from them are used in the form of tea, tinctures, extracts, and creams. At the same time, it has a great effect on the heart and circulatory system, calms the heartbeat, helps lower blood pressure, and is used for pharyngitis, laryngitis, colds, and due to sedative properties - for insomnia and stress. The oil vapor is used in cases of suffocation in the form of inhalation. Menthol ointment is used for insect bites, eczema, hemorrhoids, and muscle pain [5]. The extensive list of physiological and pharmacological properties of essential oils, such as antioxidant, anticancer, anti-allergic, anti-inflammatory, antiviral, antibacterial, antimicrobial, insecticidal, and more, is essential not only for the plants themselves but also for humans, enabling their wide range of applications and uses [6]. It should be noted that climatic factors have a significant effect on the synthesis of the essential oil of crops.

Quantitative and qualitative changes in essential oil occur both during vegetation and during the day depending on air temperature, relative humidity, light intensity, and other factors [7,8].

Hydroponic (soilless) production of medicinal and essential oil crops is a new approach to mass production and has many advantages over soil culture [9]. Hydroponics is a phytotechnology for the soilless cultivation of plants, which is carried out under controlled growing conditions, in an artificially created environment and ensures the most efficient use of human labor [10].

As a result of many years of research, a new "water-stream hydroponic system" has been developed at the Institute of Hydroponics Problems, National Academy of Sciences, Yerevan, Armenia in its three varieties (cylindrical, gully, and continuous) [11], which is based on the use of a polymer membrane and regular and continuous irreversible jet injection of a nutrient solution directly into the root layer of the plant. The use of this system excludes environmental pollution, minimizing the risk of disease outbreaks and the spread of pests. For the first time in Armenia, the content of the main components of the quality indicators of the essential oil of peppermint grown in different water-stream and classical hydroponic systems was determined.

## MATERIALS AND METHODS

**Plant material and composition:** The experiments were carried out in the Ararat Valley, the climatic conditions of which are favorable for the rapid growth and development of essential oil plants and the accumulation of high-quality essential oil [12]. In the scientific experiments, the cuttings of peppermint grown hydroponically by us were used as planting material. These materials were planted with a density of

8 plants/m<sup>2</sup> at the beginning of May in the water-stream hydroponic system with a feeding area of 8 m<sup>2</sup> (cylindrical, gully, continuous) and in the classical hydroponic system (CHS) (hydroponic experimental station) in two concrete vegetation experimental fields with an area of 5 m<sup>2</sup>. Planting density was chosen based on the results obtained from the scientific experiments of previous years, where the 6-8 plants/m<sup>2</sup> planting density provided favorable conditions for normal growth and development, as well as high efficiency of several crops [13]. In water-stream hydroponics, porous volcanic slag of natural origin with a diameter of 3-15 mm, widely spread in our country, was used as a substrate. It provides free inflow and outflow of nutrients, as well as good ventilation of the root system of plants, capable of absorbing and retaining water and nutrient salts. Plant nutrition in hydroponics was carried out with the nutrient solution proposed by G.S. Davtyan, where nutrient limits are selected to fully ensure high plant efficiency. This solution has been used with great success in outdoor hydroponics for many years [14, 15]. During the vegetation period, the content of the most important biochemical indicator the essential oil was determined in the raw material of the peppermint plant.

#### **Quantitative changes of essential oil during the day:**

The content of essential oil was determined in 3 vegetation periods. The comparative studies with the best version of water-stream hydroponics, the cylindrical system, and CHS were performed in September to reveal the quantitative changes in the essential oil of peppermint during the day. The relative humidity of the air was measured by a Psychrometer and the illumination - with the help of a light meter instrument.

The essential oil content is determined by steam distillation followed by volume measurement. For

extraction of essential oil by distillation 50g of dry leaf sample was added in 300ml of water in a round-bottomed flask. Essential oil content in absolute dry matter is given as a mass-volume percentage [16].

**Qualitative analysis of essential oil:** For the comparative analysis of the qualitative indicators of essential oils, the peppermint harvested during the 3rd harvest was selected from two different growth systems: water-stream and CH (presumably another growth system). The choice of the 3rd harvest was based on the observation that these plants had the highest accumulation of both plant raw material and essential oil among all the harvests. Qualitative analysis of essential oil was performed using a modern Gas Chromatography – mass spectrography of EM 640S brand of Bruker Daltonik firm using the "HP-5MS" method. The following temperature program was used. start temperature was 40 °C (1 min), which was increased gradually to 280 °C at the rate of 7 °C/min. The ion source temperature was 200 °C and the interface temperature was 200 °C. The carrier gas was He. The injector flow was 30 mL/min, and the injection was split less. The identity of essential oil components was determined using both NIST-MS computer library data and reference samples.

**Physical properties of peppermint essential oil:** During three months of vegetation (July, August, and September), some physical parameters (specific gravity, refractive index) of peppermint essential oil were studied in different hydroponic systems. The quality of essential oil is characterized first by organoleptic (color, smell, taste) indicators, then by physical (density, specific gravity, rotation angle, refractive index, solubility in alcohol) and chemical constants. The concentration of the essential oil may vary depending on the stage of plant development, the conditions, and

the duration of storage of the essential oil. Deviation from the established density limits affects the quality of the essential oil. Both high density and high refractive index indicate an increase in the number of oxygenated compounds in the essential oil [17].

**Determination of the specific gravity and refractive index of oil by pycnometer:** Specific gravity or relative density of essential oil is the ratio of the mass of a given volume of oil at 20°C to the mass of the same volume of distilled water at 20°C. It can be estimated by a pycnometer. Briefly, the pycnometer is washed sequentially with a chromium mixture, alcohol, and distilled water, dried in an oven, and weighed on an analytical balance. Then distilled water is slightly added to the pycnometer above the line and placed in a bath at a temperature of 20° for 30 minutes until the water level stopped changing. The pycnometer is removed from the thermostat, excess water is taken with filter paper. The water level in the pycnometer is set at the top of the meniscus. The pycnometer is carefully wiped outside and weighed on an analytical balance [17]: The mass of water in the pycnometer ( $m$ ) is calculated by the formula:

$$m = m_2 - m_1$$

where  $m_1$  is the mass of pycnometer (g),  $m_2$  is the mass of pycnometer with water(g).

The relative density ( $d_{20}^{20}$ ) is calculated by the formula:

$$d_{20}^{20} = \frac{m_3 - m_1}{m}$$

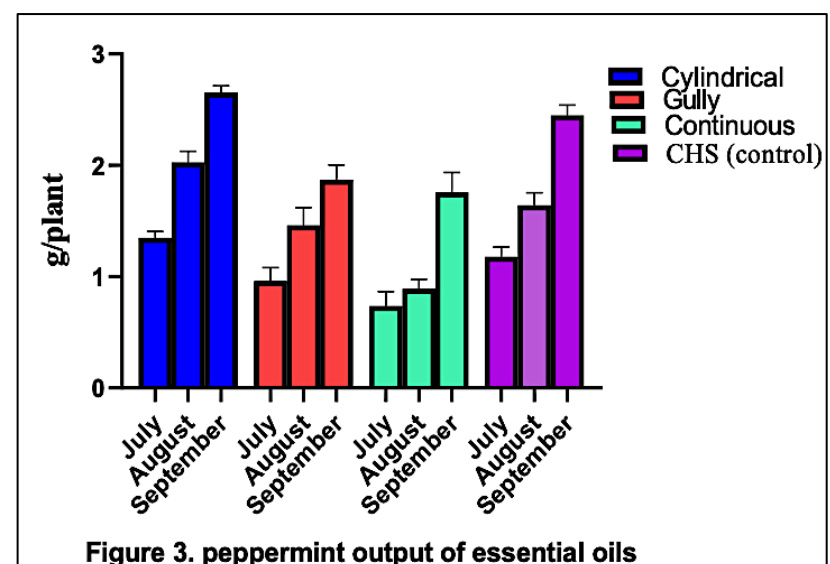
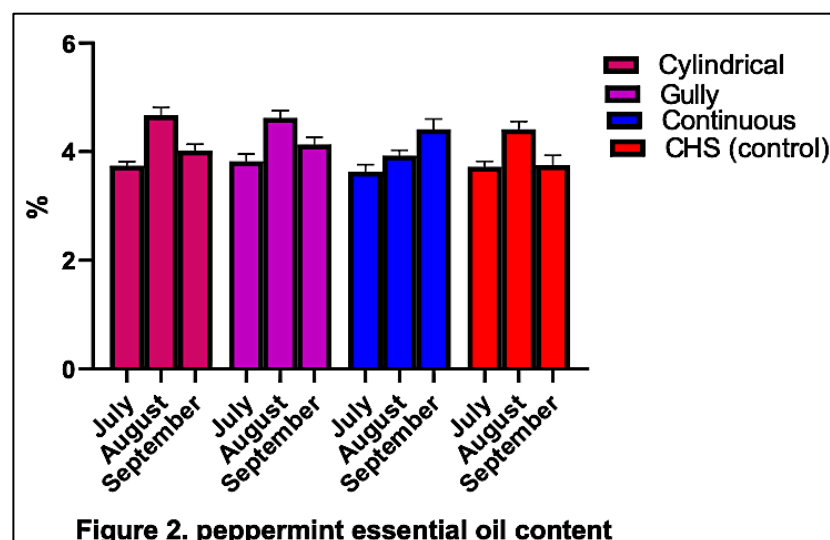
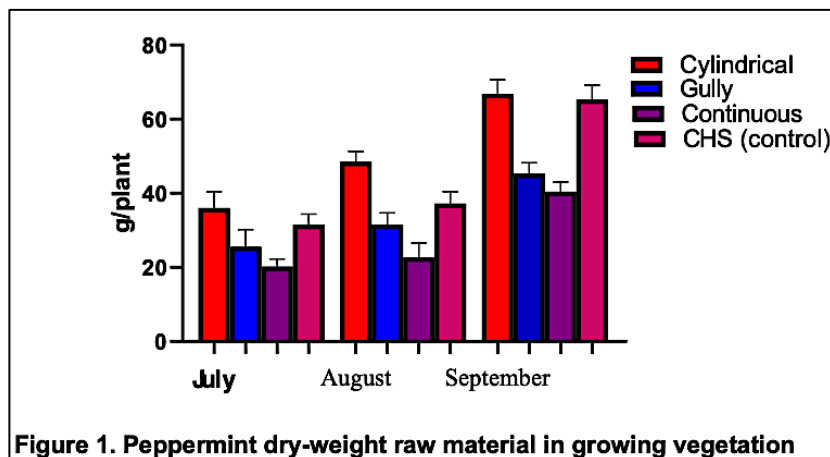
where  $m_3$  is the mass of the pycnometer with oil (g).

The refractive index of the essential oil was determined using a Carl Zeiss Jena refractometer, which requires 2 ml of sample. The refractive index is calculated with four digits of accuracy [18].

**Statistical analysis:** All analyses were carried out in triplicates and the data were expressed as means  $\pm$  standard deviations (SD). Field experiments were replicated 4-8 times and MS Excel 2013. The received results were analyzed using the statistical program GraphPad Prism 6. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (t-test).

## RESULTS AND DISCUSSION:

**Growth specificities and output yield:** During the growing season, 3 harvests were done: in July, August, and September. In all investigated variants, at the beginning of vegetation - in July, around 25% of total plant raw material was harvested, in August - 27-31%, and the largest amount of medicinal material (44-49%) was obtained in September. At the same time, the cylindrical system exceeded the other hydroponic systems in a dry weight of the raw material in July by 1.1-1.8-fold, in August by 1.2-2.0-fold, and in September it exceeded only the gully and continuous systems by 1.5-1.7-fold (Fig. 1). From the analysis, it was found that compared with the other months in August 1.1-1.3 fold intensification of the essential oil biosynthesis was observed in peppermint grown in different systems of water-stream hydroponics and classic hydroponics. The exception is for the continuous hydroponics, where a 1.1-1.2-fold higher accumulation of essential oil was observed in September, compared to the other months (Fig. 2). At the same time, compared with the other months in September 1.3-2.4-fold higher output of essential oil was recorded in all versions. Due to the high yield of the plants, the cylindrical system exceeded the other variants by 1.1-1.9 times in July, 1.3-2.4 times in August, and 1.1-1.5 times in September (Fig. 3).



**Dynamics of essential oil accumulation in leaves during**

**the day:** The study, done in Norway, showed that peppermint oil yield increased from early to full bloom and late bloom because of continuous biomass production and leaf growth. The flavor-impact compounds, menthol, and menthone, reached their optimum at full bloom (43–54 and 12–30%, respectively) [19].

In our study under the conditions of cylindrical

hydroponics, during the day a 20% increase in the biosynthesis of the essential oil of peppermint was observed at 16:00 when the air temperature was high, and the relative humidity of the air was low. In the CHS, there was no obvious change in the essential oil content of the leaves, but it was about 7% higher between 13:00 and 16:00 due to relatively high air temperature, humidity, and light intensity (Table 1).

**Table 1:** Dynamics of essential oil accumulation in fresh leaves of peppermint during the day depending on the processing conditions.

Variant	Sampling time	Air temperature, °C	Air conditioning humidity, %	Lighting, lux	Essential oil content, %
cylindrical	10	18	55	28000	0.95
	13	27	38	64000	0.93
	16	35	22	53000	1.10
	19	24	38	10000	0.90
DHS (control)	10	18	55	28000	1.11
	13	27	38	64000	1.18
	16	35	22	53000	1.18
	19	24	38	10000	1.10

**Characteristics of physical Indicators of essential oil:**

According to literature data, among the physical parameters of mint essential oil, the specific gravity varies between 0.9215 and the refractive index between 1.462 [20]. In our study (Table 2) during vegetation under the conditions of different hydroponic systems, the specific gravity of peppermint essential oil varies from 0.9114 to 0.9570 and the refractive index from 1.4609 to 1.4689. In August in all versions, the refractive

index of the essential oil increased to some extent, except for the continuous one, which is most likely explained by the change in the ratio between the different components of the essential oil. Meanwhile, at the end of vegetation, in September, a certain increase in the specific weight of essential oil was observed in the gully (2.1-3.5%) and continuous (4.5-4.7%) modules and this change was insignificant in cylindrical and classic hydroponics.

**Table 2:** Physicochemical parameters of peppermint essential oil during vegetation

Variant	Harvest time	Essential oil content, %	$d_{20}^{20}$ (Specific gravity)	$n_D^{20}$ (Refractive index)
Cylindrical	July	3.74	0,9310	1,4650
	August	4.67	0,9346	1,4689
	September	4.02	0,9310	1,4649
Gully	July	3.82	0,9114	1,4620
	August	4.62	0,9246	1,4671
	September	4.13	0,9440	1,4655
Continuous	July	3.63	0,9158	1,4659
	August	3.93	0,9137	1,4651
	September	4.41	0,9570	1,4678
CHS (control)	July	3.72	0,9307	1,4623
	August	4.41	0,9267	1,4652
	September	3.75	0,9170	1,4615

#### Comparative characteristics of quality indicators of essential oil under different growing conditions:

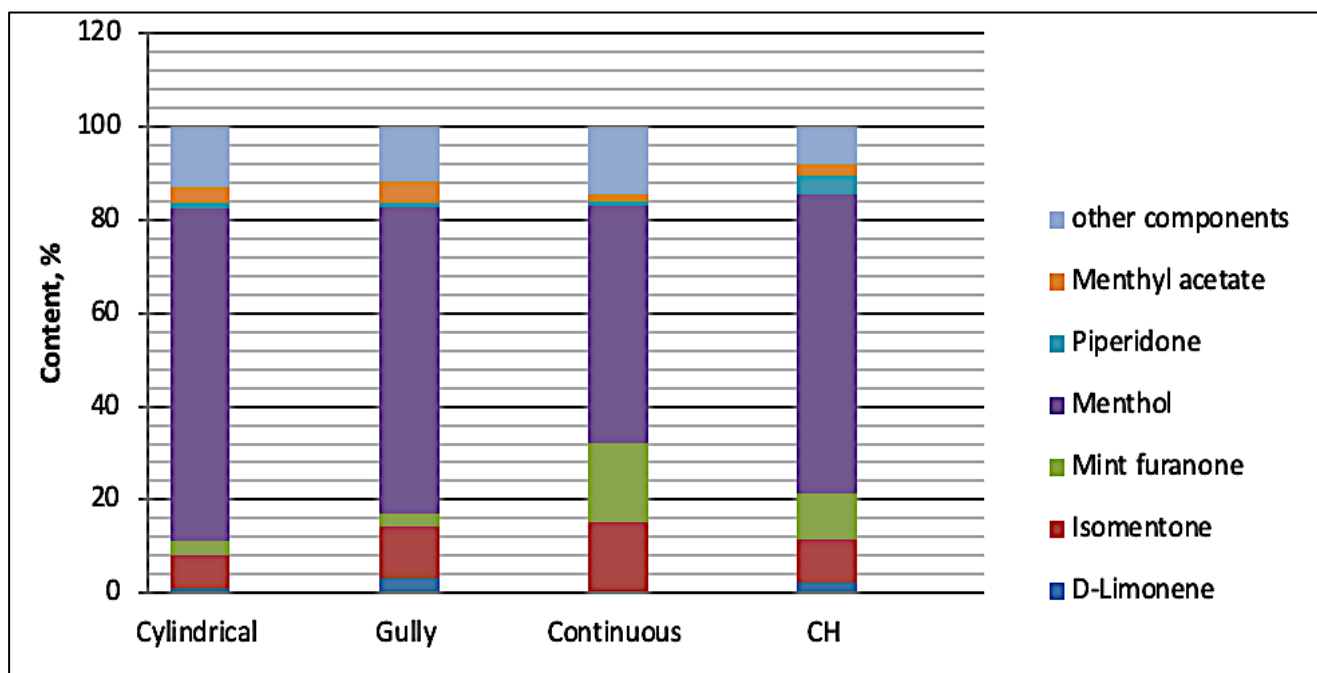
According to literature data, 26 components of peppermint essential oil were identified using gas chromatography, which increases the possibility of compound separation [21]. In our experiments, qualitative analyzes of essential oil were performed with the help of GC-MS. More than 20 components have been identified, but this work presents the main 7: menthol, isomenthone, pulegone, D-limonene, piperidone, menthyl acetate, and mint furanone. The separation of these chemical components is very important for pharmacological research.

During vegetation, the essential oil of plant leaves is subject to quantitative and qualitative changes. The analysis found that the cylindrical system outperformed

other water jet options by 1.1-1.4 times and classical hydroponics by 1.1 times with a menthol content, the essential oil's main component (Fig. 4).

The gully system exceeded other water-stream hydroponic systems by 1.3-3.0 times, and the CH by 1.4 and 1.7 times, respectively, in D-Limonene 3.6-9.7 and Menthyl acetate content. It was recorded 1.3-2.0 times higher content of Isomentone, 3.3-3.5 times higher content of Pulegone, and 5.7-6.2 times higher content of Mint furanone in continuous hydroponics compared to the other water-stream system hydroponic systems. CHS was inferior to the continuous hydroponic system in the content of the same chemicals 1.6; 2.6 and 1.7 times, respectively. CH system outperformed all water-stream options in a high content of piperidone by 3.3-4.1 times.





**Figure 4.** The main components of essential oil of peppermint in water-stream and CH conditions

## CONCLUSION

The maximum accumulation of peppermint dry raw material was observed in the water-stream hydroponics cylindrical system compared to other hydroponic systems. Although in all hydroponic variants except the widespread, essential oil biosynthesis intensification was observed in August, due to the high yield of plants, CHS and the cylindrical systems of water-stream hydroponics excelled with an increased essential oil output at all yields.

During the day, in cylindrical hydroponics, peppermint essential oil biosynthesis enhancement was observed at 16:00 when the air temperature was high, and the air relative humidity was low. In the CHS, it was about 7% higher between 13:00 and 16:00 due to relatively high air temperature, humidity, and light intensity. The cylindrical system excelled with the high content of the most important qualitative indicator of

essential oil menthol, and the gully system with a high content of D-Limonene, and Menthyl acetate. The continuous system stood out with a high content of Isomentone, Pulegone, and Mint furanone, and CHS with a high Piperidone content. The hydroponics cylindrical system provided an optimal regime for normal plant growth and development.

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**Abbreviations:** CHS – Classical hydroponic system; standard deviations (SD)

**Authors' Contribution:** All authors contributed to this study and wrote this paper.

**Competing Interests:** The authors declare no conflict of interest.

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