

## Study regarding the influence of fertilization on some physiological index and biochemical composition of peppermint oils (*Mentha piperita L.*)

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### Abstract

Essential oils and other aromatic compounds from peppermint (*Mentha piperita L.*) are widely used for their medicinal and aromatherapeutic properties in health care, traditional medicines, in preparing pharmaceuticals and cosmetics or for culinary purposes. The present study evaluated the variation of certain physiological indices (rate of photorespiration – E; stem dry matter – SDM; leaf dry matter content - LDM; chlorophyll molecules Chl; rate of photosynthesis – PSN) and quality of essential oil from peppermint cultivated under controlled environmental conditions based on Hoagland nutrient solution with addition of P and K. It was recorded the variation of physiological indexes, in relation with Hoagland solution changes. Accordingly, for rate of photorespiration was found a medium positive correlation with potassium ( $r = 0.672$ ), and a strong relationship with chlorophyll ( $r = -0.841$ ). Stem dry matter and phosphorus were strong negative correlated ( $r = -0.898$ ). The leaf dry matter was very strong negative ( $r = -0.999$ ) influenced by P, and weak correlated with K ( $r = 0.536$ ). Chlorophyll was very strong and negative influenced by K ( $r = -0.966$ ). The rate of photosynthesis and stem dry matter were weak correlated ( $r = -0.441$ ). PSN and chlorophyll were very strong negative correlated ( $r = -0.966$ ), and contrary the rate of photorespiration was strongly and positive correlated with PSN ( $r = 0.952$ ). K had a strong and positive influence on PSN ( $r = 0.866$ ). Amid these interdependencies of the nutrition conditions and physiological indices were identified high levels of special compounds in the peppermint essential oil: menthone 17%, menthol 33%, izometil acetate 5%, limonene 2%, pulegone 1.8% and from sesquiterpenes was determined  $\beta$ -caryophyllene with a 1.2% concentration.

**Keywords:** compounds, essential oil, herb, Hoagland solution, peppermint

### 1. Introduction

Peppermint (*Mentha piperita L.*) is a species of special importance in the pharmaceutical, healthcare, cosmetic, food industry, etc., for essential oils and specific compounds involved in various treatments, alternative and complementary therapies (ALI B. & al., 2015) [1]. The compounds of mint (herbs) and essential oil have been identified with antioxidant and antibacterial activity (SINGH R. & al., 2015) [36]. Peppermint has been cultivated and studied in relation to different types of nutritive media, soil, biosolids (SCAVRONI J. & al. 2005) [34], nutrient solutions for hydroponic systems or nutrient layer (GARLET T.M.B. & al. 2013) [13].

Peppermint relationship with nutrients was studied concerning the herb and oil production. Jeliaskova & al. (1999) [15] evaluated the influence of NPK in different doses on the production of peppermint oil and recorded increases between 23-86% compared to control variant.

The influence of nutrition or fertilizer has been studied both in medicinal and aromatic plants in relation to plant growth, biomass (herb), essential oils quantity and their content in nutrients (KHALID K.A. 2012) [34].

Brown & al. (2003) [34] studying the nutrients management influence in the peppermint production, found out that P and K are accumulated in peppermint plants during the growing season with a high correlation ( $R^2 = 0.838$ ) for P and ( $R^2 = 0.894$ ) for K. Tabatabaie and Nazari (2007) [34] studied the influence of nutrients concentration and salinity on some physiological indices and essential oil production in peppermint and lemon verbena, and found out that increased levels of electrical conductivity (EC) and NaCl have reduced the amount of fresh biomass in both studied species. Influence of Fe concentration in relation with the essential oil production in Japanese peppermint, was studied by Misra and Sharma (1991) [25] which found an optimum concentration of  $5.6 \mu\text{g}\cdot\text{l}^{-1}$  Fe. In Japanese mint, has been also studied the influence of water stress and was observed significant reduction in gas exchange, the assimilation area, fresh and dry matter content, chlorophyll, carotenoids, micronutrients and essential oil production. Pande & al. (2007) [30] studied the individual and combined influence of Fe and Zn on herbs production and essential oil in peppermint, highlighting the better influence of iron compared to zinc in individual application, and more favourable when both trace elements are applied at once in concentration of  $5 \text{ mg Zn kg}^{-1}$  and  $10 \text{ mg Fe kg}^{-1}$  respectively.

Characterization and purification of peppermint oils were discussed in different studies, due to the importance for the cosmetics, pharmaceutical, healthcare industry, etc. (KAVRAYAN D. and AYDEMIR T. 2001) [18]. Some studies have evaluated the alteration of the peppermint essential oils chemical composition under the influence of amendments and organic waste (KUMAR K.V. & PATRA D.D. 2012) [20]. This study aimed the assessing of potassium and phosphorus different amounts on some physiological indices and quality of peppermint oil cultivated under controlled environmental conditions based on Hoagland nutrient solution.

## 2. Materials and Methods

Plant material consists of mint genotype (*Mentha piperita L.*). Plants were grown in pots with perlite and peat mixture (ratio 1: 3) under controlled environmental conditions. Nutrients were provided by Hoagland nutrient solution in three versions: V0 - control, basic Hoagland solution; V1 - Hoagland solution with additional K and V2 - Hoagland solution with the addition of P, according to Table 1.

*Physiological indices.* The intensity of photorespiration (E) was determined by successive weighing of plant organs (leaves) at specified time intervals. The dry matter accumulated in the stems (SDM) and leaves (LDM) was determined using Kern MLS 50 thermo-balance, by the difference between the plant moisture and fresh weight.

Chlorophyll pigments (Chl) were determined quantitatively by leaf chlorophyll meter readings with Konica Minolta Chlorophyll Meter. The rate of photosynthesis (PSN) was determined by gas exchange method that involves the measuring of  $\text{CO}_2$  concentration with an infrared analyzer ( $\text{CO}_2$  ANALYSIS PACKAGE Qubit Systems - Canada).

Photosynthesis rate was determined by measuring the initial and final concentration of  $\text{CO}_2$  and reported to the unit time and per unit leaf area determined on the model proposed by

Sala & al. (2015)[33].

*Qualitative indices for volatile oils.* For determining the composition of peppermint essential oil samples were diluted with hexane (GC purity, Sigma) and to determine Kovats indices, related to volatile compounds was used a linear alkali solution of  $i\text{-C}_8\text{-C}_{20}$  (Fluka Chemie AG).

Table 1. Hoagland solution in experimental variants

Components	Supply solutions	ml Supply solutions /1L		
		V0	V1	V2
2M $\text{KN}^3$	202g/L	2.5	5	2.5
2M $\text{Ca}(\text{NO}^3)^2 \times 4\text{H}^2\text{O}$	236g/0.5L	2.5	2.5	2.5
Fier	15 g/L	1.5	1.5	1.5
2M $\text{MgSO}^4 \times 7\text{H}^2\text{O}$	493 g/L	1	1	1
1M $\text{NH}^4\text{NO}^3$	80 g/L	1	1	1
Trace elements				
$\text{H}^3\text{BO}^3$	2.86 g/L	0.5	0.5	0.5
$\text{MnCl}^2 \times 4\text{H}^2\text{O}$	1.81 g/L	0.5	0.5	0.5
$\text{ZnSO}^4 \times 7\text{H}^2\text{O}$	0.22 g/L	0.5	0.5	0.5
$\text{CuSO}^4$	0.051 g/L	0.5	0.5	0.5
$\text{H}^3\text{MoO}^4 \times \text{H}^2\text{O}$	0.09 g/L	0.5	0.5	0.5
1M $\text{KH}^2\text{PO}^4$	136 g/L	0.5	0.5	1

Analysis of peppermint essential oils were carried out by gas chromatographic method coupled with mass spectrometry detection. The equipment used was the GC Hewlett Packard HP 6890 Series with a mass spectrometer Hewlett Packard 5973 Mass Selective Detector. GC analysis conditions: injection volume of 2  $\mu\text{l}$ ; He carrier gas; injector temperature 280°C; HP-5 MS column, length 30 m, inner diameter 0.25 mm, film thickness of 0.25  $\mu\text{m}$ ; detector temperature 280°C; temperature program 50°C to 250°C at a rate of 6°C/min. For MS detector was used an energy of EI 70 eV, source temperature of 150°C, scan rate of 1  $\text{s}^{-1}$  for mass spectrometry, 50-300 amu/s scan range; spectra obtained were compared with a NIST/EPA/NIH Mass Spectral Library 2.0 database (2002). Hewlett Packard Enhanced Data Analysis software was used for data processing of gas chromatography and water spectrometry and Hewlett Packard Enhanced ChemStation software G1701BA ver. B.01.00 / 1998, was used for data acquisition.

*Determination of Kovats (KI) retention indices.* For the identification of compounds was performed GC-MS analysis, together with the identification based on MS spectra and also with Kovats retention indices (KI); in this way were identified important mono- and sesquiterpenoids. Type of columns used influences the value of the indices, while the temperature schedule, gas flow, or injector and detector temperature does not influence this parameter.

Correlation of retention time obtained from the GC analysis with conventional Kovats indices (Table 2) resulted in a standard polynomial curve used for determining the amount of the compounds studied by interpreting the corresponding retention time (resulting from GC analysis) on the KI vs RT chart. Retention time (RT) was determined after confirming (based on MS spectra) alkaline linear structures separated by the GC analysis (Figure 1).

*Experimental data processing.* Analysis of experimental data and general processing was performed by statistical module EXCEL application, series Office 2007. Correlation analysis

was performed with PAST software (HAMMER Q. & al. 2001) [16], and some graphical representations were realised by Statistics v. 10.

Tab.2. Kovats retention indices values (KI) and retention times (RT) for linear alkali C<sub>8</sub>-C<sub>20</sub>

No.	linear alkali	KI	RT (min)
1	octane, C <sub>8</sub>	800	3.07
2	nonane, C <sub>9</sub>	900	4.37
3	decane, C <sub>10</sub>	1000	6.23
4	undecane, C <sub>11</sub>	1100	8.44
5	dodecane, C <sub>12</sub>	1200	10.76
6	tridecane, C <sub>13</sub>	1300	13.06
7	tetradecane, C <sub>14</sub>	1400	15.28
8	pentadecane, C <sub>15</sub>	1500	17.39
9	hexadecane, C <sub>16</sub>	1600	19.39
10	heptadecane, C <sub>17</sub>	1700	21.3
11	octadecane, C <sub>18</sub>	1800	23.11
12	nonadecane, C <sub>19</sub>	1900	24.83
13	eicosane, C <sub>20</sub>	2000	26.48

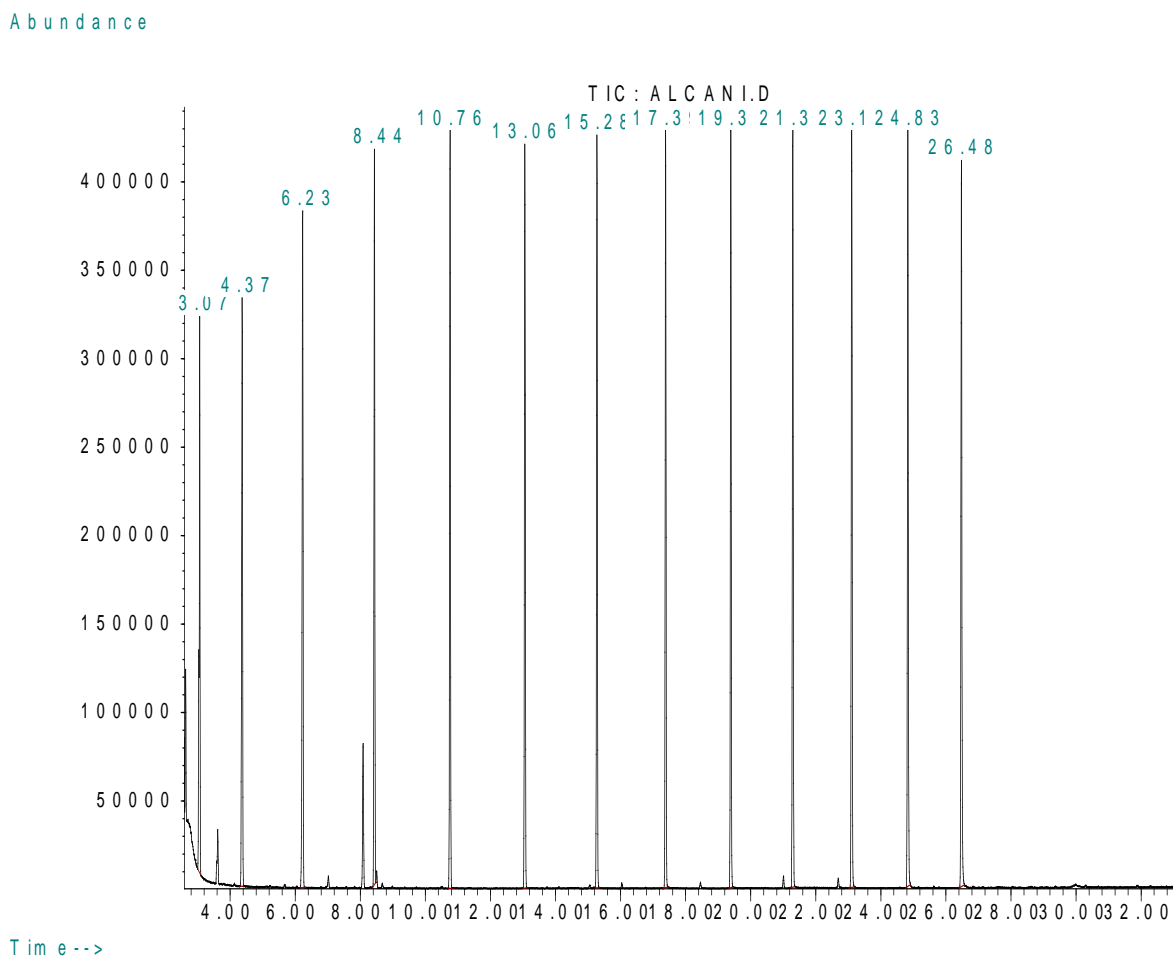


Figure. 1. Gas chromatogram of the standard mixture of C<sub>8</sub>-C<sub>20</sub> linear alkali, used to determine the Kovats retention indices

### 3. RESULTS AND DISCUSSIONS

In order to assess the performance of the plant material in accordance with differentiated conditions provided by standard Hoagland solution, and the variations created by the addition of K and P were determined indices and physiological processes.

It has been found that a higher percentage of dry matter was added to samples derived from stem as compared with those derived from the leaf. It was also observed that for the leaf fragments there were no significant differences between the experimental variants studied, while in the case of fragments from stem, a higher percentage of the dry matter was registered in variant V<sub>2</sub> with the addition of P.

Between the dry matter from the stems and phosphorus was registered a strong negative correlation ( $r = -0.898$ ) while between the dry matter from leaf was established a very strong negative correlation with P ( $r = -0.999$ ) and a weak correlation with K ( $r = 0.536$ ). The distribution of the values is graphical represented in Figure 2.

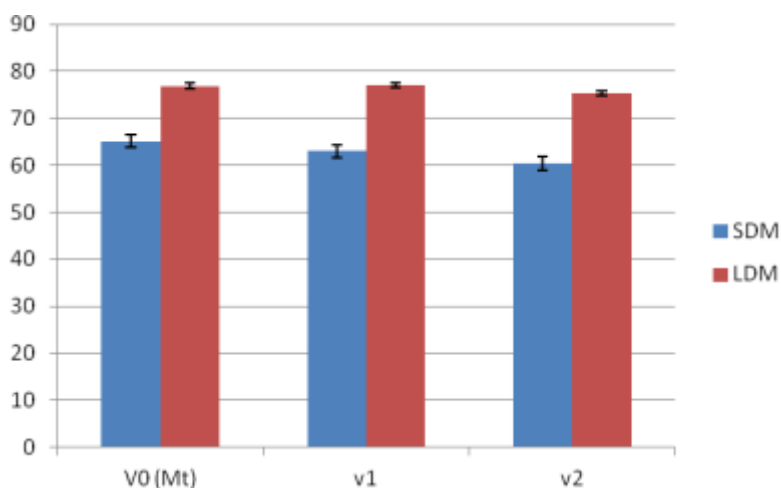


Figure 2. Percentage of dry matter - peppermint

Perspiration as physiological process was expressed by the amount of water removed reported on the weight of fresh plant per time unit (perspiration rate). In the study were found differences between the experimental variants; a higher rate of perspiration was recorded in the variant V<sub>2</sub> (with the addition of phosphorus) compared with the control, which registered a lower value (Figure 3).

For the perspiration rate was found a positive correlation of medium intensity with the addition of potassium ( $r = 0.672$ ), and a strong negative correlation with the chlorophyll ( $r = -0.841$ ). This is explained because of the phosphorus contribution in stimulating metabolic processes of plants (SALA F. 2011) [32].

As concerns the total chlorophyll content in units SPAD, has been registered a very strong negative correlation with K ( $r = -0.966$ ); a higher amount of chlorophyll was recorded for version control (34.6 SPAD units), followed by variant V<sub>2</sub>, while a lower value was recorded in variant V<sub>1</sub> (30.5 ADP), Figure 3.

The intensity of photosynthesis evaluated by the gas exchange method (CO<sub>2</sub> assimilation and O<sub>2</sub> release) had differentiated values in all three variants; a higher intensity was recorded for variant V<sub>1</sub> (with the phosphorus addition), followed by variant V<sub>2</sub> (with added K)

compared with the control, Figure 4.

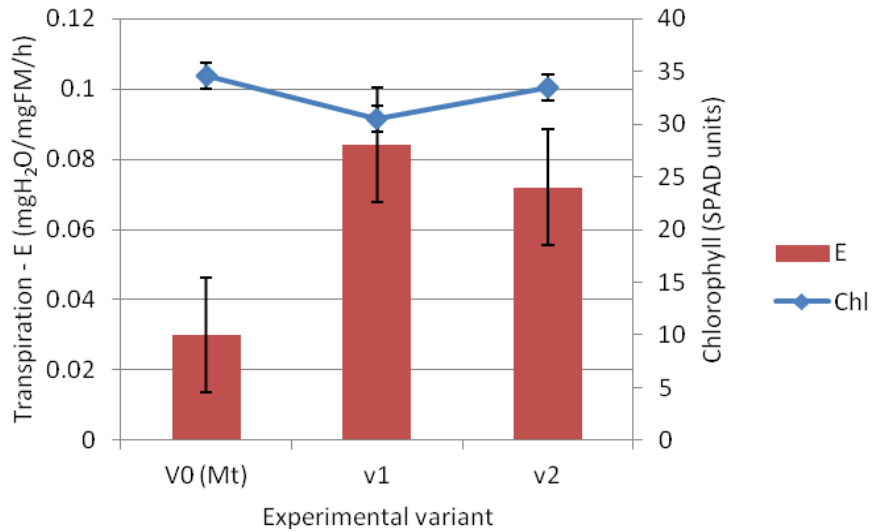


Figure 3. Content of total chlorophyll (SPAD)

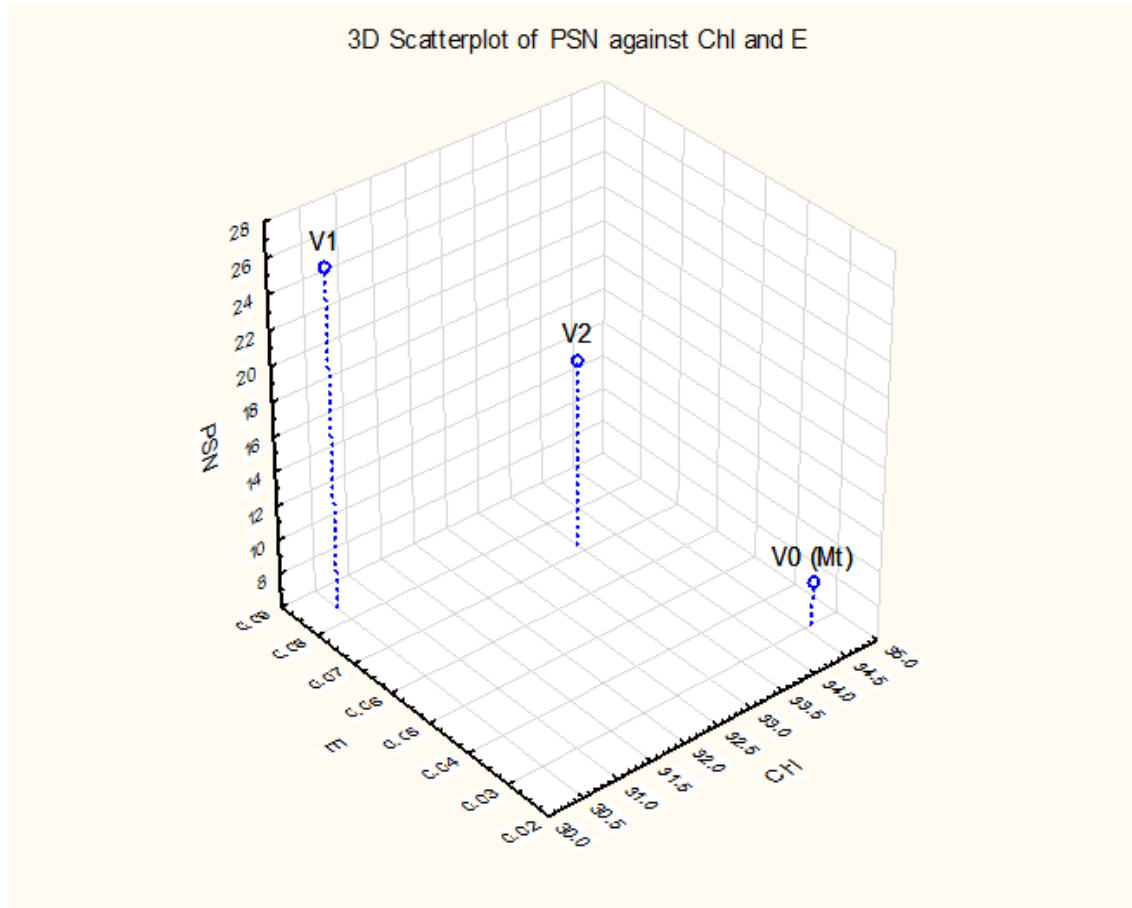


Figure 4. 3D representation of the photosynthesis intensity corresponding to the chlorophyll and respiration, after modification caused by the P and K changes in Hoagland solution

The rate of photosynthesis was weak correlated with stems dry matter ( $r = -0.441$ ), and very strong negative with chlorophyll ( $r = -0.966$ ); in the same time was strong positive

correlated with K ( $r = 0.866$ ) and very strong correlated with rate of photorespiration ( $r = 0.952$ ).

Statistical analysis of the correlation between the modified elements in the Hoagland (P and K) solution and physiological indices (SDM - stem dry matter; LDM - leaves dry matter; Chl - chlorophyll content; E - perspiration; PSN – rate of photosynthesis) facilitated the highlighting of differentiated levels of correlation between variants.

High correlations were identified between K and leaf dry matter content, and chlorophyll content respectively; high correlations between P and stems dry matter, the stems and leaf dry matter, the perspiration and chlorophyll content correlations are shown in Table 3. Were also identified other less important correlations between physiological indices and determining factors (P, K).

Table 3. Correlations among variation factors in Hoagland solution (P and K) and physiological indices

0	K	P	SDM	LDM	Chl	E	PSN
K	0	0.66667	0.95733	0.63979	0.1669	0.53093	0.33333
P	-0.500	0	0.29066	0.026878	0.83357	0.8024	1
SDM	0.067	-0.898	0	0.31754	0.87577	0.51175	0.70934
LDM	0.536	-0.999	0.878	0	0.80669	0.82928	0.97312
Chl	-0.966	0.258	0.194	-0.299	0	0.36403	0.16643
E	0.672	0.305	-0.694	-0.265	-0.841	0	0.1976
PSN	0.866	0.000	-0.441	0.042	-0.966	0.952	0

Gas-chromatographic analysis of peppermint essential oil (Figure 6) coupled with mass spectrometry has enabled the separation of more than 50 volatile compounds, of which the most important were identified based on their mass spectra and Kovats indices. Thus, were identified in high concentrations menthone (17%), menthol (33%) and limonene (2%), pulegone (1.8%) and izomentil acetate (5%). Among sesquiterpene was determined  $\beta$ -caryophyllene at a concentration of 1.2% (table 4).

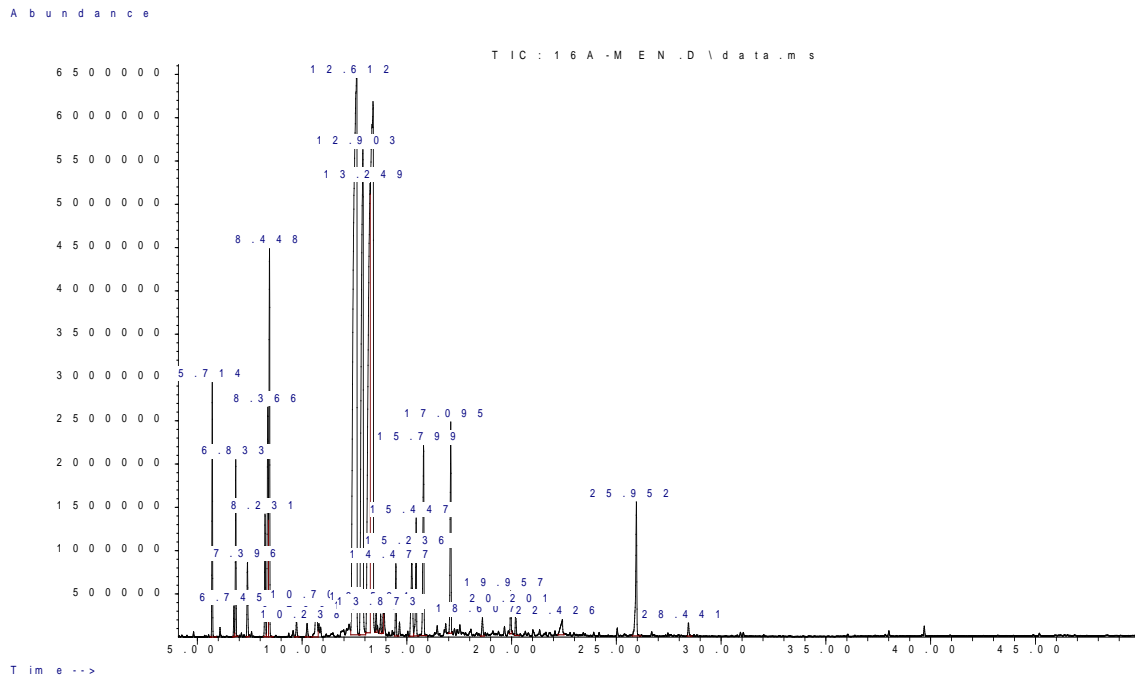


Figure. 6. Gas chromatogram of GC-MS analysis of peppermint essential oil samples

Table 4. GC-MS analysis results (MS identification and relative percentage concentrations) for the peppermint essential oil

Nr.	RT (min)	Kovats Indices	MS identification	Area (peppermint) (%)
1	5.702	936	$\alpha$ -Pinene	0.727
2	6.739	975	$\beta$ -Phellandrene	0.189
3	6.827	978	$\beta$ -Pinene	0.576
4	7.227	993	$\beta$ -Pinene	0.090
5	8.347	1030	Limonene	1.954
6	8.408	1032	Eucalyptol	0.309
7	12.911	1171	<i>p</i> -Menthone	17.223
8	13.406	1185	Menthol	32.852
9	15.291	1242	Pulegone	1.778
10	17.123	1297	Isomenthyl acetate	5.004
11	21.036	1420	$\beta$ -Caryophyllene	1.157
			<i>Other compounds</i>	38.14

Studies on the peppermint growth and development linked with the potassium variation rate were made by Valmorbidia & Boaro (2007) [39]. Their studies were on nutrient solutions with potassium between 6.0, 3.0 and 1.5 mmol l<sup>-1</sup> K limits, on Hoagland and Arnon's no.2 and have shown that plants grown under conditions of 1.5 and 3.0 mmol l<sup>-1</sup> K had the better development of leaf area, biomass and dry matter.

Similar studies were conducted by El-Masry & al. (2008) [11]. They evaluated the influence of six rates of potassium sulphate (25, 150, 217, 300, 450 and 600 g/l) on peppermint physiological indices in hydroponic conditions. Garlet & al. (2013) [13] also found the favorable influence of potassium supplemented in four doses (276, 414, 552 and 690 mg l<sup>-1</sup>), on the fresh mass of leaves, stems and biomass in peppermint and on the quality



of peppermint oil under hydroponics system NFT (Nutrient Film Technique).

The quality of the peppermint essential oil has been studied in relation to certain types of nutrients, mineral or organic fertilizers in various researches of Zheljzakov & al. (2010) [40], Costa & al. (2013) [7] and in relation to the plants age and the harvesting time of the herbs.

The variation of essential oils and their active principles according to the growing system and to nutrients was also reported in *Lavandula angustifolia* L. studies (CAMEN D. & al. 2016) [6], *Satureja hortensis* L. (ALIZADEH A. & al. 2010) [2]., *Melissa officinalis* L. (AZIZ E.E. & EL-ASHRY S.M. 2009) [3], (SHARAFZADEH S. & al. 2011), (Misra A. & Srivastava N.K. 2000) [26], *Hyssopus officinalis* L. ssp. *Officinalis* (BAJ & al. 2010) [4], *Ocimum basilicum* L. (DANESHIAN A. & al. 2009) [8]; (KANDIL M.A.M. & al. 2009) [17]; (DZIDA K. 2010) [10]; (SAID-AL AHL H.A.H. & MAHMOUD A.A. 2010) [31]; (NURZYŃSKA-WIERDAK R. & BOROWSKI B. 2011) [27], *Ocimum americanum* L. (OMER E.A. & al. 2008) [29], *Rosa damascena* Mill. (DANESHKHAH M. & al. 2007) [9], *Cymbopogon flexuosus* (GANJEWALA D. & LUTHRA R., 2007) [12], *Thymus vulgaris* L. (JABBARI R. & al., 2011) [14], *Salvia sclarea* (LATTOO S.K. & al. 2006) [21], *Foeniculum vulgare* Mill. (MAHFOUZ S.A. & SHARAF-ELDIN M.A. 2007) [22], *Acorus calamus* L. (MENEHINI A. & al. 1998) [23], *Satureja hortensis* L. cv. Saturn (MUMIVAND H. & al. 2011) [24], *Carum carvi* L. var. *annua* (VALKOVSKI N.J. & NEMETH-ZAMBORI E. 2011) [38] and various other species (NURZYŃSKA-WIERDAK R. 2013) [28].

#### 4. Conclusions

Different solutions of Hoagland through addition of P and K influence the physiological indices such as stems dry matter (SDM) and leaves (LDM), the rate of perspiration (E), the chlorophyll content (Chl) and the rate of photosynthesis (PSN) in peppermint (*Mentha piperita* L.), with statistically significance.

Given all these interdependencies between nutrition and physiological indices were identified high levels of essential oils compounds from peppermint through the gas-chromatographic method coupled with mass spectrometry. Were identified over 50 volatile compounds, of which the most important were identified based on their mass spectra and Kovats indices.

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