ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(4): 676-683
doi: 10.25083/rbl/24.4/676.683

Received for publication, January, 12, 2017
Accepted, November, 4, 2017

Original paper

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

DORIN CAMEN[1], CARMEN DRAGOMIR[1], COSMIN POPESCU[2], TIBERIU IANCU[3], SORIN STANCIU[3], ROXANA LUCA[1], NICOLETA HĂDĂRUGĂ[4], MIHAELA MOATĂR[1]*, ELEONORA NISTOR[1], ION NICOLAE[5], FLORIN SALA[6]

Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania", 119 Calea Aradului, Timişoara 300645, Romania
University of Craiova, 13 Al. I. Cuza, Craiova, 200396, Romania
Faculty of Horticulture and Forestry, [1]Genetic engineering
Faculty of Agriculture, [2]Sustainable Development and Environmental Engineering
Faculty of Farm Management, [3]Management and Rural Development Department
Faculty of Food Processing and Technology, [4]Food Science Department
Faculty of Horticulture, [5]Biology Department
Faculty of Agriculture, [6]Soil Science and Plant Nutrition

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 β-caryophyllene with a 1.2% concentration.

Keywords Compounds, essential oil, herb, Hoagland solution, peppermint.

To cite this article: CAMENk D, DRAGOMIR C, POPESCU C, IANCU T, STANCIU S, LUCA R, HĂDĂRUGĂ N, MOATĂR M, NISTOR E, NICOLAE I, SALA F. Study regarding the influence of fertilization on some physiological index and biochemical composition of peppermint oils *(Mentha piperita L.*). *Rom Biotechnol Lett*. 2019; 24(4): 676-683. DOI: 10.25083/rbl/24.4/676.683

*Corresponding author: MIHAELA MOATĂR, Faculty of Horticulture and Forestry, Genetic engineering
E-mail: mihaelamoatar@yahoo.com

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. Jeliazkova & 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 μg,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 mg Zn kg^{-1} and 10 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.

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 photo-respiration (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 CO_2 concentration with an infrared analyzer (CO_2 ANALYSIS PACKAGE Qubit Systems – Canada).

Photosynthesis rate was determined by measuring the initial and final concentration of 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 C_8-C_{20} (Fluka Chemie AG).

Table 1. Hoagland solution in experimental variants

Components	**Supply solutions**	**ml Supply solutions /1L**		
		V0	V1	V2
2M KN^3	202g/L	2.5	5	2.5
2M $Ca(NO^3)^2$ x $4H^2O$	236g/0.5L	2.5	2.5	2.5
Fier	15 g/L	1.5	1.5	1.5
2M $MgSO^4$ x $7H^2O$	493 g/L	1	1	1
1M NH^4NO^3	80 g/L	1	1	1
Trace elements				
H^3BO^3	2.86 g/L	0.5	0.5	0.5
$MnCl^2$ x $4H^2O$	1.81 g/L	0.5	0.5	0.5
$ZnSO^4$ x $7H^2O$	0.22 g/L	0.5	0.5	0.5
$CuSO^4$	0.051 g/L	0.5	0.5	0.5
H^3MoO^4 x H^2O	0.09 g/L	0.5	0.5	0.5
1M KH^2PO^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 µl; He carrier gas; injector temperature 280°C; HP-5 MS column, length 30 m, inner diameter 0.25 mm, film thickness of 0.25 µm; 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 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.

Table 2. Kovats retention indices values (KI) and retention times (RT) for linear alkali C_8-C_{20}

No.	linear alkali	KI	RT (min)
1	octane, C_8	800	3.07
2	nonane, C_9	900	4.37
3	decane, C_{10}	1000	6.23
4	undecane, C_{11}	1100	8.44
5	dodecane, C_{12}	1200	10.76
6	tridecane, C_{13}	1300	13.06
7	tetradecane, C_{14}	1400	15.28
8	pentadecane, C_{15}	1500	17.39
9	hexadecane, C_{16}	1600	19.39
10	heptadecane, C_{17}	1700	21.3
11	octadecane, C_{18}	1800	23.11
12	nonadecane, C_{19}	1900	24.83
13	eicosane, C_{20}	2000	26.48

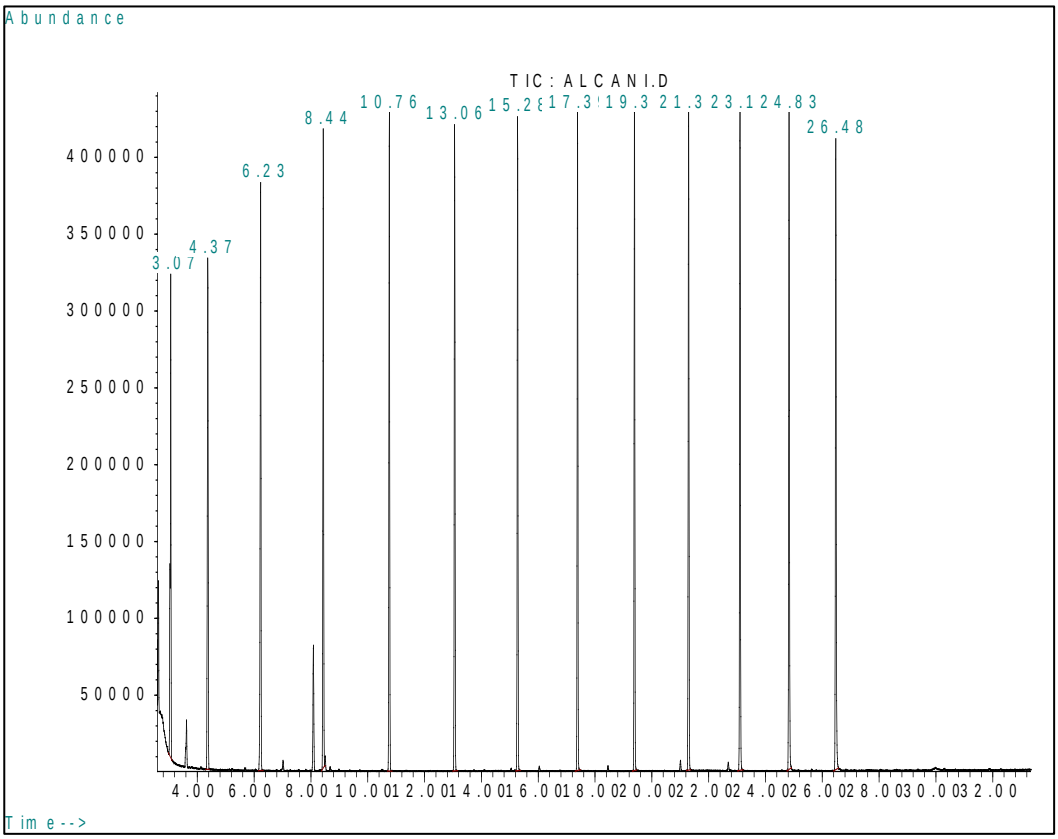

Figure. 1. Gas chromatogram of the standard mixture of C_8-C_{20} linear alkali, used to determine the Kovats retention indices.

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_2 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.

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_2 (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_2, while a lower value was recorded in variant V_1 (30.5 ADP), Figure 3.

The intensity of photosynthesis evaluated by the gas exchange method (CO_2 assimilation and O_2 release) had differentiated values in all three variants; a higher intensity was recorded for variant V1 (with the phosphorus addition), followed by variant V2 (with added K) compared with the control, Figure 4.

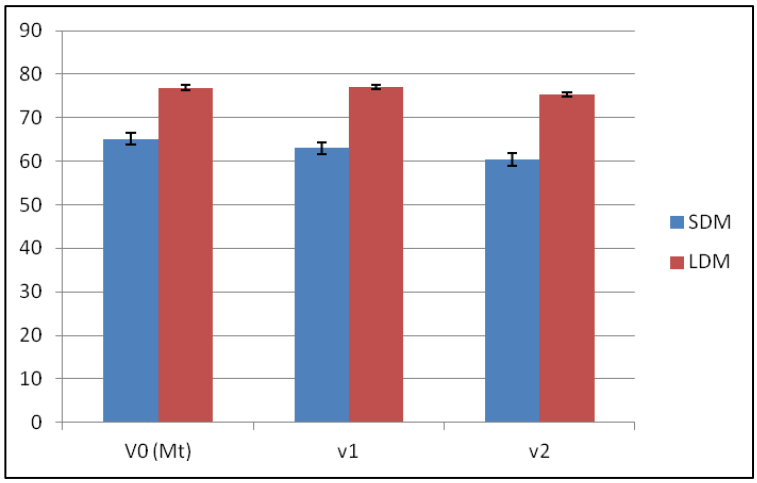

Figure 2. Percentage of dry matter – peppermint.

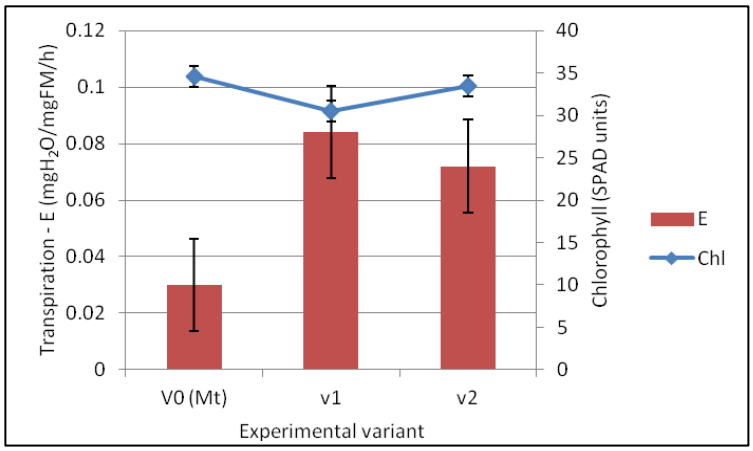

Figure 3. Content of total chlorophyll (SPAD).

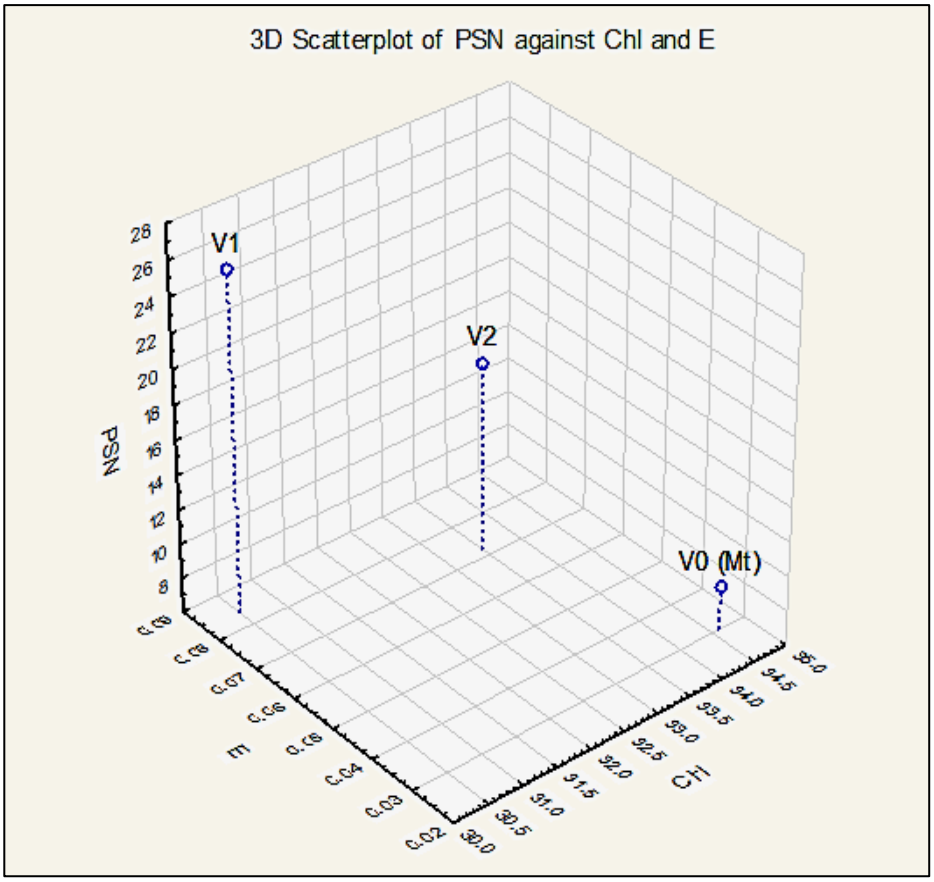

Figure. 4. 3D representation of the photosynthesis intensity corresponding to the chlorophyll and respiration, after modification caused by the P and K changes in Hoagland solution.

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

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).

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 β-caryophyllene at a concentration of 1.2% (table 4).

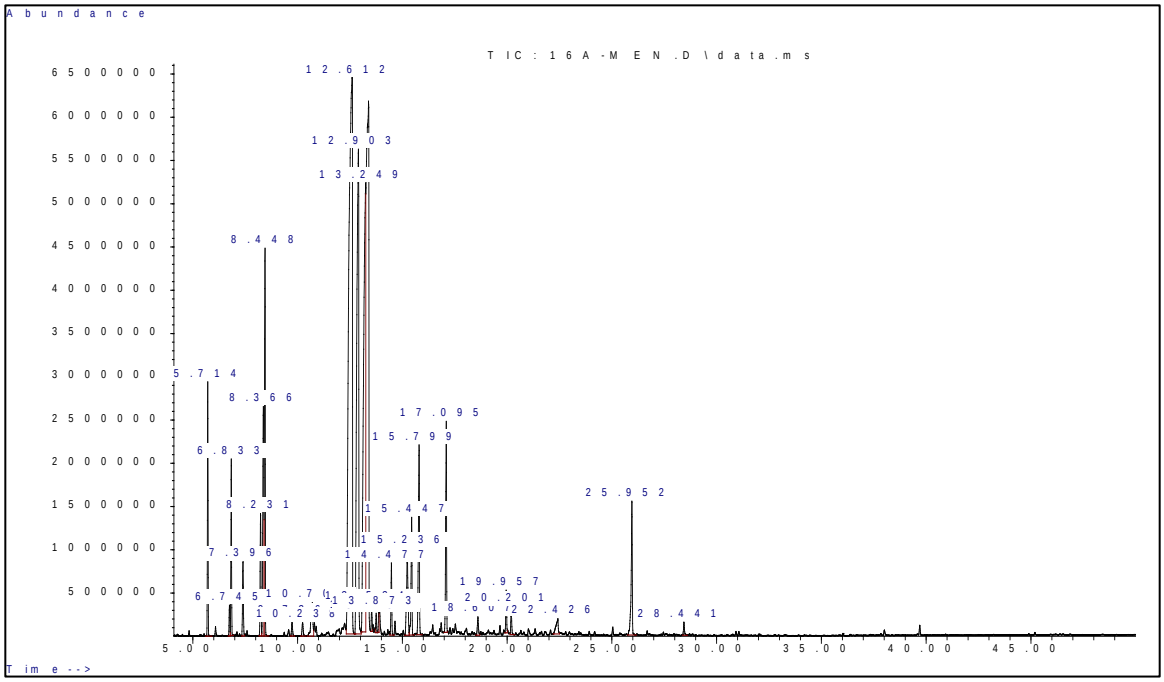

Figure. 6. Gas chromatogram of GC-MS analysis of peppermint essential oil samples.

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	α-Pinene	0.727
2	6.739	975	β-Phellandrene	0.189
3	6.827	978	β-Pinene	0.576
4	7.227	993	β-Pinene	0.090
5	8.347	1030	Limonene	1.954
6	8.408	1032	Eucalyptol	0.309
7	12.911	1171	*p*-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	β-Caryophyllene	1.157
			Other compounds	*38.14*

Studies on the peppermint growth and development linked with the potassium variation rate were made by Valmorbida & Boaro (2007) [39]. Their studies were on nutrient solutions with potassium between 6.0, 3.0 and 1.5 mmol l^{-1} 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^{-1} 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-1), 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 Zheljazkov & 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], (SHARAF-ZADEH 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. (DANESHKHAN 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. (MENEGHINI A. & al 1998) [23], *Satureja hortensis* L. cv. Saturn (MUMIVAND H. & al 2011) [24], *Carum carvi* L. var. *annua* (VALKOVSZKI N.J. & NEMETH-ZAMBORI E. 2011) [38] and various other species (NURZYĔSKA-WIERDAK R. 2013) [28].

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.

Acknowladgements

Funded research project or another internal.

References

1. B. ALI, N.A. AL-WABEL, S. SHAMS, A. AHAMAD, S.A. KHAN, F. ANWAR. Essential oils used in aromatherapy: A systemic review. *Asian Pacific Journal of Tropical Biomedicine* 5(8): 601-611 (2015).
2. A. ALIZADEH, M. KHOSHKHUI, K. JAVIDNIA, O. FIRUZI, E. TAFAZOLI, A. KHALIGHI. Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (Lamiaceae) cultivated in Iran. *J. Med. Plants Res.* 4(1), 33-40 (2010).
3. E.E. AZIZ, S.M. EL-ASHRY. Efficiency of slow release urea fertilizer on herb yield and essential oil production of lemon balm *(Melissa officinalis* L.) plant. Am-Euras. *J. Agric. Environ. Sci.* 5(2), 141-147 (2009).
4. T. BAJ, R. KOWALSKI, À. ĜWIĄTEK, M. MODZELEWSKA, WOLSKI T. Chemical composition and antioxidant activity of the essentials oil of hyssop (Hyssopus officinalis L. ssp. officinalis). Annales UMCS, sec. DDD, *Pharmacia* 23, 3(7), 55-61 (2010).
5. B. BROWN, J.M. HART, M.P. WESCOTT, N.W. CHRISTENSEN. The critical role of nutrient management in mint production. *Better Crops* 87(4): 9-11 (2003).
6. D, CAMEN, N. HADARUGA, R. LUCA, A. DOBREI, E. NISTOR, D. POSTA, A. DOBREI, F. SALA. Research concerning the influence of fertilization on some physiological processes and biochemical composition of lavender (*Lavandula angustifolia* L.). *Agriculture and Agricultural Science Procedia* 10(2016) 198-205 (2016).
7. A.G. COSTA, S.K.V. BERTOLUCCI, J.H. CHAGAS, E.O. J.E.B.P. FERRAZ. Pinto Biomass production, yield and chemical composition of peppermint essential oil using different organic fertilizer sources. *Ciência e Agrotechnologia*, 37(3): 202-210 (2013).
8. A. DANESHIAN, B. GURBUZ, B. COSGE, A. IPEK. Chemical components of essential oils from basil (Ocimum basilicum L.) grown at different nitrogen levels. *Internat. J. Nat. Eng. Sci.* 3(3), 8–12 (2009).
9. M. DANESHKHAH, K. MOHSEN, A. NIKBAKHT. Effects of different levels of nitrogen and potassium fertilizers on flower yield and essential oil content of Rosa damascena Mill. from Barzok of Kashan. *Iranian J. Hortic. Sci. Technol. Summ*. 8(2), 83-90 (2007).
10. K. Dzida. Biological value and essential oil content in sweet basil (*Ocimum basilicum* L.) depending on calcium fertilization and cultivar. *Acta Sci. Pol. Hort. Cult.* 9(4), 153-161 (2010).
11. M.H. EL-MASRY, M.R. MORALES, D.J. CHARLES, J.E. SIMON. Effect of potassium rates on hydro-ponically- grown peppermint (Mentha x piperita). *African Journal of Traditional, Complementary and Alternative medicines* (AJTCAM) 6: 426 (2009).
12. D. GANJEWALA, R. LUTHRA. Essential oil biosynthesis and metabolism of geranyl acetate and geraniol in developing *Cymbopogon flexuosus* (Nees ex Steud) Wats Mutant cv. GRL 1 leaf. *Am. J. Plant Physiol.* 2(4), 269-275 (2007).
13. T.M.B. GARLET, D. PAULUS, R. FLORES. Production and chemical composition of Mentha x Piperita var. Citrata (Ehrh.) Briq. essential oil regarding to different potassium concentrations in the hyd roponic solution. *Journal of Biotechnology and Biodiversity* 4(3): 200-206 (2013).
14. R. JABBARI, M.A. DEHAGHI, A.M.M. SANAVI, K. AGAHI. Nitrogen and iron fertilization methods affecting essential oil and chemical composition of thyme (*Thymus vulgaris* L.) medical plant. *Adv. Environ. Biol.* 5(2), 433-438 (2011).
15. E.A. JELIAZKOVA, V.D. ZHELJAZKOV, L.E. CRAKER, B. YANKOV, T. GEORGIEVA. NPK fertilizer and yields of peppermint, *Mentha* x *piperita*. *Acta Hortic.* 502, 231-236 (1999).
16. Ø. HAMMER, D.A.T. HARPER, P.D. RYAN. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1): 1-9 (2001).

17. M.A.M. KANDIL, M.E. KHATAB, S.S. AHMED, E. SCHNUG. Herbal and essential oil yield of Genovese basil (*Ocimum basilicum* L.) grown with mineral and organic fertilizer sources in Egypt. *J. Kulturpfl*, 61(12), 443-449 (2009).
18. D. KAVRAYAN, T. AYDEMIR. Partial purification and characterization of polyphenoloxidase from peppermint (*Mentha piperita*). *Food Chemistry* 74(2): 147-154 (2001).
19. K.A. KHALID. Effect of NP and foliar spray on growth and chemical compositions of some medicinal *Apiaceae* plants grow in arid regions in Egypt. *Journal of Soil Science and Plant Nutrition*, 12(3): 581-596 (2012).
20. K.V. KUMAR, D.D. PATRA. Alteration in yield and chemical composition of essential oil of *Mentha piperita* L. plant: Effect of fly ash amendments and organic wastes. *Ecological Engineering*, 47: 237-241 (2012).
21. S.K. LATTOO, R.S. DHAR, A.K. DHAR, P.R. SHARMA, S.G. AGARWAL. Dynamics of essential oil biosynthesis in relation to inflorescence and glandular ontogeny in Salvia sclarea. *Flavour Frag. Journal*, 21, 817-821 (2006).
22. S.A. MAHFOUZ, M.A. SHARAF-ELDIN. Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (*Foeniculum vulgare* Mill.). *Int. Agroph.*, 21, 361-366 (2007).
23. A. MENEGHINI, N. POCCESCHI, G. VENANZI, P.B. TOMASELLI. Effect of nitrogen fertilization on photosynthetic rate, nitrogenous metabolites and ù-asarone accumulation in triploid *Acorus calamus* L. leaves. *Flavour Fragr. J.*, 13, 319-323 (1998).
24. H. MUMIVAND, M. BABALAR, J. HADIAN, M. FAKHR-TABATABAEI. Plant growth and essential oil content and composition of Satureja hortensis L. cv. Saturn in response to calciumcarbonate and nitrogen application rates. *J. Med. Plants Res.*, 5(10), 1859-1866 (2011).
25. A. MISRA, S. SHARMA. Critical concentration of iron in relation to essential oil yield and quality parameters of Japanese mint. *Soil Science and Plant Nutrition*, 37(2): 185-190 (1991).
26. A. MISRA, N.K. SRIVASTAVA. Influence of Water Stress on Japanese Mint. *Journal of Herbs, Spices & Medicinal Plants* 7(1): 51-58 (2000).
27. R. NURZYŃSKA-WIERDAK, B. BOROWSKI. Changes in the content and chemical composition of sweet basil essential oil under the influence of fertilization of plants with nitrogen and potassium. *Annales UMCS, sec. DDD, Pharmacia* 24, 3(15), 133-145 (2011).
28. R. NURZYŃSKA-WIERDAK. Does mineral fertilization modify essential oil content and chemical composition in medicinal plants? *Acta Sci. Pol., Hortorum Cultus* 12(5): 3-16 (2013).
29. E.A. OMER, A.-G.A. ELSAYED, A. EL-LATHY, M.E. KHATTAB, A.S. SABRA. Effect of the nitrogen fertilizer forms and time of their application on the yield of herb and essential oil from Ocimum americanum L. *Herba Pol.*, 54(1), 34-46 (2008).
30. P. PANDE, M. ANWAR, S. CHAND, V.K. YADAV, D.D. PATRA. Optimal Level of Iron and Zinc in Relation to Its Influence on Herb Yield and Production of Essential Oil in Menthol Mint. *Communications in Soil Science and Plant Analysis*, 38(5-6): 561-578 (2007).
31. H.A.H. SAID-AL, A.A. AHL MAHMOUD. Effects of zinc and / or iron foliar application on growth and essential oil of sweet basil (*Ocimum basilicum* L.) under salt stress. *Ozean J. Appl. Sci.*, 3(1), 97-111. (2010).
32. F. SALA. Agrochimie, *Editura EUROBIT*, Timişoara, pp. 37-38, 288-293 (2011).
33. F. SALA, G.-G. ARSENE, O. IORDĂNESCU, M. BOLDEA. Leaf area constant model in optimizing foliar area measurement in plants: A case study in apple tree. *Scientia Horticulturae*, 193: 218-224 (2015).
34. J. SCAVRONI, C.S.F. BOARO, M.O.M. MARQUES, L.C. FERREIRA. Yield and composition of the essential oil of *Mentha piperita* L. (Lamiaceae) grown with biosolid. *Brazilian Journal of Plant Physiology*, 17(4): 345-352 (2005).
35. S. SHARAFZADEH, M. KHOSH-KHUI, K. JAVIDNIA. Effect of nutrients on essential oil components, pigments and total phenolic content of lemon balm (*Melissa officinalis L.*). *Adv. Environ. Biol.* 5(4), 639-646 (2011b).
36. R. SINGH, M.A.M. SHUSHNI, A. BELKHEIR. Antibacterial and antioxidant activities of *Mentha piperita* L. *Arabian Journal of Chemistry*, 8(3): 322-328 (2015).
37. S.J. TABATABAIE, J. NAZARI. Influence of Nutrient Concentrations and NaCl Salinity on the Growth, Photosynthesis, and Essential Oil Content of Peppermint and Lemon Verbena. *Turk J Agric For*, 31: 245-253 (2007).
38. N.J. VALKOVSZKI, E. NEMETH-ZAMBORI. Effects of growing conditions on content and composition of the essential oil of annual caraway (Carum carvi L. var. annua). *Acta Aliment.* 40(2), 235-246 (2011).
39. J. VALMORBIDA, C.S.F. BOARO. Growth and development of *Mentha piperita* L. in nutrient solution as affected by rates of potassium. *Brazilian Archives of Biology and Technology*, 50(3): 379-384 (2007).
40. V.D. ZHELJAZKOV, C.L. CANTRELL, T. ASTATKIE, M.W. EBELHAR. Peppermint productivity and oil composition as a function of nitrogen, growth stage, and harvest time. *Agron. J.*, 102(1), 124-128 (2010).