STUDIES REGARDING THE INFLUENCE OF SOME PHENOTYPIC PARAMETERS ON RAW MATERIAL'S QUALITY OF DIFFERENT GENOTYPES FROM *MENTHA* GENUS

Constantin LUNGOCI[1], Andreea Daniela VODĂ [1], Carmen Simona ȚIBULCĂ-GHIȚĂU[1], Carmenica Doina JITĂREANU[1], Teodor ROBU [1]

e-mail: constantinlungoci@uaiasi.ro

Abstract

Peppermint (*Mentha sp*.) is one of the most cultivated medicinal plant worldwide due to its diverse range of products in which it can be used. The main natural product (*Herba Menthae*) is used in pharmaceutical industry, as an additive and flavor in food industry and the extracted essential oil is used in numerous cosmetic and selfcare products. This study's purpose is to quantify a series of biometrical and physiological elements of some species which pertain to *Mentha* genus from USAMV's "Collection of Medicinal Plants", which influence the raw material's quality and the quantity of essential oils. The studied mint species are *Mentha viridis var. crispata* (Schrod) Beck Ecotype 2, *Mentha longifolia* (L.) Hudson Ecotype 2, *Mentha spicata var. viridis* L., *Mentha longifolia* (L.) Hudson Ecotip 1, *Mentha viridis var. crispata* (Schrod) Beck Ecotype 1. The study followed the influence of the leaf surface, the content of chlorophyll pigments, but also the total leaf number on the production of volatile oil. Being a very important parameter in the reception and estimation of needed quantity of raw material, drying efficiency was determined for each cultivar. The highest amount of volatile oil was obtained from two ecotypes of the *Mentha viridis var. crispata* (Schrod) Beck species, in a percentage of 0.54% and 0.58%, and the lowest amount from *Mentha longifolia* (L.) Hudson Ecotype 2. The results are showing that the quantity of volatile oil is influenced by the leaf surface, especially by the basal leaves. It is being proved that the amount of the volatile oil is influenced by the leaf surface, its placement and the chlorophyll pigments content and the cultivar factor, in the same environmental conditions.

Key words: *Mentha* spp., chlorophyll pigments, volatile oil, leaf surface

Peppermint (*Mentha* spp.*)* is one of the most important and cultivated medicinal plants from Romania, idea proved also by the fact that the first attempt of introducing it in culture was in Cluj in 1988 on a surface of 88 m^2 (Munteanu *et al*, 2016). The first peppermint crop was in 1929, on a surface of 5 ha in Bod, with seedlings from England (Păun *et al*, 1988).

The crop's importance is given especially by the aerial part (*Menthae herba*), but also by the essential oil extracted through hydrodistillation (*Menthae aetheroleum*), oil which has approximately 47-73.6% menthol in its composition (Cucu *et al*, 1982).

The concentration and chemical composition of essential oil vary depending on species and pedo-climatic conditions, some varieties containing up to 40 components. The peppermint essential oil is the 2nd most sold volatile oil extracted from plants, after the lavender essential oil.

The main method for extracting essential oil is using the water vapors. The big production countries (India, China) use this method for its convenient efficiency/quality ratio.

Peppermint species have numerous uses: pharmaceutical industry (antispastic, choleretic and carminative effect), (Stănescu *et al*, 2014), food industry (antimicrobial and antioxidant activity, flavor enhancer in food and beverages), (*Gobert et al*, 2002, Preedy *et al*, 2016), pesticide (antifungal activity for *Fusarium* and *Pyrenophora graminea*), (Kurita *et al*, 1983) and many other uses in aromatherapy, aromatizing the tabaco, personal hygiene products and veterinary medicine (Robu *et al*, 2004).

The purpose of this research is to quantify some physiological parameters (leaf surface, content in chlorophyll pigments). Chlorophyll pigments are directly involved in the photosynthetic process and indirectly in the qualitative and quantitative content of essential oil (Burzo *et al*, 2013). Through the utilized method we tried to demonstrate how these factors influence the essential oil content in some peppermint (*Mentha sp*.) ecotypes. Drying is the

[1] "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, Iași

most common and fundamental method for preserving the medicinal plants after harvesting because it allows the rapid preservation of the medicinal qualities of the plant material in a simple way (Müller Joachim, 2007). For this reason, we have realized a dynamic of natural drying to determine the drying yield, a very important factor in estimating the production and ensuring the necessary quantity of raw material.

MATERIAL AND METHOD

The research was conducted in USAMV's "Collection of Medicinal and Aromatic Plants". The 5 ecotypes of peppermint were placed in plots with a surface of 2.5 m^2 (2.5x1 m), using it during the flowering for most of the determinations. Leaf surface, a determinant factor in bioactive compounds synthesis, was determined during the flowering of mint using the AM 350 Area Meter dispositive.

The content of chlorophyll pigments was determined in two ways: once, using the portable device Opti-Sciences CCM-200 plus, directly in the field, and in the laboratory using the UV-1700 Spectrophotometer. For this method, we weigh in 0.5 grams of fresh mint leaves, grinding it and mixing with 80% concentration acetone. The obtained product is filtered using a Duran filter with 3 (16-40) µm porosity and after introduced in the spectrophotometer for analysis (Jităreanu *et al*, 2007). The processing of qualitative and quantitative data was done using the Lichtenthaler 1987 method.

Statistical processing of data resulting from biometric measurements was performed using the variance method and analysis of variance. The obtained results were processed by calculating the limit differences for the probability of transgressions of 5%, 1% and 0.1% (Jitareanu *et al*, 2006).

RESULTS AND DISCUSSIONS

The data obtained shows that in the case of leaf distribution at the 5 mint ecotypes it is differently distributed. For example, *Mentha longifolia* (L.) Hudson (ecotype 1, 2) have the leaf surface placed mostly in the middle part of the plants and in the case of those producing a large quantity of oil (*var. crispata*) the leaf surface is situated more at the base of the plant *(figure 1).*

For some species it is considered that the number of the leaves is directly proportional with the quantity of assimilated substances found in plant. At the studied peppermint ecotypes (table 2), graphically represented in figure 2, it can be observed that this rule is not applying.

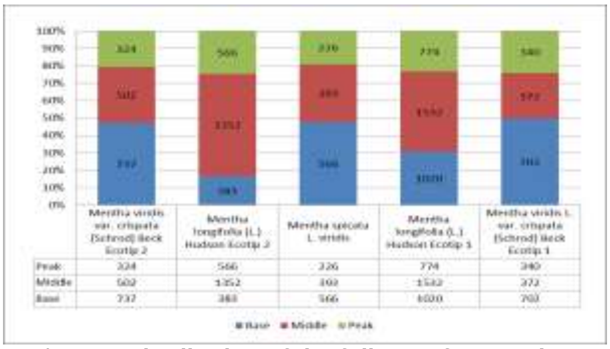


Figure 1 **Distribution of the foliar surface at plant level**

After the statistical processing it is obvious that regarding the ecotypes with a reduced number of leaves the difference is significative but negative and it's present in ecotypes with a high amount of volatile oil. The ecotypes which have 23 and 26 leaves have produced the largest amount of volatile oil.

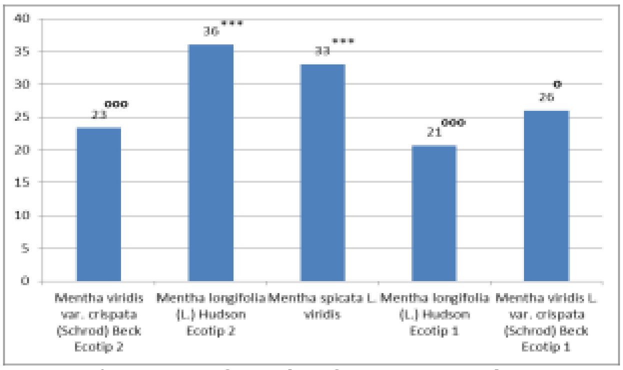


Figure 2 **Leaf number for every species**

For the statistical processing of the data obtained, the average of the 5 ecotypes taken in the study was used as a control. Positive differences significantly distinct were obtained on *M. longifolia* (L.) Hudson ecotype 2 şi *M. spicata* L. *viridis* ecotypes and significantly distinct differences but negative for *M. viridis. var. crispata* (Schrod) Beck ecotype 2 şi *M. longifolia* (L.) Hudson ecotype 1 ecotypes.

Table 1

Leaf number *for every species*

Variant	Number leaf	Differences %	Differences Nr./pl.	Significance
Mentha viridis var. crispata (Schrod) Beck Ecotip 2	23	83.81	-4.5	ooo
Mentha longifolia (L.) Hudson Ecotip 2	**36**	**129.5**	**8.2**	*******
Mentha spicata L. viridis	**33**	**118.71**	**5.2**	*******
Mentha longifolia (L.) Hudson Ecotip 1	21	74.46	-7.1	ooo
Mentha viridis L. var. crispata (Schrod) Beck Ecotip 1	26	93.53	-1.8	o
Average	**27.8**	**100**	**0**	**Control**
	DL5%	1.8 Nr/pl		
	DL 1%	2.6 Nr/pl		
	DL 0.1%	3.8 Nr/pl		

Using the CCM-Opti Science device were obtained the content in chlorophyll pigments of different foliar floors in the moment of flowering and the obtained data were graphical represented in figure 3.

It is noticed that at most ecotypes the highest content in chlorophyll pigments is in the upper leaf floors and the lowest content at the base of the plants. The highest values of upper leaves were between 45.9, 28.3 in the case of *Mentha viridis var. crispata* (Schrod) Beck and 11.6 – 19.1 at *Mentha longifolia* (L.) Hudson ecotype 2 and *Mentha spicata L. var. viridis*.

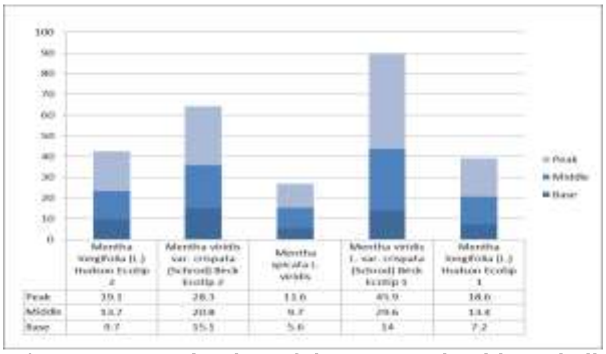


Figure 3 **Determination of the content in chlorophyll pigments with CCM - 200 plus**

By analyzing the quantity of chlorophyll conent using the spectophotometer it can be highlighted that the highst amounts of cholophyll a and b are showing at the two ecotypes of *M. viridis var. crispata* (Schrod) Beck. *(figure 4)*

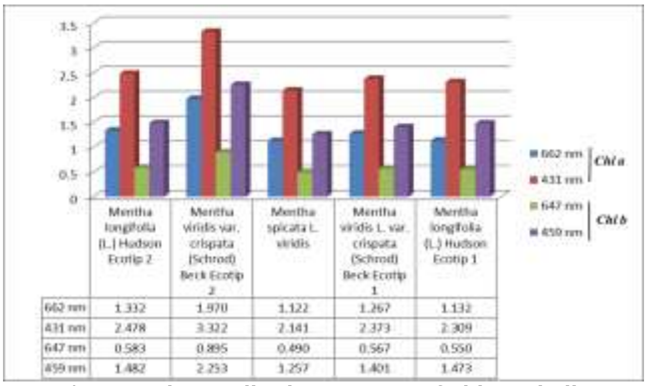


Figure 4 **The qualitative content of chlorophyll pigments**

Regarding the quantity of chlorophyll pigments calculated through the Lichtenthaler method, the dates from table 2 highlight that the two ecotypes of *M. viridis var. crispata* (Schrod) Beck have the highest amounts of chlorophyll pigments, between 21.427 mg/g fresh material (ecotype 2) and 13.816 mg/g fresh material (ecotype 1).

Similar differences were observed for chlorophyll b at the same two ecotypes.

Table 2

The quantitative content of chlorophyll pigments

	Chlorophyll a (mg / g fresh material)	Chlorophyll b (mg / g fresh material)	Total chlorophyll	Ratio a/b	Carotene (mg / g fresh material)
Mentha longifolia (L.) Hudson Ecotip 2	14.605	5.777	20.376	2.528	3.400
Mentha viridis var. crispata (Schrod) Beck Ecotip 2	21.427	9.282	30.700	2.308	4.797
Mentha spicata L. viridis	12.328	4.833	17.157	2.551	3.003
Mentha viridis L. var. crispata (Schrod) Beck Ecotip 1	13.816	5.780	19.590	2.390	3.169
Mentha longifolia (L.) Hudson Ecotip 1	12.271	6.077	18.343	2.019	5.716

Analyzing the carotenoids content it can be observed that it has higher amounts *in Mentha longifolia* (L.) Hudson (ecotype 1) with a value of 5.716 mg/g green material, followed by ecotypes 1 and 2 of *M. viridis var. crispata* (Schrod) Beck *(figure 5).*

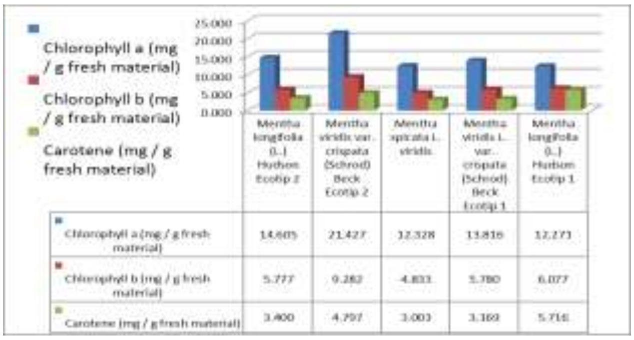


Figure 5 **The quantitative content of chlorophyll pigments**

The determinations have a direct influence on the quantity of volatile oil. The higest amount of essential oil is in ecotypes 1 and 2 of *M. viridis var. crispata* (Schrod) Beck with values between 5.8 ml/kg in ecotype 2 and 5.4 ml/kg at ecotype 1. The data are presented in *figure 6*.

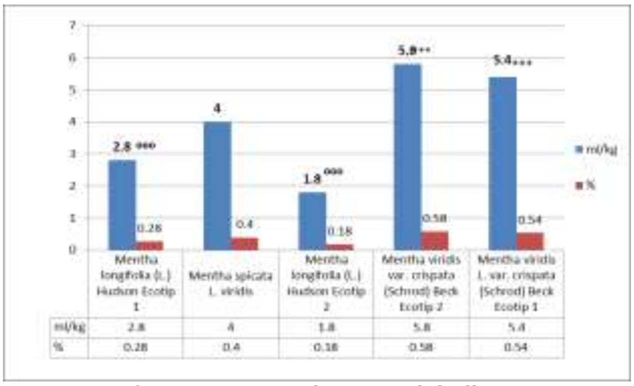


Figure 6 **Content in essential oils**

Table 2

Content in essential oils

Variant	Production ml/kg	Difference %	Difference ml/kg	Significance
Mentha longifolia (L.) Hudson Ecotip 1	**2.8**	70	-1.2	ooo
Mentha spicata L. viridis	**4**	100	0	
Mentha longifolia (L.) Hudson Ecotip 2	**1.8**	45	-2.2	ooo
Mentha viridis var. crispata (Schrod) Beck Ecotip 2	**5.8**	145	1.8	***
Mentha viridis L. var. crispata (Schrod) Beck Ecotip 1	**5.4**	135	1.4	***
Average	**4**	**100**	**0**	**Control**
DL 5%	0.3			
DL 1%	0.4			
DL 0.1%	0.6			

For some studied ecotypes of Mentha genus there is a positive correlation between the chlorophyll pigments content determined with CCM-opti Science device and the content of volatile oil determined through hydrodistillation (figure 7).

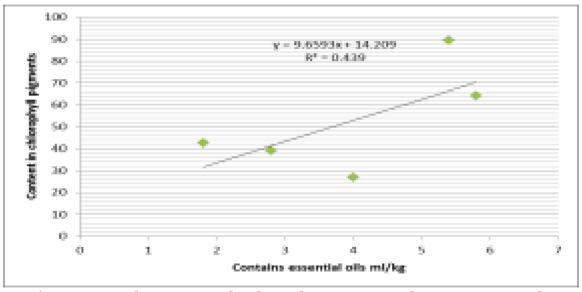
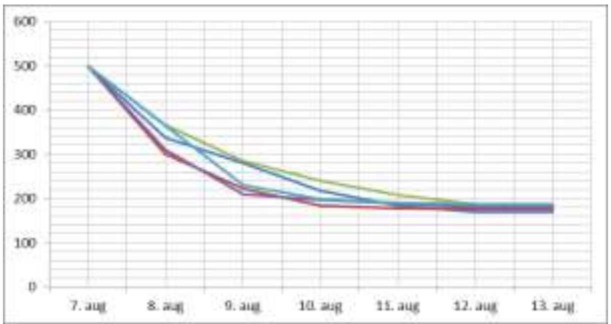


Figure 7 **The correlation between the content in chlorophyll pigments and that of the essential oil**

The drying dynamics and the fresh herb/dryied plant ratio is a imporant parameter and it's different depending on species. The drying yield was different at every ecotype, the highest being on Mentha logifolia (L.) ecotype 1 (1:2:9) and the lowest on *Mentha longifolia* ecotype 2 și *Mentha viridis var crispata* (Schrod) Beck. A very important factor in drying dynamics and drying yield was the leaves's consistance and the thickness of the drying layer (*figure* 8).



	7. aug	8. aug	9. aug	10. aug	11. aug	12. aug	13. aug	Drying efficiency
Mentha longifolia (L.) Hudson Ecotip 1	500	338	280	218	184	170	170	2.9
Mentha spicata L. viridis	500	301	222	184	178	176	176	2.8
Mentha longifolia (L.) Hudson Ecotip 2	500	367	285	240	208	187	187	2.7
Mentha viridis var. crispata (Schrod) Beck Ecotip 2	500	310	209	196	188	180	180	2.8
Mentha viridis L. var. crispata (Schrod) Beck Ecotip 1	500	365	230	199	190	187	187	2.7

Figure 8 **Drying dynamics of mint herb**

CONCLUSIONS

The studied factors influenced the content in volatile oil. The foliar surface didn't have a major involvement, it's value doesn't reflect in ecotypes with the highest amount of volatile oil. The electronic determination of the pigments (CCM-200), the high amount of pigments from the upper floors of the plants had a inluence in volatile oil synthesis.

The spectophotometer determination of quality and quantity of chlorophyll pigments highlighted that there is a positive influence in volatile oil accumulation.

ACKNOWLEGMENTS

This work was supported by a grant of the Romanian Ministery of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III

REFERENCES

Burzo Ioan, Toma Constantin, 2013 – *Țesuturile secretoare și substanțele volatile din plante*. Edit. Universității „Alexandru Ioan Cuza", Iași, pp. 52;

Cucu V., Bodea C., Cioacă C., 1982 – *Tratat de biochimie vegetală, Plante medicinale și aromatice*. Ed. Academiei Române, București;

Gobert V., Moja S., Colson M., Taberlet P., 2002 - *Hybridization in the section Mentha (Lamiaceae) inferred from AFLP markers*. Am. J. Bot. 89, 2017–2023;

Jităreanu G., 2006 – *Metode moderne de cercetare, curs*. Ed. „Ion Ionescu de la Brad", Iași;

Jităreanu Carmen Doina, 2007 – Fiziologia plantelor. Ed. „Ion Ionescu de la Brad", Iași, pp. 222;

Kurita N., Koike S., 1983 - *Synergistic antimicrobial effect of ethanol, sodium chloride, acetic acid and essential oil components*. Agric. Biol. Chem. 47, pp 67–75;

Lichtenthaler H.K., 1987 – *Chlorophylls and carotenoids: pigments of photosyntetic membranes, Methods Enzymol*, vol. 148, pp. 350-382; 390

Munteanu L. S., Tămaș M., Munteanu S., Munteanu L., DUDA M., Vârban D., Florian S., 2016 – *Tratat de plante medicinale cultivate și spontane (ediția a II-a).*Editura RISOPRINT, Cluj-Napoca, pp. 408;

Müller Joachim, 2007 - *Convective drying of medicinal, aromatic and spice plants: A review.* Stewart Postharvest Review 3(4):1-6, DOI: 10.2212/spr.2007.4.2;

Preedy R. Victor and colab. 2016 – *Essential oils in food preservation, flavor and safety*. Editura ACADEMIC PRESS, San Diego, USA, pp. 309-311;

Păun E., Mihalea A., Dumitrescu A., Verzea M., Coșocariu O., 1988 - Tratat de plante medicinale si aromatice cultivate volumul II. Editura Academiei Republicii Socialiste România, București,

Robu T., Milică C., 2004 – *Plante medicinale autohtone.* Ed. INSTITUTUL EUROPEAN, Iași, p. 181;

Stănescu U., Hăncianu M., Cioancă O., Aprotosoaie A. C., Miron A., 2014 – *Plante medicinale de la A la Z***.** Editura POLIROM, pp. 390.