EVALUATION RESULTS OF THE QUANTITATIVE-QUALITATIVE INDICATORS OF LEMON BALM AND PEPPERMINT GROWN IN THE COLD AGRO-CLIMATIC MACROREGION

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Abstract. *The aim of this experiment was to measure the impact of weather conditions on the yield of lemon balm and peppermint drug and content of rosmarinic and caffeic acid. Lemon balm and peppermint are medicinal plants from Lamiaceae family. To assess the quantitative-qualitative indicators were sampled plant material from production stands lemon balm and peppermint. Samples were collected from two cultivation plots at the site Plavnica for five years from 2004 to 2008. The production areas were located in the village Plavnica, the land units that operated company Agrokarpaty, s.r.o Plavnica. The average yield of dry drug to all variants was 1 131,55 kg ha^{-1}. Rosemary acid content in the dry tops balm was 28 613,8 mg.kg^{-1} (2007) to 36 532.4 mg.kg^{-1} (2006)The average yield of peppermint dry drug at all variant 1 621,9 kg.ha^{-1}. Rosemary acid content in the dry peppermint was recorded from 3 725.98 mg.kg^{-1} (2006 plot Pani Hura) to 5 223.0 mg.kg^{-1} (2008 plot Dziloh). Based on the results we can conclude that with increasing harvest increased the content of rosmarinic acid and caffeic acid too.*

Key words: *lemon balm, peppermint, rosmarinic acid, caffeic acid*

INTRODUCTION

Lemon balm (*Melissa officinalis*) and peppermint (*Mentha × piperita* L.) belong to the *Lamiaceae* family. Those are native extensively in central and Mediterranean Europe (Miron et al., 2013). Among the traditional growers of lemon balm in Europe are Germany, Spain, B ulgaria, Romania, Serbia, Czech republic and Slovakia. Lemon balm ranks among the most important medicinal plant species grown in agro ecological conditions of Slovakia (Habán et al., 2005). Its using is varied, such as a ingredient for the production of herb tea, phytopharmaceuticals and cosmetics (HABÁN, 2004). Peppermint is a sterile hybrid of spearmint (*Mentha × spicata*) and water mint (*Mentha × aquatica*) (RIACHI et al., 2015). Peppermint tea and peppermint essential oil are used in traditional medicines (MCKAY – BLUMBERG, 2006). Rosmarinic acid, contained in many aromatic plants (Peng et al., 2016) is classified as a phenolic acid (PETERSEN – SIMMONDS, 2003), it is characterized by antioxidant, antiviral (DING ET AL., 2010), spasmolytic (HABÁN et al., 2002), anti-inflammatory and mild antibacterial effects (HABÁN, et al., 2009). Caffeic acid belongs to a group of substances with significant antioxidant properties (SPILKOVÁ, 1997).

MATERIAL AND METHODS

The aim of this experiment was to measure the impact of weather conditions on the yield of lemon balm and peppermint drug and content of rosmarinic and caffeic acid. Under field conditions was investigated lemon balm (*Melissa officinalis* L.) and peppermint (*Mentha piperita* /L./ Huds.). Samples were collected from two cultivation plots at the site Plavnica for five years from 2004 to 2008. The production areas were located in the village Plavnica, the land units that operated company Agrokarpaty, s.r.o Plavnica. The territory belongs to the cold agroclimatic macroregion (Šiška et al., 2011).

Lemon balm (*Melissa officinalis* L., cv. Citrate) was grown in the years 2004-2008 in agroecological conditions of the location Plavnica (Stará Ľubovňa) on two parcels Mrs. Hura (p1) and Dziloh (p2).

Table 1

Yield of dry balm drug - *Melissae herba* (kg. ha^{-1}) at the locality Plavnica

Drug	Name plots	Repetition	Growing year				
			2004	2005	2006	2007	2008
Melissae herba	Pani hura (p_1)	(r_1)	1 290,5	1 650,0	1 020,0	715,5	755,0
		(r_2)	1 399,5	1 800,0	815,0	695,5	715,0
		(r_3)	1 451,5	1 650,0	790,0	704,0	750,0
		Average	1 380,5	1 700,0	875,0	705,0	740,0
	Dziloh (p_2)	(r_1)	1 470,0	2 030,0	1 050,0	685,0	745,0
		(r_2)	1 380,0	1 975,0	1 050,0	680,0	775,0
		(r_3)	1 500,0	1 800,0	1 050,0	720,0	760,0
		Average	1 450,0	1 935,0	1 050,0	695,0	760,0

Plavnica village is located southeast of the town Stara Lubovna at a distance of 11 km. territory belongs to Ľubovnianska and Levočska Highlands. The area is characterized by a strongly rugged terrain with large relative height and considerable erosive activity. The altitude varies from 300-900 m. Most of the land has a northwestern exposure. The average annual temperature is between 7-9 ° C and 640-790 mm rainfall (KOŠŤALIK, 1989).

Plant samples were taken during the dry, windless weather. Within each type, three samples were taken, which are then dried, homogenized. After homogenization, the portion of prepared extract. Contents of caffeic and rosmarinic acid was determined using the method HPLC by modified methodical procedure, which state WANG et al. (2004).

RESULTS AND DISCUSSION

Multi-factorial analysis of variance was confirmed statistically high significant effect of growing year and growing area for crops dry drugs, while repetition were statistically insignificant. Tukey's contrast inconclusive difference was observed between 2007 and 2008.

Statistically highly significant difference was found between 2004, 2005, 2006 and the years 2007, 2008 (Tab. 5.19). It had also been confirmed by a highly significant difference between production lots. The average yield of dry drug all variants was 1 131,55 kg ha $^{-1}$.

Table 2

Statistical evaluation of yield *Melissae herba* by analysis of variance

Examined factor	Level of factor	F-test	Level of significance	Average	Significant ($\alpha = 0,01$)
Year	2007	186,037	0,0000 + +	700,00	A
	2008			750,00	A
	2006			962,50	B
	2004			1 415,25	C
	2005			1 817,50	D
Plot	(p_1)	9,782	0,0049 + +	1 080,10	A
	(p_2)			1 178,00	B
Repetition	(r_3)	0,189	0,8291 -	1 117,55	A
	(r_2)			1 128,50	A
	(r_1)			1 141,10	A

Analogous results were achieved in peppermint yield - *Menthae piperitea herba*. The average yield of dry drug to all variants was 1 621.9 kg ha -1. In 2004, the peppermint in February damaged by frost, causing a fall out of the crop and reduce the average yield per 1820 kg ha^{-2} on a plot Dziloh (p2), respectively 2100 kg ha^{-2} on a plot of Mrs. Hura (p1). In 2005, the regeneration of vegetation and increase yields. In this climatically favorable year when it was sunny during harvesting operations, thermally balanced weather, have been reported record yield 2,565 kg ha $^{-1}$. In the third to fifth growing year there is a fall harvest peppermint. One of the reasons is also contesting stand mint by rust (*Puccinia menthae* Pers.) And a reduction of fitness condition through multi-year crop grown on the same land.

Table 3

Yield of peppermint dry drug - *Menthae piperitae herba* (kg ha^{-1}) at the locality Plavnica

Drug	Name of parcel	Repetition	Growing year				
			2004	2005	2006	2007	2008
Menthae	Pani hura	(r_1)	2 200,5	2 550,0	1 305,5	1 255,0	1 205,5

piperitae herba	(p_1)	(r_2)	2 055,0	2 710,0	1 400,0	1 200,0	1 210,5
		(r_3)	2 044,5	2 630,0	1 134,5	1 190,0	1 214,0
		Average	2 100,0	2 630,0	1 280,0	1 215,0	1 210,0
	Dziloh (p_2)	(r_1)	1 700,0	2 430,5	1 030,0	1 200,0	1 050,0
		(r_2)	1 840,0	2 524,0	1 190,5	1 130,0	1 226,0
		(r_3)	1 920,0	2 545,5	1 190,5	1 165,0	1 210,0
		Average	1 820,0	2 500,0	1 137,0	1 165,0	1 162,0

Multi-factorial analysis of variance was confirmed statistically high significant effect of year and growing area for yield of dry drugg, while repetition were statistically insignificant. Interactions between year and plot were statistically insignificant, therefore Table 4. states the results of tests without major factor interactions. Tukey's contrast was found inconclusive difference between 2006 and 2008. Statistically highly significant difference was observed between 2004 and 2005 and following the growing season. It had also been confirmed a highly significant difference between cultivation plots, with higher yields were achieved on a plot of Pani Hura (p1). Even the experiment with mint peppermint been through multi-grown on the same plot recorded lower yields and reduce overall state of fitness stand.

Table 4

Statistical evaluation of yield *Menthae piperitae herba* by analysis of variance

Examined factor	Level of factor	F-test	Level of significance	Average	Significant ($\alpha = 0,01$)
Year	2007	291,631	0,0000 + +	1 186,00	A
	2008			1 190,00	A
	2006			1 208,50	A
	2004			1 960,00	B
	2005			2 565,00	C
Plot	(p_2)	15,935	0,0006 + +	1 556,80	A
	(p_1)			1 687,00	B
Repetiton	(r_1)	0,985	0,3893 -	1592,70	A
	(r_3)			1624,40	A
	(r_2)			1648,60	A

Rosmarinic acid content in the dry tops of balm was evaluated during the last three growing years, ranging from 28 613,8 mg.kg^{-1} (2007) to 36 532.4 mg.kg^{-1} (2006). Both values were from samples collected from the area Dziloh (tab. 6).

Table 6

Rosmarinic acid content (mg.kg-1) of *Melissae herba* harvested in the locality Plavnica

Drug	Name of parcel	Growing year		
		2006	2007	2008
Melissae herba	Pani hura (p_1)	36350,0	29673,2	33352,2
	Dziloh (p_2)	36532,4	28613,8	31425,0

Analysis of variance confirmed a statistically significant effect on content of rosmarinic acid in dry peppermint - *Menthae piperitae herba*. Impact of area was statistically inconclusive. Tukey's contrast confirmed significant difference between the year 2006, 2007 and 2008 (tab. 7). Contrast between rosmarinic acid content due to growing plots was statistically inconclusive.

Table7

Statistical evaluation of rosmarinic acid content in *Menthae piperitae herba* by analysis of variance

Examined factor	Level of factor	F-test	Level of significance	Average	Evidence supporting (α = 0,01)
Year	2006	415,37	0,0024 + +	3771,37	A
	2007			3888,75	A
	2008			5105,81	B
Plot	(p_1)	14,01	0,0645 -	4176,96	A
	(p_2)			4333,67	A

Contents of caffeic acid in lemon balm tops was determined by liquid chromatography. Its contents fluctuated in the range from 726.8 (2007 plot Pani Hura) to 1 377.7 mg.kg^{-1} (2006 plot Dziloh). Analogous results were confirmed in peppermint, which was the lowest concentration of caffeic acid also in 2007 and the plot Pani hura with value 1 439.84 mg.kg $^{-1}$. The largest amount of caffeic acid containing plants harvested from plots Pani Hura in 2006, where content was recorded at 1 817.42 mg.kg^{-1} (tab. 8).

Table 8

Content of caffeic acid (mg.kg-1) of *Melissae herba* in the locality Plavnica

Drug	Name of parcel	Growing year		
		2006	2007	2008

Melissae herba	Pani hura (p_1)	1023,6	726,8	868,1
	Dziloh (p_2)	1377,7	792,6	951,8

From a statistical evaluation of caffeic acid content in the dry tops balm harvested from the agro-climatic conditions of cold agro-ecological locality Plavnica, show that the impact between the content and the aging of cultivation, as well as the content and growing parcel was statistically inconclusive (tab. 9).

Table 9

Statistical evaluation of the caffeic acid content in *Melissae herba* by analysis of variance

Examined factor	Level of factor	F-test	Level of significance	Average	Evidence supporting ($\alpha = 0,05$)
Year	2007	7,7039	0,1149	759,70	A
	2008		-	909,95	A
	2006			1200,65	A
Plot	(p_1)	3,2400	0,2137	872,83	A
	(p_2)		-	1040,70	A

Table 10

Caffeic acid content in *Menthae piperitae herba* at the lokality Plavnica

Drug	Name of parcel	Growing year		
		2006	2007	2008
Menthae piperitae herba	Pani hura (p_1)	1817,42	1439,84	1758,17
	Dziloh (p_2)	1596,67	1582,37	1475,62

From a statistical evaluation of caffeic acid content in the dry peppermint harvested from agro-climatic conditions of cold agro-ecological locality Plavnica shows, that the impact between the content and the aging of cultivation, as well as the content and growing parcel was statistically inconclusive (tab. 11).

Table 11

Statistical evaluation of the caffeic acid content in *Menthae piperitae herba* by analysis of variance

Examined factor	Level of factor	F-test	Level of significance	Average	Evidence supporting ($\alpha = 0,05$)
Year	2007	0,7294	0,5782	1511,105	A
	2008		-	1616,895	A
	2006			1707,040	A
Plot	(p_2)	0,8225	0,4601	1551,55	A
	(p_1)		-	1671,81	A

Correlation analysis indicated a positive correlation between yield of *Melissae officinalis herba* and production of rosmarinic acid (r = 0.86 ++). Linear regression analysis of the rosemary acid production from the yield may be represented by a linear equation y = 21,029x + 15 747, with a determination index 73.8%. With increasing harvest increases the rosemary acid content.

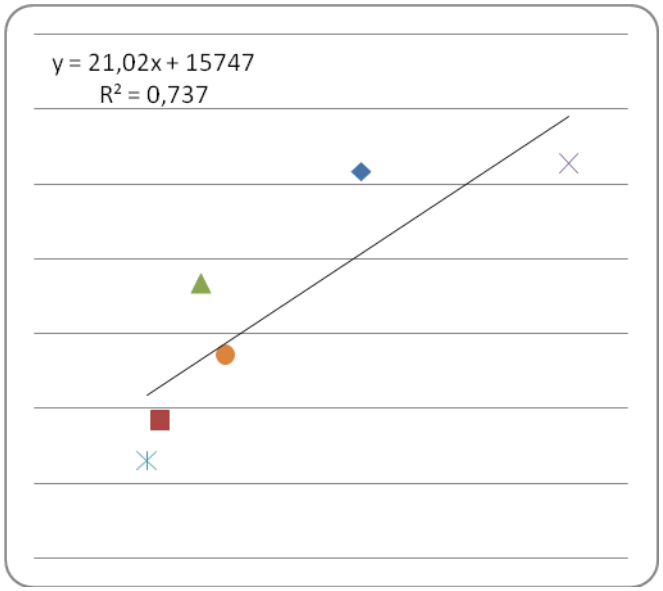

Figure 1. Graph linear regression analysis of the rosmarinic acid content for balm yield

Correlation analysis indicated a high positive correlation between the yield *Melissae officinalis herba* and production of caffeic acid (r = 0.98 ++). Linear regression analysis of caffeic acid production may be represented by a linear equation y = 1,6602x - 378.32 with determination index of 95.4%. With increasing harvest increases the content of caffeic acid.

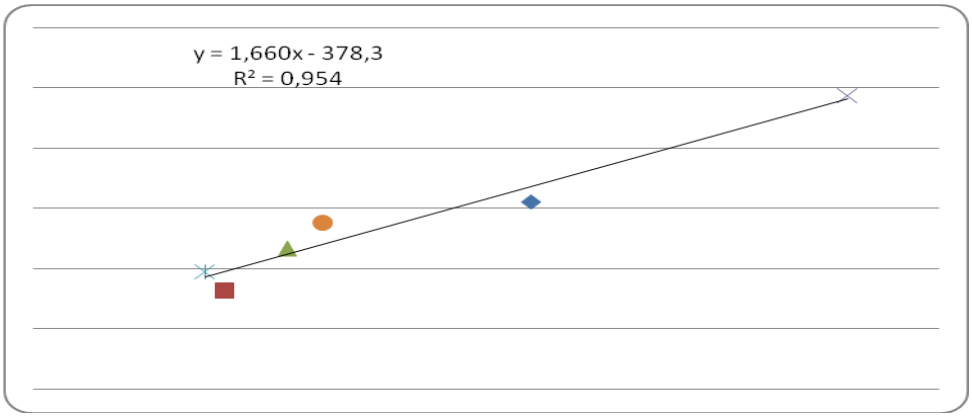

Figure 2. Graph linear regression analysis of the caffeic acid content for balm yield

In the case of rosmarinic acid production (r = -0.24 -) and caffeic acid (r = 0.58 -) in *Menthae piperitae herba* has not been established correlation dependence.

The results of rosemary acid content in different of medicinal and aromatic plants harvested from the agro-ecological conditions of heat and cold agro-climatic macroregion Slovakia are initial, are within the purview of the results achieved several scientific teams engaged in a hydroxycinnamic acid derivatives (Parhnham and Kesselrig, 1985; Fecka et al., 2002). Recently, the acid content in rosemary lemon balm grown in Slovakia interested Tóth et al. (2005), who also confirmed the variability from ontogenetic stages of growth. For this reason it will be necessary to verify the fluctuation rosmarinic acid, caffeic acid and chlorogenic acid in subsequent growing years.

CONCLUSION

In a field experiment with lemon balm grown in the years 2004-2008 in the agro-ecological conditions of cold agro-climatic macroregion at the locality Plavnica (Stará Ľubovňa) was confirmed statistically high significant effect of year and growing area for yield of dry drugs. The average yield of dry drug to all variants was 1 131,55 kg ha -1. Rosemary acid content in the dry tops balm was 28 613,8 mg.kg^{-1} (2007) to 36 532.4 mg.kg^{-1} (2006), which was confirmed by a statistically significant effect on rosemay acid content in the dry tops lemon balm.

In field trials with peppermint grown in the years 2004-2008 in agroecological conditions of cold agro-climatic macroregion Plavnica (Stará Ľubovňa), the average yield of dry drug at all variant 1 621,9 kg ha^{-1}. It was confirmed statistically high significant effect of year and growing area for yield. Rosemary acid content in the dry peppermint was recorded from 3 725.98 mg.kg^{-1} (2006 plot Pani Hura) to 5 223.0 mg.kg^{-1} (2008 plot Dziloh) and was confirmed a statistically significant effect of year on the rosemary acid content, the impact of area was

statistically inconclusive. Rosemary acid content in peppermint grown at locality in the cold agro-climatic macroregion were two and a half times lower in comparison with the concentration of the tops grown from hot agroclimatic macroregion.
Correlation analysis indicated a positive correlation between yield of dry tops balm and rosemary acid production (r = 0.86 ++). With increasing harvest increased the rosmarinic acid content.
Correlation analysis indicated a high positive correlation between the yield of dry tops lemon balm and production of caffeic acid (r = 0.98 ++) too. With increasing harvest increased the content of caffeic acid.

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