HERBAGE PRODUCTION AND CONTENT OF ROSEMARY ACID IN LEMON BALM (*MELISSA OFFICINALIS* L.) GROWN IN CULTURAL CONDITIONS OF WARM AGRI-CLIMATIC MACROREGION

ÚRODA VŇATE A OBSAH KYSELINY ROZMARÍNOVEJ V MEDOVKE LEKÁRSKEJ (*MELISSA OFFICINALIS* L.) PESTOVANEJ V KULTÚRNYCH PODMIENKACH TEPLEJ AGRO-KLIMATICKEJ MAKROOBLASTI

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Abstract: *In the solving year of 2005 – 2006 the analysis of agri-ecological conditions of Lemon balm cultivation was realized at Kolinany locality (Nitra district, Slovakia). Yield components were analyzed at Department of Sustainable Agriculture and Herbology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra. Lemon balm var. ´Citra´ was used as a testing material. Harvesting of the plant material has been carried out during whole vegetation period. The content of rosemary acid was determined in the samples. During the years 2005 – 2006 these experimental factors were tested: (1) the influence of the way of planting or sowing, e.g. the way of setting up the stand, (2) applied plant nutrition and (3) harvest time, on the reached level of aboveground herbage crop yield and content of rosemary acid in the drug, e.g. quality of air-dried drug. The average yields of Lemon balm drug (386.5 – 493.6 g.m^{-2}) and rosemary acid content (2.39 – 4.49 %) were found and statistically determined depending on sowing system, organic fertilization, and harvest time during two years of the experiment. High content of rosemary acid (over the required 4.0 % content) was confirmed in samples within both fertilization variants when stand was established from seedlings.*

Abstrakt: *V rokoch 2005 – 2006 bola realizovaná analýza agroekologických podmienok pestovania medovky lekárskej na lokalite Kolíňany (okres Nitra, Slovensko). Úrodotvorné prvky medovky lekárskej boli analyzované na Katedre udržateľného poľnohospodárstva a herbológie, Fakulty agrobiológie a potravinových zdrojov, Slovenskej poľnohospodárskej univerzity v Nitre. Ako rastlinný materiál bola použitá medovka lekárska, kultivar ´Citra´. Zber rastlinného materiálu bol vykonávaný v priebehu celého vegetačného obdobia. Vo vzorkách bol zisťovaný obsah kyseliny rozmarínovej. Počas obdobia 2005 – 2006 boli experimentálne sledované tieto faktory: (1) spôsob založenia porastu, t.j. výsadba z priesad alebo priama sejba, (2) aplikovaná výživa pre rastliny a (3) termín zberu, na dosiahnutú úrodu nadzemnej biomasy a obsah kyseliny rozmarínovej v droge, t.j. kvalita tzv. vzduchosuchej drogy. Priemerné úrody drogy medovky lekárskej (386,5 – 493,6 g.m^{-2}) a obsah kyseliny rozmarínovej (2,39 – 4,49 %) boli zistené a štatisticky vyhodnotené v závislosti od spôsobu založenia porastu, organického hnojenia a termínu zberu. Súčasne boli stanovené obsahy kyseliny rozmarínovej, ktoré potvrdili jej vysoké zastúpenie (nad požadovaných 4,0 %) v rastlinnej hmote a to najmä vo vzorkách obidvoch variantoch hnojenia, kde bol porast založený z priesad.*

Key words**:** *essential oil, growing, Lemon balm (Melissa officinalis L.), rosemary acid*
Kľúčové slová: *silica, pestovanie, medovka lekárska (Melissa officinalis L.), kyselina rozmarínová*

INTRODUCTION

Therapeutic effects of Lemon balm (*Melissa officinalis* L.) are described mainly due to essential oil content and therefore many studies have been directed to the analysis of volatile substances obtainable from the plant. One of the important medicinal plant species grown in agri-ecological conditions of Slovakia is Lemon balm (*Melissa officinalis* L.), which is used as

a basic raw material for the production of herb tea, phytopharmaceuticals and cosmetics (Habán, 2004). For this reason, this study was arranged to measure and analyze productive characteristics of this medicinal plant grown in warm agri-climatic conditions (macro-region) of Slovakia (Kováč et al. 2005, Macák et al. 2006). Rosemary acid is perspective nature based chemical substance isolated from the most plant species of *Lamiaceae* family (Spilková 1997, 1998). This substance is used in pharmaceutical industry as an antioxidant for medicinal cosmetics, and inflammation or antimicrobial component in appliance for oral hygiene. It is possible to use rosemary acid in cosmetics, food and pharmaceutical industry mostly in 0.5 – 2.0 % concentrations. Pure rosemary acid is not a component of any pharmaceuticals yet. However, there are many phytotherapeuticals, which contain extracts standardized to these chemical substances (Holzmannová 1996). Using of rosemary acid as an antioxidant is less suitable for food industry from the economy point of view, however, it will be perspective after improve of technological procedures. Biotechnology of rosemary acid production from plant cell-tissue cultures was proposed by Petersen & Simmonds (2003).

Lemon balm (*Melissa officinalis* L.) belongs to the plant resources with higher content of rosemary acid, which can be grown and produced in Central European agri-ecological conditions or obtained from plant natural resources. Isolation of rosemary acid from Lemon balm was managed on Food Research Institute in Bratislava – Biocentre Modra, Slovakia.

MATERIAL AND METHODS

Plant material

During the experimental fieldwork plant material of Lemon balm (*Melissa officinalis* L.) cultivar ´Citra´ (Czech Republic) was used.

Characteristics of the locality

The area, in which the experimental field is situated, geomorphologically belongs to Žitavská pahorkatina (hills) as a part of Podunajská nížina (lowland). Geomorphologic shape of 19 ha experimental station is plain with mild declination to the South (1 – 2°). Altitude is 170 – 180 m above sea level (Slovík & Libant, 1996). The University Farm of Slovak University of Agriculture in Kolíňany village manages these experimental fields. This locality belongs to a maize production type and a barley subtype.

Climatic conditions

The area where experimental work was carried out can be divided according to agri-climatic conditions (Špánik et al. 1995) into:

- Macro-area – warm with temperature sum t > 10°C; within 3,100 – 2,400°C;
- Area – mostly warm with temperature sum t > 10°C, within 3,000 – 2,800°C;
- Sub-area – very dry with rate of the climatic irrigation indicator in VI – VIII months = 150 mm;
- Small area – mostly mild winter with average absolute minimum T_{min} = -18 to – 21°C.

Average air temperature – the highest average temperature (23.1°C) per month was recorded in July 1994 and a year temperature (11.1°C) in 1994 and 2000. The lowest temperature per month (-3.2°C) was in January 2000 and a year temperature (9.0°C) in 1996. The average air temperature within years 1961-1990 was 9.8°C (between 8.4-10.8°C), within years 1991-2000 it was 10.2°C (between 9.0-11.1°C).

Atmospheric rainfalls – average total rainfall per year in 1961-1990 periods was 532.5 mm (350-761 mm), within years 1991-2000 it was 539 mm (436-680 mm). While the

average rainfall was not very different the average air temperature in the last 10 years was 0.4°C. The highest total rainfall per month occurred in September 1998 (149.6 mm), the lowest in February 1998 (0.0 mm). Total rainfall during a year changed from 436.1 mm (1991) to 680.4 mm (1995).

Setting up the experiment

The polyfactorial field experiment was set up and experimentally tested within two vegetative years (2005, 2006). The experiment was held in three individual blocks. The structure of experimental factors and variants was as follows:

A – the way of setting up the stand
- a1 – via seeds
- a2 – via seedlings

B – fertilizing
- b1 – non-manured
- b2 – manured

T – Year of the experiment
- T1 – 2005
- T2 – 2006

R – repeating of the experiment
- r1 – 1st repetition
- r2 – 2nd repetition
- r3 – 3rd repetition

Crop evaluation by quantitative ecology method

Reached crop yields were evaluated by analysis of plant structure with the use of quantitative ecology method, i.e. the quantity of dry biomass per area unit. Particular pickings of herbs were realized on productive fields by a destructive method. The area of 1 m^2 was marked in the cover. Plant material was harvested in the beginning of flowering ontogenetic growing phase. Stems of the plant were cut by scissors. The picking of samples was done at three places in one area. The mass of fresh phytomass was weighed in laboratory conditions. Drying of samples was done right after weighing in temperatures to 35 – 40°C in a dark room and after that in well-aired laboratory drying room. The gained dry biomass was weighed on scales type Sartorius.

Rosemary acid isolation

Isolation of rosemary acid from Lemon balm using HPLC method was managed at Department of Sustainable Agriculture and Herbology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovakia. Principle of the isolation technology is given in details by Wang et al. (2004).

RESULTS AND DISCUSSION

The results show that the highest crop yield of Lemon balm (*Melissa officinalis* L.) was obtained in the stand of manured plants established from seedlings (493.6 $g.m^{-2}$, A_2b_2) in 2005. The lowest yield was marked in the manured stand set up from seedlings (386.5 $g.m^{-2}$, A_2b_2) in the year 2006. Statistical evaluation of dry herbage crop yield shows no influence of the way of the setting up the stand and nor influence of application of organic manure (Table 1). However, better results, from the point of view setting up the cover, were reached when seedlings were used (433.5 $g.m^{-2}$) in comparison to the direct seeding (430.1 $g.m^{-2}$).

Lemon balm also reacts to fertilization with organic manure. Higher crop yields were obtained when these plants were manured (437.7 $g.m^{-2}$) in comparison with non-manured plants (425.8 $g.m^{-2}$). The influence of vegetative year on the level of crop yield was also statistically determined. These results were highest in the first year of experiment (2005: 454.2 $g.m^{-2\ ++}$) and in the following year they were decreasing (2006: 409.4 $g.m^{-2\ ++}$). The results reported by Vaverková et al. (2003) correspond with our results. The authors who dealt with productive characteristics of the Lemon balm stand (Kišgeci et al., 1987, Stepanović, 1998, Habán et al., 2004) reported that the aboveground herbage crop yields ranged from 150.0 to 250.0 $g.m^{-2}$. The herbage crop yield of Lemon balm grown in warm agri-climatic conditions within our experiment reached the amount of 386.5 $g.m^{-2}$ to 493.6 $g.m^{-2}$ as it is mentioned above.

Table 1

Dry aboveground crop yields of Lemon balm [$g.m^{-2}$] in the years 2005 – 2006

The way of setting the cover	Manure	Year 2005 (T1)	Year 2006 (T2)	Average 2005 - 2006
Direct-seeds (a1)	without manure (b1)	440.0 (r1)	405.3 (r1)	422.7
		421.0 (r2)	410.3 (r2)	415.7
		433.0 (r3)	438.9 (r3)	436.0
		431.3	418.1	424.7
	with manure (b2)	400.0 (r1)	415.6 (r1)	407.8
		475.0 (r2)	429.2 (r2)	452.1
		469.0 (r3)	426.0 (r3)	447.5
		448.0	422.6	435.3
Average (a1)		439.7	420.4	430.1
Seedlings (a2)	without manure (b1)	450.0 (r1)	440.0 (r1)	445.0
		440.5 (r2)	380.3 (r2)	410.4
		440.0 (r3)	410.0 (r3)	425.0
		443.5	410.1	426.8
	with manure (b2)	491.5 (r1)	344.5 (r1)	418.0
		500.0 (r2)	400.0 (r2)	450.0
		489.5 (r3)	415.5 (r3)	452.5
		493.6	386.5	440.1
Average (a2)		468.6	398.3	433.5
Total average		454.2 a	409.4 b	431.8

Legend: A – the way of setting up the stand: a1 – via seeds, a2 – via seedlings; B – fertilizing: b1 – non-manured, c2 – manured; T – Year of the experiment: T1 – 2005, T2 – 2006; R – repeating of the experiment: r1 – 1st repetition, r2 – 2nd repetition, r3 – 3rd repetition.

The analysis of rosemary acid content in the Lemon balm drug shows that the highest content was obtained from the samples of manured plants established from seedlings (4.70 %, A_2b_2) in 2005. The lowest content of rosemary acid was marked in the non-manured stand set up from seedlings (2.21 %, A_2b_1) in the year of 2006. Statistical evaluation of rosemary acid content in Lemon balm plant samples shows high influence of the organic manure application

(Table 2). The higher content of rosemary acid corresponds with organic fertilization. Significantly higher content was obtained when samples were originated from plants that were manured (3.73 % $^{++}$) in comparison with non-manured plants (3.24 % $^{++}$). The influence of vegetative year on the rosemary acid content was also statistically determined. These results were highest in the first year of experiment (2005: 4.20 % $^{++}$) and in the following year they were decreasing (2006: 2.77 % $^{++}$), similarly as it was in aboveground Lemon balm crop yields. There were no statistical differences determined from the point of view setting up the cover when analyze the rosemary acid content: 3.44 % when seedlings were used in comparison to the direct seeding 3.53 %.

Table 2

The content of rosemary acid in Lemon balm [%] in the years 2005 – 2006

The way of setting the cover	**Manure**	**Year 2005 (T1)**	**Year 2006 (T2)**	**Average 2005 – 2006**
Direct-seeds (a1)	without manure (b1)	3.64 %	2.83 %	3.24 % b
	with manure (b2)	4.16 %	3.46 %	3.81 % a
Average (a1)		3.90 %	3.15 %	3.53 %
Seedlings (a2)	without manure (b1)	4.27 %	2.21 %	3.24 % b
	with manure (b2)	4.70 %	2.57 %	3.64 % a
Average (a2)		4.49 %	2.39 %	3.44 %
Total average		4.20 % a	2.77 % b	3.49 %

Legend: A – the way of setting up the stand: a1 – via seeds, a2 – via seedlings; B – fertilizing: b1 – non-manured, c2 – manured; T – Year of the experiment: T1 – 2005, T2 – 2006.

CONCLUSIONS

Lemon balm (*Melissa officinalis* L.) cultivar ´Citra´ is suitable for growing in the tested warm agriclimatic macro-area. This was approved by results of two-year experiment during 2005 – 2006 growing seasons. The average yields of Lemon balm (*Melissa officinalis* L.) drug (386.5 – 493.6 g.m^{-2}) and rosemary acid content (2.39 – 4.49 %) were found and statistically determined depending on sowing system, organic fertilization, and harvest time during two years of the experiment. According to the presented results it is recommended to continue in the research of the influence of intensification factors (the way of setting up the stand, nutrition, etc.) on the content of active ingredients in grown plants. Choosing of suitable genotypes with minimum required content of this acid in drug (4 %) cultivated in cultural agri-ecological conditions could increase the profitability of rosemary acid isolation.

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