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Note

Qualitative properties of *Melissa officinalis* after the application of Rastim 30 DKV

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ABSTRACT: *Melissa officinalis* L. (lemon balm) is one of the important medicinal plant species. It is important to determine what conditions the plant needs for its optimum growth and development and when the plant contains the most high-quality oils. The aim of our work was to investigate the content and composition of essential oil in various samples of the plants at various developmental stages of growth and to monitor the effect of the auxinoid growth regulator Rastim 30 DKV. Rastim 30 DKV treatment did not change the quality of lemon balm essential oil in comparision with control plants.

Key words: *Melissa officinalis* L., essential oil, ontogenesis, leaf inserts, Rastim 30 DKV [3-(benzyloxycarbonyl-methyl)-2-benzothiazolinone]

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UDK

Lemon balm (*Melissa officinalis* L.) is grown mainly for its essential oil which is located in the trichome glands. It can be obtained from fresh or dried flowers, leaves, and branches of this plant. The main components of the esential oil are citronellal (2-40%) and citral (neral and geranial) 10-30%, along with β-caryophyllene, germacrene D, ocimene and citronellol (Tittel *et al.* 1982; Enjalbert *et al.* 1983; Schultze *et al.* 1989; Sarer & Kőkdil 1991; Adzet *et al.* 1992; Kreis & Mosandl 1994; Sorensen 2000; Blum & Lorenz 2005).

These components of lemon balm essential oil are monoterpenes and sesquiterpenes. Terpenes are divided into oxygen-containing compounds and hydrocarbons. The hydrocarbon content is considerably lower than the content of oxygen-containing compounds (Tittel *et al.*1982). The most important hydrocarbon is β-caryophyllene, accompanied by α-humulene, δ-cadineme, α-copaene, α-cubebene, and β-bourbonene (Enjalbert *et al.* 1983). Most of the oxidised products are citrals a, b (geranial and neral), which represent a cis/trans isomeric pair (Tittel *et al.* 1982; Sorensen 2000).

Several studies have analysed the total essential oil content and its composition in *Melissa officinalis L.* of different origin. Considerable variability in both these parameters can be found due to the influence of several factors, which also affect the essential oil pharmaceutical quality. These differences may be due to external conditions during plant growth ((Tittel *et al.* 1982; Hose et al. 1997; Carnat et al. 1998; Sari & Ceylan 2002) as well as genetic variability (Wolf *et al.* 1999).

This study was conducted to examine the content and composition of essential oil in *M. officinalis* throughout the growing season and to compare the content of essential oils in different parts of the plant. In the experiment we have used Rastim 30 DKV (a growth regulator from the auxinoid group), which is primarily used for the treatment of agricultural crops to accelerate flowering, but is recommended also for lemon balm cultures. We compared Rastim 30 DKV-treated plants with control plants.

The experimental material was *M. officinalis* grown in the region of Nitra. The field surface area was 10 square

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metres. The plants were grown in 40 x 40 cm plots. Some plants were treated with the growth regulator Rastim 30 DKV to accelerate the germination and the onset of flowering. All samples were taken in the third year of vegetation. Collection of plant material was carried out throughout the growing season in the morning. Leaves were collected by hand, while stem foliage was cut a few cm above the ground. Dates of collection were selected with respect to the plant development stage (flower initiation, flushing, onset of flowering, full bloom, biological maturity).

The whole plant was dried in the open air. After drying, the leaves and other organs were separated from stems to prevent their fragmentation.

Determination of essential oil content was carried out by steam distillation of the drug according to the Slovak Pharmacopoeia SL (Slovenský liekopis, 1977) and the European Pharmacopoeia (2005) for 4 h. The content was calculated from 3 parallel analyses and expressed in % V/m (i.e. ml per 100g of drug).

Analysis of the oil samples was done using a gas chromatograph CHROME 61, FID with a recorder and integrator CI 100 (Laboratorní přístroje, Prague, Czech Republic). A capillary column DB - 17 (30 m: 0.32 mm, film thickness 0.25 μm) quartz (Quadrex, USA) was used. The temperature programme was as follows: temperature of column was held at 60 °C for 2 min, then 4 °C/min to 220 °C for 15 min.; temperature of injector 210 °C, temperature of detector 250 °C. Carrier gas N 1 ml/min, injected sample 0.2μl (microsyringe Hamilton, U.S.A.). Mass spectra were recorded at EI of 70 eV.

Components of the oils were identified by comparison of their mass spectra with those from databases NBS 75K, Hewlett Packard, INRA MASS WILEY 275. An important factor influencing the content of plant essential oils is the developmental phase of the plant. Our results indicated that the essential oil content of *M. officinalis* changed during the vegetation period. The total amount of essential oil ranged between 0.06 to 0.26% (V/m). During the early development of the plants the oil content was relatively low. In the period before flowering, the content of oil was increasing with a peak at the onset of flowering (0.18 to 0.26%). This maximum was observed for both treated and control plants and their oil content decreased with gradual aging. At the stage of full bloom the plant ceases to grow and its essential oil content decreased (Table 1). Our findings are in agreement with the results of other authors (Hefendehl 1970; Sari & Ceylan 2002; Patora *et al.* 2003; Basta *et al.* 2005).

When comparing the main components of essential oil in particular leaf inserts, we found the greatest content of citronellal in the basal leaves (51% in control plants, 48.2% in treated plants) and the lowest content of citronellal in the apical leaves (10.3% in control plants, 9.3% in treated plants). The highest content of citral a and b was in the apical leaves: citral a (39.6% in control plants, 36.9% in treated plants), citral b (37.8% in control plants, 29.1% in treated plants). The lowest content of citral was found in the basal leaves, citral a (15.2% in control plants, 16.3% in treated plants), citral b (12.4% in control plants, 17.0% in treated plants). The highest proportion of β-caryophyllene was located in the basal leaves (14.9% in control plants, 16.5% in treated plants) and the lowest in the apical leaves (10.5% in control plants, 12.9% in treated plants) (Table 2, 3), which is consistent with the results of Hefendehl (1970), Tittel *et al.* (1982), Werker *et al.* (1985), Schulze *et al.* (1989), Carnat *et al.* (1998), Sari & Ceylan (2002), Sandraei *et al.* (2003).

Table 1. Essential oil content from the above-ground parts of control and treated lemon balm plants in different developmental phases

Ontogenesis phase	Essential oil content ml	(control) % (V/m)	Essential oil content ml	(treated) % (V/m)
Flower initiation	0.006 0.008 0.007	0.07	0.008 0.007 0.003	0.06
Flushing	0.010 0.012 0.017	0.13	0.016 0.014 0.021	0.17
Onset of flowering	0.020 0.017 0.017	0.18	0.030 0.024 0.024	0.26
Full bloom	0.009 0.016 0.020	0.15	0.020 0.014 0.020	0.18
Biological maturity	0.007 0.010 0.010	0.09	0.009 0.013 0.011	0.11

Table 2. Content of the main essential oil components in various parts of control plants

	citronellal	geranial (citral a)	neral (citral b)	β-caryophyllene
Apical leaves	10.3	39.6	37.8	10.5
Central leaves	25.9	26.5	30.1	12.7
Basal leaves	51.0	15.2	12.4	14.9

Table 3. Content of the main essential oil components (%) in various parts of plants treated with Rastim 30 DKV

	citronellal	geranial (citral a)	neral (citral b)	β-caryophyllene
Apical leaves	9.3	36.9	29.1	12.9
Central leaves	26.7	25.8	28.7	13.1
Basal leaves	48.2	16.3	17.0	16.5

Table 4. Content of essential oil (%)in various parts of control plants and plants treated with Rastim 30 DKV

	Apical leaves	Central leaves	Basal leaves
Control plants	0.21	0.15	0.03
Treated plants	0.26	0.17	0.07

Some authors (Adzet *et al.* 1992; Hose *et al.* 1997; Basta *et al.* 2005) reported that young leaves contain larger amounts of the essential oil than older, fully developed leaves, on which the secerning glands and gland trichomas start to degenerate. Our results regarding the essential oil content in lemon balm leaf inserts from control plants and plants treated with Rastim 30 DKV are in agreement with these findings. The highest essential oil content was found in the apical leaves and the lowest essential oil content in the basal leaf inserts both in the control and treated plants while the essential oil content in treated plants was slightly higher in all groups of leaves (Table 4).

The experimental work, subsequent analysis of samples, and evaluation of results led to conclusions important from the viewpoint of significant changes in the content and composition of the essential oil taking place in the plant in the course of the vegetation period.

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Botanica SERBICA

REZIME

Kvalitativna svojstva matičnjaka (*Melissa officinalis* L.) nakon primene Rastim 30 DKV

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Melissa officinalis L. (matičnjak) je važna lekovita biljka. Uslovi rasta i razvoja ove biljke kad ona sadrži najkvalitetnija ulja nisu jasni. Cilj ovog rada bio je da se utvrdi sadržaj i sastav esencijalnih ulja matičnjaka kod različitih primeraka u različitim razvojnim fazama i da se prati uticaj primene Rastim 30 DKV. Rastim 30 DKV nije značajno uticao na sadržaj eteričnih ulja matičnjaka u odnosu na kontrolne biljke koje njime nisu tretirene.

Ključne reči: *Melissa officinalis*, esencijalna ulja, ontogeneza, Rastim 30 DKV [3-(benziloksikarbonil-metil)-2-benzotiazolinon]