Phytotoxicity and phenolic compounds content in soil during long-term cultivation of lemon balm (*Melissa officinalis* L.) and its effect on herb yield and essential oil content

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Summary

The object of experiments carried out in 2007 and 2008 were plants of lemon balm (*Melissa officinalis* L.) which were at that time in the 1st, 3rd and 4th year (2007) and in the 2nd, 4th and 5th year (2008) of growing. The soil from plots on which the plants were grown was studied as well. The yield of fresh and dry matter of the herbal raw material as well as the essential oil content in the raw material were determined. Furthermore, the soil phytotoxicity and phenolic compounds content in the soil were studied. Maximum yield of the fresh and dry weight of the herbal raw material were obtained in the 3rd and 4th year of plant cultivation and it was maintained on an unchanged level in the 4th and 5th year of growing. As the time of lemon balm growing was elongated, the increase of essential oil yield was observed. Phytotoxicity of soil occurred seasonally – in spring and in autumn and did not depend on the year of growing duration. On the other hand, the content of phenolic compounds in the soil increased gradually with the long-term cultivation. However, the phenolic compounds did not accumulate in such amount as to exert a negative effect on the plants.

Key words: *lemon balm, long-term cultivation, soil phytotoxicity, phenolic compounds, herb yield, essential oil*

INTRODUCTION

In long-term cultivations, decrease of yielding caused by soil sickness is frequently observed. A number of biotic and abiotic factors are responsible for this phenomenon [1]. The reason is either the accumulation of compounds with a phytotoxic character secreted by plants in the soil or their development as a result of the decomposition of post-harvest residues, whereby, they most frequently consist of phenolic compounds [2, 3]. Studies referring to these problems were carried out mainly in long-term cultivations of agricultural and fruit plants. However, there are no studies dealing with medicinal plants.

The objective of the presented studies was the investigation of the yielding and the essential oil content in the raw material of lemon balm depending on the plant age and a verification whether in the long-term cultivation of this species the soil sickness is caused by the accumulation of phytotoxic compounds.

MATERIAL AND METHODS

In 2007 and 2008, the experiment was conducted in the Research Institute of Medicinal Plants in Poznań, Poland. In spring 2007, on the field where lemon balm had been cultivated for two and three years, plots of 5 m^2 were marked out for the exact experiment. At the same time, on a field beside, in a place, where lemon balm had not been grown earlier, on plots of 5 m^2, lemon balm seedlings were planted in 45 x 45 cm spacing. The experiment was established in 3 replications. One replication was represented by a plot of 16 plants. The objects of studies were lemon balm plants in the ages of: 1 and 2, 3 and 4 as well 4 and 5 years, and the soil on which they were grown. The harvest was done manually in the first decade of July in each of the studied years. The yield of fresh and dry matter of herbal raw material, as well as the essential oil content in the raw material were determined. The essential oil was isolated from the herb without stems by hydrodistillation in the Dering's apparatus according to methods recommended by Polish Pharmacopoeia VI [4]. The essential oil yield obtained from area unit was calculated basing on oil content in the raw material and on the yield of dry raw material.

The phytotoxicity degree and the content of phenolic compounds in the soil were studied three times during vegetation season – in April, July and October. Phytotoxicity of soil was estimated in bioassay [5]. Soil extracts were prepared according to the method described by Politycka et al. 1989 [6]. Phenolic compounds were extracted from soil according to the method of Politycka and Wójcik-Wojtkowiak [7]. The total contents of phenolic compounds were determined spectrophotometrically using Folin and Ciocalteu's Phenol Reagent (Sigma-Aldrich) with *p*-coumaric acid as a standard.

Results were subjected to one-way analysis of variance and significant differences were estimated by Duncan's test at the level of $p<0.05$, and were presented as mean values of two-year studies.

RESULTS AND DISCUSSION

Fresh and dry matter yield of raw material in the 1st and 2nd year of cultivation were not high (fig. 1). In the 3rd and 4th year of studies, the fresh matter yield increased three-fold and it was maintained on that level in the 4th and 5th year of cultivation. At the same time, the yield of raw material dry matter increased twice, in comparison with the yield in the 1st and 2nd year of growing. Similar increase of yield was obtained by Saglam et al. [8] who compared lemon balm herb obtained in the 2nd and 3rd year of cultivation. However, those authors did not find any differences in the essential oil content depending on the age of plants which oscillated from 0.20 to 0.28% and it was similar to the content found by Kordana et al. [9] and by Holla et al. [10]. In our experiment, a similar content of essential oil was found in lemon balm raw material (fig. 2). It oscillated within the limits from 0.22 to 0.28%. Despite the fact that no significant differences were proven depending on the long-term cultivation, a tendency indicating that the content of oil increased with the age of plants could be observed. This fact exerted an influence on the essential oil yield from area unit. In the 3rd and 4th year of cultivation, the oil yield increased by 100% and in the 4th and 5th year it increased by about 150% as compared with the oil yield obtained in the 1st and 2nd year.

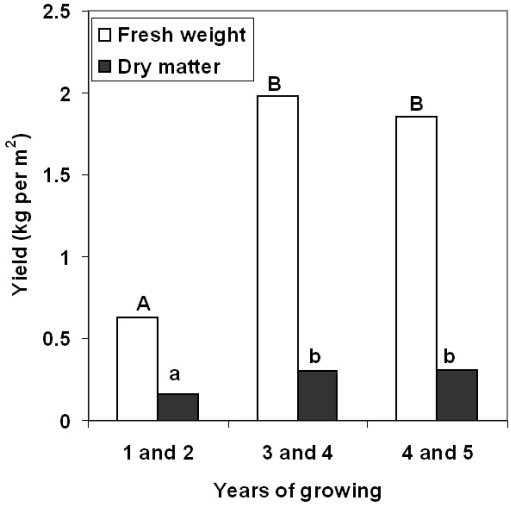

Figure 1. Yield of fresh and dry lemon balm raw material during long-term growing. Values marked with the same letters do not differ significantly

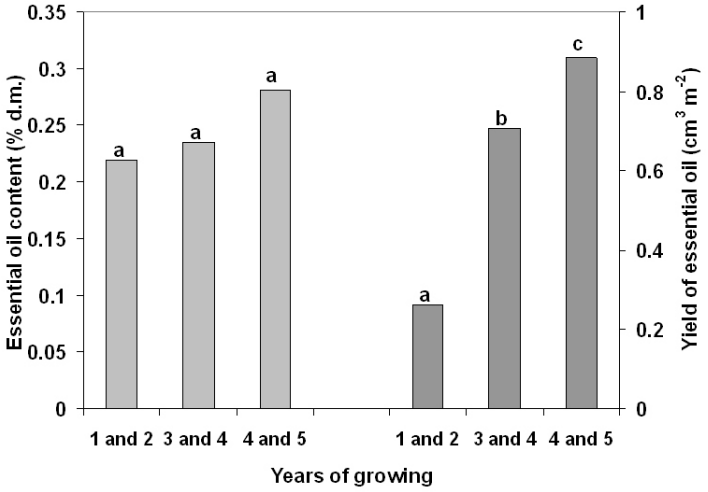

Figure 2. Total content and yield of lemon balm essential oil during long-term growing. Values marked with the same letters do not differ significantly.

Secondary metabolites present in plant tissues can be secreted by plants or can be released from the dead plant parts and accumulated in the soil. This is one of the reasons of soil sickness which may cause a negative after-effect in successive plants or it may lead to autotoxicity in long-term cultivations and monocultures [10]. Results obtained by Kato-Nogushi [11, 12] proved that lemon balm can contain compounds of phytotoxic properties. That author found an inhibitory effect of extracts from herb of lemon balm on the germination and growth of several species of cultivated plants and weeds. In our experiment, the phytotoxicity of soils from lemon balm fields was estimated (fig. 3). It was found that phytotoxic effect appeared seasonally – in spring and autumn, and its intensity was small (9–14% of inhibition in relation to the control bioassay). On the other hand, soil collected in the summer showed a weak stimulating effect (by 6% on the average in relation to control). This effect was observed independently from the duration of plant growing. As reported by An et al. [3], phytotoxic compounds released from plant residues in low concentration show a stimulatory effect of plant growth, while in higher concentrations they reveal an inhibitory action. Seasonal changes in the level of phytotoxic compounds can be explained by the fact that secondary metabolites occurring in high amounts in lemon balm tissues represent volatile substances whose diffusion from the soil increases with the increase of temperature, i.e. in summer [13]. Next to the phytotoxicity, the soil was also analysed regarding its content of phenolic compounds (tab. 1). Their level remained stable during the vegetation season; however, it increased when the cultivation was elongated. In the 3rd and 4th year of cultivation, the content of phenolic compounds was higher by 30%, while in the 4th and 5th year, it was higher by 60%, in comparison with the

cultivation in the 1st and 2nd year. However, these compounds did not accumulate in the soil in amounts which could exert a negative effect on the plants, because no decrease in lemon balm yield or any dependence between the phytotoxicity degree of soil and the phenolic compounds content were found.

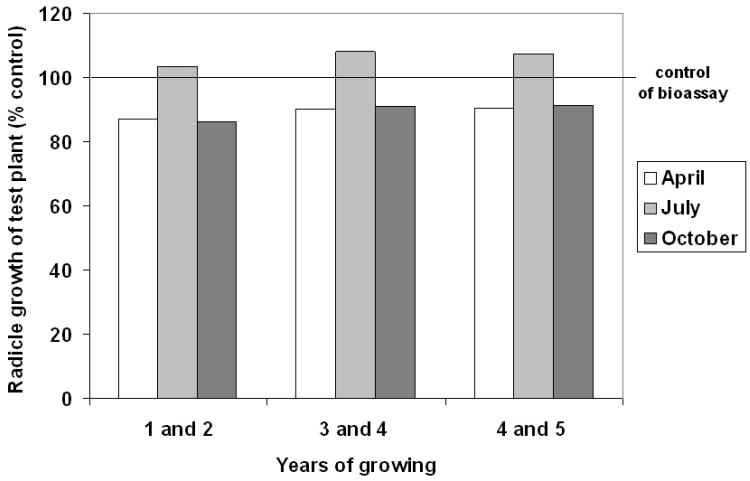

Figure 3. Phytotoxicity of soil during long-term growing of lemon balm

Table 1.

Phenolic compounds content in soil during long-term growing of lemon balm ($\mu g\ g^{-1}$ dry matter of soil)

year of growing	time of sampling			mean
	April	July	October	
1st and 2nd	22.99 a*	21.45 a	24.71 a	23.05
3th and 4th	29.86 b	31.79 b	29.52 b	30.39
4th and 5th	40.50 c	34.50 bc	35.50 bc	36.83
Mean	31.12	29.25	29.91	

*Values marked with the same letters do not differ significantly.

CONCLUSIONS

1. As a result of long-term cultivation of lemon balm no decrease in the yield of raw material was found. However, the yield of essential oil increased.
2. When the cultivation of lemon balm was extended for more years, no phytotoxic substances were found in the soil which could exert a negative effect on the growth and development of plants. The content of phenolic compounds was increasing, however, in the period of five years, the amount of these compounds did not reach any level that could be toxic to the plants.

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FITOTOKSYCZNOŚĆ I ZAWARTOŚĆ ZWIĄZKÓW FENOLOWYCH W GLEBIE PODCZAS WIELOLETNIEJ UPRAWY MELISY LEKARSKIEJ (*MELISSA OFFICINALIS* L.) I JEJ WPŁYW NA PLON ZIELA I ZAWARTOŚĆ OLEJKU ETERYCZNEGO

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S t r e s z c z e n i e

Obiektem badań w doświadczeniu przeprowadzonym w latach 2007 i 2008 były rośliny melisy w 1., 3. i 4. (2007) oraz 2., 4. i 5. roku (2008) uprawy oraz gleba z poletek, na których rosły. Oznaczano plon świeżej masy części nadziemnych i suchej masy surowca zielarskiego oraz zawartość olejku eterycznego w surowcu. Ponadto badano fitotoksyczność oraz zawartość związków fenolowych w glebie. Maksymalny plon świeżej masy części nadziemnych i plon suchej masy surowca zielarskiego osiągnięto w 3. i 4. roku uprawy roślin i utrzymywał się on na niezmienionym poziomie w 4. i 5. roku uprawy. W miarę przedłużania czasu uprawy bazylii stwierdzono zwiększanie plonu olejku. Fitotoksyczność gleby występowała sezonowo – wiosną i jesienią – i nie była zależna od czasu trwania uprawy. Natomiast zawartość związków fenolowych w glebie zwiększała się stopniowo wraz z czasem trwania uprawy. Nie gromadziły się one jednak w ilościach, w których mogłyby negatywnie oddziaływać na rośliny.

Słowa kluczowe: *melisa lekarska, uprawa wieloletnia, fitotoksyczność gleby, związki fenolowe, olejek eteryczny, plon*